

# Insect herbivores increase mortality and reduce tree seedling growth of some species in temperate forest canopy gaps

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Insect herbivores help maintain forest diversity through selective predation on seedlings of vulnerable tree species. Although the role of natural enemies has been well-studied in tropical systems, relatively few studies have experimentally manipulated insect abundance in temperate forests and tracked impacts over multiple years. We conducted a three-year experiment (2012-2014) deterring insect herbivores from seedlings in new treefall gaps in deciduous hardwood forests in Maryland. During this study, we tracked recruitment of all tree seedlings, as well as survivorship and growth of 889 individual seedlings from five tree species: *Acer rubrum*, *Fagus grandifolia*, *Fraxinus* spp., *Liriodendron tulipifera*, and *Liquidambar styraciflua*. Insect herbivores had little effect on recruitment of any tree species, although there was a weak indication that recruitment of *A. rubrum* was higher in the presence of herbivores. Insect herbivores reduced survivorship of *L. tulipifera*, but had no significant effects on *A. rubrum*, *Fraxinus* spp., *F. grandifolia*, or *L. styraciflua*. Additionally, insects reduced growth rates of early pioneer species *A. rubrum*, *L. tulipifera*, and *L. styraciflua*, but had little effect on more shade-tolerant species *F. grandifolia* and *Fraxinus* spp. Overall, by negatively impacting growth and survivorship of early pioneer species, forest insects may play an important but relatively cryptic role in forest gap dynamics, with potentially interesting impacts on the overall maintenance of diversity.

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2 **of some species in temperate forest canopy gaps**

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## 21 **Introduction**

22           Insect herbivores can directly and indirectly influence plant community composition by  
23 altering the recruitment, mortality, or individual growth rates of plant species (Maron and Crone  
24 2006; Kim et al. 2013). In old fields, for example, insect herbivores indirectly increase the cover  
25 of subdominant plant species by reducing the growth of competitively superior species (Brown  
26 and Gange 1992; Davidson 1993; La Pierre et al. 2015). In tropical forests, insect herbivores  
27 increase the diversity of tree seedling recruits (Dyer et al. 2010; Swamy and Terborgh 2010;  
28 Terborgh 2012; Bagchi et al. 2014). In temperate forests, however, the most well-documented  
29 and obvious impacts of insects come from pest species outbreaks, like mountain pine beetles,  
30 which cause widespread mortality of dominant tree species (Romme et al. 1986). However, we  
31 know relatively little about the role of insects in temperate forests during non-outbreak scenarios  
32 (Maron and Crone 2006), because there have been few experimental manipulations of non-  
33 outbreaking insect herbivores in temperate forests.

34           The first pathway by which herbivorous insects might influence forest dynamics is  
35 altered germination and emergence (hereafter termed recruitment). Unfortunately, few data  
36 exist regarding the effects of herbivores on seedling emergence. Recruitment is suppressed by  
37 herbivores for many tree species via predation on newly germinated seedlings (Meiners et al.  
38 2000; Dulamsuren et al. 2008). Reduced germination, caused by insect herbivores, can alter  
39 forest successional trajectories over long time scales by modifying initial community composition  
40 (Bagchi et al. 2014). Indeed, fast-growing pioneer species are often the most susceptible to  
41 herbivory (Coley et al. 1985). Insect herbivores can also increase spatial heterogeneity in  
42 species composition by restricting the locations in which particular species can establish (Fine  
43 et al. 2004; Bagchi et al. 2014). Here, we hypothesized that insect herbivores will reduce  
44 recruitment of palatable, fast-growing tree species, potentially increasing community diverse  
45 over longer time scales.

46 Insect herbivores can also influence forest dynamics via mortality of emerged and  
47 established seedlings. Norghauer & Newbery (2013, 2014), for example, demonstrated that  
48 herbivorous insects significantly alter forest community composition through selective predation  
49 on particular seedling species. Such selective predation can allow for coexistence of  
50 competitively inferior seedlings by reducing growth rates of competitively superior species (Fine  
51 et al. 2004; Fine et al. 2006). This mechanism is thought to be at least partly responsible for the  
52 maintenance of forest diversity (Swamy and Terborgh 2010; Terborgh 2012; Bagchi et al. 2014).  
53 Insect herbivores can also cause immediate plant death, tripling normal seedling mortality rates  
54 (Prittinen et al. 2003), whereas in other cases insect-caused seedling mortality may occur up to  
55 several years following initial defoliation (Eichhorn et al. 2010). Similarly, chronic foliar damage  
56 can exacerbate competition-induced mortality among seedlings (Meiners and Handel 2000),  
57 highlighting the need for relatively long-term studies of insect impacts on tree seedling  
58 dynamics. We hypothesized that early pioneer species, being the most palatable, would suffer  
59 the highest mortality rates of common species in a temperate forest.

60 Finally, herbivores reduce growth of numerous tree species. In particular, fast-growing,  
61 palatable species suffer the greatest reduction in growth following herbivory (Fine et al. 2004);  
62 whereas less-palatable, slow growing species are often more tolerant of herbivory (Siemann  
63 and Rogers 2003). Indeed, herbivores can equalize growth rates among species that vary in  
64 competitive ability (Norghauer and Neberry 2013) and subdominant species often only persist in  
65 communities with insect herbivores (Brown and Gange 1992; Davidson 1993; La Pierre et al.  
66 2015). Herbivory can, therefore, restrict species to particular habitats or soil types, ultimately  
67 increasing beta diversity in tropical forests (Fine et al. 2004, 2006). We tested the hypothesis  
68 that insect herbivores would reduce growth of a subset of early successional, pioneer species  
69 whereas slower growing, late successional species would be more tolerant of insect herbivory.

70 The effects of insects on tree seedlings might be particularly strong within treefall gaps.  
71 Treefall gaps maintain forest diversity by increasing light availability and nitrogen mineralization

72 rates (Denslow 1987; Mladenoff 1987; Hubbell et al. 1999), leading to abundant germination  
73 and recruitment of a diverse assemblage of tree species (Denslow 1987). In addition, insect  
74 herbivores are attracted to the warmth and light in treefall gaps, increasing local abundances  
75 and thus their potential impacts on seedling growth and mortality (Richards and Windsor 2007;  
76 Norghauer and Newbery 2013). Moreover, early successional plant species are generally  
77 thought to possess high growth rates at the expense of anti-herbivore defenses, which might  
78 make them particularly susceptible to insect herbivory (Coley et al. 1985; Shure and Wilson  
79 1993). Thus, recruitment in forest gaps may be partially mediated by insect herbivore  
80 consumption of palatable new seedlings, but little information exists regarding the role of insect  
81 herbivores in forest regeneration in treefall gaps.

82         Here, we conducted a three-year experiment to assess the influence of insect herbivores  
83 on community dynamics within treefall gaps in a temperate forest in the eastern United States.  
84 We used a paired design, where each treefall gap consisted of two plots: a control plot and a  
85 plot sprayed with pesticide to remove insect herbivores. We then tracked seedling recruitment,  
86 mortality, and growth in each plot for three years.

87

## 88 **Materials and Methods**

89         We conducted this experiment in an intensively studied forest at the Smithsonian  
90 Environmental Research Center (SERC; Edgewater, Maryland USA. 38°53' N, 76°33'W). SERC  
91 comprises a 2650 acre protected research facility encompassing a variety of early-, mid-, and  
92 late-successional forests. Most forests at SERC, including those in this study, are mid-  
93 successional forests 75-120 years old that have been federally protected since the 1960s  
94 (Parker et al. 2010). This forest was typical of those in the mid-Atlantic United States, dominated  
95 by *Carya* spp., *Fagus grandifolia*, *Fraxinus* spp., *Liquidambar styraciflua*, *Liriodendron tulipifera*,  
96 and *Quercus* spp (Brush et al. 1980; McMahon et al. 2012). Insect communities in hardwood

97 forests of North America are dominated by only a few groups of insects: diptera, hymenoptera,  
98 homoptera, coleoptera, and lepidoptera (Rohr et al. 2009). Of these groups, roughly 20%  
99 individuals are phytophagous (Ingwell et al. 2012).

100         In May 2012, we surveyed the forest for new treefall gaps. We only recorded gaps that  
101 appeared during the previous winter, restricting our experiment to new forest regeneration. We  
102 found six new treefall gaps large enough to include two 1.5 x 1.5 m experimental plots. This plot  
103 size is within the range of sizes common in studies of insect herbivory (Brown and Gange 1992;  
104 La Pierre et al. 2015). In May 2013, we added one additional gap that opened during the winter  
105 of 2012-2013, resulting in seven total gaps. All gaps were interspersed within a forest area of  
106 approximately 16 ha. In each gap we established two 2.25 m<sup>2</sup> plots, each entirely surrounded by  
107 1 m high chicken wire to exclude deer, as we were solely interested in the impacts of insects in  
108 this experiment. Previous experiments at SERC have used identical plot designs to successfully  
109 eliminate deer browsing, where deer densities are low (3-8 individuals km<sup>-2</sup>) compared to many  
110 parts of the United States (Cook-Patton et al. 2014), and no evidence of deer browsing was  
111 observed in any plot over the course of this experiment. Plots within each gap were located at  
112 least 5 m apart, minimizing the possibility of pesticide spray drifting between plots. Plots were  
113 not cleared or weeded in order to leave all natural vegetation intact.

114         Within each gap, one plot was randomly designated as the 'Control' treatment and the  
115 other was designated as a 'Pesticide' treatment. 'Pesticide' plots were sprayed every 2-3 weeks  
116 with 0.5 L of ASANA XL (DuPont) pesticide, diluted to a concentration of 40 µL/L . ASANA XL is  
117 an esfenvalerate insecticide that contains very little nitrogen, such that fertilization effects on  
118 plant growth should be minimal. Furthermore, ASANA XL is highly resistant to both rainfall and  
119 UV degradation and remains as a highly effective residue on leaf surfaces for several weeks  
120 after the initial application (DuPont 2006). It is therefore commonly used to examine the effects  
121 of insect herbivory on plant communities (Siemann and Rogers 2003; Heath et al. 2014).

122 Control plots were sprayed with an equivalent amount of water to control for possible  
123 watering effects. Pesticide applications began each year in May and continued through October,  
124 encompassing the entire growing season. Pesticide was applied for three years, beginning in  
125 2012 and ending in 2014. We sprayed the entirety of each plot, including ground cover, in order  
126 to judge how insects affect recruitment. To judge the efficacy of the pesticide treatment, we  
127 surveyed each seedling in each plot for insect damage at the end of the 2012 growing season.  
128 We examined the top four leaves of each seedling, categorizing damage as 0%, 25%, 50%,  
129 75%, or 100% damage and then averaged the values over all seedlings in each plot (Palmer  
130 and Brody 2013; Johnson et al. 2016).

131 In May of each year, each seedling was identified, measured for initial height, and  
132 tagged with a unique identification number. In October of each year, plots were censused again  
133 to record seedling final height, mortality, and recruitment over the growing season. Seedlings  
134 were only recorded as dead if an empty tag was found within the plot. New seedlings without  
135 tags were recorded as new recruits in both May and October, although only recruitment over the  
136 growing season (*i.e.* recruits appearing in October) was examined here, as recruits over the  
137 winter were not exposed to pesticide treatment. Seedlings were considered dead if they had no  
138 measurements for at least two consecutive census periods and did not reappear in the plot at a  
139 later date. In total, we tagged and measured 1,173 seedlings over the course of the experiment.

140

#### 141 *Data Analysis*

142 We examined pesticide effects only on seedlings  $\leq 15$  cm in height because these were  
143 the newest recruits and thus the most likely to experience mortality from herbivory. The term  
144 seedling has a broad and inconsistent usage, referring to plants anywhere between 0 – 4 years  
145 old (Hanley et al. 2004), but we use seedling to refer any individual  $\leq 15$  cm in height. Of the  
146 original dataset, 924 individuals (78%) met this criterion. We further restricted our analyses to  
147 the five most common species that occurred in enough plots to provide reasonable replication at

148 the species level: *Acer rubrum*, *Fagus grandifolia*, *Fraxinus* spp., *Liriodendron tulipifera*, and  
149 *Liquidambar styraciflua* (Table 1). These five species comprised 82% of observed seedlings  
150 (76% of all tagged individuals) and are dominant species in the eastern United States (Parker et  
151 al. 1989).

152 We conducted all analyses using Bayesian methods, which allowed us to incorporate  
153 prior information that prevented us from overestimating effect sizes. Small sample sizes, as  
154 used here ( $n = 7$  gaps), often lead to overestimates of the true effect sizes when using  
155 traditional analytical methods. Such overestimates have been a persistent problem in biological  
156 research (Button et al. 2013). Bayesian methods allowed us to place weakly informative priors  
157  $N(0, 1)$  on all parameters, which shrink parameter estimates towards 0 and help prevent  
158 overestimating effect sizes during the analysis of small sample sizes (Kruschke 2010; Button et  
159 al. 2013).

160 We analyzed total recruitment using Bayesian hierarchical Poisson regression, with  
161 species as random effects (Appendix 1). Because recruitment in any given year was absent for  
162 many species, we calculated the total number of new recruits for each of the five species in  
163 each plot to yield total number of recruits observed for each species over the entire  
164 experimental duration.

165 We used a hierarchical Bayesian model to examine the influence of pesticide treatment  
166 and gap age on seedling mortality. We analyzed mortality rate using logistic regression that  
167 included pesticide treatment, gap age, and the interactions of these two categorical variables as  
168 predictors (Appendix 1). We did not use a paired model because not all species were present in  
169 all gaps during each year, yielding an unbalanced design. This limitation means that we cannot  
170 ascribe some variance in the response to spatial variation among treefall gaps, thereby making  
171 it more difficult to detect effects of insect herbivores. Plant species were random effects, with  
172 species-level coefficients varying around overall coefficients. Overall coefficients describe the  
173 aggregate influence of pesticide and gap age on overall seedling mortality. The correlation

174 matrix between species-level parameters was given an uninformative Wishart prior. Variances  
175 of mortality rate were allowed to differ among species, relaxing the assumption of homogenous  
176 variances among species.

177 We analyzed relative growth rate [ $100 * (\text{Height}_{\text{end}} - \text{Height}_{\text{start}}) / \text{Height}_{\text{start}}$ ] using a similar  
178 hierarchical Bayesian model as described above (Appendix 1). To account for the fact that  
179 individual seedlings were not independent within a plot, growth of individual seedlings within a  
180 plot varied around the mean growth of their respective species in that particular plot and year.  
181 The plot-level means were a function of pesticide treatment, gap age, and their interactions;  
182 species-level coefficients varied around the overall coefficients as described above. (Appendix  
183 1).

184 Posterior distributions for all parameters were constructed using 5,000 'burn-in' iterations  
185 that were discarded followed by 5,000 sampling iterations for each chain, saving every 10<sup>th</sup>  
186 posterior draw (4 chains, 2,000 estimates per parameter). Response variables, except total  
187 recruitment, were standardized prior to analyses by subtracting the mean from each observation  
188 and dividing by the standard deviation in order to allow the use of weakly informative priors as  
189 described above. We examined model assumptions and fit using standardized residual plots.  
190 We report all statistics as probability of an effect (Pr), where higher probabilities denote more  
191 certainty regarding the importance of an effect. For example, Pr = 0.95 indicates that 95% of a  
192 coefficient's posterior distribution lay above or below zero. There is therefore a 95% chance that  
193 the coefficient is either negative or positive and therefore important. In contrast, Pr = 0.50  
194 indicates that a coefficient is equally likely to be either positive or negative and therefore  
195 unimportant. To facilitate interpretation of probabilities, we provide effect sizes and 95% credible  
196 intervals for all effects. Credible intervals were calculated as the interval between the 2.5% and  
197 97.5% quantiles for each posterior distribution (all posterior distributions were normal and  
198 symmetric). All analyses were conducted in Python v2.7. Bayesian models were built using  
199 STAN accessed via Pystan (Stan Development Team 2015).

200

201 **Results**

202 At the beginning of the experiment in May 2012, plots contained an average of 20  
203 seedlings each (range: 9 to 163 seedlings per plot). The five common species analyzed here  
204 accounted for ~80% of all initially tagged seedlings, with *L. tulipifera* being the most common  
205 (36.3%) followed by *Fraxinus* spp. (15.8%), *F. grandifolia* (15.1%), *L. styraciflua* (8.5%), and *A.*  
206 *rubrum* (8.5%) (Table 1). Pesticide application reduced foliar damage by approximately half.  
207 Percent of leaf area removed in 'Control' plots was  $7.38 \pm 0.83\%$ , whereas leaf area removal in  
208 'Pesticide' plots was  $4.27 \pm 0.76\%$  ( $\text{Pr}(\text{Control} > \text{Pesticide}) = 0.99$ ). *Fagus grandifolia* suffered  
209 the highest insect damage ( $16.5 \pm 5.7\%$ ), followed by *A. rubrum* ( $9.7 \pm 5.9\%$ ), *L. styraciflua* ( $5.6$   
210  $\pm 2.7\%$ ), *Fraxinus* spp. ( $3.7 \pm 3.7\%$ ), and *L. tulipifera* ( $0.64 \pm 0.34\%$ ).

211

212 *Recruitment*

213 In general, recruitment over the duration of the experiment was relatively low. Most  
214 species averaged fewer than two recruits during the growing season per plot over the duration  
215 of the experiment. *Liriodendron tulipifera* had the most recruits of any species, with plots  
216 averaging > 10 new recruits over the entire three years (Fig. 1). Surprisingly, long-term  
217 recruitment of two species, *A. rubrum* and *Fraxinus* spp., was higher in control plots compared  
218 to plots sprayed with pesticide ( $\text{Pr}(\text{Control} > \text{Pesticide}) \geq 0.95$  for both species) (Fig. 1, Table 2).  
219 Pesticide application did not affect the recruitment of the other three species ( $\text{Pr}(\text{Control} <$   
220  $\text{Pesticide}) < 0.84$  for all species) (Fig. 1, Table 2).

221

222 *Mortality*

223 Pesticide had negligible effects on seedling mortality averaged across all species  
224 ( $\text{Pr}(\text{Control} > \text{Pesticide}) = 0.66$ ). However, species varied in their responses to pesticide.

225 Pesticide did not alter mortality rates of *A. rubrum*, *F. grandifolia*, *Fraxinus* spp., or *L. styraciflua*  
226 ( $\text{Pr}(\text{Control} > \text{Pesticide}) \leq 0.79$  for all four species, Fig. 2, Table 3). In contrast, pesticide  
227 application marginally reduced mortality for *L. tulipifera* ( $\text{Pr}(\text{Control} > \text{Pesticide}) = 0.93$ ) (Fig. 2,  
228 Table 3). Mortality of *L. tulipifera* was 33.1% higher in plots without pesticide compared to  
229 control plots ( $\text{CI}_{95} = 9.9\%$  lower – 76.3% higher). Weak interaction coefficients suggest that  
230 pesticide effects were consistent across all years for all species (Table 3). Insects therefore  
231 marginally decreased survival of one common, temperate tree species, *L. tulipifera*.

232 Overall, total seedling mortality declined between the one and three year old gaps  
233 ( $\text{Pr}(\text{Year 3} < \text{Year 1}) = 1.0$ ), and this effect was consistent across all species except *L.*  
234 *styraciflua* (Table 3). Mortality of *F. grandifolia* and *L. styraciflua* marginally declined as gaps  
235 aged (Table 3). *Fraxinus* spp. mortality significantly decreased between one and three year old  
236 gaps ( $\text{Pr}(\text{Year 3} < \text{Year 1}) = 0.98$ , Table 3). Mortality of *A. rubrum* and *L. tulipifera* declined  
237 rapidly between the first and second year (Table 3, Fig. 2) and between the second and third  
238 year for *L. tulipifera* ( $\text{Pr}(\text{Year 3} < \text{Year 1}) = 1.00$ ). Mortality did not decline further between two  
239 and three year old gaps for *A. rubrum* ( $\text{Pr}(\text{Year 3} < \text{Year 2}) = 0.88$ ) (Fig. 2).

240

#### 241 *Relative Growth Rates*

242 Exposure to insects slightly decreased overall relative growth rates of seedlings  
243 ( $\text{Pr}(\text{Control} < \text{Pesticide}) = 0.91$ ), and this effect also varied among species (Table 4). *Acer*  
244 *rubrum* grew 23.7% faster ( $\text{CI}_{95} = 4.1\% - 45.6\%$ ) in one-year-old gaps sprayed with pesticides  
245 ( $\text{Pr}(\text{Control} < \text{Pesticide}) = 0.99$ , Fig. 3). However, pesticide did not affect *A. rubrum* growth rates  
246 in either two or three year old gaps ( $\text{Pr}(\text{Control} < \text{Pesticide}) < 0.69$  for both years, Fig. 3, Table  
247 4). Herbivory had little effect on relative growth rates of *F. grandifolia* ( $\text{Pr}(\text{Control} < \text{Pesticide}) <$   
248  $0.80$  for all years) or *Fraxinus* spp. ( $\text{Pr}(\text{Control} < \text{Pesticide}) < 0.85$  for all years) (Fig. 3, Table 4).  
249 *Liriodendron tulipifera* growth increased markedly in two year old gaps ( $\text{Pr}(\text{Year 2} > \text{Year 1}) =$   
250  $1.00$ ) but was unaffected by pesticide ( $\text{Pr}(\text{Control} < \text{Pesticide}) < 0.85$  for all years, Fig. 3, Table

251 4). Insects suppressed growth of *L. styraciflua* by 19.3% ( $CI_{95} = 1.3\% - 38.2\%$ ) in one and three  
252 year old gaps ( $\text{Pr}(\text{Control} < \text{Pesticide}) = 0.998$ ). In two year old gaps, insects had no detectable  
253 effect on *L. styraciflua* growth rates ( $\text{Pr}(\text{Control} < \text{Pesticide}) = 0.33$  in year two).

254

## 255 Discussion

256 Insects can kill > 50% of canopy trees during outbreak years (Romme et al. 1986). Even  
257 during non-outbreak years, insect herbivores play important roles in forest ecosystem function.  
258 For example, over one quarter of annual nitrogen deposition in tropical forests derives from  
259 insect frass (Metcalf et al. 2014). Insect herbivores can also govern community composition on  
260 decadal time scales by changing the abundance and diversity of tree seedling recruits in tropical  
261 forests (Dyer et al. 2010; Swamy and Terborgh 2010; Terborgh 2012; Bagchi et al. 2014). In  
262 contrast, we know comparatively little about the role of insect herbivores in temperate forest  
263 regeneration in non-outbreak years. Our study demonstrates that insects play a relatively minor  
264 role in structuring temperate forest communities. Most seedling species were resistant or  
265 tolerant of insect herbivores, as insects decreased growth of only three early pioneer tree  
266 species (*A. rubrum*, *L. styraciflua*, *L. tulipifera*) and decreased survival of only *L. tulipifera* during  
267 the initial phase of forest regeneration in temperate treefall gaps.

268 Contrary to our initial hypotheses, recruitment of most species (*F. grandifolia*, *L.*  
269 *tulipifera*, and *L. styraciflua*) was unaffected by insects. Surprisingly, pesticide application  
270 appeared to reduce recruitment of both *A. rubrum* and *Fraxinus* spp, albeit weakly. These  
271 patterns are similar to those reported by Meiners *et al.* (2000), where the presence of insect  
272 herbivores occasionally resulted in higher germination rates of *A. rubrum*. In our study,  
273 increased cumulative recruitment of *A. rubrum* exposed to insects was largely driven by  
274 recruitment during the last year, in which two plots contained 13 new recruits, 57% of the total  
275 number of *A. rubrum* recruits observed over the entire study duration. We therefore cannot

276 discount the possibility that our results are driven by abnormally high recruitment in two plots  
277 independent of insect presence. Indeed, insects exerted only weak, if any, effects on  
278 recruitment for both *A. rubrum* and *Fraxinus* spp., as insects increased recruitment by ~ 1  
279 individual over the course of three years for both species. Given our small sample size and a  
280 *priori* hypothesis that recruitment should decrease in the presence of insects, it is likely that  
281 these patterns result from either Type S (wrong sign – *i.e.* finding a positive effect that should be  
282 negative) or Type M (*i.e.* overestimating the magnitude of an effect) errors (Lemoine et al.  
283 2016). We therefore conclude that insects have little influence on the recruitment of tree species  
284 studied in this forest.

285         As predicted, insects increased mortality of *L. tulipifera*, but had little effect on the  
286 survival of most temperate forest species examined here, like *A. rubrum*, *Fraxinus* spp., and *F.*  
287 *grandifolia* (Meiners et al. 2000; Siemann and Rogers 2003). Many other temperate tree  
288 species, like *Quercus* spp. or *Prunus serotina*, are also resistant to herbivory or pathogens  
289 unless simultaneously subject to high densities of conspecific neighbors and, as a result,  
290 intense intraspecific competition (Packer and Clay 2000; Bell et al. 2006; Burt et al. 2014).  
291 Seedling density is therefore likely an important predictor of herbivore-driven mortality (Paine et  
292 al. 2012). Indeed, insects increased the mortality of only one species, *L. tulipifera*, which had the  
293 highest seedling densities of any species examined here. *Liriodendron tulipifera* produces  
294 exceedingly high numbers of seeds, releasing up to an order of magnitude more seeds m<sup>-2</sup> than  
295 most other coexisting species, except *A. rubrum* (Greene and Johnson 1994; Hille Ris Lambers  
296 et al. 2005). *Acer rubrum*, however, has a substantially smaller seed bank than *L. tulipifera* (Hille  
297 Ris Lambers et al. 2005). As a result, *L. tulipifera* can produce large numbers of seedlings in  
298 any given year and likely experiences stronger density-dependent mortality than other tree  
299 species. In our study, *L. tulipifera* averaged 11.35 individuals m<sup>-2</sup>, substantially lower than the  
300 densities of seedlings observed in either Burt et al. (2014) or Bell et al., (2006) (10 – 80 or 100 –  
301 1000 individuals m<sup>-2</sup>, respectively) but similar to densities of *P. serotina* recorded by Packer &

302 Clay (2000) (1 – 15 individuals m<sup>-2</sup>). Our results therefore suggest that *L. tulipifera* is subject to  
303 density-dependent mortality even at relatively low seedling densities.

304         Likewise, insect damage did not affect growth of most species examined here, but rather  
305 reduced the growth of only *A. rubrum* and *L. styraciflua*. Importantly, reduced growth was only  
306 observed in the youngest life stages; the impact of insects on growth of *A. rubrum* and *L.*  
307 *styraciflua* declined as gaps and seedlings aged. The declining effects of insects over time  
308 might be attributed to changes in insect abundance over time or, more likely, increased  
309 tolerance to insect damage by older seedlings. Indeed, seedlings alter their resource allocation  
310 patterns to maximize survival at any given demographic stage (Zhang and Jiang 2002). Older  
311 plants have the carbohydrate reserves necessary to withstand loss of photosynthetic tissue  
312 (Hanley et al. 1995), thus making them more tolerant to herbivory. Additionally, foliar damage at  
313 our study site was generally low, ranging from 4 – 7% leaf area removal. This is, however,  
314 consistent with estimates of foliar damage on the same species (5 – 12%, Siemann and Rogers  
315 2003), across eastern North America (0-12%, Adams and Zhang 2009), and within tropical  
316 forests (0-10%, DeWalt et al. 2004). Such increased tolerance to foliar damage likely mediates  
317 the declining impact of insects as gaps and seedlings age and might be responsible for the  
318 overriding effects of gap age on seedling growth.

319         As expected, plant life-history strategy appears to be an important determinant of the  
320 effects of insects. Early pioneer species, like *A. rubrum*, *L. tulipifera*, and *L. styraciflua*, all  
321 suffered reduced growth rates when exposed to insects. Herbivory may therefore help maintain  
322 forest diversity by decreasing the survival and growth of abundant, fast-growing seedlings:  
323 although *L. tulipifera* and *A. rubrum* comprised 36.3% and 8.5% of all tagged seedlings, the  
324 adult community is more diverse; *A. rubrum* and *L. tulipifera* comprise only 7.71% and 0.34% of  
325 adult trees in this study system (Parker et al. 1989). In contrast, *F. grandifolia* is a slow-growing,  
326 shade-tolerant, late-successional species that accounts for 20.77% of adult trees in our forest  
327 (Parker et al. 1989). In our study, *F. grandifolia* experienced the highest level of herbivory but

328 remained largely unaffected by the pesticide, suggesting that species that resist or tolerate  
329 herbivory during the seedling stage can become dominant members of the overstory  
330 community. Variable susceptibility to herbivory among seedlings of temperate tree species  
331 therefore indicates that insects might influence trajectories of temperate forest succession by  
332 selectively preying on seedlings of specific species; abundant but palatable seedlings comprise  
333 less of the forest overstory than do abundant, non-palatable seedling species.

334         Here, we examined the role of insects at non-outbreak levels on temperate forest  
335 regeneration in treefall gaps. Although several studies have characterized the effects of insect  
336 herbivores on seedling growth and mortality in treefall gaps (Norghauer and Newbery 2014),  
337 few track the effects of insects over multiple years. Interannual studies are especially important  
338 given yearly fluctuations in insect abundances, temperature, and rainfall (Burt et al. 2014;  
339 Norghauer and Newbery 2014). We show that gap age is an important determinant of the  
340 effects of insects on tree seedlings; insects had weaker impacts in older gaps and on older  
341 seedlings. Importantly, our study examines the youngest life stage of tree seedlings (<15 cm  
342 tall), whereas many other studies of insect herbivory on tree seedlings use older, well-  
343 established seedlings that can withstand defoliation and loss of photosynthetic capacity (e.g  
344 Myster & McCarthy, 1989). By conducting a multi-year study that examines the smallest  
345 seedling life stages, we demonstrated that insects have relatively weak effect on seedling  
346 survival and growth. Any effects of insect herbivore on forest succession are likely due to  
347 increased mortality of a single, dominant species: *L. tulipifera*. Long-term studies should  
348 examine whether or not insect herbivores can increase forest diversity by reducing the  
349 abundance of such competitively dominant tree species in temperate forests.

350

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355

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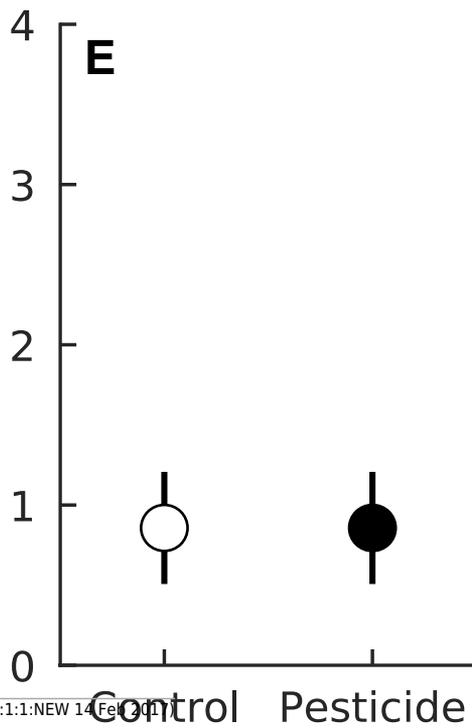
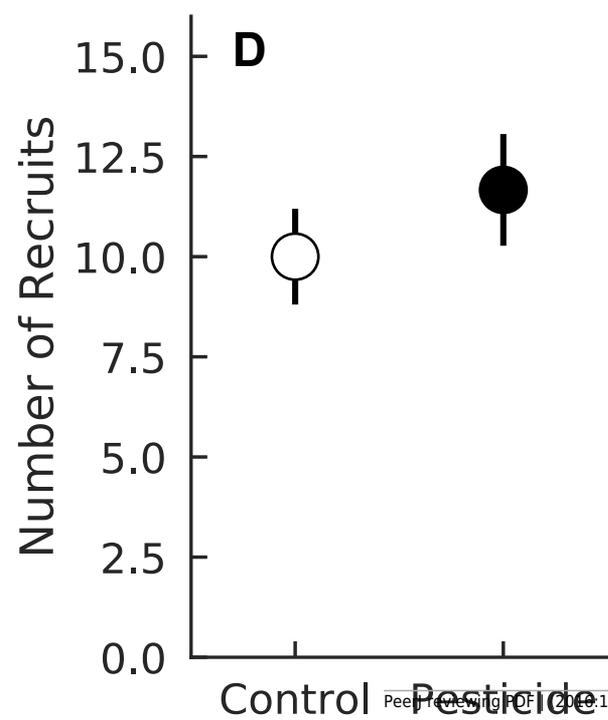
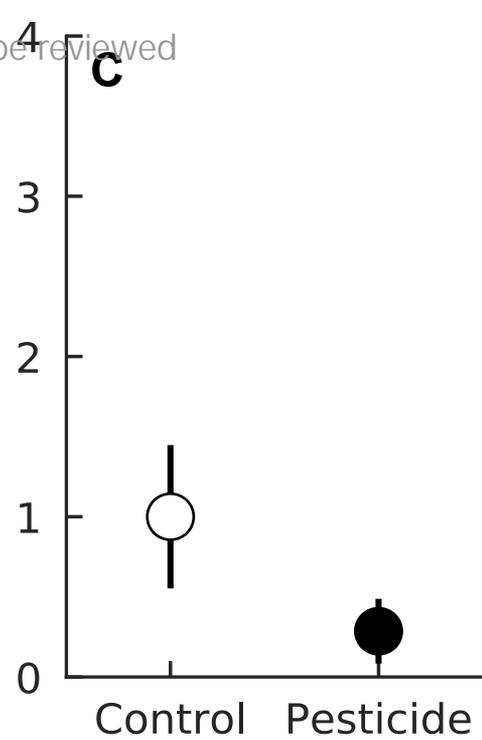
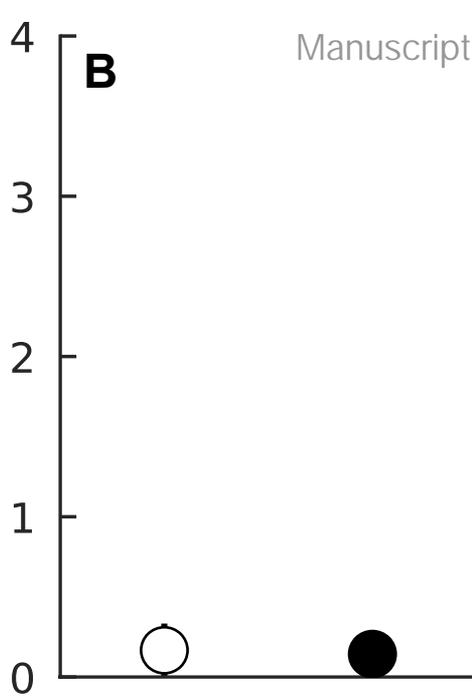
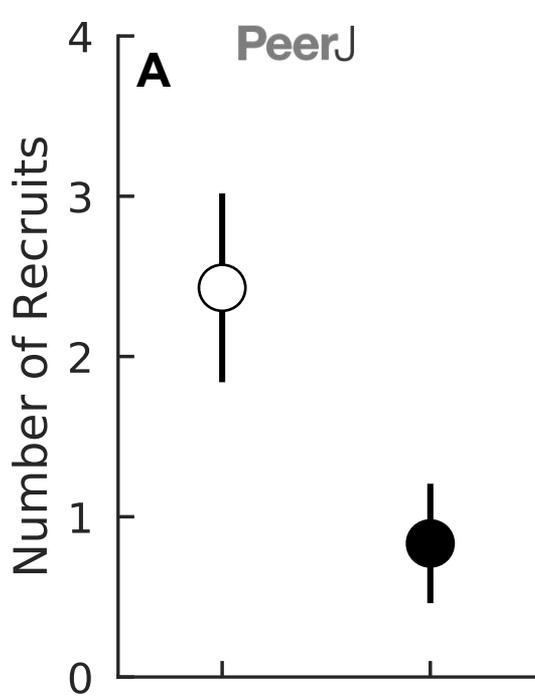
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**Figure 1**(on next page)

Number of recruits of five tree species in plots exposed to and protected from insect herbivores at the Smithsonian Environmental Research Center (Edgewater, MD) from 2012-2014.

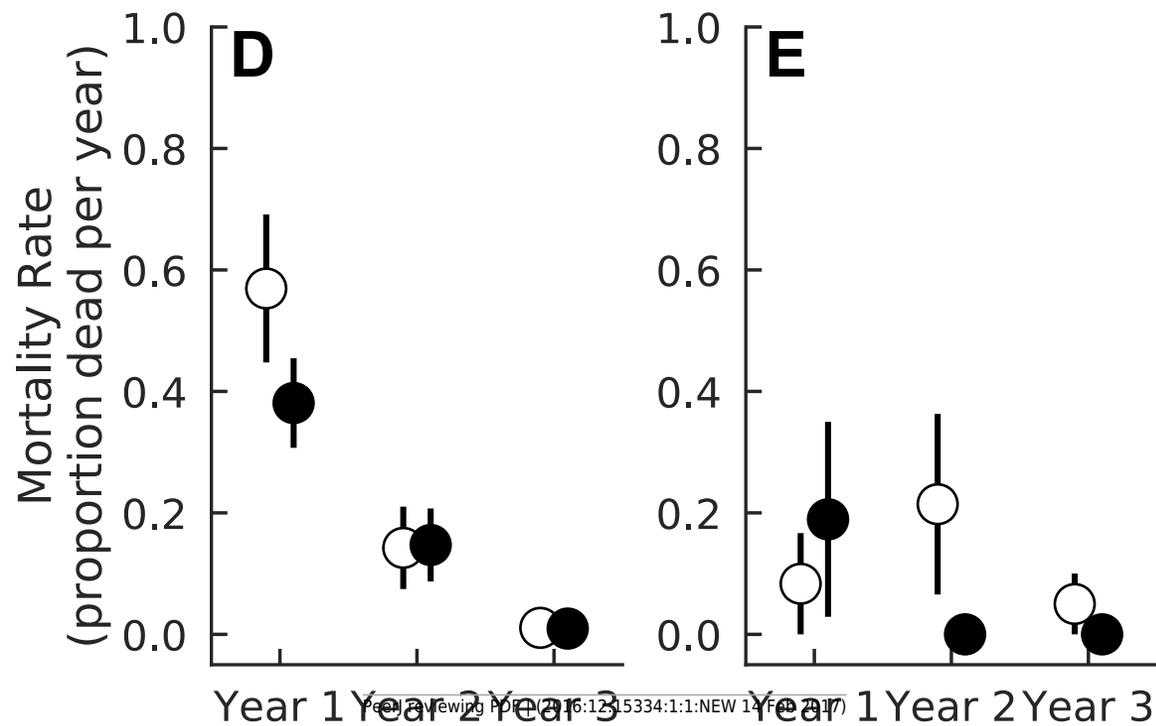
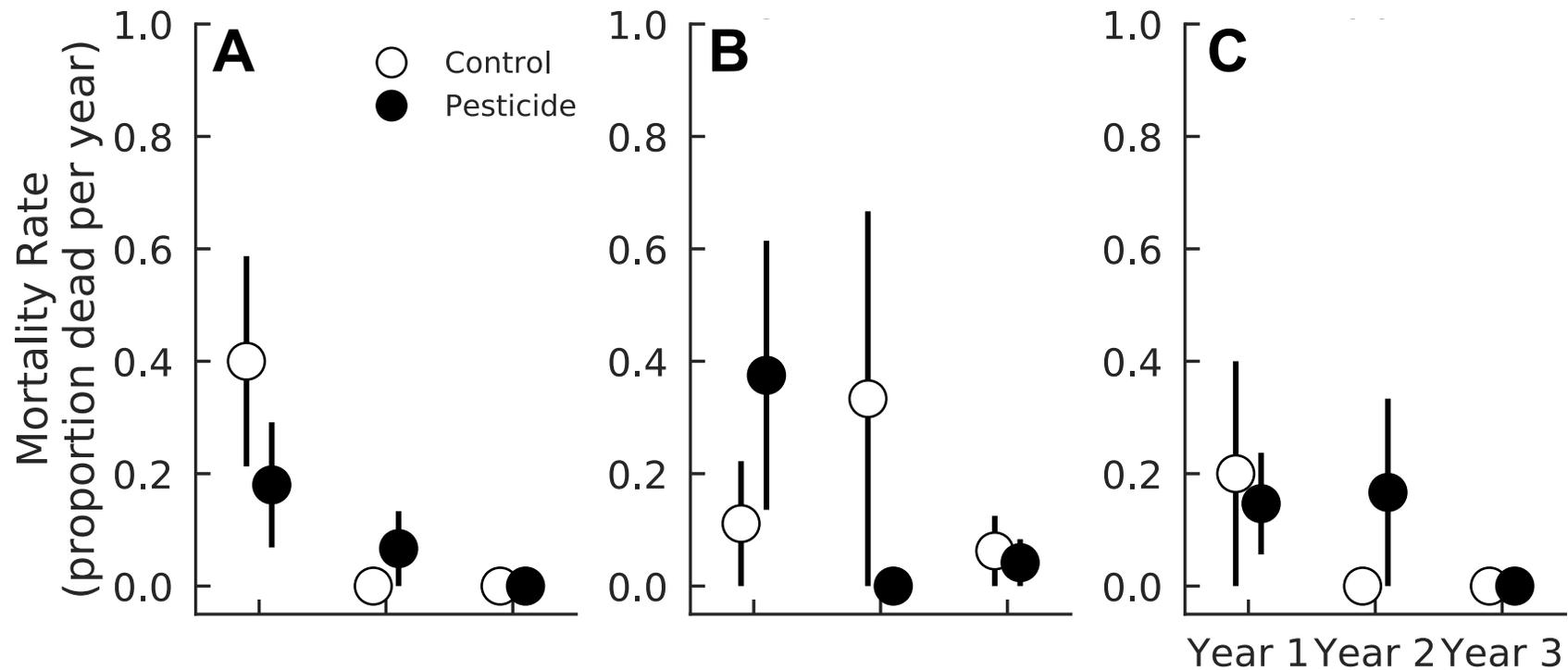
**A)** *Acer rubrum*, **B)** *Fagus grandifolia*, **C)** *Fraxinus* spp., **D)** *Liriodendron tulipifera*, **E)** *Liquidambar styraciflua*. Points show means  $\pm$  1 SE. Data were pooled by each plot ( $n = 7$  plots per treatment) prior to calculating treatment means and standard errors.



**Figure 2**(on next page)

Mortality rate of five tree species in plots exposed to and protected from insect herbivores at the Smithsonian Environmental Research Center (Edgewater, MD) from 2012-2014.

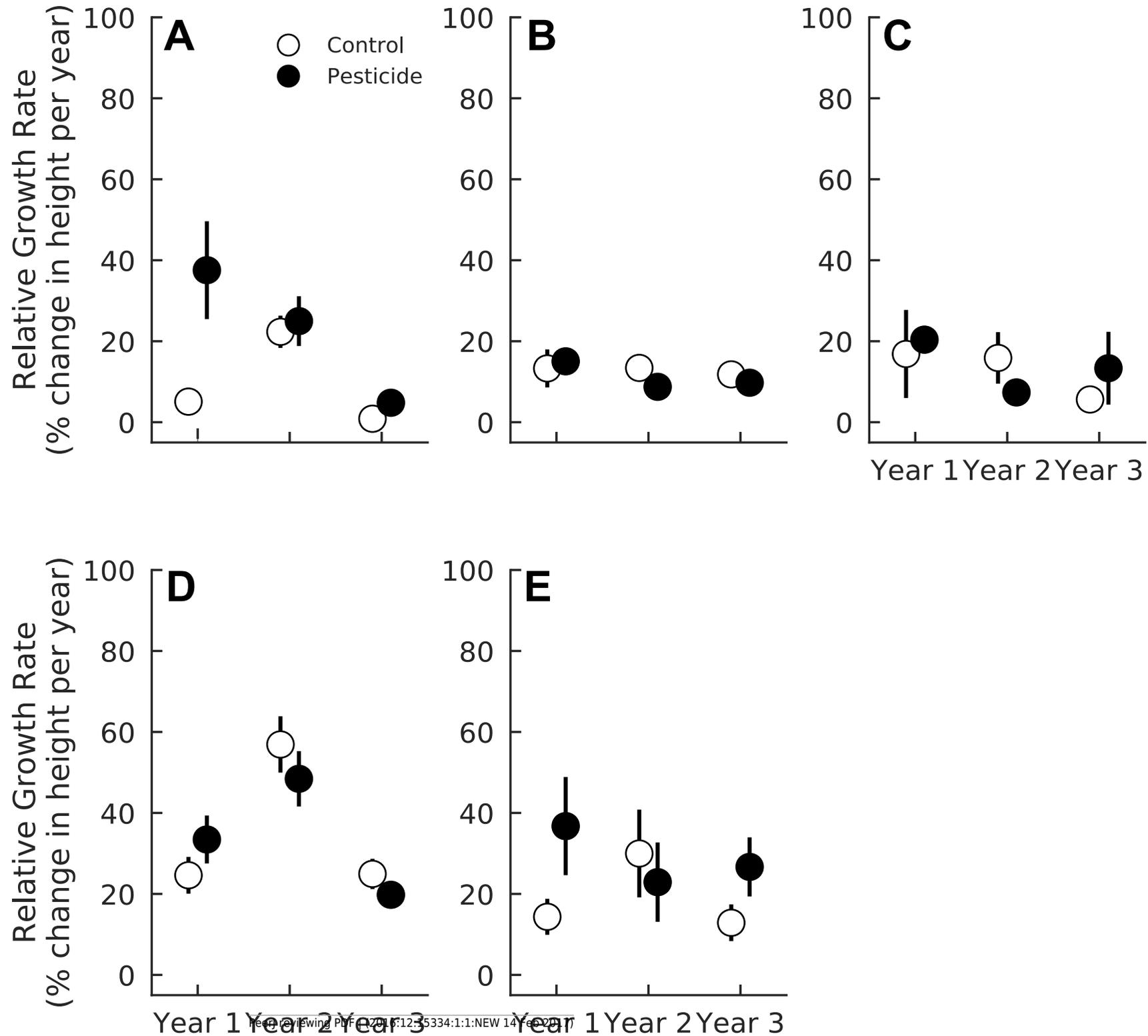
**A)** *Acer rubrum*, **B)** *Fagus grandifolia*, **C)** *Fraxinus* spp., **D)** *Liriodendron tulipifera*, **E)** *Liquidambar styraciflua*. Points show means  $\pm$  1 SE. We calculated the proportion of mortality of each species in each plot ( $n = 7$  plots per treatment) prior to calculating treatment means and standard errors.



**Figure 3**(on next page)

Relative growth rates of five tree species in plots exposed to and protected from insect herbivores at the Smithsonian Environmental Research Center (Edgewater, MD) from 2012-2014.

**A)** *Acer rubrum*, **B)** *Fagus grandifolia*, **C)** *Fraxinus* spp., **D)** *Liriodendron tulipifera*, **E)** *Liquidambar styraciflua*. We calculated mean growth rate of each species within each plot ( $n = 7$  plots per treatment) prior to calculating treatment means and standard errors.



**Table 1** (on next page)

Numbers of seedlings (individuals < 15 cm tall) observed for each species during each year for each of the five focal species at the Smithsonian Environmental Research Center.

1  
2

<b>Species</b>	<b>Year 1 2012</b>	<b>Year 2 2013</b>	<b>Year 3 2014</b>
<i>Acer rubrum</i>	29	23	22
<i>Fagus grandifolia</i>	22	24	30
<i>Fraxinus</i> spp.	42	41	28
<i>Liriodendron tulipifera</i>	332	278	191
<i>Liquidambar styraciflua</i>	55	51	14

3

**Table 2** (on next page)

Probability that each recruitment coefficient is greater or less than zero for each of the five species analyzed here.

Probabilities denote  $\max[\Pr(\text{coefficient}) > 0, \Pr(\text{coefficient}) < 0]$ , such that sign of the coefficient was ignored and the probabilities simply represent the probability of the coefficient being important in the model. Bold denotes  $\Pr(\text{coefficient}) \geq 0.90$ .

1  
2

<b>Recruitment Coefficient</b>	<i>Acer rubrum</i>	<i>Fagus grandifolia</i>	<i>Fraxinus spp.</i>	<i>Liriodendron tulipifera</i>	<i>Liquidambar styraciflua</i>
Pesticide	<b>0.99</b>	0.74	<b>0.95</b>	0.82	0.58

3  
4

**Table 3** (on next page)

Probability that each mortality coefficient is greater or less than zero for each of the five species analyzed here.

Probabilities denote  $\max[\Pr(\text{coefficient}) > 0, \Pr(\text{coefficient}) < 0]$ , such that sign of the coefficient was ignored and the probabilities simply represent the probability of the coefficient being important in the model. Bold denotes  $\Pr(\text{coefficient}) \geq 0.90$ .

1

<b>Mortality Coefficient</b>	<i>Acer rubrum</i>	<i>Fagus grandifolia</i>	<i>Fraxinus spp.</i>	<i>Liriodendron tulipifera</i>	<i>Liquidambar styraciflua</i>
Pesticide	0.79	0.68	0.65	<b>0.93</b>	0.56
Year 2	<b>0.99</b>	<b>0.93</b>	<b>0.97</b>	<b>1.00</b>	<b>0.91</b>
Year 3	<b>0.99</b>	<b>0.93</b>	<b>0.98</b>	<b>1.00</b>	0.87
Pesticide x Year 2	0.63	0.84	0.71	0.78	0.85
Pesticide x Year 3	0.66	0.63	0.72	0.59	0.70

2

3

**Table 4**(on next page)

Probability that each growth coefficient is greater or less than zero for each of the five species analyzed here.

Probabilities denote  $\max[\Pr(\text{coefficient}) > 0, \Pr(\text{coefficient}) < 0]$ , such that sign of the coefficient was ignored and the probabilities simply represent the probability of the coefficient being important in the model. Bold denotes  $\Pr(\text{coefficient}) \geq 0.90$ .

1

<b>Growth Coefficient</b>	<i>Acer rubrum</i>	<i>Fagus grandifolia</i>	<i>Fraxinus spp.</i>	<i>Liriodendron tulipifera</i>	<i>Liquidambar styraciflua</i>
Pesticide	<b>0.99</b>	0.77	0.84	0.84	<b>0.98</b>
Year 2	0.87	0.76	0.71	<b>0.99</b>	<b>0.93</b>
Year 3	0.79	0.51	0.67	0.54	0.58
Pesticide x Year 2	<b>0.94</b>	<b>0.91</b>	<b>0.94</b>	<b>0.92</b>	<b>0.97</b>
Pesticide x Year 3	<b>0.90</b>	0.75	0.67	0.84	0.72

2