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William Seth Ratliff
East Tennessee State University

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Demography and Disease in Populations of the Rare Shrub *Buckleya distichophylla* (Santalaceae) in Northeastern Tennessee

By

William Seth Ratliff

An Undergraduate Thesis Submitted in Partial Fulfillment
of the Requirements for the
Midway Honors Scholars Program
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William Seth Ratliff Date

Dr. Foster Levy, Thesis Mentor Date

Dr. Aruna Kilaru, Reader Date

Dr. Timothy McDowell, Reader Date

ABSTRACT

Piratebush (*Buckleya distichophylla* (Nutt.) Torr.) is a rare, hemiparasitic shrub with the only extant populations in western North Carolina, northeastern Tennessee, and southwestern Virginia. The preferred natural hosts of piratebush, Carolina and eastern hemlocks, have seen sharp declines over the last decade due to the invasive hemlock woolly adelgid. Virginia pine, another important host of piratebush, is also susceptible to disease, specifically *Cronartium appalachianum*, a rust fungus for which piratebush is the secondary host. This study described and analyzed current demographic parameters of three Tennessee piratebush populations. Additionally, spatial patterns of disease and demographic characters were analyzed. These data were compared to data from previous censuses to infer the impacts of diseases on piratebush and its host. All three populations were relatively stable in numbers and age structure over the past thirty years. Plant height and stems per shrub were similar among populations and stable over time. Seedlings represented 14%-19% of populations and non-flowering plants 33%-41% of populations. Two populations had an equal sex ratio and one population was male-biased. Disease prevalence was similar among populations but disease was more severe at Temple Ridge. The effects of hemlock decline were most acute at the Temple Ridge population where areas of high hemlock decline were associated with lower vigor piratebush individuals. Piratebush individuals near Virginia pines were more likely to be infected by *C. appalachianum*, and individuals infected by the rust fungus were more likely to have lower vigor. If hemlock decline is causing a piratebush host shift toward Virginia pine, piratebush populations may also decline because of potential enhanced infection by *C. appalachianum*.

Treatment to prevent HWA infestation may be needed because of its effectiveness in improving the health of both hemlock and piratebush populations.

INTRODUCTION

Background

Buckleya distichophylla (Nutt.) Torr., commonly called piratebush, is a rare, hemiparasitic shrub (Fig. 1). The species is dioecious, exhibiting either male or female flowers, both of which are pale green in color. It is a member of the *Thesium* clade within the sandalwood family (Santalaceae), one of the largest parasitic plant families (Carvell and Eshbaugh 1982). The genus *Buckleya* comprises four species in two sister clades, with *B. distichophylla* and *B. graebneriana* in one clade and *B. lancelolata* and *B. henryi* in the other (Li et al. 2001). Piratebush is the most geographically and genetically divergent species in the genus *Buckleya*, and is the only species not endemic to Asia (Carvell and Eshbaugh 1982; Li et al. 2001). This eastern Asia-eastern North America biogeographic pattern is seen in many plant groups.

Piratebush is a rare species with the only known natural populations occurring sporadically in the mountains of northeastern Tennessee, southwestern Virginia, and western North Carolina (Kadis 2010; Musselman 1982). Piratebush is listed as a federal species of concern and is considered threatened and imperiled in Tennessee (Crabtree 2014). While the populations in Virginia have been more thoroughly described, particularly in regards to the functions they play in the forest plant community, the populations in Tennessee and North Carolina have been less investigated (Huish et al. 2015; Leahy et al. 2006; McCoy 2010; Musselman 1982). Periodic censuses have been conducted on the populations in Tennessee, but details about the biology of the species are few (Jennison 1935; McCoy 2010). Similarly, few attempts have been made to understand how environmental factors and disease may affect populations over time.

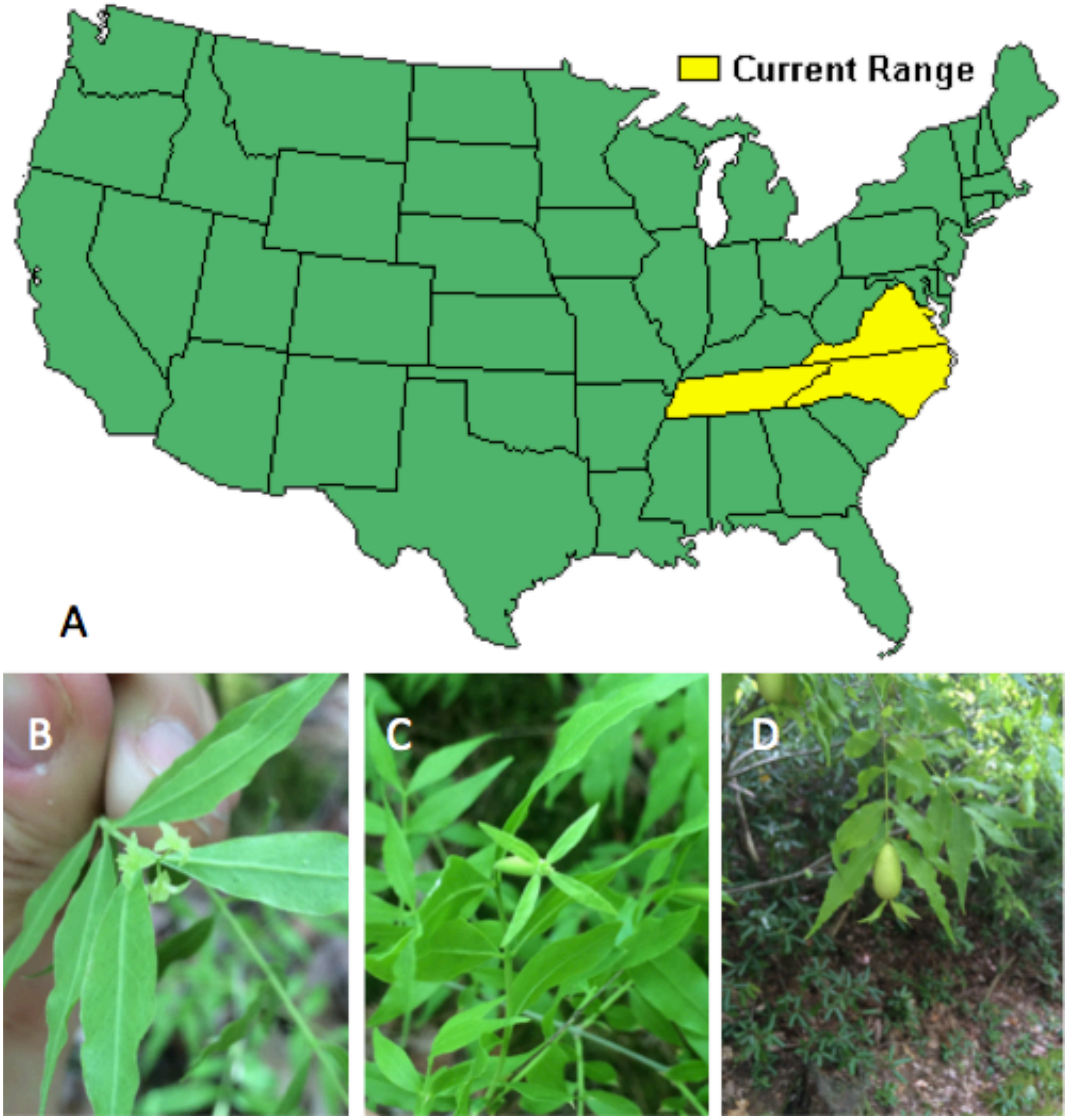

Figure 1. Current range of natural piratebush populations by state highlighted in yellow (A)(Kadis 2010), piratebush male (B) and female (C) flowers, and fruit (D).

Piratebush, like many plants in the Santalaceae, is a hemiparasite, and the adult plants extract sugars and nutrients from the roots of host trees (Fineran 1974; Rao 1942).

Root parasites such as piratebush use haustoria, specialized root structures that penetrate the roots of neighboring plants (Piehl 1965; Toth and Kuijt 1977). These structures break through and around the root cell walls of host plants, enter the host's vascular tissue, and take up the nutrients contained within the host cells (Heide-Jørgensen and Kuijt 1993; Musselman and Dickison 1975; Szabo and Bushnell 2001; Toth and Kuijt 1977). It was originally thought that piratebush would form parasitic relationships with only Carolina hemlock (*Tsuga caroliniana*) or eastern hemlock (*T. canadensis*), but it has since been shown in both nature and culture that piratebush will attach to some oak (*Quercus*) and pine (*Pinus*) species as well as other genera (Musselman and Mann 1979; Piehl 1965). In nature, piratebush primarily parasitizes hemlock species, but rather than host preference, it is thought this is a function of common host-parasite environmental requirements (Leopold and Muller 1983; Piehl 1965).

The two hemlocks of eastern North America are susceptible to infestation by the invasive hemlock woolly adelgid (HWA)(*Adelges tsugae*), a species of insect native to Japan, which was introduced into the eastern United States in the 1950s (Bennett 2013; Havill et al. 2006; Orwig et al. 2003). Since then, HWA has become the most important cause of mortality in eastern and Carolina hemlock (Krapfl et al. 2011; Levy and Walker 2014; McClure et al. 2001; Orwig et al. 2003). In the Southern Appalachian Mountains, HWA has caused significant disease and decline in both species of hemlock (Cleaves 2008; Krapfl et al. 2011; Vose et al. 2013). Because hemlock can be considered a keystone species in Southern Appalachian forests, the impact of decline in populations on the entire forest ecosystem is of concern (Cleaves 2008; Krapfl et al. 2011; Orwig and Foster 1998; Vose et al. 2013).

The outlook for hemlock reestablishment is also uncertain. It has been suggested that native and invasive opportunistic herbaceous species may flourish due to reduced canopy coverage resulting from hemlock mortality, and that hemlock seedlings may be outcompeted (Orwig and Foster 1998). Several strategies (fire, chemical, fungal, predatory) intended to control HWA have been proposed and implemented with varying degrees of success (Davis et al. 2012; Havill et al. 2014; Vose et al. 2013). Since piratebush is still thought to primarily parasitize hemlocks in nature, the effect that a decrease in hemlocks will have on piratebush populations is unclear.

Another factor that may impact the size and vigor of piratebush populations is the occurrence of the rust fungus, *Cronartium appalachianum*, sometimes called *Peridermium appalachianum* (Vogler and Bruns 1998). Rust fungi have the most elaborate and plastic life cycles of any fungal groups (Cummins 2003; Johnson 1986; Mohanan 2010; Peterson 1974). Their life cycle consists of up to five reproductive stages (spermagonia, aecia, uredinia, telia, and basidia) and may require two hosts and several years to complete (Cummins 2003; Johnson 1986; Peterson 1974). Rusts, such as *C. appalachianum,* that have life cycles consisting of all five reproductive stages and that parasitize two hosts are said to be macrocyclic (Peterson 1974). *Cronartium appalachianum* is an obligate parasite infecting its primary host, Virginia pine (*Pinus virginiana*), with basidiospores originating from teliospores that germinate on piratebush, the rust's secondary host (Hepting 1957; Johnson 1986; Vogler and Bruns 1998). Up to several years after infection, spermatia (also called pycniospores) form on pine stems (Hepting 1957; Johnson 1986; Vogler and Bruns 1998). These spermatia then form pustules that eject aeciospores, which can infect piratebush (Hepting 1957; Johnson 1986; Vogler and Bruns 1998). After approximately two weeks, the infection on piratebush begins to produce uridiniospores, dikaryotic vegetative spores that can infect other piratebush individuals (Hepting 1957; Peterson 1974; Vogler and Bruns 1998). Several weeks later, hairlike projections that produce the teliospores begin to form from which basidiospores arise and are ejected repeating the life cycle (Hepting 1957; Peterson 1974).

Cronartium appalachianum, like other species within the genus *Cronartium*, colonizes the leaves and stems of its secondary hosts (Hepting 1957). Similar to piratebush, rust fungi utilize haustoria to invade host cells and extract nutrients (Johnson 1986; Tainter 1973; Voegele et al. 2001). Oftentimes, rust infection causes host defoliation and deformation, and can be fatal (Hepting 1957; Tainter 1973). The impact of *C. appalachianum* on piratebush has not been extensively examined. While it is known that *Cronartium* rusts can take several years to kill their larger primary hosts, it is uncertain what long-term health effects *C. appalachianum* has on the growth and vigor of piratebush (Hepting 1957; Johnson 1986).

Purposes

Because of the diseases associated with piratebush and its hosts, and because of the small population sizes and rarity of the species, this study seeks to investigate the demographics of piratebush populations in eastern Tennessee. It is uncertain how *Cronartium* rust affects piratebush populations and their hosts, but if the infection is similar to that of *Puccinia* in the grain plants of North America, local to complete decimation and/or extinction of piratebush is conceivable (Cummins 1971; Kolmer 2005).

The devastation of hemlock populations caused by the hemlock woolly adelgid is also of concern in piratebush populations. The photosynthetic ability of piratebush has never been evaluated, but, because it is a facultative parasite, its relative capacity for autotrophism could be diminished (Young and Wolf 1997). The ability of piratebush to switch hosts as an adult is also unknown. These facts compound concern for the persistence and growth of current piratebush populations that have been historically primarily parasitizing hemlocks.

An updated demography of piratebush populations will be compared with previous censuses of the same populations in order to determine if and how these epidemiological factors have affected populations. The data may be used as a reference for how best to maintain stability in piratebush populations where *Cronartium* rust or HWA are prevalent.

MATERIALS AND METHODS

Populations

Three geographically separate populations of *Buckleya distichophylla* were censused in northeastern Tennessee. The populations were chosen because of proximity to East Tennessee State University and because they had all been examined and censused previously, particularly before the decline of the populations of piratebush's preferred host (hemlock) due to hemlock woolly adelgid infestation.

The smallest and northernmost population examined was near Wilbur Lake (Fig. 2) along on the Watauga River in Carter County, Tennessee (coordinates: 36.339133, -82.120439). Wilbur Lake is a small, horseshoe shaped reservoir created by the construction of Wilbur Dam (WD). Although the lake measures about five kilometers in length, the piratebush population spans an area of about 0.81 hectares on the south side of the lake's inner peninsula. This area of the shore is primarily composed of rocky cliffs descending 10-20 m. The vegetation associated with the cliffs is dominated by Virginia pine (*Pinus virginiana)* and great rhododendron (*Rhododendron maximum*), with some red maple (*Acer rubrum*) and a variety of oaks (*Quercus* spp.). Because of the steepness of the cliffs, not all piratebush individuals present in this population were included in this survey. Approximately 50 plants were counted, but only 14 were accessible for full data collection.

The southernmost study site was located within the Cherokee National Forest near Paint Rock (Fig. 3) in Greene County, Tennessee (coordinates: 35.946092, -82.897887). The population was near the Tennessee-North Carolina state line where Paint Creek (PC) empties into the French Broad River. This population occupies an area of about 0.81 hectares of sandstone hills above Paint Creek and is dominated by hemlocks (*Tsuga canadensis*) and pines (*Pinus strobus*, *P. virginiana*) as well as several oak species. All individuals of this population were growing away from the riverbank, and, as a result, all individuals were accessible and counted. A total of 96 individuals were located.

The largest, most spatially dispersed population that was examined occurs near Chestoa along the Nolichucky River (Fig. 4) in Unicoi County, Tennessee (coordinates: 36.103751, -82.449517). It is sometimes referred to as the Temple Ridge (TR) population although the south section of this populations is restricted to Cliff Ridge rather than Temple Ridge. This population is divided into two subpopulations by the Nolichucky River, both along the Appalachian Trail. The population covers approximately 10 hectares, but the clusters of individuals are sporadic. This area is similar to the other study sites in that there are many large rocky outcrops on which the piratebush are numerous. The difference between this site and the others is that parts of this population grow at an elevation of 915+ meters (3000+ feet), approximately 460 meters (1500 feet) higher than the other sites. An abundance of pines (*P. virginiana, P. strobus, P. pungens*) and hemlocks (*T. canadensis, T. caroliniana*) cover slopes on both sides of the river, in addition to several species of oaks and maples. Hemlocks are the dominant trees in the area, but on and near the cliffs, pines become more abundant. Again, the cliffs caused several individuals to be inaccessible, and those individuals were not included in this study. The total number of individuals censused was 246.

Demography

From early May to mid-July several trips were made to the three study sites and data was collected on the piratebush populations. All three populations were included in earlier demographic surveys of piratebush in eastern Tennessee (Levy and Walker 2007; McCoy 2010; Mowbray 1985). To locate the specific areas of piratebush occurrences at each study site, preliminary reconnaissance trips were made in the early spring and regions of interest were noted. Later, several trips were made to each study site for data collection.

Piratebush is a dioecious shrub and therefore gender of individual plants was easily distinguishable when flowers were present and was recorded for each individual. Non-flowering plants were also tallied. In addition to noting the sex of each plant, seedlings were identified by the absence of woody stems and/or the occurrence of primary leaves and were noted as such.

Two different size measurements were made for each individual. First, a categorical height measure was noted for each plant. If the longest stem of the plant was shorter than one meter, the plant was assigned to category one. If the longest stem of the plant was longer than one meter, the plant was assigned to category two. Also, the number of stems for each individual plant was counted.

Because piratebush is a root parasite and attaches itself to nearby trees, the distance of each individual to the nearest tree as well as the species of that tree was noted. Because of time, resource constraints, and rare plant regulations, parasitic relationships between individual piratebush plants and their nearest trees were presumed but not confirmed. Also, distance measurements were approximated and not exact.

Coordinates of individual piratebush plants were recorded using a Garmin eTrex HCx hendheld Global Positioning System (GPS) and Garmin Basecamp software. Each plant was assigned a waypoint with coordinates. Piratebush individuals within approximately one meter from each other were marked with a single waypoint and given the same coordinates because the resolution of the GPS system was not accurate at smaller distances. After each data collection trip, appropriate demographic data was assigned to each waypoint indicating the sex, size, health, and nearest tree for each piratebush individual.

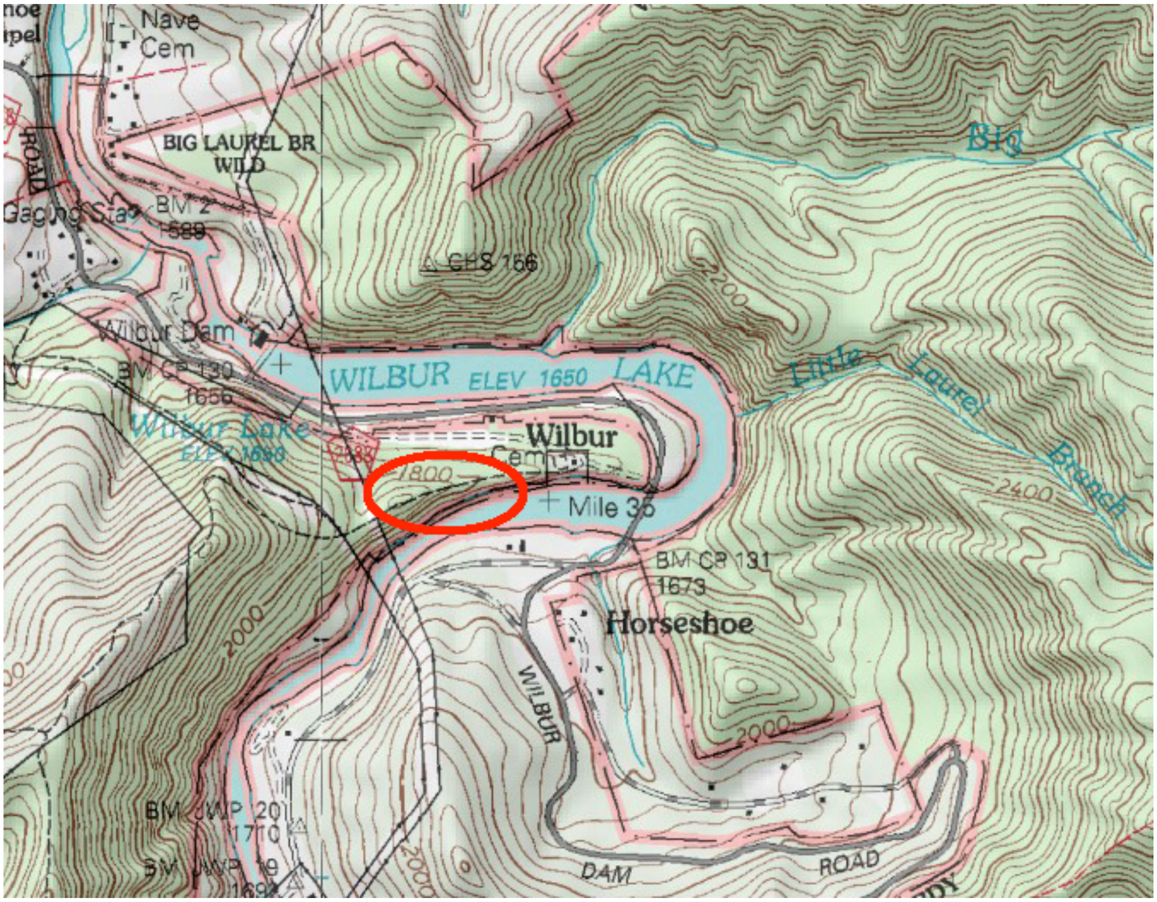

Figure 2. Wilbur Dam quadrangle showing area containing piratebush individuals censused.

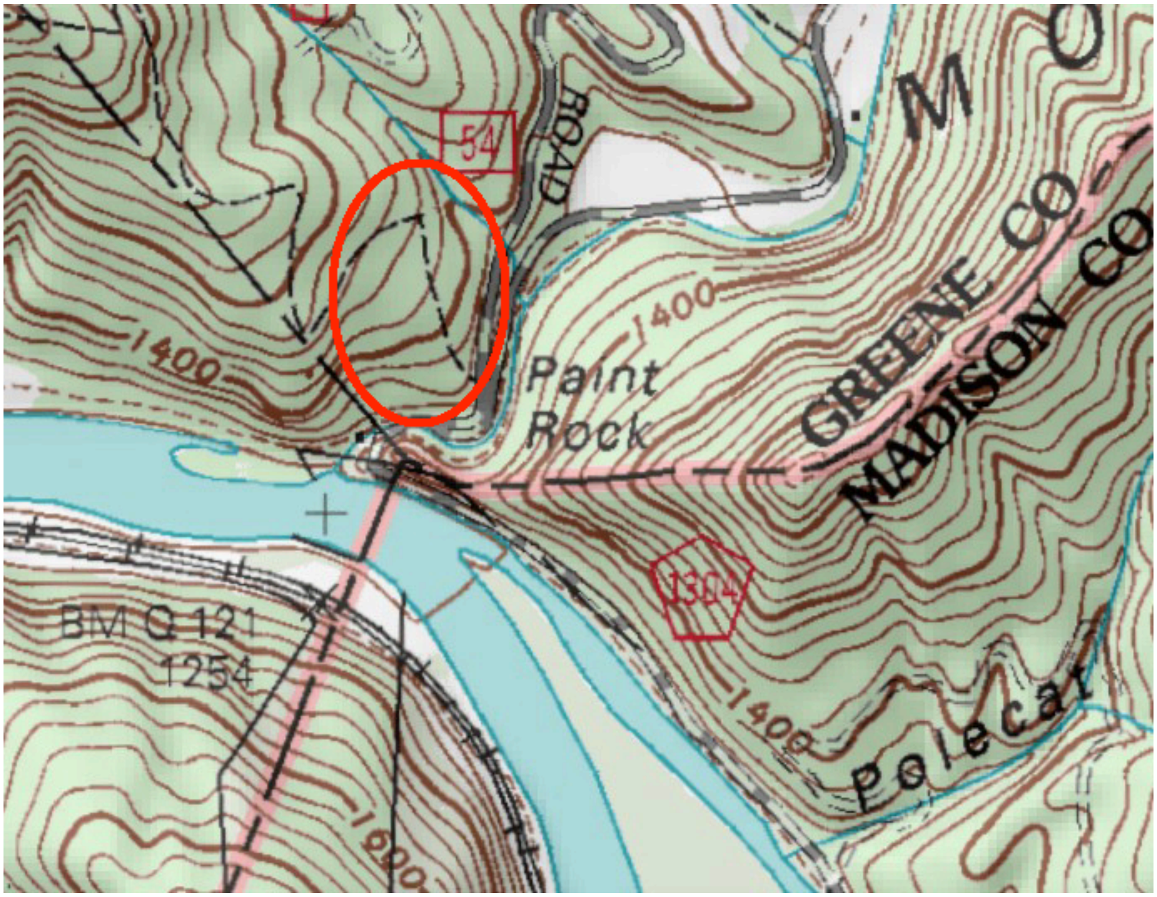

Figure 3. Paint Creek quadrangle showing area containing piratebush individuals censused.

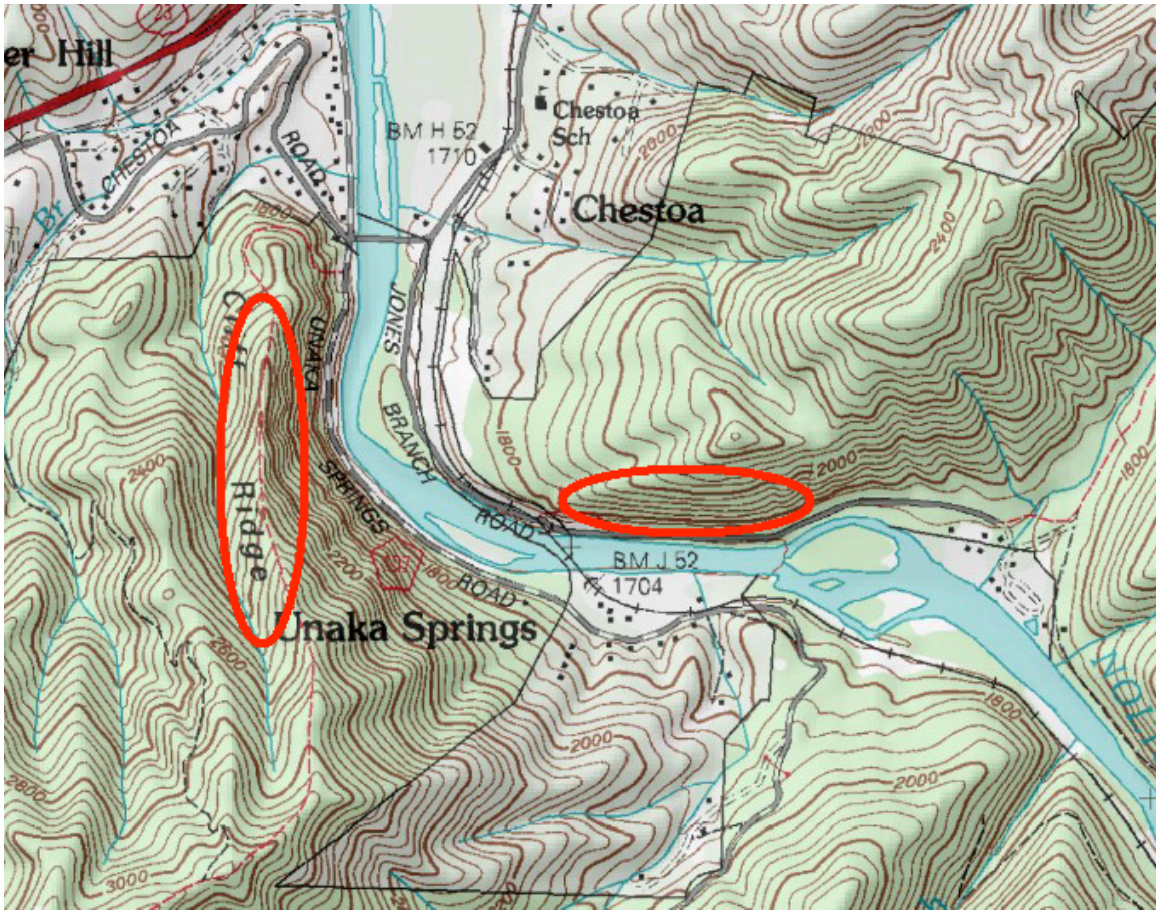

Figure 4. Temple Ridge quadrangle showing areas containing piratebush individuals censused.

Disease

The vigor of each plant was recorded in the early summer as each piratebush plant was examined and demographic information was collected. Vigor was rated based on a whole number 3-point scale. Plants that exhibited no signs of disease were assigned a vigor rank of 1. Plants with chlorotic or withered leaves were assigned a vigor rank of 2. Plants with sparsely leafed stems or dead stems were assigned a vigor rank of 3. The level of vigor of each plant was then attached to the appropriate waypoint.

Piratebush is the secondary host of a rust fungus (*Cronartium appalachianum*). This rust does not exhibit fruiting bodies until late into the host plant's growing season. Several more outings were made to these populations in late summer and early fall to record the presence and amount of rust infection for each plant. The amount of rust infection was rated on a whole number 5-point scale with 1 representing very slight infection and 5 assigned to plants whose leaves had nearly complete rust coverage.

Virginia pine trees that may host piratebush may also serve as the primary host for *C. appalachianum*. Because of this, the presence of rust on presumed piratebush pine hosts was also noted.

The sex of each diseased plant was also recorded if flowers and/or fruits were present. Additionally, the light level (shade, partial shade, light) in which each plant was growing was also recorded. All instances of rust were mapped using GPS waypoints and each of the above mentioned characters were assigned to the appropriate waypoint.

Data Analysis

Sex ratios were calculated for each population independently and were compared to the 1:1 (male:female) expected ratio using chi-square goodness-of-fit (Paint Creek and Temple Ridge) and Fisher's exact tests (Wilbur Dam). A chi-square test for heterogeneity was then used to compare sex ratios between the Temple Ridge and Paint Creek populations (the Wilbur Dam population was omitted due to small sample size).

Descriptive statistics were used to characterize the number of stems of plants within populations as well as the distance to the presumed host. An ANOVA was then used to compare mean stem number and mean distance to the presumed host between populations. A t-test was used to compare mean stem number between relative height categories for all observations.

The ratio of seedlings to adults was compared among populations using a chi-square test for heterogeneity. Chi-square tests for heterogeneity were used to compare vigor categories and relative plant height within and among populations. Additionally, a chi-square test for heterogeneity was used to analyze the relationship between plant height and presence of flowers.

A chi-square test for goodness-of-fit was used within populations to determine if there was an even distribution of presumed hosts. A chi-square test for heterogeneity was used to determine if there was a difference in the identities of presumed hosts among populations. This was done to investigate if piratebush exhibits a host preference in natural environments. A chi-square test of heterogeneity was also utilized to determine variation in plant vigor between populations.

Several characters relating to disease were also analyzed. Prevalence of rust on piratebush, severity of rust on piratebush, and light level of diseased plants were analyzed using chi-square tests of heterogeneity to test for differences in these characters between populations. Additionally, the medians of disease severity were compared between the Paint Creek and Temple Ridge populations using a Mann-Whitney U test. Chi-square tests of heterogeneity were used to see if disease in piratebush was correlated with presumed host species, plant vigor, stem number, age, and relative height.

The relationship of sex and disease was analyzed within each population with a chi-square test for goodness-of-fit (Wilbur Dam was omitted because of small sample size), and among populations with a chi-square test for heterogeneity.

The scan statistic was used to test for clusters of disease, disease severity, sex, host, plant vigor, and seedlings. The cluster analyses were performed using SaTScan software v.

9. 2. (Kulldorf et al. 2005). SaTScan utilizes the scan statistic to determine if significant clustering occurs. It does this by simulating a window over the range of the coordinates provided, and scanning that range while tallying the number of observed occurrences for the character of interest. The view window that gives the highest number of occurrences in the actual data is the scan statistic. This process is done with the actual data and then with randomly generated distributions of the same data to arrive at a probability associated with that scan statistic. In the randomly generated data sets, the number of times that the occurrences within the view window exceeds the actual data scan statistic are divided by the total number of view window trials in the randomly generated data sets. The resulting number is the p-value associated with the scan statistic.

RESULTS

Demography

Population Dynamics

Sex ratios for the Temple Ridge (TR) and Wilbur Dam (WD) populations did not deviate significantly from the expected 1:1 ratio (TR: $\chi^2=0.01$, $p=0.94$; WD: $p=0.51$) (Fig. 5), but the Paint Creek (PC) sex ratio was significantly skewed toward a higher fraction of male plants (69% male; $\chi^2=8.02$, $p<0.01$) (Fig. 5). Sex ratios of the Paint Creek and Temple Ridge populations were significantly different from one another with more males at Paint Creek ($\chi^2=5.15$, $p=0.02$). There was no difference between sex ratios in Wilbur Dam and Paint Creek ($p=1.0$) or between Wilbur Dam and Temple Ridge ($p=0.50$). The proportion of non-flowering individuals was similar at each population with 41% at Paint Creek, 33% at Temple Ridge, and 36% at Wilbur Dam ($\chi^2=2.88$, $p=0.24$).

Plants were not significantly clustered by sex at Paint Creek or Wilbur Dam. Temple Ridge had two significant clusters, one cluster (~40 m^2; 36.101551, -82.450583; $p<0.01$) of 43 plants that contained 41 (95%) non-flowering individuals and another (~8,200 m^2; 36.100603, -82.450828; $p<0.01$) with 105 plants dominated by 95 flowering individuals (59% male, 42% female).

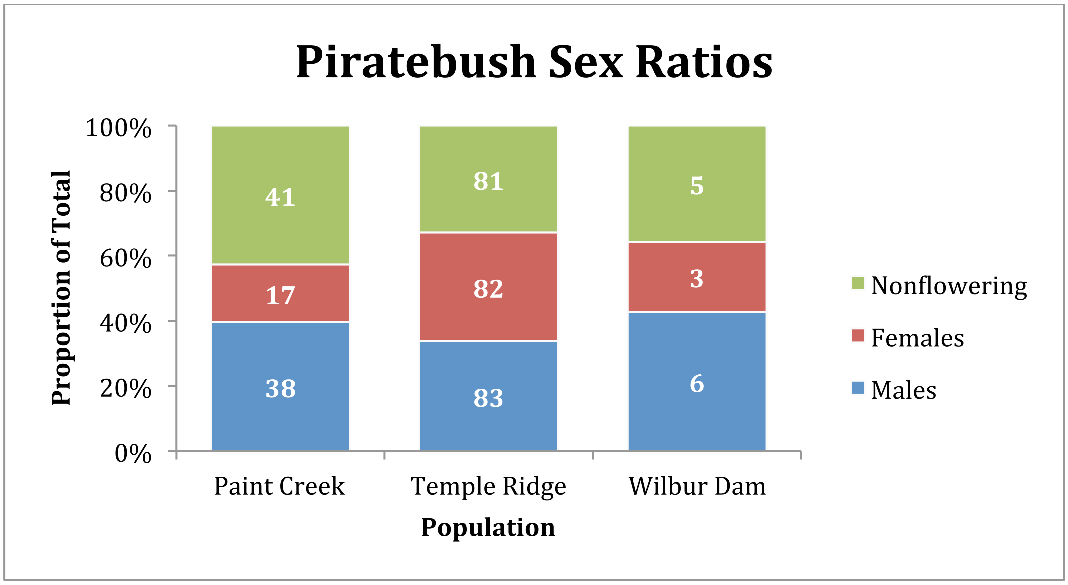

Figure 5. Sex ratios for three populations of piratebush. Number of plants overlaid on their representative section of proportion bars.

The seedling to adult ratio was strongly skewed toward a greater proportion of adult plants, and this proportion, which ranged from 81-86%, was similar among all populations ($\chi^2=0.33$, $p=0.85$)(Fig. 6).

One seedling cluster (35.945462, -82.897579; $p<0.01$) spanning approximately 800 m^2 was found at Paint Creek. In this cluster, 14 of 29 plants (48%) were seedlings. At Temple Ridge, one cluster of seedlings (40 of 43 (93%) in an area of approximately 40 m^2) (36.101551, -82.450583; $p<0.01$) and three clusters (36.100783, -82.450549; 36.100508, -82.450922; 36.101202, -82.450721; all $p<0.01$) of adult plants were found.

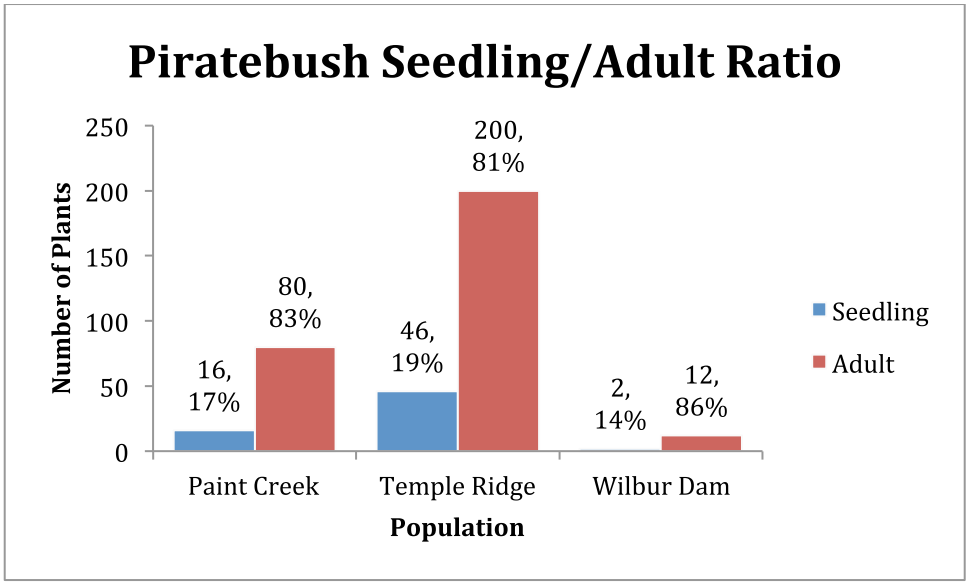

Figure 6 Proportion of seedling and adult plants for three piratebush populations. Number of plants and percentages are shown above each bar.

Plant Size and Vigor

Plant vigor in all populations was significantly skewed toward high vigor plants (PC: χ^2=192.0, p<0.01; TR: χ^2=395.3, p<0.01; WD: χ^2=7.61, p=0.02). There was a significant difference in the plant vigor between populations because there were no low vigor plants found at the Paint Creek site (χ^2=19.5, p<0.01)(Fig. 7).

Plants were not significantly clustered by high or low vigor at either Paint Creek or Wilbur Dam, but at Temple Ridge there was a cluster of 122 high vigor plants (~9,500 m^2; 36.101235, -82.450685; p=0.02) and cluster of 11 lower vigor plants (~8,200 m^2; 36.100071, -82.450535; p=0.02).

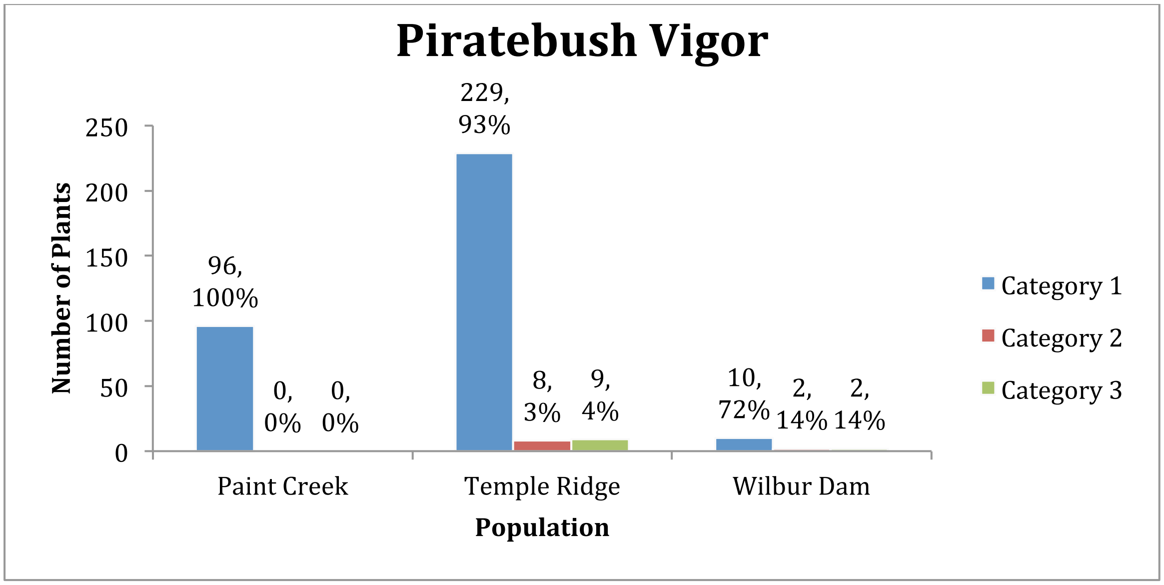

Figure 7. Vigor proportions in three piratebush populations. Number of plants and percentages above each bar. Category 1=most vigorous, Category 3=least vigorous.

Relative plant height (taller or shorter than one meter) was not skewed at either the Paint Creek or Wilbur Dam populations (PC: χ^2=1.50, p=0.22; WD: p=0.18). There were significantly taller plants in the Temple Ridge population (χ^2=13.68, p<0.01). In all these populations, taller plants were more numerous, with the most extreme difference at Temple Ridge. Nevertheless, relative plant height was not significantly different between populations (χ^2=1.59, p=0.451)(Fig. 8). Additionally, smaller plants were much less likely to be in flower than were larger plants (χ^2=185.0, p<0.01)(Fig. 9).

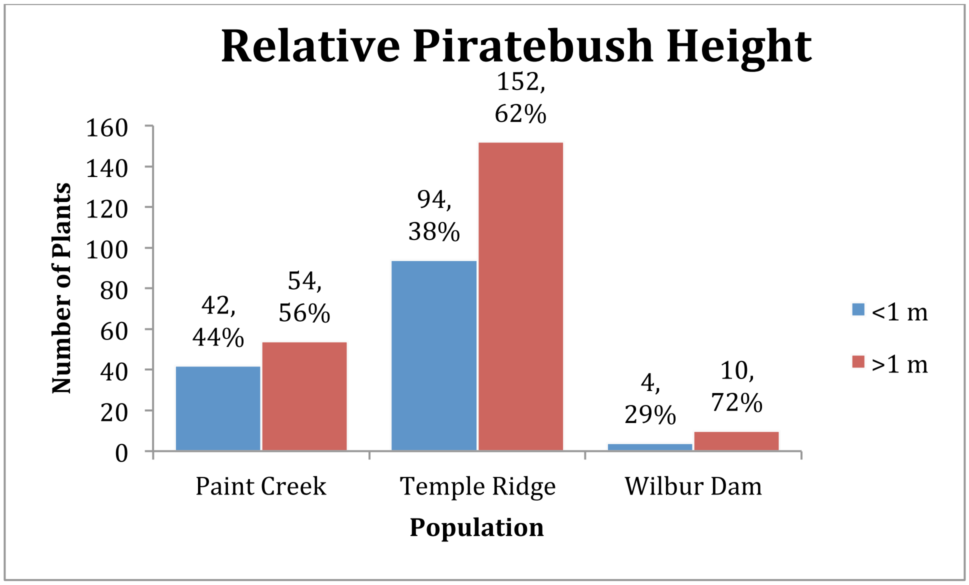

Figure 8. Relative height proportions for three piratebush populations. Number of plants and percentages above each bar.

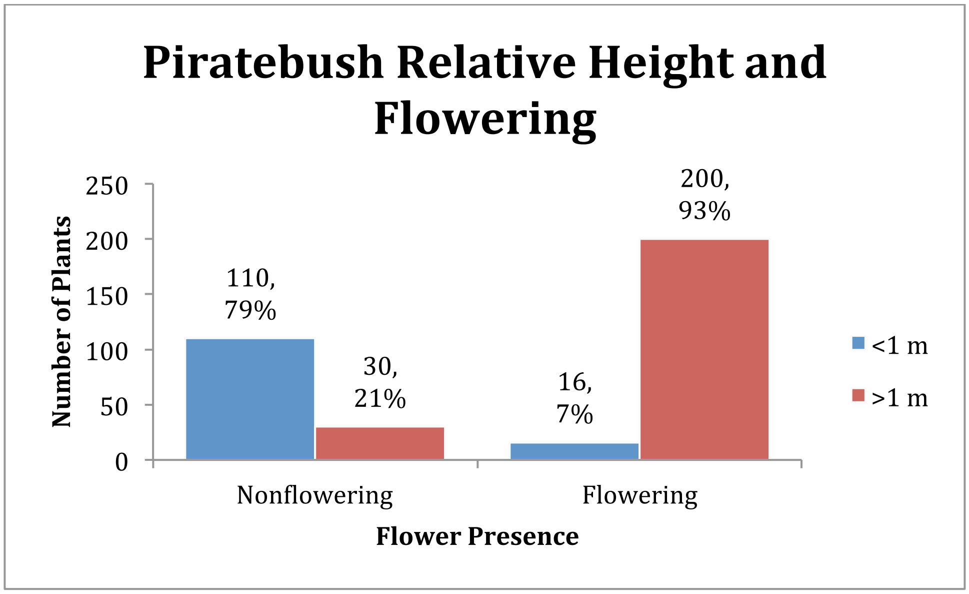

Figure 9. Combined relative height proportions for flowering and non-flowering categories of piratebush individuals in all three populations. Number of plants and percentages above each bar.

The mean number of stems per plant was not significantly different between populations (F=0.49, p=0.61)(Fig. 10). However, mean stem number was significantly lower among smaller plants (t=-9.7, p<0.01)(Fig. 11).

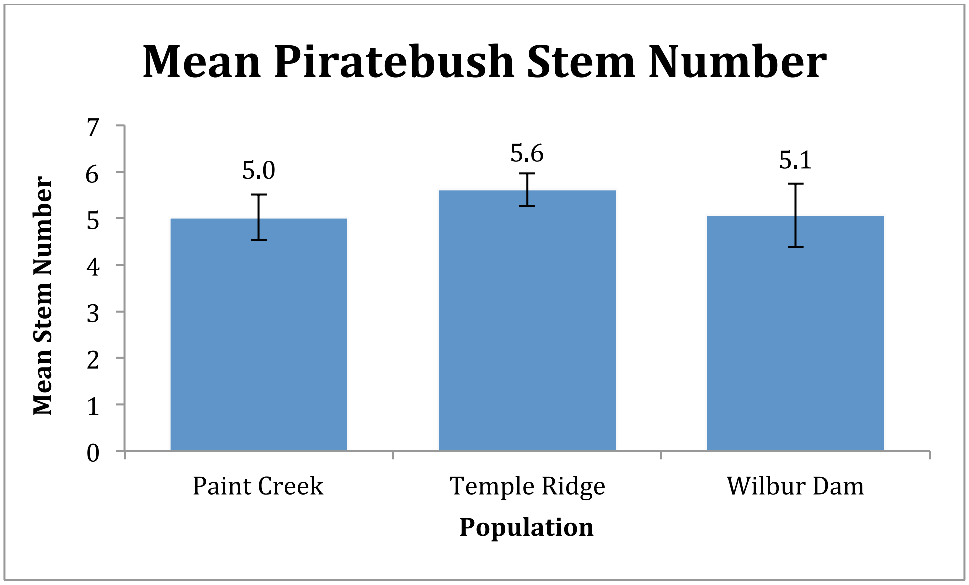

Figure 10. Mean number of stems per plant with standard error of the mean for three piratebush populations.

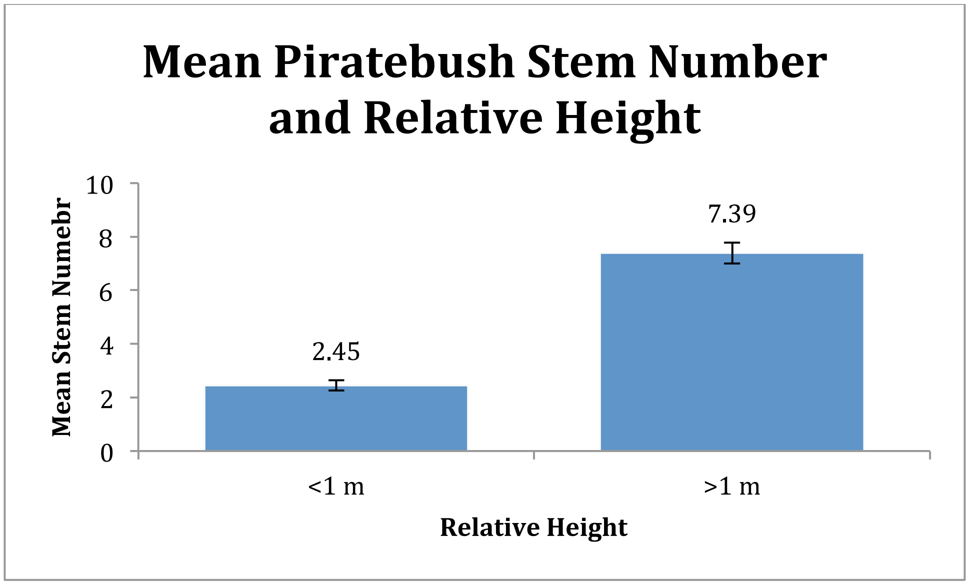

Figure 11. Mean number of stems per plant with standard error of the mean for two relative height categories

Nearest Tree/Host Interactions

The mean distance to the nearest tree, i.e., presumed host species, was 1.1-1.3 m in all populations, and when the means were compared among populations there were no significant differences (F=0.13, p=0.88)(Fig. 12).

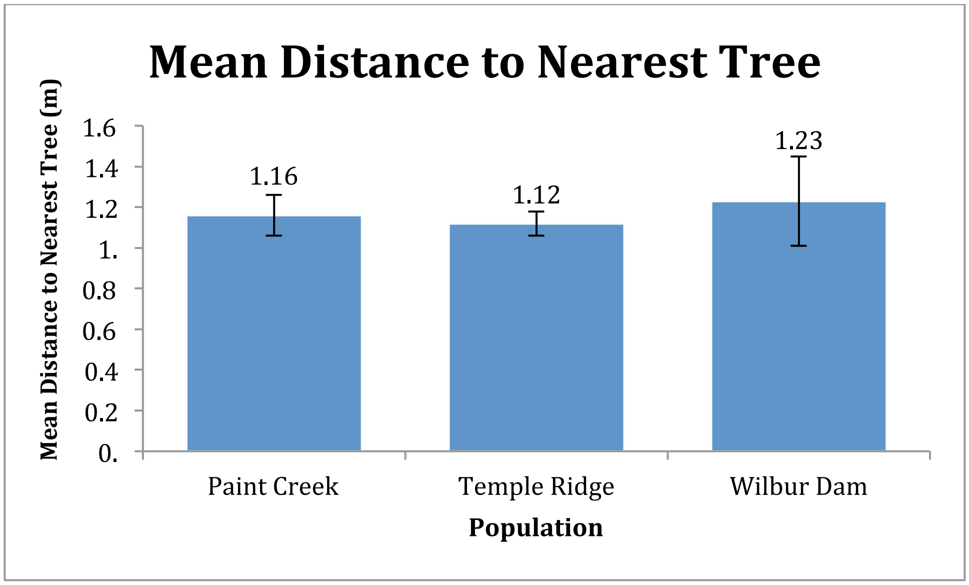

Figure 12. Mean distance to nearest tree (m) with standard error of the mean for three piratebush populations.

If the assumption that the nearest tree is the actual host is correct, then the Paint Creek and Temple Ridge populations displayed significant host preferences (PC: χ^2=84.5, p<0.01; TR: χ^2=516.7, p<0.01), but the Wilbur Dam populations did not (χ^2=11.0, p=0.09). *Tsuga canadensis* and *Pinus virginiana* were the most common presumed host species at Paint Creek while *T. caroliniana* and *T. canadensis* were the most common presumed host species at Temple Ridge. Presumed host preference was significantly different between populations (p<0.01)(Fig. 13).

There were two significant clusters of presumed host species at Paint Creek. The first was a cluster of *Acer rubrum* (~10,200 m^2; 35.945967, -82.897222; p<0.01) and the second was a cluster of *T. canadensis* (~800 m^2; 35.945355, -82.897525; $p<0.01$). Similarly, at Temple Ridge, a cluster (~3,000 m^2; 36.101531, -82.450613; $p<0.01$) of *T. caroliniana* and a cluster (~2.21 km^2 36.099669, -82.441453; $p<0.01$) of pines (*P. strobus* and *P. virginiana*) were found. The Wilbur Dam population exhibited no host species clustering.

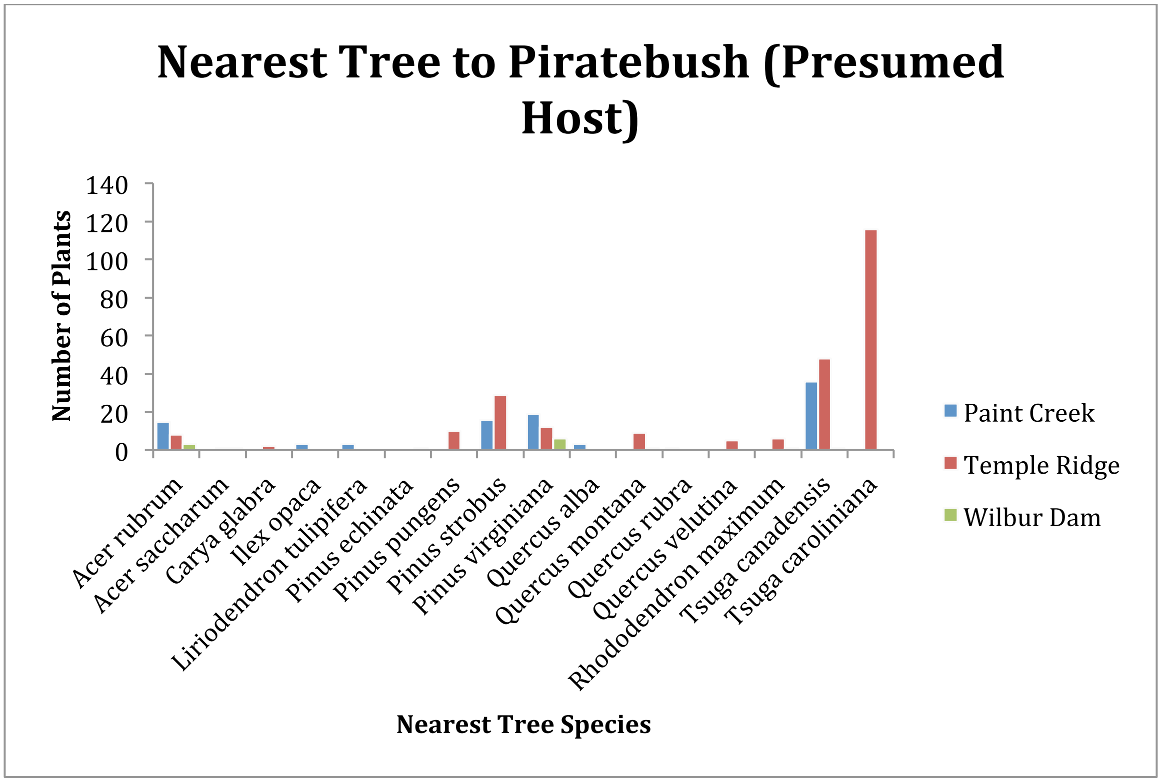

Figure 13. Distribution of presumed hosts for three piratebush populations.

Disease

Disease Prevalence and Severity

All populations displayed comparable ratios of diseased (19-29%) to healthy plants with no significant differences in the prevalence of *Cronartium* rust between populations ($\chi^2=0.79$, $p=0.67$)(Fig. 14). One Virginia pine was found with *Cronartium* rust at the Temple Ridge population. No other population yielded evidence of rust in primary host plants, but the season for observing visual evidence of infection in those host plants was past when the data were recorded. At all sites, disease prevalence was randomly distributed throughout the population as no significant clusters of diseased or healthy plants were found.

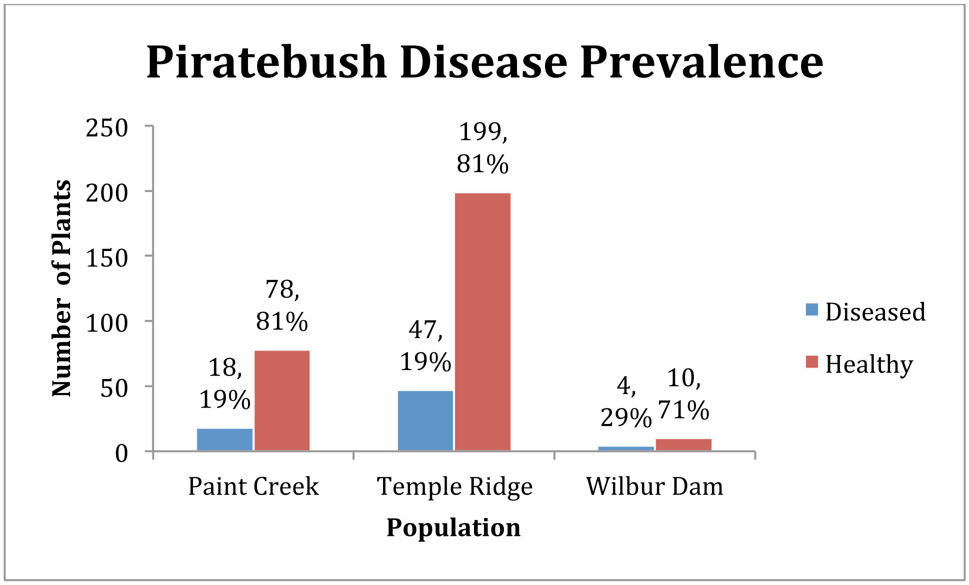

Figure 14. Proportions of diseased and healthy plants for three piratebush populations. Number of plants and percentages above each bar.

The Temple Ridge population had the highest disease severity of all populations and was the only population with plants of the two most severely diseased categories. The comparison of disease severity showed a significant difference between populations ($p<0.01$)(Fig. 15). Additionally, the medians of the Temple Ridge and Paint Creek populations were significantly different (Medians: TR=3; PC=2; $p<0.01$). No clusters of more or less severely diseased plants were found.

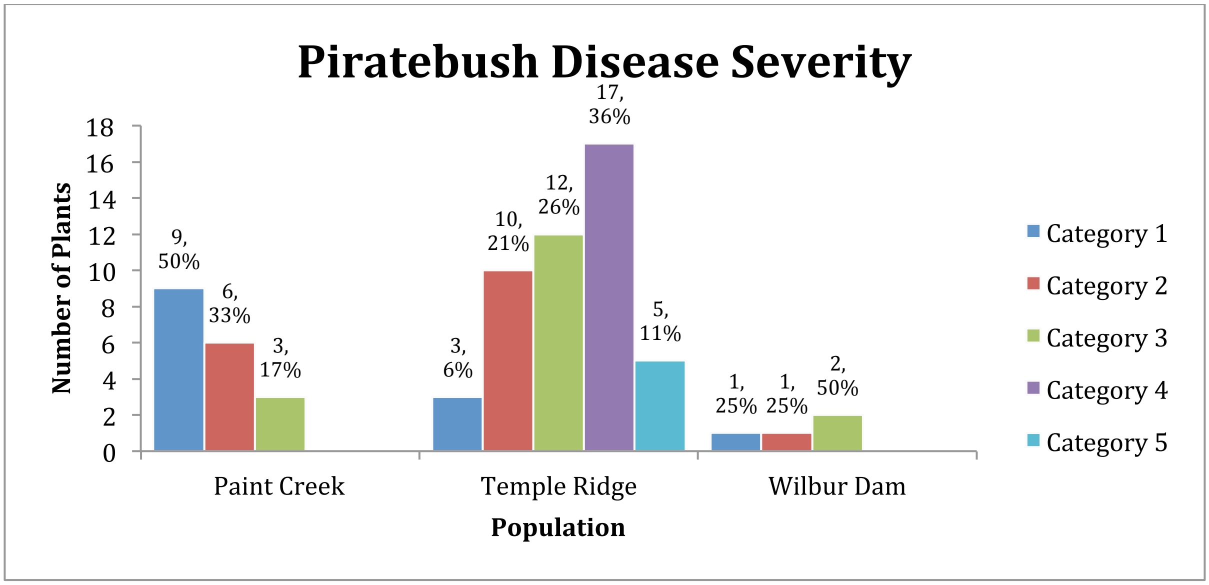

Figure 15. Disease severity distribution for three piratebush populations. Number of plants and percentages above each bar. Category 1=least severe, category 5=most severe.

Disease and Sex

Plants with rust were more likely to be male or non-flowering than female in all populations, but no more so than expected based on the proportion of male to female plants within each population. (PC: p=0.58; TR: p=0.16). The Wilbur Dam population was not analyzed for an association between sex and disease because no diseased females were found. There was no significant difference between the Paint Creek and Temple Ridge populations in the association between disease and sex (p=0.46)(Fig. 16).

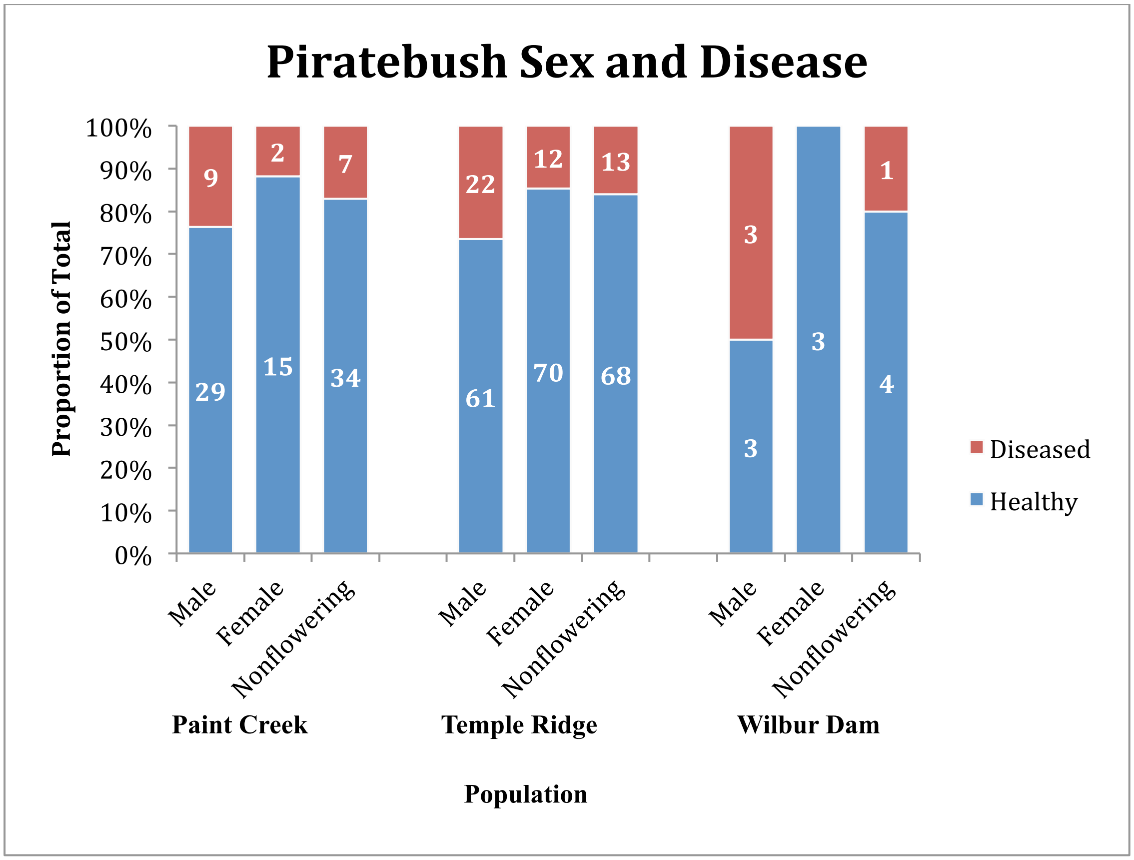

Figure 16. Proportion of disease prevalence in piratebush for each sex category in three populations. Number of plants is overlaid on their representative sections of bars.

Disease, Size, and Age

The characters of relative plant height (PC: p=0.43; TR: p=0.86; WD: p=0.35) and mean stem number (PC: p=0.98; TR: p=0.06; WD: p=0.29) had no association with disease in any population. However, there was a marginally significant difference in the distribution of disease between seedlings and adults in the Paint Creek and Temple Ridge populations with a larger proportion of disease in adult plants (PC: p=0.07; TR: p=0.06; WD: p=0.56)(Fig. 17-19).

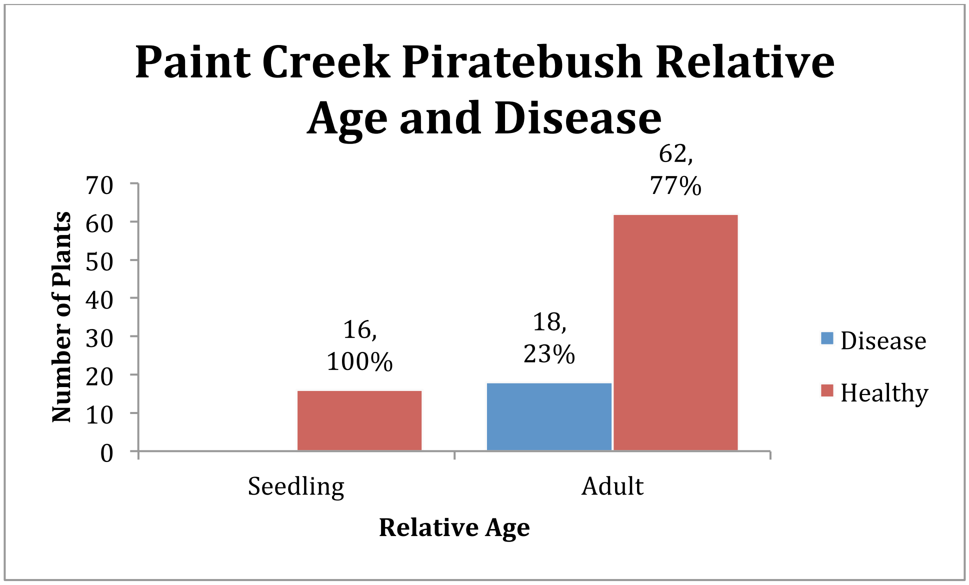

Figure 17. Comparison of disease in piratebush among seedlings and adults within the Paint Creek population.

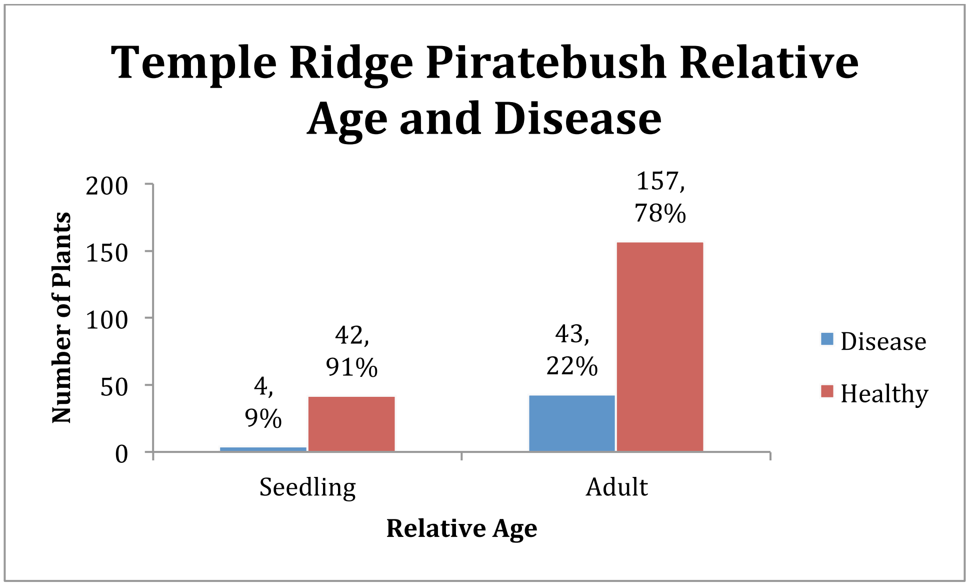

Figure 18. Comparison of disease in piratebush among seedlings and adults within the Temple Ridge population.

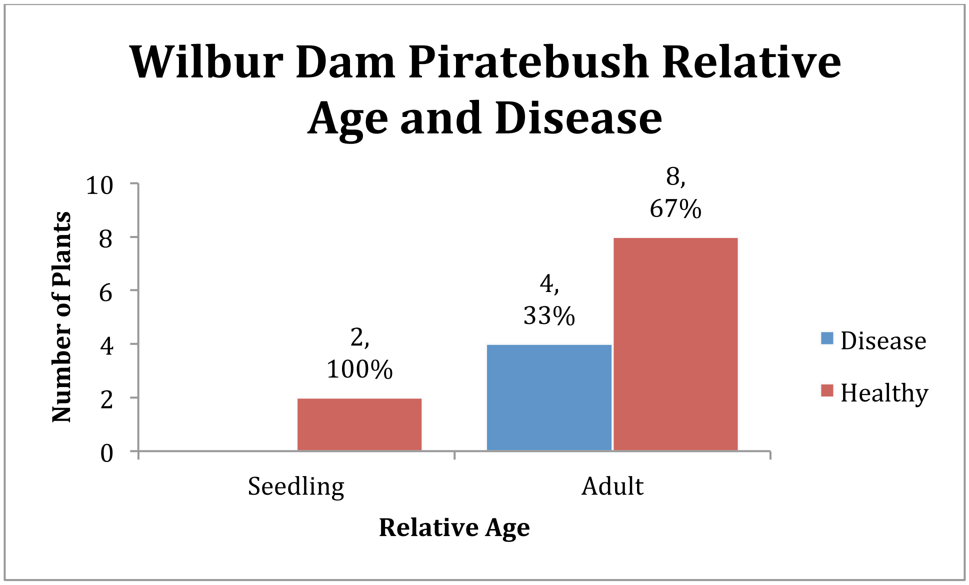

Figure 19. Comparison of disease in piratebush among seedlings and adults within the Wilbur Dam population.

Disease and Vigor

In the Paint Creek and Wilbur Dam populations, no association was found between plant vigor and disease (PC: p=1.00; WD: p=0.13)(Fig. 20, 22). In the Temple Ridge population, disease prevalence was significantly skewed toward plants with lower vigor (vigor categories 2 and 3) (p=0.01)(Fig. 21). However, lower vigor plants were not more likely to have a higher severity of disease (p=0.57).

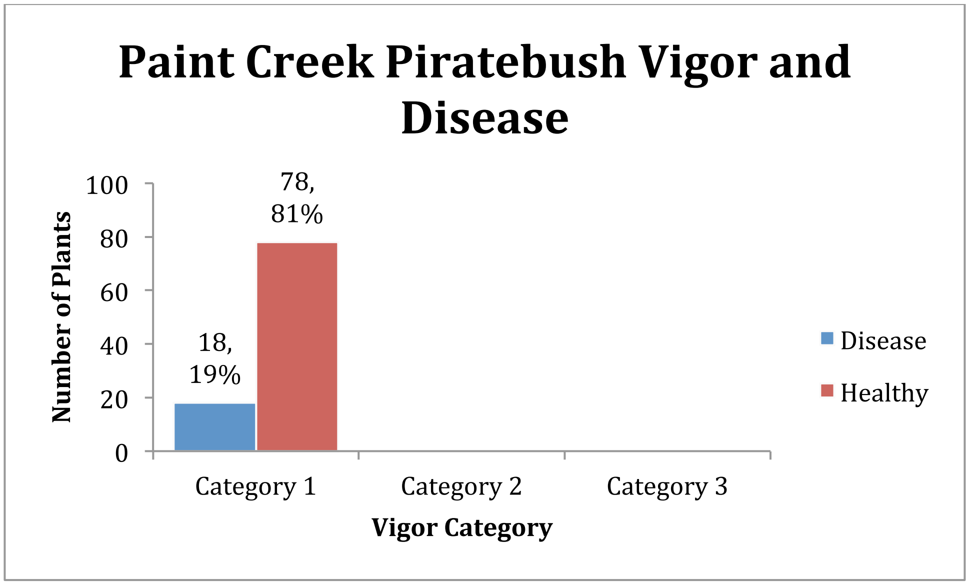

Figure 20. Comparison of piratebush disease prevalence and vigor at Paint Creek. Number of plants and percentage above each bar. Category 1=most vigorous, Category 3=least vigorous.

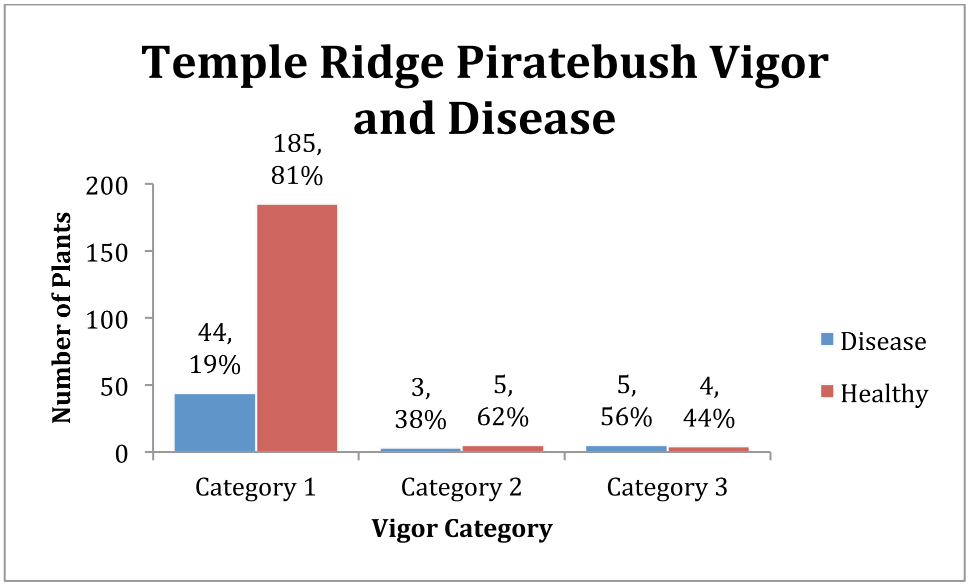

Figure 21. Comparison of piratebush disease prevalence and vigor at Temple Ridge. Number of plants and percentage above each bar. Category 1=most vigorous, Category 3=least vigorous.

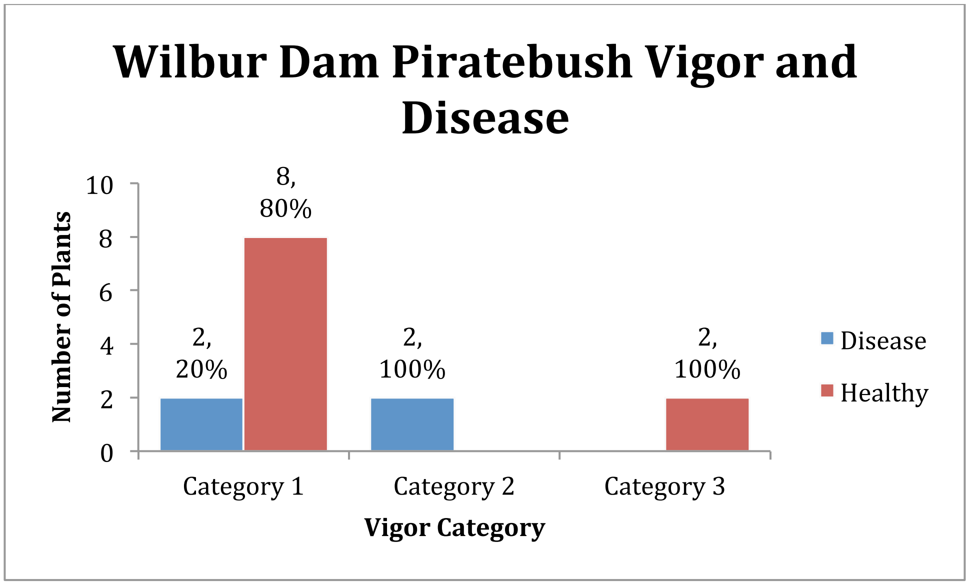

Figure 22. Comparison of piratebush disease prevalence and vigor at Wilbur Dam. Number of plants and percentage above each bar. Category 1=most vigorous, Category 3=least vigorous.

Disease and Presumed Host

In the Paint Creek and Wilbur Dam populations there was no significant association of presumed hosts with rust disease (PC: χ^2=7.10, p=0.42; WD: χ^2=7.47, p=0.28)(Fig. 23, 25). The Temple Ridge population showed a significant difference between the expected (based on number of individuals of a host species) and observed (based on number of diseased piratebush on a host species) disease-host association (χ^2=24.59, p<0.01)(Fig. 24). Among the top four presumed hosts at Temple Ridge (*P. strobus, P. virginiana, T. canadensis, T. caroliniana*), disease prevalence was significantly different with higher levels of disease associated with *P. virginiana* (χ^2=8.40, p=0.04)(Fig. 27).

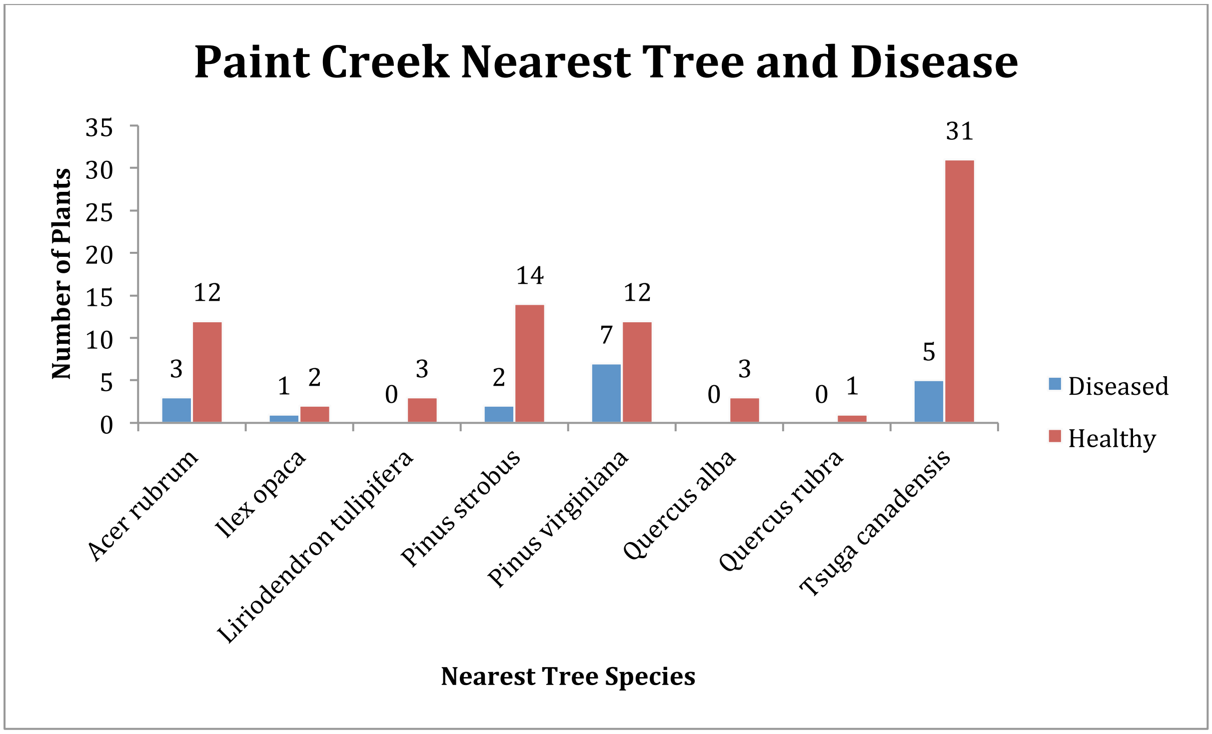

Figure 23. Distribution of diseased and healthy piratebush plants for each presumed host at Paint Creek. Number of plants above each bar.

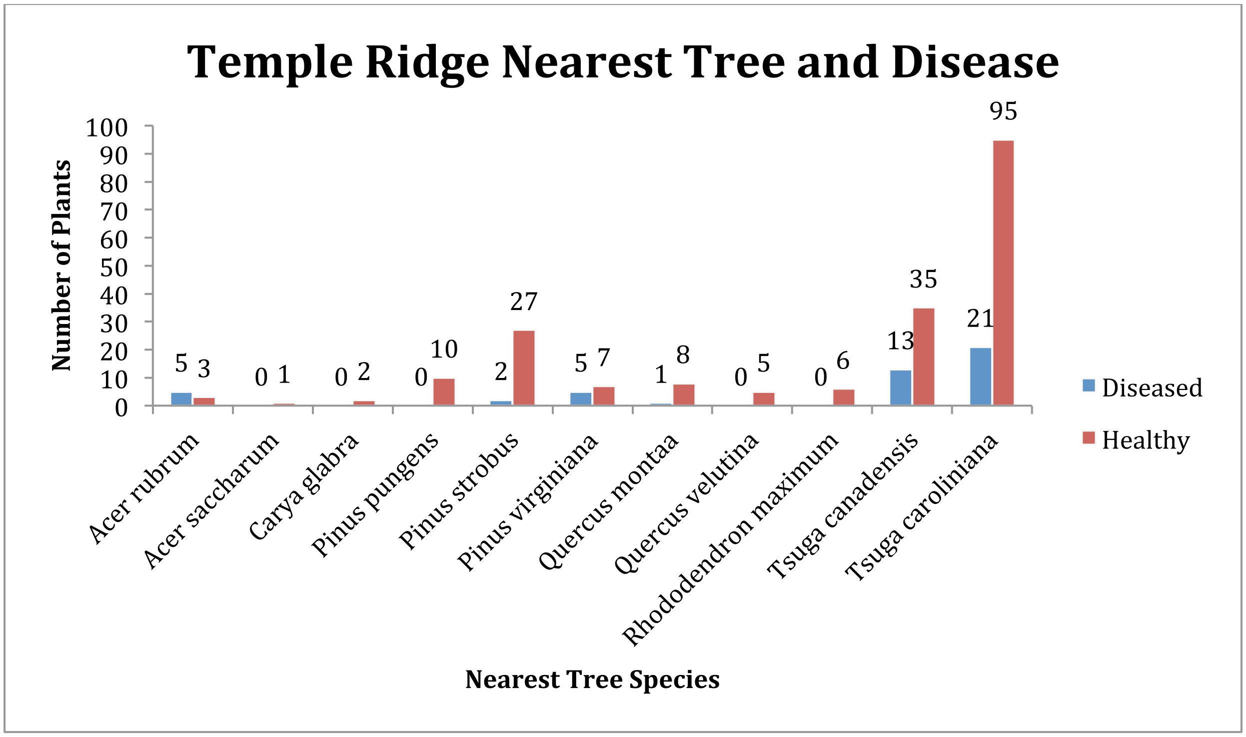

Figure 24. Distribution of diseased and healthy piratebush plants for each presumed host at Temple Ridge.

Number of plants above each bar.

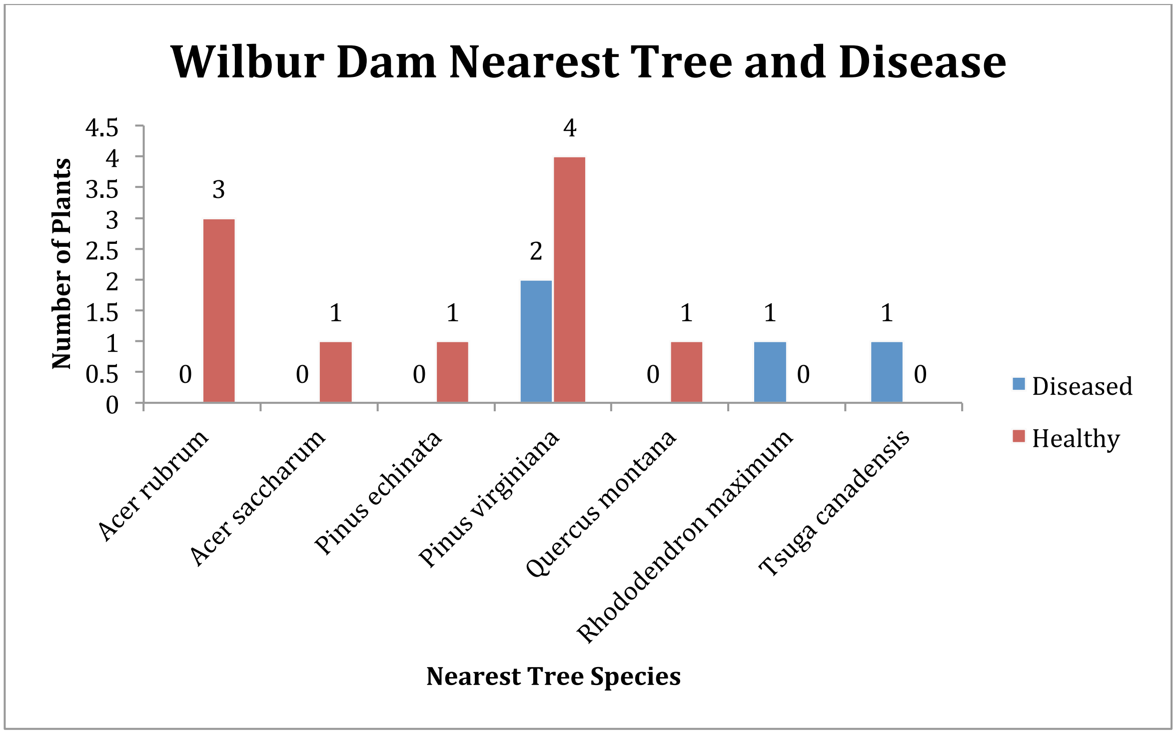

Figure 25. Distribution of diseased and healthy piratebush plants for each presumed host at Wilbur Dam. Number of plants above each bar.

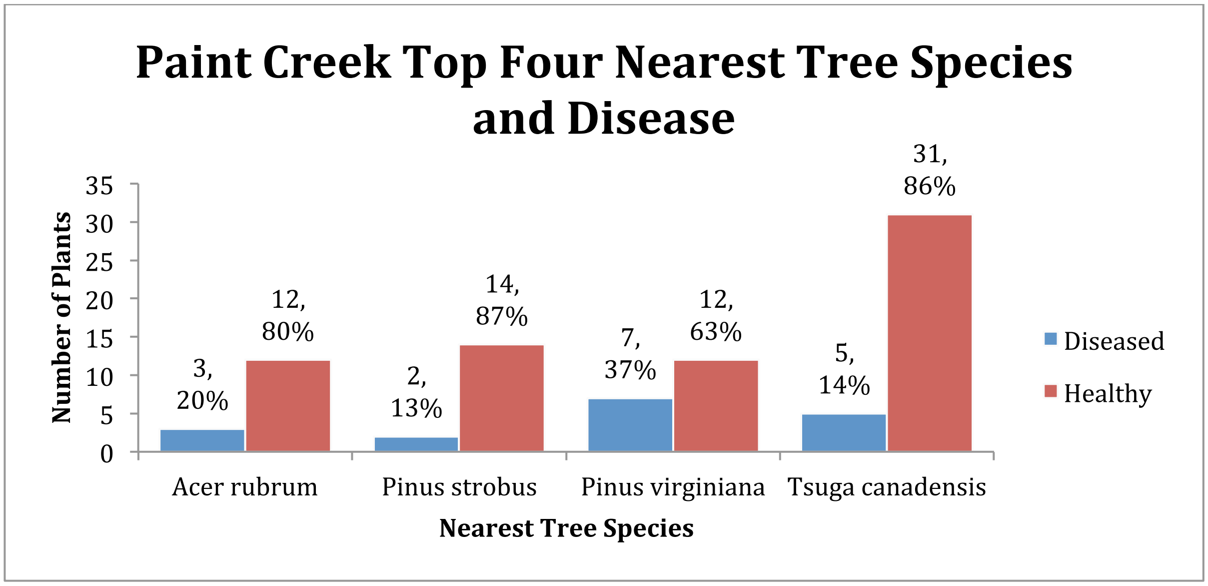

Figure 26. Distribution of diseased and healthy piratebush plants for each of the top 4 presumed host species at Paint Creek. Number of plants and percentage above each bar.

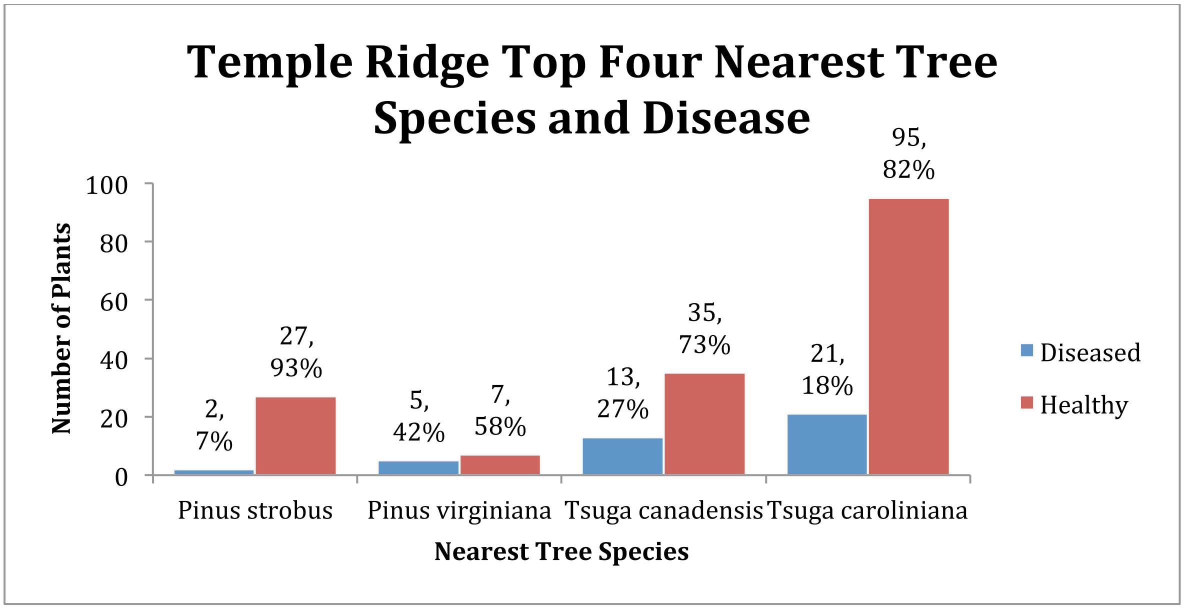

Figure 27. Distribution of diseased and healthy piratebush plants for each of the top 4 presumed host species at Temple Ridge. Number of plants and percentage above each bar.

Disease and Light

The amount of light in which a piratebush individual was growing had no significant effect on the prevalence of disease in any of the three populations (PC: χ^2=2.33, p=0.31; TR: χ^2=0.30, p=0.86; WD: χ^2=3.50, p=0.17). When disease prevalence by light category was compared between populations, there was no significant difference (χ^2=1.26, p=0.53)(Fig. 28).

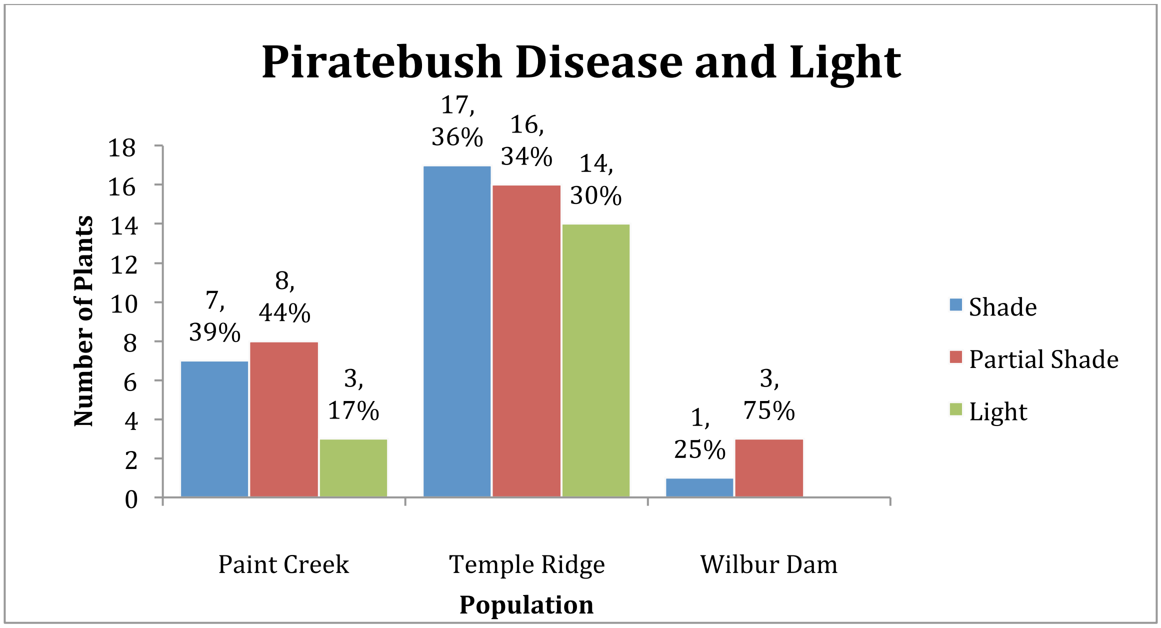

Figure 28. Light treatment of diseased plants in three populations of piratebush. Number of plants and percentage above each bar.

DISCUSSION

Demography

In a Virginia population, male piratebush plants had a flowering period of approximately one month, from late April to late May, and they tended to begin flowering one to two weeks before female plants (Huish et al. 2015). Though two of the three populations studied in Tennessee were predominantly male, a sex-based difference in floral phenology could be responsible for why a significant difference was only observed in the Paint Creek population. The Paint Creek population was the first site visited in early to mid-May, while the Temple Ridge and Wilbur Dam sites were first visited in late May. Possibly, many male plants at the latter populations had abscised their flowers by that point causing those plants to be counted among the non-flowering. However, this possibility is not likely due to the fact that, even after floral abscission, the peduncle of the male inflorescence often persists, thus making sex identification relatively easy.

Two of the three populations studied (Temple Ridge & Wilbur Dam) did not differ from the expected equal representation of sex. However, it is still unclear whether or not a prescriptive sex ratio can be assigned to piratebush due to the fact that the only sex ratios that were significantly different from one another were the male-biased Paint Creek ratio and near equal Temple Ridge ratio. The Wilbur Dam ratio was similar to both the Paint Creek and Temple Ridge ratios. Both a male-biased and equal or female-biased ratio have been reported in previous studies. While piratebush populations in Virginia have been described as exhibiting a male-biased sex ratio, the sex ratios of other species within Santalaceae have been shown to be either equal or female-biased (Field et al. 2013a; Field et al. 2013b; Huish et al. 2015; Rottenberg 1998). It has also been noted that, in dioecious species, female reproductive structures require more energy to produce, and this contributes to a greater number of non-flowering individuals by delaying female plants from flowering until later adulthood (Barrett et al. 2010). This would lend support to the proposition of Huish et al. (2015) that not only is size an indicator of age in piratebush, the absence of flowers on an individual may indicate juvenility, as well.

In a recent study by Huish et al. (2015), non-flowering piratebush individuals were often found to be significantly smaller than flowering individuals. Thus, two characters, small size and absence of flowers, were considered signs of juvenility (Huish et al. 2015). Our findings support the assertion that smaller plants (<1 m) were significantly less likely to produce flowers than larger plants. This suggests that smaller individuals have not yet reached reproductive maturity. Furthermore, smaller relative heights were strongly associated with a smaller mean stem number, which suggests that stem number may also be an indicator of age and maturity. In combination, the three characters of height, flower presence, and stem number may be used as indicators of juvenility and maturity of piratebush individuals.

The ability to identify seedlings and young piratebush individuals is of great importance for future conservation studies. Long term and/or periodic investigations of Tennessee piratebush populations will be needed to better understand the specifics of population growth and stability in piratebush. Many models have been designed to predict whether or not a population size is stationary. The stable population model states that if fertility and death rates are fixed and net migration equals zero, then the population will be stationary (Kuhn 2004). More recently this model has been updated to account for immigration stating that if fertility rate is less than death rate, immigration may be higher than zero in stationary populations (Espenshade et al. 1982). Still another model (stage distribution) has been produced that may be applicable to piratebush. This model suggests that, specifically in plants, it may be better to gauge population stability by the distribution of morphological and developmental stages rather than age (Werner and Caswell 1977). With insight on the relationship between piratebush size, stem number, and flowering, the stage model may be the more accurate model for understanding population growth trends in piratebush. Whichever model is found to be most applicable, it seems that each population's demographics are variable depending on life history and community factors as well as many others.

Though it is unknown what fertility rate is necessary for stability in piratebush, seedling establishment was observed in all populations. Furthermore, little variation was found in the proportion of seedlings between populations. Whether or not this suggests stable populations is unknown, but the fact that seeds are establishing and growing is promising. Moreover, since the species is dioecious, there is heightened potential for adaptation to environmental challenges through sexual recombination. Further investigation into seedling establishment is needed, however, particularly in regards to the probability of a seedling finding a suitable host. It was noted while collecting data in all populations that many seedlings were growing very near to the base of their parent plant. This raises the question of whether or not a piratebush seedling can parasitize a piratebush adult. Though this strategy may not prove successful in the long-term, it may be an effective method of establishing seedlings quickly, especially if piratebush has the ability to switch hosts later in development.

Perhaps the greatest evidence of piratebush population growth and stability is in the Paint Creek population. This is the type location and was first discovered nearly 200 years ago (Nuttall 2010). Paint Creek was the most vigorous population and contained the greatest number of short plants, the largest proportion of non-flowering individuals, and lowest mean stem number. If characters of size, flowering, and stem number can be used to identify young individuals, this may suggest that the Paint Creek population is, in fact, stationary. However, in 2007 this population was severely burned, which could be responsible for the abundance of small non-flowering individuals. Long-term demographics of this population, as well as the Temple Ridge and Wilbur Dam populations, are needed to confirm population stability.

With regards to host data in east Tennessee piratebush populations, all information recorded by this study is based on the assumption that the nearest tree to each piratebush individual is the most likely host tree. This assumption may be more reliable when the distance to the nearest tree is minimal, but as distance increases, the probability of that assumption being accurate may decrease. This assumption is also increasingly speculative when multiple potential hosts occur nearby. But, to the extent that this assumption is valid, our host data shows that piratebush exhibits host preferences.

At sites where hemlock species were abundant, piratebush was much more likely to be in proximity to them, and presumably, to parasitize them. However, if hemlocks were few or absent, pines appeared to be the preferred host. The only site that was dominated by hemlocks was the Temple Ridge site, particularly on the south side of the Nolichucky River. This is noteworthy because this was the only location where the hemlocks were chemically treated to prevent hemlock woolly adelgid infestation and where Carolina hemlock was common. At this site, hemlocks were the predominant presumed hosts. The presumed hosts at all other locations showed either an equal distribution of pine and hemlock or were predominantly pine. This may indicate a shift from the historically preferred piratebush host (hemlock) to pine. Long-term studies are needed to see if a host shift trend exists.

When compared to a 1985 survey of all known piratebush populations, some differences can be noted at all three populations (Mowbray 1985). At that time it was noted that both the Paint Creek and Wilbur Dam populations were quite stable (Mowbray 1985). Mowbray described the Paint Creek population as “extremely vigorous” with 80 adult plants and 20 seedlings, and indicates that the number of seedlings suggests a growing population (Mowbray 1985). The data collected in this study is nearly identical to that of Mowbray’s and the seedling to adult ratios are similar (p=0.58). Mowbray reported a total of 55 plants, 48 adults and 7 seedlings, at the Wilbur Dam site (Mowbray 1985). Similar to the Paint Creek population, his description is very near to the current state of this population with no significant differences in seedling to adult ratio (p=1.00). Although the current area of piratebush coverage at Wilbur Dam is similar to that reported by Mowbray (0.40-0.81 hectares), the current Paint Creek population seems to be concentrated into a smaller area (0.81 acres) than stated by Mowbray (Mowbray 1985).

Mowbray referred to the Temple Ridge population as moderate in size with low density resulting from variation in overstory coverage due to topographic heterogeneity (Mowbray 1985). He reports 54 adults and 12 seedlings covering an area of 2.0 hectares, which is quite different from the current state of this population of 246 individuals (200 adults and 46 seedlings) covering approximately 20 hectares (Mowbray 1985). However, the ratio of seedling to adult is similar to that reported in Mowbray's (1985) survey (p=1.00).

Of the two additional surveys conducted on these populations of piratebush (Levy & Walker 2007; McCoy 2010), only the 2007 study contains data that can be compared with the data from this study. The McCoy (2010) survey contained descriptions and broad population estimates, but not enough information was given to permit quantitative comparisons.

In the 2007 study, characters of plant height, stem number, and nearest conifer were recorded for Paint Creek, Temple Ridge, and Wilbur Dam (Levy and Walker 2007). In our survey, Paint Creek was found to have more plants, while Temple Ridge and Wilbur Dam have fewer plants than the 2007 survey. The distribution of stem number was similar for both studies at Temple Ridge and Wilbur Dam (TR: p=0.21; WD: p=0.27), while the current Paint Creek population is now significantly skewed toward plants with lower stem number ($p<0.01$). Relative height (<1 m or >1 m) was similar for all populations (PC: $p=0.52$; TR: $p=0.20$; WD: $p=0.72$) when the current information was compared with that collected in 2007. Presumed host data between surveys was not compared because Levy and Walker (2007) only recorded nearest conifers while this study recorded all species of trees.

In evaluating the development of the three censused populations over the past thirty years, a few demographic characteristics are noteworthy. Firstly, at Paint Creek, the number of piratebush individuals has remained similar with 100 plants in 1985 and 96 plants currently. This suggests that the Paint Creek population is stable, and possibly a growing population due to the significant increase in small sized plants since 2007. The Wilbur Dam population also seems to be quite stable due to its thirty-year consistency in population size.

The Temple Ridge population has changed most drastically over the past thirty years. Since Mowbray's census, the population has increased by at least 180, but this population number may be an underestimate considering the fact that many plants at Temple Ridge were omitted from this study due to inaccessibility (Mowbray 1985). Additionally, the hemlock trees south of the Nolichucky have been chemically treated to eliminate HWA, whereas the trees on north side of the Nolichucky River have been left untreated. When presumed host locations tested for clusters, all hemlocks were found to be clustered on the south side of the Nolichucky River and many of the pines were found to be clustered on the north side of the Nolichucky River. Additionally, the clusters of plants with high vigor contained many points that overlapped with the hemlock cluster. These data suggest that a loss of hemlock host in the north subpopulation due to HWA has caused a reduction in the vigor of many piratebush individuals in that area.

Disease

Over the past decade, the hemlock woolly adelgid has devastated the hemlock populations of eastern North America, specifically in the southern Appalachian Mountains (Cleaves 2008; Krapfl et al. 2011; Vose et al. 2013). With hemlocks representing a primary wild host of piratebush, this decline may negatively affect piratebush populations in ways similar to those hypothesized for the northern Temple Ridge subpopulation. Additional information is needed to better understand exactly what effects the will have on piratebush, but this threat of host extinction may severely disrupt populations.

Much of this concern is motivated by the lack of knowledge regarding the parasitic habits of piratebush. It is unknown how strongly piratebush relies on parasitism versus photosynthesis. It is also unknown if adult piratebush plants can parasitize more than one host or switch host in the middle of life. If hosts are continually necessary and adult plants parasitize only one host, the danger of piratebush decline may be a likely possibility resulting from hemlock devastation. More information is needed to better understand the parasitism habits of piratebush as well as the effects of hemlock devastation by HWA on piratebush in the wild. However, because the south subpopulation at Temple Ridge was generally much larger and more vigorous than the north subpopulation, and because the population and vigor decline in the north subpopulation corresponds with loss of hemlocks in that area, chemical treatment of hemlocks that serve as hosts to piratebush should be considered for the conservation of both plant species.

If piratebush can switch hosts or parasitizes multiple hosts simultaneously, then it may be likely that, without human interference, the primary host of piratebush will change from hemlocks to Virginia pine, another significant piratebush host in nature. This is important because of the complex relationship between piratebush, Virginia pine, and the rust fungus, *Cronartium appalachianum*. In this complicated host-parasite-pathogen system, piratebush needs Virginia pine for nutrients while *C. appalachianum* needs both piratebush and Virginia pine to complete its life cycle. However, the rust can be fatal to Virginia pine. If *C. appalachianum* kills the Virginia pine, that leaves piratebush with fewer hosts but reduces canopy coverage and increases light availability for piratebush in the understory. Further studies regarding the nutrients acquired via photosynthesis versus those acquired via parasitism in piratebush will be needed to understand how this host loss and light gain will affect populations.

Identification of *C. appalachianum* was done by observing the presence of uredia and telia on the underside of piratebush leaves. This was considered sufficient because *C. appalachianum* is the only rust known to parasitize piratebush and its gross morphology is diagnostic (Hepting 1957). There was a report of an occurrence of *C. comandrae*, a rust infecting *Pinus taeda* (loblolly pine) and *Comandra umbellata* (bastard toadflax), in east Tennessee, but these sightings were made further west on the Cumberland Plateau (Cordell and Knighten 1969). Additionally, no loblolly pines were found near any of the piratebush populations studied and *C. umbellata* does not occur in the study regions. For these reasons, mistaking *C. comandrae* for *C. appalachianum* was not of concern.

Aside from the original identification by Hepting in 1957 and a phylogenetic study of pine stem rusts, little work has been done regarding *C. appalachianum* (Hepting 1957;

Vogler and Bruns 1998). Specifically, it is unknown if the rust has any period of dormancy within the secondary host or if it is chronic in the secondary host. The leaves of piratebush were the only visibly infected parts of the plants, and, if this is the only part of the plant that is colonized by the rust, it is unlikely that the infection is chronic or systemic. It is known that the rust does infect the primary host's stems, so persistent infection of the primary host does occur (Hepting 1957).

It is also unknown if *C. appalachianum* negatively affects the health and development of piratebush. Often, other *Cronartium* rusts will cause defoliation and deformation in their secondary hosts (Johnson 1986; Tainter 1973). The association of rust prevalence with low vigor piratebush plants at Temple Ridge indicates that *C. appalachianum* may cause negative health effects in its secondary host. However, this correlation may simply indicate that *C. appalachianum* opportunistically infects weaker piratebush individuals. If *C. appalachianum* is harmful in piratebush, there may be some cause for concern with 19% of individuals at Paint Creek and Temple Ridge and 29% of individuals at Wilbur Dam infected to some degree. Long-term studies would better identify what precise effects, if any, are caused by rust on piratebush. If it is found that mortality is an effect of rust, mortality should be compared with long-term data on seedling establishment and growth to better understand the specific factors at play in the population dynamics of piratebush. Additionally, more precise information should be gathered about the effects of rust on piratebush and the type of infection (chronic, systemic, etc.) should be better characterized.

Because rust prevalence and severity was randomly distributed in all populations, *C. appalachianum* is considered effective at dispersing throughout a population where both its primary and secondary hosts occur. The fact that so many piratebush individuals were infected by rust is attributed to the rust's ability to infect piratebush with at least two types of reproductive spores (aesciospores and uridiniospores). Additionally, in the random distribution of disease, many uninfected plants would be growing in the middle of a cluster of infected plants. Furthermore, the infected plants showed no clusters of disease severity in any population. These facts suggest variation in resistance to rust infection among piratebush individuals in all populations. More information is needed to better understand the method of rust infection as well as rust immunity and defense in piratebush.

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