ABSTRACT

Title of Thesis:	A GOOD NEIGHBORHOOD TO RAISE A BROOD: TREE SPECIES DIVERSITY DECREASES PERIODICAL CICADA OVIPOSITION AND TREE RESPONSE
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Natural systems contain diverse assemblages of plants, providing a matrix of potential hosts that herbivores must navigate. Insect-plant host choice patterns are crucial to understanding both herbivore outbreaks and the consequences of outbreaks for plant hosts. Here, I follow the 2021 Brood X periodical cicada mass emergence event in the BiodiversiTREE forest diversity experiment at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD, to uncover whether tree diversity influences cicada oviposition preferences or tree responses to oviposition (flagging), for 15 tree species grown in plots of single species or 12-species mixtures. While cicadas demonstrate clear tree species preferences, the diversity of the surrounding tree neighborhood plays at least as important a role in determining oviposition preference and tree flagging responses. Cicadas were threefold more likely to oviposit in trees grown in single species vs. mixed species plots. While overall, I find a concomitant decrease in tree flagging in diverse plots. I also document that species flag at different rates in response to the same oviposition scar density. Even when accounting for differential oviposition rates, surrounding

tree diversity remains an essential additional predictor of tree flagging responses with trees in diverse plots less likely to flag at the same density of scars, suggesting a differential capacity of trees to tolerate damage when growing in single species plots. This study creates a richer understanding of the importance of tree context, specifically surrounding tree diversity, in shaping the ecological ramifications of a mass insect emergence event.

A GOOD NEIGHBORHOOD TO RAISE A BROOD: TREE DIVERSITY DECREASES PERIODICAL CICADA OVIPOSITION AND TREE RESPONSE

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2023

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Table of Contents

List of Tables and Figures

Introduction

The context in which plants live dramatically affects their relationship with insect herbivores. Plant diversity and the identity of neighboring species, i.e., associational effects, can strongly influence an ovipositing female insect's choice of host (Holmes et al., 2023). This has been established in agricultural systems where intercropping and polyculture (high diversity planting) have been used to combat pest insects and encourage beneficial insects (Andow, 1991; Eigenbrode et al., 2016; Letourneau et al., 2011). In natural systems, the directional effects of diversity on herbivory rates are mixed, with evidence of forest diversity both increasing and decreasing herbivory (Barbosa et al., 2009; Haase et al., 2015; Jactel & Brockerhoff, 2007), while other studies found differing effects for different insect species within a single system in which the direction of the diversity effect depends on the method of feeding used by the herbivore (Plath et al., 2012; Sobek et al., 2009).

Despite our general understanding of the importance of diversity in agricultural and natural systems, mechanisms through which associational effects influence plant-insect interactions are variable, often system-specific, and poorly understood in forested systems where the manipulation of diversity is challenging (Underwood et al., 2020). Neighboring tree diversity changes environmental conditions experienced by the trees as they grow and by the herbivores as they seek their hosts in the environment. These associational effects may be a mechanism that alters oviposition decisions (Plath et al., 2012). In particular, studying cyclical outbreaks of forest herbivores can provide a window into how plant diversity alters host choice and thus shapes the impact of the outbreak on plant hosts and their associated communities. Host choice is often related to larval performance, though this pattern has been primarily investigated in oligophagous herbivores such as Lepidoptera (Gripenberg et al., 2010; Menacer et al., 2021). Despite the complexity of navigating a matrix of potential hosts among diverse plant assemblages in natural systems, studies assessing the impact of plant diversity on host choice patterns are rare in forested natural landscapes. In this study, I expand our understanding of these processes by investigating oviposition choice during a mass emergence event of periodical cicadas within a tree diversity experiment.

In North American deciduous forests, periodical cicadas, (Hemiptera: *Magicicada* spp.), emerge en masse, becoming the most abundant arthropod within each brood's particular range during emergence years. During their synchronous emergence, adults select the host trees upon which to lay eggs. Their offspring will feed and develop beneath them for 13 or 17 years. These oviposition choices greatly influence the likelihood of offspring survival (Karban, 1984; Williams & Simon, 1995) and potentially have long-term influences on individual trees and the ecosystems that comprise them (Flory & Mattingly, 2008). Periodical cicada-tree interactions are a model system for observing tree response and ecology during a large-scale mass emergence event. Their synchrony and density make periodical cicadas a great model for host preference in woody tree species that are significant components of temperate North American ecosystems.

The life history and ecology of periodical cicadas

Periodical cicadas have an extraordinary life cycle; North American Magicicada spp. spend 13 or 17 years developing underground on host plant roots before emerging to live as adults for only a few short weeks. Their emergence is highly synchronized, with billions of individuals from multiple species emerging in high densities over a 6-8 week window. Brood X is among the largest of 15 extant cicada broods in North America, one of the twelve 17-year cohorts. It covers 15 states in the mid-Atlantic and Midwest regions of the United States and includes *Magicicada septendecim* Linnaeus, 1758, *M. septendecula* Alexander and Moore, 1962*,* and *M. cassinii* Fisher, 1852 (Simon et al., 2022; Williams & Simon, 1995). Adults of Brood X began appearing in mid-May 2021, and had all but died off by the end of June.

Periodical cicadas lay their eggs in the branches of trees, damaging them in the process. They begin ovipositing approximately 2 weeks after eclosing and mating. During oviposition, female cicadas insert their sclerotized ovipositors into twigs and small branches to deposit a cache of eggs within the outer layers of woody stems. A single female might lay between 20 and 30 eggs per nest, with many nests on the same branch (White & Lloyd, 1981). These linear rows of punctures can damage the vessels and sieve tubes of the xylem and phloem, respectively, adversely affecting the growing tissues distal to the damage. When this damage is extensive, the living distal tissues can wither and die, referred to as "flagging" (White, 1980). Moreover, extensive damage to the branches and stems can compromise their structural integrity, making them susceptible to breakage.

As flagging kills most egg nests distal to the branch breakage, ovipositing females alter their behavior to reduce breakage (White & Lloyd, 1981). As such, only a small proportion of oviposition sites result in flagging despite high numbers of egg nests in a single tree. While personal observation has shown that some tree species flag dramatically more than others during an emergence, it is still being determined if this is due to cicada oviposition preference and, thus scar density or morphological traits that facilitate flagging. Cicada host choice, and subsequent flagging seem to be determined by a complex mixture of tree species identity, tree morphology, and environmental cues.

Both environmental factors and tree-level cues strongly direct cicada oviposition choice of host trees. Cicadas preferentially oviposit in trees that receive high levels of sunlight, which is hypothesized to predict long-term host tree survival and quality for the female cicadas (Yang, 2006). Consequently, cicadas preferentially oviposit in undisturbed edge habitats (Rodenhouse et al., 1997) and trees growing in low density stands (Cook et al., 2001).

While there is no direct evidence, it is possible that the surrounding diversity context of a tree may be an additional predictor of oviposition preference, as diversity and species composition are the environmental factors known to be important for ovipositing insects and/or may make host-finding more difficult. Indeed, there are hints that tree diversity may also play a role in preferences in natural forests, but such work includes the confounding factors of different tree ages and environment-tied diversity differences. Female periodical cicadas favor some tree species and morphologies over others as oviposition sites in a given habitat. They show a preference for native trees and non-native trees with native congeners (Brown & Zuefle, 2009; Mattingly & Flory, 2011), with this trend being driven by tree morphology. Specifically, cicadas prefer ovipositing in thicker (3-7mm) and longer branches than expected based on availability (Flory & Mattingly, 2008). This was true for both native and invasive trees, but since the native trees in the experiment had thicker and longer branches, cicadas oviposited more in native trees (Mattingly & Flory, 2011), providing a potential explanation for cicada preference for native trees. Regardless of origin, trees with more dense and bushy branches (i.e., higher structure index) were more likely to have oviposition scars present, but overall densities of oviposition scars were lower (Brown & Zuefle, 2009). This is likely because bushy branches might impede access and oviposition, and because higher numbers of branches might dilute oviposition density. Conversely, there is strong evidence that cicadas avoid conifers whose sap might suffocate eggs (e.g., red cedar; (Cook et al., 2001; Maier, 1982), plants with corky bark (e.g., *Euonymus*; (Smith & Linderman, 1974),) and trees with extremely hard wood (e.g., Osage orange; (White, 1980).

Tree size, location (edge or interior, sun or shade), presence of other alternative hosts, and density of cicadas can all affect a tree's use for oviposition. A tree favored in one habitat may not be available in another or may be less preferred when near a more attractive alternative. While there have been a few attempts to quantify oviposition preference of *Magicicada* spp. (Brown & Zuefle, 2009; Cook et al., 2001; Mattingly & Flory, 2011), more robust comparisons exploring the surrounding forest context will deepen our understanding of plant-insect interactions.

While the adverse effects of tissue feeding by insect herbivores on plants have been extensively documented (Crawley, 1989; Simms & Rausher, 1987), oviposition can also damage plants, particularly during insect outbreaks. Various species of sawflies, beetles, and true bugs (e.g., leafhoppers and cicadas) deposit eggs directly into plant structures, including twigs, stems, petioles, and leaves, which can damage these plant structures directly and provide a conduit for pathogens (Hilker & Meiners, 2002). While small amounts of oviposition-related damage are unlikely to cause significant impacts on affected plants, some insects, including periodical cicadas and some species of wood-boring beetles (Feller 2002) can inflict substantial damage to the stems of woody plants that serve as nurseries for their developing offspring (Cook & Holt, 2002; White, 1980). Because periodical cicadas emerge in such massive numbers, the branches and twigs of individual trees and shrubs, and in particular those of vigorously growing plants, are often left scarred from hundreds of unique ‘egg nests’ following synchronized oviposition events of 1-3 co-occurring species (Cook & Holt, 2002). At the same time, the flagging that predictably results from each 13- or 17-year cicada brood emergence is often a cause for public concern. The wounds created by cicada oviposition can increase the introduction of pathogens that can cause infections (Ostry & Anderson, 1983). However, studies examining the effects of *Magicicada* spp. oviposition damage on tree growth or reproductive success has been equivocal, with many woody plants exhibiting high tolerance to even extensive damage/flagging (Clay et al., 2009a, 2009b; Cook & Holt, 2002).

Focal questions and experimental approach of the thesis

Here, I explore how neighboring tree species richness influences periodical cicada oviposition and the trees' response to this oviposition using a large-scale 8-year-old tree diversity experiment. This experimental approach isolates the effect of tree stand diversity while controlling for tree age and species. Because the installment of the experiment occurred (1) many years after the last Brood X emergence and (2) in fields previously used for corn agriculture, I can be reasonably sure that all ~18,000 trees in the experiment lacked previous exposure or colonization by cicadas through a previous event. In this study, I specifically ask:

1. Does tree diversity or species identity affect periodical cicada oviposition preference?
2. Does tree diversity or species identity affect tree response (flagging) to periodical cicada damage?
3. Do tree species growing in diverse plots respond differently to similar levels of cicada oviposition?

Overall, I predict trees growing in more diverse plots will experience lower cicada oviposition and damage rates due to associational resistance gained from the presence of diverse neighbors. Further, because trees have different physiological tolerance and resistance traits, I predict that tree species will experience different thresholds for responding to cicada-induced damage by flagging.

Materials and Methods

<u>Study location:</u>

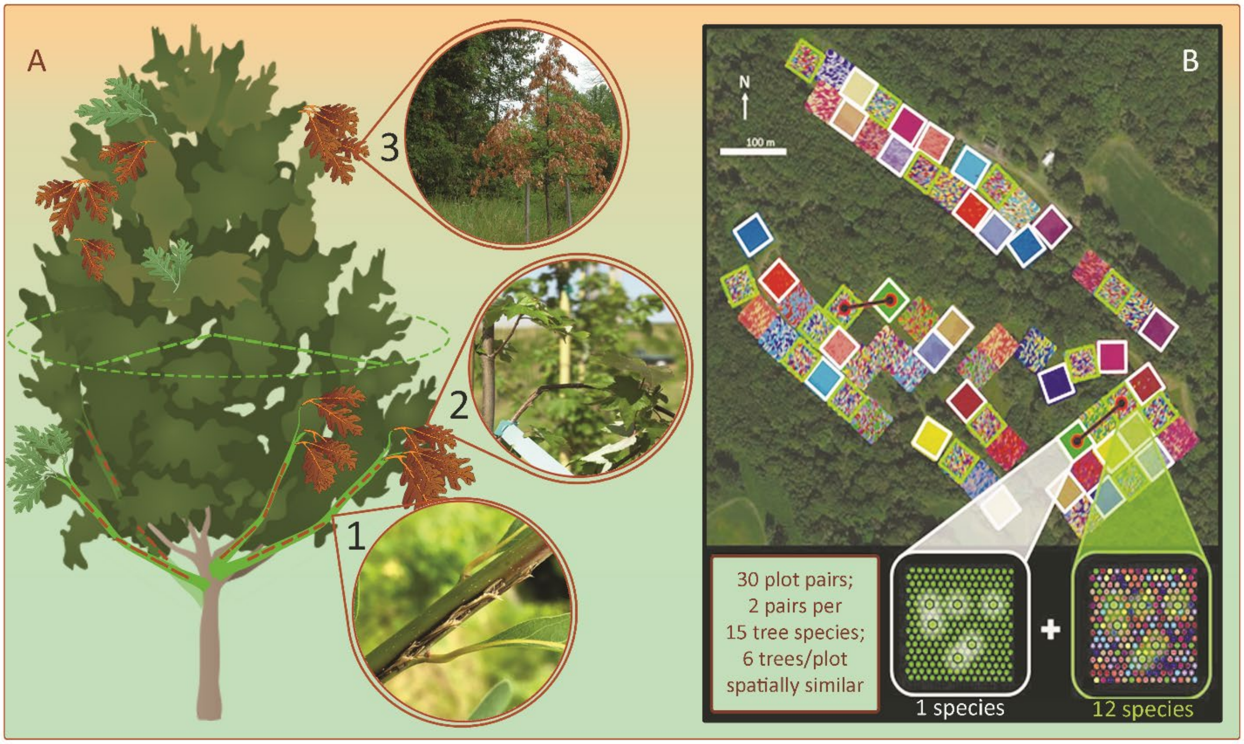

Figure 1: Cicada study site and experimental design at BiodiversiTREE. Using a spatially paired design, I counted cicada oviposition scars (panel A-1), and recorded individual branch flagging (panel A-2), as well as overall canopy flagging (panel A-3) from 360 trees of 15 tree species (panel B). Each single species plot was paired with the nearest and most environmentally similar 12-species mixture containing the focal species. This resulted in 30 sampled plot pairs with six individual trees of the focal species sampled within each plot.

I examined cicada oviposition patterns within the BiodiversiTREE tree diversity and forest restoration experiment at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD, USA. The site was established in 2013 when 17,850 one-year-old saplings from 16 locally abundant native species were planted into a classic diversity-ecosystem function experimental design consisting of 1, 4, and 12 species plots, each 35 m x 35 m, each with 255 trees at the time of planting (Fig. 1b). Extensive soil sampling has revealed similar soil nutrients, bulk density, and moisture across the site, likely owing to minimal changes in topography and a shared agricultural land-use history (Parker, *unpublished data.*). The BiodiversiTREE site is thus an ideal, large-scale common garden experiment to examine how cicada oviposition preference and damage differ by tree species identity and local community tree diversity.

4-letter Code	Tree Species	Common Name
ACRU	*Acer rubrum*	Red maple
CAAL	*Carya alba*	Mockernut hickory
CACA	*Carpinus caroliniana*	Ironwood
COFL	*Cornus florida*	Dogwood
FAGR	*Fagus grandifolia*	Beech
FRPE	*Fraxinus pennsylvanica*	Green ash
LIST2	*Liquidambar styraciflua*	Sweetgum
LITU	*Liriodendron tulipifera*	Tulip poplar
NYSY	*Nyssa sylvatica*	Blackgum
PLOC	*Platanus occidentalis*	Sycamore
QUAL	*Quercus alba*	White oak
QUPA	*Quercus pagoda*	Cherrybark oak
QURU	*Quercus rubra*	Northern red oak
QUVE	*Quercus velutina*	Black oak
ULAM	*Ulmus americana*	American elm

Table 1. Tree species included in the study, by 4 letter abbreviation code, species name and common name.

Experimental Design- paired plot focal tree sampling

I used a targeted focal tree sampling approach that allowed us to partition the effects of tree diversity (single species vs. mixed species) relative to species-specific differences while controlling for variation in other factors (e.g., distance to nearby forests/fields and within-plot spatial arrangement) that might also affect cicada preferences. First, monoculture plots containing one of 15 focal species were paired

with the nearest 12-species polyculture plot containing the same focal species. The distance to field/forest edges and location within-plot matched accordingly. Within each paired plot, six individual trees of the focal species were sampled for cicada egg nest density and flagging responses. Each of the 15 focal tree species had two replicate pairs across the experiment, resulting in 360 focal trees (n=15 tree species x 2 planting diversities (1 vs. 12) x 2 spatial blocks per species x 6 trees per plot = 360 trees). Across the experiment, eight individual trees originally planned for sampling could not be sampled because they died before cicada emergence.

Individual tree measurements

I quantified periodical cicada oviposition and damage on focal trees planted in single- and 12-species plots between July 12th and 15th, 2021. These dates were approximately 2 weeks after the last adults were observed at my site; hence, this sample captures the full extent of Brood X cicada oviposition and damage at this location. I took multiple measurements on each of the focal trees, specifically:

Branch-level oviposition measurements

I counted the number of individual oviposition scars (egg nests) on one branch per tree quadrant (northeast, southeast, southwest, and northwest) for a total of four branches per tree. I noted if no branch occurred in a quadrant, and no branch was sampled. Branches were selected by standing in each quadrant and sampling the highest reachable limb. Oviposition scars were counted along the main stem of each branch from the trunk distally to the tip of the branch; secondary branches were disregarded unless split evenly, in which case the observer asked the notetaker to randomly select a direction (left or right) to follow. Using calipers, I measured the branch diameter to the nearest 0.1mm at the position of the most proximal scar and the most distal scar. I recorded the linear distance between them to the nearest centimeter. To measure branch girdling, I recorded whether oviposition scars occurred in a branch cross-section in 1 quadrant, 2 adjacent, 2 opposite, 3- or all 4 quadrants. On small trees, I also recorded scar density measurements on the main trunk if present, as this damage can contribute to flagging on distal branches even if there are no oviposition scars on the branches themselves. The raw number of oviposition scars per branch were standardized by the average branch length of an individual tree to generate a scar density metric (scars per cm) that helped account for the different tree sizes across species.

Damage (Flagging) estimation

I visually estimated overall branch flagging as a percent of leaves flagged out of the total tree canopy. Two observers independently assessed the percent of damage in the tree canopy from 0-100% in 5% increments(Robinson et al., 2023). If their assessments differed by more than five percent, the average of the two estimates was recorded. For a small subset of trees, no substantive branches were available in any quadrant to assess branch-level oviposition. In this case, I recorded any oviposition on the main trunk/stem, but these trees were excluded from the branch-level oviposition analysis to allow consistent comparisons across species. However, these trees were included in the tree-level damage (flagging) analysis.

Tree size metrics

I used data from the BiodiversiTree trophic tree annual tree size census for average branch length, canopy volume, and tree height. These data are collected in the late winter dormant period to capture the full extent of the previous growing season. The majority of yearly determinant branch growth was complete by the time I measured cicada damage in summer 2021, so the late winter 2022 dormant season data best represents tree branch radius and size at the time of data collection.

Statistical Analysis

I performed all analyses in R version 4.2.3 (R Core Team, 2023).

Cicada Oviposition (Q1) and Tree Damage (Q2) Models:

I built a series of linear mixed-effects models using the lmer() command in the lme4 package (Bates et al., 2015). I log-transformed the response variables of scar density and overall percent flagging to ensure a normal distribution of model residuals. For each model, I used the anova() function in the lmerTest package (Kuznetsova et al., 2017) to perform F-tests using Type III Sums of Squares with Satterthwaite's method to calculate degrees of freedom. I built a similar model for both Q1 and Q2 response variables with fixed effects of tree species identity, plot diversity, and their interaction. I included a random intercept to account for the paired plot design with tree species identity nested within the replicate block. The complete model specification is *response variable ~ plot diversity + tree species + plot diversity× tree species + (1 | replicate block/tree species)*. If the fixed effect of tree species or an interaction including tree species was a significant predictor, I used the emmeans package (Searle et al., 1980) to calculate the estimated marginal mean for each species and the pairs command to complete pair-wise post-hoc Tukey tests between species or within significant interactions to account for unplanned multiple comparisons.

Tree Species Flagging Response to Scar Density (Q3) Model

Next, to examine how different tree species responded to similar scar densities, I used a generalized linear binomial model built for proportion data (family=log-link) using the glm()command in the stats package in base R. The weights argument accounted for the number of branches available for sampling on each tree. I used the anova() command to perform likelihood ratio tests using the chi-square distribution to test the significance of each fixed effect. The complete model specification is *proportion of branches flagged ~ scar density +tree species +plot diversity* weighted by the number of branches examined on the tree. For the model for Q3 only, convergence issues prevented the examination of interactions between fixed effects or the inclusion of random effects. One species, *Acer rubrum,* was included in the total flagging response analysis. However, as no branches fulfilling protocol requirements were available to sample within one of the diversity treatments, this species was not included in the scar density or individual branch response analysis.

Results

General results

In total, I sampled 351 individual trees for cicada oviposition and damage. Cicadas oviposited on all 15 tree species sampled in the experiment. Similarly, all tree species exhibited some damage as indicated by a tree branch flagging response. Total scars per branch ranged from zero to 195. A dogwood tree, *Cornus florida,* had the most scars on one branch 195. Mean scars per branch for an individual tree ranged from zero to 118. A sycamore tree, *Platanus occidentalis*, had the highest mean scars per branch sampled, with 118. I found that scar densities were at a maximum of 3.53 scars per cm. The total flagging for trees ranged from no flagging to a maximum of 70% of total branches flagged. The proximal diameter of the branches sampled ranged from 2.4-15.6mm, while the distal diameter ranged from 1.2-10.5mm.

Q1- Cicada oviposition preference

I found lower oviposition scar density on trees growing in 12 species plots (0.094 ± 0.22 mean scars/cm ± SE) compared to the cohort of trees growing in single species plots (0.26 ± 0.033mean scars/cm ±SE), ($F_{1, 271}$ =52.52, p<0.001). Oviposition scar density varied across tree species ($F_{13, 13.4}$ = 3.19, p = 0.02). While the overall effect of plot diversity was to decrease oviposition, a significant interaction between tree species and plot diversity indicates that the degree and direction of the impact of plot diversity differs across species ($F_{13, 268}$ = 4.62, p = 0.001). Higher plot tree diversity decreased oviposition in *Quercus alba* (*t*(265)= 3.401, *p*<0.001)*, Cornus florida* (*t*(266) = 5.375, *p*<0.001)*, Quercus velutina* (*t*(265) = 3.683, *p*<0.001)*, Fagus grandifolia* (t(266) = 4.339, *p*<0.001)*, Quercus rubra* (t(265) = 2.281, *p*=0.0233)*, Liquidambar styraciflua* (*t*(275) = 3.545, *p*<0.001), and *Ulmus americana* (*t*(271) = 3.308, *p*=0.001). I found weak evidence to support the opposite trend in *Quercus pagoda,* which had increased oviposition in 12-species plots (*t*(266) = -1.869, *p* = 0.062).

Q2-Cumulative Tree Flagging Response

Similarly, I found a lower incidence of flagging on trees grown in diverse 12-species plots (1.28± 0.33% flagging ± SE) versus single-species plots (6.92 ± 1.04%

flagging ± SE) ($F_{1,\,311}$ =59.79, p<0.001). Total flagging differed across tree species ($F_{14,\,14}$ = 3.60, p=0.01). I also found that the interaction between tree species and plot diversity influenced flagging ($F_{14,\,298}$ =6.86, p<0.001). Specifically, diversity has a negative effect on flagging for many species such as *Quercus alba* ($t(300) = 5.874$, $p<0.001$), *Cornus florida* ($t(301) = 6.666$, $p<0.001$), *Quercus velutina* ($t(300) = 4.366$, $p<0.001$), *Fagus grandifolia* ($t(300) = 5.912$, $p<0.001$), *Quercus rubra* ($t(300) = 3.094$, $p=0.002$), and *Liquidambar styraciflua* ($t(300) = 2.295$, $p=0.022$) but not for all species. *Quercus pagoda* again drove the interaction between diversity and species identity with significantly increased flagging when growing in 12 species plots ($t(300) = 2.523$, $p=0.012$).

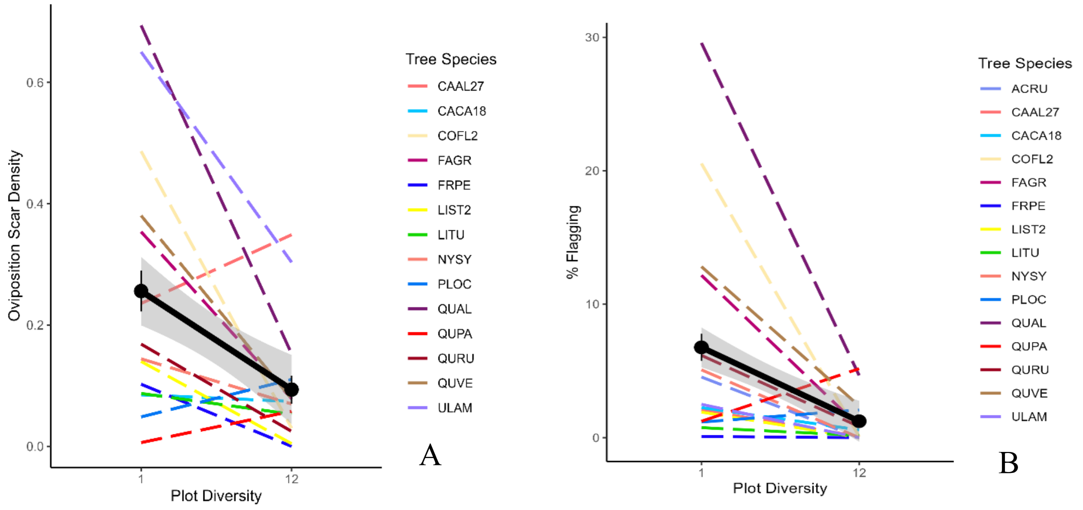

Figure 2: Trees growing in diverse plots with 12 tree species generally had fewer oviposition scars per cm of branch tissue (A) and lower total tree flagging percentages (B).

Q3: Do tree species respond differently to the same level of oviposition

As expected, scar density is a strong predictor of flagging (χ^2 =73.35; p< 0.001). When controlling for scar density and plot diversity, I found that tree species responded differently to cicada oviposition (χ^2 = 83.53, p<0.001). Some species were more sensitive to cicada oviposition (i.e., more likely to flag at low scar densities), and others less likely to flag even at high scar densities (Figs. 3, S5). For example, post-hoc pairwise comparisons between species indicate higher sensitivity of *Quercus alb*a compared to both *Ulmus americana* (z = 4.003, p<0.05) and *Fraxinus pennsylvanicus* (z=-3.518, p<0.05). I also found weak evidence that *Q. alba* was more sensitive than *Q. rubra* (z=3.246, p=0.07), *Cornus florida* (z=-3.247, p=0.07), and *Carpinus caroliniana* (z=-3.230, p=0.08). *Ulmus americana* was also less sensitive than *Platanus occidentalis* (z= 3.318, p=0.04), *Quercus velutina* (z=3.215, *p* = 0.06), and *Cornus florida* (z= -3.155, p=0.07). Notably, across all species, trees grown in polyculture flagged less often at the same scar density than trees growing in monocultures (χ^2 =21.43; p < 0.001).

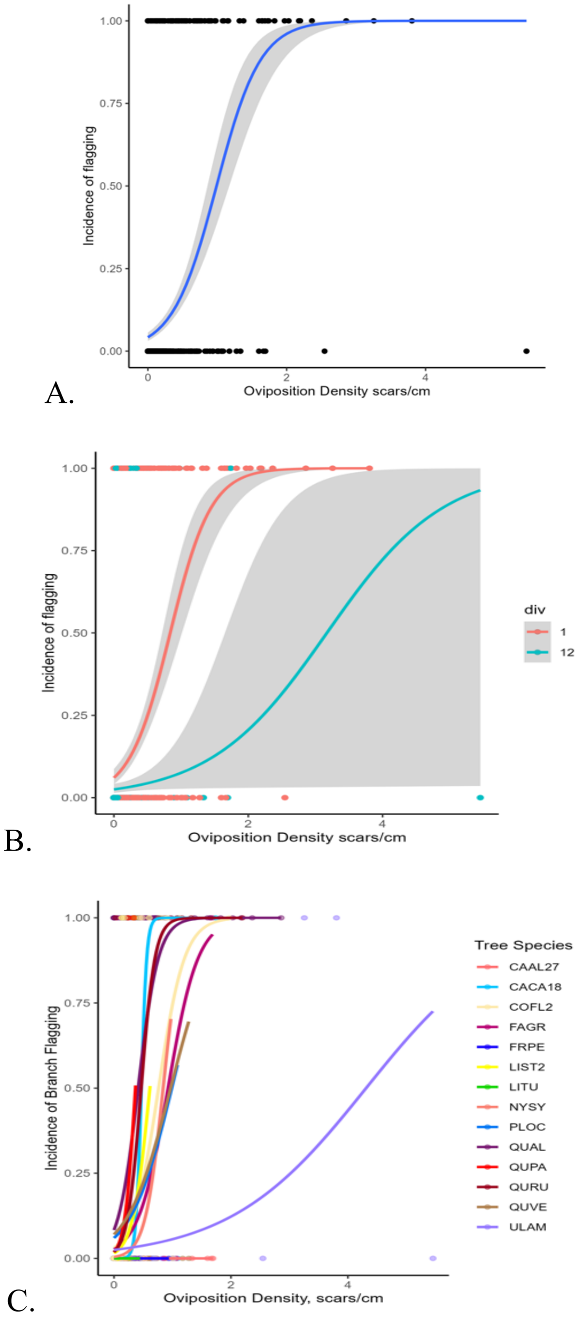

Figure 3: Generally, higher oviposition scar density increases the chance of a measured branch flagging on a tree (A). When a tree grows in a diverse plot, it is less likely to flag even at high oviposition density (B). Overall, tree species also differed in the sensitivity of their flagging response (C), with some species, such as *Carya alba* (CAAL17), *Fraxinus pennsylvanica* (FRPE), *Ulmus americana* (ULAM)*,* and *Liriodendron tulipifera* (LITU) much less likely to flag than other species with the same oviposition density of scars.

Discussion

By documenting the oviposition choices of brood X cicadas in a large-scale tree diversity experiment during a mass emergence event, I demonstrate that the richness of neighboring tree species shapes both insect preference and tree response in this plant-insect interaction. Twelve-species plots had 64% lower cicada oviposition in trees in diverse plots compared to trees growing in single-species stands (Fig. 2A). This decrease in overall oviposition was coupled with 81% lower overall canopy branch flagging for trees in diverse plots (Fig. 2B). While on average, I found strong overall negative effects of diversity across species, I also documented a significant interaction between tree species and plot diversity for both oviposition density and flagging (Fig. 2A&B), indicating variation in the strength or direction of the diversity effect across tree species. I also found evidence that cicadas prefer ovipositing in certain tree species over others (Fig. 2). These patterns are similar when analyzed using scar density, mean scars per branch, and the raw count of oviposition sites per branch. However, I found that cicada oviposition preference may be decoupled from the trees' flagging response, with tree species responding differently to cicada oviposition at similar densities (Fig. 3C). This suggests that cicada preference studies that rely on tree flagging to estimate preference may misstate preference patterns that will later correlate to below-ground feeding by cicada nymphs in subsequent years. Lastly, the branches of trees in diverse plots that received the same density of cicada oviposition scars as those in single species plots were less likely to exhibit a flagging response where the branch wilted and died at the tip (Fig. 3B). Overall, my study is a unique demonstration that the context of species diversity shapes both the host choice preferences of an iconic insect and the response of plants to oviposition by these insects.

There are multiple, non-mutually exclusive, potential explanations for the striking preference of periodical cicadas to oviposit on trees growing in single species rather than diverse plots. First, while not able to be directly evaluated with this study, it may be advantageous to the future growth of cicada offspring for the female to lay eggs near a clump of preferred hosts (i.e., in a single species plot) as it is more likely the offspring will feed on the roots of the same species in which they were oviposited after dropping to the group beneath the tree (Gripenberg et al., 2010). Secondly, there may be increased plant apparency or host-finding of preferred species by cicadas in single species plots. These plots contain 255 individual trees of the same species and may lead to aggregations of cicada adults that are less likely to occur in diverse plots with only 21 individuals of the same species interspersed in a plot. Similarly, plant apparency may be lower in multi-species plots due to variation in plant structure and microclimate. While Feeny’s apparency hypothesis (Feeny, 1976) was initially formulated to explain increased quantitative plant defenses in mature large trees that were “bound to be found” by herbivores compared to rare small species, the concept may also prove helpful to explain generalist cicada preferences within this study.

Specifically, while my results confirm other reports in the literature (Brown & Zuefle, 2009; Cook & Holt, 2002; Dybas & Lloyd, 1974; Flory & Mattingly, 2008; Maier, 1982) that cicadas are broadly generalized in oviposition choice by utilizing all 15 tree species I examined, there was a clear preference to oviposit in certain species over others (Figure 2A). In fact, faster-growing species in the experiment (sycamore-*Platanus occidentalis,* tulip tree-*Liriodendron tulipifera,* cherrybark oak-*Quercus pagoda,* and sweetgum-*Liquidambar styraciflua*) were relatively less preferred for oviposition than shorter, slower-growing species such as white oak, *Quercus alba,* and American elm, *Ulmus americana*. At the same time, cicadas oviposited on trees spanning a large range of tree heights in the study from approximately 0.3 to over 14 m. I recorded cicada oviposition on trees as small as 32cm in single-species red maple *Acer rubrum* plots, in which the trees are small enough to be surrounded by herbaceous plants and tall grasses that cicadas must navigate through to reach viable branches in which to lay eggs. As these small trees are blocked from aerial view by other plants, it implies that the host-finding of female cicadas likely includes cues other than just light and visual signals. It may also indicate that these signals are amplified when many trees of the same species are growing in close proximity. Conversely, these cues could be dampened in diverse forests. For example, I found no *Acer rubrum* with oviposition scars in 12-species plots.

My results suggest that these faster-growing, less preferred species may obscure more preferred hosts in a diverse plot. Specifically, trees grown in single-species plots tend to have similar height, spread, and general structure across a plot (Schmitt, unpublished data). In contrast, those growing in 12-species plots varied in these aspects from each other and their neighbors of different species, creating more canopy rugosity in diverse plots that adult cicadas must navigate (Parker, unpublished data). Thus, factors such as light, phytochemicals, and temperature may be more consistent in single-species plots, facilitating the location of preferred species. In contrast, these potential host-finding signals could be disrupted in diverse plots, making it more difficult for female cicadas to locate their host of choice (Root, 1973; Stephens & Myers, 2012).

Overall, this negative effect of diversity on oviposition broadly aligns with associational resistance being conferred by non-preferred hosts in diverse plots (Barbosa et al., 2009). For example, *Quercus alba, Cornus florida,* and *Ulmus americana* experience high rates of oviposition on average overall and also have the steepest decrease in oviposition from 1- to 12-species plots (Fig. 2A). Therefore, my results suggest that growing in a mixture of other species may offer some resistance for preferred tree species. However, the interaction I document between tree species and plot diversity also provides evidence for the associational susceptibility of some non-preferred species in diverse plots. Here, I see the overall diversity effect reversed for one of the 15 individual tree species, *Quercus pagoda* (Figs. 2, A and B). While *Quercus pagoda* is a less preferred host species overall than average, when growing in diverse plots, it has higher oviposition rates than conspecifics in single species plots (Figure 2A). Notably, this also coincides with more flagging in diverse plots. At the same time, I document some evidence of increasing associational susceptibility for less preferred species.

As expected, the pattern of higher oviposition density on trees in single species plots was also reflected in higher total tree flagging percentage in single species plots. I found damage ranging from 0-70% of tree branches flagged. While this may damage or cause decreased growth in young or small trees if the flagging is extensive (Smith & Linderman, 1974), periodical cicadas do not typically cause tree death or long-term effects on tree health (Cook & Holt, 2002; Yang & Karban, 2019) in established trees. The flagging response has even been hypothesized to positively affect plant health through self-pruning (Cook & Holt, 2002) or as an adaptive response decreasing future root damage (Perkovich & Ward, 2022; Yang & Karban, 2019). For the latter, a robust flagging response could negatively affect cicada offspring if dead and dying branches cause eggs to dry out before hatching (White & Lloyd, 1981). Thus, tree response could offer some protective effect to the tree in addition to suggesting removal of plant tissue. It is beyond the scope of the current study to evaluate these hypotheses, although it would be possible to do so by revisiting trees over time and assessing growth rates.

My study documents that trees of the same species tend to flag more easily (i.e., are more sensitive to oviposition damage) in single-species plots compared to trees growing in 12-species plots. When assessing the likelihood of a branch to flag based on the oviposition density, I see a distinct increase in flagging at a lower density in single species plots versus 12 species plots (Fig 3B). While a surprising result, it is possible that trees in diverse plots can draw on different resources (light, nutrients etc.) to shape their response to oviposition damage compared to those going in single species plots. Trees in diverse forest stands experience spatial and temporal resource partitioning above-and below ground, which confers advantages during adverse events and/or conditions (Grossiord, 2020; Hooper, 1998). Additionally, other work within BiodiversiTree has documented differences in tree shape across treatments that may correspond to different branch widths and, thus alter the tendency of a branch to flag (Schmitt unpublished). These differences in architecture might make it more likely that a larger number of branch quadrants were damaged, increasing the chance that an individual branch is girdled by oviposition, cutting of the vascular flow of materials down the branch. Overall, this suggests that forest diversity matters in terms of trees' ability to tolerate large-scale insect emergence events.

Implications for forest conservation, restoration, and management

These results have clear implications for human-managed forests and planting efforts. All the trees in this study were 8 years old and are established in this site, Younger, more recently planted trees may not yet have established energy reserves, and are thus are at a higher risk of injury or death from oviposition damage during. Planting a single-age in-ground tree nursery or forest plantation crop, or initiating a forest restoration project in the year before an emergence event could put trees at risk for higher damage and potential mortality. Urban and plantation plantings are also often done on a large scale but with a single uniform species, which is often considered more aesthetically pleasing (for street and park trees) and convenient (for plantations). My results suggest that one way to mitigate this risk is by planting trees in diverse assemblages in these settings rather than single species planting often favored by developers and forest plantation owners. Homeowners and urban foresters could avoid also avoid planting lone specimen trees in the year before an emergence, which could be vulnerable to attack, especially if it is among the species that cicadas prefer.

Major cicada emergence events occur on long-time scales from a human perspective but not from the perspective of a tree's lifespan. For example, oaks typically live 75-150 years and likely experience many cicada emergence events and subsequent underground root-feeding by cicada nymphs across their lifetimes. While my study did not find any mortality in 9-year-old trees a year after the cicada emergence event, the dynamics and oviposition preferences I document likely do have effects on the spatial distribution of resources through resource pulse events (Setälä et al., 2022; Yang, 2013) and many ramify out to affect the populations of many wildlife species in area forests (Getman-Pickering et al., 2023).

Appendix

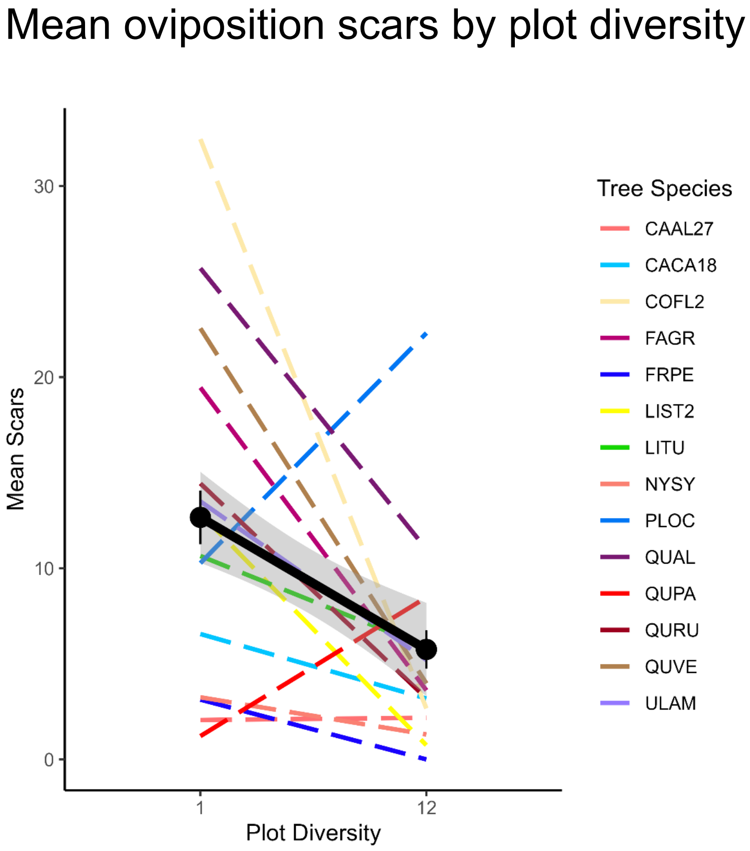

Figure S4. Trees growing in diverse plots with 12 tree species generally had lower mean oviposition scars.

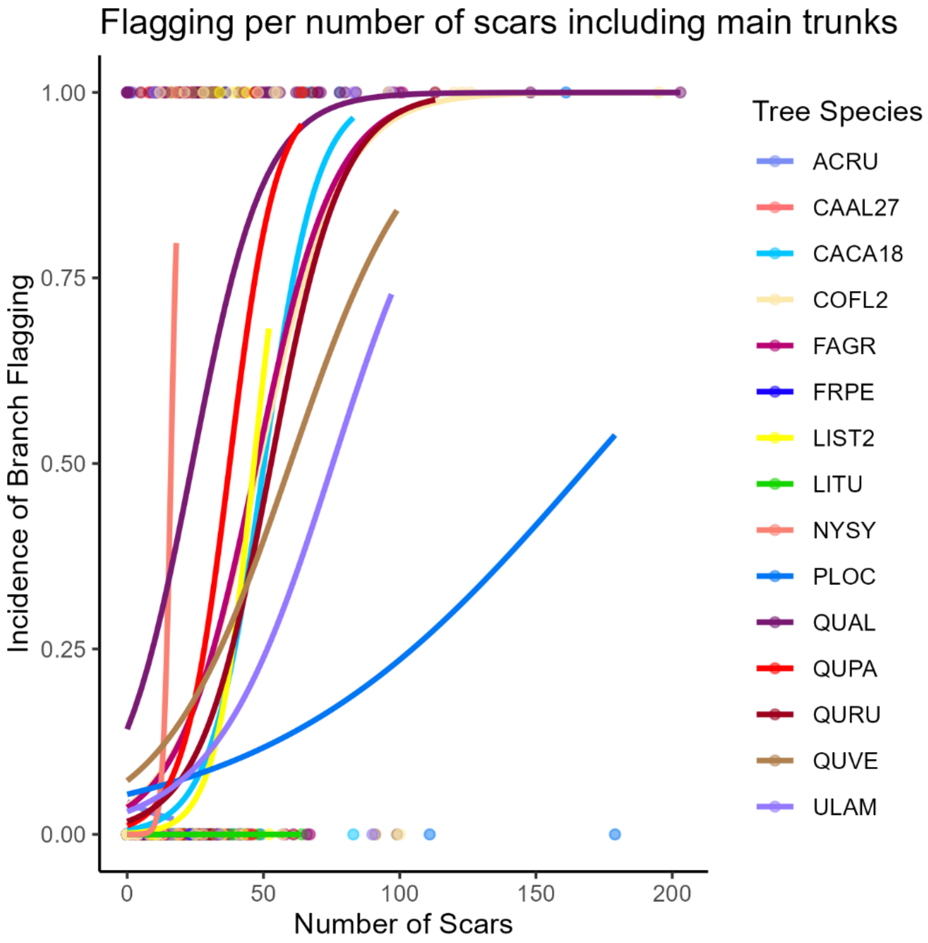

Figure S5. In general, more oviposition increases the chance of a measured branch flagging. However, tree species differed in the sensitivity of their flagging response.

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