SEASONAL ACTIVITY AND PHORESY RATES OF SAP BEETLES (COLEOPTERA: NITIDULIDAE) IN OAK WILT INFECTION CENTERS, VOLATILE ORGANIC COMPOUNDS RELATED TO THE OAK WILT CYCLE, AND LONG TERM EVALUATION OF RED OAK PROVENANCES.

By

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ABSTRACT

SEASONAL ACTIVITY AND PHORESY RATES OF SAP BEETLES (COLEOPTERA: NITIDULIDAE) IN OAK WILT INFECTION CENTERS, VOLATILE ORGANIC COMPOUNDS RELATED TO THE OAK WILT CYCLE, AND LONG TERM EVALUATION OF RED OAK PROVENANCES.

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In chapter one of this thesis, the seasonal timing of Nitidulidae beetle activity and contamination by the fungus (*Bretziella fagacearum*) that causes oak wilt were monitored, along with development of red oak xylem vessels. Activity for all species was highest in May and June, which coincided with the main contamination period. Of the 23 nitidulid species captured, six were found to be carrying viable *B. fagacearum* spores.

In chapter two, volatile organic compounds (VOCs) associated with oak wilt were collected and identified from samples of healthy, unwounded red oak trees, healthy red oak trees with a fresh wound, *B. fagacearum* mycelial mats on dead red oak trees, and pure colonies of *B. fagacearum* grown on Petri dishes. Identity and abundance of compounds differed among sample types. Compounds that were especially abundant and characteristic of each sample type could be potentially useful for future lure development.

In chapter three, I evaluated survival and growth of red oak trees in 22 provenances in a plantation at MSU's W.K. Kellogg Experimental Forest in southwest Michigan. These trees were planted in 1962-1963 and were 55-56 years old at the time of evaluation. Survival differed among provenances; all trees from three provenances had died by 2018. Overall, 17% of the trees originally planted in the plantation remained alive in 2018. Diameter at breast height (DBH) and height of trees did not differ among provenances.

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CHAPTER 1: SEASONAL PATTERNS IN SAP BEETLE (COLEOPTERA:NITIDULIDAE) ACTIVITY AND PHORESY RATES IN RELATION TO RISK OF OAK WILT TRANSMISSION

Introduction

Oak wilt, an important disease affecting oak (*Quercus* spp.) trees across much of the Upper Midwestern US, is caused by the fungus *Bretziella fagacearum* (Bretz), formerly known as *Ceratocystis fagacearum* (de Beer et al. 2017). Oak wilt was first recognized as an important forest pathogen in 1944 in Wisconsin and has since been detected in 24 states (Juzwik et al. 2008). The origin of *B. fagacearum* is unknown, although Juzwik et al. (2008) speculated it may have been introduced from Central America, where oak diversity is high. A vascular disease, oak wilt causes wilting in the upper crown when hyphae grow into and plug xylem vessels in infected trees, affecting water transport from roots to the crown and eventually leading to tree death (Garske 2013).

Although red, white, and live oak species can be infected by *B. fagacearum*, vulnerability varies among species (Gearman & Blinnikov 2019, Juzwik et al 2004). Red oaks (Sect. *Lobatae*) are highly susceptible to infection and trees can be killed in as little as three weeks following infection (Hayslett et al 2008, Appel et al. 1986, Gibbs & French 1980). Infection in white oaks (Sect. *Quercus*) is less severe and rarely results in mortality, although some dieback may occur (Hayslett et al 2008, Juzwik et al. 2011, Juzwik et al 2004). Resilience of white oak reflects their smaller vessel size strong ability to compartmentalize the disease by producing tyloses in xylem vessels, which inhibit movement of the fungal hyphae through the tree (Gearman & Blinnikov 2019, Hayslett et al 2008). In red oaks, however, this response is weaker and the fungus typically spreads faster than trees are able to occlude hyphal growth (Jacobi & MacDonald 1980). Red oaks may be particularly vulnerable to oak wilt infection in spring and early summer when xylem is comprised of large diameter earlywood vessels that transport water to the growing canopy, compared with later in the summer when thick-walled latewood vessels, with relatively small diameters, are produced (Carteni et al 2018, Torbenson et al. 2016, Juzwik personal communication 03 May 2019). Earlywood vessels are utilized by the tree to transport a higher volume of sap in the beginning of the growing season following bud burst (Kitin & Funada 2016). Latewood vessel production begins after the leaves are fully expanded and offer protection against embolisms caused by drought (Kitin & Funada 2016).

Once trees are infected, oak wilt can be transmitted to healthy trees underground via root grafts, which results in expansion of existing disease centers, while new infection centers occur as a result of overland spread of the fungus by insect vectors (Ambourn et al. 2005). Mortality from oak wilt because of root graft transmission increases as the percent of red oak trees in forests increases (Juzwik 2007). Although root graft transmission may be responsible for up to 95% of new oak wilt infections, insect vectoring is concerning because it can introduce the fungus to uninfected areas (Robert 1989). Past studies have shown native sap beetles (Coleoptera: Nitidulidae) are the primary vectors of oak wilt in states including Wisconsin and Minnesota (Jagemann et al. 2018, Juzwik et al. 2011, Ambourn et al. 2005, Cease et al. 2001, Juzwik et al. 2001). Tiny nitidulid beetles, especially those within the subfamilies Carpophilinae, Cryptarchinae, and Nitidulinae, feed on fungi and decaying organic matter (Skalbeck 1976, Gazit et al 1982), as well as sap emanating from fresh wounds on oaks (Norris 1956, Dorsey et al. 1953). At least some nitidulid species are also attracted to volatile organic compounds (VOCs) associated with young mycelial mats produced on infected trees by the oak wilt fungus (Kyhl et al. 2002, Lin & Phalen 1992, Shelstad et al. 1991). Mycelial mats are most commonly produced in the spring, although fresh mats can be found in summer and fall (Carlson et al. 2001). Nitidulids may feed, mate, and oviposit on these mats (Wermelinger et al. 2012, Loughner et al. 2007, Juzwik et al 2004, Kyhl 2004, Lin & Phalen 1992). Hydrophobic spores, which are produced for two to three weeks on fresh mycelial mats, readily adhere to insect exoskeletons, thus contaminating nitidulids upon contact (Wilson 2005, Malloch & Blackwell 1993). Healthy oak trees become infected by oak wilt when contaminated nitidulids visit fresh wounds with exposed xylem (Gibbs & French 1980).

New oak wilt infections, therefore, require multiple circumstances to occur. One or more nitidulids must visit an actively sporulating mycelial mat, become contaminated, then locate and feed on sap emanating from a relatively fresh wound that is deep enough to expose xylem on a healthy tree. Vulnerability of trees to infection may also vary seasonally, as xylem transitions from earlywood to latewood.

In this study, we identified and monitored temporal activity and fungal contamination rates of nitidulid species from spring through autumn over two years, using baited traps placed in oak wilt infection centers. We also tracked seasonal xylem development of red oaks to determine the onset of earlywood and latewood production. Identifying peak activity periods of the nitidulid species active in these areas, determining the proportion of beetle species bearing viable fungal spores, and documenting availability of earlywood in red oaks provides information essential for identifying relative risks of overland oak wilt transmission. Other states in the Great Lakes region currently restrict activities that could potentially wound red oaks, such as pruning, utility right-of-way maintenance and partial or selective harvests (Bonello 2019, Wisconsin Dept. of Natural Resources 2019, Juzwik et al 2018), but there are no relevant data from Michigan forests to support such guidelines. Additionally, previous studies have largely focused on two nitidulid species, *Carpophilus sayi* (Parsons 1943) and *Colopterus truncatus*

Randall 1838), that were implicated as dominant oak wilt vectors in Wisconsin and Minnesota (Jagemann et al. 2018, Juzwik et al. 2004, Khyl et al. 2002). Less is known about other nitidulid species that may be present in red oak stands in Michigan, their activity periods, and their propensity for becoming contaminated with fungal spores.

Methods

Study Sites

Three research sites on state forest land with active oak wilt infection centers in northern lower Michigan were selected in 2017: Nessen Road, Bunker Hill, and Coyote Road. An additional site, Bass Lake, was added to the study in 2019 (Table 1.1., Figure 1.1.). At each of the 2018 sites, we selected and tagged at least 80 apparently healthy red oak trees (DBH > 10 cm). At the smaller Bass Lake site, we selected, measured, and recorded GPS coordinates of 20 healthy red oaks. Another site consisted of a 0.25 ha red oak plantation in MSU's W.K. Kellogg Experimental Forest in Kalamazoo County in southwest lower Michigan. While oak wilt was previously detected in this county (Michigan Dept. of Natural Resources, 2019), there was no detectable active oak wilt infection in the plantation or in other nearby oak compartments.

Cumulative growing degree days recorded by MSU's extensive Enviroweather meso-network were acquired from stations nearest each site, in addition to daily mean, minimum, and maximum temperature, and weekly precipitation. Northern sites were 14 to 85 km from the weather station while the Kellogg Forest site was 8 km from the weather station. Degree days were calculated with the Baskerville-Emin method with a start date of 1 January and a base threshold of 10 ^{o}C (https://enviroweather.msu.edu/).

Monitoring Nitidulids

Nitidulid beetles were captured using baited, wind-oriented funnel traps (Dowd et al. 1977). In 2018, satellite images of all sites were acquired and overlaid with a grid, dividing each site into six relatively equal areas. One trap was installed in each of the six areas. Three traps were hung from a lower canopy branch on a healthy red oak, approximately 20 m high, while the other three traps were hung 1.5 meters high from a bent length of rebar embedded in the ground. Traps were deployed on 8 March 2018. Canopy and ground traps were alternated among the six areas within each site. Captured beetles were collected in glass vials on each trap, which were filled with crinkled brown craft paper (Wrap N Style^{TM}, Michaels Stores, Irving, TX) to limit any contact and potential cross-contamination of beetles.

Each trap was baited with approximately 10 mL of fermenting whole wheat bread dough (WWBD), along with a male-produced aggregation pheromone lure for either *Carpophilus sayi* ((2E,4E,6E,8E)-3,5-dimethyl-7-ethyl-2,4,6,8- undecatetraene and (2E,4E,6E,8E)-3,5,7-trimethyl-2,4,6,8-undecatetraene), *Colopterus truncatus* ((2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene, (2*E*,4*E*,6*E*)-4,6-dimethyl-2,4,6-nonatriene, and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene), or both (Great Lakes IPM, Vestaburg, MI) (Bartelt et al. 2004, Khyl et al. 2002). At any given time, one canopy and one ground trap at each site had a *Ca. sayi* lure, a *Co. truncatus* lure, or both lures, in addition to the WWBD. Lures in traps were replaced on each trap check date, cycling through a *Ca. sayi* lure, followed by a *Co. truncatus* lure, followed by a combination of the two. Collection vials with captured nitidulids, lures and WWBD were replaced at two week intervals from 22 March to 3 November 2018 at the three northern sites and from 5 April through 25 October at the Kellogg Forest site in southwest Michigan.

In 2019, trapping methods were modified, in part because of the addition of the Bass Lake site. Four ground traps were deployed at each of the five sites; no canopy traps were used in 2019. Trap locations were determined by dividing each site into four areas of similar size and with similar numbers of red oak trees. One trap was installed near the center of each area. All traps were baited with WWBD, along with either a *Ca. sayi* pheromone lure (two traps) or the *Co. truncatus* pheromone lure (two traps). Traps were checked at two week intervals. On each trap check date, bread dough was replaced and *Ca. sayi* lures were replaced with *Co. truncatus* lures, and vice versa. Traps were deployed on 26 March 2019. Nitidulids were trapped at the four northern sites from 7 April through 26 October and at the Kellogg Forest site from 3 April through 17 October 2019.

In 2018 and 2019, captured insects were transported in collection vials in coolers with ice to the Michigan State University Forest Entomology lab. All beetles were handled and identified using sterile techniques to avoid cross contaminating individuals. Beetles were carefully removed from vials with forceps, placed in individual microcentrifuge tubes, then frozen until they could be identified. After beetles were identified to species, they were returned to the same individual tubes (Downie & Arnett 1996). A minimum of 10 beetles of each nitidulid species captured were mounted and submitted to the Albert J. Cook Arthropod Research Collection (ARC) at Michigan State University.

Nitidulid Contamination by *Bretziella fagacearum*

Adult nitidulid beetles were identified to species and stored individually in sterilized, centrifuge tubes at -20ºC until processed. For each collection date, up to 10 individuals from each captured species, per trap, per site were submitted for *B. fagacearum* screening (Table 1.2.). If less than two beetles of a species were captured, none were submitted for testing to ensure enough specimens would be available for mounting and submission to the Albert J. Cook Arthropod Research Collection (ARC) at Michigan State University.

Presence of the *B. fagacearum* fungus on the collected insects was determined by plating beetle 'rinsate' using serial dilution plating techniques. Beetles collected in spring 2018 were processed following the protocol developed by Ambourn et al. (2005), while remaining beetles were processed using our modified protocol. Five hundred µl of sterile water were added to each microcentrifuge tube containing a beetle specimen. Each specimen was crushed for 3-5 seconds using a tip sonicator to dislodge fungal propagules. The resulting suspension was serially diluted to make additional two, 10-fold dilutions, then 900 µl aliquots of each dilution were spread onto duplicate lactic acid-amended potato dextrose agar (PDA). One additional petri dish was plated with 100 µl of undiluted 'rinsate' of each beetle. Original suspensions (sonicated beetle in 500 ul water) and further 10-fold dilutions were vortexed for 3-5 secs to homogenize samples before collecting aliquots for subsequent 10-fold dilutions and plating. A total of five Petri dishes were cultured from 'rinsates' of each insect. Sonicator tips were sterilized after processing each insect specimen by dipping it into 70% ethanol for 5 seconds, then rinsed with sterile water. Cultured Petri dishes were incubated at 25ºC in the dark and inspected periodically for 14 days. Colony morphology and endoconidia characteristics were used to identify *B. fagacearum* colonies (de Beer et al. 2017).

Mycelial Mats

In 2017 and 2018, a related study conducted by collaborators (Chahal et al., *in preparation*) involved inoculating red oak trees to study seasonal susceptibility and confirm Koch's postulates. From August to October 2017, and again from April to November 2018, one tree in each of the three northern field sites (Nessen Road, Bunker Hill, and Coyote Road) was inoculated every month with 2 spore suspensions that each spore suspension containing 1 × 106 endoconidia. Similarly, a second tree was inoculated at each location per month with sterile water as a negative control. Production of mycelial mats on inoculated trees were considered as satisfying Koch's postulates along with other methods of detecting *B. fagacearum* (Chahal et al., *in preparation*). These sites also have natural infestations of oak wilt. Mycelial mats were observed on naturally infected trees and inoculated trees.

Xylem Development

To monitor development of earlywood, an increment borer was used to collect approximately 3 cm long cores with at least 4-5 years of xylem from trees that appeared healthy. Cores were collected 1.3 m above ground on the north-facing side of the trunk of two trees at each site every two weeks, corresponding to trap check dates. Following core collection from a tree, the hole created was sealed with silicone. Cores were stored with 50% ethanol in individual glass vials until they could be examined. Each core was dried then examined under a stereomicroscope to identify presence of earlywood or latewood vessels. Cores were collected until latewood vessels were observed on all trees on two consecutive samples. Cores were collected from 22 March through 20 June 2018 and from 4 April through August 8 in 2019.

Statistical Analysis

Total nitidulid captures on each date in each of the northern sites were analyzed using a Type III two-way ANOVA (PROC MIXED) (SAS 9.4) with collection date and site as fixed factors to identify periods when nitidulid activity was highest and to evaluate potential differences in captures among sites. Nitidulid data from 2018 and 2019 were analyzed separately. Assumptions of normality were tested with the Shapiro-Wilks test and residual plots, while homogeneity of variance was evaluated with Levene's test. Nitidulid counts in 2018 and in 2019 were log transformed to meet ANOVA assumptions. When ANOVA results were significant ($P<0.05$), the Tukey-Kramer adjustment for multiple comparisons was applied.

Nitidulid captures at the Kellogg Forest site in southwest Michigan, where oak wilt was not present, were analyzed separately from captures at the northern sites each year. For 2018 capture data, transformations did not correct normality, so the nonparametric Kruskal-Wallis test was used to assess differences in beetle captures among trap check dates. The 2019 capture data was normalized with a log transformation then analyzed with a one-way ANOVA to evaluate nitidulid activity among dates.

We further analyzed seasonal activity of the five most abundant nitidulid species captured in both 2018 and 2019; beetle data from northern sites and the Kellogg Forest site were analyzed separately. Captures were log transformed then analyzed with a 2-way ANOVA to evaluate differences among sites and trap check dates for each of the five species.

Binary logistic regression was used to analyze presence–absence of viable oak wilt spores on subsets of the nitidulid beetles captured on each collection date from the northern sites using PROC GLIMMIX (SAS 9.4). Model terms were collection date and site, with the Satterthwaite approximation for calculating denominator degrees of freedom. In 2018, there was only one group of consecutive dates of contamination and further contrasts were unnecessary. In 2019, an *a priori* contrast to compare contamination rates was performed between the first group of contaminated dates (22 May-5 June; 5 June -21 June) and the second group of contaminated dates (14-29 August).

To determine if we needed to combine the *Ca. sayi* and *Co. truncatus* lures in traps in 2019 as in 2018, we conducted an Indicator Species Analysis (ISA) with species data from the 2018 traps. We were interested in whether any nitidulid species was a significant indicator of traps where both lures were used. If so, we would continue to bait a portion of traps in 2019 with both lures. If not, we would rotate the pheromone lures among the traps in each site in 2019. An indicator value, which can range from 0 – 100, was calculated for each nitidulid species to evaluate its association with each lure and combination of lures.

Non-metric multidimensional scaling (NMS) was conducted in PC-ORD (PC-ORD 6.08, MJM Software, Gleneden Beach, OR) to compare abundance and diversity of nitidulid species captured in ground traps versus canopy traps in the three northern sites in 2018. For the NMS, 100 iterations of real and randomized data were run on the autopilot mode using the "slow and thorough" and the Sorenson (Bray-Curtis) method of measuring distance was applied. The resulting NMS scree plot was assessed to determine dimensionality. The number of axes was determined by efforts to decrease stress without creating distortion by including too many axes. For NMS ordinations, final stresses of 5 – 15 are considered ideal, while a final stress greater than 25 would not be interpretable (Peck 2016). Using the dimensionality from the scree plot, the ordination was re-run with the appropriate number of axes. Trap location (ground or canopy) for counts of nitidulid species were overlaid for graphical comparison. In the event of a significant difference between canopy and ground trap captures, indicator species analysis (ISA) was performed in PC-ORD to identify which species were caught predominantly in ground versus canopy traps.

Results

Nitidulid Captures at Northern Sites

2018: A total of 1188 nitidulids representing 19 species were captured in the 18 traps at the three northern sites from 24 April (12 DD_{10C}) to 3 November (1490 DD_{10C}) 2018 (Table 1.3.). Number of nitidulid captures differed among dates of trap checks (Figure 1.2.a) (F=5.80;

df =14,137; *P* < 0.0001). More than half (58%) of all nitidulid captures in the northern sites occurred between 8 May (61 DD_{10C}) and 6 June (247 DD_{10C}), peaking on 22 May 2018 (112 DD_{10C}), when 327 nitidulids (27.5% of the total) were collected. Captures generally decreased after 6 June until a peak in activity on 24 July (782 DD_{10C}), when 106 nitidulids were collected, accounting for 9% of the total captures in 2018.

The number of nitidulids captured in traps varied among the three northern sites (F = 6.47; df = 2,137; *P* = 0.0021) and was highest at the Bunker Hill site, which accounted for 45% of all captures in 2018. On average (± SE), 6.0 ± 1.19 beetles per trap per collection date were captured at the Bunker Hill site, compared to 3.2 ± 0.83 and 4.0 ± 0.82 beetles per trap per collection date at the Coyote Road and Nessen Road sites, respectively. Bunker Hill traps captured more beetles than traps at Coyote Road (t = 2.21; DF = 2, 253; *P* = 0.0278) but did not differ from traps at Nessen Road (t = 1.57; DF = 2,253; *P* = 0.1179).

At all three northern sites, *Ca. sayi* was the most frequently captured species in 2018, comprising 39%, 19% and 25% of total nitidulid captures at Bunker Hill, Coyote Road and Nessen Road sites, respectively (Figure 1.3.a). Captures of *Ca. sayi* differed among trap check dates (F = 4.55; df = 13, 75; *P* < 0.0001) and among the three sites (F = 6.02; df = 2, 75; *P* = 0.0038). This species was captured more frequently at Bunker Hill (t = 3.44; DF = 2, 75, *P* = 0.0009) than at Coyote Road but similar numbers were captured at Nessen Road (t = 1.46; DF = 2, 75; *P* = 0.1482). Traps at Coyote Road also captured significantly more *Ca. sayi* than Nessen Road (t = -2.14; DF = 2, 75; *P* = 0.0360). Captures of *Ca. sayi* were significantly higher from 22 May through 24 July, when 87% of these beetles were trapped, than on other dates.

The second most commonly captured species in 2018 was *Ca. brachypterus*, which accounted for 16% of the total. Captures of this species did not differ among collection dates (F

= 1.70; df = 13, 40; $P = 0.0983$) or sites (F = 3.16; df = 2, 40; $P = 0.533$), but 79% of *Ca. brachypterus* beetles were collected in ground traps (IV = 21.1; $P = 0.0034$) (Figure 1.3.a).

Colopterus truncatus captures represented 16% of the total and differed among collection dates (F = 2.18; df = 11, 42; $P = 0.0344$) and sites (F = 10.83; df = 2, 42; $P = 0.0002$) in 2018. This species was captured more frequently at the Bunker Hill site than at Coyote Road (t = 4.47; DF = 2, 42; $P < 0.0001$) and Nessen Road (t = 3.32; DF = 2, 42; $P = 0.0024$), while captures at Coyote Road and Nessen Road did not differ from each other (t = -1.33; DF = 2,42; $P = 0.1911$). Most (68%) of these beetles were collected from 8 May through 6 June and captures were highest on the 22 May 2018 trap check (Figure 1.3.a). Most (89%) of *Co. truncatus* were captured in ground traps (IV = 29.6; $P = 0.0002$).

Captures of *Glischrochilus fasciatus* represented 14% of total captures in 2018 (Figure 1.3.a), did not differ among collection dates (F = 0.72; df = 11, 25; $P = 0.7131$) or sites (F = 0.88; df = 2, 25; $P = 0.4285$) and 92% of these beetles were captured in ground traps (IV = 19.8; $P = 0.0002$) (Figure 1.3.a). Similarly, captures of *G. sanguinolentus* represented 6% of total captures in 2018 and did not differ among collection dates (F = 0.42; df = 10, 23; $P = 0.9217$) or sites (F = 1.02; df = 2, 23; $P = 0.3746$) and nearly all (93%) were captured in ground traps (IV = 22.0; $P = 0.0002$) (Figure 1.3.a).

2019: A total of 735 nitidulids representing 18 species were captured in 2019. Trap collections occurred from 24 April (26 DD_{10C}) through 26 October (1200 DD_{10C}) (Table 1.3.). Nitidulids captured at the four northern sites differed by collection date (F = 5.54; df = 13, 95; $P < 0.0001$) and among sites (F = 2.82; df = 3, 95; $P = 0.0432$). Activity was greatest from 22 May (71 DD_{10C}) through 3 July (338 DD_{10C}), when 65% of the total beetles were captured (Figure 1.2.b). Captures peaked on 21 June (212 DD_{10C}) when 231 nitidulids, representing 31%

of the total captures, were trapped. Captures remained relatively low following 21 June and a second peak later in the summer was not observed in 2019 as in 2018.

In 2019, traps at Bunker Hill and Coyote Road captured more nitidulids than traps at Nessen Road (t = 2.63; DF = 3, 95; $P = 0.0100$) and Bass Lake (t = 2.26; DF = 3, 95; $P = 0.0264$), but did not differ from each other (t = 1.55; DF = 3, 95; $P = 0.1247$). On average (± SE), each trap at Bunker Hill, Nessen Road, Coyote Road and Bass Lake captured 5 ± 1.35, 5 ± 1.35, 3 ± 0.88 and 1 ± 0.35 beetles, respectively. As in 2018, *Ca. sayi* was the most frequently captured species at three northern sites, comprising 42% of the captures at Bunker Hill, 48% at Coyote Road, and 46% at Nessen Road. At Bass Lake, however, the most frequently captured species was *Ca. brachypterus,* representing 25% of all captures.

Five species were represented by more than 25 trap captures (Figure 1.3.b). However, only one species, *G. sanguinolentus,* comprised 14% of the total captures, differed among dates (t = 4.19; df = 11, 26; $P = 0.0003$) and sites (F = 0.25; df = 3, 11; $P = 0.8568$). More *G. sanguinolentus* were captured on 22 May (71 DD_{10C}) than on other collection dates and in traps in the Bunker Hill site than in other sites (t = 5.32; df = 2, 26; $P < 0.0001$). Captures of *Ca. sayi, Ca. brachypterus, Co. truncatus, G. fasciatus,* which represented 42%, 12, 13 and 5% of the total captures, respectively, did not differ among collection dates nor sites.

On 22 May, cumulative degree days were higher in 2018 (112 DD_{10C}) than in 2019 (71 DD_{10C}), and the captures standardized by trap reflect this lag in accumulated degree days. For the first collections of the high activity period, 2018 and 2019 had similar accumulated degree days although their calendar dates were 2 weeks apart, with 61 DD_{10C} and 71 DD_{10C}, respectively. The first collections for the high activity period all occurred in May, where cumulative degree days ranged from 61 to 124 (10C). For *Ca. sayi*, the first capture occurred at

61 DD_{10C} (2018) and 39 DD_{10C} (2019), and *Co. truncatus*' first capture occurred at 12 DD_{10C} (2018) and 26 DD_{10C} (2019).

Canopy versus Ground Traps at the Northern Sites

Trap data from the three northern sites were used to evaluate diversity and abundance of nitidulids captured in canopy versus ground traps. Results of the PERMANOVA showed ground traps caught a significantly higher diversity and number of beetles than canopy traps (F = 7.80; *P* = 0.0006). Canopy traps captured an average (± SE) of 2 ± 0.22 beetles per trap per collection date over the eight month trapping season, compared to ground traps, which averaged 7 ± 0.39 beetles per trap per collection date. Over the 2018 trapping season, canopy and ground traps captured 14 and 19 nitidulid species, respectively. Overall, ground traps in 2018 captured 78% of all nitidulids collected from northern sites. Results of the NMDS ordination were consistent with PERMANOVA results, indicating that nitidulid species and numbers of individuals captured by canopy traps were also represented in ground trap captures (Figure 1.4.). Our final stress for a 2-dimentional solution was 10.8, which is within the optimal range for stress. Indicator Species Analysis (ISA) demonstrated that of the 21 species captured at the northern sites in 2018, only *Colopterus maculatus* (IV = 3; *P* = 0.1252) and *Cryptarcha concinna* (IV = 3.4; *P* = 0.5613) were indicators for canopy traps. The low Indicator Values f and high P values reflect the low number of captures of both species even in the canopy traps.

Pheromone Lures

The Indicator Species Analysis (ISA) demonstrated that nitidulid species captured in traps baited with either a *Ca. sayi* pheromone lure, the *Co. truncatus* pheromone lure or both types of lures did not differ (*P* = 0.457). *Ca. sayi* was the only species captured in significantly higher numbers in traps baited with the *Ca. sayi* lure. However, no species was captured in higher numbers in traps baited with the *Co. truncatus* pheromone compared to traps baited with both lures or with the *Ca. sayi* lure. With the exception of *Ca. sayi*, Indicator Values for the nitidulid species were very low, ranging from 0.6 – 10.8 (Table 1.4.). The low IVs and the high *P* values indicate that the combination of lures did attract unique species; all species captured in traps with both lures were also captured in traps baited with just the *Ca. sayi* or the *Co. truncatus* lure.

Nitidulid Contamination by ***B. fagacearum***

2018: Mycelial mats were not observed on any of the trees at the three northern sites on any trap check date in 2018. A total of 791 beetles representing 12 species of nitidulids were captured in high enough numbers at the northern sites to be evaluated for contamination with *B. fagacearum* in 2018 (Table 1.2.). Overall, 20 beetles, belonging to four species, *Ca. sayi, Ca. dimidiatus, Co. truncatus,* and *G. fasciatus*, were contaminated with viable oak wilt fungal spores in 2018 (Table 1.5.). All contaminated beetles were captured between 8 May and 6 June. Percentage of tested beetles contaminated with viable spores ranged from 4% to 50% per species. We note, however, that one of the two *Ca. dimidiatus* beetles that were screened was contaminated, inflating the overall proportions. Contamination rates for the other three nitidulid species ranged from 4 to 7% of the 10 to 85 beetles screened per species.

2019: We observed 50 trees in the northern sites producing mats in 2019 and counted 222 mats on those trees (Table 1.6.). Bass Lake had the most (168) mycelial mats recorded while Nessen Road had the fewest (5). All mycelial mats at Bass Lake were the result of natural infection. Mycelial mats tallied in May and June 2019 represented 44% of all mycelial mats observed from April through November in 2019. No mats were observed in any site in April or July in 2019 and none of the screened nitidulids collected during these months were contaminated. Mycelial mats were noted on two trees at Bunker Hill and on seven trees at Bass Lake in August 2019, coinciding with the low levels of contamination detected on beetles from those sites.

Of the 487 beetles screened in 2019, 76 beetles representing six species of nitidulids were contaminated with *B. fagacearum* spores in 2019. This included the same four species that were contaminated in 2018, plus *Ca. brachypterus* and *G. sanguinolentus* (Table 1.5.). Nearly all (96%) contaminated beetles were captured between the 22 May and 21 June trap checks. One contaminated beetle was captured in a trap at Bunker Hill checked on 14 August, while two beetles were captured in a trap at Bass Lake checked on 29 August. Contamination rate varied among trap collection dates (F = 1.85; df = 12,403; *P* = 0.0395) and was higher for the 22 May to 5 June and 5 June to 21 June period than for the August period (t = 2.14; df = 403; *P* = 0.0330). Contamination rates did not differ among sites (F = 0.25; df = 3,43; *P* = 0.8592) . The 50% contamination rates for *Ca. dimidiatus* and *G. fasciatus* were high relative to other species but only two and four individuals, respectively, were screened. Contamination rates for the other four species ranged from 31% for *Co. truncatus* and 30% for *Ca. sayi*, down to 15% for *G. sanguinolentus*. Traps at Bunker Hill had both the highest number of captured beetles in 2019 and the highest proportion of contaminated beetles, while traps at Bass Lake captured the lowest number of beetles and had the lowest contamination rate.

Nitidulid Captures at Kellogg Forest

2018: The six traps at the Kellogg Forest site in southwest Michigan captured 464 beetles representing 16 species in 2018 (Table 1.3.). Total nitidulid captures varied significantly among trap check dates (F = 2.88; df = 12, 92; *P* = 0.0031). Activity was higher between 2 May and 18 May, when 74% of all the nitidulids were captured, than on other trap check dates.

Captures of five species, *Co. truncatus, Ca. sayi, G. sanguinolentus, Ca, brachypterus,* and *Ca. lugubris*, ranged from 26 to 220 individuals, while 11 species were represented by less than 25 individuals (Figure 1.6.a). *Colopterus truncatus* represented 47% of all nitidulid captures. While *Co. truncatus* were captured throughout the season, numbers differed among collection dates (F = 3.54; df = 5,10; *P* = 0.0423). Captures of *Co. truncatus* peaked on the 2 May trap check, when 85% of the total beetles were collected. The second most abundant species, *Ca. sayi,* represented 21% of total captures at Kellogg Forest. Captures differed among collection dates (F = 2.84; df = 12,65; *P* = 0.0035), peaking on the 18 May trap check date, when 58% of the total *Ca. sayi* beetles for Kellogg Forest were captured. Captures of *G. sanguinolentus,* which comprised 6% of total captures, were also significantly different among collection dates (F = 3.66; df = 12,65; *P* = 0.0003). Captures were highest on 15 June, when 37% of these beetles were collected. Captures of *Ca. brachypterus* represented 6% of the total, differed among trap check dates (F = 4.58; df = 12,65; *P* > 0.0001) and were highest on 2 May, when 43% of the *Ca. brachypterus* were collected. Kellogg Forest was the only site where *Ca. lugubris* (6% of total captures) was frequently captured in 2018 and captures did not differ among trap check dates (F = 1.45; df = 12,65; *P* = 0.1653).

2019: A total of 480 nitidulids from 17 species were collected in the four traps monitored at Kellogg Forest in 2019 (Figure 1.5.b). Nitidulids were captured at Kellogg Forest from the first trap check on 18 April (42 DD_{10C}) through 17 October (1659 DD_{10C}). Nitidulid captures at Kellogg Forest differed significantly by date (F = 4.98; df = 11, 86; *P* = 0.0011). More nitidulids were captured between 16 May (124 DD_{10C}) and 28 May (217 DD_{10C}), when 63% of the total beetles were trapped than later in the season (Figure 1.5.b). Peak captures occurred on 16 May, accounting for 48% of all the beetles collected in 2019 at Kellogg Forest.

Twelve species were represented by less than 25 beetles in 2019 (Figure 1.6.b). The most frequently captured species were *Ca. sayi*, *Co. truncatus*, *G. quadrisignatus*, *Ca. brachypterus*, and *G. fasciatus*, which represented 27%, 11%, 6%, 5%, and 4%, respectively, of the total nitidulid captures at Kellogg Forest (Figure 1.6.b). Captures differed by trap check dates for *Ca. sayi* (F = 2.79; df = 12,39; $P = 0.0077$), *Co. truncatus* (F = 3.39; df = 12,39; $P = 0.0019$), *G. quadrisignatus* (F = 2.15; df = 12,39; $P = 0.0357$) and *G. fasciatus* (F = 8.19; df = 12,39; $P < 0.0001$). Captures of all four species peaked on 16 May, while captures of *Ca. brachypterus* did not differ among dates (F = 1.78; df = 12,39; $P = 0.0858$).

Canopy versus Ground Traps at Kellogg Forest

At Kellogg Forest, canopy and ground traps captured 13 and 14 different nitidulid species, respectively, in 2018. In contrast to traps at the three northern sites, only 26% of the nitidulid specimens were captured by ground traps. This pattern, however, was driven by two canopy traps that captured 320 beetles belonging to 12 species over the trapping season, representing 79% of total nitidulid captures. In contrast, the other canopy trap captured only 0.2% of the remaining nitidulids, including the only *Ca. discoideus* captured at Kellogg Forest. Of the five most frequently captured species at this site, only *G. sanguinolentus*, was captured mostly by ground traps, which accounted for 70% of the total of this species. Three species, *Ca. sayi, Ca. brachypterus,* and *Co. truncatus,* were 58 to 95% more abundant in canopy traps than in ground traps, while *Ca. lugubris* was captured at similar rates in both trap types.

Xylem Development

In 2018, red oaks in the three northern sites produced earlywood from 8 May (61 DD_{10C}) through 6 June (247 DD_{10C}) (Table 1.7.). Current-year earlywood cells were first observed on a core collected on 24 April (12 DD_{10C}) from a tree at Nessen Road. Earlywood was not consistently present on cores from other trees and sites until 8 May, when all but one core from a tree at Bunker Hill were producing earlywood. Earlywood production continued until 19 June (365 DD_{10C}), when latewood cells were observed on all cores from trees at all sites. One core from a tree at Kellogg Forest had current-year earlywood on 2 May (66 DD_{10C}) but earlywood cells were consistently observed from 18 May (170 DD_{10C}) to 1 June (326 DD_{10C}) 2018 (Table 1.7.). Latewood was presented in all cores collected after 1 June.

In 2019, earlywood was produced from 22 May (71 DD_{10C}) through 16 July (478 DD_{10C}). One tree at Coyote Road had earlywood cells on 7 May (39 DD_{10C}) but consistent earlywood production was not observed until 22 May at the northern sites. Earlywood vessels continued to be produced until trees were sampled on 15 July, when all latewood cells were present in all sampled trees. Cooler temperatures in April and May of 2019 compared to 2018, may have delayed the onset of earlywood production in 2019 (Figure 1.7.) Additionally, weekly precipitation was greater in June 2019 than in June 2018, which may have caused the longer earlywood production in 2019. Precipitation for the northern sites during the last week of June 2018 totaled 2.4 cm, compared to the last week of June 2019, where 52 cm of precipitation fell (Figure 1.8.). Trees at Kellogg Forest in 2019 produced earlywood from 3 May (78 DD_{10C}) through 9 July (670 DD_{10C}) (Table 1.7.). Latewood vessels were present in cores collected from trees on 27 July (922 DD_{10C}) and thereafter.

Discussion

Seasonal activity of nitidulids captured in the northern sites and the Kellogg Forest site in the southwest followed similar trends in both years. The slight lag in the beginning of captures in 2019 compared to 2018 might be attributable to the lower temperatures in April and May of 2019 (Figure 1.7.). Despite this, three species were consistently active early in the spring (24

April – 7 May), including *Ca. brachypterus, Ca. sayi,* and *Co. truncatus*, and their activity peaks coincided on similar degree days across both years. *Ca. sayi* and *Co. truncatus* have been consistently reported to be important vectors of oak wilt in the Great Lakes region (Cease & Juzwik, 2001, Juzwik et al. 2004, Ambourn et al. 2005, Juzwik et al. 2011, Jagemann et al. 2018). Activity peaks in July, as we observed in 2018, were reported in Wisconsin in 2015 and 2016 for *Ca. sayi* (Jagemann et al. 2018). In Wisconsin, the first captures of *Ca. sayi* corresponded to approximately 200 DD_{10C} (18 April 2016), lagging behind captures of *Co. truncatus*, which corresponded to 50 DD_{10C} (14 March 2016) by roughly two weeks (Jagemann et al. 2018). Although latitudes of the Wisconsin sites and our sites were similar, most of our *Ca. sayi* and *Co. truncatus* beetles were trapped at similar time periods, from May to early June in 2018 and in June through mid-July in 2019. Other studies in the Great Lakes region similarly reported nitidulid activity, as evidenced by trap captures, was highest in May and June (Juzwik et al. 2004, Ambourn et al. 2005, Jagemann et al. 2018).

Other studies that captured and screened nitidulids in the Great Lakes region reported numbers and proportions of beetles contaminated with viable *B. fagacearum* spores were consistently higher in May and June than later in the summer (Ambourn et al. 2005, Juzwik et al. 2004). Our contamination rates for individual species are similar to those reported in studies with similar collection methods (Jagemann et al. 2018), although lower than rates reported in studies in which niditulids were collected directly from mycelial mats (Cease & Juzwik 2001, Juzwik & French 1985). Jagemann et al. (2018) reported 5% of *Co. truncatus* and 7% of *Ca. sayi* were contaminated in 2015 in Wisconsin, similar to our 4% contamination of *Co. truncatus* and 7% of *Ca. sayi* in 2018. In 2019, overall contamination rates in our study were much higher; 46% of the *Co. truncatus* and 31% of the *Ca. sayi* beetles screened were contaminated. Time periods when nitidulids were contaminated in our sites and in other studies consistently align with peak production of mycelial mats on dead trees. In an Illinois study, germination of *B. fagacearum* spores was positively correlated with increasing temperature and precipitation in spring (Curl 1955).

We identified all captured nitidulids to species, regardless of whether the species was previously indicated as a vector of *B. fagacearum*. Other studies that focused on one or two nitidulid species or genera (Hayslett et al. 2008, Ambourn et al. 2005, Jagemann et al. 2018) seem likely to have overlooked potentially important vectors. In addition to *Ca. sayi* and *Co. truncatus*, four other nitidulid species were contaminated with *B. fagacearum* spores in our sites. Excluding evaluation of other species of nitidulids could result in underestimating their role as oak wilt vectors. Identifying all species of nitidulids shows an array of species could serve as potential vectors, plus provides information on seasonal activity for species that have not been thoroughly studied.

We observed a notable increase in the percentage of beetles that yielded viable spores between 2018 (6% of screened beetles) and 2019 (25% of screened beetles). This likely reflects both the abundance of mycelial mats at the Bass Lake site plus mats production by trees inoculated in 2018 in the other three sites (Table 1.6.) (Chahal et al *in preparation*). The abundance of mycelial mats in 2019, particularly in May and June, obviously increased the availability of inoculum and may also have attracted nitidulids from surrounding stands. The low levels of contamination we detected in August 2019 presumably reflect mycelial mats produced in August. Only 14% of the beetles screened for viable fungal spores in 2019 were captured in August. The three contaminated beetles from the August captures represented only 3% of the total number of contaminated beetles in 2019. Monitoring mat production also indicates that while nitidulids were active in July, the risk for vectoring in July is relatively low, as no mycelial mats were detected at any site for that month. Oaks that wilt in the beginning of the summer (through mid-July) may produce mats in August or September, while oaks that wilt later in the summer produce mats the following spring (Gibbs 1980). Artificial monthly inoculations, as well as progression of the Bass Lake infection center likely resulted in mat formation in August. Mats typically form in the spring rather than in the fall because the moisture content of the wood is higher and supports mat formation (Tainter & Gubler 1973). Given that only 19% of all nitidulids captured in 2019 were captured after 16 July and the low production of mycelial mats in July and subsequent months, risk for overland spread of oak wilt is low in late summer compared to spring and early summer.

Our traps were baited with aggregation pheromones of *Ca. sayi* and *Co. truncatus*, a practice used in previous studies on *B. fagacearum* vectors (Jagemann et al. 2018, Hayslett et al. 2008, Ambourn et al. 2005). Both species are consistently identified as important vectors of oak wilt and these pheromone lures are commercially available. The aggregation pheromone lures used to bait the traps, however, can attract multiple niditulid species (Bartelt et al 2004). Whether the abundance of species captured in the baited traps accurately represents the species assemblage of nitidulids in our sites and more broadly, in oak forests (Cosse & Bartelt 2000) is largely unknown. Further research to identify specific compounds associated with mats that attract nitidulids would be helpful in vector monitoring. Many species were captured in low numbers in our traps, possibly because they were only attracted to the fermenting whole wheat bread dough. For example, *Ca. dimidiatus* was captured in low numbers in our traps but beetles were contaminated in both years. Research in Minnesota also indicated *Ca. dimidiatus* was associated with *B. fagacearum* (Juzwik & French 1983). This species could potentially play an important role as a vector of *B. fagacearum* despite being underrepresented in trap collections. Evaluating multiple nitidulid species also enabled us to monitor differences in contamination rates overall and by individual species occur over the season. Although mycelial mats were not abundant in late summer and fall, there would still be some risk that nitidulid beetles could acquire viable spores and infect wound on healthy trees. Evaluating multiple species and learning more about their role as vectors of *B. fagacearum* will facilitate more accurate assessments of relative risks of overland spread throughout the year.

Development of earlywood vessels in xylem of ring-porous trees typically begins one to four weeks before bud break, with the first vessels fully expanded at bud break (Kitin & Funada 2016). Earlywood development in relation to accumulated degree days was similar at all sites in both 2018 and 2019, although the duration of earlywood production differed between years. Large diameter (60 – 80 µl) earlywood vessels may be more vulnerable to oak wilt infection because their size allows for easier movement of oak wilt hyphae through the xylem than ilater in the summer or fall when narrow-diameter latewood vessels (20 – 40 µl) are produced (Massart 2017, Kitin & Funada 2016, Sousa et al. 2016). Latewood vessel formation begins as leaf expansion is completed and is negatively correlated with mean maximum monthly temperatures (Jewett 2016, Kitin & Funada 2016). Although it seems reasonable to expect red oaks are more vulnerable to *B. fagacearum* before earlywood vessels give way to latewood formation, this relationship requires further research.

We originally expected different nitidulid species might be captured in canopy traps versus ground traps. Studies with other forest insects such as cerambycid beetles have shown species composition differed substantially between baited traps hung on branches in tree canopies and traps hung on rebar poles at ground level directly below the canopy traps (Engelken and McCullough 2020, Graham et al. 2012). In our northern sites, all nitidulid species collected by the canopy traps were also collected in ground traps, although two *Ca. discoideus* beetles were captured only in a canopy trap at the southwestern site. Nitidulid beetles are generalist feeders capable of utilizing a range of food sources, including fruit, nectar, woody materials, detritus, along with fungi (Gaszit et al. 1982, Phelan & Lin 1991, Zeran et al. 2006). Several of these food sources occur on or near the ground, suggesting beetle activity may be less likely to occur in the upper canopies of large trees. This could be reflected in the higher abundance and diversity of beetles captured in the ground traps compared to the canopy traps in 2018. Overwintering sites or larval development may also affect the spatial or vertical distribution of nitidulids in a given site. If adult beetles emerge from leaf litter in the spring, they might be more attracted to ground traps compared to canopy traps due to their closer proximity. Relatively little information on nitidulid behavior and development in forested sites is available, however, presumably because of the difficulty of attempting to monitor these tiny beetles in a natural environment (Bai et al. 2017, Eliceche et al. 2017). In agricultural fields, where nitidulid beetles are sometimes considered to be pests, they have been found to overwinter in leaf litter and uncultivated areas along agricultural fields (Levesque & Levesque 1992, Noronha & Mason 2017).

While the seasonal risk of overland spread of the *B. fagacearum* fungus has not been previously studied in Michigan, guidelines to reduce the risk of oak wilt infection were developed by the Michigan Department of Natural Resources based on research in other states (MIDNR 2017, Cook 2018, Sakalidis et al. 2020). These recommendations suggest suspending activities that might create wounds on healthy oak trees when trees are particularly vulnerable to oak wilt infection, when mycelial mat production is high, or when activity of potential nitidulid vectors is high. Current guidelines, which identified the period between mid-April and mid-July as high risk (WIDNR 2012, MIDNR 2017), have been challenged by arborists who believe the high risk period extends well into fall. Conversely, utility foresters and others prefer to be able to conduct line clearing, thinning and other activities throughout the year. Factors determining risk include local weather conditions, mycelial mat and spore production, nitidulid abundance, activity and attraction to mycelial mats, and fresh wounds that expose xylem on a living oak tree. Nitidulid captures, which presumably represent beetle activity, were highest at our sites in May and June in both years. This period also corresponded to the time frame when most contaminated beetles (96%) were captured. This, along with the abundance of mycelial mats observed in 2019 by Chahal et al. (*in preparation*) during this period suggests the risk for nitidulids acquiring viable spores is highest in May and June. Wounds exposing xylem created on living red oak trees provide germination sites for the fungal spores introduced following nitidulid beetles' visitation to mycelial mats. Assuming earlywood vessel production is associated with higher vulnerability of red oaks to *B. fagacearum* infection, the period of relatively high risk could vary somewhat with weather. Trees we sampled produced earlywood in May and June in 2018, but in 2019, earlywood production continued through mid-July, possibly a result of greater precipitation in July 2019 compared to July 2018 (Figure 1.8.). In previous studies, aggregations of nitidulid beetles were observed on mycelial mats in April and May (Cease & Juzwik 2001, Juzwik & French 1983). Our highest nitidulid captures and contamination rates coincided with the period of earlywood production, indicating a higher risk for overland infection by *B. fagacearum*. Following management policies to limit human-made wounds on red oak trees during periods when mycelial mat production and nitidulid contamination will help reduce the spread to healthy trees. Additionally, in landscape settings where root grafts are unlikely to be present, removing oak wilt-killed trees as soon as possible will reduce mycelial mat production and availability of the fungal inoculum. Results from our research are consistent with and support the high risk period and guidelines put forth by the MI DNR for reducing the likelihood of overland spread of oak wilt.

APPENDICES

APPENDIX A.

Tables and Figures

Table 1.1. Location, size and oak wilt status of sites, total nitidulid captures and mean (±SE) nitidulid captures per trap (6 traps per site in 2018; 4 traps per site in 2019) and mean (±SE) diameter at breast height (DBH) (1.3 m aboveground) of red oaks at each study site. Northern sites (latitude > 44) were on state forest land managed by the Michigan Dept. of Natural Resources. The W.K. Kellogg Experimental Forest in southwest Michigan is owned and managed by Michigan State University.

						2018		**2019**		
Site	**County**	**Latitude**	**Longitude**	**Infection Center**	**Size (ha)**	**Total Captures**	**Capture per trap ± SE**	**Total captures**	**Capture per Trap ± SE**	**Mean red oak DBH ± SE**
Nessen Road	Grand Traverse	44.55913	-85.7990833	Yes	20	363	60.5 ± 13.54	156	39.0 ± 8.22	26.91 ± 1.28
Bunker Hill	Grand Traverse	44.7351944	-85.4437778	Yes	10	531	88.5 ± 33.23	282	70.5 ± 18.25	27.19 ± 1.59
Coyote Road	Roscommon	44.5093889	-84.8372778	Yes	10	294	49.0 ± 12.94	236	59.0 ± 15.87	37.25 ± 2.46
Bass Lake	Grand Traverse	44.605959	-85.812356	Yes	3.5	-	-	69	17.25 ± 7.15	25.80 ± 1.86
Kellogg Forest	Kalamazoo	42.368705	-85.361303	No	0.25	465	77.5 ± 25.95	477	119.25 ± 36.24	33.47 ± 1.33

Table 1.2. Number of nitidulid beetles screened for viable *Bretziella fagacearum* spores on each collection date. Cumulative degree days (10°C) are included for each collection date. Cumulative degree days for the northern sites were recorded by the MSU Enviroweather station at Williamsburg and by the Hickory Corners station for Kellogg Forest.

				2018				
DD$_{10C}$	**Collection Date**	**Bunker Hill**	**Coyote Road**	**Nessen Road**	**Bass Lake**	**DD$_{10C}$**	**Collection Date**	**Kellogg Forest**
12	24-Apr	0	0	0	-	16	18-Apr	0
61	8-May	33	48	48	-	66	2-May	64
112	22-May	111	90	32	-	170	18-May	58
247	6-Jun	39	35	34	-	326	1-Jun	12
365	19-Jun	40	9	10	-	455	15-Jun	8
422	26-Jun	24	6	10	-	599	29-Jun	10
615	10-Jul	28	19	17	-	852	17-Jul	4
782	24-Jul	39	4	23	-	1027	2-Aug	3
937	7-Aug	0	0	0	-	1200	16-Aug	1
1115	22-Aug	7	5	13	-	1295	26-Aug	0
1290	7-Sep	15	3	4	-	1600	24-Sep	2
1384	19-Sep	2	2	10	-	1704	11-Oct	3
1452	4-Oct	5	4	1	-	1710	25-Oct	3
1485	17-Oct	6	3	6	-			
1490	3-Nov	3	1	2	-			

				2019				
DD$_{10C}$	**Collection Date**	**Bunker Hill**	**Coyote Road**	**Nessen Road**	**Bass Lake**	**DD$_{10C}$**	**Collection Date**	**Kellogg Forest**
26	24-Apr	0	6	0	0	42	18-Apr	2
39	7-May	8	4	10	5	78	3-May	4
71	22-May	46	15	8	2	124	16-May	68
133	5-Jun	0	25	7	12	217	28-May	25
212	21-Jun	45	22	33	6	346	12-Jun	25
338	3-Jul	20	11	15	8	670	9-Jul	11
478	16-Jul	2	1	9	3	922	27-Jul	0
664	2-Aug	12	7	5	0	1082	9-Aug	15
788	14-Aug	6	5	4	1	1240	22-Aug	5
919	29-Aug	2	9	6	4	1365	6-Sep	0
1008	12-Sep	7	0	2	3	1499	19-Sep	23
1141	27-Sep	6	6	2	0	1625	3-Oct	3
1188	10-Oct	3	1	1	3	1659	17-Oct	1
1200	26-Oct	0	0	2	0			

Table 1.3. Counts of nitidulid species by subfamily captured in six traps at each research site in 2018 and four traps at each site in 2019.

Subfamily / Species	**Bunker Hill**		**Coyote Road**		**Nessen Road**		**Bass Lake**	**Kellogg Forest**		**Total**
Carpophilinae	2018	2019	2018	2019	2018	2019	2019	2018	2019	
Carpophilus antiquus Melsheimer	1	0	1	0	1	1	0	0	0	4
Carpophilus brachypterus Say	67	18	26	27	99	25	17	26	29	334
Carpophilus dimidiatus Fabricus	2	0	0	3	3	0	2	0	5	15
Carpophilus discoideus LeConte	0	0	2	1	0	1	1	2	2	9
Carpophilus freemani Dobson	0	1	0	0	0	2	1	1	0	5
Carpophilus fumatus Boheman	0	0	0	0	0	0	0	6	0	6
Carpophilus hemipterus Linnaeus	13	0	0	0	8	0	0	1	0	22
Carpophilus humeralis Fabricus	0	0	0	0	1	0	0	0	218	219
Carpophilus lugubris Murray	5	0	2	3	14	5	1	26	21	77
Carpophilus sayi Parsons	193	118	99	111	131	71	9	98	167	997
Cillaeinae										
Colopterus maculatus Erichson	0	0	1	1	4	0	0	2	1	9
Colopterus semitectus Say	6	0	1	0	5	0	0	0	0	12
Colopterus truncatus Randall	116	44	29	19	41	17	15	220	67	568
Colopterus unicolor Say	0	1	2	14	3	2	3	0	2	27
Cryptarchinae										
Cryptarcha ampla Erichson	4	5	7	2	2	1	4	16	14	55
Cryptarcha conicinna Melsheimer	12	0	3	3	6	0	0	17	1	42
Glischrochilus fasciatus Olivier	46	3	92	21	29	11	3	11	26	242
Glischrochilus quadrisignatus Say	5	2	1	0	5	12	2	9	39	75
Glischrochilus sanguinolentus Olivier	49	74	18	19	4	0	8	27	8	207
Glischrochilus siepmanni Brown	0	1	5	0	1	4	3	1	4	19
Epuraeinae										
Epurea aestiva Linnaeus	0	1	0	0	0	1	0	0	0	2
Epurea peltoides Horn	12	12	5	6	6	3	0	0	1	45
Nitidulinae										
Stelidota germinata Say	0	0	0	0	0	0	0	1	5	6

Table 1.4. Indicator Species Analysis (ISA) results supporting the discontinuation of combining the aggregation pheromone lures of *Ca. sayi* and *Co. truncatus* in traps. Indicator Values (IV) range from 0 – 100.

Species Captured	**Lure Group**	**Value (IV)**	***P* - *v*alue**
Carpophilus sayi	*Ca. sayi*	20.4	0.0236
Carpophilus lugubris	*Co. truncatus*	2.3	0.7187
Carpophilus hemipterus	Both	3.2	0.5807
Carpophilus freemani	Both	0.6	1.0
Carpophilus dimidiatus	*Ca. sayi*	1.3	0.786
Carpophilus humeralis	Both	1.1	1.0
Carpophilus discoideus	Both	1.1	1.0
Carpophilus brachypterus	Both	14.7	0.0724
Carpophilus antiquus	*Co. truncatus*	3.3	0.1096
Colopterus truncatus	Both	9.7	0.5421
Colopterus semitectus	Both	3.2	0.3011
Colopterus unicolor	*Ca. sayi*	2.0	0.6189
Colopterus maculatus	*Ca. sayi*	2.0	0.3141
Glischrochilus sanguinolentus	Both	6.7	0.5145
Glischrochilus fasciatus	*Co. truncatus*	7.8	0.4763
Glischrochilus quadrisignatus	Both	1.8	0.804
Glischrochilus siempmanni	Both	1.5	0.7782
Cryptarcha concinna	Both	3.8	0.2607
Cryptarcha ampla	*Co. truncatus*	3.4	0.2805
Lobiopa undulata	*Co. truncatus*	1.1	1.0
Epuraea peltoides	Both	2.9	0.6009

Table 1.5. Number of nitidulid beetles screened, number of screened beetles with viable *Bretziella fagacearum* spores and dates corresponding to captures of screened beetles. A dash indicates no beetles from that species were captured on that date.

	8 May 2018		**22 May 2018**		**6 June 2018**					
Contaminated Species	**Tested**	**Positive**	**Tested**	**Positive**	**Tested**	**Positive**				
Ca. dimidiatus	-	-	2	1	-	-				
Ca. sayi	5	0	78	4	63	6				
Co. truncatus	31	2	42	1	20	1				
G. fasciatus	35	3	49	2	11	0				
	22 May 2019		**5 June 2019**		**21 June 2019**		**14 August 2019**		**29 August 2019**	
	Tested	**Positive**	**Tested**	**Positive**	**Tested**	**Positive**	**Tested**	**Positive**	**Tested**	**Positive**
Ca. brachypterus	10	3	14	4	8	0	1	1	6	0
Ca. dimidiatus	-	-	2	1	-	-	-	-	3	1
Ca. sayi	18	11	16	9	57	7	1	0	-	-
Co. truncatus	17	5	5	3	10	2	1	0	-	-
G. fasciatus	1	0	2	1	1	1	3	0	3	0
G. sanguinolentus	24	3	2	2	14	1	4	0	2	1

Table 1.6. Earlywood development in red oak trees at the three northern sites and the Kellogg Forest site for 2018 and 2019. An “X” in the earlywood column indicates that samples collected demonstrated earlywood vessels were being produced at the time of collection. Both collection date and cumulative degree days (10°C) are included.

Northern Sites						**Kellogg Forest Site**					
2018	**DD$_{10C}$**	**Earlywood**	**2019**	**DD$_{10C}$**	**Earlywood**	**2018**	**DD$_{10C}$**	**Earlywood**	**2019**	**DD$_{10C}$**	**Earlywood**
24 Apr	12		24-Apr	26		18-Apr	42		18-Apr	16	
8-May	61	X	8-May	39		3-May	78	X	2-May	66	
22-May	112	X	22-May	71	X	16-May	124	X	18-May	170	X
6-Jun	247	X	5-Jun	133	X	28-May	217	X	1-Jun	326	X
19-Jun	365		21-Jun	212	X	12-Jun	346	X	15-Jun	455	
26-Jun	422		3-Jul	338	X	9-Jul	670	X	29-Jun	599	
10-Jul	615		16-Jul	478	X	27-Jul	922		17-Jun	852	
24-Jul	782		2-Aug	664		9-Aug	1082		2-Aug	1027	
7-Aug	937		14-Aug	788		22-Aug	1240		16-Aug	1200	
22-Aug	1115		29-Aug	919		6-Sep	1365		26-Aug	1295	
7-Sep	1290		12-Sep	1008		19-Sep	1499		24-Sep	1600	
19-Sep	1384		27-Sep	1141		3-Oct	1625		11-Oct	1704	
4-Oct	1452		10-Oct	1188		17-Oct	1659		25-Oct	1710	
17-Oct	1485		26-Oct	1200							
3-Nov	1490										

Table 1.7. Numbers of inoculated trees that were successfully infected with *Bretziella fagacearum* and numbers of mycelial mats observed on inoculated trees at the four northern research sites in 2019.

	Bunker Hill		**Coyote Road**		**Nessen Road**		**Bass Lake**		
	No. trees	**No. mats**	**No. trees**	**No. mats**	**No. trees**	**No. mats**	**No. trees**	**No. mats**	**Total mats per month**
April	0	0	0	0	0	0	0	0	0
May	2	12	1	4	1	2	4	38	56
June	3	4	2	9	1	3	8	48	64
July	0	0	0	0	0	0	0	0	0
August	2	9	0	0	0	0	7	41	50
September	0	0	0	0	0	0	9	30	30
October	2	6	0	0	0	0	5	9	15
November	1	5	0	0	0	0	2	2	7

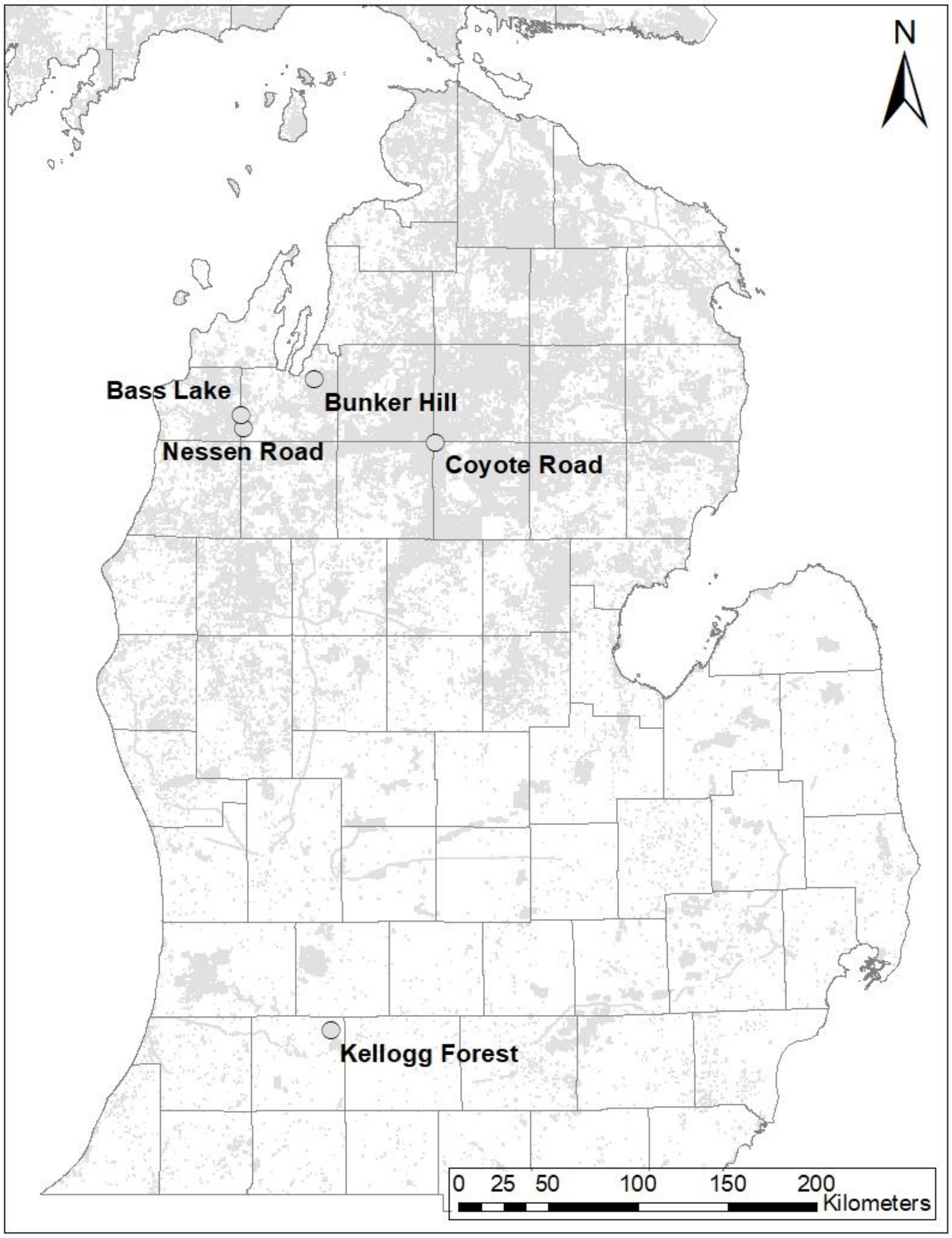

Figure 1.1. Locations of research sites in lower Michigan. Northern sites had active oak wilt infections while the Kellogg Forest site in southwestern Michigan had no active oak wilt infections. Shaded areas represent state forest land managed by the Michigan Dept. of Natural Resources.

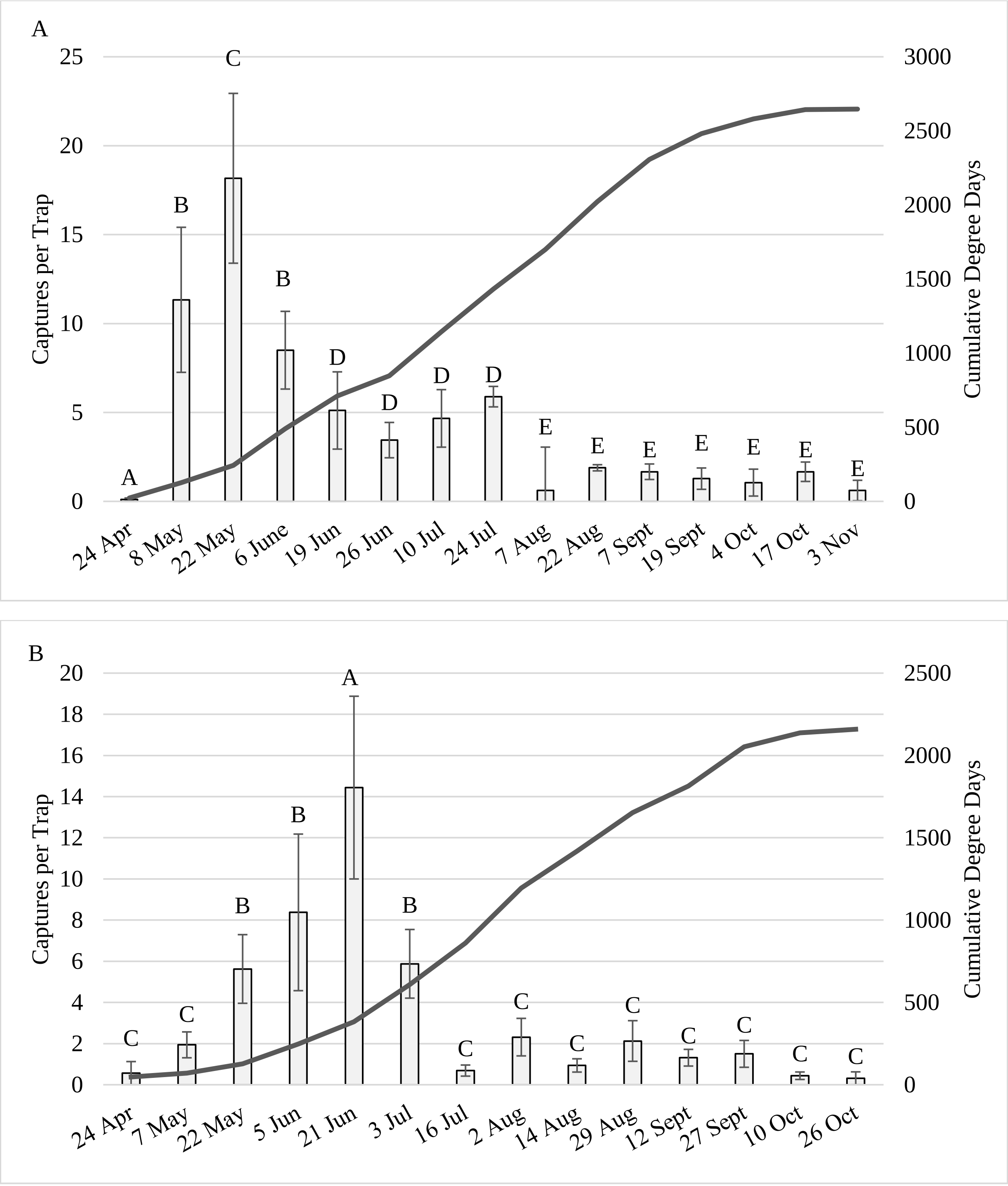

Figure 1.2. Total number of nitidulid beetles captured on each trap check date along with growing degree day accumulation (base 10 C) at (A) three northern sites in 2018 (18 traps) and (B) four northern sites in 2019 (16 traps total). Different letters above columns indicate significant differences among dates; ($P < 0.05$).

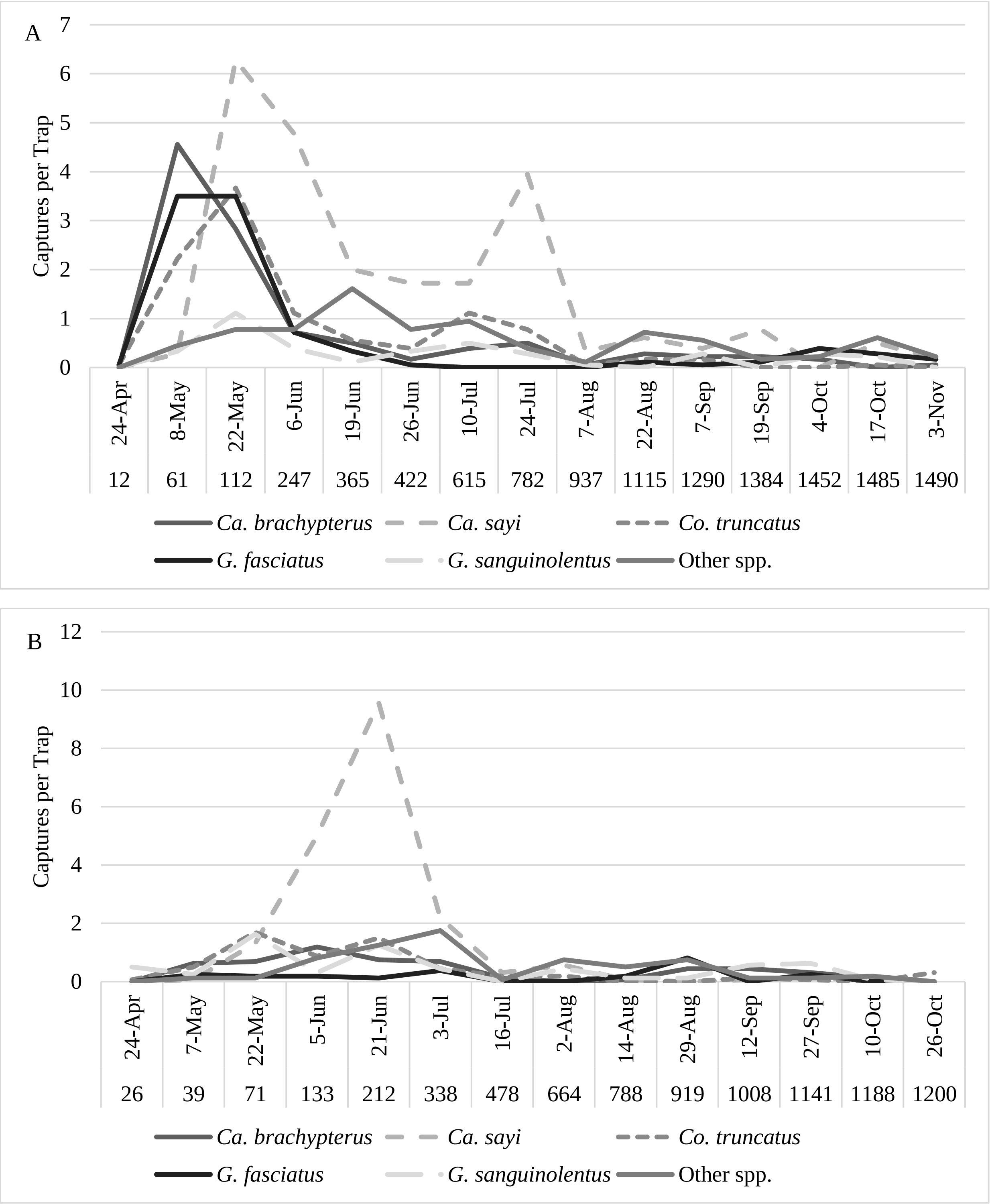

Figure 1.3. Number of nitidulid beetles captured per trap for the top five most frequently captured species and all other species combined in the northern sites by trap check date and corresponding cumulative growing degree days (base 10°C) in 2018 (A) and 2019 (B). A total of 18 traps and 16 traps were monitored in these sites in 2018 and 2019, respectively.

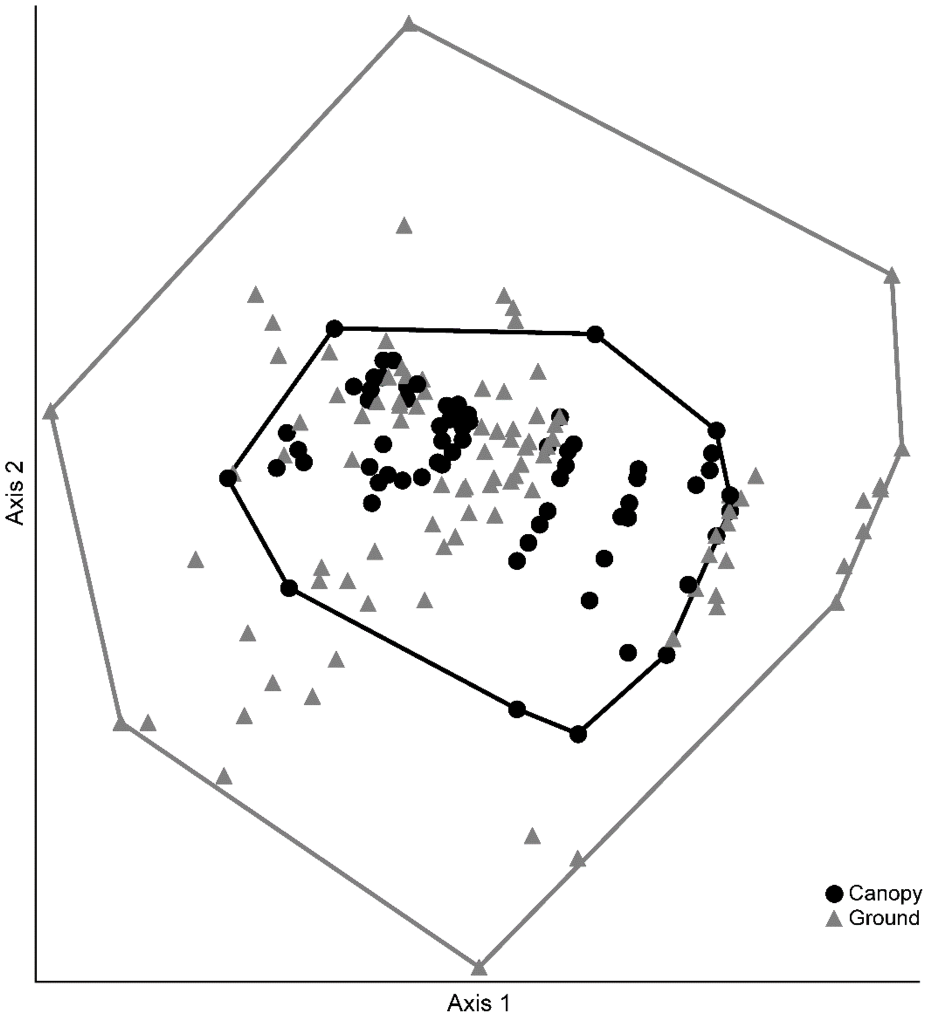

Figure 1.4. Nonmetric multidimensional scaling (two dimensional) for nitidulid species captured by three canopy traps and three ground traps in three northern sites in 2018. Final stress = 10.8.

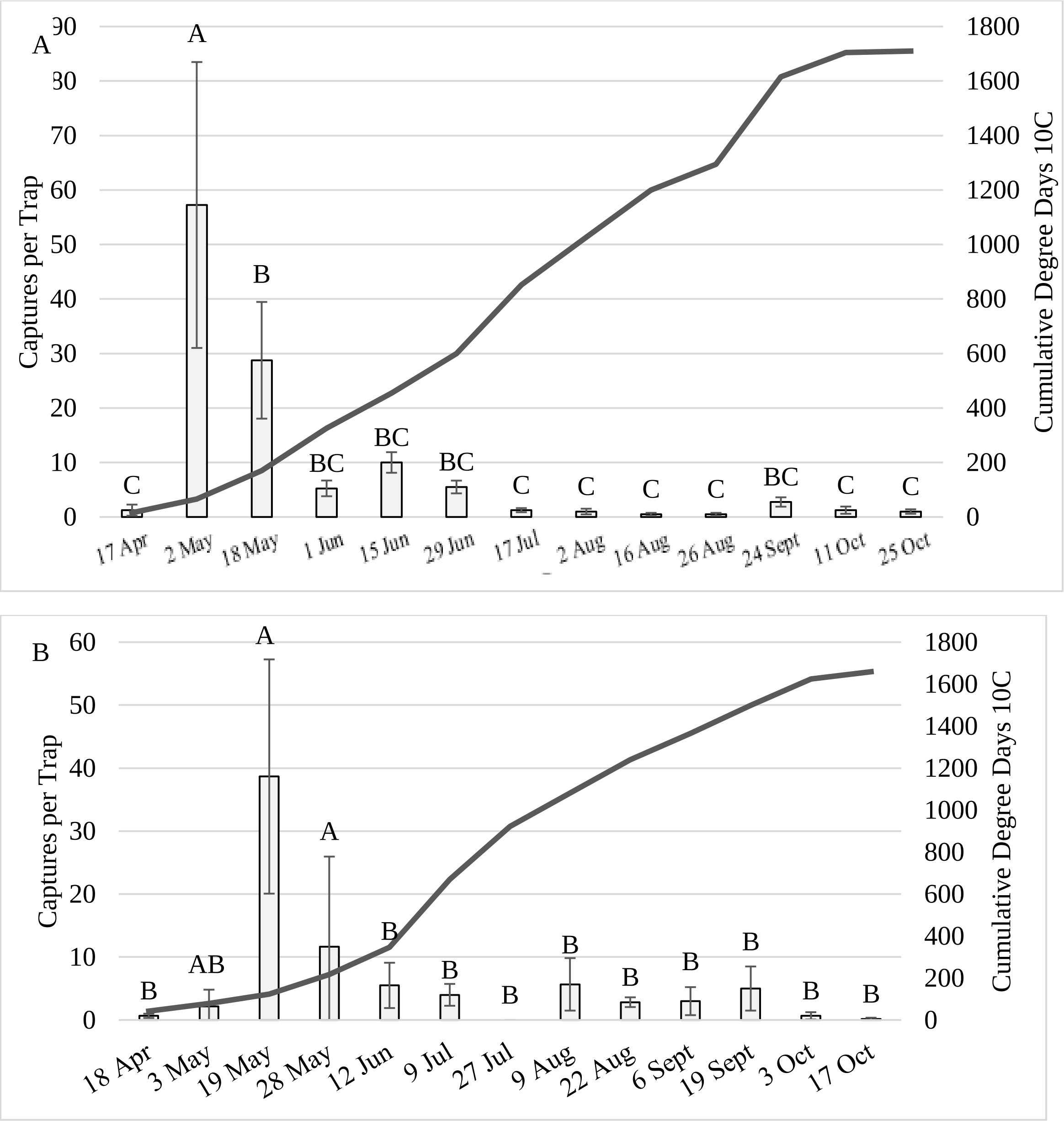

Figure 1.5. Number of nitidulid beetles per trap captured on each trap check date along with cumulative growing degree days (base 10 C) at the Kellogg Forest site in southwest Michigan in (A) 2018 (6 traps) and (B) 2019 (4 traps). Different letters above columns indicate significant differences among dates; ($P < 0.05$).

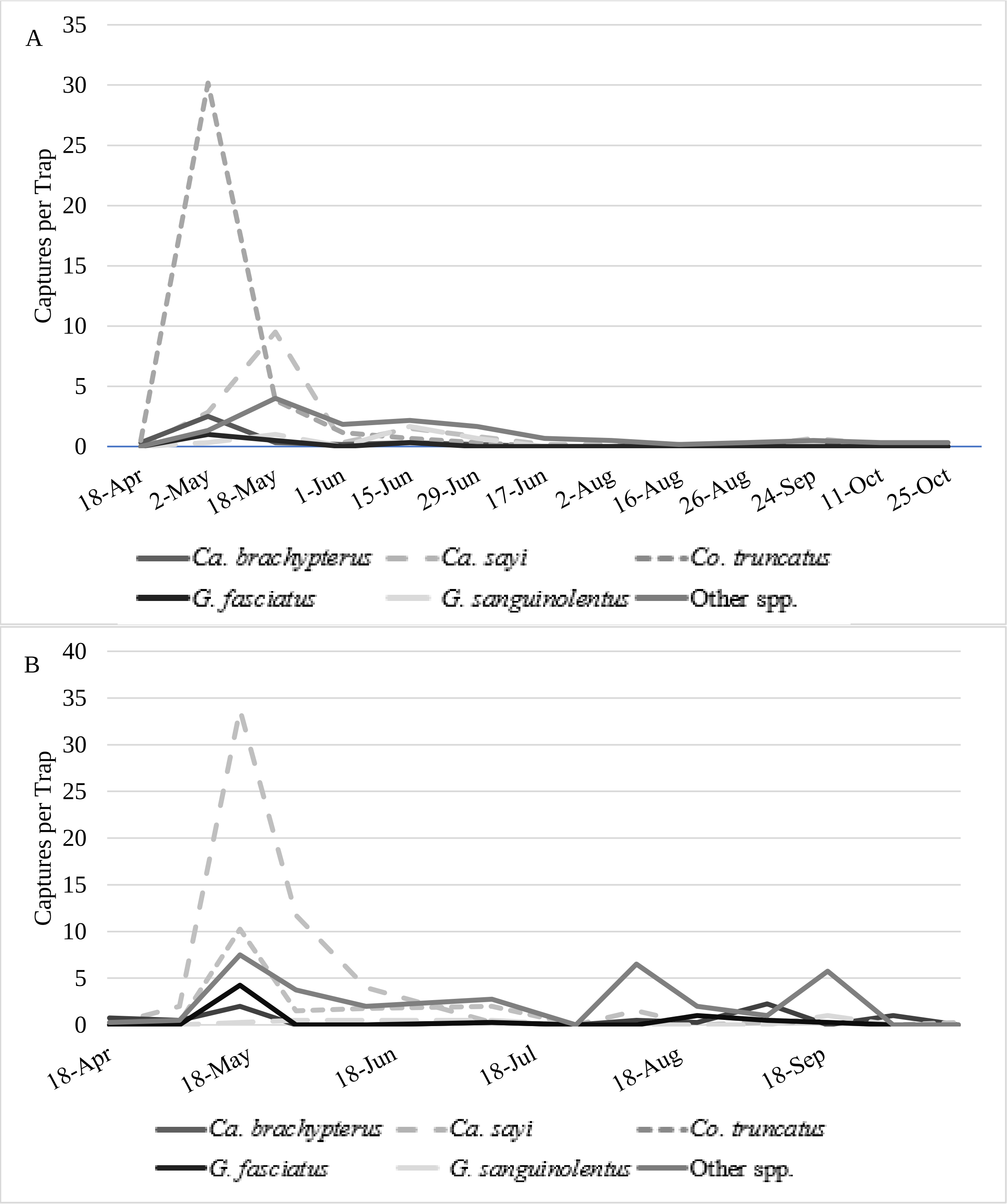

Figure 1.6. Number of beetles per trap captured on each trap check date for the top five most frequently captured species and all other species at Kellogg Forest in 2018 (A) (6 traps) and 2019 (B) (4 traps).

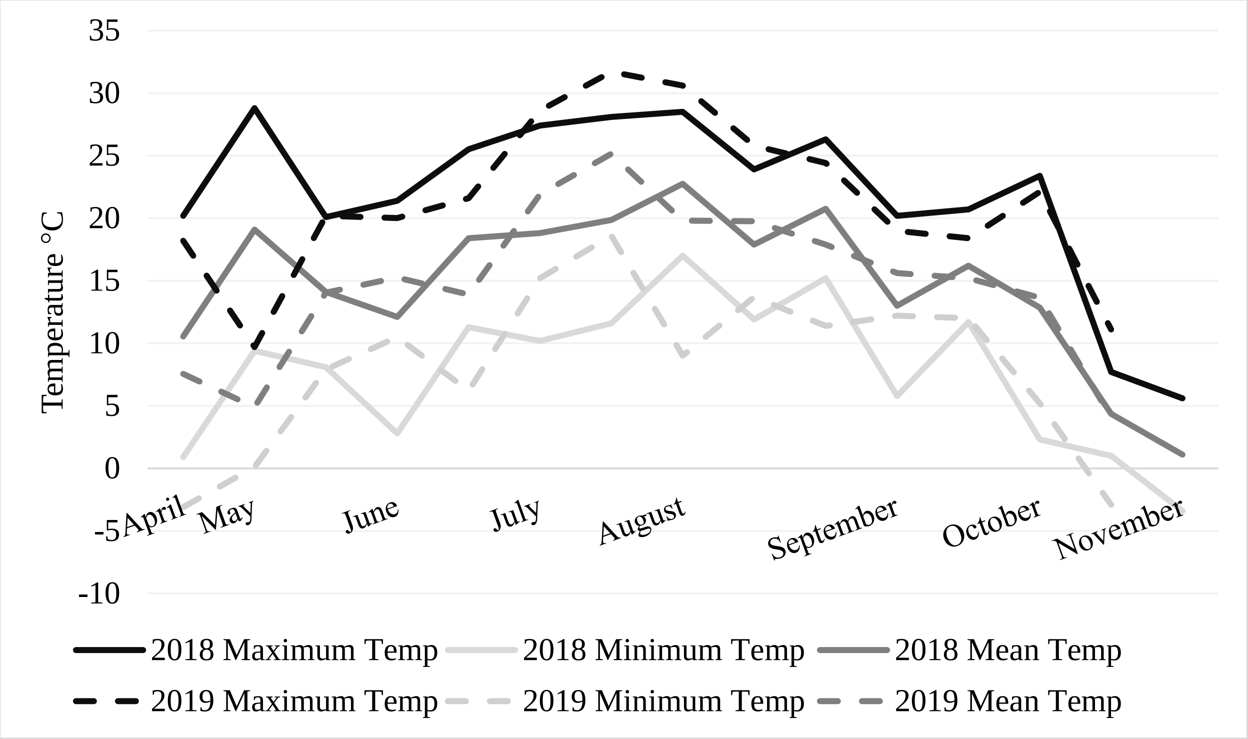

Figure 1.7. Mean, minimum, and maximum temperature for 2018 and 2019 were recorded by the MSU Enviroweather station at Williamsburg. Solid lines represent 2018 data while dotted lines represent 2019 data.

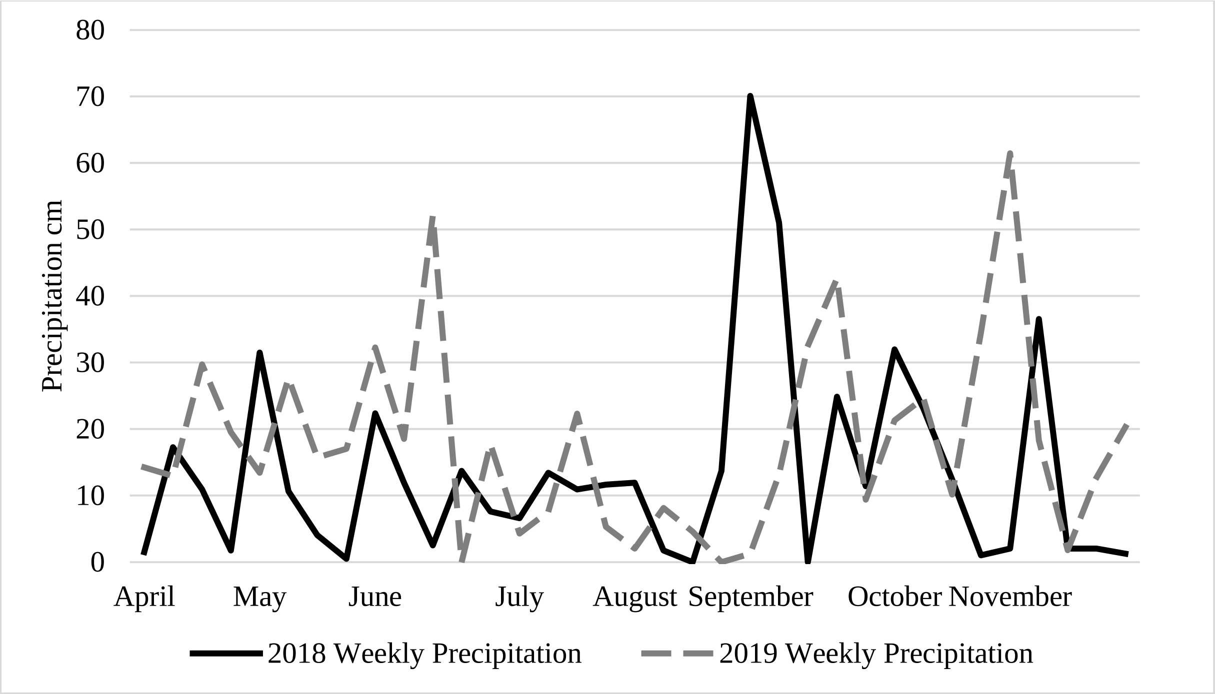

Figure 1.8. Weekly rainfall for 2018 and 2019 was recorded by the MSU Enviroweather station at Williamsburg. Solid lines represent 2018 data while dotted lines represent 2019 data.

APPENDIX B.

Record of Deposition of Voucher Specimens

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2020-12

Author and Title of thesis: Olivia Rose Morris

Seasonal activity and phoresy rates of sap beetles (Coleoptera: Nitidulidae) in oak wilt infection centers, volatile organic compounds related to the oak wilt cycle, and long term evaluation of red oak provenances.

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Nitidulidae	*Carpophilus sayi*	Adult	10	Pointed
Nitidulidae	*Carpophilus brachypterus*	Adult	10	Pointed
Nitidulidae	*Colopterus truncatus*	Adult	10	Pointed
Nitidulidae	*Glischrochilus sanguinolentus*	Adult	10	Pinned
Nitidulidae	*Glischrochilus fasciatus*	Adult	10	Pinned

CHAPTER 2: ANALYSIS OF VOLATILE ORGANIC COMPOUNDS RELATED TO THE OAK WILT DISEASE

Introduction

Many pathogens of trees rely in varying levels on insect vectors to infect new hosts (Humble & Allen 2006, Faccoli & Santini 2016, Jagemann et al. 2018). These include Dutch elm disease (*Ophiostoma ulmi* Melin & Nannf.), ash yellows (*Candidatus fraxini* Griffiths), and oak wilt (*Bretziella fagacearum* Bretz) (Hill & Sinclair 2000, Jernelöv 2017). Successful attraction of insect vectors to the reproductive structures of these pathogens can be an integral aspect of pathogen dispersal and facilitates continuation of the pathogen disease cycle. Understanding how pathogens attract insect vectors can lead to options for disease management to reduce damage to forest and landscape trees.

Volatile organic compounds (VOCs) refers to chemicals produced and released by plants and other organisms into the air (Rodrigues-Saona et al. 2011, Cappellin et al. 2017). VOCs help plants attract pollinators , defend against herbivores, and serve as signals to neighbor plants. They may be exploited by herbivores or parasitic insects to locate hosts. Pathogens, however, may alter VOC’s released by the infected tree (Shapiro et al. 2012). Additionally, VOC’s may be released by the pathogen itself (Shapiro et al. 2012, Cordoven et al. 2017).

Oak wilt, caused by the fungus *Bretziella fagacearum* (Bretz), is an important disease affecting oak (*Quercus* spp.) across the eastern United States. Oak wilt causes vascular wilt in oak trees, and while all oaks are susceptible, red oaks (Sect. *Lobatae*) are much more susceptible than white oaks (Sect. *Quercus*) (Juzwik et al. 2011). White oaks may experience dieback but an oak wilt infection rarely causes death, while red oaks are highly susceptible and may die within as early as three weeks of infection (Hayslett et al. 2008). Oak wilt is spread from infected trees to healthy trees by two pathways: underground via root grafts and overland via insect vectors. The origin of the oak wilt fungus remains unknown, although genetic analysis and the ongoing spread of the *B. fagacearum* pathogen indicate it is not native to the United States (Juzwik et al. 2008).

Phoresy rates reported in previous studies of nitidulids in the upper Midwest and Texas are inconsistent (Hayslett et al. 2008, Ambourn et al. 2005, Cease & Juzwik 2001). For *Carpophilus sayi* (Parsons 1943), a species found to be an important vector of oak wilt in the Midwest, contamination rates of *Ca. sayi* beetles captured in June 2002 and 2003 ranged from 0% to 83% (Ambourn et al. 2005). This inconsistency may reflect the lack of a co-evolved relationship between *B. fagacearum* and native nitidulids.

Nitidulid beetles are commonly knowns as sap beetles, because of their propensity for feeding on plant sap (Lee et al. 2020), which contributes to their role as vectors of oak wilt (Lin & Phelan 1992, Guenther et al. 1995, Arimura et al 2004, Kim et al. 2011, Portillo-Estrada et al. 2015). Overland spread of the oak wilt fungus occurs when nitidulid beetles visit a sporulating mycelial mat on a dead oak killed by oak wilt, then feed on a fresh wound on a live red oak tree (Gibbs 1980). Mycelial mats, which produce ascospores, form on xylem of dead oaks that have been killed by oak wilt and emit a distinct odor usually described as overripe fruit (MacDonald & Hindal 1981). As the mat grows, cushion-like tissues form a pressure pad, which exerts pressure and eventually results in a small vertical crack in the outer bark (Juzwik et al. 2011). Nitidulid beetles are attracted to the mycelial mats, presumably as a food source, and may also encounter potential mates while visiting the mats (Juzwik & French 1983, Blackmer & Phelan 1995, Cease & Juzwik 2001). Since the mats are not apparent on the outside of the tree and can only be accessed via small cracks in the bark, beetles presumably are attracted to VOCs produced by fresh pads (Lin & Phalen 1992). If beetles become contaminated with viable fungal spores from the mat, then visit a fresh sap-producing wound on a healthy red oak, the spores are introduced into the xylem where they germinate (Hayslett et al. 2008), leading to the eventual death of the tree.

For oak wilt vector monitoring, the use of male-produced aggregation pheromone lure for *Carpophilus sayi* ((2E,4E,6E,8E)-3,5-dimethyl-7-ethyl-2,4,6,8- undecatetraene and (2E,4E,6E,8E)-3,5,7-trimethyl-2,4,6,8-undecatetraene) and *Colopterus truncatus* ((2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene, (2*E*,4*E*,6*E*)-4,6-dimethyl-2,4,6-nonatriene, and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene), are common (Great Lakes IPM, Vestaburg, MI)(Bartelt et al. 2004, Khyl et al. 2002). However, there may be nitidulids that are not attracted to these species' aggregation pheromones, such as *Cryptarcha* spp. or *Glischrochilus* spp. The deployment of a lure that uses compounds emitted from stages of the oak wilt cycle may provide better tools for monitoring all potential species of vectors.

Identifying compounds associated with mycelial mats and wounded oak trees could provide the framework for developing options to better monitor vectors of oak wilt and perhaps mitigate its impact. We sought to identify common and abundant compounds produced by (1) healthy, unwounded red oak trees, (2) healthy red oaks wounded to expose the xylem (sapwood), and (3) mycelial mats produced by the *B. fagacearum* fungus. We also collected VOCs from colonies of pure *B. fagacearum* growing on Petri dishes in the laboratory.

Methods

Study Sites

Volatile organic compounds (VOCs) were collected in 2018 from live red oak trees at four sites on state forest land in northern Lower Michigan where state forest health personnel had previously confirmed oak wilt infections (Table 2.1., Figure 2.1.). There were at least 80 healthy red oak trees (DBH > 10 cm) recorded at each site. Other overstory species at the sites included *Acer rubrum* (L.), *A. saccharum* (Marshall), *Populus grandidentata* (Michx.), *Pinus strobus* (L.), *P. resinosa* (Aiton), *Quecus rubra* (L.), and *Q. alba* (L.). Mean red oak DBH (± SE) at the sites ranged from 25.8 ± 1.86 cm to 37.25 ± 2.46.

Sample Collection

At the Bass Lake site, we collected VOCs from six healthy, unwounded red oaks, six healthy red oaks that we wounded just before VOC collection, and from fresh mycelial mats on six infected (dead) red oaks. At Nessen Road, Coyote Road, and Bunker Hill sites, we collected VOC samples from three healthy, unwounded red oaks and three healthy, wounded red oaks. Our ability to collect samples from fresh mycelial mats at each site was limited by the availability of the mats, which varied among sites and seasonally. Wounds were created using a cordless drill with a 2.5 cm diameter dozier drill bit to removed outer bark and phloem, exposing sapwood on the trunk, 1.4 m above ground. Similarly, the drill bit was used to removed bark and phloem over a relatively fresh mycelial mat on infected trees. Drill bits were immersed in 70% ethanol then flamed between each tree to sterilize the bits and prevent contamination.

We wrapped a 48 cm by 59 cm sheet of heat resistant nylon (oven turkey bags, Reynolds Kitchen, Lake Forest, IL) around the circumference of the trunk of each sampled tree ensuring that wounds or mycelial mats were centered under the wrap. A glass tube with 30 mg of Super-Q absorbent material (Alltech Associates Inc., Chicago, IL), was inserted into the nylon wrap directly over the wound, mycelial mat or in the center of the wrapped area on unwounded trees. The small hole in the nylon wrap for the glass tube was sealed with parafilm. The glass tube was connected with rubber tubing to an air pump (Sensidyne, Clearwater, FL) powered by a

12V8.0Ah battery. Pumps pulled air across the Super-Q tubes for at least four hours. After pumps were shut off, the Super-Q tubes were retrieved using nitrile gloves, wrapped individually in aluminum foil, placed into a cooler with ice and transported to the Forest Entomology Lab at Michigan State University. Super-Q tubes were kept in a freezer (20°C) until compound extraction in the Forest Service Lab at Michigan State University.

In addition to field samples from red oak trees, we also collected VOCs in the lab from pure colonies of *B. fagacearum* grown on PDA media in Petri dishes. Two genetic strains of the oak wilt fungus were provided by collaborators at the Forest Pathology Laboratory at Michigan State University. Strain FCC was isolated from Grand Traverse County, MI in 2017, and strain 519 was isolated from Sherburne County, MN in 1992. Four Petri dishes of each strain were sampled on 18 March 2018. The Petri dishes were uncovered and placed individually under a 4 L glass cylinder (30.5 cm x 15.2 cm; Analytical Research Systems, Inc., Gainesville, FL). Air, generated by a 2 Hp compressor (Grainger, Northbrook, IL), was purified trough activated charcoal and pushed through the top opening of the glass chamber. Volatiles were collected by adsorbent Super-Q filter tubes (Alltech Associates Inc., Chicago, IL) attached to the base of the glass chambers and connected by tubing to a ¼ Hp vacuum pump. Each tube was then wrapped individually in aluminum foil as with the field samples and placed in a freezer (20°C) until they could be analyzed.

VOC Extraction

Each Super-Q was flushed with 150 µl of a pentane/hexane mixture with 3.5 ng/µl added as an internal standard and pushed through with N_2 gas into a glass vial. The extracted liquid was concentrated down by blowing the N_2 gas over the top of the vial, until the volume was reduced to approximately three µl.

After extraction, two µl from each sample were injected into a Thermo Scientific Trace gas chromatograph equipped with a DSQ-II mass spectrometer and a 30 mm x 0.25 mm TR-1MS column using splitless injections with helium as the carrier gas. The oven temperature began at 40°C for one minute then increased by 8°C per minute to 300°C and held for five minutes. Compounds were tentatively identified based on matches of spectral data to those in the NIST Library. Quantities of all compounds were estimated by comparing their peak areas to that of the internal standard. Tentative compound identifications were recorded with the direct matching factor (SI) as an indicator of the strength of the NIST Library match, where any score over 900 is considered excellent, and a score of 870 or greater is considered good (Gujar et al. 2018). Chemical standards are listed in Table 2.2.

Statistical Analysis

To evaluate similarity of VOC composition collected from healthy trees, fresh wounds, mycelial mats, and laboratory cultures of *B. fagacearum,* the estimated quantities compounds tentatively identified in each of the four sample types were analyzed using non-metric multidimensional scaling (NMS) with PC-ORD software (PCORD 6.08, MJM Software, Gleneden Beach, OR). For the NMS, 100 iterations of real and randomized data were run on the autopilot mode using the “slow and thorough” mode and the Sorenson (Bray-Curtis) method of measuring distance was applied. The resulting NMS scree plot was assessed to determine dimensionality. Number of axes was determined by efforts to decrease stress without creating distortion by including too many axes. For NMS ordinations, final stresses of 5 – 15 are considered ideal, while a final stress greater than 25 would not be interpretable (Peck 2016). Using the dimensionality from the scree plot, the ordination was re-run with the appropriate number of axes. Each sample type and its chemical drivers were overlaid to visualize similarity of compounds collected from the four types of samples. An Indicator Species Analysis (ISA) test was conducted (using PC-ORD) to identify compounds associated with specific types of samples. The Indicator Value (IV), which ranges from 0 to 100, was calculated for each compound most closely associated with specific sample types.

Results

Comparisons among samples from the NMS indicate that the compounds associated with each sample type differed (Figure 2.2., Table 2.3.). A pattern developed on the NMS ordination regarding the clustering of compounds in mycelial mats nearest those from fungal colonies, and the clustering of compounds in wounded trees closest to compounds emitted from unwounded trees, visually demonstrating the differences in volatile profiles. Only four compounds were identified in all types of samples including unwounded and wounded trees, mycelial mats, and pure *B. fagacearum* colonies. These compounds were tentatively identified as 9-Hexadecen-1-ol,(Z)-, benz[i]aceanthrylene,3-methyl-, gamabufotalin, and undecanoic acid. While undecanoic acid was detected in all sample types, it was only abundant in samples from the *B. fagacearum* colonies.

A total of 56 significant peaks were tentatively identified in samples from the healthy unwounded red oaks, including 16 compounds that were not found in any other sample type based on retention times and mass spectra (Table 2.4.). Abundant compounds characteristic of unwounded oaks were determined through ISA and were tentatively identified as *N*-[(7*S*)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5*H*-benzo[a]heptalen-7-yl]acetamide, or colchicine, and (*Z*,12*R*)-12-hydroxyoctadec-9-enoic acid, known as ricinoleic acid (Table 2.3.). This compound was also present in three samples from wounded trees and eight samples from mycelial mats.

Samples collected from the fresh wounds on the otherwise healthy but wounded trees yielded 47 significant peaks including ten found only in samples collected from wounded trees based on their retention times and mass spectra (Table 2.6.). The most abundant compound in these samples was tentatively identified as 2,3,5-trichloro-6-[(3,4,6-trichloro-2-hydroxyphenyl)methyl]phenol, or hexachlorophene (Table 2.3.). Hexachlorophene was also detected in one sample from unwounded red oak trees and in three samples from unwounded trees. In samples from wounded trees, hexachlorophene had SI scores ranging from 448 to 474, indicating a weak match. Therefore, further tests are needed to confirm compound identification. Eight compounds were found in samples from both unwounded and wounded trees but were not found in samples from mycelial mats or *B. fagacearum* colony samples. Two compounds, colchicine and hexachlorophene, were abundant among wounded tree samples.

Samples from mycelial mats on dead red oak trees yielded 45 significant peaks, including eight that were unique to the mycelial mats based on retention times and mass spectra (Table 2.7.). The most abundant compounds from mycelial mats on trees were tentatively identified as tetradecan-1-ol, or 1-tetradecanol and (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenal, or 9-cis-retinal (Table 2.3.). There were six samples from mycelial mats that yielded 1-tetradecanol, along with samples from six wounded trees, while 11 unwounded trees had very low quantities of the compound. In mycelial mat samples, 1-tetradecanol had SI scores ranging from 489 to 600. Five samples from mycelial mats contained 9-cis-retinal, as did three samples from wounded oaks and a sample from one unwounded tree. In mycelial mat samples, 9-cis-retinal had SI scores ranging from 444 to 535. The los SI's from the tentative identifications of compounds from mycelial mats indicate weak matches to NIST Library spectra and the need for further analysis for identification. Three compounds in samples of wounded red oak trees and mycelial mats were not found in other sample types but were present at low levels.

We identified 35 significant peaks produced by *B. fagacearum* colonies grown on Petri dishes in the laboratory, including 14 compounds detected exclusively in these samples (Table 2.8.). In the 519 *B. fagacearum* colony, undecan-1-ol, or 1-undecanol was the most abundant compound, while undec-10-enoic acid, or undecanoic acid, was most abundant in the FCC strain of *B. fagacearum* (Table 2.3.). In addition to being detected in four of the *B. fagacearum* 519 colony samples, 1-undecanol was detected in samples from one unwounded red oak tree and two wounded trees. From the 519 *B. fagacearum* colony, the 1-undecanol had SI scores ranging from 519 to 595. Undecanoic acid, the third most common compound across all sample types, was detected in 12 samples from *B. fagacearum* colonies along with samples from eight mycelial mats, seven unwounded oaks, and two wounded oaks. In the FCC colony samples, undecanoic acid had SI scores ranging from 480 to 602. The low SI scores from compounds tentatively identified from *B. fagacearum* colonies indicate weak matches to NIST Library spectra, with further analysis needed to confirm identifications. Three compounds in samples from mycelial mats, ethyl cyclopropanecarboxylate, m-tolyl iscothiocyanate, and pyrene, were also identified in samples from the *B. fagacearum* colonies, but only at low levels.

Discussion

Our results indicate that blends of compounds emitted from healthy and wounded oaks, mycelial mats and fungal cultures differed significantly with several unique compounds characteristic of each group. However, low SI scores for our tentatively identified compounds suggests that our matches to standard spectra in the NIST Library were weak, and further analysis is necessary to confirm our identifications more confidently.

Volatile compounds emitted from the samples of mycelial mats on dead red oak trees and pure colonies of *B. fagacearum* may be key to understanding the attraction of insect vectors to the causal agent of the oak wilt disease. Attraction of nitidulid beetles to mycelial mats, especially species identified as important vectors of oak wilt, such as *Ca. sayi* and *Co. truncatus*, may be critical element of overland spread of the oak wilt fungus (Cease & Juzwik, 2001, Juzwik et al. 2004, Ambourn et al. 2005, Juzwik et al. 2011, Jagemann et al. 2018). Further studies are needed to confirm identification of key compounds and conduct behavioral bioassays to determine if any are attractive to nitidulid beetles. If nitidulids are consistently attracted to compounds from fungal cultures or mycelial mats, lures could be formulated to improve our ability to monitor nitidulid activity. Compounds associated with, samples from wounded trees and mycelial mat samples should be investigated further. While these compounds were not abundant in our samples, they may play a role in the ability of nitidulids to differentiate between wounds on live trees, unwounded trees, and mycelial mats.

In our study, VOCs were collected in the field within a four-week period from 30 May through 20 June. There may be seasonal differences in the VOCs released by trees, which our study would not have captured. However, our samples of VOCs were collected within the high risk period for overland oak wilt transmission according to Michigan’s Dept. of Natural Resources (MIDNR 2017,) which indicates they are likely to be more relevant than compounds collected earlier or later in the year.

Comparing VOCs from various ages of mycelial mats might also provide information about which compounds are attractive to specific nitidulid species. Previous research has documented different species of nitidulids are attracted to different stages of mycelial mat growth (Cease & Juzwik 2001). For monitoring potential oak wilt vectors, VOCs from older mats that are not producing viable spores would presumably be less useful because these mats pose no risk for spreading oak wilt (Curl 1955). We attempted to only sample mycelial mats that were fresh or mature, as opposed to declining or deteriorating mats. Similarly, compounds present in samples from mats, wounds, and unwounded oaks but not in the samples from the *B. fagacearum* colonies may be less likely to be useful for monitoring nitidulid vectors.

Pheromones are used for monitoring and trapping a variety of insects, including nitidulid beetles. Lures currently used to bait traps use sex pheromones produced by *Ca. sayi* and *Co. truncatus*, which also attract other species (Bartelt et al. 2004, DiGirolomo et al. 2020). If nitidulids exhibit attraction to the compounds identified from mycelial mats and pure oak wilt colonies, including these compounds in lures could enhance trapping and efforts to monitor nitidulid vectors. This could enable pest managers to monitor nitidulid species that can vector the fungus but are repelled or do not respond to the pheromones from the *Ca. sayi* or *Co. truncatus* lures. Assessing relative risks of overland transmission of oak wilt is often predicated on captures of nitidulids contaminated with oak wilt fungal spores in traps. Using VOCs to increase attraction to baited traps could contribute to improved oak wilt management.

APPENDIX

Table 2.1. Study site location, stand size and number of volatile samples collected in 2019 from healthy, unwounded red oaks, healthy and wounded red oaks, and mycelial mats on trees previously infected with oak wilt.

Site	**Nessen Road**	**Bunker Hill**	**Coyote Road**	**Bass Lake**
County	Grand Traverse	Grand Traverse	Roscommon	Grand Traverse
Latitude	44.55913	44.7351944	44.5093889	44.605959
Longitude	-85.7990833	-85.4437778	-84.8372778	-85.812356
Size	20 ha	10 ha	10 ha	3.5 ha
Mean Red Oak DBH ± SE	26.91 ± 1.28	27.19 ± 1.59	37.25 ± 2.46	25.80 ± 1.86
Healthy Samples	3	3	3	6
Wounded Samples	3	3	3	6
Mycelial Mat Samples	0	1	1	6
Sample Date	26 June	20 June	5 June	30 May

Table 2.2. Purity and source of the chemical standards used to confirm tentative NIST Library identifications of compounds.

Compound	**Chemical Formula**	**Purity**	**Company**	**Location**
Retinal, 9-cis-	$C20H28O$	98%	AKSci	Union City, CA
Benzene 2-propenyl	$C9H10$	98%	AKSci	Union City, CA
Undecanoic acid	$C11H22O2$	≥99.0%	Sigma Aldrich	St. Louis, MO
Undecylenic acid	$C11H20O2$	≥99.0%	Sigma Aldrich	St. Louis, MO
Ricinoleic acid	$C18H34O3$	≥99.0%	Sigma Aldrich	St. Louis, MO
Colchicine	$C22H25NO6$	≥99.0%	Sigma Aldrich	St. Louis, MO
1-Tridecene	$C14H30O$	≥99.0%	Sigma Aldrich	St. Louis, MO

Table 2.3. Abbreviated results of the non-metric multidimensional scaling (NMS) comparison of compounds collected from unwounded red oaks, wounded red oaks and mycelial mats on dead red oaks killed by oak wilt.

Sample		**Sample**	**t-value**	***P*-value**
Unwounded	vs.	Wounded	4.744	0.0006
Unwounded	vs.	Mycelial Mat	3.9911	0.0004
Unwounded	vs.	Pure Colony	9.4684	0.0012
Wounded	vs.	Mycelial Mat	2.8869	0.0074
Wounded	vs.	Pure Colony	5.931	0.0006
Mycelial Mat	vs.	Pure Colony	8.1989	0.0006

Table 2.4. Tentatively identified compounds detected only in samples from healthy, unwounded red oak trees, including retention time (RT) and standard indicator (SI) values for match to mass spectra in NIST Library.

Compound Name	**Chemical Formula**	**RT (min)**	**SI**
2',5'-Dichloro-4'-nitroacetanilide	C8H6Cl2N2O3	5.98	431
4,5-Dimethyl-ortho-phenylenediamine	C8H12N2	13.28	552
6-Bromohexanenitrile	C6H10BrN	20.96	537
7H-Dibenzo(a,g)carbazole,12,13-dihydro-	C20H15N	15.94	674
Benzene, 1,3-dichloro-	C6H4Cl2	28.54	432
Benzene, 2-propenyl-	C9H10	9.99	624
Benzene, 4-ethyl-1,2-dimethyl-	C10H14	9.21	361
Benzenemethanol, 2,5-dimethyl-	C9H12O	10.05	573
Ergoline-8-carboxamide, 0,10-didehydro-6-methyl-,(8a)-	C16H17N3O	6.17	413
Hydrogen bromide	BrH	10.12	236
Naphthalene, 1-(1-methylethyl)-	C13H14	14.86	500
n-Heptyl acrylate	C10H18O2	13.96	569
Phenol, 2,2'methylenebis(3,5,6-trichloro-	C13H16Cl6O2	6.04	361
Phenol, 2-methyl-	C7H8O	13.37	256
Phenol, 2-methyl-6-(2-propenyl)-	C10H12O	14.9	520
Retinol, acetate	C22H32O2	20.07	546

Table 2.5. Characteristic compounds identified by ISA in samples from unwounded red oaks, wounded red oaks and mycelial mats on red oaks killed by oak wilt, based on abundance, volume, and the range of retention times. Indicator values range from 0 to 100 and represent the measure of exclusiveness for a compound in a sample type.

Sample Type	**Characteristic Compound(s)**	**IUPAC Name**	**Chemical Formula**	**Retention Time**	**Mean ± SE ng/g**	**Indicator Value**	***P* - value**
Unwounded Red Oak	Colchicine	*N*-[(7*S*)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5*H*-benzo[a]heptalen-7-yl]acetamide	C22H25NO6	20.71 - 24.9	0.000028 ± 0.0000059	57.1	0.0046
	Ricinoleic acid	(*Z*,12*R*)-12-hydroxyoctadec-9-enoic acid	C18H34O3	11.17 - 28.56	0.00011 ± 0.000038	40.3	0.0492
Wounded Red Oak	Hexachlorophene	2,3,5-trichloro-6-[(3,4,6-trichloro-2-hydroxyphenyl)methyl]phenol	C13H6Cl6O2	6.15 - 6.26	0.00055 ± 0.000077	42.9	0.028
Mycelial Mats	1-Tetradecanol	tetradecan-1-ol	C14H30O	12.05 - 13.3	0.00021 ± 0.0000761	33	0.0366
	9-cis-Retinal	(2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenal	C20H28O	20.07 - 22.55	0.000096 ± 0.0	45.5	0.0192
519 Oak Wilt Colony	1-Undecanol	undecan-1-ol	C11H24O	7.01 - 13.9	0.0031 ± 0.0016	42.9	0.0262
FCC Oak Wilt Colony	Undecanoic acid	undecanoic acid	C11H20O2	4.55 - 13.9	0.016 ± 0.013	82.4	0.004

Table 2.6. Tentatively identified compounds, including their retention times (RT) and standard indicator (SI) values for match to spectra in NIST Library, detected only in samples collected from fresh wounds on healthy red oak trees.

Compound Name	**Chemical Formula**	**RT (min)**	**SI**
1-Nonadecene	$C_{19}H_{38}$	21.81	559
1-Octadecanol	$C_{18}H_{38}O$	10.92	518
2-Butynedinitrile	C_4N_2	8.98	271
2-Methylpyrrolidine	$C_5H_{11}N$	12.06	548
2-Pyridinecarboxylic acid, 5-butyl-	$C_{10}H_{13}NO_2$	8.88	466
3-Furanmethanol	$C_5H_6O_2$	20.97	478
4-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	21.48	533
Botran	$C_6H_4Cl_2N_2O_2$	6.01	405
c Dodecalactone	$C_{12}H_{22}O_2$	13.29	527
Phenol, 4-fluoro-	C_6H_5FO	12.62	535

Table 2.7. Tentatively identified compounds, including their retention times (RT) standard indicator (SI) values for match to spectra in NIST Library, detected only in samples collected from mycelial mats on red oak trees killed by oak wilt.

Compound Name	**Chemical Formula**	**RT (min)**	**SI**
2(3H)-Furanone, 5-heptyldihydro-	C11H20O2	8.19	554
4a,7-Methano-4aH-naphth[1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl-	C15H24O	14.53	521
4-Isoprophylphenylacetic acid	C11H14O2	16.49	427
9-(Chloromethyl)anthracene	C15H11Cl	24.56	438
Acetic acid, 2-methylpropyl ester	C6H12O2	4.47	641
Benzo[b]triphenylene	C22H14	11.21	426
Hexadecane	C16H34	13.27	529
Phenprobamate	C10H13NO2	10.37	557

Table 2.8. Tentatively identified compounds, including their retention times (RT) and standard indicator (SI) values for match to spectra in NIST Library, detected only in samples collected from *B. fagacearum* colonies grown on PDA-media in Petri dishes.

Compound Name	**Chemical Formula**	**RT (min)**	**SI**
1-Butanol, 3-methyl-, formate	$C_6H_{12}O_2$	7.03	484
2-Amino-2-ethyl-1,3-propanediol	$C_4H_{11}NO_2$	6.97	502
4-Methyloctanoic acid	$C_9H_{18}O_2$	4.53	482
a-D-Glucopyranoside, a-D-glucopyranosyl	$C_{12}H_{22}O_{11}$	4.54	539
Benzene, (1-bromoethyl)-	C_8H_9Br	7.00	502
Benzene, 1,1'-[ethylidenebis(oxy-2,1-ethanediy)]bis-	$C_{18}H_{22}O_2$	4.54	514
Benzoic acid, methyl ester	$C_8H_8O_2$	17.68	566
Methyl nicotinate	$C_7H_7NO_2$	17.68	554
N,N'-Diisopropylethylenediamine	$C_8H_{20}N_2$	17.16	532
Octadecane, 1-isocyanato-	$C_{19}H_{37}NO$	13.14	517
Penicillamine	$C_5H_{11}NO_2S$	13.16	481
Phensuximide	$C_{11}H_{11}NO_2$	13.16	491
Styrene	C_8H_8	13.13	454
Undecanoic acid, 2-methyl-	$C_{12}H_{24}O_2$	4.53	536

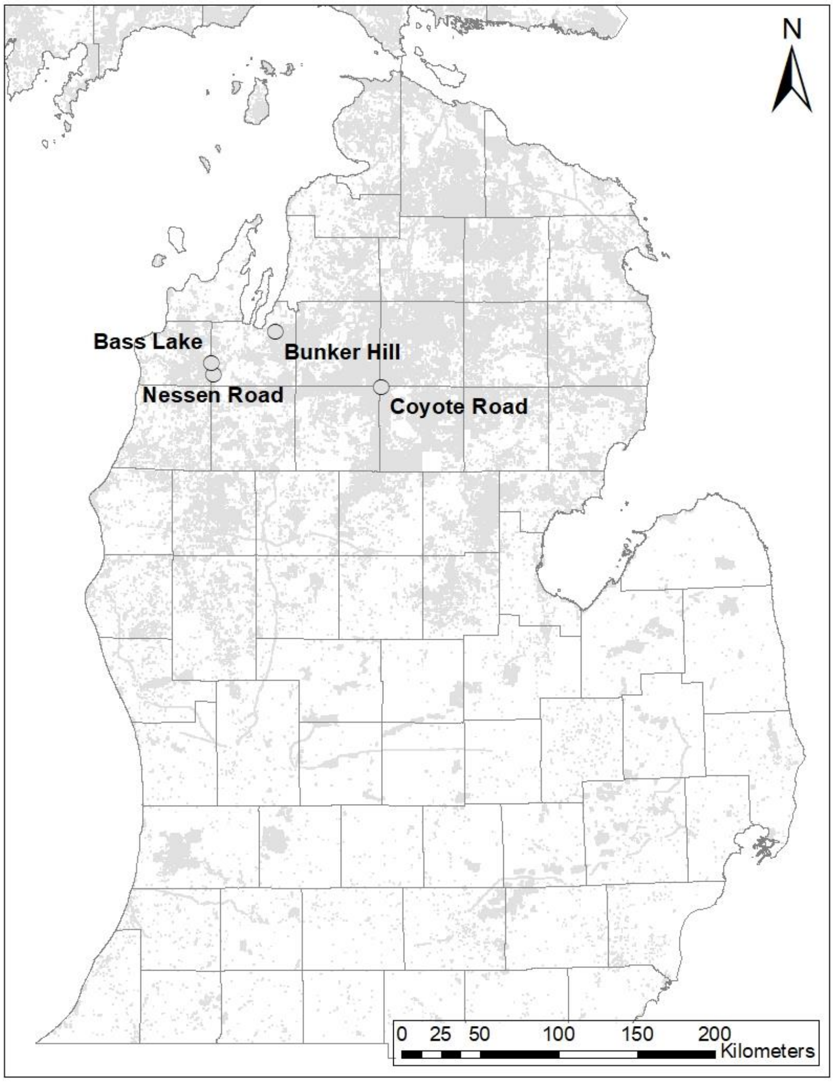

Figure 2.1. Locations of research sites in northern Lower Michigan. Each site had active oak wilt infections confirmed by Michigan Dept. of Natural Resources personnel. Shaded areas indicate state land owned and managed by the Michigan Dept. of Natural Resources.

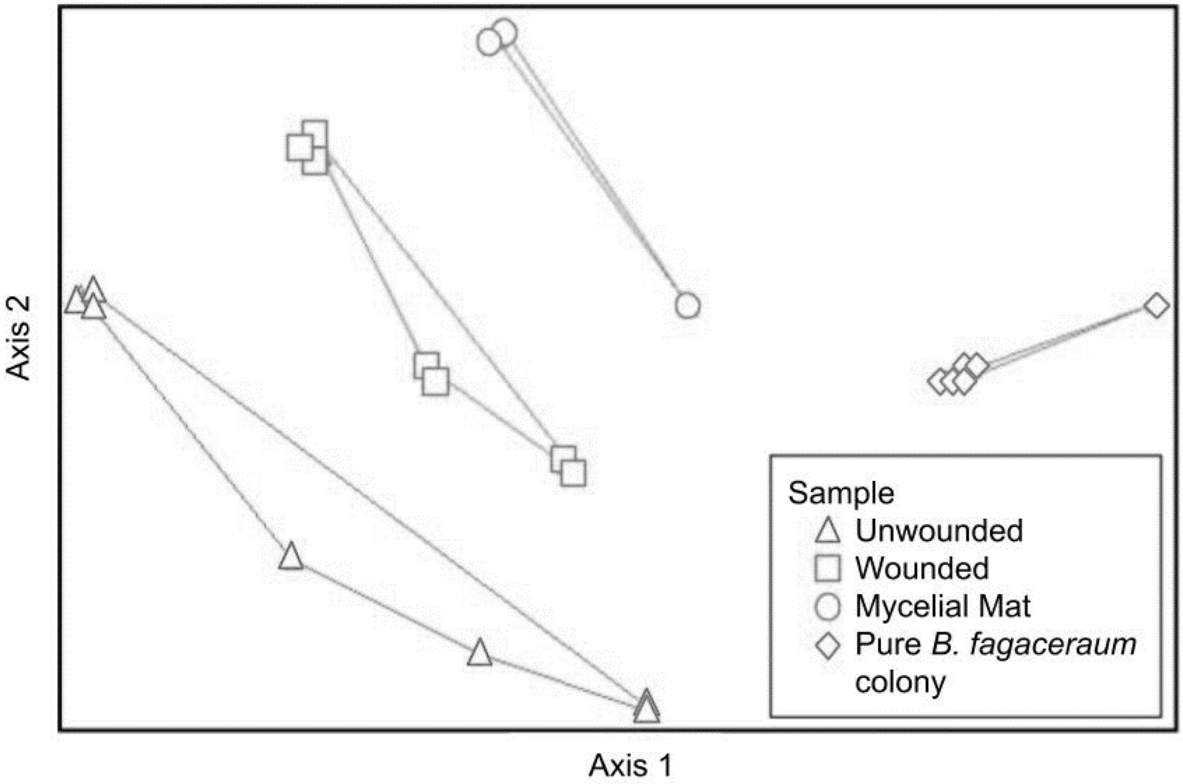

Figure 2.2. Results from non-metric multidimensional scaling (NMS) of compounds associated with unwounded red oaks, wounded red oaks, mycelial mats on red oaks killed by oak wilt and pure *Bretziella fagacearum* colonies grown on Petri dishes. Compounds from the two strains of *B. fagacearum* were combined for analysis. Final stress = 11.2.

CHAPTER 3: SURVIVAL AND GROWTH OF 55-YEAR OLD NORTHERN RED OAK (*QUERCUS RUBRA* L.) TREES FROM 22 PROVENANCES

Introduction

Provenance plantations, where seed or seedlings originating from different parent populations are planted in a common location, function as reservoirs of genetic material and provide an opportunity to monitor survival and growth of trees over time (Mazer 1999). Traits of trees from different geographic origins can be compared to those of trees from a local and presumably well adapted population (Kriebel et al. 1976, 1988, Lu et al. 2016), providing a basis to assess genotypic variability. These plantations are often established for valuable timber species to provide information on relative growth rates, tree form, and resistance to major pests or pathogens (Kriebel et al. 1988, Petkova et al. 2014, Pelleri et al. 2020). Moreover, as climate change advances, provenance plantations may become increasingly important as a means to screen and select candidates for assisted migration programs (O'Neil et al. 2008, Leech et al. 2011, Isaac-Renton et al. 2014).

Northern red oak (*Quercus rubra* L.) is a major overstory component in 24 forest cover types (Tirmenstien 1991) and a minor component in several other cover types across much of the Upper Midwest and eastern United States, along with southern Ontario and Quebec, Canada (Sander 1990). Net volume of red oak in the northeast and Great Lakes regions of the United States was estimated at more than 540 million m^3 (19,071 mill ft^3) in 2017 (USDA Forest Service 2017). Red oak is an important source of high value lumber and wood products (Chapman and Bessette 1990, Millers, Shriner, and Rizzo 1989). Recent stumpage prices in Michigan ranged from 264 to 700 USD per MBF (MI DNR 2018) and nationally, red oak accounted for 28.9% of hardwood exports in 2017 (Caldwell 2018). Mature red oaks, i.e., ≥ 50 years old, produce large acorn crops at irregular 2 to 5 year intervals (Sander 1990), providing hard mast for wildlife (Tirmenstien 1991). Like other Fagaceae species, red oaks have marcescent leaves, meaning not all leaves are dropped in autumn (Karban 2007), which provides winter habitat and browse for deer and other wildlife (Telfer 1972, Wakeland and Swihart 2009).

Red oaks in the Great Lakes region are challenged by numerous biotic and abiotic factors. An array of native and invasive pests feed on red oaks, including defoliators such as gypsy moth (*Lymantria dispar* L.), two-lined chestnut borer (*Agrilus bilineatus* Weber), a native phloem-borer that colonizes stressed oaks, and oak wilt (*Bretziella fagacearum* Bretz.), which causes tree mortality and continues to spread throughout the region (Michigan Dept. of Natural Resources, 2017). Oaks, like other native trees, are affected by changing climatic conditions, including warmer winter temperatures, and altered precipitation patterns. Climate change may provide more favorable conditions for pests if, for example, insects develop more rapidly and complete more generations annually or sustain less winter mortality (Hlasny & Turcani et al 2008, McKee & Aukema 2015, Bentz et al. 2016). Projected distributions of forest cover types suggest red oak distribution will advance to the north by 2040-2050, shifting regions that were on the outer boundary of the range into more central positioning (Iverson et al 2008**,** Prasad et al. 2014). Adaptive migration, e.g., intentionally establishing populations originating from warmer or drier locations, represents a potential option to help mitigate negative impacts on northern red oak populations that are presumably not well adapted to projected mid-century climate conditions (Renwik & Rocca 2015).

A northern red oak provenance test established in 1962-1963 at the Michigan State University W.K. Kellogg Experimental Forest in Kalamazoo County, in southwest Michigan (Kriebel et al. 1988), could provide useful information for managing this important species.

Oaks in the plantation originated from acorns collected in 22 locations across 11 US states, including a local population only 63 km from the Kellogg Forest plantation, plus two locations in southern Ontario, Canada (Table 3.1., Figure 3.1.). In 1984, Kriebel et al. (1988) evaluated the 23-year-old trees. They reported survival rates were similar among provenances but documented differences in tree diameter and height. Subsequently, the plantation was occasionally revisited to remove dead trees or for thinning. Tree survival and diameter were re-measured in 2000 but data were not published.

In 2018, we re-surveyed the plantation to assess the 55-year-old trees to evaluate differences in survival and size of trees from different provenances. We also determined whether distance from the Kellogg Forest plantation to parent populations affected provenance performance. We hypothesized that variation among provenances in survival would increase over time as trees encountered occasionally severe weather and maladapted trees died. We predicted differences in growth would diminish as trees matured, given their common planting location and exposure to the same environment. Finally, we hypothesized that trees from the local provenance would outperform trees originating from more distant populations.

Methods

Plantation Establishment

Original methods for planation establishment were reported by Kriebel et al. (1988). Briefly, acorns were collected from 22 northern red oak populations in 1961 extending across much of the current range of northern red oak (Table 3.1., Figure 3.1.). Each provenance was assigned a unique identification number reflecting the origin of the parent population. Acorns were collected from multiple parent trees within stands that ranged from 1 to approximately 10 ha. The exception was provenance MI:77; acorns were collected from a single tree in Volinia, Michigan (Table 3.1.), 63 km (38 miles) from the Kellogg Forest plantation. After germination, seedlings were grown in a nursery bed in Green Springs, Ohio. Seedlings were planted at Kellogg Forest in 1962 and 1963 in 18 columns and 38 rows, spaced 1.8 × 3 m. Odd numbered columns were planted in 1962 and even numbered columns were planted in 1963. Number of trees planted per provenance ranged from 8 to 48, presumably reflecting variation in germination rates. Borders of red oak trees of undocumented origin were planted around the plantation to limit edge effects. In 1978-1980 and again in 2009, the plantation was thinned by removing the smallest tree from each provenance.

Tree Measurements

Diameter at breast height (DBH), measured 1.3 m aboveground and total height (to the top of the canopy) of live trees were measured in 1984, when trees were 23 years old (Kriebel et al. 1988). Live tree DBH was re-measured and survival recorded in 2000 by MSU Dept. of Forestry personnel. In 2018, we tallied survival, re-measured DBH and used a digital clinometer (VL402 Vertex Laser, Haglöf, Sweden) to measure total height of all live trees.

Statistical Analysis

Differences in survival among provenances in 2000 and 2018 were assessed using binary regression (1 for live trees, 0 for dead trees) (PROC GLIMMIX, SAS ver 9.4, Cary, NC). When the test was significant ($P<0.05$), survival between MI:77 trees and trees from other provenances was compared with Dunnett's adjustment for multiple comparisons with a control. Only provenances with at least one live tree were included in the survival analysis.

Measurements of tree DBH recorded in 1984, 2000, and 2018 were analyzed separately. Normality of the data was evaluated by examining residual q-q plots and the likelihood ratio test was used to assess homogeneity of variance (PROC GLIMMIX). Residuals for DBH data from

1984 and 2000 were normally distributed and variances were equal. Tree DBH measured in 2018 was log transformed to normalize the data. To assess differences in DBH among provenances, we used a one-way ANOVA with provenance as the fixed effect in PROC GLIMMIX. If ANOVA results were significant, the Dunnett adjustment for multiple comparisons of treatment means with a control mean was used to compare the local provenance MI:77 with the other provenances (Ekpenyong 2019). Provenances TN:1, MN:2, and MN:20 were excluded from analysis of 2018 DBH measurements no living trees remained. Tree height measurements were similarly evaluated using one-way ANOVA with PROC GLIMMIX with provenance as the fixed effect. Differences between M:77 and other provenances were compared if ANOVA results were significant. Multiple forward stepping linear regression was conducted to determine if growth, survival, or geographic origin were significant predictors of DBH, height, or survival (PROC REG in SAS 9.4). Potential predictors included percentage survival, average height and DBH, latitude, longitude, and elevation of provenance origin, and the linear distance (km) from the origin to the Kellogg Forest plantation.

Results

Survival

Kriebel et al. (1988) reported that survival rates in 1984 averaged (± SE) 49% ± 1.25, ranged from 38% (ME:6) to 57% (MI:77, NY:19), but did not differ among provenances (Table 3.1.). In 2000, 35% of the trees originally planted remained alive. Survival among all provenances averaged (± SE) 31 ± 2.73%. Survival rates in 2000 ranged from 16% for the MO:10 provenance to 56% for MI:77 and NY:19 and differed significantly among provenances (F = 1.67; df = 21,501; *P* = 0.0318), Survival rate of the MO:10 provenance was lower than that of MI:77, the local provenance, but other provenances did not differ from MI:77. Between 1984 and 2000, 31% of the trees from MO:10 died. There was no mortality of trees from ME:6, MI:77, and NY:19 between 1984 and 2000, while mortality rates of trees from other provenances during this period ranged from 9% (ME:29, WI:26) to 67% (MO:10) (Table 3.1.).

When we surveyed the plantation in 2018, only 17% of the originally planted trees were alive. Survival in 2018 differed among provenances (F = 2.51; df = 18,440; *P* = 0.0006). None of the trees from the TN:1, MN:2, and MN:20 provenances were alive (Table 3.1.); these three provenances were thus excluded from further analyses. Only 8% of the trees from provenances IA:9 and WI:12 were alive, a rate that was significantly lower than the 40% survival of the MI:77 trees. Between 2000 and 2018, 50% of the trees from six provenances (ME:29, WI:22, ON:24, MN:23, IL:11) died. Other provenances sustained lower mortality rates, ranging from no mortality (ON:21, OH:31) to 30% mortality (WI:26).

Relationships between survival rate and the latitude, longitude, and elevation of the parent population were insignificant for all years except in 2000, when elevation had a significant, positive linear relationship with survival ($R^2 = 0.49$). When a multiple regression model was fitted to predict percent survival of red oak trees in 2018 (Table 3.2.), height was the only term that significantly entered the model ($R^2 = 0.32$). The final model to predict percent survival of red oak trees was:

$$\text{Percent survival} = -0.67224 + 0.88527*\text{height}$$

Tree Diameter and Radial Growth

Kriebel et al. (1988) reported DBH of live trees in the plantation in 1984 averaged (± SE) 14.5 ± 0.44 cm, ranged from 4.3 to 22.6 cm and varied among provenances (F = 4.96; df = 21,226; *P* <0.0001). Trees from the local MI:77 provenance, which averaged 15.2 ± 0.39 cm in DBH, were significantly larger than trees from WI:12, ME:3, and IA:9, which averaged 11.2 ±

0.68 cm, 11.2 ± 0.88 cm and 11.9 ± 0.66 cm, respectively. Trees from MI:17, however, had an average DBH of 18.9 ± 0.46 cm, which was larger than that of the MI:77 trees (Table 3.3., Figure 3.2.a). Trees from WI:12 were significantly smaller than trees from the MI:77 provenance.

In 2000, DBH of live trees in the plantation averaged 21.2 ± 0.81 cm (Table 3.3.). Mean DBH differed among provenances (F = 3.40; df = 21,144; *P* <0.0001), ranging from 14.9 ± 1.9 cm for the AR:5 trees to 29 ± 1.8 cm for trees from MI:17. Trees from the MI:17 provenance, which averaged 39.7 ± 1.34 were significantly larger than the MI:77 trees, which averaged 21.5 ± 1.2 cm. No other significant differences between MI:77 and other provenances were detected. Overall, between 1984 and 2000, DBH of trees in the plantation increased by an average of 52.0 ± 2.47%, ranging from a 29% increase for trees from AR:5 to a 72% increase for trees from OH:31. Trees from the local MI:77 provenance increased by 42%. The five provenances with the greatest radial growth ranging from 63% - 72% (WI:26, MO:10, IL:11, MN:23, OH:31) were from latitudes between 37.7 to 44.8 degrees, which encompassed all but the northern-most (MN:20, MN:22, WI:26, MI:13) and southern-most provenances (AR:5, IL:11, TN:1), and from -93.5 to -73.3 degrees longitude, which excluded three provenances from Maine (ME:3, ME6, ME:29) and the provenance furthest to the west (MN:20). Diameter of trees from several provenances increased by ≥60% between 1984 and 2000, including OH:31, IN:7, IL:11, MO:10, MN:23, and WI:26.

In 2018, DBH of live trees in the plantation averaged 33.4 ± 0.95 cm and differences among provenances were not significant (F = 1.65, df = 16,66; *P* = 0.1081) (Table 3.3.). On average, the IA:9 trees were smallest (27.8 ± 0.99 cm), while the two surviving ME:3 trees had a DBH of 56.1 cm and 29.8 cm (average of 43.0 ± 13.15 cm). Between 2000 and 2018, DBH of the ME:3 trees increased by 187%, while, in comparison, DBH of trees from IL:11 and WI:26 increased by only 32%. Diameter of trees from five provenances increased by ≥60% between 2000 and 2018 (ON:24, ME:29, MI:13, WI:12, and ME:3). These provenances were from latitudes ranging from 35.9° to 46.5° degrees, excluding MN:20, and from longitudes between -93.3° to -83.2°, which excluded provenances in the northeast United States and southern Canada. The MI:77 trees did not have the largest DBH in 2018 nor did they exhibit the highest radial growth. Mean DBH of MI:77 trees in 2018 were slightly above the overall average for the plantation (Figure 3.2.a). In contrast, average diameter growth of trees from ME:3 and OH:31 was more than one standard deviation above the plantation mean (Figure 3.2.a). Diameters of trees from ME:6, IN:7, MI:17, NY:19, and ON:21were also greater than the plantation average.

A multiple regression model ($F = 8.4$; df = 2,15; $P = 0.0104$) was fitted to predict average DBH of provenances using trees measured in 2018. Height ($R^2 = 0.35$) was the only term that significantly entered the model (Table 3.2.). The final model to predict DBH was:

$$\text{DBH} = 19.219 + 0.5284*\text{height}$$

Tree Height

In 1984, trees from two provenances, ON:21 and MI:17, were taller than trees from other provenances, averaging (± SE) (11 ± 0.22 m) and (12 m ± 0.13 m), respectively (Kriebel et al. 1988). Height of the MI:77 trees, which were not reported by Kriebel et al. (1988), averaged 12 ± 0.23 m while the overall plantation mean was 10.64 ± 0.13 m (Table 3.4.).

Height of live trees in 2018 averaged 28.2 ± 0.34 m, ranged from 25.6 m (MN:23) to 31.7 m (IN:7) and did not differ among provenances ($F = 1.70$; df = 18,69; $P = 0.0602$) (Table 3.4.). Trees from ten provenances, particularly IN:7, were taller than the overall average height of trees in the plantation, while average height of trees from six provenances (IA:9, IL:11, WI:22, MN:23, ON:24, WI:26) was lower than the overall mean (Figure 3.2.b). From 1984 to 2018, trees from MI:13 had the greatest increase in height, growing from an average of 9.6 ± 0.81 m in 1984 to 29.8 ± 0.55 m in 2018. Trees from MI:77 had the lowest height growth, increasing from an average of 11.58 ± 0.23 m in 1984 to 26.51 ± 1.01 m in 2018. Average height of MI:77 trees was 1.7 m less than the plantation average in 2018 (Figure 3.2.b). Average height of trees from IN:7 and OH:31 were more than one standard deviation above the plantation mean. On average, trees from provenances ME:3, AR:5, ME:6, MO:10, MI:13, MI:17, NY:19, and ON:21 were also taller than the plantation mean (Figure 3.2.b).

A multiple regression model to predict average tree height measured in 2018 using DBH, survival and variables related to the origin of provenances was significant (F = 6.20; df = 5,15; $P = 0.0109$). Two predictors, including DBH ($R^2 = 0.35$), and percent survival ($R^2 = 0.45$), entered the model (Table 3.2.). The final model was:

$$\text{height} = 3.77 + 0.46279*\text{DBH} + 0.23483*\text{percent alive}$$

Discussion

The plantation at W.K. Kellogg Experimental Forest was originally established to assess variation in growth and survival among populations originating across the native range of red oak and to provide a basis for selecting high vigor seed sources for future planting. When the trees were measured in 1984, geographic origin (latitude) of the provenances was correlated with tree height. Kriebel et al. (1976) also evaluated other red oak plantations established 1962-1964 in Kansas, Nebraska, Illinois, Ohio, and Indiana, which included several of the same provenances established at Kellogg Forest. They reported variation in tree height of 14-year-old trees was more strongly related to stand factors and genetic influences than geographic origins and speculated that trees from southern provenances may have been more drought tolerant.

We originally hypothesized that survival rates would vary over time as provenances encountered challenging weather or site conditions. In 1984, survival rates for the local MI:77 trees were similar to survival rates of other provenances. In 2000, one provenance, MO:10, had significantly lower survival than MI:77 but 20 to 56% of trees from the other provinces were still alive. By 2018, all the trees from three provenances (TN:1, MN:2, MN:20) were dead and trees from IA:9 and WI:12 had significantly lower survival rates than MI:77. It was perhaps not surprising that all the TN:1 trees died, as it was one of the most southern provenances and 695 km from the Kellogg Forest plantation. However, 100% mortality of trees from the high latitude MN:2 and MN:20 provenances, which originated 715 km (MN:2) and 904 km (MN:20) northwest of the plantation, was somewhat unexpected. The origins of the two MN provenances have temperatures similar to or even more extreme than those at Kellogg Forest. For example, mean annual temperatures from 1984 to 2018 in the area encompassing the MN populations ranged from 4 to 6 °C compared to 8 to 10 °C at the Kellogg Forest plantation. During this same time period, average annual minimum temperatures of the MN provenances ranged from -4 to -2 °C, compared to more moderate average minimum temperatures of 3 to 4 °C at the plantation (PRISM Climate Group). Similarly, survival of trees from the Marquette, MI provenance, which also experiences cold winters (average Jan-Feb temperatures of -10 C) (PRISM Climate Group) was only 10%. We hypothesized the local MI:77 provenance would be well adapted to local weather conditions and should exhibit the highest survival. In 2018, however, the survival rate of MI:77 trees was lower than that of MI:17, ON:21 and NY:19 trees.

Differences in original planting numbers and the 2009 thinning likely affected survival in 2000 and 2018 to some extent. Number of seedlings originally planted at Kellogg Forest ranged from eight to 48 trees per provenance (Table 3.1.), so effects of felling the smallest tree from each provenance varied considerably. For example, in 2000, only five of the original 24 trees (21%) from MN:2 were alive while 18 of the original 32 MI:77 trees (56%) were alive. Removing one tree from each provenance in 2009 when the plantation was thinned obviously had a greater effect on the MN:2 survival rate than on the MI:77 survival rate.

We hypothesized that as the trees matured, variability among provenances in tree diameter and height would decrease given that trees experienced the same site and weather conditions. Differences in DBH among provenances were detected in 1984 (Kriebel et al. 1988) and 2000, but not in 2018. Similarly, while height differed among provenances in 1984, there were no differences by 2018, consistent with our hypothesis.

Given the relatively close proximity of the origin of the MI:77 trees to the Kellogg Forest plantation, we expected the MI:77 trees would out-perform trees from more distant provenances in both survival and growth. While MI:77 survival was relatively high, survival rates of other provenances were often as high or even higher. Provenances with higher survival rates than MI:77 were from latitudes between 44.1° to 44.5°, encompassing a small area between Ohio and Michigan's Upper Peninsula. High survival of trees from provenances originating from this region likely reflects adaptation to climate and weather patterns similar to those at Kellogg Forest. However, in light of the 100% mortality of trees originating from the two Minnesota provenances, adaptation to regional climatic conditions may be less important than other factors.

In an Indiana provenance study with 23-year-old white oak (*Quercus alba* L) trees, Huang et al. (2015) reported that height growth of locally sourced provenances was not better than that of distantly sourced provenances, although parameters used to define "local" white oak provenances were not identified. They suggested genetics exerted stronger effects on height growth than geographic distance. Evaluation of a provenance test of 22-year-old black walnut

(*Juglans nigra* L.) in Kansas led to recommendations for sourcing planting stock from 100 – 200 km south of the planting location (Bresnan et al. 1992). Similarly, in a white ash (*Fraxinus americana* L.) provenance planting in Illinois, seven-year-old trees from populations distant to the planting location were taller than trees from nearby populations (Bresnan et al. 1994, Clausen 1984). Kriebel et al. (1976) reported that geographical origins for provenances of 14-year-old red oak had less effect on tree growth and survival than genetics of the parent population. In contrast, the Kellogg Forest data showed that differences in growth diminished over time and at maturity (55-years-old), tree diameter and height were similar among all provenances.

Measurements of the trees in the Kellogg Forest plantation illustrate the importance of retaining provenance plantations for long term monitoring, particularly to assess survival, as well as growth or related variables. Radial and height growth of mature trees is generally limited by resource availability (Köhl & Lotfiomran 2017). Thus, the relatively similar size of the 55-year-old trees growing in the Kellogg Forest plantation is perhaps not surprising. Conversely, differences among provenances in survival were substantial and could be important to consider when selecting stock for planting.

Currently, the northern limit of the red oak range extends from northern Minnesota through Maine and southern Ontario. Under current climatic conditions, provenances from populations originating in Maine, New York, Ontario, Ohio, Indiana, and the lower peninsula of Michigan all had reasonably high survival and growth rates. Projected changes in mean annual temperatures, however, indicate distribution of suitable climatic conditions for red oak are likely to move northward (Prasad et al. 2014). Results from the Kellogg Forest plantation suggest seed (acorns) originating in an area extending from northern Ohio through northern lower Michigan should be relatively well adapted for establishing red oak in the projected northern range of red oak. Populations originating in this area appear likely to survive to maturity although growth rates are more difficult to project. Genetic variation within parent populations was not addressed when the Kellogg Forest plantation was established but is also likely to play a role in determining red oak persistence over time. Ensuring persistence of genetically different parent populations across the current and future range of red oak should provide resilience to changing climatic conditions.

APPENDIX

Table 3.1. Location of parent populations represented in the provenance plantation established in 1962-1963 at the MSU W.K. Kellogg Experimental Forest, Michigan. The local provenance, MI:77, is in bold font.

County/State	Provenance ID	Latitude	Longitude	Elevation (m)	Distance (km) to Kellogg Forest	Total Planted	No. and percentage of trees alive		
							1984	2000	2018
Newton, AR	AR:5	35.9167	-93.2500	610	989	20	8 (40%)	6 (30%)	1 (5%)
Jackson, IL	IL:11	37.5000	-88.4167	165	601	20	10 (50%)	4 (20%)	2 (10%)
Lawrence, IN	IN:7	38.5000	-86.5000	244	441	8	4 (50%)	3 (38%)	1 (13%)
Boone, IA	IA:9	41.6833	-91.3500	314	500	48	4 (8%)	3 (6%)	1 (2%)
Penobscot, ME	ME:3	44.8333	-68.5000	52	1387	20	9 (45%)	6 (30%)	2 (10%)
Cumberland, ME	ME:6	43.5000	-70.5833	90	1197	24	9 (38%)	9 (38%)	3 (13%)
York, ME	ME:29	43.5000	-70.7500	90	1211	20	9 (45%)	9 (45%)	3 (15%)
Marquette, MI	MI:13	46.5000	-87.3333	235	484	20	9 (45%)	6 (30%)	2 (10%)
Missaukee, MI	MI:17	44.2500	-85.3333	420	205	20	11 (55%)	13 (65%)	9 (45%)
Cass, MI	**MI:77**	**42.0117**	**-85.9590**	**880**	**65**	**32**	**18 (56%)**	**18 (56%)**	**13 (41%)**
Carver, MN	MN:2	44.8333	-93.5833	274	715	24	12 (50%)	6 (25%)	0 (0%)
Cass, MN	MN:20	47.3333	-94.5000	396	904	20	10 (50%)	5 (25%)	0 (0%)
Winona , MN	MN:23	44.0833	-92.0833	297	577	36	18 (50%)	12 (33%)	6 (17%)
Dent, MS	MO:10	37.6667	-91.5000	387	741	32	15 (47%)	5 (16%)	2 (6%)
Essex, NY	NY:19	44.1667	-73.3333	100	991	16	9 (56%)	9 (56%)	8 (50%)
Allen, OH	OH:31	40.7500	-84.1667	269	206	8	4 (50%)	3 (38%)	3 (38%)
Anderson, TN	TN:1	36.1667	-84.1667	303	695	20	10 (50%)	4 (20%)	0 (0%)
Vernon, WI	WI:12	43.5833	-90.8333	396	466	48	24 (50%)	15 (31%)	4 (8%)
Pierce, WI	WI:22	45.7500	-92.6667	423	694	35	16 (46%)	14 (40%)	7 (20%)
Oneida, WI	WI:26	45.5833	-89.3333	488	476	20	11 (55%)	10 (50%)	7 (35%)
Simcoe, ON	ON:21	44.5000	-80.0000	183	495	12	6 (50%)	5 (42%)	5 (42%)
Algoma, ON	ON:24	46.2500	-83.1667	190	463	20	10 (50%)	8 (40%)	4 (20%)

Table 3.2. Summary of results from forward stepping multiple regression to predict survival, DBH, and height of northern red oak trees measured in 2018. Potential predictor variables included latitude, longitude, and elevation of origin, distance of origin to the final planting location, survival, 2018 DBH measurements and 2018 height measurements.

Response Variable	**Predictor Variables**	**Estimate**	**R^2**	**F Value**	***P* Value**
Survival	Intercept	0.67224		0.01	0.9347
	Height	0.88527	0.32	7.40	0.0151
Height	Intercept	3.77066		0.29	0.5953
	DBH	0.46279	0.35	8.43	0.0104
	Survival	0.23483	0.45	2.94	0.1070
DBH	Intercept	19.21916		17.79	0.0007
	Height	0.52841	0.35	8.43	0.0104

Table 3.3. Mean (± SE) diameter at breast height (DBH) (cm) of trees from 22 provenances measured in 1984, 2000 and 2018. Dashes indicate all trees had died. Standard errors were not reported when only one live tree remained. An asterisk indicates DBH of trees from that provenance differed from DBH of MI:77, the local provenance (bolded).

	Mean (± SE) DBH (cm)				
Provenance	**1984**		**2000**		**2018**
AR:5	12 ± 0.82		15 ± 1.91		33 ± 0.00
IA:9	12 ± 0.66	*	19 ± 1.14		28 ± 1.00
IL:11	13 ± 0.72		22 ± 2.73		29 ± 4.40
IN:7	16 ± 2.41		25 ± 2.74		40 ± 0.00
ME:29	14 ± 0.88		19 ± 2.31		33 ± 3.19
ME:3	11 ± 0.88	*	15 ± 1.97		43 ± 13.15
ME:6	14 ± 0.76		22 ± 2.17		34 ± 2.11
MI:13	12 ± 1.44		19 ± 3.84		33 ± 2.4.
MI:17	19 ± 0.46	*	29 ± 1.82	*	40 ± 2.37
MI:77	**15 ± 0.39**		**22 ± 1.16**		**33 ± 1.96**
MN:2	13 ± 0.71		20 ± 0.88		-
MN:20	14 ± 0.70		21 ± 0.77		-
MN:23	12 ± 0.82		21 ± 1.31		29 ± 0.92
MO:10	12 ± 0.73		20 ± 2.04		30 ± 3.50
NY:19	17 ± 1.00		25 ± 2.17		34 ± 3.14
OH:31	17 ± 2.42		28 ± 3.53		41 ± 5.27
ON:21	17 ± 1.22		28 ± 3.09		40 ± 6.51
ON:24	13 ± 1.19		19 ± 2.70		31 ± 4.31
TN:1	14 ± 0.88		19 ± 0.55		-
WI:12	11 ± 0.68	*	17 ± 1.63		32 ± 1.12
WI:22	13 ± 0.72		21 ± 1.68		30 ± 2.25
WI:26	14 ± 1.08		23 ± 1.51		31 ± 2.07

Table 3.4. Mean (± SE) height (m) measured in 1984 and 2018 for trees from 22 provenances. Missing values indicate no live trees remained. Standard errors were not reported when only one live tree remained. An asterisk indicates height of trees from that provenance differed from height of MI:77 trees, the local provenance (bolded).

	Mean (± SE) height (m)		
Provenance	**1984**		**2018**
AR:5	10 ± 0.38		28 ± 0.00
IA:9	10 ± 0.32		27 ± 0.71
IL:11	11 ± 0.50		26 ± 2.10
IN:7	11 ± 0.53		32 ± 0.00
ME:29	10 ± 0.45		28 ± 0.78
ME:3	11 ± 0.49		29 ± 0.70
ME:6	11 ± 0.26		30 ± 0.97
MI:13	10 ± 0.81	*	30 ± 0.55
MI:17	12 ± 0.13		29 ± 0.41
MI:77	**12 ± 0.23**		**27 ± 1.01**
MN:2	10 ± 0.23		-
MN:20	10 ± 0.30		-
MN:23	10 ± 0.33	*	26 ± 1.18
MO:10	10 ± 0.32	*	28 ± 1.80
NY:19	11 ± 0.29		28 ± 0.63
OH:31	11 ± 0.55		30 ± 0.62
ON:21	11 ± 0.22		30 ± 0.77
ON:24	10 ± 0.61		26 ± 2.15
TN:1	10 ± 0.31	*	-
WI:12	10 ± 0.37	*	28 ± 0.51
WI:22	10 ± 0.25		27 ± 0.69
WI:26	11 ± 0.39		27 ± 0.90

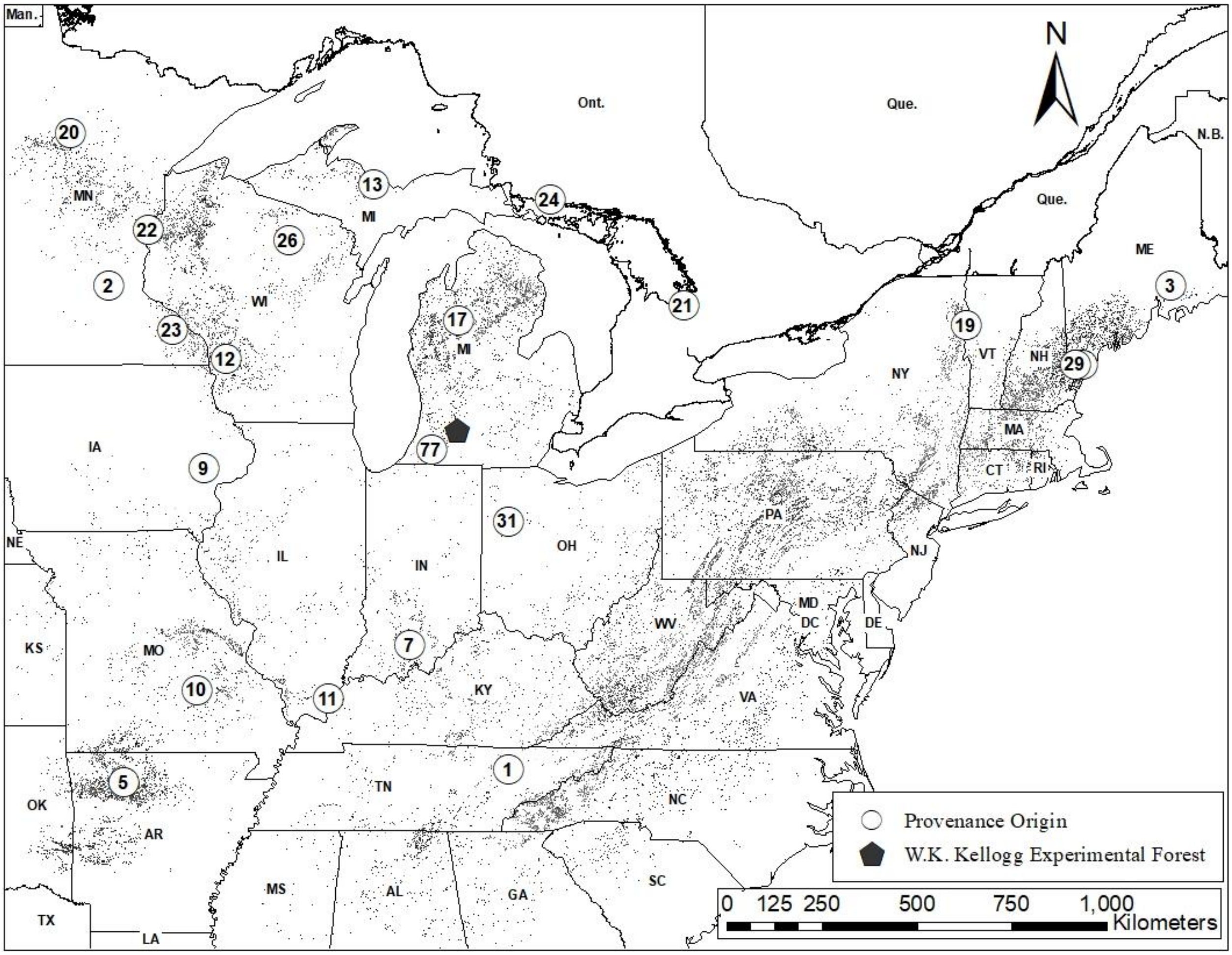

Figure 3.1. Provenance identification number and geographic distribution of parent populations of the 22 red oak provenances established at the Kellogg Forest plantation. Grey shading represents northern red oak (*Quercus rubra*) basal area (m^2 per ha) (USDA 2002). Red oak inventory data for Ontario provenances was not available.

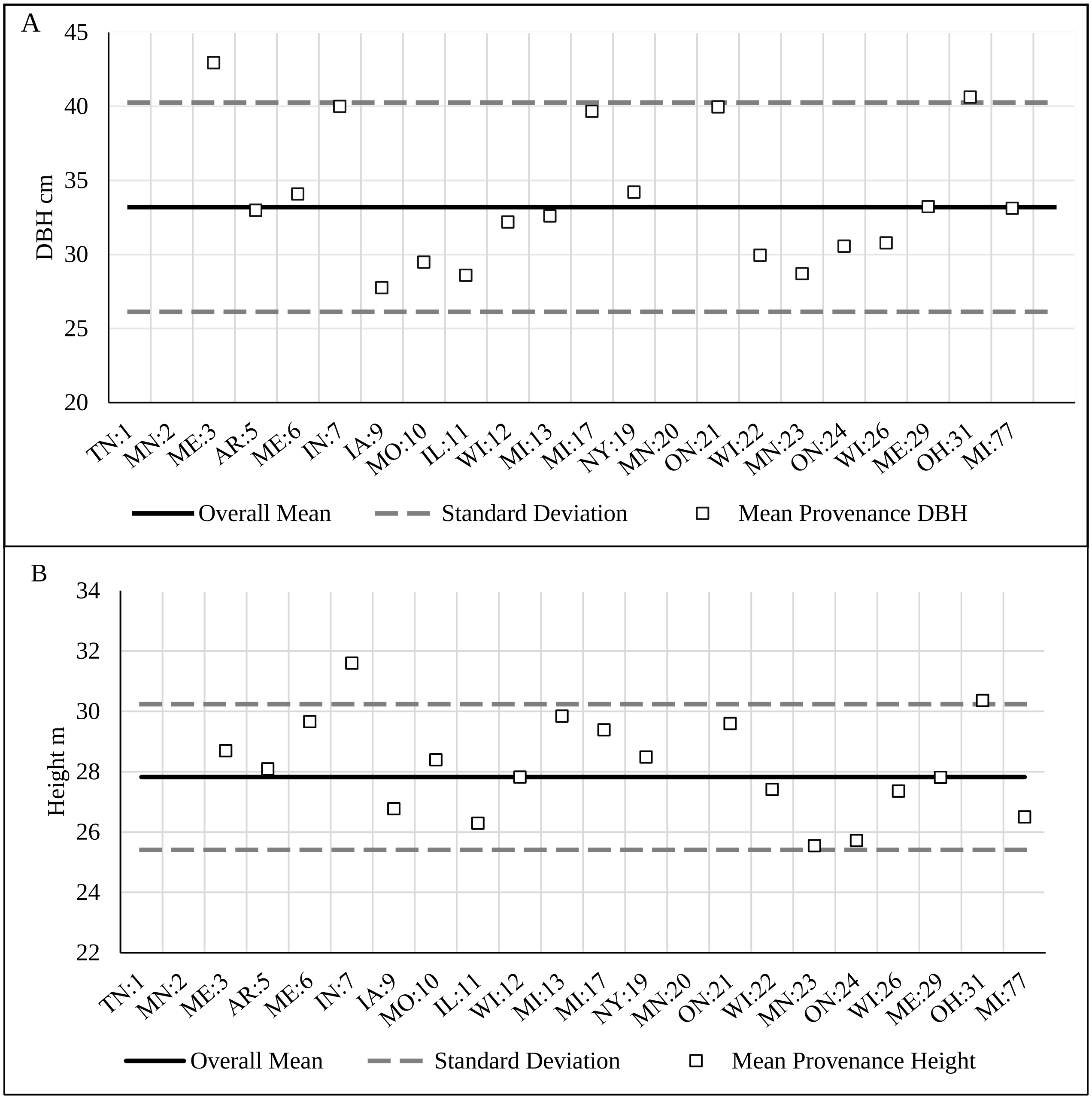

Figure 3.2. Mean (±SE) values for DBH (cm) (A) and height (m) (B) by provenance of trees measured in 2018. Values calculated from 1 to 13 live trees per provenance; all trees from 3 provenances were dead. Solid lines indicate the overall mean for all trees in the plantation (n=88 trees) and dashed lines represent ± 1 standard deviation.

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