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Running title: Within-host selection by a sessile herbivore

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Abstract

1.   Evolutionary theory predicts that ovipositing females that can actively disperse should select hosts that increase offspring performance. However, for organisms that are exclusively passively-dispersed feeding site selection is only possible at the within-host level. This should be particularly important for their offspring which have strong temporal and spatial dispersal constraints within a host. Such constraints will be likely magnified by tissue quality heterogeneity caused by competing herbivores.

2.   We investigated within-host feeding site selection of passively-dispersed neonates of a sessile herbivore, the hemlock woolly adelgid (*Adelges tsugae* Annand) when it shares its eastern hemlock (*Tsuga canadensis* Carrière) host, with another sessile herbivore, the elongate hemlock scale (*Fiorinia externa* Ferris). Within-host feeding site selection was studied at the (a) shoot level (with or without *F. externa*) using choice tests and (b) needle level by scoring insect presence in field surveys.

3.   *A. tsugae* avoided *F. externa*-colonized foliage in both the choice tests and field surveys. Since *A. tsugae* has no efficient predators or parasitoids in the study area, we conclude that our results are due to the presence of the competing herbivore.

4.   Even though *A. tsugae* cannot actively disperse among hosts, we showed that within-host feeding site selection is an important mechanism to minimize the co-occurrence with a competing herbivore that has known negative impacts on *A. tsugae* population densities. Studying within-host feeding site selection in a multiple-species context could assist in understanding and predicting the impact of destructive pests such as *A. tsugae* and the co-evolution with their novel hosts.

24   **Introduction**

25   Evolutionary theory predicts that ovipositing females capable of discriminating between

26 hosts should lay eggs on those hosts that increase the performance of their offspring (Thompson,

27 1988; Gripenberg *et al.*, 2010). Most studies on herbivore behavior to date have focused on

28 quality differences at the whole-plant level, comparing adult and/or offspring's preference

29 among different genotypes or phenotypes within a plant species and the relationship between

30 preference and offspring performance (Horner & Abrahamson, 1992; van Leur *et al.,* 2008;

31 Kleine & Müller, 2011; Nyman *et al.,* 2011; Soler *et al*., 2012). Although selecting a high-

32 quality host is an important first step in maximizing the performance of future offspring,

33 assessing multiple hosts is impossible for some organisms such as passively-dispersed

34 herbivores. As a result, the selection of suitable feeding locations for such organisms is only

35 possible at the within-host level. Because of ubiquitous within-plant heterogeneity in food

36 quality (Shelton, 2004) and its potential impact on plant-herbivore interactions (Awmack &

37 Leather, 2002), the ability to select feeding sites within a given host may prove crucial to

38 offspring performance. Evidence shows that herbivores display different preferences between

39 tissues within a host and this can be affected by prior herbivory or other factors extrinsic to the

40 host plant (McAuslane & Alborn, 2000; Anderson & Agrell, 2005; Gómez *et al.,* 2007; 2008). In

41 the case of passively-dispersed organisms, we expect they will be subjected to particularly strong

42 selective pressure to evolve mechanisms to discriminate quality differences within the host plant

43 upon landing. To our knowledge, not much is known about preference of passively-dispersed

44 herbivores and the link to offspring performance.

45   Within a given host, variation in resource quality (and subsequent herbivore preference)

46 can be driven by ontogenetic differences in the foliage. Young foliage is richer in nitrogen and

47   water content (Mattson, 1980) and usually more palatable than older foliage (Coley & Barone,

48   1996; Fenner *et al.*, 1999; Gómez *et al.*, 2008). Variation in within-host resource quality can also

49   be affected by the presence of other conspecific or heterospecific herbivores. Feeding by

50   competing herbivores can change host quality via altered resource concentration and

51   composition and/or the induction of defense compounds (Karban & Baldwin, 1997). These

52   changes can be especially important in interactions between temporally-separated herbivore

53   species. Later-arriving herbivores can experience suboptimal growth and development on a host

54   that has suffered prior herbivory (Inbar *et al.*, 1999; Soler *et al.*, 2007) because changes in

55   foliage quality can persist over extended periods of times (Gómez *et al.*, 2010; Underwood,

56   2012).

57   Within-host feeding site selection is especially important for herbivores whose active

58   dispersal within a host is constrained by spatial and/or temporal limitations (Burstein & Wool,

59   1993). Herbivores with limited spatial mobility across all life stages might experience strong

60   intraspecific competition due to limited resource availability. Similarly, time-limited dispersers,

61   such as those that suffer high mortality rates within hours after hatching (Itioka & Inoue, 1991)

62   will be forced to settle in suboptimal feeding sites if a suitable location is not found after an

63   initial habitat discrimination phase is over (Ward 1987). Such spatial and temporal constraints

64   apply to the hemlock woolly adelgid (*Adelges tsugae* Annand). *A. tsugae* is a destructive non-

65   native hemipteran pest introduced to the eastern United States from Japan. It is currently present

66   in 18 states, covering over 45% of the eastern hemlock's range (Morin *et al*., 2011). In the

67   invaded range, *A. tsugae* feeds exclusively on the native eastern and Carolina hemlocks (*Tsuga*

68   *canadensis* Carrière and *T. caroliniana* Engelm, respectively), killing its hosts in as little as four

69   years (McClure, 1991). Stands of these long-lived and shade-tolerant conifers create cool and

70   moist microclimates, critical habitat for a wide range of plant and animal species (Orwig *et al.*,

71   2012). The invasion by *A. tsugae* is devastating hemlock forests and dramatically altering the

72   surrounding forest ecosystems (Orwig & Foster, 1998; Jenkins *et al.*, 1999; Stadler *et al.*, 2005;

73   Cobb, 2010). Because viable *A. tsugae* neonates ('*crawlers*') in the invaded range are wingless,

74   between-host movement of *A. tsugae* occurs via passive dispersal by wind or animals (McClure,

75   1991; Turner *et al.*, 2011). *A. tsugae* dispersal is also temporally limited since crawlers are only

76   mobile for several days; once they settle and begin feeding, they are sessile for the remainder of

77   their life cycle, including the mature stages (McClure, 1987). Despite the limitations imposed on

78   between-host dispersal, the newly-hatched crawlers are mobile and capable of active within-host

79   dispersal, typically settling on the youngest hemlock foliage (McClure, 1989). Because sessile *A.*

80   *tsugae* adults must oviposit *in situ*, within-host selection of feeding sites by the newly-hatched

81   crawlers may be crucial for their survival and future fitness.

82   In its invaded range, *A. tsugae* frequently co-occurs on eastern hemlock with a second non-

83   native hemipteran pest, the elongate hemlock scale (*Fiorinia externa* Ferris). *A. tsugae* and *F.*

84   *externa* overlap throughout much of their invaded range, and are found in the same hemlock

85   stands and on the same individual trees (Preisser *et al.*, 2008). *F. externa* also feeds preferentially

86   on eastern hemlock and, like *A. tsugae*, continues to expand its invaded range (Preisser *et al.*,

87   2008; 2011). At low to moderate densities, *F. externa* has minimal impacts on its host (Miller-

88   Pierce *et al.*, 2010; Radville *et al.*, 2011; Gonda-King *et al.*, 2012); at high densities, however, it

89   may be able to kill already-stressed trees (McClure, 1980). There is strong evidence of

90   interspecific competition between *A. tsugae* and *F. externa* (Preisser & Elkinton, 2008). Because

91   *A. tsugae* crawlers are produced mid-summer, 1-2 months after *F. externa* crawlers have settled

92   and begun to feed, *F. externa*-induced changes in host quality could alter *A. tsugae* performance.

93   This hypothesis is consistent with the results of a previous study showing a 40% decrease in *A.*

94   *tsugae* population density in trees previously infested with *F. externa* for two years (Miller-

95   Pierce & Preisser, 2012). If this difference in *A. tsugae* performance can alter its settling

96   behavior, a selective advantage will accrue to crawlers capable of detecting and avoiding *F.*

97   *externa*-infested foliage within a host.

98   Here we examine tissue selection and distribution of *A. tsugae* crawlers in the presence and

99   absence of *F. externa*. Because crawlers are small, few studies have explored patterns of

100   distribution within or between hosts (but see Evans & Gregoire, 2007, Turner *et al.*, 2011), and

101   to our knowledge no previous studies have addressed active within-host feeding site selection.

102   Because adult *A. tsugae* have no effective predators in the invaded range (Wallace & Hain, 2000;

103   Havill *et al.* 2011), within-tree crawler movement is likely driven by the need to find a

104   permanent feeding site and by the site-specific likelihood of competition with other herbivores.

105   Specifically, we hypothesized that *A. tsugae* prefers uninfested eastern hemlock foliage over *F.*

106   *externa*-infested foliage. We tested our hypothesis by conducting laboratory choice tests and

107   field surveys.

108

109   **Materials and methods**

110   *Herbivores: A. tsugae* has two generations a year in its invaded range (McClure, 1987).

111   Briefly, the April-to-June progrediens generation emerges in early spring and settles on

112   previously-produced hemlock foliage, where it produces ~75 eggs/female. In contrast, crawlers

113   from the July-to-April sistens generation settle on newly-produced foliage in mid-summer. They

114   aestivate until late fall, then feed throughout the winter, producing ~300 eggs/female that will

115   become the next progrediens generation. *F. externa* has only one generation in the northern part

116   of the invaded range; *F. externa* crawlers emerge in late spring and are the first herbivores to

117   settle on newly-produced hemlock foliage (Abell, 2010). The mobile *F. externa* crawlers also

118   settle on young foliage and reduce host nitrogen levels (McClure, 1980; Gómez *et al.*, 2012).

119   While *A. tsugae* feeds at the base of hemlock needles by inserting its stylet bundle into ray

120   parenchyma cells at the base of the needle cushion (Young *et al.*, 1995), *F. externa* crawlers

121   settle on the underside of the needles, where they suck fluids from the mesophyll cells (McClure,

122   1980). Both herbivores' crawlers remain sessile once they settle.

123   ***Laboratory choice tests:*** We conducted choice tests to test whether *A. tsugae* crawlers

124   showed preference for uninfested vs. *F. externa*-infested foliage within the same tree. In April

125   2012, forty-seven eastern hemlocks naturally infested with *F. externa* were haphazardly selected

126   in the Middlesex Fells Reservation (Winchester, MA). In each tree, two ~5 cm shoots produced

127   during the previous growing season were selected, one collected from an uninfested branch and

128   the other having at least five adult *F. externa* settled on the needles (= 47 replicates). All of the

129   selected foliage was carefully inspected for the presence of *A. tsugae* or any other non-*F. externa*

130   herbivores; there was no evidence of *A. tsugae* or other herbivores on any of the shoots.

131   In addition to investigate *A. tsugae* preference in naturally-infested trees, we conducted a

132   second set of laboratory choice tests using foliage sources from nursery-purchased trees to

133   control for unknown factors governing *F. externa* infestation in the field. These choice tests were

134   conducted following the same procedure as above but using uninfested and infested foliage

135   (minimum two scale present) from eastern hemlock saplings manually inoculated yearly with *F.*

136   *externa* in June 2011 and 2012. The saplings were planted in the understory of a mixed conifer-

137   deciduous forest in Kingston, Rhode Island. By enforcing the infestation of *F. externa* on a given

138   set of experimental trees rather than using naturally-infested trees we can rule out the possibility

139 that the *F. externa* foliage used in the choice tests were successfully infested by *F. externa* due to

140 unknown traits of the host rather than by chance and the possibility that *A. tsugae* behavioral

141 patterns in feeding site selection were due to innate differences in preference/survival across

142 hosts (or tissues within hosts) by these two insects. In a subset of those tests (n=30), *F. externa*

143 remained intact on the infested foliage, while in a second subset (n=32) *F. externa* was manually

144 removed from the foliage to determine whether *A. tsugae's* preference is driven by the physical

145 presence of the scale or by *F. externa*-induced changes in the foliage quality.

146   In all choice tests, the base of each shoot was placed inside an eppendorf tube with a

147 pierced lid filled with deionized water to avoid desiccation. Both tubes were taped to the bottom

148 lid of a 9 cm diameter petri dish. Eastern hemlock foliage with *A. tsugae* egg masses was

149 collected from naturally-infested trees and a small stem fragment with three *A. tsugae* egg

150 masses was placed inside each petri dish touching both shoots (Fig. 1b). The petri dishes were

151 closed and sealed with parafilm and placed on their sides so that the shoots were in an upright

152 position at 21°C and natural light conditions. We inspected each petri dish daily for the presence

153 of *A. tsugae* crawlers. Once *A. tsugae* crawlers were detected, the inoculants were removed and

154 the crawlers allowed to choose between the two foliage types. Seven days after *A. tsugae*

155 crawlers were first detected, the number of *A. tsugae* crawlers on each shoot was counted and the

156 percentage of crawlers on each choice was calculated. A sign test was used to assess whether *A.*

157 *tsugae* crawlers prefer uninfested foliage by comparing the number of replicates with a higher

158 percentage of crawlers on uninfested versus *F. externa*-infested foliage. The second set of choice

159 tests (experimental trees) was analyzed by using a repeated measures ANOVA on the amount of

160 crawlers present on either choice at the end of the test, using infestation by *F. externa* (uninfested

161   vs infested foliage) as the within-subjects factor and the removal of *F. externa* from the infested

162   foliage (yes/no) as the between-subjects factor.

163   ***Field surveys:*** We conducted three surveys of *A. tsugae* preference in the field. Three

164   hemlock stands were selected based on their moderate densities of naturally-occurring *A. tsugae*

165   and *F. externa* (11-100 insects/m branch) as shown by 2011 field surveys (Preisser *et al.*, 2011).

166   The stands were located in Massachusetts (Belchertown and Hampden; sampled on June 21st,

167   2012) and Connecticut (Suffield; sampled on July 2nd, 2012). In each site we selected 25 trees

168   infested with both insects. In each tree, we selected two ~5 cm new-growth shoots (i.e., produced

169   during the 2012 growing season) that were infested with at least three *A. tsugae* and three *F.*

170   *externa* individuals. We counted the number of needles on each shoot with neither insect, *A.*

171   *tsugae* only, *F. externa* only, or both insects. The results from the two shoots per tree were

172   averaged and the expected distribution of needles in each category analyzed using a 2x2

173   contingency table. Because there were no statistical differences among sites (Likelihood ratio

174   test; site effect: $\chi^2 = 0.9261$, P = 0.629), data from the three sites were pooled in the analysis.

175

176   **Results**

177   ***Laboratory choice tests:*** *A. tsugae* crawlers chose uninfested foliage over *F. externa*-

178   infested foliage from the same tree in both naturally- and artificially-infested with *F. externa*

179   trees. In choice tests using naturally-infested trees, 32 of 47 (68%) choice tests, the majority of *A.*

180   *tsugae* crawlers were present on the uninfested foliage (one-tailed Sign test P = 0.015; Fig 1a).

181   Although there was considerable variation in the percentage of *A. tsugae* crawlers choosing

182   uninfested foliage, 23 of 47 choice tests had between 51-75% of crawlers on the uninfested

183   foliage (Fig 1c). Interestingly, the percentage of *A. tsugae* crawlers on *F. externa*-infested foliage

184   was not correlated with the amount of *F. externa* present on the foliage (Pearson correlation = -

185   0.087, P = 0.561; Fig 1d). In choice tests using foliage from experimentally inoculated trees, *A.*

186   *tsugae* crawlers also exhibited an overall preference for uninfested foliage (Insect infestation P

187   <0.0001), regardless of whether *F. externa* was left intact on the foliage or manually removed

188   (Insect infestation x Insect removal P = 0.194; Fig 2).

189   ***Field surveys:*** Our field surveys found that *A. tsugae* and *F. externa* co-occurred less often

190   than expected (χ^2 = 19.04, P < 0.0001; Table 1). Had *A. tsugae* been randomly distributed with

191   respect to *F. externa*, 3.1% of needles should have contained both insects. Our results show that

192   *A. tsugae* and *F. externa* only co-occurred on 1.8% of needles, meaning that the observed value

193   was 42% lower than expected. Conversely, *A. tsugae* and *F. externa* occurred alone more often

194   than expected if both species were randomly distributed with respect to the other.

195

196   **Discussion**

197   We found both experimental and survey evidence for active within-host feeding site

198   selection in *Adelges tsugae*. Specifically, we found that *A. tsugae* crawlers preferred foliage that

199   lacks the competing herbivore *F. externa* in both laboratory choice tests and field surveys of

200   naturally-infested trees. The lower preference for *F. externa*-infested foliage was driven by plant-

201   mediated changes on the foliage caused by *F. externa's* attack rather than the physical presence

202   of this insect. This demonstrates that relying on passive between-host dispersal does not preclude

203   *A. tsugae* (and, presumably, many other small-bodied insects) from actively choosing a suitable

204   feeding site within a given host.

205   Insect performance will inevitably differ across hosts of the same species because of

206   varying genotypic and ontogenetic host quality, which is in turn influenced by abiotic factors

207   such as nutrient availability and by biotic factors such competing herbivores (Cronin &

208   Abrahamson, 1999). While differences in host quality are important for all herbivores, they are

209   perhaps most critical for sessile species that must quickly assess host quality before settling

210   irrevocably in a single location (Ward 1987). In such organisms, the success of newly-hatched

211   juveniles thus depends upon the quality of their natal host or being dispersed to a new suitable

212   host.

213   Our results showed that *A. tsugae* crawlers could discriminate between *F. externa*-infested

214   foliage at the shoot level (choice tests) and at the needle level (field survey). In the laboratory,

215   68% of the choice tests had more *A. tsugae* crawlers on uninfested foliage than on *F. externa*-

216   infested foliage from *F. externa* naturally-infested trees. This preference was particularly evident

217   in choice tests conducted on experimentally infested trees, with a known prior history of no

218   infestation, where on average approximately twice as many *A. tsugae* crawlers showed

219   preference for uninfested foliage. In the field, the co-occurrence of *A. tsugae* and *F. externa* on

220   the same needle occurred 42% less often than expected if both insects were randomly distributed

221   with respect to each other. Because *F. externa* crawlers settle on the surveyed newly-produced

222   foliage earlier in the summer than *A. tsugae* crawlers, the most likely explanation for our results

223   is that the presence of settled *F. externa* drives *A. tsugae* preference and settlement through

224   plant-mediated changes in foliage quality. The possibility of apparent competition can also be

225   ruled out since *A. tsugae* has no efficient predators in the invaded range or parasitoids worldwide

226   (Wallace & Hain, 2000; Havill *et al.*, 2011).

227   Our results showed that *A. tsugae* has a lower preference for *F. externa*-infested shoot

228   regardless of the number of *F. externa* present on the infested shoot. This suggests that the

229   foraging cue *A. tsugae* uses to avoid *F. externa* is not density-dependent. Instead, a

230   presence/absence cue might be used as a longer-range detection mechanism to identify (and

231   subsequently avoid) a broader tree area infested with *F. externa*. This is advantageous because in

232   tree hosts where *F. externa* density is not high, their distribution is initially very patchy within

233   the tree (Preisser, *unpublished data*). Although we found that *A. tsugae* has a lower preference

234   for *F. externa*-infested foliage, we still observed co-occurrence in the field and especially in the

235   laboratory choice-tests. One possible explanation is that *A. tsugae* has not yet adapted to avoid *F.*

236   *externa*-infested foliage to a larger extent. While *A. tsugae* invaded New England in the late

237   1980s (McClure & Cheah, 1999), the rapid spread of *F. externa* through this area began in the

238   early 2000s (Preisser *et al.*, 2008); as a result, the co-occurrence of high-density populations of

239   both species is a relatively new event. It is also possible that while *A. tsugae* prefers to settle on

240   *F. externa*-free foliage, it will settle in a suboptimal location rather than incur the risk of

241   increased mortality associated with continued searching for a feeding location.

242   The fact that *A. tsugae* is obligately asexual in its invaded range (McClure, 1987) might

243   constrain selection for *F. externa* avoidance; since all offspring are clones of their parent, the

244   only source of genetic variation is randomly-occurring mutations in a given individual. The lack

245   of sexual reproduction, and the recombination that results, might limit the adelgid's ability to

246   adapt to avoid a competing herbivore. Despite this limitation, there is some evidence that

247   adelgids in the invaded range have differentiated in response to strong selection; specifically,

248   northern adelgid clones are more tolerant to cold winter temperatures than their southern

249   counterparts (Butin *et al.*, 2005). The authors of this paper suggest that the extremely high rates

250   of adelgid fecundity - a single female can produce >300 eggs in one generation – might produce

251   so many individuals that even 'rare' random mutations could often occur. If avoiding *F. externa*

252   foliage increases *A. tsugae* fitness, as suggested by higher *A. tsugae* population growth rate on

253   uninfested versus *F. externa*-infested trees (Miller-Pierce & Preisser, 2012), mutations that

254   promote *F. externa* avoidance (or quality discrimination in general) could provide a substantial

255   fitness advantage to the clonal line.

256   Despite being the more recent invader in New England, *F. externa* has now exceeded the

257   northern range of *A. tsugae* and is present in areas that currently lack the adelgid. If *F. externa*

258   can lower *A. tsugae* densities (Miller-Pierce & Preisser, 2012), and therefore tree mortality

259   (Preisser *et al.*, 2008), hemlock stands previously colonized by *F. externa* might derive some

260   benefit from the presence of this second invasive herbivore. Alternately, the ability of *A. tsugae*

261   to efficiently discriminate against *F. externa*-infested foliage within a host might minimize the

262   potential benefits of *F. externa* infestation. Future research should investigate what herbivore

263   induced plant-mediated traits influence within-host feeding site selection and whether they are

264   similar in response to hetero- and conspecific herbivores.

265

266   **Acknowledgements**

271

272   **Contribution of authors**

273   SG designed the project with intellectual contributions from all authors, SG and LGK

274   collected and analyzed the data and all authors contributed to the writing of the manuscript.

275

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Figure Legends

Figure 1. (a) Percentage of choice tests with the majority of *A. tsugae* crawlers on uninfested foliage (white) or *F. externa*-infested foliage (gray); (b) Typical choice test experimental set-up; (c) Frequency distribution of choice tests with different ranges of *A. tsugae* crawler percentage on uninfested foliage; (d) Correlation between *A. tsugae* crawlers on *F. externa*-infested foliage and number of *F. externa* present on the infested foliage.

Figure 2. Average number (+ standard error) of *A. tsugae* crawlers on uninfested (white) or *F. externa*-infested (gray) foliage sampled from manually inoculated trees in laboratory dual choice tests. *F. externa* was left intact on the foliage in a subset of choice tests (left bars; n=30) and it was manually removed from the infested foliage in a second subset (right bars; n=32).
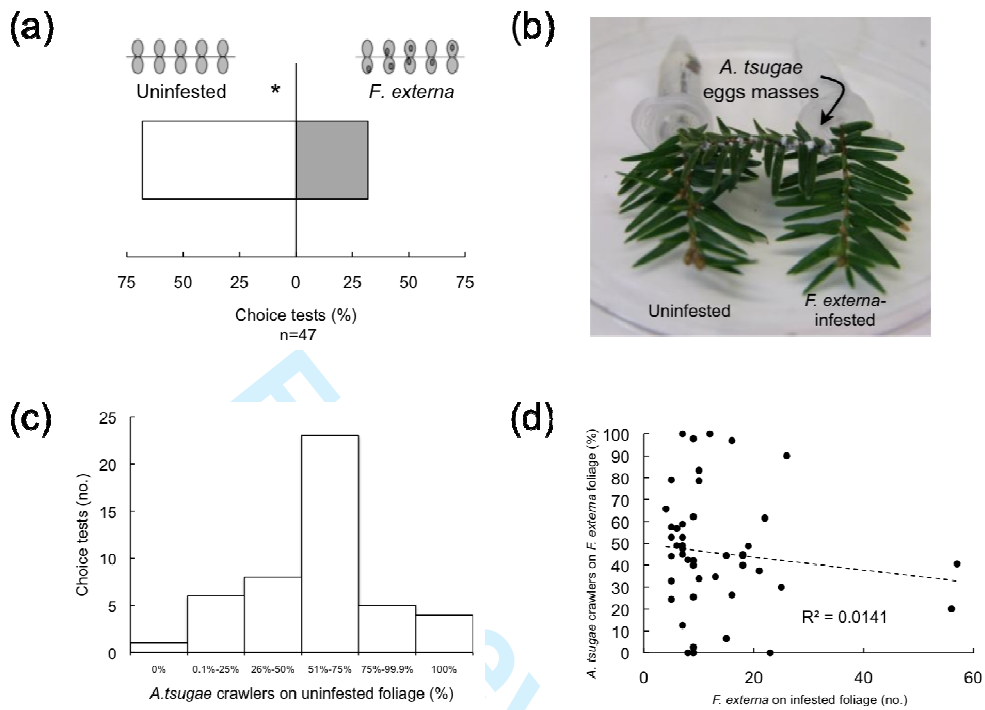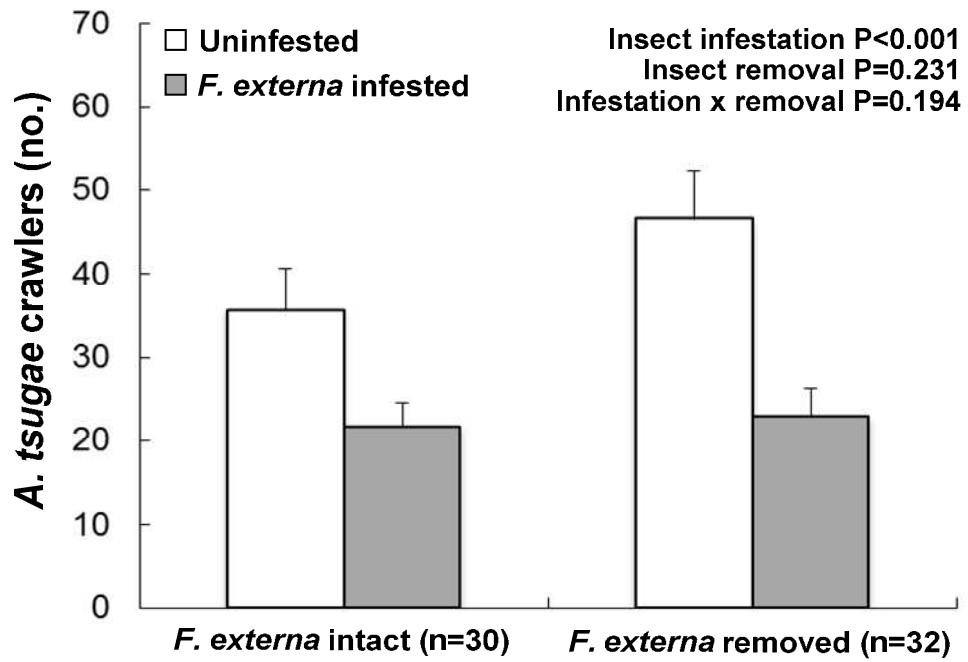
Figure 1

(a)

Uninfested   *   *F. externa*

Choice tests (%)
n=47

(b)

A. tsugae
eggs masses

Uninfested   *F. externa*-
infested

(c)

Choice tests (no.)

A.tsugae crawlers on uninfested foliage (%)

(d)

A. tsugae crawlers on *F. externa* foliage (%)

R² = 0.0141

F. externa on infested foliage (no.)

Figure 2

Table 1. Observed counts of *A. tsugae* and *F. externa* on individual needles of eastern hemlock naturally occurring in the field. Numbers in brackets represent the expected values.

A. tsugae

		Absent	Present
F. externa	Absent	1556 (1586)	339 (308)
	Present	408 (377)	43 (73)

Both insects absent

A. tsugae only present ○

F. externa only present ⬭

Both insects present