Forest stand characteristics of Jefferson National Forest that observed gypsy moth defoliation from 2015-2019 in southwestern Virginia

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ABSTRACT

The gypsy moth (*Lymantria dispar* L., Lepidoptera: Erebidae) is an invasive forest pest that has greatly impacted oak dominated forest stands in the northeastern United States. The pest, introduced in the 1860s in Massachusetts, has spread significantly north, south, and west. The National Gypsy Moth Slow the Spread (STS) limits its spread by targeting isolated, low-density gypsy moth populations with aerial applications of insecticides and/or pheromones. Other activities by state and federal agencies include conducting aerial and ground-truth surveys of forest stand injury, resulting from gypsy moth defoliation. Annually, thousands of forested acres across the Commonwealth of Virginia are defoliated by gypsy moths. This survey was conducted to compare and identify some forest stand characteristics of infested (outbreak) and uninfested (control) areas of Virginia that could be used to predict further gypsy moth defoliation. All of the research sites were located in southwestern Virginia in close proximity to the Jefferson National Forest. The survey found stand density index, proportions of defoliation per site, and total basal area per acre of preferred host trees with gypsy moth injury to be significant factors in examining forest susceptibility.

This research should be continued to further analyze these forest characteristics and help predict where future stand defoliation could occur.

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Introduction

The gypsy moth, *Lymantria dispar L. (*Lepidoptera: Erebidae), is a major invasive forest pest that has plagued the eastern United States for more than 150 years. Introduced into the United States in Medford, Massachusetts in 1869, gypsy moth has spread north, south, and west across various terrains along the Appalachian Mountains, the Great Lakes Region, and into Canada. Gypsy moth populations have spread through natural and artificial means. Naturally, early-instar gypsy moth larvae can disperse off silken threads by strong winds (Schweitzer 2004). Gypsy moth females deposit egg masses on tree bark or household articles (USDA 2020). Anthropogenically, these trees or articles are transported to other locations where new populations can become established (Liebhold et al. 1995).

Currently, gypsy moth is established throughout the northeastern U.S. and the Great Lakes (Figure 1). It first arrived in Virginia in the early 1980s (Roberts 2001), and is now present in most of the state. With normal winter temperatures, researchers found that gypsy moth spread at an average of 12.9 miles/year (20.8 km/year) (Liebhold et al. 1992). The United States Department of Agriculture (USDA) has established a national gypsy moth quarantine that limits the movement and spread of gypsy moth lifestages (USDA 2020). This quarantine requires all woody materials and household articles to be inspected for gypsy moth lifestages before exiting the quarantined areas.

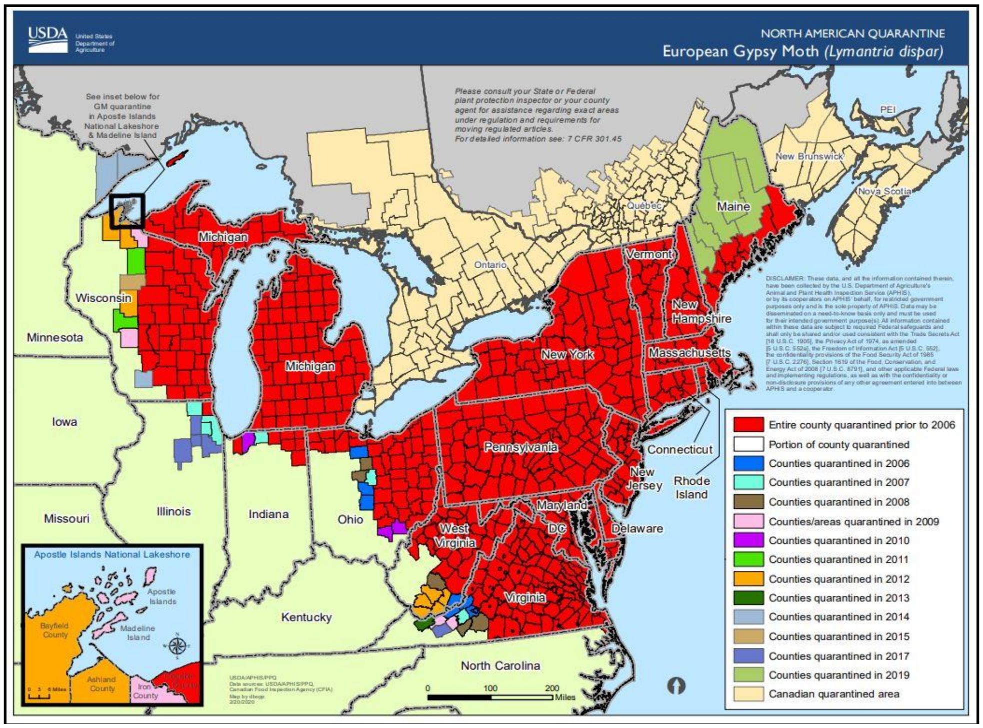

Fig 1. The current North American European gypsy moth quarantine map (USDA 2020).

State and federal agencies work together to manage all areas that are or maybe affected by gypsy moth. National Gypsy Moth Slow the Spread Program (STS), established in 2000, reduces the rate of gypsy moth spread into uninfested areas (GMSTS 2020). STS monitors annual gypsy moth trap catches in the transition zone between established and unestablished gypsy moth populations (GMSTS 2020). The STS program limits the gypsy moth spread through aerial treatments applied to landscapes that contain isolated gypsy moth populations (Coleman et al. 2020). Over 9 million acres (>3,600,000 ha) were since 2000 using mating disruption and biologically-based larvicides (GMSTS 2020).

Gypsy moth larvae are the source of the gypsy moth-related damage. These larvae defoliate trees and shrubs in both the forest and landscape settings. They are polyphagous and feed on over 300 species of trees and shrubs (Liebhold et al. 1995). This makes management difficult for many state and federal organizations. Although they have a wide host range, gypsy moth larvae prefer oak (*Quercus* spp.), primarily white oaks. They also prefer apple (*Malus* spp.), aspen (*Populus* spp.), basswood (*Tilia* spp.), river and white birch (*Betula* spp.), hawthorn (*Crataegus* spp.), hazelnut (*Corylus* spp.), hornbeam (*Carpinus* spp.), serviceberry (*Amelanchier* spp.), sweetgum (*Liquidambar* spp.) and willows (*Salix* spp.) (Hoyle 2007). Not all of these species exist in the mountainous regions of southwestern Virginia, where the survey presented in this report was conducted.

Southwestern Virginia is home to several oak-dominated forest types. Oak (*Quercus* spp.) and hickory (*Carya* spp.) forests represent nearly 60% of forestland in Virginia (Rose, 2008; Asaro and Chamberlin, 2015). According to data collected by the USDA Forest Service Forest Inventory and Analysis program (FIA), approximately 35% of the wood volume of southwestern Virginia is oak (Fig. 2) (Miles 2014; Asaro and Chamberlin, 2015). *Quercus* spp. provide many ecological services. Currently, there is a high demand and value of select oak timber for lumber and other industries in Virginia (Asaro and Chamberlin, 2015). Also, their mast serves are an important food source for many wildlife species (Coleman et al. 2020). Oaks are a major host of forest lepidopteran species, more than any other genus in the eastern United States (Asaro and Chamberlin, 2015). In turn, lepidopteran adults and larvae provide a food source for various animal species, both vertebrate (birds, mammals) and invertebrate (spiders). Overall, oak forests are important for humans and the environment. Thus, oak mortality and decline of oak-dominated forests can pose a real problem for the future health of eastern forests.

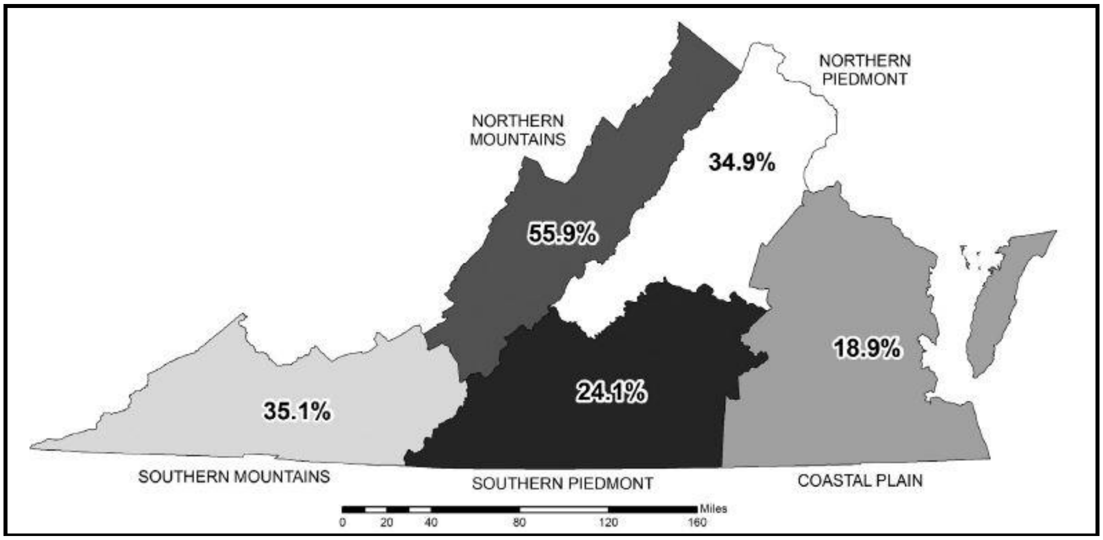

Fig 2. Total estimated percentage of oak volume in five major regions of Virginia. Data for figure obtained from the USDA-FIA (Forest Inventory and Analysis) program (Asaro and Chamberlin, 2015; Miles 2014).

A number of models have been created using forest inventory data collected by the USDA Forest Service to map forest stand susceptibility (Liebhold et al. 1997). Oak susceptibility can be affected by a number of different factors. The most influential factor in determining forest susceptibility appears to be the percentage of basal area in a stand represented by preferred tree species (Fig. 3) (Herrick and Gasner 1986; Liebhold et al. 1997). Predisposing factors like forest characteristics, such as slope, aspect, and elevation, have also been correlated with host species susceptibility (Herrick and Gasner 1986). For example, Walter et al. (2015) studies the correlation between topography changes and gypsy moth invasion dynamics. Additionally, inciting factors, such as tree stress, other pest outbreaks, or extreme weather conditions, can contribute to oak susceptibility and decline.

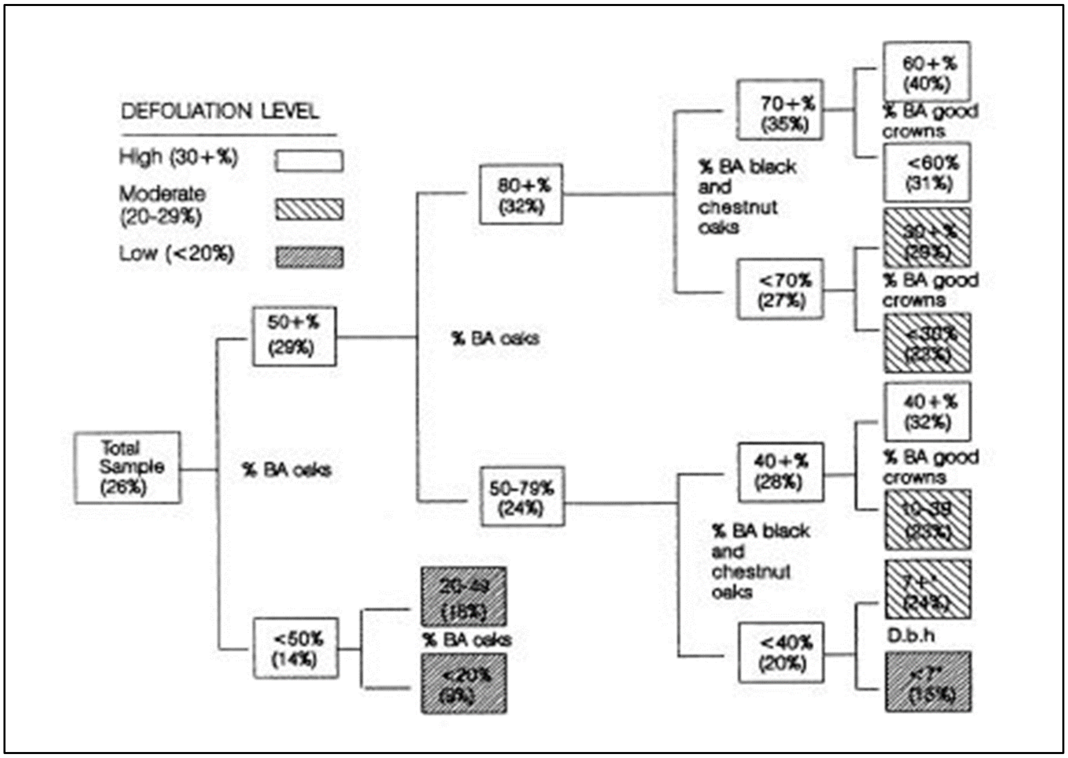

Fig 3. Gypsy moth defoliation predictor model based on percentage of basal area of preferred tree species (Herrick and Gasner 1986).

When gypsy moth populations are high, extensive tree damage and injury is likely. Annual gypsy moth populations are cyclical, going for long periods of time with small populations then populations rapidly increase (Davidson et al. 1999). Due to its cyclic nature, periodic outbreaks of gypsy moth populations have occurred in Virginia, leading to extensive defoliation events. During outbreaks, gypsy moth larvae can consume thousands of acres in foliage and result in severely stressed and defoliated forests stands. Two or more consecutive years of heavy to severe defoliation (>60%) by gypsy moth leads to host mortality (Asaro and Chamberlin 2015). After a defoliation event, tree mortality can be difficult to predict, since there are many factors that contribute to mortality including climatic conditions, tree health, tree species, age, etc. Gypsy moth has been linked to a large amount mature oak decline in the eastern United States, particularly in the mountains of Virginia, where many of the forests are oak-dominant (Asaro and Chamberlin 2015). The mountainous regions of Virginia are home to many oak species including black oak (*Quercus velutina* Lamarck), blackjack oak (*Quercus marilandica* Muenchhausen), chestnut oak (*Quercus prinus* L.), northern red oak (*Quercus rubra* L.), post oak (*Quercus stellata* Wangenheim), scarlet oak (*Quercus coccinea* Muenchhausen), and white oak (*Quercus alba* L.) (VDOF 2007).

Typically, pest defoliation is surveyed and reported annually by states and federal agencies across the United States. Gypsy moth defoliation surveys are conducted by aerial surveys. Due to the difficulty of plotting data in aircrafts, aerial surveys are quickly followed by ground-truth operations to confirm the defoliation was caused by gypsy moths. In Virginia, these surveys are conducted by USDA Forest Service, Virginia Department of Forestry (VDOF), and Virginia Department of Agriculture and Consumer Services (VDACS). Due to the lack of resources and the COVID-19 pandemic, defoliation surveys were not conducted in Virginia in 2020.

Project Objectives

Research on factors leading to gypsy moth outbreaks and the role of host preferences has not been addressed recently. Most of the gypsy moth outbreak and host preference data collection and research was conducted between the 1970s and 1990s in the northeastern United States (Herrick and Gasner 1986; Gribko et al 1995; Liebhold et al. 1997). Recently, state and federal agencies have turned their attention to newly introduced pests such as the spotted lanternfly (*Lycorma delicatula* White, (Hemiptera: Fulgoridae). Due to the lack of gypsy moth-related stand research, it is difficult to predict future defoliation sites in southwestern Virginia. Therefore a survey in southwest Virginia provides a great opportunity to examine the forest structure of defoliated sites where gypsy moth is currently advancing. The objectives of the reported in this report were: 1) compare the characteristics of forest stands that observed multiple years of gypsy moth defoliation to the characteristics of the uninfested stands; 2) compare the forest composition between uninfested areas of southwestern Virginia and areas infested with gypsy moth; 3) document any present gypsy moth lifestages for all sites; 4) compare forest injury between infested and uninfested stands; and 5) compare forest floor regeneration between infested and uninfested sites.

Methods

To conduct this survey, USDA Forest Service was contacted to obtain information on previous defoliation in southwestern Virginia. Obtained shapefiles were then used to plot some previously defoliated sites from 2015-2019 on a map using ArcGIS 10.1 (Fig. 4). Map layers created by USDA Forest Service from aerial surveys included observed defoliation from 2015-2017 (blue), 2018 (red), and 2019 (yellow). The sites were concentrated in Bland, Giles, Pulaski, Tazewell, and Wythe counties.

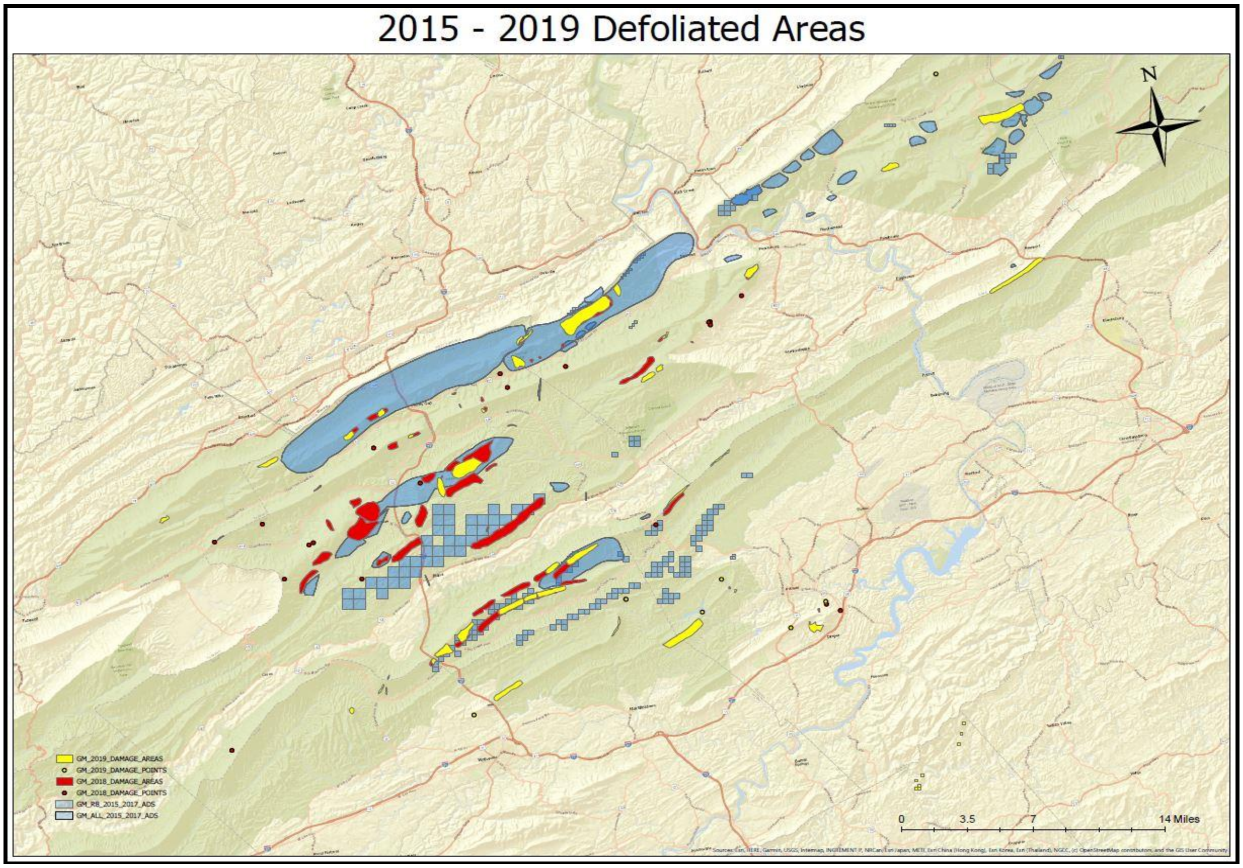

Fig 4. A map of areas defoliated by gypsy moth from 2015-2019. Created using shapefiles from the USDA Forest Service using ArcGIS 10.1 ®.

We identified areas that received multiple years of gypsy moth defoliation (overlapping layers) and chose 14 study sites, here referred to as “outbreak” sites. Additionally, 10 sites were chosen, to serve as controls (Fig. 5). To locate uninfested control sites, we examined treatment locations from the last decade found on STS Project ArcGIS webpage (2010-2020). STS treatments are conducted when isolated pockets of gypsy moth populations in transition zone are revealed by trapping.

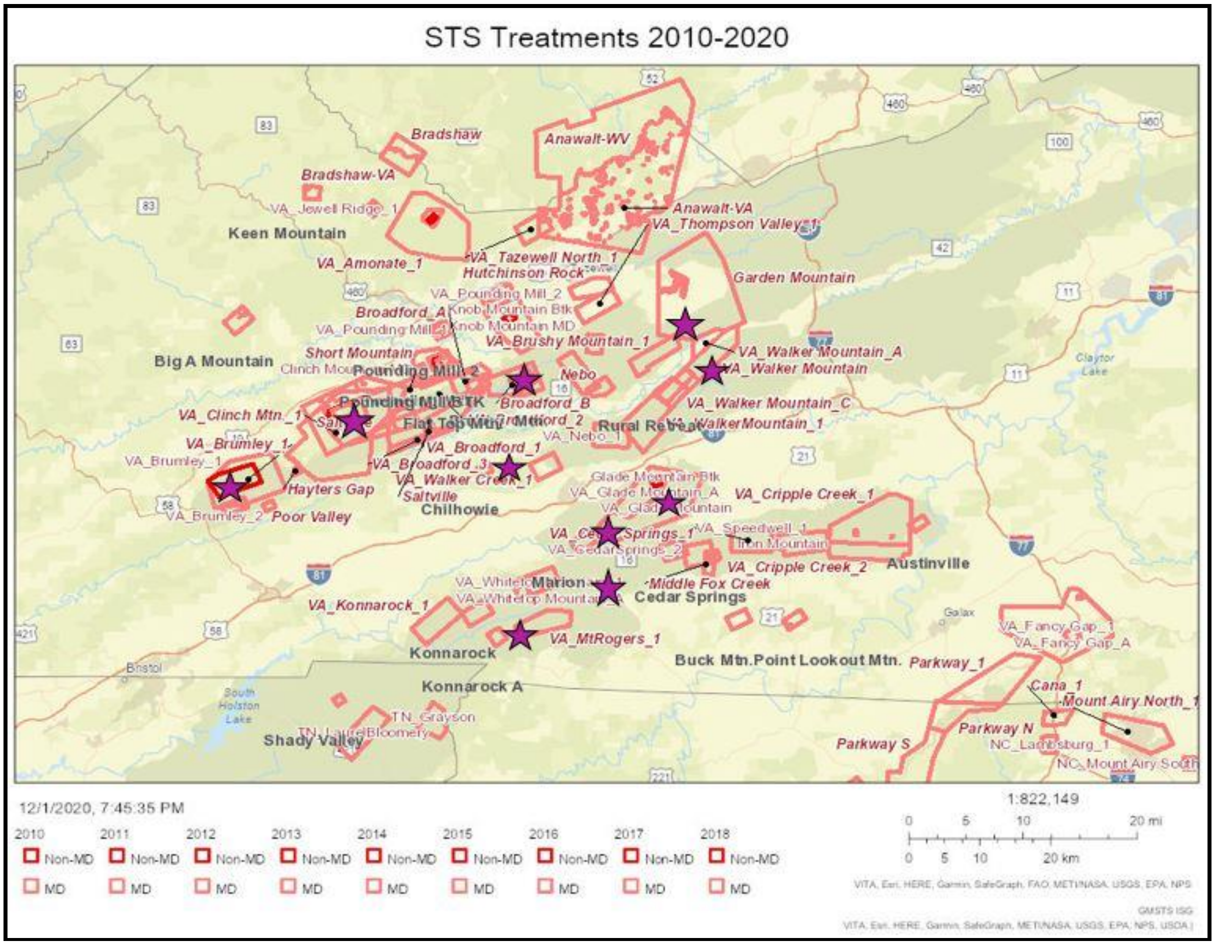

Fig 5. Survey control site locations in reference to STS Treatment sites from 2010-2020. Purple stars represent control site locations. Created using shapefiles from the National Gypsy Moth Slow the Spread Program's treatment archive.

A total of 24 study sites were chosen (Fig. 6). The ArcGIS® collector application was used to record locations of the study sites (purple stars), navigate, plot points, and collect some geographical data. In these sites, we targeted mountain ridgetops where preferred tree species were more prevalent. The majority of all research in this survey was conducted on public land in Jefferson National Forest in southwestern Virginia. The remainder of the research sites were on private or state-owned land. This survey was conducted between July and September of 2020, after full leaf development.

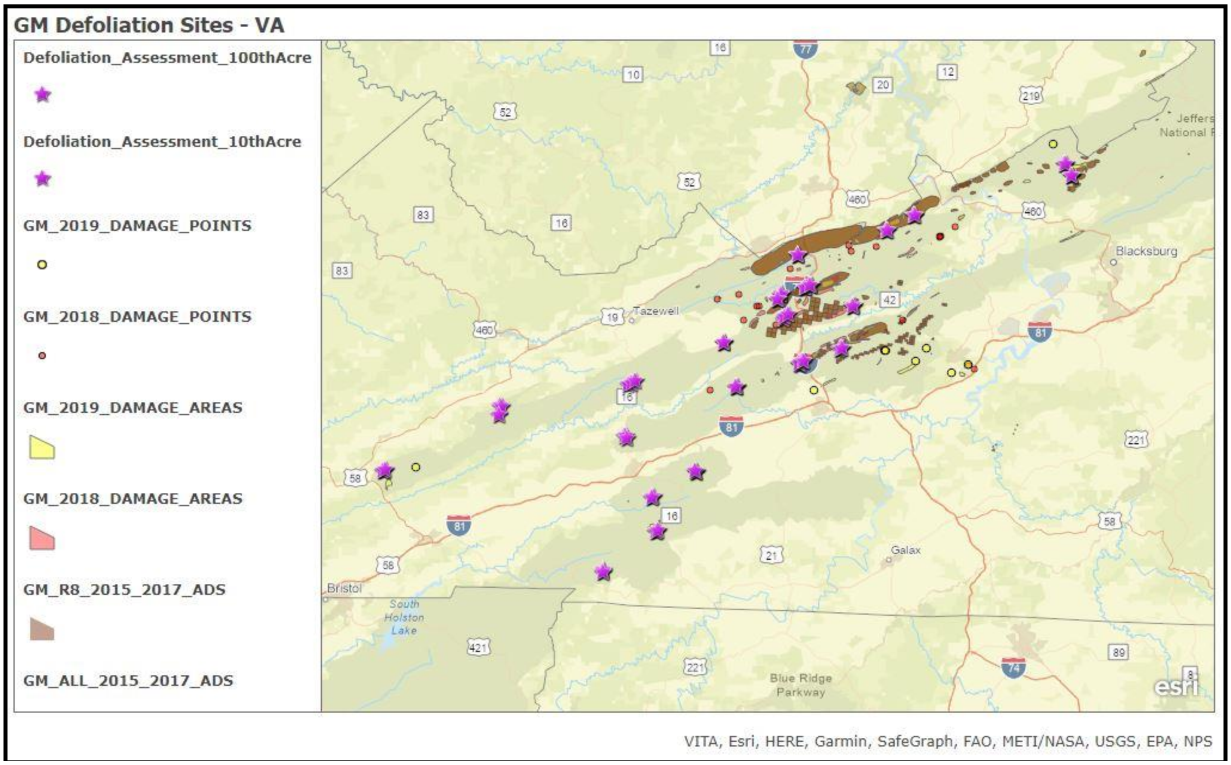

Fig 6. The locations of the 24 gypsy moth defoliation research sites in southwest Virginia. The layers of map included observed defoliation from 2015-2017 (brown), 2018 (red), and 2019 (yellow). Purple stars represent site locations. Created using ArcGIS® Online.

Within each of the 24 sites there were three 0.1-acre (0.04 ha) and three imbedded 0.01-acre (0.004 ha) fixed radius plots (Fig. 7), for a total of 72 0.1-acre and 0.01-acre plots. For fixed-radius plots, only trees within established circular plot areas were measured. To determine the circular plot area, we first established a plot center, determined a plot boundary using a transect tape (for a 0.1-acre plot, the radius is 37.2 ft) and then marked various shrubs and landmarks using flagging tape to mark the plot boundary. This process was repeated several times to distinguish what trees were within the plot boundary. This procedure was repeated for each of the plots in the survey.

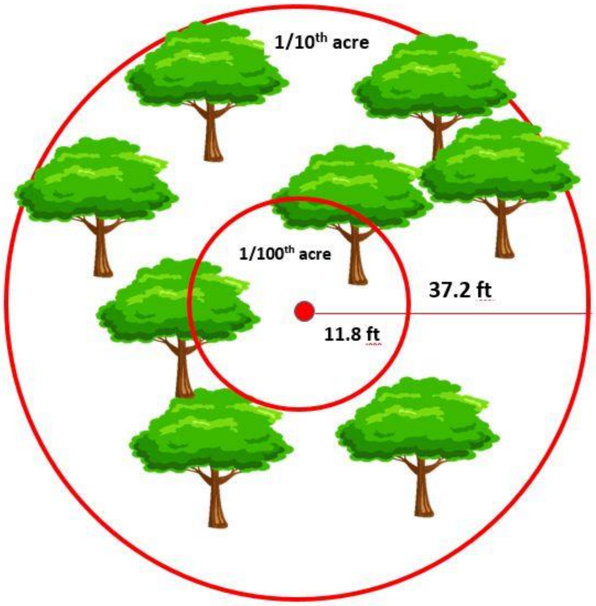

Fig 7. An example of the size of a 0.1-acre and 0.01-acre fixed radius plot.

In each 0.1-acre plot, we recorded information on location (longitude and latitude), elevation, aspect, and slope of the plot. Other inventory data collected in each plot included tree species present, tree condition (living or dead), tree diameter at breast height in inches (DBH) using a diameter tape, crown class (Dominant, Codominant, Intermediate, Overtop), amount of gypsy moth defoliation in the canopy (light (<30%), moderate (30-60%), or heavy (>60%) defoliation of leaves), and presence of egg masses. Egg masses were observed using visual surveys of each plot tree's canopy using sight and/or binoculars then recorded on a datasheet. Gypsy moth damage and defoliation was observed by thoroughly examining canopy condition and symptoms of typical gypsy moth damage (skeletonized leaves, nearby lifestages, and historical gypsy moth catch), then recorded in our datasheets. Each tree in the plot had to have a 5 inch (12.7 cm) minimum diameter at breast height (DBH) in order to be recorded.

In each imbedded 0.01-acre plot, we reused the plot center of the 0.1-acre plots for location information (longitude and latitude), elevation, aspect, and slope of the plot. We examined tree regeneration by recording the species present and the count of each species. We only reported tree seedlings with a minimum height of 6 inches (15.24 cm).

Once collected, we analyzed the data using t-tests (two-sample assuming unequal variances) and F-tests using Microsoft Excel. Also, Excel was used to calculate the mean DBH (calculated by averaging the mean DBH of each site), mean DBH of preferred tree species, percentage of preferred tree species based off the number of stems within each site, percentage of preferred tree species that had defoliation, preferred tree mortality per acre, total basal area (BA) per acre (ft^2), total basal area per acre of preferred trees (ft^2), total basal area per acre of defoliated, preferred trees (ft^2), total basal area per acre of dead, preferred tree (ft^2), trees per acre (TPA), trees per acre of preferred trees, trees per acre of preferred trees with gypsy moth defoliation, trees per acre (TPA) of dead, preferred trees, and stand area index (SDI) of each site within the 0.1-acre plots. TPA is calculated by multiplying the total numbers of trees in a plot by the expansion factor or number of trees per acre a given plot tree represents (Plot size divided by number of plots). Total basal area is determined by multiplying the tree basal area by the expansion factor (EF). The other computations are calculated by separating and organizing species composition. It is important to note when surveying 0.1-acre (EF = 3.33) and 0.01-acre (EF = 33.3) plots, the expansion factors are different.

We decided to further analyze forest injury data by separating and organizing the amount of observed defoliation in study plots. In the field, we categorized these data sets by estimating the amount of gypsy moth defoliation on preferred tree species within both outbreak and control sites. The categories were light (<30%), moderate (30-60%), and heavy (>60%) defoliation. Within the 0.01-acre plots, we calculated the average number of preferred and unfavored tree seedlings and tree seedlings per acre. Also, we chose to analyze the species composition within the 0.01-acre plots.

Results

General Stand Information – 0.1-acre plots

Mean DBH (in), total basal area/acre (sqft), trees per acre (TPA), stand density index (SDI), slope (%), and elevation (ft), are general forest stand measures commonly used forest managers and considered important for this evaluation (Table 1). The results of the analysis indicated that there was no significant difference between the mean DBH of the outbreak sites ($\bar{X}$ = 10.27 in, SD = 0.94 in) and the control sites ($\bar{X}$ = 10.71 in, SD = 1.16 in), t(17) = -0.98, p = 0.34.

There was a marginally significant difference in the total site basal area per acre between outbreak ($\bar{X}$= 148.12 ft^2, SD = 27.01 ft^2) and the control sites ($\bar{X}$= 174.80 ft^2, SD = 37.02 ft^2), t(16) = -1.94, p = 0.07. There was no significant difference between trees per acre of outbreak sites ($\bar{X}$= 224.06, SD = 50.69) and control sites ($\bar{X}$= 241.76, SD = 39.47), t(22) = -0.96, p = 0.35. Stand Density Index (SDI) values were significantly lower in outbreak sites ($\bar{X}$= 261.85, SD = 44.07) than control sites ($\bar{X}$= 359.717, SD = 71.582), t(14) = -3.84, p = 0.002). There was no significant difference in slope between outbreak sites ($\bar{X}$= 25.34%, SD = 7.71%) and the control sites ($\bar{X}$= 24.73%, SD = 4.21%), t(21) = 0.25, p = 0.81. There was no significant difference in elevation of the outbreak sites ($\bar{X}$= 3181.24 ft., SD = 567.33 ft.) and the control sites ($\bar{X}$= 3497.82 ft., SD = 543.54 ft.), t(20) = -1.38, p = 0.18.

Table 1. Stand measures from gypsy moth outbreak and control survey sites in southwest Virginia taken in 2020.

	Mean (± SD) of Outbreak Plots	**Mean (± SD) of Control Plots**
DBH (in)	10.27 ± 0.94	10.71 ± 1.16
Total basal area/acre (ft^2)	148.12 ± 27.01	174.80 ± 37.02
Trees per acre (TPA)	224.06 ± 50.69	241.76 ± 39.47
Stand Density Index (SDI)	261.85 ± 44.07	359.72 ± 71.58
Slope (%)	25.34 ± 7.71	24.73 ± 4.21
Elevation (ft.)	3,181.24 ± 567.33	3497.82 ± 543.54

Species Composition – 0.1-acre

We only found a few different preferred tree species within all outbreak and control sites, including bigtooth aspen (*Populus grandidentata* Michaux), black oak (*Quercus velutina* Lamarck), chestnut oak (*Quercus prinus* L.), northern red oak (*Quercus rubra* L.), scarlet oak (*Quercus coccinea* Muenchhausen), and white oak (*Quercus alba* L.) We observed 491 preferred trees (52.12%) in all outbreak sites and 305 preferred trees (42.15%) within all control sites. Although the outbreak sites ($\bar{X}$ = 51.37%, SD = 11.45%) had a numerically higher mean percentage of preferred trees species than control sites ($\bar{X}$= 42.01%, SD = 20.80%) it was not significantly different, t(13) = 1.29, p = 0.22. There was no significant difference in mean DBH of preferred host species between the outbreak sites ($\bar{X}$= 11.90, SD = 2.17) and the control sites ($\bar{X}$= 11.37, SD = 1.94), t(21) = 0.63, p = 0.54. Additionally, there was no significant difference in total basal area of preferred tree species, t(12) = 1.52, p = 0.15, despite outbreak sites ($\bar{X}$=

97.24 ft^2, SD = 18.84 ft^2) having a numerically higher basal area value than control sites ($\bar{X}$= 77.52 ft^2, SD = 37.71 ft^2). Furthermore, the outbreak sites ($\bar{X}$= 115.84, SD = 41.74) had more preferred tree species per acre than the control sites ($\bar{X}$= 101.57, SD = 48.75), but the difference was not significant (t(18) = 0.75, p = 0.46).

Table 2. A comparison of preferred tree species data between gypsy moth outbreak and control sites in southwestern Virginia in 2020.

	Outbreak Sites	**Control Sites**
Mean (%) Preferred tree species (± SD)[1]	51.37 ± 11.45	42.01 ± 20.80
Mean DBH of preferred tree species (in) (± SD)	11.90 ± 2.17	11.37 ± 1.94
Mean total basal area per acre of preferred tree species (ft^2) (± SD)	97.24 ± 18.84	77.52 ± 37.71
Mean preferred trees per acre (± SD)	115.84 ± 41.74	101.57 ± 48.75

[1] Based on a total of 491 and 305 trees for outbreak and control sites, respectively.

Mean (± SD) percentage of individual preferred tree species were evaluated and results are shown in Fig. 8. Results of individual preferred species composition indicate that there is no significant difference between the outbreak ($\bar{X}$= 8.67%, SD = 9.18%) and the control site ($\bar{X}$= 7.00%, SD = 6.66%), f(5) = 1.89, p = 0.25. Within both outbreak and control sites, chestnut oak was the most prevalent gypsy moth-preferred tree species. Chestnut oak averaged 23.67% of the total reported trees with the outbreak sites, whereas it averaged 17.22% of the total trees reported in control sites. Control sites (57.99%) had a higher average percentage of unfavored tree species than outbreak sites (47.98%).

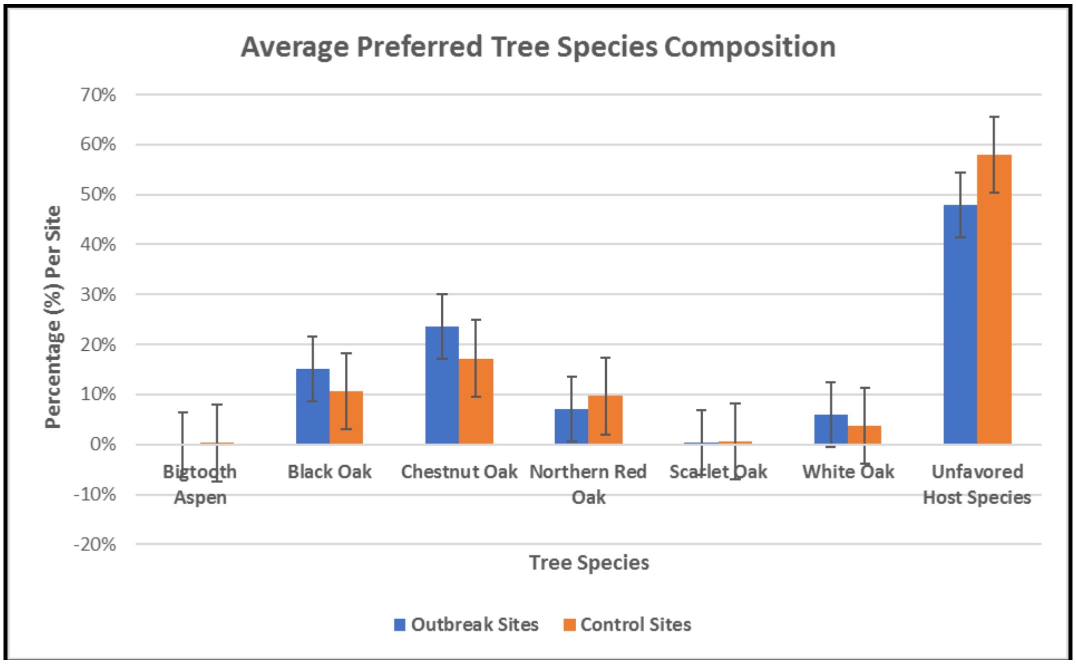

Fig. 8. Mean (± SD) of individual preferred trees species composition in gypsy moth outbreak and control sites in southwestern Virginia in 2020.

Forest Injury – 0.1-acre

All of the outbreak sites showed signs of gypsy moth-related injury. Some forest injury was also observed in some of the control sites. We determined some of the tree injury in control plots were indeed caused by gypsy moth due to the distinctive skeletonized leaves and nearby egg masses outside of the 0.1-acre plots. We determined that the observed forest injury statistics for both outbreak and control sites are provided in Table 3. A total of 533 injured trees (57%) were observed within all of the outbreak sites, whereas control sites only had 162 injured trees (22%). Of the 491 preferred trees in the outbreak sites, 476 trees were injured as a result of gypsy moth defoliation. Of the 305 preferred trees in the control sites, 75 preferred host trees appeared to show signs of gypsy moth defoliation. Results of the data analysis indicated a highly significant difference between the total basal area per acre of preferred, defoliated trees in the outbreak ($\bar{X}$= 91.31, SD = 18.30) versus control sites ($\bar{X}$= 26.12, SD = 24.39), t(16) = 7.14, p < 0.001. The mean of preferred trees species per acre with signs of gypsy moth defoliation was significantly greater in outbreak ($\bar{X}$= 113.22, SD = 43.57) than control sites ($\bar{X}$= 24.98, SD = 20.18), t(19) = 6.65, p < 0.001.

Table 3. Some general forest injury statistics in both gypsy moth outbreak and control sites in southwestern Virginia in 2020.

	Outbreak Sites	Control Sites
Total trees with injury	533	162
Total number of preferred trees with gypsy moth injury or defoliation	476	75
Total basal area per acre of preferred defoliated trees (ft^2)	91.31*	26.15*
Mean (± SD) preferred trees per acre with gypsy moth defoliation	113.22* ± 43.57	24.98* ± 20.18

* was significantly different in my t-tests.

Results of forestry injury analysis indicated that all three levels of defoliation differed significantly between outbreak and control plots (Fig. 9). Light defoliation was significantly greater (t(18) = 3.98, p < 0.001) in outbreak ($\bar{X}$= 20.07, SD = 12.55) compared to control sites ($\bar{X}$= 5.50, SD = 4.67). Moderate defoliation was significantly greater (t(19) = 4.94, p < 0.001) in outbreak ($\bar{X}$= 8.36, SD = 4.67) compared to control sites ($\bar{X}$= 1.40, SD = 2.07). Lastly, heavy defoliation was significantly greater (t(17) = 5.08, p < 0.001) in outbreak ($\bar{X}$= 5.43, SD = 3.32) compared to control sites (X̄= 0.60, SD = 1.07). Overall, the outbreak sites had significantly more defoliation than control sites.

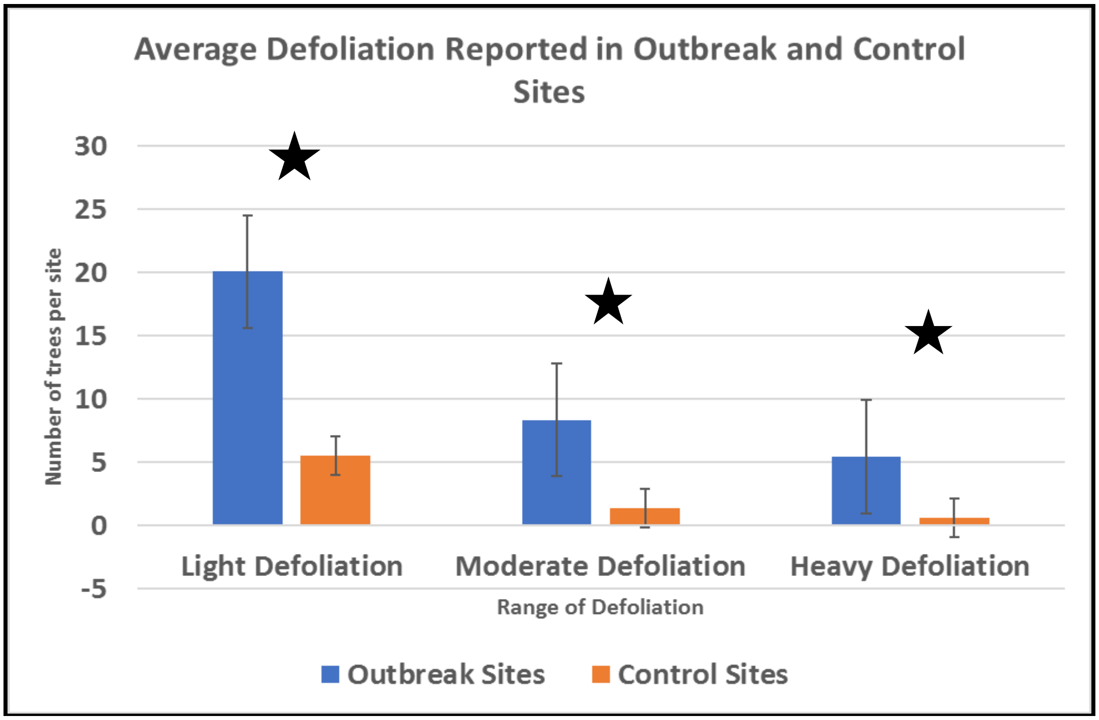

Fig 9. Graph of the estimated gypsy moth defoliation of preferred trees within outbreak and control sites in southwestern Virginia in 2020. Stars within each defoliation category indicate significant differences between outbreak and control sites.

Additionally, using my data I calculated mean basal area in oaks (50.95%) and mean basal area in black and chestnut oak (35.81%). I used these calculations in the gypsy moth defoliation predictor model (Fig. 3) and found that the control sites have an estimated to receive moderate defoliation.

The results of the tree mortality analysis between outbreak and control sites indicate no significant difference (t(19) = 1.47, p = 0.16) in the mean number of dead trees between outbreak (X̄= 4.71, SD = 3.91) and control (X̄= 3.00, SD = 1.63) (Table 4). There was no significant difference (t(21) = 1.52, p = 0.14) in the average number of dead, preferred trees between outbreak ($\bar{X}$= 2.71, SD = 2.64) and control sites ($\bar{X}$= 1.40, SD = 1.58). No significant difference was observed (t(21) = 1.52, p = 0.14) in the mean number of dead trees per acre between outbreak ($\bar{X}$= 9.04, SD = 8.80) and control sites ($\bar{X}$= 4.66, SD = 5.25). A marginally significant difference was observed in the mean basal area per acre of dead trees (t(20) = 1.89, p = 0.08) between outbreak ($\bar{X}$= 6.59 ft^2, SD = 7.05 ft^2) and control sites ($\bar{X}$= 2.59 ft^2, SD = 3.34 ft^2).

Table 4. General tree mortality statistics in both gypsy moth outbreak and control sites in southwestern Virginia in 2020.

	Outbreak Sites	**Control Sites**
Mean (± SD) number of dead trees per site	4.71 ± 3.91	3.00 ± 1.63
Mean (± SD) number of dead preferred trees per site	2.71 ± 2.64	1.40 ± 1.58
Mean (± SD) dead trees per acre	9.04 ± 8.80	4.66 ± 5.25
Mean (± SD) dead tree basal area per acre (ft^2)	6.59 ± 7.05	2.59 ± 3.34

Pest Life Stages

A total of forty-three new and old gypsy moth egg masses were found in the outbreak sites, whereas only one egg mass was found within all the control sites. Throughout the survey, we observed signs or lifestages of other forest pests, including emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), periodical cicada, *Magicicada septendecim* L. (Hemiptera: Cicadidae), eastern tent caterpillar, *Malacosoma americanum* F. (Lepidoptera:

Lasiocampidae), and Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). These species could contribute to forest injury and decline within those sites.

Forest Regeneration – 0.01-acre

The 0.01-acre plot collected data on tree seedlings by species counts, with a minimum height of 6 inches. There was no significant difference between mean number of preferred tree seedlings per site outbreak ($\bar{X}$= 42.21, SD = 31.26) and control sites ($\bar{X}$= 54.60, SD = 35.70), t(19) = -0.91, p = 0.37 were observed (Table 5). Also, there was no significant differences in the mean number of unfavored tree seedlings per site, t(21) = -1.25, p = 0.22 (Outbreak: $\bar{X}$= 72.29, SD = 28.54; Control: $\bar{X}$= 85.90, SD = 24.42). After altering the expansion factor for 0.01-acre plots, there was no significant difference between mean tree seedlings per acre for outbreak ($\bar{X}$= 1405.74, SD = 1041.08) compared to control sites ($\bar{X}$= 1818.18, SD = 1126.44), t(19) = -0.91, p = 0.37. Additionally, the mean unfavored, tree seedlings per acre calculation were not significantly different either, t(21) = -1.25, p = 0.22 (Outbreak: $\bar{X}$= 2407.11, SD = 950.47; Control: $\bar{X}$= 2860.47, SD = 813.33).

Table 5. Some general forest regeneration statistics in gypsy moth outbreak and control sites in southwestern Virginia in 2020.

	Outbreak Sites	**Control Sites**
Mean (± SD) number of preferred tree seedlings per site	42.21 ± 31.26	54.60 ± 35.70
Mean (± SD) number of unfavored tree seedlings per site	72.29 ± 28.54	85.90 ± 24.42
Mean (± SD) preferred tree seedlings per acre	1407.74 ± 1041.08	1818.18 ± 1126.44

Mean (± SD) unfavored tree seedlings per acre	2407.11 ± 950.47	2860.47 ± 813.33

After observing the understory, very few preferred tree species were present in both outbreak and control sites. Possibly, due to the small size of the 0.01-acre plots (r = 11.8 ft.), not all preferred tree species had seedlings. Analysis conducted to compare the seedling species composition indicated no significant difference between the outbreak ($\bar{X}$= 20.00%, SD = 26.28%) and the control sites ($\bar{X}$= 20.00%, SD = 25.40 %), f (4) = 1.07, p = 0.47. Figure 10 shows a visual comparison of the percentages that preferred tree seedlings contributed to the species composition within the 0.01-acre outbreak and control sites.

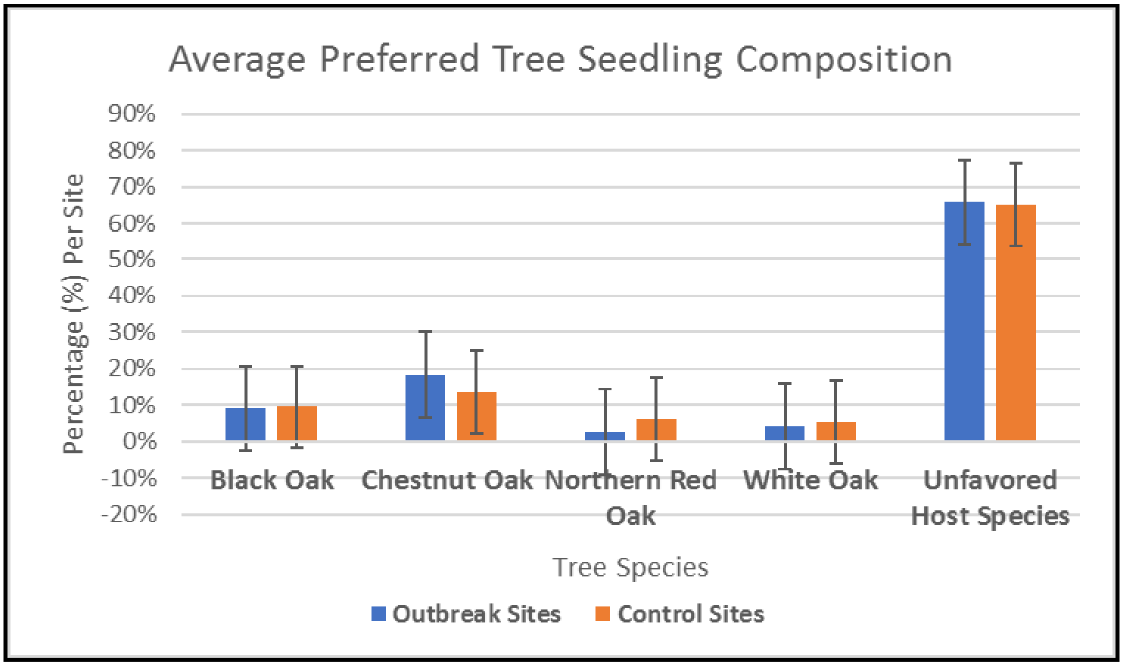

Fig 10. Mean (± SD) preferred tree seedlings composition within the 0.01-acre outbreak and control sites.

Discussion

Gypsy moth outbreaks have greatly impacted oak-dominated stands of the Appalachian Mountains in the last century (Sharov et al. 1996). Gypsy moth outbreaks have disrupted forest ecology throughout the Appalachian mountain range causing tree mortality, loss of food sources for vertebrate and invertebrate species, and the spread of many undesired plants in the understory (Schweitzer 2004). Gypsy moth dispersal and outbreaks are difficult to predict since populations vary annually. Isolated populations become established beyond the expanding front due artificial movement (Tobin and Whitmire 2005, USDA 2020). As a result, it is important to study and assess some general forest stand characteristics ahead of the expanding gypsy moth front to help predict future forest susceptibility.

Due to the varying forest characteristics of the central and southern Appalachians, more studies are needed to fully characterize and compare the forest stand characteristics of infested and uninfested areas in southwestern Virginia. In this study, we compared general stand characteristics such as, species composition, forest injury, the presence of pest lifestages, and forest regeneration.

Our results showed stand data between outbreak and control sites did differ in some measures but not in others. Stand density index value was significantly greater in control than outbreak sites. Also, total basal area per acre computation showed only marginal significance. Species composition did not appear to differ between the outbreak and control sites. Differently, outbreak sites were significantly greater than control sites in total basal area per acre of defoliated preferred trees, the average preferred trees per acre with gypsy moth defoliation, and the average number of trees with light, moderate, or heavy defoliation per site. The total basal area per acre of preferred, dead trees was marginally greater in outbreak than control sites.

Significantly more gypsy moth egg masses were found in the outbreak sites than in control sites. Lastly, there were no significant differences in the forest regeneration measures.

In general, control sites were denser than outbreak plots, thinning through Timber Stand Improvement (TSI) is recommended after a gypsy moth defoliation event (Gottschalk 1993). Cochran et al. (1993) explains that a higher basal area and stand density increases a stands risk of pest impacts like defoliation. There are two types of thinning recommended after a defoliation event, salvage and sanitation thinning. Salvage thinning refers to the removal of dead or dying trees from a defoliated stand, trying to “salvage” the rest of the stand. Whereas, sanitation thinning refers to the removal of preferred host species prior-to or post outbreak to reduce the spread of gypsy moth damage. It is unlikely that USDA Forest Service conducted TSI thinning to improve stand quality as a result of a major gypsy moth outbreak since outbreak site locations are spread over a large area and thinning is expensive. I contacted a USDA silviculturist in Blacksburg, Virginia, that manages Jefferson National Forest near my outbreak sites, to obtain any specific information on silvicultural techniques on forest stands affected by gypsy moth outbreaks. After a gypsy moth defoliation event, he typically prescribes regeneration cuts to remove infested trees, reduce stand density, and promote forest regeneration. The significance in tree density between outbreak and control sites could be due to the small sample size of this study.

It was anticipated that the forest injury statistics would have the most significance between outbreak and control sites. Mean preferred trees per acre with gypsy moth defoliation and mean defoliation reported per site (light, moderate, and heavy) were highly significant. This corroborates the idea that gypsy moth is not yet established in the control sites.

Throughout all the computations, basal area showed the marginal potential for significance. Both the total basal area per acre (general stand information) and total basal area per acre of preferred trees with gypsy moth defoliation (tree injury) showed a marginally, significant difference. In the forest injury statistics, total basal area per acre of preferred trees with gypsy moth defoliation was highly significant. This result agrees with results of previous studies, other researchers have used basal area of preferred host species as a measure for forest susceptibility (Liebhold et al. 1997) and have created gypsy moth defoliation predictor models based on basal area of preferred species and number of host refuges in a stand (Herrick and Gasner 1986, Gottschalk 1993, Gribko et al. 1995).

Lastly, species composition in the outbreak sites was very similar to the species composition in the control sites. Within these 0.01-acre plots, shade-tolerant species like red maple, striped maple, and sassafras were the most prominent. Preferred tree species are in competition with these shade-tolerant species for resources. Only time will tell of what will become of these seedlings.

This survey had many limitations. The entire project was funded solely by myself. This included transportation costs, overnight travel, and meals. Additionally, there were many incidences with the 2020 COVID-19 pandemic. Many private landowners did not want to have a face-to-face conversation or allow researchers onto their property. Also, I was only available to conduct this survey on weekends due to my job and other classwork. After addressing these challenges, I was able to finish the survey just prior to abscission, or leaf drop.

Although this survey baseline data on forest stand characteristics of the mountains of southwestern Virginia, further research is needed. Additional site sampling would improve statistical analysis and provide a larger sample of southwestern Virginia. Stand characteristics should continue to be monitored. State and Federal agencies should work together to conduct aerial and ground-truth defoliation surveys to map the spread of gypsy moth defoliation along the southern Appalachian Mountains. This research may inspire future studies or raise more questions on forest characteristics and gypsy moth defoliation. Future studies should consider basal area as an important factor in predicting gypsy moth defoliation. A future study could be to examine site conditions in Slow-the-Spread treatment areas (with low-density populations) and compare it to the outbreak areas in this study.

Literature Cited

Asaro, C. and Chamberlin, L.A. Outbreak history (1953-2014) of spring defoliators impacting oak-dominated forests in Virginia, with emphasis on gypsy moth (*Lymantria dispar* l.) and fall cankerworm (*Alsophila pometaria* Harris). *Am. Entomol.* 61(3): 174-185.

Cochran, P.H.; Geist, J.M.; Clemens, D.L.; Clausnitzer, Rodrick R.; Powell, David C. 1993. Suggested stocking levels for forest stands in northeastern Oregon and southeastern Washington. Res. Note PNW-RN-513. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 26 p.

Coleman, T.W., Haavik L.J., Foelker, C., and Liebhold A.M. 2020. Gypsy Moth (FIDL). Forest Insect & Disease Leaflet 162. U.S. Dept. of Agriculture, Forest Service, State & Private Forestry

Davidson, C.B., Gottschalk, K.W., and Johnson, J.E. 1999. Tree mortality following defoliation by the European gypsy moth (*Lymantria dispar L.*) in the United States: a review. *For. Sci.* 45:74–84.

Gottschalk, K.W. 1993. Silvicultural guidelines for forest stands threatened by gypsy moth. General Technical Report NE-171. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 50 p.

Gribko, L.S., Liebhold, A.M., and Hohn, M.E. 1995. Model to predict gypsy moth (Lepidoptera: Lymantriidae) defoliation using kriging and logistical analysis. *Environ. Entomol*. 24(3): 529-537.

Herrick, O.W. and Gasner, D.A. 1986. Rating forest stands for gypsy moth defoliation. Research Paper NE-583. Radnor, PA: U.S. Department of Agriculture, Forest Service.

Hoyle, Z. 2007. The gypsy moth invasion can silviculture save the day? U.S. Department of Agriculture, Forest Service, Southern Research Station, Compass. 28 p. Accessed on 12/2/20. https://www.srs.fs.usda.gov/compass/issue9/issue9.pdf

Liebhold, A. M., Halverson, J.A., and Elmes, G.A. 1992. Gypsy moth invasion in North America: a quantitative analysis. *J. Biogeogr*. 19: 513-520.

Liebhold, A.M., Gottschalk, K.W., Mason, D. A. and Bush, R.R. 1997. Forest susceptibility to the gypsy moth. *J. Forest.* 95: 20-24.

Liebhold, A.M., Macdonald, W.L., Bergdahl D., and Mastro V.C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. *Forest Science Monographs*. 30, 49 p.

Miles, P.D. 2014. Forest inventory EVALIDator web-application version 1.6.0.01. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northern Research Station.

National Gypsy Moth Slow the Spread Program (GMSTS). 2020. Gypsy moth slow the spread program story map. U.S. Department of Agriculture, Forest Service. Accessed on 12/31/2020. https://gmsts.maps.arcgis.com/apps/MapSeries/index.html?appid=38cd5cbbb3364b1195e3b812f68d004b

Roberts, E.A. 2001. Gypsy moth in Virginia: an update. Virginia Cooperative Extension, Publication 444-750. pp. 1-4. Accessed on 1/3/21. https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/444/444-750/444-750_pdf.pdf

Rose, A.K. 2008. The status of oak and hickory regeneration in forests of Virginia, pp. 70-79. In Jacobs, D.F., and C.H. Michler (eds.). Proceedings, 16th Central Hardwood Forest Conference; 2008 April 8-9; West Lafayette, IN. Gen. Tech. Rep. NRS-P-24. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Schweitzer D.F. 2004. Gypsy moth (Lymantria dispar): Impacts and Options for Biodiversity-Oriented Land Managers. NatureServe: Arlington, Virginia. 59 p.

Sharov, A.A., Liebhold A. M., and Roberts, E.A. 1996. Spread of gypsy moth (Lepidoptera: Lymantriidae) in the Central Appalachians: comparison of population boundaries obtained from male moth capture, egg mass counts, and defoliation records. *Environ. Entomol*. 25: 783-792.

Tobin, C.T. and Whitmire, S.L. 2005. Spread of gypsy moth (Lepidoptera: Lymantriidae) and its relationship to defoliation. *Environ. Entomol.* 34(6): 1448-1455.

U.S. Department of Agriculture. 2020. Gypsy moth pest and disease webpage. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Accessed on 11/29/2020. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/gypsy-moth

Virginia Department of Forestry. 2007. Common native trees of Virginia: tree identification guide. *Timber*. pp. 1-136. Accessed on 11/4/2020. https://www.dof.virginia.gov/infopubs/Native-Tree-ID_2020.pdf

Walter, J.A., M.S. Meixler, T. Mueller, W.F. Fagan, P.C. Tobin and K.J. Haynes. 2015. How topography induces reproductive asynchrony and alters gypsy moth invasion dynamics. *J. of Animal Ecology*. 84:188-198.