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7 **Tree Resin Flow Dynamics during an Experimentally Induced Attack by *Ips avulsus*, *I.***
8 ***calligraphus*, and *I. grandicollis***

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27 **ABSTRACT:** Bark beetles' tree colonization success depends on their ability to overcome host
28 tree defenses, which includes resin exudation, and toxic chemicals, which deter bark beetle
29 colonization. Resin defenses during insect outbreaks are challenging to study *in situ*, since
30 outbreaks are stochastic events that progress quickly and thus preclude the establishment of
31 baseline observations of non-infested controls. We use synthetic aggregation pheromones to
32 demonstrate that confined *Ips* bark beetle herbivory can be successfully initiated to provide
33 opportunities for studying interactions between bark beetles and their hosts, including the
34 dynamics of constitutive and induced resin exudation. In *Pinus taeda* L. plantations between 12
35 and 19 years old in North and South Carolina, U.S., trees were affixed with pheromone lures,
36 monitored for evidence of bark beetle attacks, and resin samples were collected throughout the
37 growing season. Baiting increased beetle herbivory to an extent sufficient to produce an induced
38 resin response. Attacked trees exuded ~ 3 times more resin at some time than control trees. This
39 supports previous work that demonstrates information on constitutive resin dynamics alone
40 provide an incomplete view of a host tree's resistance to bark beetle attack.

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42 **KEY WORDS:** Bark beetle outbreak, pheromones, *Pinus taeda*, plant defense, resin flow

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49 1. INTRODUCTION

50 Bark beetles (Coleoptera: Curculionidae: Scolytinae) are some of the most devastating insect
51 pests in the coniferous forests of the southeastern U.S. Their damage results in massive
52 economic losses, changes in ecosystem structure and function, and creates challenges for natural
53 resource policy (Werner et al., 2006; Raffa et al., 2008; Price et al., 2010; Coulson & Meeker,
54 2011; Pye et al., 2011; Meddens et al., 2012). Global climate change is predicted to result in
55 conditions that are favorable to bark beetle fitness (Gan, 2004) and range expansion into new
56 areas and/or new hosts (Cudmore et al., 2010; Lesk et al., 2017).

57 Bark beetle species vary in terms of their reproduction dynamics and how aggressively they
58 attack trees. Attacks by Southern Pine Beetle (*Dendroctonus Frontalis* Zimmerman, heretofore
59 SPB), for example, can quickly evolve into widespread outbreaks that kill healthy trees. Species
60 of *Ips* bark beetles (*Ips avulsus* [Eichhoff], *Ips calligraphus* [Germar], and *Ips grandicollis*
61 [Eichhoff]), on the other hand, generally attack stressed or weakened trees, but pose a threat to
62 healthy trees when vigor is reduced, occasionally causing tree mortality (Drooz, 1985; Riley &
63 Goyer, 1988; Fettig et al., 2007; Schowalter, 2012; Clarke, et al., 2000). Despite these
64 differences in reproduction dynamics and host preference, however, bark beetles tend to invade
65 and colonize trees by similar mechanisms. Adult bark beetles breed, create nuptial chambers, and
66 excavate egg galleries in the host tree's phloem and outer xylem (Raffa et al., 2014), and larvae
67 consume phloem tissue, a process which halts the transport of carbohydrates and water. A tree
68 can tolerate some loss of this conductive tissue, but a loss of too much conductive tissue,
69 combined with deleterious effects of symbiotic blue stain fungi on tree xylem, can result in tree
70 mortality. Because *Ips* bark beetles are less aggressive and more likely to attack weakened trees
71 (Hain et al., 2011; Cognato, 2014), they could be used to investigate tree defense responses

72 under various conditions of environmental stress and study the links between tree physiology and
73 defense against attacking bark beetles.

74 The exudation of resin (also called oleoresin) by pine trees represents both a physical and
75 chemical defense against invading bark beetles. This resin can physically eject or entomb the
76 beetles, and the containment of beetles and/or associated fungi allows the tree an opportunity to
77 withstand low-density beetle attacks and prevent or delay the onset of outbreaks (Knebel et al.,
78 2008; Klepzig and Hofstetter, 2011). Resin also contains monoterpenes and phenolic
79 compounds that may be toxic to attacking beetles and their mutualistic fungi (Franceschi et al.,
80 2005; Bohlmann, 2012) and that may affect host selection (Fischer et al., 2010). Viscosity of the
81 resin is determined by the ratio of all classes of monoterpenes, including sesquiterpenes and
82 diterpene acids; the latter have insecticidal properties contributing to the resistance to
83 colonization (Phillips & Croteau, 1999). Chemical components and exudation rates are highly
84 variable between individual trees, and some chemical defenses occur ancillary to resin exudation.
85 Resin flow may be 'constitutive', representing resin synthesized prior to attack, or 'induced',
86 representing newly synthesized resin after phloem wounding (Ruel et al., 1998; Lombardero et
87 al., 2000). During active bark beetle attacks, trees can reallocate resources to actively mobilize
88 newly synthesized (i.e., induced) resin for defense, resulting in higher exudation rates until the
89 insect threat has passed, available resources in the tree are depleted, or the tree is otherwise
90 compromised (Lombardero et al., 2000; Franceschi et al., 2005; Luchi et al., 2005; Knebel et al.,
91 2008). The induced, secondary, resin contains toxic levels of monoterpenes in addition to acting
92 as a physical barrier (Martin et al., 2002; Boone et al., 2011). Once a tree's resin defenses are
93 overcome, the level of bark beetle colonization can lead to tree mortality (Boone et al., 2011).

94 Lower constitutive resin flow has been correlated with lower resistance to bark beetle attack,
95 though mechanistic drivers of variability in both constitutive and induced resin flow are not well
96 characterized (Lombardero et al., 2000; Knebel et al., 2008; Novick et al., 2012). Resin
97 production and flow rate varies inter- and intra-specifically, depending on site-specific factors,
98 including management and water availability (Hodges & Lorio, 1975; Hodges et al., 1977;
99 Franceschi et al., 2005). One popular conceptual framework for predicting constitutive resin
100 flow responses is the growth-differentiation balance hypothesis (GDBH, Herms & Matson,
101 1992), which predicts a tradeoff between growth and defense, particularly when trees experience
102 mild resource deficits. Under limited resource availability, such as low soil nutrients and
103 moderate water stress, photosynthate allocation may be shifted from growth to secondary
104 metabolites (Lorio & Sommers, 1986), increasing constitutive reserves of resin (Warren et al.,
105 1999). In contrast, when nutrient or moisture limitations are alleviated, carbohydrates are
106 preferentially allocated to growth and constitutive resin reserves are reduced (Warren et al.,
107 1999). However, moderate water stress affects the chemical composition of resin, with
108 decreased acids and increased monoterpene hydrocarbons rendering them more susceptible to
109 bark beetle attack (Hodges & Lorio, 1975). Finally, severely drought stressed trees often
110 demonstrate a reduction in resin production (Lombardero et al., 2000; Gaylord et al., 2013;
111 Netherer et al., 2015) when carbohydrate supply is highly compromised.

112 It is less clear, however, how well the GDBH can predict the dynamics of induced resin
113 flow. Expression of inducible resin defenses may be limited to rapidly growing (i.e. nutrient
114 rich) plants suggesting that fertilization, while possibly decreasing constitutive resin reserves,
115 may increase the ability of host trees to induce resin flow during herbivory (Herms & Mattson,
116 1992). Complicating matters is the fact that studies on induced resin flow are less common than

117 on constitutive flow since bark beetle outbreaks are difficult to predict and often occur quickly,
118 thus challenging efforts to observe pine tree defense dynamics during active herbivory. Previous
119 efforts to promote an induced resin response have included mechanical wounding and fungal
120 inoculation techniques. These techniques have been successfully deployed to understand resin
121 defense and bark beetle attack (Lombardero et al., 2006), environmental stress effects on terpene
122 and phenolic accumulation (Klepzig et al., 1995), and prior wounding effects on acquired
123 resistance induction (Christiansen et al., 1999). However, they are labor intensive (Ruel et al.,
124 1998; Knebel et al., 2008), and physical wounding by itself does not always promote an induced
125 resin flow response (Lombardero et al., 2000; Klepzig et al., 2005; Luchi et al., 2005; Mason et
126 al., 2017). Moreover, when the treatments are confined to the accessible lower portion of the
127 bole, they cannot promote direct wounding and subsequent fungal invasion in the upper portion
128 of the tree, where many bark beetles prefer to attack (Anderson and Anderson, 1968; Birch,
129 1987). This may unintentionally exclude important host tree-bark beetle interactions (i.e. direct
130 severing of resin ducts in the upper branching).

131 Here, we explore the utility of using synthetic pheromones to lure bark beetles to attack study
132 trees with the specific goal of measuring resin flow throughout the course of an active bark
133 beetle attack. Much previous work has relied on the use of synthetic pheromones to attract bark
134 beetles to study a range of processes, including crown scorch effects on colonization success
135 (Wallin et al., 2003), effect of crown density and tree vigor on host susceptibility (Waring &
136 Pitman, 1983), and aggregation behavior in response to synthetic pheromones (Vite & Pitman,
137 1969). However, to our knowledge, none have relied on the application of pheromones to attract
138 bark beetles with the particular goal of eliciting an induced resin flow response. Given the
139 ecological and economic importance of bark beetles and their pine tree hosts in the southeastern

140 U.S., developing a broader set of tools for inducing studying the links between host tree
141 physiology, and constitutive and induced resin defenses, would improve our ability to develop
142 effective 21st century management approaches.

143 The specific objective of this work was to develop an experimental baiting approach that
144 allows us to assess the *in-situ* response of pine trees to bark beetle attacks, and to apply the
145 approach in two experiments to better understand temporal and spatial dynamics of resin flow
146 during active bark beetle attack. In the first pilot experiment, our objective was to test whether
147 the use of aggregation pheromones would successfully attract *Ips* bark beetles into a localized
148 forested area. This could offer an opportunity to study the tree's defense response from initial
149 beetle colonization through the establishment phase. We focused on *Ips* bark beetles because
150 they physically bore into the phloem and xylem of trees, but usually do not cause extensive
151 outbreaks like SPB, mitigating the risk that our methods would lead to insect damage outside of
152 the study domain.

153 A second experiment, designed to more precisely characterize the resin flow response to
154 invading bark and woodboring insects, was replicated in two study locations. Our first objective
155 was to demonstrate whether this technique could produce an induced resin flow response to
156 better understand and disentangle the relationship between constitutive and induced resin flow.
157 Our second objective was to explore possible differences in the constitutive and induced resin
158 flow as a function of management (e.g., fertilization) and climatic variability. In particular, we
159 test if non-fertilized plots demonstrate greater constitutive resin flow than fertilized plots as
160 suggested in the resource availability hypothesis (Coley, 1987) and the growth-differentiation
161 balance hypothesis (Herms & Mattson, 1992); and further, if fertilized plots produce greater
162 inducible resin exudates in line with the resource availability hypothesis (Coley, 1987; Endara &

163 Coley, 2011). This study explores the physical aspect of pine tree resin defense from the initial
164 onset of attack throughout herbivory and provides insight into the relationship between
165 constitutive and induced resin response to beetle attack.

166 **2. MATERIALS AND METHODS**

167 **2.1. Study Sites**

168 We conducted studies in two managed loblolly pine plantations on the Atlantic Coastal Plain
169 in the southeastern U.S. – one in South Carolina, and one in North Carolina. Study trees were
170 between 12 and 19 years old, and sites were situated on sandy soil with a similar climate (Table
171 1). As a proxy for local soil water availability, we calculated a seven-day moving window of
172 total precipitation using data from the North Carolina Climate Retrieval and Observations
173 Network of the Southeast (CRONOS) database (<http://climate.ncsu.edu/cronos/>).

174 The pilot study and one of the replicated resin response experiments took place at the U.S.
175 Department of Energy's Savannah River Site (SRS), a National Environmental Research Park, in
176 Aiken County, SC, in 2011 and 2012. Trees were kept weed and pest free via mowing weeds
177 between tree rows and chemical pest management techniques when appropriate, including
178 glyphosate, carbaryl, and imidacloprid. Study trees had not received any chemical pest
179 management treatments for the previous five years. From planting in spring 2000 through the
180 2010 growing season, separate plots received either irrigation (I; 3.0 cm water wk⁻¹), fertilization
181 (F; 120 kg N⁻¹ ha⁻¹ yr⁻¹), irrigation and fertilization (I + F), or were untreated (control) (Coleman
182 et al., 2004; Coyle et al., 2016). The second replicated response experiment was conducted at
183 the Southeast Tree Research and Education Site II (SETRES-II) in Scotland County, NC, where
184 fertilization plots received 79 N, 8 P, 8 K, 0.6 Ca, 5 Mg, and 15 S kg ha⁻¹ yr⁻¹. Control of non-

185 pine vegetation was maintained since 1992 through mechanical and chemical (glyphosate)
186 methods (Albaugh et al., 1998).

187 **2.2. Pilot study at SRS**

188 During the summer of 2011, we tested the efficacy of aggregation pheromones and tree stress
189 volatiles to initiate a controlled infestation of *Ips* bark beetles. The experiment occurred in three
190 plots receiving the I + F treatment (one plot in each of three blocks). In each plot, two trees,
191 each ~ 25 cm diameter at breast height (DBH, 1.4 m) and separated by at least 25 m, were
192 selected to receive baits. On 7 June 2011, we attached ipsenol (+ 50/-50 bubble capsule 40 mg
193 lure), ipsdienol (+ 50/-50 bubble capsule lure 40 mg), and kairomones (alpha-pinene and ethanol
194 ultra-high release [UHR] lures, all from Contech Enterprises, Victoria, BC, Canada) to each tree.
195 Baited and adjacent trees (located within 3 m of the baited trees) were monitored on 14 June, 28
196 June, and 23 August for holes resulting from insect entrance and/or exit. In some cases, entrance
197 holes had the typical pitch tube associated with a successful bark beetle attack, while in other
198 cases the tree was unable to produce enough resin to cause a pitch tube. Exit holes were always
199 without a pitch tube. Since some entrance holes may have looked identical to exit holes, we
200 were unable to separate the two hole types, and thus lumped them into one metric hereafter
201 called “bark beetle holes”.

202 We recorded bark beetle holes (primarily from *Ips* species, though it is possible a SPB may
203 have been present. SPB are known to persist in very low populations on weakened, injured, or
204 *Ips* beetle attacked trees during their endemic phase), often indicated by the presence of sawdust
205 on the bark or ground), and concave egg niches carved in the bark by the pine sawyers
206 *Monochamus titillator* (Fabricius) and *M. carolinensis* (Olivier) (egg niches appear identical for
207 these two species). Yellow multigard sticky traps (15 × 10 cm, Scentry Biologicals Inc.,

208 Billings, MT) that did not contain any attractants were installed allowing for beetle identification
209 using a microscope to ensure no SPB were landing. On 14 June, we estimated insect holes on
210 the lower 3 m of the bole. We quantified these holes on three vertical locations on the bole (1.4
211 m, 2 m, 3 m) on 28 June (using a 13 × 18 cm square), and on two sides of the tree at DBH level
212 and at ~ 3 m on 23 August (using a 22 × 16 cm square). We explored relationships between bark
213 beetle hole density low and high on the bole.

214 **2.3. Resin Response Study at SRS and SETRES-II**

215 Following a successful pilot experiment (see Section 3.1), in 2012 an experiment was
216 conducted at SRS to include a larger number of study trees, to monitor insect landing, insect
217 entrance / exit holes, and to measure resin flow. The resin response experiment at SRS was
218 designed to compare fertilized and non-fertilized treatments (Fig.1). We established three
219 replicated blocks, each consisting of three plots (3 blocks × 2 treatments × 3 plots). Within each
220 plot, fifteen trees (i.e. three sets of five) were selected for a total of 90 sample trees. One tree
221 within each set was baited with synthetic aggregation pheromones (as described in the pilot
222 study) and three trees located adjacent (≥ 8 m) to the baited tree (termed “bait-adjacent” trees)
223 were monitored to determine the spatial extent of baiting. One additional tree > 13 meters from
224 the bait was monitored as the control, with the expectation that the effect of baiting would not
225 affect trees at this distance. Beetle presence in the region is widespread, and small numbers of
226 bark beetle holes were sometimes observed in non-baited trees. We did not attempt to exclude
227 beetles from our control trees. Instead, we leveraged information about bark beetle holes on
228 control trees to set a baseline of damage for trees operationally classified as “attacked” as
229 described in more detail below.

230 In 2013, a second resin response experiment was conducted at SETRES-II. The procedures
231 at SETRES-II were similar to SRS, with fertilized and non-fertilized treatments (Fig. 1). We
232 established two replicated blocks, each consisting of 4 plots (2 blocks \times 2 treatments \times 4 plots).
233 An additional fifth plot was established in one non-fertilized treatment totaling 17 plots (Fig.1).
234 Within each plot, we sampled five trees: one baited, three bait-adjacent, and one control > 13
235 meters away from baited for a total of 85 trees.

236 **2.3.1. Insect landing and bark beetle holes**

237 During the growing season, we visually assessed bark and woodboring beetle damage on the
238 lower 2 m (from base to 2 m high) of each study tree using methods identical to those used in the
239 pilot study. In the SETRES-II experiment, we also used Swedish climbing ladders to assess
240 damage to approximately 4 m high around the entire stem. Holes by the black turpentine beetle,
241 (*Dendroctonus terebrans* (Olivier); BTB), identifiable by large resinous pitch tubes on the lower
242 bole, were also recorded. Yellow multigard sticky traps (15 \times 10 cm, Scentry Biologicals Inc.,
243 Billings, MT) that did not contain any attractants were installed on all baited and control trees to
244 quantify *Ips* bark beetle abundance. Traps were installed approximately 2.4 to 3 m from the base
245 of the tree and mounted directly on the stem. Traps captured *I. avulsus*, *I. calligraphus*, and *I.*
246 *grandicollis*. Beetles were identified using the taxonomic key in Wood (1982) and attack density
247 was derived from bark beetle holes. Quantification of insect damage was similar at both sites,
248 though trap collection dates differed (Table 1).

249 We pooled bark beetle hole data from SRS and SETRES-II to the determine threshold of
250 bark beetle activity beyond which trees were operationally classified as “attacked.” We
251 classified all trees with six or fewer holes as non-attacked, as six was the maximum number of
252 insect holes observed on trees located > 13 m from the baited trees. All trees with greater than

253 six holes were classified as attacked. Our results are not particularly sensitive to the magnitude
254 of the threshold differentiating attacked from non-attacked trees; had the attack threshold been
255 set at two standard deviations (nine holes) above the established level, it would have resulted in
256 no fewer trees classified as attacked at SRS and only two fewer at SETRES-II.

257 Among trees located more than 13 m from the baited tree, the mean number of observed bark
258 beetle holes in the lower 2 m of the bole was 0.95 (SD = 1.6), ranging from zero to six (Fig. 2).
259 We selected the maximum number of bark beetle holes on these trees, which represents six times
260 the mean number of holes, to represent the threshold level for ambient beetle activity in non-
261 attacked trees. In contrast, mean beetle holes on baited trees were 21.9 (SD = 15.2), ranging
262 from zero to sixty-four (Fig. 2). At SRS, 18 of the 18 baited trees (100%) exceeded the threshold
263 and were considered attacked, whereas none of the bait-adjacent trees were attacked. Baited
264 trees at SETRES-II had a greater total number of trees attacked than SRS. The majority of baited
265 trees exceeded the attack threshold (14 of 17 trees, or 82%) plus 5 of 51 bait-adjacent trees were
266 characterized as attacked (~ 10%). We emphasize that the majority of bait-adjacent trees were
267 not attacked.

268 **2.3.2. Volumetric observations of resin flow**

269 Resin flow sampling began when trees were baited and continued through multiple sampling
270 events over the remainder of the growing season (Table 1). Each sampling date, resin flow rate
271 (g day^{-1}) was measured in the five trees that were selected to be monitored as bait, bait-adjacent,
272 and control; with two samples per tree collected from opposite sides of the stem. Following
273 standard practice (Lombardero et al., 2000; Knebel et al., 2008; Novick et al., 2012), resin flow
274 was measured by removing a 0.8 cm diameter disk of bark to the phloem-xylem interface at
275 DBH, and installing resin samplers around the wound to direct the flow of resin into pre-weighed

276 collection vials for a 24 ± 1.5 -hour period. Vials were installed on all trees between 0800 and
277 1200 to minimize time-of-day effects on resin flow. Measurements were repeated on the same
278 set of trees, and care was taken to ensure that new wounds were laterally displaced 5-10 cm from
279 previous wound sites. Once collected, tubes were sealed and taken to the laboratory to be
280 weighed. Resin flow collection methodology was identical at each site; however, on day 15 after
281 baiting during the SETRES-II field campaign, the site experienced an extreme rain event that
282 contaminated the resin flow vials; those data were removed from the analysis.

283 *2.3.3. Statistical Analysis*

284 Statistical analysis was performed using SAS 9.3 (SAS Inc., Cary, NC) and we considered an
285 alpha-level of 0.05 significant for all analyses. A random effects mixed model analysis of
286 variance (ANOVA) was used to test for the effects of fertilization treatment and baiting for bark
287 and woodboring beetles on amount of resin. The model for fixed effects, including interaction
288 terms, took the form:

$$290 \quad Q = \mu + D + T + A + DT + DA + TA + DTA + \varepsilon \quad [1]$$

291
292 where Q is the resin flow metric of interest, μ is the overall mean resin flow, D is the day effect
293 (categorical variable), T is the treatment effect (non-fertilized or fertilized), A is the beetle attack
294 (attacked or not attacked), and ε is the error associated with the model with expectations of
295 normal independent distribution (NID; $\sim 0, \sigma^2_\varepsilon$). Random effects were assigned based on the
296 nested design of trees (individuals) within a plot (replicate) within a fertilization treatment within
297 a block. Statistical analysis was performed separately because the sites were of different age and
298 size, and studies were performed in different years with varying climate conditions. Both sites

299 were *Pinus taeda* plantation sites, however, sites used different provenances of the species as
300 well as having different applications of fertilizer treatments and controls of understory growth.

301 We analyzed several resin flow metrics in an effort to understand treatment effects on both
302 constitutive and induced resin flow. We compared treatment effects on flow collected prior to
303 baiting (Q_{initial}) and on the final sampling day of each experiment (Q_{final}). Since the highest flow
304 rates were not always observed on the final day of sampling, we also analyzed maximum Q
305 (Q_{max}) during the study. Finally, we investigated maximum induced resin flow ($Q_{\text{maxinduced}}$), the
306 difference between Q_{max} and Q_{initial} . Welch's t-tests were used to determine differences of Q_{final}
307 and Q_{max} between attacked and non-attacked trees as well as $Q_{\text{maxinduced}}$ for attacked trees. To
308 analyze the success of aggregation pheromones in attracting bark and woodboring beetles, a
309 general linear model approach was used to test for differences between baited and control trees
310 in the fertilization treatments.

311 3. RESULTS

312 3.1. *Bark beetle response to baiting – Experiment 1*

313 One week after lures were installed, between 10 and 25 bark beetle holes were observed on
314 the boles of all baited trees growing in SRS. Two *Monochamus* sp. egg niches were observed on
315 each of two trees. After 21 days, baited trees continued to sustain an increased number of holes
316 (ranging from 6 to 54 additional holes), but no adjacent trees showed any signs of insect damage.
317 After ~ 70 days, all six baited trees were dead. Within a distance of 5 meters from each baited
318 tree, bark beetle holes were observed on between 4-11 nearby trees, and mortality (assessed as
319 crown discoloration or lack of needles) was observed for 7 of these neighboring trees on 23
320 August.

321 We compared beetle attack intensity at different heights on the stem to determine whether
322 sampling at breast height was representative of the entire tree. Bark beetle holes observed on
323 upper portions of the stem (≥ 2 m; A_{high}) showed a strong linear correlation (Fig. 3; $R^2 = 0.88$, P
324 < 0.0001 , $A_{\text{high}} = 1.451 A_{\text{low}} + 0.541$) with those observed near the base of the stem (< 1.4 m;
325 A_{low}). There were approximately 50% more observed holes on the upper stem than holes
326 observed near the base of the stem.

327 **3.2. Temporal beetle attack- Experiments 2&3**

328 During the second experiment occurring at SRS, bark beetle attacks occurred later and at a
329 lower intensity than in the first experiment. Bark beetle holes were observed 25 days after baits
330 were deployed, with 13 of 18 (72%) baited trees attacked by this date. Little change occurred in
331 the number of bark beetle holes until the final sampling date (86 days), at which point 100% of
332 all baited trees, and zero non-baited study trees, were attacked. Attacked trees were located
333 within 10 m of the bait tree, and bark beetle hole occurrence decreased with increasing distance
334 from the bait (Fig. 2a). We observed six-fold more bark beetle holes on baited trees than all
335 others (mean \pm SE: 15.6 ± 1.4 , compared to 2.6 ± 0.7 and 2.1 ± 0.3 on bait-adjacent and control
336 trees, respectively; Fig 4). *Ips* species were captured much more frequently on baited than
337 control trees ($t_{17} = 8.66$; $P < 0.01$; Table 2). Total number of *Ips avulsus*, *calligraphus*, and
338 *grandicollis* were 2567, 72, and 242, respectively (Table 2). We observed BTB pitch tubes on
339 12 trees (mean \pm SE: 3.5 ± 0.84); of which 3 trees had > 7 pitch tubes 25 days after bait
340 deployment.

341 Bark beetle attacks in response to baiting progressed less quickly at SETRES-II than SRS.
342 Beginning 42 days after baits were deployed (DOY 145), bark beetle holes were observed on
343 only 16 trees, and only two trees had > 3 holes m^{-2} (Fig 4b). Bark beetle hole counts increased

344 dramatically by DOY 189, when 16 trees had > 6 holes m^{-2} . By the final sampling day (DOY
345 214), 20 trees had > 10 holes m^{-2} , eight of which had > 15 holes m^{-2} . The number of beetle bark
346 beetle holes on non-baited trees decreased with increasing distance from the bait (Fig. 2b).
347 Attacked trees (with > 6 holes) were limited to approximately 5 meters from the bait. Of the 17
348 trees that were baited, 88% were considered attacked. Additionally, 29% of the 51 bait-adjacent
349 trees that were monitored experienced bark beetle holes.

350 Baited trees had over 13 times bark beetle holes than bait-adjacent and control trees (mean \pm
351 SE: 35.3 ± 6.4 compared to 2.6 ± 0.3 and 0.7 ± 0.4 , respectively; Fig 4). Sticky trap data
352 revealed a significantly higher presence of *Ips* spp. ($t_{16} = 6.60$; $P < 0.01$) on baited trees
353 compared to control trees. Total number of *Ips avulsus*, *calligraphus*, and *grandicollis* were 173,
354 524, and 129, respectively (Table 2). We observed BTB pitch tubes on 23 trees (22.3 ± 4.2); 14
355 of these had > 20 pitch tubes 118 days after bait deployment.

356 **3.3 Resin flow dynamics**

357 **3.3.1 Resin flow response to bark beetle attack**

358 At SRS, attacked trees experienced a significant increase in resin flow over the course of the
359 experiment ($F_{1,344} = 12.97$, $P = 0.0004$), indicating that beetle attack resulted in induced resin
360 flow. Specifically, we observed three times as much resin flow in attacked trees 86 days post
361 bait deployment (Day of Year (DOY) 244) than of non-attacked trees (Fig. 5c).

362 Overall, final resin flow (Q_{final}) was higher in attacked trees than non-attacked trees, 4.2 g d^{-1}
363 versus 1.3 g d^{-1} , respectively (Fig 6a; $t_{18} = 4.77$, $P < 0.01$). Q_{max} was also higher among attacked
364 trees than non-attacked trees, 4.9 g d^{-1} versus 2.3 g d^{-1} , respectively (Fig 6c; $t_{19} = 4.47$, $P < 0.01$).
365 Overall, attacked trees had higher $Q_{maxinduced}$ (i.e., a greater difference between $Q_{initial}$ and Q_{max})
366 than non-attacked trees, 3.0 g d^{-1} versus 0.2 g d^{-1} , respectively (Fig. 6e; $t_{18} = 4.75$, $P < 0.01$).

367 At SETRES-II, attacked trees at SETRES-II experienced a significant increase in resin flow
368 over the course of the experiment ($F_{1,324} = 19.13$, $P < 0.0001$). Resin flow of non-attacked trees
369 remained low ($< \sim 1 \text{ g d}^{-1}$) throughout the experiment. In contrast, resin flow was considerably
370 elevated in attacked trees. Q_{final} was higher in attacked trees than non-attacked trees, 2.3 g d^{-1}
371 versus 1.6 g d^{-1} , respectively (Fig. 6b; $t_{21} = 3.02$, $P < 0.01$). Q_{max} was also higher among attacked
372 trees compared with non-attacked trees, 3.2 g d^{-1} versus 0.6 g d^{-1} , respectively; as well as
373 $Q_{\text{maxinduced}}$, 1.9 g d^{-1} versus 0.3 g d^{-1} , respectively (Fig. 6d; $t_{21} = 3.0$ and 6f; $t_{21} = 2.59$, $P < 0.01$,
374 respectively).

375 **3.3.2 Fertilization effects on resin flow**

376 At SRS, Q_{initial} was higher in fertilized trees than non-fertilized trees ($t_{86} = 1.99$, $P < 0.05$) but
377 no difference at SETRES-II ($t_{80} = 1.99$, $P = 0.51$). At SRS, fertilization had no effect on Q_{final} or
378 Q_{max} in non-attacked trees (Fig. 6a,c). Within fertilized plots, attacked trees had significantly
379 higher Q_{final} than non-attacked fertilized trees ($t_7 = 2.51$; $P = 0.03$); however, no significant
380 fertilization treatment effect was observed when comparing Q_{final} between fertilized and
381 unfertilized plots ($t_{15} = 0.91$; $P = 0.38$). Attacked non-fertilized trees experienced increased resin
382 flow one sampling period earlier (approximately 21 days) than fertilized trees, despite similar
383 timing of beetle attack (Fig. 5a,c). Fertilization had no effect on Q_{max} of non-attacked ($t_{68} = 0.61$;
384 $P = 0.54$ and $t_{70} = 1.38$; $P = 0.17$) or attacked trees ($t_{15} = 0.58$; $P = 0.57$ and $t_{15} = 1.30$; $P = 0.22$).
385 Similarly, we observed no fertilization effect on $Q_{\text{maxinduced}}$ of attacked trees ($P > 0.05$).

386 Again, at SETRES-II, fertilization had no effect on Q_{final} or Q_{max} in non-attacked trees ($t_{47} =$
387 0.68 ; $P = 0.50$ and $t_{47} = 1.11$; $P = 0.27$, respectively). Q_{final} was higher in attacked fertilized trees,
388 although results were not significant ($t_{17} = 1.09$; $P = 0.29$). Fertilization had no effect on Q_{max} in
389 attacked trees ($t_{17} = 0.52$; $P = 0.61$).

390 4. DISCUSSION

391 *4.1 Effects of synthetic pheromone lures on bark beetle attacks*

392 Our research objective was to determine whether using baits to create localized bark beetle
393 attacks could facilitate mechanistic studies of induced pine tree resin defenses. Both our pilot
394 study and replicated studies support previous successes for using aggregation pheromones as a
395 method to attract bark and wood boring insects into an area of interest and promote beetle attack.
396 Results from the pilot study demonstrate this technique was capable of producing localized
397 pockets of mortality in baited trees and their neighbors. Mortality did not occur in the replicated
398 studies. Importantly, however, an induced resin flow response was observed in both study sites.
399 The spatial extent of herbivory in response to the baiting was limited to target trees and adjacent
400 trees, with trees greater than 10 m from the bait showing no evidence of increased beetle activity
401 (Fig. 2).

402 Bark beetle attack was achieved quickly after aggregation pheromone installation. While
403 bark beetle hole abundance was not particularly high when compared to previous experimental
404 baiting studies (Schlyter et al., 1987; Schlyter & Anderbrant, 1989), the level of attack was
405 sufficient to produce an increase in resin flow. It should be recognized that different species of
406 *Ips* beetles colonize different sections of the host tree (Paine et al., 1981). However, a high
407 number of *I. avulsus* were identified on sticky traps even though this species tends to occupy the
408 upper stem and crown (Connor & Wilkinson, 1983). We observed a strong linear correlation
409 between holes on the lower and upper stem, suggesting that total beetle activity affecting the
410 stem would be greater than what was observed on the ground, especially considering that some
411 species preferentially attack the crown.

412 We emphasize that in this study, the primary goal was to see if baits could produce an
413 induced resin flow response over the course of a growing season. The sampling plan did not
414 include long term monitoring to determine the extent of colonization or sustained populations but
415 investigating the potential for this approach to produce long-term changes in beetle populations
416 and resin production is a good avenue for future research.

417 **4.2 Resin Flow Response**

418 In the replicated experiments at SRS and SETRES-II, an induced resin flow response was
419 observed for the attacked trees. At both sites, we observed that resin flow increased in trees after
420 they were attacked, and the attacked trees produced consistently more resin than their non-
421 attacked neighbors (Figs. 5c,d & 6). No difference between initial and final resin flow was
422 observed in non-attacked trees (Fig. 5c,d), suggesting higher final resin flow in attacked trees
423 was in response to the beetle attack and not seasonal variation or ancillary effects of mechanical
424 wounding. The spike in resin flow of attacked trees at the end of the growing season in both
425 SRS and SETRES-II (Fig. 5c, d) suggests an induced response of newly synthesized resins, in
426 order to continue defense during the bark beetle attack (Lombardero et al., 2000). Because much
427 of what is known about resin flow dynamics concerns constitutive flow rates only (Hodges et al.,
428 1977; Warren et al., 1999; Gaylord et al., 2007), the approach described here has the potential to
429 enhance our understanding of the mechanisms of resin flow production before and during bark
430 beetle herbivory. Here, we limited the scope of our analysis to focus on the physical aspect of
431 resin defense, which is an important but ultimately incomplete description of the integrated
432 defense system of pine trees. Future work could use this approach with a greater emphasis on
433 the chemical composition of resin exudates, both in terms of constitutive and induced resin
434 chemical composition. Furthermore, by modifying this approach to target specific insects with

435 different types of bait, future research could help to improve our understanding of how tree
436 defense responses may depend on the attacking species.

437 ***4.2.1 Fertilization effects on resin flow***

438 The observed effects of fertilization on resin flow were subtle and differed among sites. At
439 SRS, the temporal pattern of beetle arrival was nearly identical among fertilization treatments
440 until the final sampling date, when more bark beetle holes were observed in non-fertilized trees.
441 Similarly, at SETRES-II, fertilized and non-fertilized trees followed a similar pattern of bark
442 beetle arrival for the first two sampling events. However, unlike SRS, once bark beetle arrival
443 passed our metric for threshold of attack, fertilized trees showed consistently higher bark beetle
444 hole counts than non-fertilized trees.

445 Within the scope of our experiment, our results only partially support the growth-
446 differentiation balance hypothesis (GDBH). In contrast to the GDBH, but consistent with
447 previous results observed by Novick et al. (2012), Klepzig et al. (2005), van Akker et al. (2004),
448 and Lombardero et al. (2000), we found no consistent significant effect of fertilization treatments
449 on constitutive resin flow at SETRES-II. However, at SRS, fertilized trees produced ~60%
450 higher initial flow than non-fertilized trees; in contrast to results presented by Warren et al.
451 (1999), reporting that fertilized trees produced as much as 50% lower resin flow than non-
452 fertilized trees. The results from SRS are inconsistent with predictions from the GDBH, which
453 states when conditions (like nutrient availability) are favorable for growth; carbohydrates are
454 preferentially allocated for growth processes rather than defense (Herms & Mattson, 1992). We
455 would expect that initial flow would be greater for non-fertilized trees compared with fertilized
456 trees which is not what we observed at SRS and suggests growth was limited by something other

457 than nutrients (i.e. energy) allowing the fertilized trees to allocate resources to secondary
458 processes. No significant fertilizer effect was observed for SETRES-II.

459 Research from Moreira et al. (2015) suggest trade-offs between growth and defense are
460 determined not only by resource availability (i.e. nutrients) but also by genetics and genetic-
461 nutrient interactions. Sampedro et al. (2010) demonstrate that phenotypic variation in leaf
462 volatile terpene concentrations is affected by genetic variation and not nutrient (in this case
463 phosphorus) availability in certain pines. This could explain why the results from SRS and
464 SETRES-II were different, with fertilized trees exuding more initial resin and less final resin
465 than non-fertilized trees at SRS and no difference in initial resin flow and higher final resin flow
466 at SETRES-II. Further, in non-attacked trees at both SRS and SETRES-II, we reported no
467 differences in resin flow throughout the experiment between fertilized and non-fertilized trees
468 suggesting that fertilization has no effect on constitutive flow.

469 **5. CONCLUSION**

470 We modified the utility of synthetic pheromone lures to attract bark beetles into a localized
471 pocket of attack to elicit a resin flow defense response without the threat of a major outbreak.
472 Attack levels were sufficient to cause mortality in the first experiment; in the subsequent resin
473 flow experiments, attack levels were not sufficient to cause mortality, but were clearly sufficient
474 to elicit an increased resin response. We achieved increased resin flow rates in attacked trees,
475 and confidently attribute these increased resin flow rates to bark beetle invasion. Future studies
476 may utilize this method to further investigate environmental and management effects on
477 inducible physiological defenses in conifer trees. With this approach, we are better equipped to
478 design experiments investigating invading bark beetle- host tree defense responses and the
479 factors or disturbances (i.e. fire, water stress) that may exacerbate or hinder this response.

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489 REFERENCES

- 490
491
492 Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W., and J.S.King. 1998. Leaf area and
493 above and belowground growth responses of loblolly pine to nutrient and water additions.
494 For. Sci. **44**: 317-328.
- 495 Anderson, N.H., and Anderson, D.B. 1968. Ips bark beetle attacks and brood development on a
496 thlighting-struck pine in relation to its physiological decline. Fla. Entomol. **51**(1): 23-30.
- 497 Birch, M.C. 1978. Chemical communication in pine bark beetles: The interactions among pine
498 bark beetles, their host trees, microorganisms, and associated insects for a system superbly
499 suited for studying the subtlety and diversity of olfactory communication. Am. Sci. **66**: 409-
500 419.
- 501 Bohlmann, Joerg. 2012. Pine terpenoid defences in the mountain pine beetle epidemic and in
502 other conifer pest interactions: specialized enemies are eating holes into a diverse, dynamic
503 and durable defence system. Tree Phys. **00**: 943-945.
- 504 Boone, C.K, B.H. Aukema, J. Bohlmann, A.L. Carroll, K.F. Raffa. 2011. Efficacy of tree
505 defense physiology varies with bark beetle population density: a basis for positive feedback
506 in eruptive species. Can. J. For. Res. **41**: 1174-1188.

- 507 Bryant, C.M., Kulhavy, D.L., Billings, R., and Clarke, S.R. 2006. Characteristics of bark beetle
508 infestations in east Texas during a period of low southern pine beetle activity. *Southwest*
509 *Entomol.* **31**(3): 187-199.
- 510 Christiansen, E., Krokene, P., Berryman, A.A., Franceschi, V.R., Krekling, T., Lieutier, F.,
511 Lonneborg, A., and Solheim, H. 1999. Mechanical injury and fungal injection induce
512 acquired resistance in Norway spruce. *Tree Physiol.* **19**: 399-403.
- 513 Clarke, S. R., R. E. Evans, and R. F. Billings. 2000. Influence of pine bark beetles on the west
514 gulf coastal plain. *Tex. J. Sci.* **52**(4) Supplement: 105-126.
- 515 Clarke, S.R., and Nowak, J.T. 2009. Southern pine beetle. Forest and Disease Leaflet 49. US
516 Forest Service. Washington, DC.
- 517 Cognato, A.I. 2014. Biology, systematics, and evolution of *Ips*, pp 351-370. *In* Vega, F.E.,
518 Hofstetter, R.W. (Eds.), *Bark Beetles: Biology and Ecology of Native and Invasive Species*.
519 Academic Press. London, UK.
- 520 Coleman M.D., Coyle, D.R., Blake, J., Britton, K., Buford, M., Campbell, B., Cox, J., Cregg, B.,
521 Daniels, D., Jacobson, M., Johnson, K., McDonald, T., McLeod, K., Nelson, E., Robison, D.,
522 Rummer, R., Sanchez, P., Stanturf, J., Stokes, B., Trettin, C., Tuskan, J., Wright, L., and
523 Wullschleger, S. 2004. Production of Short-Rotation Woody Crops Grown with a Range of
524 Nutrient and Water Availability: Establishment Report and First Year Responses. USDA
525 Forest Service Southern Research Station. Asheville, NC.
- 526 Coley, P.D. 1987. Interspecific variation in plant anti-herbivore properties: the role of habitat
527 quality and rate of disturbance. *New Phytol.* **106**: 251-263.
- 528 Connor, M.D., and Wilkerson, R.C. 1983. *Ips* Bark Beetles in the South. Forest Insect &
529 Disease Leaflet 129. US Foerst Service. Washington, DC.

- 530 Coulson, R.N., and Meeker, J.R. 2011. Social and Political Impact of the Southern Pine Beetle,
531 pp. 235-243. *In* Coulson, R.N., and Klepzig, K.D. (eds.). Southern Pine Beetle II, General
532 Technical Report-SRS-140. U.S. Department of Agriculture, Forest Service, Southern
533 Research Station, Asheville, NC.
- 534 Cudmore, T.J., Björklund, N., Carroll, A.L, and Lindgren B.S. 2010. Climate change and range
535 expansion of an aggressive bark beetle: evidence of higher beetle reproduction in naïve host
536 tree populations. *Journal of Applied Ecology*. **47**(5): 1036-1043.
- 537 Drooz, A. 1985. Insects of eastern forests. United States Dept. of Agriculture Forest Service.
538 Washington, DC.
- 539 Endara, M.J. and Coley, P.D. 2011. The resource availability hypothesis revisited: a meta-
540 analysis. *Func. Ecol.* **24**:389-398.
- 541 Fettig, C. J., K. D. Klepzig, R. F. Billings, A. S. Munson, T. E. Nebeker, J. F. Negrón, and J. T.
542 Nowak. 2007. The effectiveness of vegetation management practices for prevention and
543 control of bark beetle infestations in coniferous forests of the western and southern United
544 States. *For. Ecol. Manage.* 238: 24-53
- 545 Fischer, M.J., Waring, K.M., Hofstetter, R.W., and Kolb, T.E. 2010. Ponderosa pine
546 characteristics associated with attack by the round headed pine beetle. *For. Sci.* 56 (5):473-
547 483.
- 548 Franceschi, V.R., Krokene, P., Christiansen, E., and Krekling, T. 2005. Anatomical and
549 chemical defense of conifer bark against bark beetle and other pests. *New Phytol.* **167**: 353-
550 376.
- 551 Gan, J. 2004. Risk and damage of southern pine beetle outbreaks under global climate change.
552 *For. Ecol. Manage.* **191**: 61-71.

- 553 Gaylord, M.L., Kolb, T.E., Wallin, K.F., and Wagner, M.R. 2007. Seasonal dynamics of
554 tree growth, physiology, and resin defenses in a northern Arizona ponderosa pine forest. *Can.*
555 *J. For. Res.* **37**: 1173-1183.
- 556 Gaylord, M.I., Kolb, T.E., Pockman, W.T., Plaut, J.A., Yopez, E.A., Macalady, A.K.,
557 Pangle, R.E., and McDowell, N.G. 2013. Drought predisposes pinyon-juniper woodlands to
558 insect attacks and mortality. *New Phytol.* **198**: 567-578.
- 559 Hain, F. P., Duehl, A.J., Gardner, M.J., and T.L. Payne. 2011. Natural History of the Southern
560 Pine Beetle. In: Coulson, R.N., Klepzig, K.D. (eds.). *Southern Pine Beetle II, General*
561 *Technical Report-SRS-140*. U.S. Department of Agriculture, Forest Service, Southern
562 Research Station, Asheville, NC, pp. 13-24.
- 563 Herms, D.A., and Mattson, W.J. 1992. The dilemma of plants: to grow or defend. *Quart. Rev.*
564 *Biol.* **67**: 283-335.
- 565 Hodges, J.D., and Lorio, P.L. Jr. 1975. Moisture Stress and Composition of Xylem Oleoresin in
566 Loblolly Pine. *Forest Sci.* **21**: 283-290.
- 567 Hodges, J.D., Elam, W.W., Watson, W.F., and Nebeker, T.E. 1977. Physical properties of the
568 oleoresin system of the four major southern pines. *Can. Entomol.* **7**: 520-525.
- 569 Hodges, J.D., Elam, W.W., Watson, W.F., and Nebeker, T.E. 1979. Oleoresin characteristics
570 and susceptibility of four southern pines to southern pine beetle (Coleoptera:scolytidae)
571 attacks. *Can. Ent.* **111**: 889-896.
- 572 Klepzig, K.D., Kruger, E.L., Smalley, E.B., and Raffa, K.F. 1995. Effects of biotic and abiotic
573 stress on induced accumulation of terpenes and phenolics in red pines inoculated with bark
574 beetle-vectored fungus. *J. Chem Ecol.* **21**(5): 601-626.

- 575 Klepzig, K.D., Robison, D.J., Fowler, G., Minchin, P.R., Hain, F.P., and Allen, H.L. 2005.
576 Effects of mass inoculation on induced oleoresin response in intensively managed loblolly
577 pine. *Tree Physiol.* **25**: 681-688.
- 578 Klepzig, K.D. and Hofstetter, R.W. 2011. From attack to emergence: interactions between
579 southern pine beetle, mites, microbes and trees, NC, pp. 141-152. *In*: Coulson, R.N., and
580 Klepzig, K.D. (eds.). Southern Pine Beetle II, General Technical Report-SRS-140. U.S.
581 Department of Agriculture, Forest Service, Southern Research Station, Asheville.
- 582 Knebel, L., Robison, D.J., Wentworth, T.R. and Klepzig, K.D. 2008. Resin flow responses to
583 fertilization, wounding and fungal inoculation in loblolly pine (*Pinus taeda*) in North
584 Carolina. *Tree Physiol.* **28**: 847-853.
- 585 Lesk, C., Coffel, E., D'Amato, A.W., Dodds, K., and Horton, R. 2017. Threats to North
586 American forests from southern pine beetle with warming winters. *Nat. Clim. Change* **7**: 713-
587 718.
- 588 Lombardero, M.J., Ayres, M.P., Lorio, P.L. Jr., and Ruel, J.J. 2000. Environmental effects on
589 constitutive and inducible resin defenses of *Pinus taeda*. *Ecol. Lett.* **3**: 329-339.
- 590 Lombardero, M.J., Ayres, M.P., and Ayres, B.D. 2006. Effects of fire and mechanical wounding
591 on *Pinus resinosa* resin defenses, beetle attacks, and pathogens. *For. Ecol. Manage.* **225**:
592 349-358.
- 593 Lorio, P.L. Jr., and Sommers, R.A. 1986. Evidence of competition for photosynthates between
594 growth processes and oleoresin synthesis in *Pinus taeda* L. *Tree Physiol.* **2**: 301-306.
- 595 Luchi, N., Ma, R., and Capretti, P. 2005. Systemic induction of traumatic resin ducts and resin
596 flow in Austrian pine by wounding and inoculation with *Sphaeropsis spinea* and *Diplodia*
597 *scrobiculata*. *Planta* **221**: 75-84.

- 598 Martin, D., Tholl, D., Gershenzon, J. and Bohlmann, J. 2002. Methyl jasmonate induces
599 traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing
600 xylem of Norway spruce stems. *Plant Physiol.* **129**: 1003–1018.
- 601 Mason, C.J., Villari, C., Keefover-Ring, K., Jagemann, S., Zhu, J., Bonello, P., and Raffa, K.F.
602 2017. Spatial and temporal components of induced plant responses in the context of
603 herbivore life history and impact on host. *Funct. Ecol.* **00**: 1-17. DOI: 10.1111/1365-
604 2435.12911
- 605 Meddens, A.J., Hicke, J.A., and Ferguson, C.A. 2012. Spatiotemporal patterns of observed bark
606 beetle caused tree mortality in British Columbia and the western United States. *Ecol. Appl.*
607 **22**: 1876-1891.
- 608 Moreira, M., Zas, R., Solla, A., and Sampedro, L. 2015. Differentiation of persistent anatomical
609 defensive structures is costly and determined by nutrient availability and genetic growth-
610 defence constraints. *Tree Physiol.* **35**: 112-123.
- 611 Netherer, S., Matthews, B., Katzensteiner, K., Blackwell, E., Henschke, P., Hietz, P.,
612 Pennerstorfer, J., Rosner, S., Kikuta, S., Schume, H., and Schopf, A. 2015. Do water-limiting
613 conditions predispose Norway spruce to bark beetle attack? *New Phytol.* **205**: 1128-1141.
- 614 Novick, K.A., Katul, G.G., McCarthy, H.R., and Oren, R. 2012. Increased resin flow in
615 mature pine trees growing under elevated CO₂ and moderate soil fertility. *Tree Physiol.* **32**:
616 752-763.
- 617 Paine, T.D., Birch, M.C., and Svihra, P. 1981. Niche breadth and resource partitioning by four
618 sympatric species of bark beetles (Coleoptera: Scolytidae). *Oecologia* **48**: 1-6.
- 619 Phillips, M.A., and Croteau, R.B. 1999. Resin-based defenses in conifers. *Trends Plant Sci.* **4**(5):
620 184-190.

- 621 Price, J.I., McCollum, D.W., and Berrens, R.P. 2010. Insect infestation and residential property
622 values: a hedonic analysis of the mountain pine beetle epidemic. *For. Pol. Econ.* **12**: 415-422.
- 623 Pye, J.M., Holmes, T.P., Prestemon, J.P., and Wear, D.N. 2011. Economic impacts of the
624 southern pine beetle, pp. 213-234. *In*: Coulson, R.N., and Klepzig, K.D. (eds.). *Southern Pine
625 Beetle II*. US Forest Service Southern Research Station General Technical Report. SRS-140.
626 US Forest Service Southern Research Station, Asheville, NC.
- 627 Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G., and Romme,
628 W.H. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic
629 amplification: the dynamics of bark beetle eruptions. *BioScience* **58**: 501-517.
- 630 Raffa, K.F., Gregoire, J.C., and Lindgren, B.S. 2014. Natural history and ecology of bark beetles,
631 pp. 1-40. *In* Vega, F.E., and Hofstetter, R.W. (Eds.) *Bark Beetles: Biology and Ecology of
632 Native and Invasive Species*, Academic Press. London, UK.
- 633 Riley, M.A., and Goyer, R.A. 1988. Seasonal abundance of beneficial insects and *Ips* spp.
634 engraver beetles in felled loblolly and slash pines in Louisiana. *J. Entomol. Sci.* **23**: 357-365.
- 635 Ruel, J.J., Ayers, M.P., and Lorio, P.L. Jr. 1998. Loblolly pine responds to mechanical
636 wounding with increased resin flow. *Can. J. For. Res.* **28**: 596-602.
- 637 Sampedro, L., Moreira, X., Llusia, J., Penuelas, J., and Zas, R. 2010. Genetics, phosphorus
638 availability, and herbivore-derived induction as sources of phenotypic variation of leaf
639 volatile terpenes in a pine species. *Journal of Experimental Botany* **61**: 4437-4447.
- 640 Schowalter, T. D. 2012. Ecology and management of bark beetles (Coleoptera: Curculionidae:
641 Scolytinae) in southern pine forests. *J. Integ. Pest Manage.* **3**(2): 7 pp.

- 642 Schlyter, F., Bigersson, G., Byers, J.A., Lofqvist, J., and G. Bergstrom, G. 1987. Field
643 response of spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates. J.
644 Chem. Ecol. **13**: 701-716.
- 645 Schlyter, F. and Anderbrant, O. 1989. Mass attack of trees by *Ips typographus* induced by sex-
646 specific pheromone: a model of attack dynamics. Holarctic Ecol. **12**: 415-426.
- 647 van Akker, L., Alfaro, R.I., and Brockley, R. 2004. Effects of fertilization on resin canal
648 defences and incidence of *Pissodes strobi* attack in interior spruce. Can. J. For. Res. **34**: 855-
649 862.
- 650 Vite, J.P., and Pitman, G.B. 1969. Aggregation behaviour of *Dendroctonus brevicomis* in
651 response to synthetic pheromones. J. Insect Physiol. **15**(9):1617-1622.
- 652 Wallin, K.F., Kolb, T.E., Skov, K.R., and Wagner, M.R. 2003. Effects of crown scorch on
653 Ponderosa pine resistance to bark beetles in Northern Arizona. Env. Entomol. **32**(3): 652-
654 661.
- 655 Waring, R.H., and Pitman, G.B. 1983. Physiological stress in lodgepole pine as a precursor for
656 mountain pine beetle attack. J. Applied Entomol. **96**: 265-270.
- 657 Warren, J.M., Allen, H.L., and Booker, F.L. 1999. Mineral nutrition, resin flow and phloem
658 phytochemistry in loblolly pine. Tree Physiol. **19**: 655-663.
- 659 Werner, R.A., Holsten, E.H., Matsuoka, S.M, and Burnside, R.E. 2006. Spruce beetles and
660 forest ecosystems in south-central Alaska: a review of 30 years of research. For. Ecol.
661 Manage. **227**: 195-206.
- 662 Wood, D.L. 1982. The role of pheromones, kairomones, and allomones in the host
663 selection and colonization behavior of bark beetles. Annu. Rev. Entomol. **27**: 411-446.

664 Table 1. Weather, abiotic variables, stand conditions, and experimental details at the two study
 665 sites used in this study.

	Study Sites	
	SRS†	SETRES-II*
County and State	Aiken, SC	Scotland, NC
Coordinates	33 ° N, 81 ° E	34 ° N, 79 ° W
Annual temp. average	18 ° C	17 ° C
Physiographic Region	Aiken Plateau Sandhills	Georgia/ Carolina Sandhills
Climate	Humid Continental: warm summers, mild winters	Humid continental: warm summers, mild winters
Soil Series	Blanton	Wakulla
Annual Precip. average	1320 mm	1210 mm
DBH (mean(SD) cm)	19.75 (3.18)	16.79 (3.87)
Age	12	19
Fertilizer Treatment	120 kg N ha ⁻¹ yr ⁻¹ †	79 N, 8 P, 8 K, 0.6 Ca, 5 Mg, and 15 S kg ha ⁻¹ yr ⁻¹ ‡
Spacing	2.5 × 3 m	1.5 × 2.1 m
Tree Density	1,333 trees/ha	3,076 trees/ha
Plot Size	0.22 ha	0.25 ha
Bait Deployment	7 June 2012	7 April 2013
Resin Flow Samples	Day 1, 7, 25, 62, and 86 after bait deployment	Day 1, 15, 48, 92, 108, and 117 after bait deployment
Sticky Collections	Day 10, 17, and 22 post bait deployment	Day 48 and 92 post bait deployment
Average Holes	~16	~36
Duration	86 days	117 days

666 * Albaugh et al. 1998

667 † Coleman et al. 2004

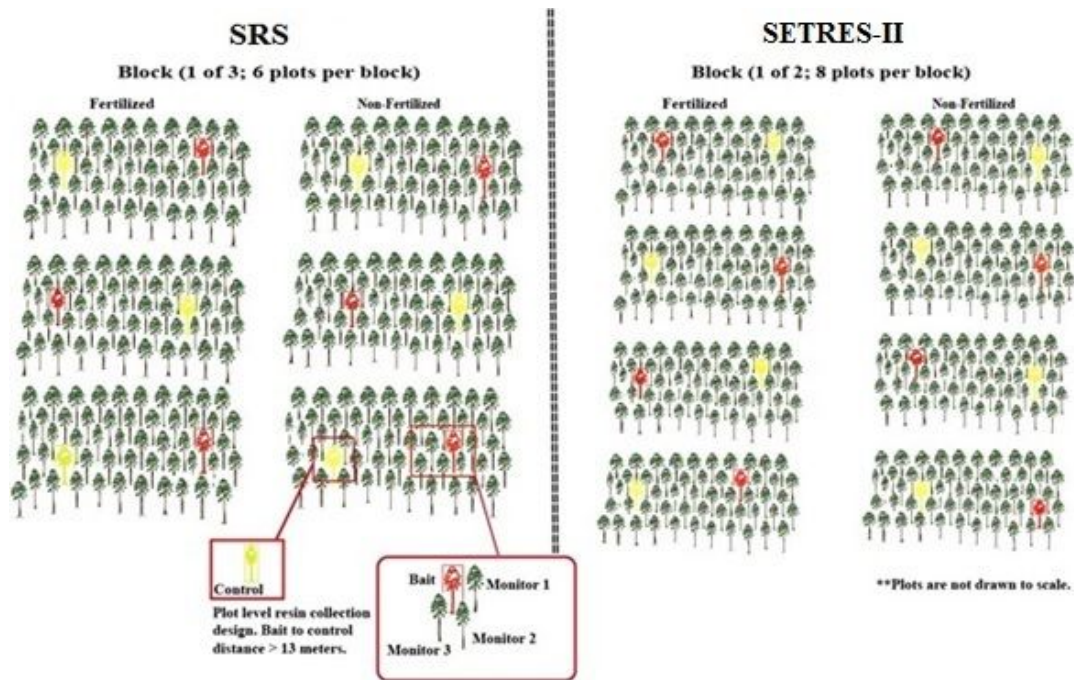
668 ‡ From 1994 to 2007, annual fertilizer treatments varied with this average nutrient application (S.
 669 McKeand, personal communications, 2012)

670

671 Table 2. *Ips* spp. sticky trap collections at the two study sites: SRS (2012) and SETRES-II
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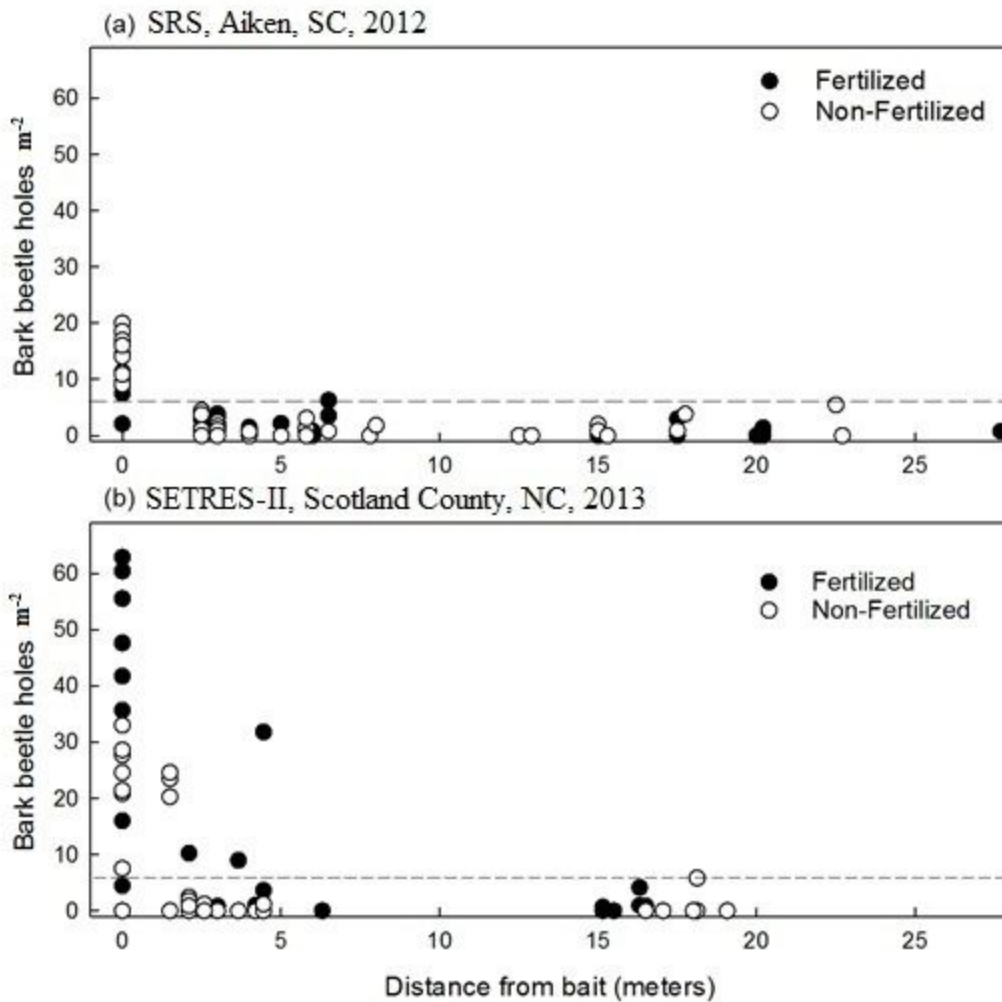
Mean and total number of <i>Ips</i> caught					
Adult Beetles, Mean (SE)					
SRS	Tree Bait	Dates	<i>Species</i>		
			<i>Ips avulsus</i>	<i>Ips calligraphus</i>	<i>Ips grandicollis</i>
		June 8-14	36.1 (4.23)	0.5 (0.15)	1.53 (0.24)
		June 15-22	27.2 (3.08)	1.17 (0.98)	3.08 (0.35)
		June 22-25	8.02 (1.39)	0.33 (0.38)	2.11 (0.40)
	Control	June 8-14	0.03 (0.12)	0 (0)	0 (0)
		June 15-22	0 (0)	0 (0)	0 (0)
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SETRES-II					
	Bait	7 April- 23 May	3.76 (0.47)	8.29(0.87)	1.76(0.25)
		24 May- 7 July	7.06(1.59)	21.41 (3.88)	5.82 (1.08)
	Control	7 April- 23 May	0.18 (0.09)	0.29 (0.14)	0.06(0.06)
		24 May- 7 July	0.06(0.06)	0.53 (0.15)	0 (0)
Adult beetles, Total					
SRS			2567	72	242
SETRES- II			173	524	129

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676 Figure 1. Experimental design for Savannah River Site (SRS), 2012 (left) and Southeastern Tree
677 Research and Education Site II (SETRES-II), 2013 (right).



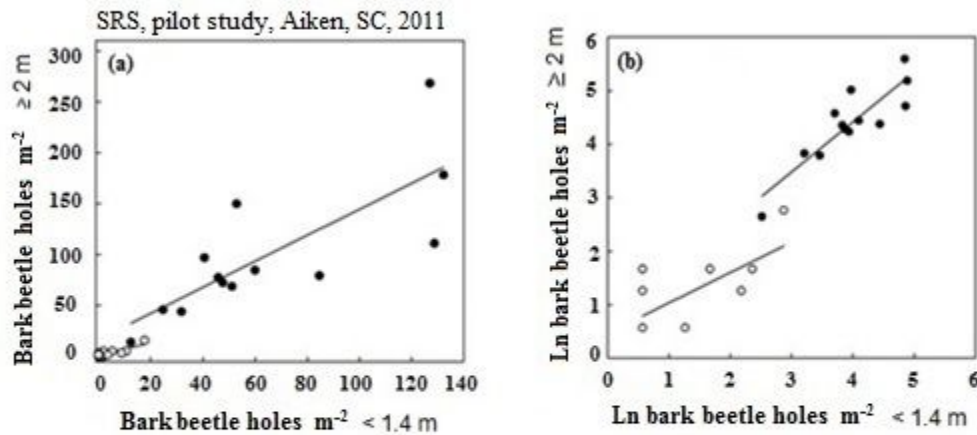
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679 Figure 2. Bark beetle holes in relation to distance (m) from pheromone baits at (a) SRS and (b)
 680 SETRES-II for non-fertilized (open circles) and fertilized (closed circles) trees.

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686 Figure 3. Regression for insect holes observed below 1.4 m and above 2 m using data from our

687 pilot study. Insect holes for live (open circles) and dead (solid circles) trees (a) ($R^2 = 0.88$; A_{high} 688 $= 1.45 A_{\text{low}} + 0.54$; $P < 0.01$); log transformation of insect holes for live and dead trees (b) ($R^2 =$ 689 0.90 ; $A_{\text{high}} = 1.10 A_{\text{low}} - 0.091$; $P < 0.01$).

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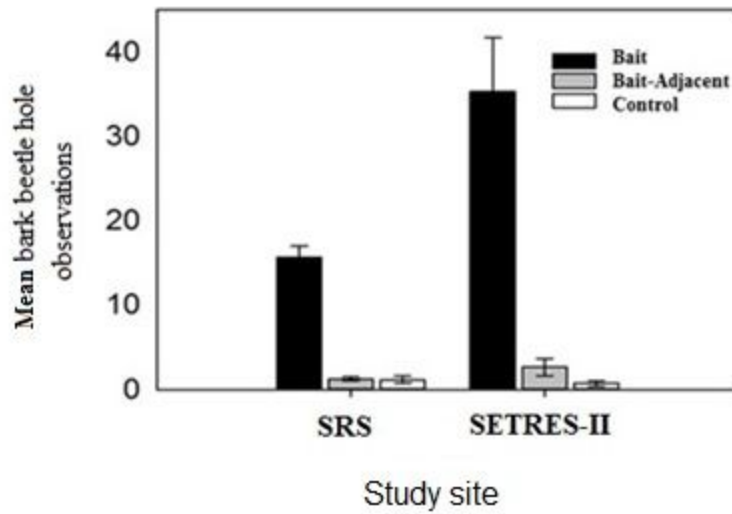
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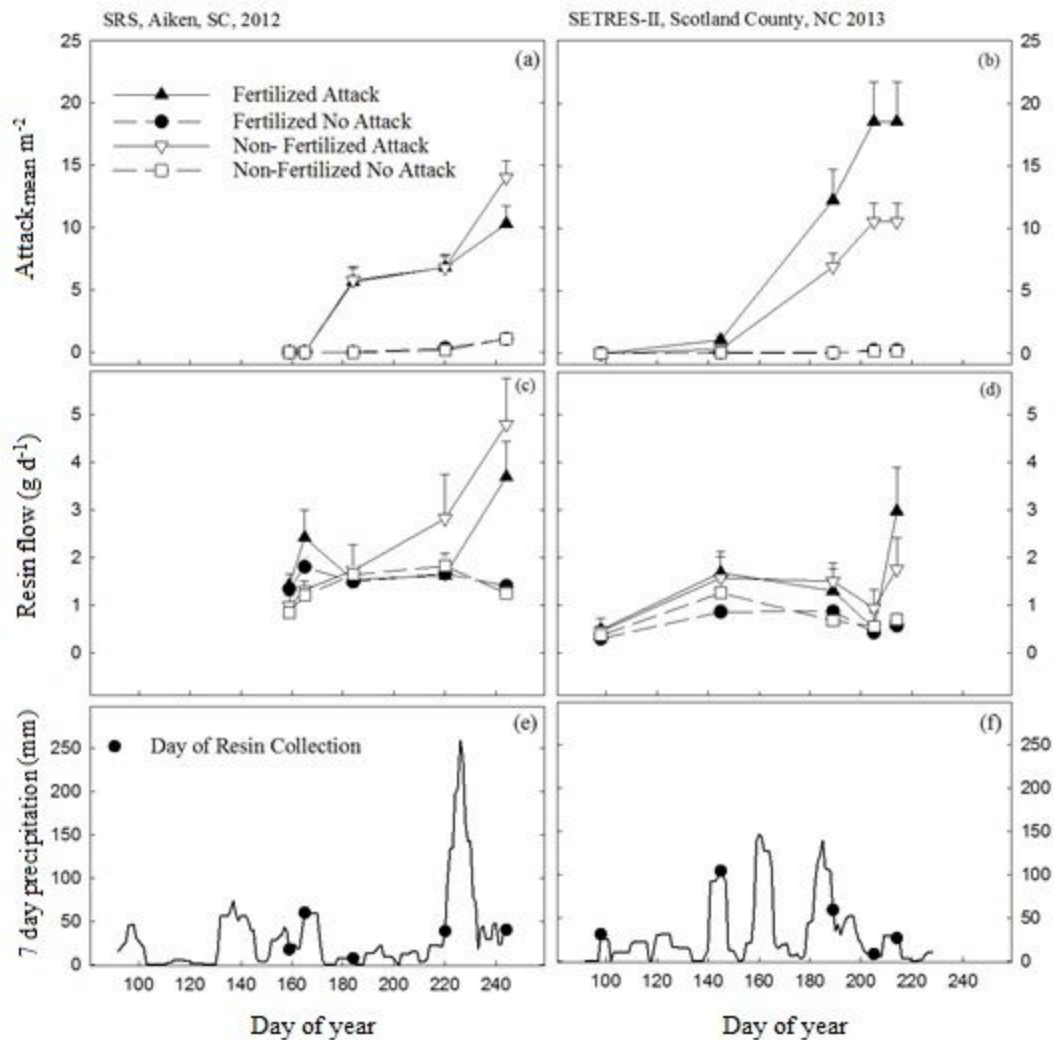
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708 Figure 4. Bark beetle bark beetle holes observed along the stem of trees sampled for resin flow.
709 Baited trees experienced significantly more holes than adjacent or control trees, highlighting the
710 localized nature of the bark beetles being the primary wounding agent for this study.

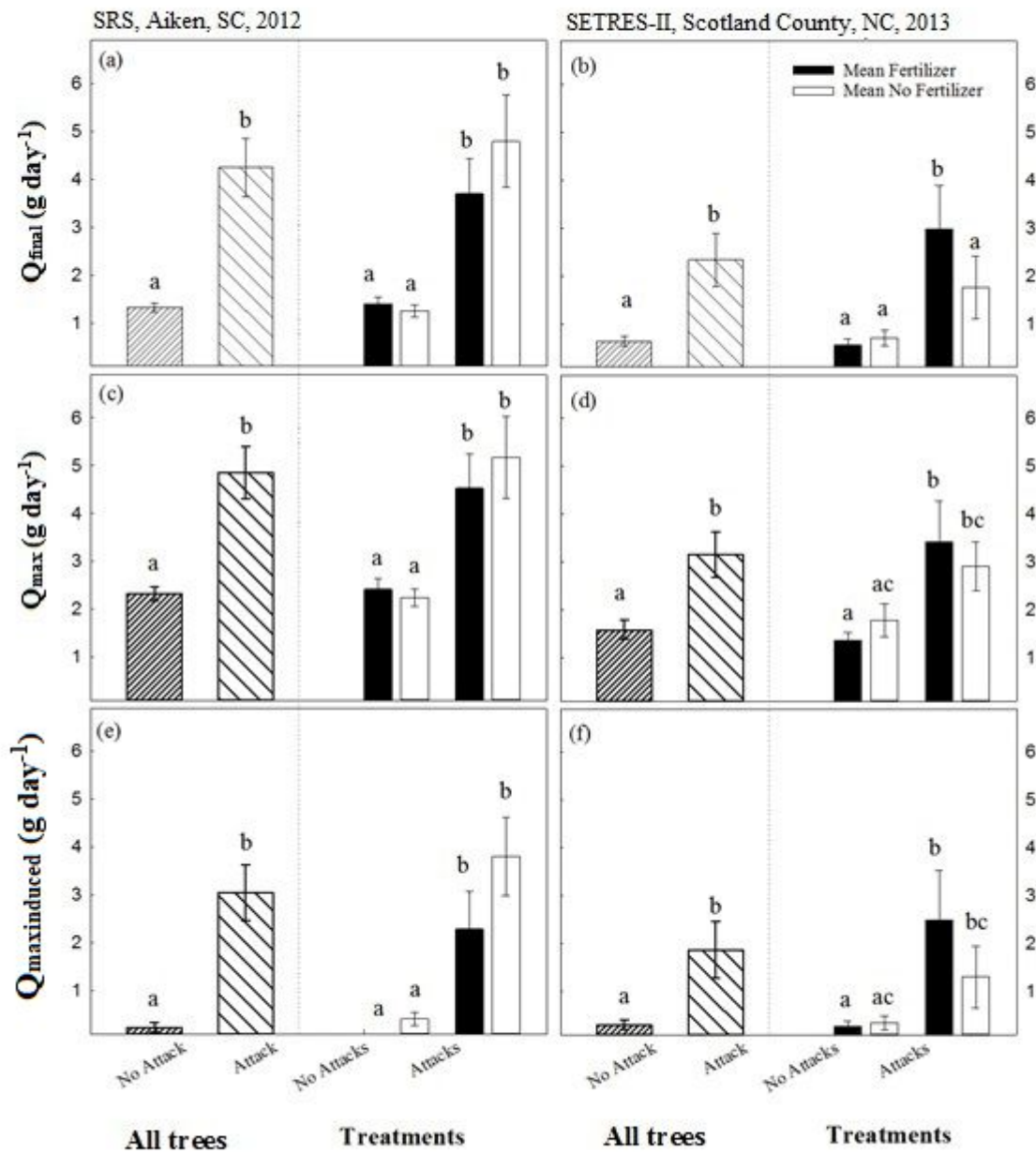
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727 Figure 5. Mean bark beetle attack per square meter at SRS (a), and SETRES-II (b), Mean resin
 728 flow by site treatment for non-attacked and attacked trees at SRS (c) and SETRES-II (d); Total of
 729 prior 7 day precipitation totals for SRS (e) and SETRES-II (f).

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731
 732 Figure 6. Q_{final} at SRS (a) and SETRES-II (b), Q_{max} at SRS (c) and SETRES-II (d), $Q_{maxinduced}$ at
 733 SRS (e) and SETRES-II (f). All trees non-attacked (fine stripes) and attacked (thick stripes).

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Table 1. Weather, abiotic variables, stand conditions, and experimental details at the two study sites used in this study.

	Study Sites	
	SRS†	SETRES-II*
County and State	Aiken, SC	Scotland, NC
Coordinates	33 ° N, 81 ° E	34 ° N, 79 ° W
Annual temp. average	18 ° C	17 ° C
Physiographic Region	Aiken Plateau Sandhills	Georgia/ Carolina Sandhills
Climate	Humid Continental: warm summers, mild winters	Humid continental: warm summers, mild winters
Soil Series	Blanton	Wakulla
Annual Precip. average	1320 mm	1210 mm
DBH (mean(SD) cm)	19.75 (3.18)	16.79 (3.87)
Age	12	19
Fertilizer Treatment	120 kg N ha ⁻¹ yr ⁻¹ †	79 N, 8 P, 8 K, 0.6 Ca, 5 Mg, and 15 S kg ha ⁻¹ yr ⁻¹ ‡
Spacing	2.5 × 3 m	1.5 × 2.1 m
Tree Density	1,333 trees/ha	3,076 trees/ha
Plot Size	0.22 ha	0.25 ha
Bait Deployment	7 June 2012	7 April 2013
Resin Flow Samples	Day 1, 7, 25, 62, and 86 after bait deployment	Day 1, 15, 48, 92, 108, and 117 after bait deployment
Sticky Collections	Day 10, 17, and 22 post bait deployment	Day 48 and 92 post bait deployment
Average Holes	~16	~36
Duration	86 days	117 days

* Albaugh et al. 1998

† Coleman et al. 2004

‡ From 1994 to 2007, annual fertilizer treatments varied with this average nutrient application (S. McKeand, personal communications, 2012)

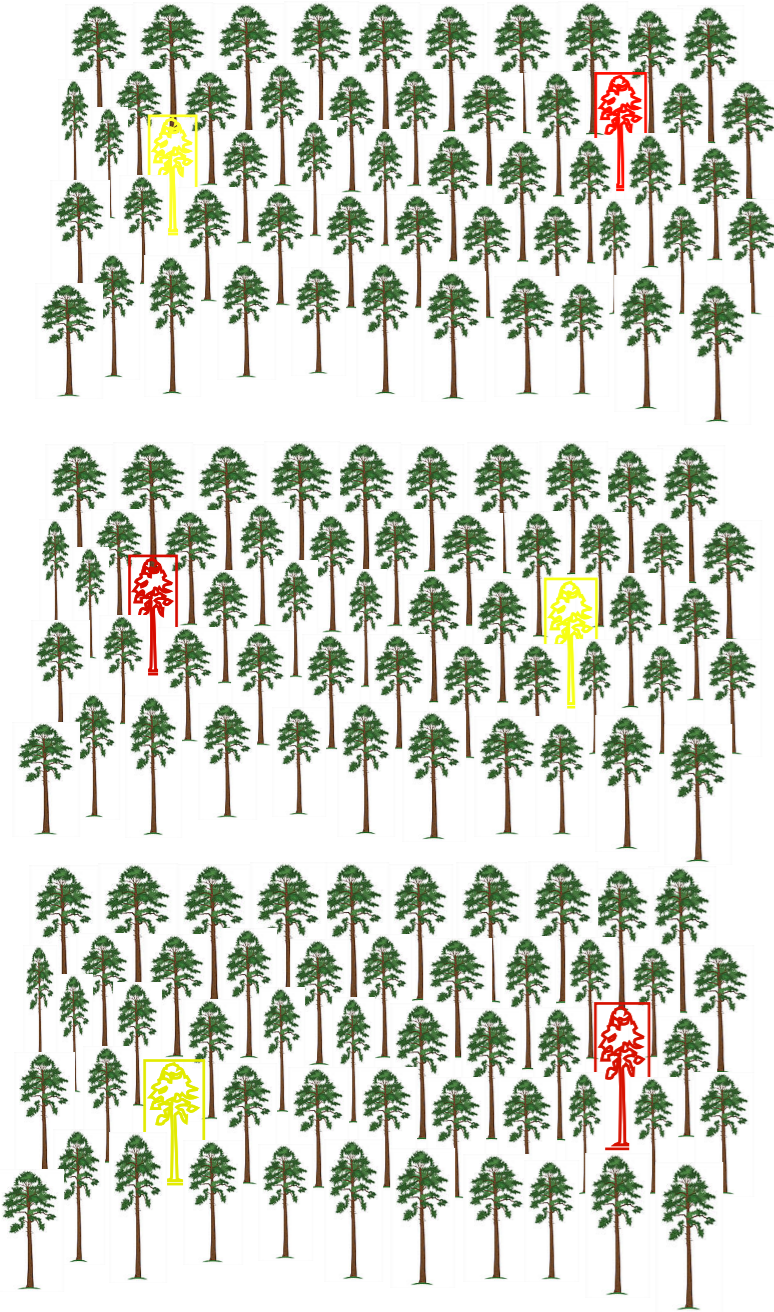
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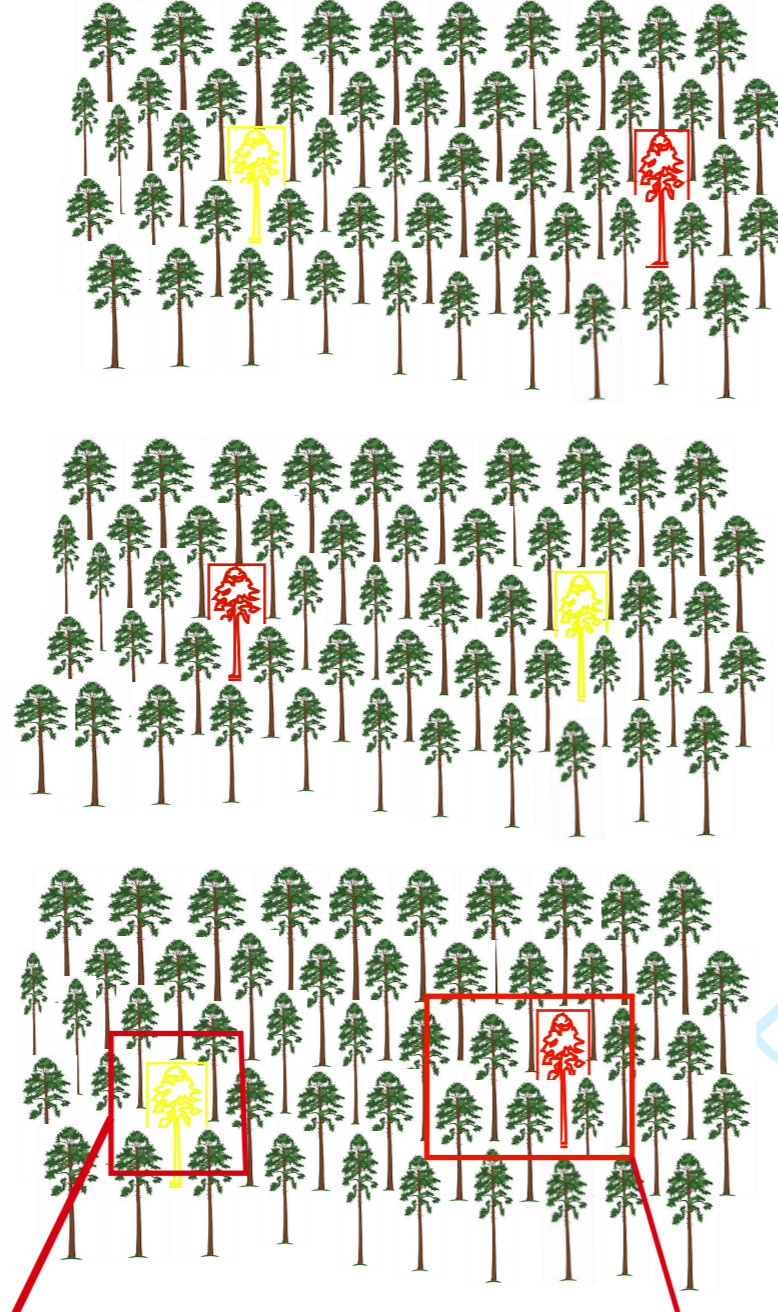
SRS

Block (1 of 3; 6 plots per block)

Fertilized

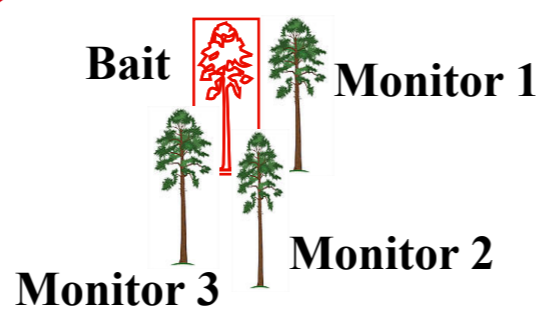


Non-Fertilized



Control

Plot level resin collection design. Bait to control distance > 13 meters.

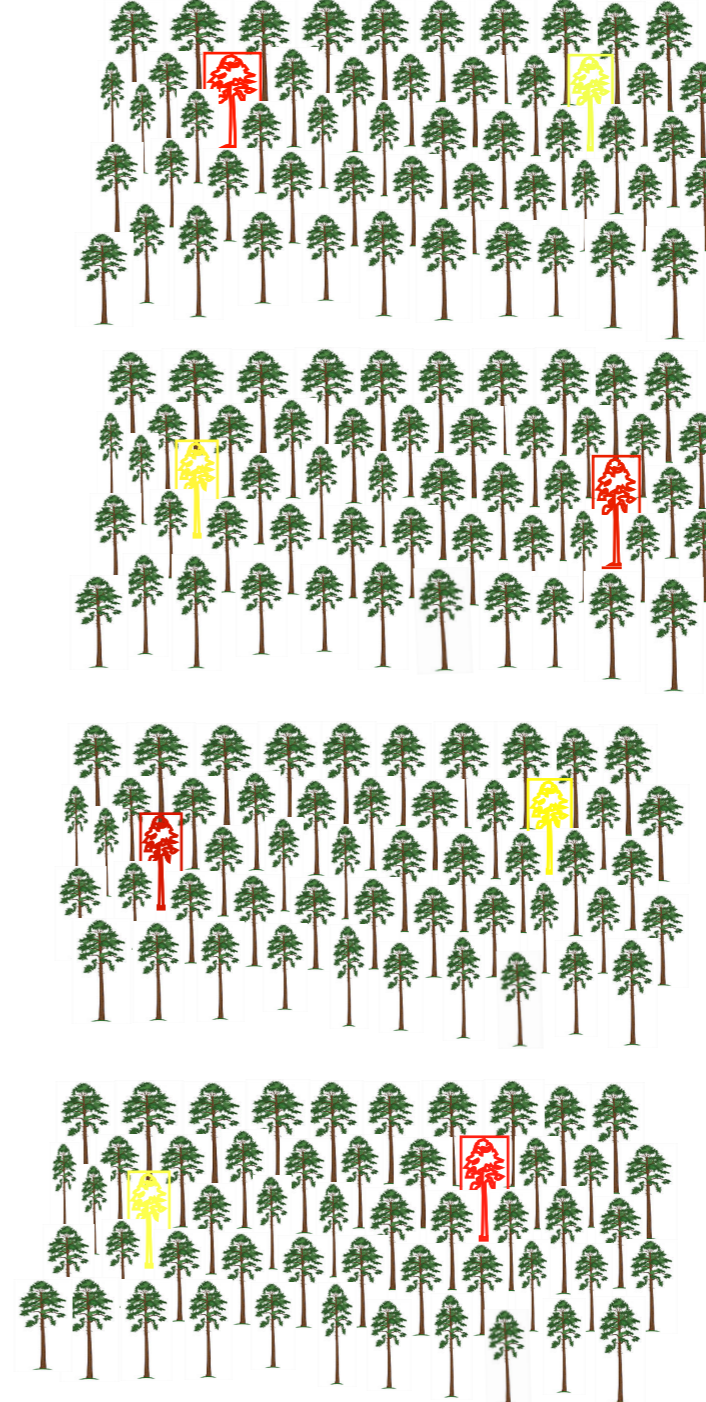


Bait **Monitor 1**
Monitor 2 **Monitor 3**

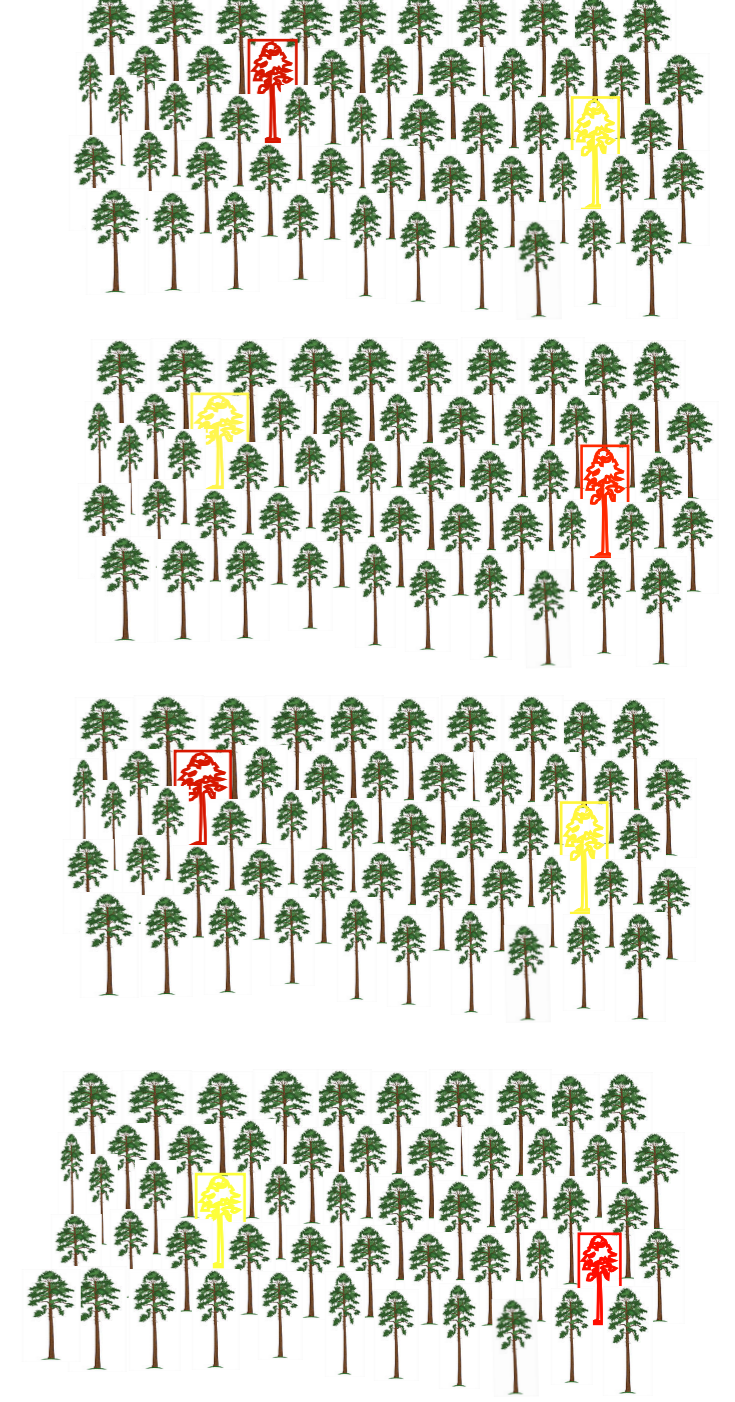
SETRES-II

Block (1 of 2; 8 plots per block)

Fertilized

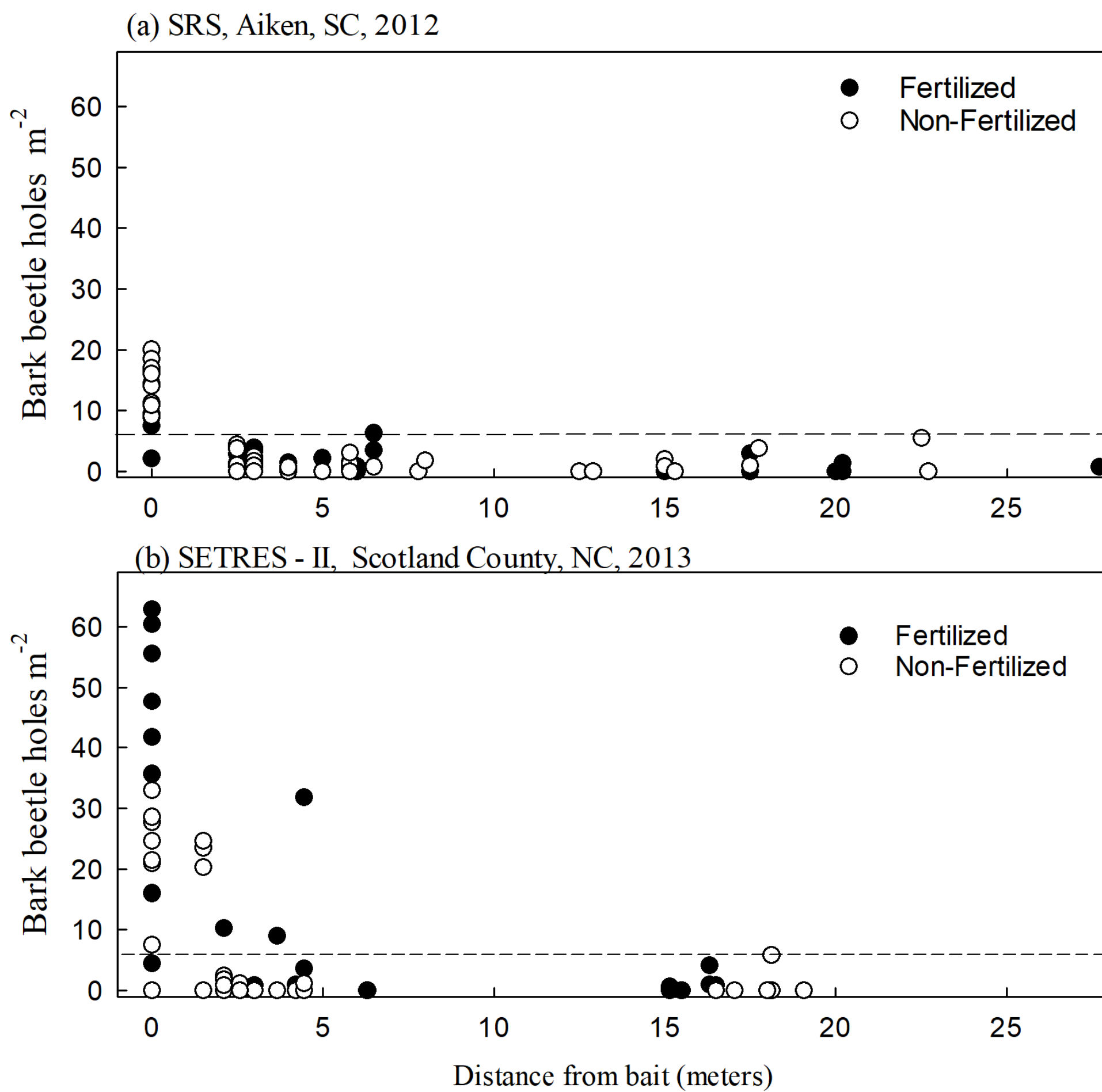


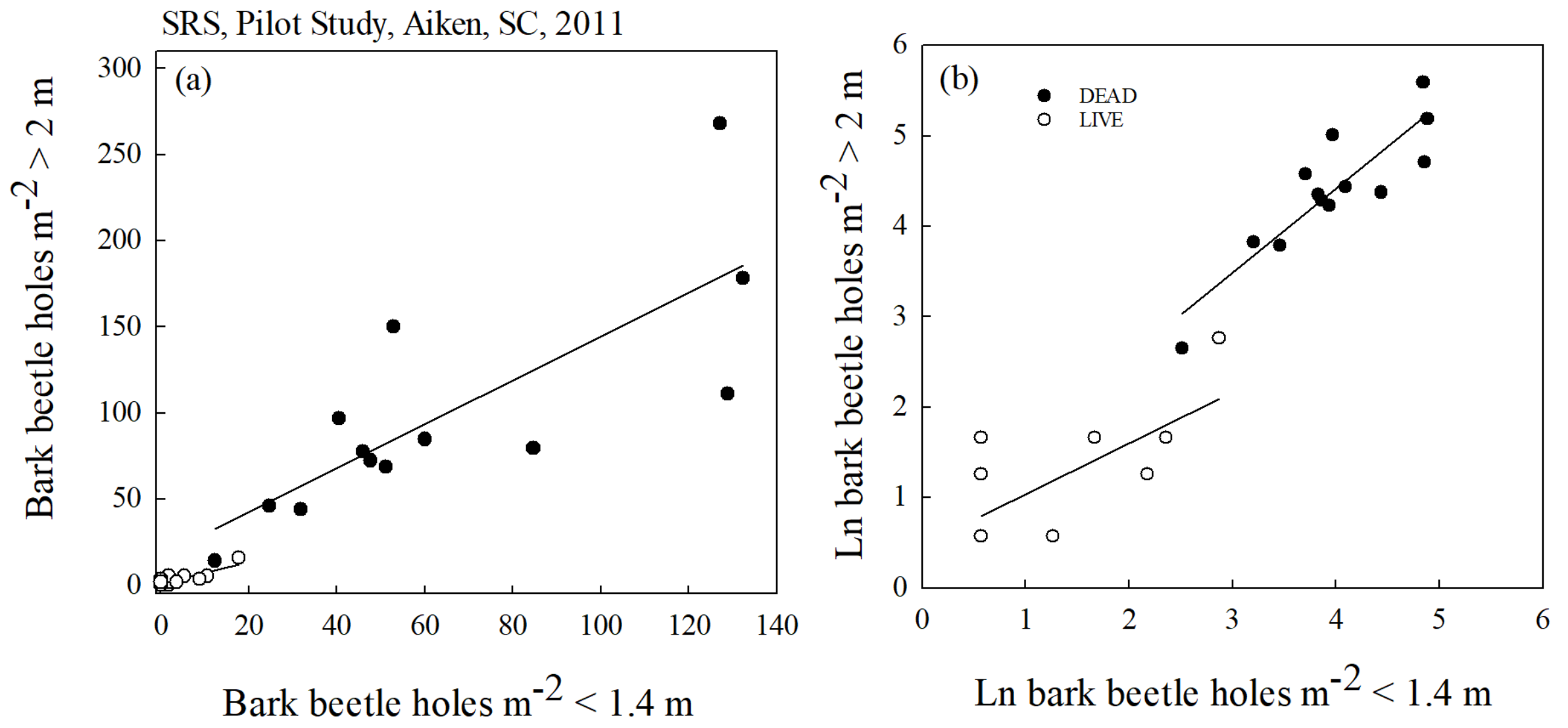
Non-Fertilized

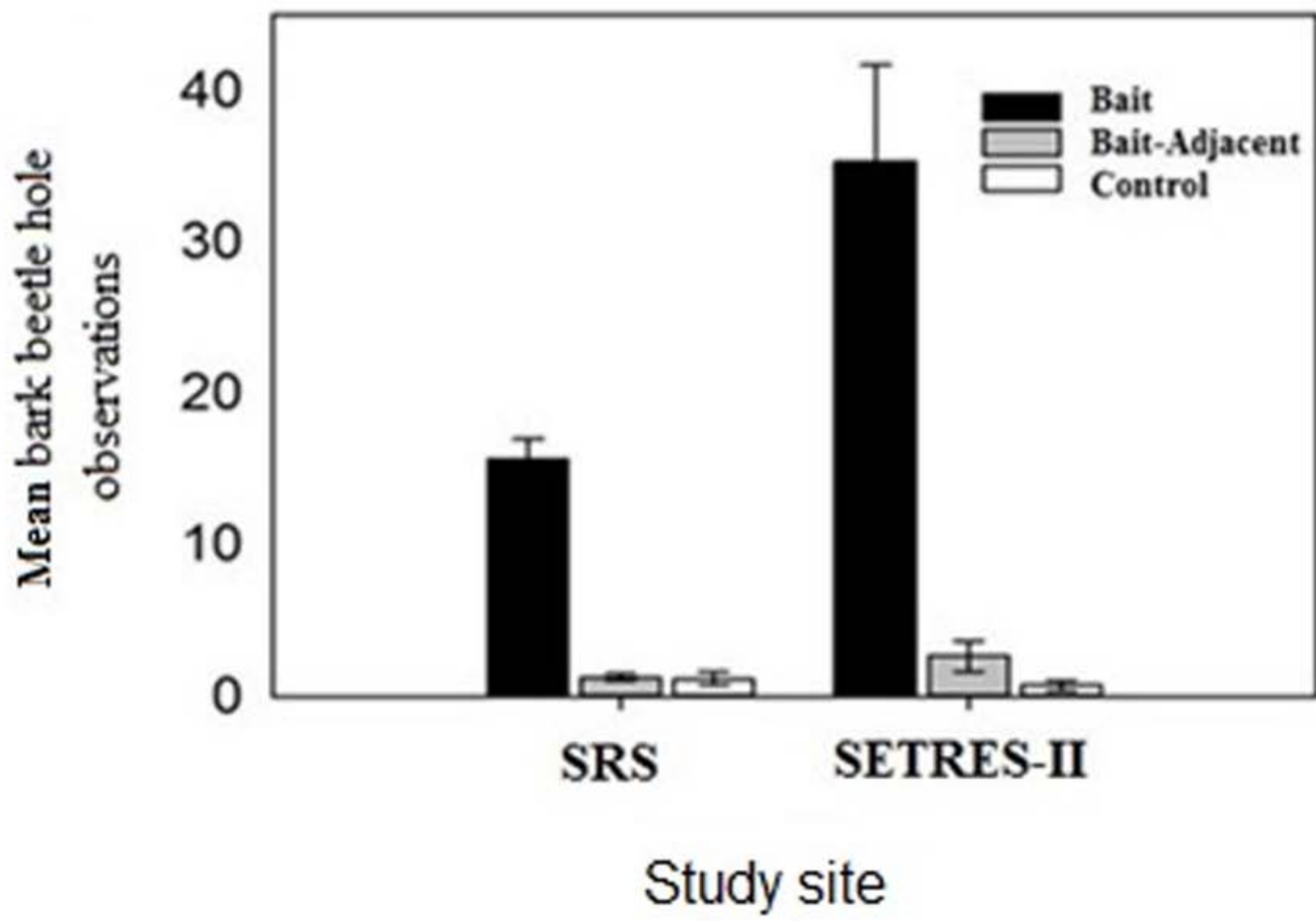


****Plots are not drawn to scale.**

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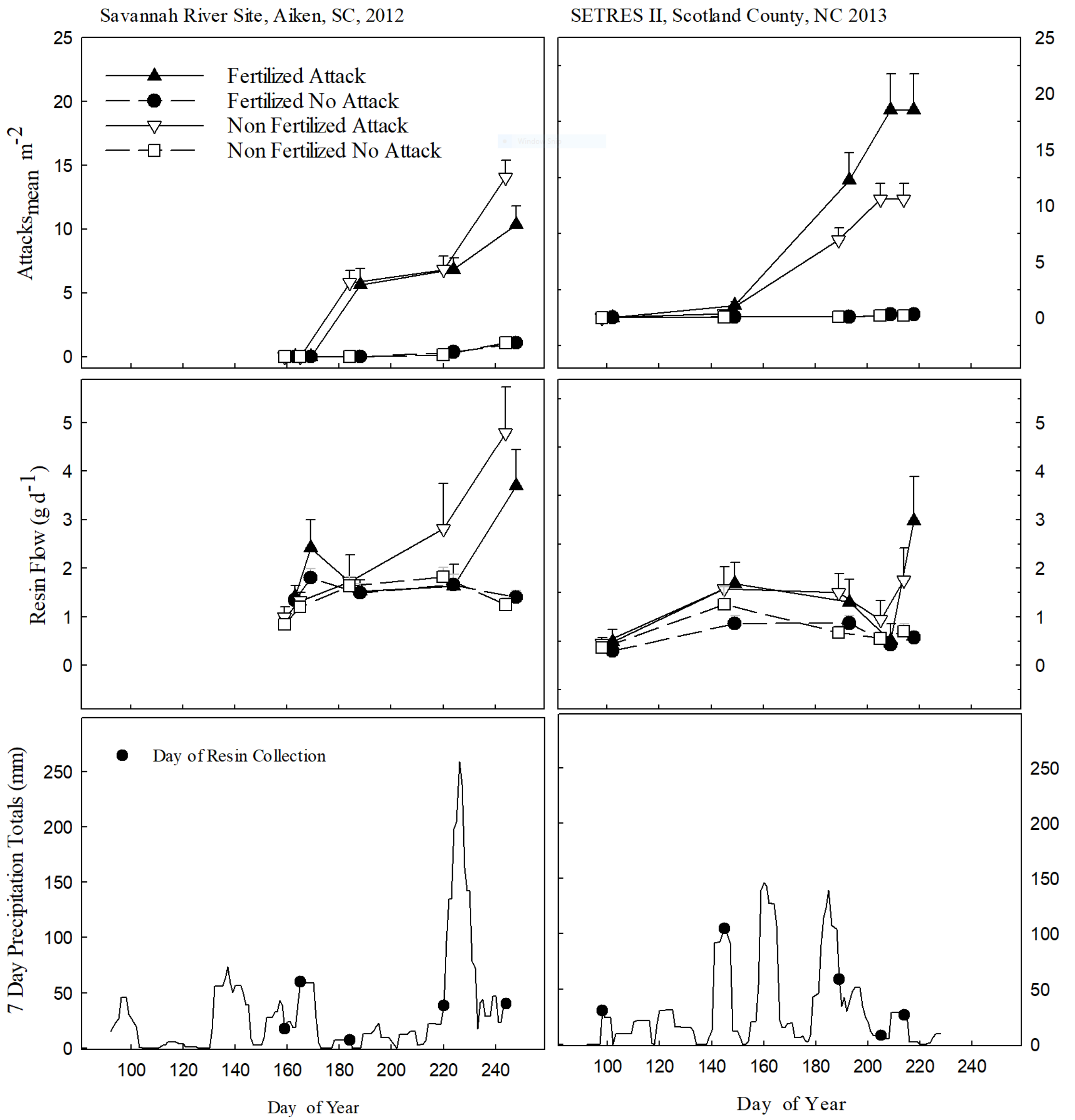






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