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1 Variation in levels of acceptance, developmental success, and abortion of
2 *Halyomorpha halys* eggs by native North American parasitoids

3

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Abstract

~~In order for native parasitoids to adapt to an unsuitable invasive host, there must be intraspecific variation in traits that could be selected upon to enable exploitation or avoidance.~~ Using a native North American parasitoid species (Hymenoptera: Scelionidae) that often unsuccessfully attacking the eggs of the invasive brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae) ~~as a case study~~, we assessed ~~the~~ variation in traits that may determine the parasitoids' ~~future~~ ability to adapt to the invasive host by either exploiting or avoiding *H. halys* eggs (acceptance, developmental success). ~~The aim of surveying these~~ We also assessed variation in the parasitoids' ability to induce *H. halys* host egg abortion, which may contribute to biological control of *H. halys* in invaded areas. parasitoid's variations is to assess their, and their current contribution to their biological control (ability to induce host egg abortion) of *Halyomorpha halys*. The first set of experiments evaluated intra- and interspecific variation using standardized laboratory tests with iso-female lines of *Telenomus podisi* and *Trissolcus euschisti* that included matching of detailed behavioural observations of acceptance with developmental outcomes. In a second set of experiments, we assessed how variation in developmental ability and abortion induction may affect levels of biological control by indigenous parasitoid species. We examined a broader sample of parasitoids that emerged from field collections of egg masses of an indigenous north American stink bug *Podisus maculiventris* in a region newly invaded by *H. halys*. Results from the first set of experiments showed high levels of acceptance of *H. halys* eggs among iso-female lines of parasitoids, but offspring development success was almost zero. *H. halys* egg abortion due to unsuccessful parasitism was often very low and varied among iso-female lines only for *T. podisi*. In the second set of experiments we never observed increases in abortion levels of *Halyomorpha halys* eggs above natural levels, even for the two species (*T. euschisti* and *T. podisi*) that were observed to oviposit in and abort *H. halys* eggs in the first set of experiments. We conclude that while there may be some variation in behavioural and physiological parameters mediating acceptance and abortion of *H. halys* eggs by native North American egg parasitoids, there does not appear to be significant variation in developmental success. Moreover, ~~and that their~~ current biological control impact of *H. halys* eggs via host egg abortion is likely very low.

Keywords: Intraspecific variation, biological control, acceptance behaviour, evolutionary trap, Scelionidae, *Telenomus podisi*, *Trissolcus euschisti*

61 1.1 Introduction

62 The introduction of invasive species exposes native species to novel biotic selective pressures. The
63 establishment, spread, and proliferation of invasive species in new environments can have important
64 ecological consequences such as niche displacement, competition and in some cases, extinction of
65 native species (Mooney and Cleland, 2001; Sax and Gaines 2008; Ekesi et al., 2009; Wilson and
66 Holway, 2010; San Sebastián et al., 2015; Kenis et al., 2017). However, new and frequent
67 interactions with invasive species may lead to novel behavioural responses and trophic interactions.
68 For example, native natural enemies may begin to exploit the invasive species as a prey resource
69 (Carroll and Dingle, 1996, Mooney and Cleland, 2001). In some cases, invasive species may initially
70 be unsuitable resources for native natural enemies that commonly attempt to exploit them, resulting
71 in evolutionary traps (Schlaepfer et al., 2005). Nevertheless, the adaptation of native species to
72 successfully exploit invasive species can occur over a range of timescales. Native species can adapt
73 either by avoiding the unsuitable invasive species or by evolving traits that increase the suitability
74 of the invasive species as prey (reviewed in Berthon, 2015).

75 For insect-egg parasitoids to adapt to an initially unsuitable invasive host (i.e., an escape
76 from the evolutionary trap), there needs to be genetic variation in traits that mediate the parasitoid's
77 levels of host acceptance (oviposition inside the eggs of the invasive host) or the ability of the
78 parasitoid's offspring to develop once accepted. Selection on variation in behaviours that mediate
79 acceptance could lead to avoidance of the unsuitable host, while selection on variation in
80 developmental success could lead to increased host suitability (Keeler and Chew, 2008; Nelson et
81 al., 2011; Abram et al., 2014). In addition, when an invasive species that acts as an evolutionary
82 trap for native parasitoids is the target of biological control programs, it may be important to
83 consider; variation in the ability of parasitoids to kill the invasive host when they unsuccessfully
84 attack it ("non-reproductive effects"; reviewed in Abram et al., 2019a) ~~may be important to~~
85 ~~consider~~. The mortality of the invasive pest resulting from non-reproductive effects could
86 theoretically contribute to biological control of the invasive pest via both direct and indirect
87 ecological effects (Kaser et al., 2018; Abram et al., 2019a; Stahl et al., 2019a). Assessing
88 intraspecific variation in native parasitoid acceptance, developmental success, and the ability to
89 induce host mortality can provide insight into the potential for the adaptation of native species to
90 new invasive hosts, while also forming and form the basis for genetic improvement through
91 breeding programs for native biological control agents (Lommen et al., 2017; Kruitwagen et al.,
92 2018).

93 The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is
94 a polyphagous insect pest native to Asia that has rapidly spread throughout North America and

95 Europe (Haye et al., 2015a; Leskey and Nielsen, 2018, Maistrello et al., 2018), causing economic
96 losses in a number of different crops (Leskey et al., 2012; Maistrello et al., 2017; Bosco et al.,
97 2018). Many studies have been conducted in invaded areas to survey for native parasitoid and
98 predator species ~~with the potential potentially able~~ to contribute to the biological control of *H. halys*
99 ~~biological control~~ (e.g. Ogburn et al., 2016; Dieckhoff et al., 2017; Haye et al., 2015b; Herlihy et
100 al., 2016; Roversi et al., 2016; Cornelius et al., 2016a, b; Abram et al., 2017a; Costi et al., 2019).
101 Overall, egg parasitoids in the family Scelionidae (mostly species of *Telenomus* Haliday and
102 *Trissolcus* Ashmead) are the most common group of natural enemies attacking *H. halys* in invaded
103 areas. Several studies have found that these parasitoids readily accept *H. halys* eggs for oviposition,
104 but their offspring rarely develop successfully (Abram et al., 2014; Haye et al., 2015b; Konopka et
105 al., 2018, 2019, 2020). This combination of high levels of acceptance and low developmental
106 suitability suggests that *H. halys* represents an evolutionary trap for these native scelionid
107 parasitoids (Abram et al., 2014; Schlaepfer et al., 2005). However, a few studies have shown that
108 between 15–25% of attacked *H. halys* eggs abort development as a result of unsuccessful parasitoid
109 attack by parasitoids in invaded areas, potentially as a result of a lack of shared evolutionary history
110 (Abram et al., 2014, 2016; Haye et al., 2015b). Recent studies using molecular diagnostic markers
111 have confirmed that the unsuccessful attack of *H. halys* eggs by native scelionid egg parasitoids is
112 common in nature and have ~~shown that observed~~ *H. halys* egg mortality that could be due to
113 parasitoid-induced host egg abortion (Konopka et al., 2018; Garipey et al., 2019). Thus, aborted
114 host egg development induced by native parasitoids may contribute to biological control of *H. halys*
115 in invaded areas, although the magnitude of the effect is unclear.

116 Some variation in the ability of indigenous parasitoids to develop in and abort *H. halys* eggs
117 has previously been observed in a number of field and laboratory studies (Abram et al., 2014;
118 Cornelius et al., 2016a, b; Herlihy et al., 2016; Ogburn et al., 2016; Dieckhoff et al., 2017; Konopka
119 et al., 2019; Tognon et al., 2017, 2019; Abram et al., 2014; Haye et al., 2015b; Abram et al., 2016;
120 Abram et al., 2017b). However, laboratory studies have either been restricted in taxonomic breadth,
121 and within-species genetic variation (e.g., Abram et al., 2014, 2016; Konopka et al., 2018, 2020;
122 Haye et al., 2015b), or have lacked direct behavioural observations of parasitoids and unexposed
123 control egg masses to relate levels of acceptance to developmental success and egg abortion (e.g.,
124 Tognon et al., 2017, 2019). In field studies, because most ovipositions by native parasitoids are
125 unsuccessful (Abram et al., 2014, 2016, 2017b), the true incidence of *H. halys* egg attack by
126 genetically variable pools of native parasitoids and how it might explain variation in *H. halys* egg
127 mortality is still mostly unclear (Haye et al., 2015b). Previous studies have suggested that native
128 parasitoids might eventually adapt to be able to develop in *H. halys* eggs (Abram et al., 2014, 2016;

129 Tognon et al., 2017) but none have assessed whether there is ~~enough~~ intraspecific variation in
130 relevant traits ~~that would be needed~~ for natural selection to proceed. Standardized laboratory studies
131 that consider the role of intra- and inter-specific variation in acceptance, offspring development, and
132 host egg abortion induction in field populations of parasitoids could improve understanding of the
133 range of impacts that indigenous parasitoids may have on *H. halys* in different invaded regions,
134 both in the short and long terms.

135 The goal of this study is to understand intra and interspecific variation in egg parasitoid
136 traits that may determine their ability to adapt to exploiting or avoiding *H. halys* eggs (acceptance,
137 developmental success), and their current contribution to its biological control (ability to induce
138 host egg abortion). We took two approaches. First, we conducted standardized tests ~~with narrow~~
139 ~~inter-lines genetic samples comparing among~~ (iso-female lines) of two common native North
140 American parasitoid species, ~~attacking~~ *H. halys* that included matching of detailed behavioural
141 observations of acceptance with developmental outcomes. Second, we screened a broader
142 taxonomic and genetic sample of parasitoids that emerged from field collections of egg masses of
143 an indigenous stink bug to assess how variation in developmental ability and abortion induction
144 may affect levels of biological control by indigenous parasitoid species in a region newly invaded
145 by *H. halys*.

147 1.2 Material & Methods

148 1.2.1 Insect colonies

149 The spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), a predatory
150 species native to North America, was used to produce egg masses for rearing native parasitoids and
151 “sentinel” egg masses to collect parasitoids in the field. The *P. maculiventris* colony, established
152 from individuals collected in Ontario and Quebec, Canada in 2015, was reared continuously in
153 mesh cages with access to potted fava bean plants (*Vicia faba* L.) and mealworms (*Tenebrio molitor*
154 L.). Egg masses laid on the sides of cages and on plants were collected daily and glued to strips of
155 filter paper with white non-toxic glue (~~Elmer’s school glue~~) and offered to parasitoid females to
156 maintain parasitoid colonies. Egg masses laid on strips of Reemay fabric (Avintiv, USA), which
157 were provided as additional oviposition substrate, were stored for up to 4 days at 8°C before
158 deployment as sentinel egg masses. This short period of cold storage does not affect parasitism or
159 parasitoid offspring emergence (PKA, unpublished observations).

160 *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae) (~~native to North America~~) and
161 *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) (~~native to Southeastern USA~~) are ~~solitary,~~
162 ~~sexually reproducing idiobiont and generalist parasitoids of stink bugs species of economic~~

163 importance and egg parasitoids distributed throughout North America that both have broad host
164 ranges within the Pentatomidae. They are also the most common scelionids attacking eggs of the
165 brown marmorated stink bug in North America (Abram et al., 2017b). We established five strains
166 of *Tr. euschisti* (provided by Tara Garipey, London Research and Development Centre, Agriculture
167 and Agri-Food Canada; Jacques Brodeur and Mathilde Gaudreau, Université de Montréal) and five
168 strains of *T. podisi* (provided by Jacques Brodeur and Mathilde Gaudreau, Université de Montréal;
169 James Walgenbach and Emily Ogburn, North Carolina State University). Strains tested in our study
170 likely represent a small sample of the range present in North America, as our collections are based
171 on a small number of localities. However, our goal was not to be comprehensive. Rather, we aimed
172 to conduct a preliminary assessment of whether there could be inter-line variation in the parameters
173 we measured. Single females from each collection were used to establish an iso-female lines for
174 each strain, which were reared in climate-controlled rooms for at least 5 generations of sib-mating
175 before testing. Using this iso-female line approach, any phenotypic differences among lines should
176 be due mostly to genetic differences (Beukeboom and Zwaan, 2007), although we cannot exclude
177 the possibility that other differences such as microbiomes (e.g. facultative or obligate microbial
178 endosymbionts), long-term rearing effects, or maternal effects could also be a factor, especially
179 because detailed genetic analyses of these lines has not been performed. Table 1 shows the
180 parasitoid lines tested and their origins. Fresh (<24h since laying) *P. maculiventris* eggs were
181 offered to the parasitoids on a regular basis for colony maintenance. To collect parasitoid females
182 for use in experiments, parasitized egg masses were kept in 1.5 mL plastic microtubes and observed
183 daily for newly emerged female parasitoids. Females were always paired with males from the same
184 strain.

185 *Halyomorpha halys* individuals were originally collected from two locations in Chilliwack,
186 British Columbia, in 2016 and were reared thereafter at the Agassiz Research and Development
187 Center (BC). Stink bugs were maintained in mesh cages provided with potted pepper (*Capsicum*
188 *annuum* L.) plants and store-bought corn and carrots as food sources. Eggs laid by females on
189 Reemay fabric, plant leaves, paper towels, and the sides of the cages were collected daily for
190 laboratory experiments.

191 All insect colonies were maintained at $23 \pm 2^\circ\text{C}$, 40-60% relative humidity, and a
192 photoperiod of 16L:8D.

193

194 1.2.2 Intraspecific variation in acceptance and development

195 The goal of the first experiment was to compare the level, latency, and rate of behavioural
196 acceptance of *H. halys* eggs among strains of *T. euschisti* and *T. podisi*. Prior to use in experiments,

197 newly emerged parasitoids from each species and strain were isolated daily in 1.2 mL Eppendorf
198 tubes with a drop of honey water (70% honey and 30% water) for food, kept with a male for 24
199 hours to allow mating, and then kept alone for one week to allow time for ovariole maturation. For
200 each trial, individual female parasitoids were transferred to small petri dishes (diameter: 5.0 cm,
201 height: 1.0 cm) where a fresh *H. halys* egg mass containing 28 eggs was secured to the bottom of
202 the dish with a small amount of white, non-toxic glue. The trials were recorded for 5 h using a mini-
203 button CCD camera connected to a digital microscope (Dino-Lite, London, Ontario, Canada). Each
204 treatment conducted in a climatic chamber (25°C and 16L:8D) was replicated between 17 and 22
205 times for each strain of each parasitoid species. The experiment was performed in 42 testing days
206 over a total period of 129 days. For each set of egg masses exposed to parasitoids (i.e. on each day
207 of the experiment), unexposed egg masses (controls) were subjected to the same conditions as the
208 treatment egg masses except that they were not exposed to a parasitoid. These unexposed egg
209 masses (n=85) allowed the measurement of baseline levels of *H. halys* egg abortion that was not
210 due to parasitoids (see Abram et al., 2016).

211 After the recording period, parasitoids were removed, and both exposed and unexposed egg
212 masses were maintained under standard rearing conditions for at least 30 days, observing them daily
213 to record the number of host nymphs and parasitoids emerging. After 30 days, any unhatched eggs
214 were dissected and classified as: (i) emerged or fully developed but unemerged *H. halys* nymphs or
215 (ii) aborted, containing only undifferentiated liquid contents. The dissections yielded no fully
216 developed parasitoid adults that failed to emerge.

217 When analyzing each video (n=198), a single observer recorded the occurrence and timing
218 of ovipositions, indicated by the parasitoid inserting its ovipositor into a host egg followed by
219 marking behaviour (see Abram et al., 2014). This allowed us to determine the level of acceptance of
220 the host eggs (the total number of eggs accepted), the latency to the first successful oviposition, and
221 the total time period over which parasitism took place (time between the first and last ovipositions).
222 Self-superparasitism was relatively uncommon in our trials (5.2% of all ovipositions for *T.*
223 *euschisti*; 6.8% of all ovipositions for *T. podisi*), and the total number of ovipositions was almost
224 perfectly correlated with the number of eggs accepted for both *T. euschisti* ($r = 0.99$) and *T. podisi*
225 ($r = 0.99$). Thus, superparasitism events were disregarded in the analysis, and the number of eggs in
226 which at least one oviposition was observed was used as the metric of behavioural acceptance.

227 We expected the level of acceptance to indicate the readiness of native parasitoids to
228 oviposit in *H. halys* eggs. We anticipated that the time until the first oviposition occurred could be a
229 proxy for the time necessary for native parasitoids to recognise the eggs of the host and accept it as
230 a resource. Finally, we expected acceptance rate, calculated as the total time to complete parasitism

231 of the *H. halys* egg mass divided by the number of eggs attacked, to measure the efficiency with
 232 which the different strains of the two species of parasitoids exploited *H. halys* eggs. Moreover, the
 233 acceptance rate would be driven by other behaviours that decrease this efficiency, such as increased
 234 time required to drill into eggs, host rejections, superparasitism, and short patch leaving bouts (Field
 235 1998).

236 Next, we determined the level of *H. halys* egg abortion associated with unsuccessful
 237 ovipositions by comparing the number of host eggs the parasitoids oviposited into and the
 238 developmental outcomes of eggs. The number of eggs aborted due to oviposition in each replicate
 239 was calculated with the following formula (see also Abram et al. 2016):

$$240 \text{ eggs aborted due to oviposition} = AbE - \left(\frac{AbC * TotE}{TotC} \right) \quad (\text{eqn. 1})$$

242
 243 Where AbE and AbC are, respectively, the number of aborted eggs observed in the exposed and
 244 control egg masses, and TotE and TotC are the total number of eggs in the exposed and control,
 245 respectively. Abortion levels in each exposed egg mass (AbE and AbC) were adjusted using pooled
 246 values of TotE and TotC from paired egg masses set up on the same date.

247 Two factors contribute to the numbers of aborted *H. halys* eggs among parasitoid strains.
 248 The first is variation in the number of eggs attacked; the second is variation in the per-attack
 249 probability of aborting host development. To investigate whether parasitoid strains differed with
 250 respect to the per-attack probability of inducing *H. halys* egg abortion, we applied the following
 251 formula to calculate abortion efficiency, calculating the number of eggs aborting due to oviposition
 252 (for eggs accepted by parasitoids only), and then dividing it by the total number of accepted
 253 eggs. ~~first calculated the corrected number of eggs aborted in each replicate (eqn. 1), considering~~
 254 ~~only accepted eggs.~~

$$255 \text{ Abortion efficiency} = \frac{AbE_A - \left(\frac{AbC * TotE_A * TotE}{TotC} \right)}{TotE_A * TotA}$$

256
 257 Where AbE_A and TotE_A are the number of aborted eggs accepted by parasitoids, and the total
 258 number of eggs accepted by parasitoids, respectively.

259 ~~First, we calculated the corrected number of eggs aborted in each replicate (eqn. 1), considering~~
 260 ~~only accepted eggs. We then we divided this by the total number of eggs accepted by the parasitoid~~
 261 ~~(TotA), giving the corrected number of aborted eggs per host accepted, which we term “abortion~~
 262 ~~efficiency”.~~

263

264 *1.2.3 Laboratory tests of field-collected parasitoids on H. halys eggs*

265 To complement detailed laboratory estimates of parasitoid developmental success and abortion
266 induction with narrow [inter-linesgenetiinter-line comparisonse samples withinof](#) two species, we
267 investigated variation in these parameters with a larger number of parasitoid species. We assumed
268 that these encompassed a broader genetic diversity, because individuals were collected from sites
269 across a relatively wide geographic region, although we did not directly assess the true amount of
270 genetic variation present with molecular tools. The goal of these experiments was to explore to what
271 extent the evolutionary trap posed by *H. halys* for native parasitoids generally applies across species
272 present in British Columbia, and to what degree egg abortion caused by native parasitoids may be
273 contributing to biological control of *H. halys*.

274 We used sentinel egg mass surveys, deploying eggs of the native species *P. maculiventris*, to
275 investigate the diversity of egg parasitoids of stink bugs present in British Columbia and obtain
276 parasitoids for laboratory screening against *H. halys*. From the beginning of May to the end of
277 August of 2017, 1,264 *P. maculiventris* egg masses (21,310 eggs) were set out and retrieved at 10
278 sites in coastal (Fraser Valley) and interior (Okanagan Valley) British Columbia (Table 2), [on](#)
279 [between 2 and 8 different dates per site through the sampling period, on an opportunistic basis](#). All
280 sites were semi-urban, urban, or suburban areas with mixed woody and herbaceous vegetation
281 where breeding populations of *H. halys* had previously been detected (Abram et al., 2017a8; Abram
282 et al., 2019b). Fabric squares containing egg masses (see *Insect Colonies* above) were stapled to the
283 undersides of leaves of a variety of woody trees (e.g., *Prunus* spp., *Davidia* spp., *Acer* spp.,
284 *Gleditsia* spp., *Ailanthus* spp., *Sorbus* spp.) and shrubs (e.g., *Rubus* sp., *Mahonia* spp.,
285 *Symphoricarpos* spp., *Rosa* spp.). The egg masses were then retrieved before nymphs emerged, 3–7
286 days after being set out. Because of the variable retrieval intervals, parasitism rates measured here
287 are intended only to determine which parasitoid species were present and to serve as sources of
288 parasitoids for laboratory tests.

289 Retrieved egg masses were placed in Petri dishes (50 mm diameter, 9 mm depth) and kept
290 under ambient laboratory conditions until parasitoid emergence. Emerging parasitoids were given
291 cotton wicks with 10% sucrose as a food source. Newly emerged parasitoids from the Okanagan
292 Valley collections were shipped overnight in a cooler with an ice pack (temperature ~8–10°C) to the
293 Agassiz Research and Development Centre (Agassiz, BC) where samples from the Fraser Valley
294 were located, and where all subsequent tests took place in the laboratory. Emerging parasitoids from
295 [a subset of 139](#) parasitized egg masses were separated into 1.5 ml plastic Eppendorf tubes at least
296 one day before they were tested. [A subset of females was tested \(n = 139\), with the subset](#)
297 [determined by the availability of fresh H. halys eggs when parasitoids were of the correct age for](#)

298 testing (see Table 2 and Figure 4 for per-species sample sizes). All females used in tests emerged in
 299 the presence of males from the same egg mass and were thus assumed mated. In all cases,
 300 parasitoids were 7–14 days old when used in these tests, enough time for post-emergence ovariole
 301 maturation to take place but well before the end of their typical lifespan, ~~which is underin the~~
 302 laboratory ~~conditions is (greater than >40 days)~~ (see Abram et al., 2016). ~~For each parasitoid~~
 303 ~~species a~~ At least 5 replicates were conducted for each parasitoid species. Individual females were
 304 transferred from plastic tubes to Petri dish arenas (50 mm diameter, 9 mm depth) containing an *H.*
 305 *halys* egg mass of between 6 and 32 eggs (mean \pm SE: 20.6 ± 0.52 , $n=191$). The size of the egg
 306 masses used depended on the availability of fresh *H. halys* eggs on each testing day; egg masses
 307 were divided into smaller patches when availability of host material was limited, ~~depending on the~~
 308 ~~relative availability of *H. halys* eggs and adequately aged parasitoids.~~ However, we accounted for
 309 variation egg mass size by including it as a covariate in the statistical analysis (see below). In all
 310 replicates, parasitoids were removed from dishes after 24 h. Egg masses were then incubated under
 311 standard rearing conditions and monitored for the emergence of host nymphs and parasitoids. After
 312 being incubated for at least 1 month, they were dissected to record unemerged parasitoids and
 313 aborted eggs, using the same classification criteria as the first experiment (see above). Throughout
 314 the period of the tests, a set of similarly-sized egg masses (24.3 ± 1.0 eggs, $n=29$) were set up in
 315 Petri dish arenas but not exposed to parasitoids as controls, inclubated, and then dissected to record
 316 levels of nymph emergence and egg abortion. These trials were run concurrently with the iso-
 317 female line experiments described above, under the same abiotic conditions and using *H. halys* egg
 318 masses of the same age from the same laboratory colony.

319 All parasitoids emerging from sentinel egg masses, including those used in laboratory tests
 320 with *H. halys* eggs, were subsequently preserved in 95% EtOH, point-mounted, and identified based
 321 on morphological characters (Talamas et al., 2015). Vouchers are deposited in the Florida State
 322 Collection of Arthropods ~~and Nematodes~~ and the Canadian National Collection of Insects,
 323 Arachnids, and Nematodes.

325 1.2.4 Statistical analysis

326 For laboratory trials where *H. halys* eggs were exposed to different iso-female lines of *T. podisi* and
 327 *T. euschisti*, the number of eggs accepted, acceptance latency, acceptance rate, the corrected number
 328 of eggs aborted, and abortion efficiency were compared among iso-female lines with Kruskal-
 329 Wallis tests followed by Dunn's Kruskal-Wallis multiple comparisons with Benjamini-Hochberg
 330 adjustments. -These non-parametric tests were selected after finding that the response variables did
 331 not meet the assumptions of generalized linear models (with either Gaussian or Poisson error

332 distributions) in most cases. To test whether exposure to parasitoids from each iso-female line of the
 333 two species was associated with higher-than-baseline *H. halys* egg mortality, the corrected number
 334 of eggs aborted when exposed to each iso-female line was compared to zero with a Wilcoxon test.

335 The statistical approach was modified for the experiment measuring levels of egg abortion
 336 in *H. halys* eggs exposed to parasitoids emerging from field-collected *P. maculiventris* egg masses.
 337 First, because egg mass size varied among replicates, the proportion (rather than number) of aborted
 338 eggs was used as the response variable, and egg mass size (number of eggs) was included as a
 339 covariate in the analysis. Second, because multiple parasitoids were sometimes tested from the
 340 same sites and natal egg mass (which could result in pseudoreplication if not accounted for), “natal
 341 egg mass” and “collection site” were included in the statistical model as random effects. Third,
 342 because paired unexposed control egg masses could not be run for ~~each~~ every exposed egg mass
 343 due to limited *H. halys* egg availability on some days, the global level of abortion in unexposed egg
 344 masses set up over the course of the experiment was compared to levels of abortion in egg masses
 345 exposed to each parasitoid species. Thus, the proportion of *H. halys*-egg eggs that aborted when
 346 exposed to different species of parasitoids or were not exposed to parasitoids, was compared with a
 347 generalized linear mixed model (GLMM) with a binomial error distribution, with parasitoid species
 348 and egg mass size as fixed effects and natal egg mass as a random effect. Observation-level random
 349 effects (Harrison, 2014) were also included to account for overdispersion.

350 All statistical analyses were analyzed using R version 3.6.0 (R Development Core Team,
 351 2019).

352

353 1.3 Results

354

355 1.3.1 Intraspecific variation in acceptance and development

356 All iso-female lines of both *T. euschisti* and *T. podisi* oviposited in *H. halys* eggs, but the number of
 357 *H. halys* eggs in which parasitoids oviposited varied significantly among lines for both *T. euschisti*
 358 ($\chi^2_4 = 26.19$, $p < 0.0001$) and *T. podisi* ($\chi^2_4 = 29.32$, $p < 0.0001$), with the median number of eggs
 359 accepted ranging from 15 to 27 for *T. euschisti* and 7 to 28 eggs for *T. podisi*, out of the 28 *H. halys*
 360 eggs offered (Figure 1). Median acceptance latency by *T. podisi* ranged between 0.34 and 1.68
 361 hours but did not differ among lines ($\chi^2_4 = 26.19$, $p = 0.11 < 0.0001$) (Figure 2). However,
 362 acceptance latency did vary significantly among lines for *T. euschisti* ($\chi^2_4 = 10.80$, $p = 0.029$), with
 363 median time until the first observed oviposition ranging from 0.09 to 0.67 hours (Figure 2). The
 364 oviposition rate did not clearly differ among the lines of *T. podisi* ($\chi^2_4 = 8.00$, $p = 0.092$; medians

365 7.8 to 12.0 ovipositions/hour) or *T. euschisti* ($\chi^2_4 = 8.81$, $p = 0.066$; medians 5.6 to 10.0
 366 ovipositions/hour).

367 There was no evidence in variation in the developmental success of parasitoid offspring
 368 among iso-female lines of either parasitoid species. In fact, only a single parasitoid offspring, a *T.*
 369 *euschisti* (line Te_3) emerged from one of the parasitized *H. halys* egg masses, despite a total of
 370 1,907 eggs being oviposited in by *T. euschisti* across all replicates. We never observed the
 371 emergence of *T. podisi* offspring from *H. halys* eggs, which were observed ovipositing in a total of
 372 1,588 *H. halys* eggs across all replicates.

373 After being corrected for natural levels of *H. halys* egg mortality, the number of eggs
 374 aborted by *T. podisi* varied among iso-female lines ($\chi^2_4 = 12.22$, $p = 0.016$), with all lines causing at
 375 least some *H. halys* egg abortion; median corrected numbers of aborted eggs ranged from 0.5–4.0
 376 (Figure 3). These differences in *H. halys* egg abortion induction among lines were maintained when
 377 accounting for the number of ovipositions; abortion efficiency also varied among lines of *T. podisi*
 378 ($\chi^2_4 = 18.11$, $p = 0.0012$) (Figure 3), with per-strain medians of between 1.7% and 40.6% of
 379 attacked eggs aborting. For *T. euschisti*, numbers of aborted eggs did not vary among lines ($\chi^2_4 =$
 380 2.67, $p = 0.61$), and medians were generally lower (range: 0.5–1.0 eggs), with only three out of five
 381 lines causing abortion levels significantly above zero (Figure 3). Likewise, abortion efficiency did
 382 not vary significantly among the lines of *T. euschisti* ($\chi^2_4 = 3.43$, $p = 0.49$), with an intraspecific
 383 mean of 6.0% of attacked *H. halys* eggs aborting.

384 385 1.3.2 Laboratory tests of field-collected parasitoids on *H. halys* eggs

386 Out of the total of 1,264 *P. maculiventris* sentinel egg masses deployed in the field, 15.3% were
 387 parasitized by scelionid egg parasitoids (Table 2). The percentage of egg masses with parasitoid
 388 emergence varied among sites from 8.4–24.0%. Of the five scelionid species emerging from
 389 sentinel eggs, *T. euschisti* was present at the greatest number of sites (10/10 sites), followed by *Te.*
 390 *podisi* (9/10 sites), *Trissolcus cosmopeplae* (Gahan) (3/10 sites), *Tr. utahensis* (Ashmead) (2/10
 391 sites), and *Tr. hullensis* Harrington (1/10 sites). The latter three *Trissolcus* spp. were only found at
 392 sites in the Okanagan Valley; *T. euschisti* and *T. podisi* were found in both the Fraser Valley and
 393 Okanagan Valley regions. The only non-scelionid parasitoid reared from *P. maculiventris* eggs was
 394 a single individual in the family Encyrtidae (Table 2).

395 A total of 191 parasitoids, representing the five scelionid species emerging from *P.*
 396 *maculiventris* eggs, were tested on *H. halys* eggs in the laboratory. Oviposition was commonly
 397 observed at the beginning of these trials by all species tested, and post-oviposition brood guarding
 398 was often observed afterwards (P. Abram, personal observations). These informal observations,

399 together with the direct observations on two of these species (*T. podisi* and *T. euschisti*) in the iso-
400 female lines experiment under similar conditions, suggest that host acceptance occurred in many of
401 these trials. However, of the 3,935 eggs exposed to these parasitoids, only a single *T. euschisti*
402 individual emerged from an *H. halys* egg.

403 Levels of *H. halys* egg abortion in these experiments were very low, often with a median of
404 zero (Figure 4). The proportion of *H. halys* egg abortion was not significantly higher in eggs
405 exposed to any of the parasitoid species than in unexposed egg masses and did not vary
406 significantly among parasitoid species to which they were exposed (GLMM, $\chi^2_{4,81} = 2.92$, $p = 0.57$)
407 (Figure 4). Levels of egg abortion in exposed egg masses were not associated with variation in *H.*
408 *halys* egg mass size ($\chi^2_{1,81} = 0.013$, $p = 0.91$) or its interaction with exposure treatment ($\chi^2_{4,81} = 2.60$,
409 $p = 0.63$).

410

411 **1.4 Discussion**

412 Our study adds to a growing body of evidence from laboratory and field studies indicating that
413 native scelionid parasitoids in North America readily oviposit in the eggs of *H. halys* but rarely
414 develop successfully (Abram et al., 2014; Haye et al., 2015b; Ogburn et al., 2016; Cornelius et al.,
415 2016a, b; Dieckhoff et al., 2017; Abram et al., 2017b; Garipey et al., 2019; Konopka et al., 2018,
416 2019, 2020). In fact, under our standardized laboratory conditions, the developmental success of
417 parasitoid offspring occurred only twice out of the thousands of *H. halys* eggs that we exposed to
418 parasitoids. In addition, we provide the first evidence that, in at least two native parasitoid species,
419 behavioural parameters related to acceptance of *H. halys* eggs can vary intraspecifically. Finally,
420 our results suggest that the biological control effects of parasitoid-induced abortion of *H. halys*
421 eggs, when considering a broader range of native parasitoid genotypes than tested in past studies, is
422 minimal. These findings have implications for the short-term biological control impact of native
423 parasitoids on *H. halys* as well as their longer-term potential to adapt to this invasive host.

424 Several studies have suggested that native North American parasitoids may eventually adapt
425 to be able to develop in *H. halys* eggs (Abram et al., 2014; Konopka et al., 2018; Tognon et al.,
426 2019), and there are other examples where native natural enemies have eventually adapted to
427 successfully exploit invasive prey or hosts (Keeler and Chew, 2008; Berthon et al., 2015). However,
428 in order for natural selection to promote this shift in physiological host range, there would need to
429 be genetic variation present in populations of native parasitoids for traits that influence their ability
430 to successfully complete development in *H. halys* eggs. The low levels of emergence of native
431 parasitoids, including *T. podisi* and *T. euschisti*, observed in past field studies using sentinel *H.*
432 *halys* egg masses (e.g. Dieckhoff et al., 2017; Ogburn et al., 2016; Herlihy et al., 2016; Cornelius et
433 al., 2016a, b), including in the area where parasitoids were collected for our second experiment
434 (Abram et al., 2019b), indicates that this variation may exist. However, it is also possible that other
435 abiotic and biotic factors in the field (e.g., unfertilized eggs, extreme temperatures, desiccation)
436 could kill *H. halys* eggs at variable levels, making them more suitable for parasitoid development
437 (similar to freeze-killing; e.g. Haye et al., 2015b), which would overestimate the ability of native
438 parasitoids to develop in viable *H. halys* eggs. In addition, what proportion of *H. halys* eggs are
439 located and unsuccessfully parasitized by native parasitoids in the field is unknown unless
440 molecular diagnostic tools are used (Garipey et al., 2019; Stahl et al., 2019b). Thus, to properly
441 assess intraspecific variation in developmental success, standardized laboratory studies that observe
442 parasitoid behaviour, ~~and using *H. halys* eggs with low levels of baseline mortality,~~ are needed in
443 conjunction with *H. halys* eggs with low levels of baseline mortality. In the current study, we found
444 no evidence to suggest that there is significant intraspecific variation in the ability of *T. podisi*, *T.*

445 *euschisti*, or any of the other scelionid species we tested, to complete development in viable *H.*
446 *halys* eggs. Across both experiments, only two individuals of *T. euschisti* emerged. It is, of course,
447 possible that the hypothesized variation in developmental success exists in some populations of
448 North American scelionids; our study did not include any such strains. ~~However, even if initially~~
449 ~~very rare, after several years of strong selection, parasitoid these variants capable of developing in~~
450 ~~*H. halys* eggs would be expected to spread rapidly in areas where *H. halys* is common. But also, in~~
451 ~~terms of variation, it's possible that some traits of an appropriate strains may needs different years~~
452 ~~of strong selections before showing their adaptation or avoidance to the target host. Even if initially~~
453 ~~rare, these variants would be expected to spread rapidly in areas where *H. halys* is common.~~
454 However, ~~De~~during the period of 2013–2016, when our parasitoid lines were established, there is no
455 evidence to suggest that there is significant intraspecific phenotypic variation in the ability of native
456 North American scelionid parasitoids to consistently develop in viable *H. halys* eggs. However, the
457 data and methodology presented here provide a basis for assessing how this situation could change
458 in the future, and when considering more comprehensive samples of parasitoid strains present in
459 areas invaded by *H. halys*.

460 There may be a significant fitness penalty for native parasitoids when they expend energy,
461 time, and eggs while unsuccessfully exploiting *H. halys* eggs (Abram et al., 2014). Thus, natural
462 selection could act on variation in parasitoid behaviours that mediate acceptance of *H. halys* eggs,
463 leading to avoidance of the evolutionary trap by native parasitoids. We did observe variation in
464 acceptance levels, measured as the number of eggs parasitized in 5 h, of both *T. podisi* and *T.*
465 *euschisti*. Likewise, we observed significant variation in the time it took different iso-female lines
466 of *T. euschisti*, but not *T. podisi*, to begin ovipositing in *H. halys* eggs. We did not observe any
467 variation in the speed at which either parasitoid species oviposited in *H. halys* eggs once parasitism
468 began. On the one hand, these results could provide preliminary evidence that intraspecific variation
469 exists in behaviours or physiological variables that determine acceptance of *H. halys* eggs by native
470 parasitoids. For example, greater latency to the first oviposition could indicate that native
471 parasitoids are less able to locate *H. halys* egg masses at a short range or are more likely to delay
472 oviposition once the egg mass is encountered. Likewise, fewer total ovipositions by females from
473 some iso-female lines of the two species could indicate that some strains had a greater tendency to
474 leave *H. halys* egg masses before fully exploiting them. On the other hand, the variation in
475 acceptance and oviposition latency we observed could be underpinned by variation in other aspects
476 of parasitoid behaviour or physiology that are unlikely to be acted upon by natural selection to
477 reduce the level of exploitation of *H. halys* by native parasitoids. For example, some iso-female
478 lines may simply have lower fecundity than others leading to fewer ovipositions before their egg

479 load is exhausted; or lower locomotor activity leading to a delay in the amount of time required to
480 find and begin ovipositing in *H. halys* eggs. We would not anticipate natural selection to act on
481 these types of traits specifically to avoid the evolutionary trap posed by *H. halys*, as they would also
482 affect their ability to exploit more suitable native host species. Any eventual behavioural avoidance
483 or rejection of *H. halys* eggs by native parasitoids would need to be mediated by modified
484 sensitivity to specific semiochemical, visual, or physical cues that are specific to *H. halys* eggs.
485 Future studies that examine intraspecific variation in parasitoid behaviour throughout the process of
486 host searching are needed to better understand the potential for the evolution of behavioural
487 avoidance of *H. halys* eggs. [In particular, experiments comparing the behaviour of different strains
488 on a suitable, native host species in addition to *H. halys* \(e.g., Abram et al. 2014\) could help to
489 determine whether behavioural variation of parasitoids in response to *H. halys* is due to specific
490 parasitoid avoidance or increased acceptance of *H. halys* specifically, versus non-specific parasitoid
491 life history variation.](#)

492 Although almost none of the oviposition events by *T. podisi* and *T. euschisti* we observed in
493 this study resulted in offspring production, some of them caused increases in the number of aborted
494 *H. halys* eggs. In our first experiment, all five iso-female lines of *T. podisi* (1.7–40.6% of attacked
495 eggs aborted) and three out of five lines of *T. euschisti* (7.4 – 10.0% of attacked eggs aborted)
496 caused a significant number of *H. halys* eggs to abort above natural levels (Figure 3). Thus, [the
497 unsuccessful attack of *H. halys* eggs by *T. podisi* and *T. euschisti* in some invaded areas may be
498 contributing to increasing its egg mortality.](#) Past studies using a single laboratory strain of *T. podisi*
499 have recorded increased abortion levels of between approximately 24 and 30% (Abram et al., 2014;
500 Abram et al., 2016) in parasitized eggs, while one other study on two native European *Trissolcus*
501 spp. estimated abortion rates of about 10-15% on unsuccessfully attacked *H. halys* eggs (Haye et
502 al., 2015b). Abortion levels observed in past studies are thus within the range measured in our first
503 experiment. Additionally, our results demonstrate that abortion levels vary between species, and, in
504 some cases, within species. The number of *H. halys* eggs aborted, as well as abortion efficiency,
505 varied among our iso-female lines of *T. podisi*. In contrast, there was no clear intraspecific variation
506 in *H. halys* egg abortion caused by *T. euschisti*, with only three out of five lines causing at least
507 some *H. halys* eggs to abort and two lines causing no abortion whatsoever. In combination with past
508 studies, the results of our first experiment imply that for *H. halys*, the biological control effect of
509 egg abortion by native parasitoids is likely to vary widely in time and space depending not only on
510 parasitoid species composition but also on the relative frequency of different intraspecific variants
511 causing different levels of egg abortion.

512 Although some parasitoid lines we tested aborted a significant number of *H. halys* eggs,
513 after taking into account variation in acceptance levels and intraspecific variation in abortion
514 efficiency, the absolute numbers of eggs aborted were low overall. While the most efficient strain of
515 *T. podisi* aborted, on average, about 40% of attacked *H. halys* eggs (= 11.3 eggs per completely
516 exploited egg mass of 28 eggs) (Figure 3), many egg masses were not completely exploited (Figure
517 1), and it was more typical for *T. podisi* and *T. euschisti* to abort fewer than four eggs per exposed
518 egg mass (Figure 3). Considering that less than 50% of *H. halys* egg masses are located and
519 oviposited within by native parasitoids in the field (Garipey et al., 2019; Konopka et al., 2018), our
520 results indicate that the biological control effect of egg abortion induced by native parasitoids on *H.*
521 *halys* in North America is probably limited or even negligible. In fact, in our second set of
522 experiments testing the capacity of native parasitoids emerging from sentinel egg masses of *P.*
523 *maculiventris* in British Columbia, we never observed increases in abortion levels of *H. halys* eggs
524 above natural levels – even for the two species (*T. euschisti* and *T. podisi*) that were observed to
525 oviposit in and abort *H. halys* eggs in the first laboratory experiment. This result could indicate that
526 the populations we sampled in British Columbia are composed of strains of native parasitoids that
527 are ineffective at aborting *H. halys* eggs. Because we did not conduct detailed, comprehensive
528 behavioural observations in this experiment, however, we cannot rule out that acceptance levels of
529 *H. halys* eggs may have been lower than in the first experiment, and that this may be responsible for
530 the low abortion levels observed. This would be unexpected, however, given that (i) qualitative
531 behavioural observations (oviposition, patch guarding) indicated frequent acceptance; and (ii) the
532 exposure period in the second experiment (24h) was almost five times longer than the first
533 experiment (5h), giving parasitoids much more time to exploit the available eggs under similar
534 abiotic conditions. In any case, our results suggest that native scelionid parasitoids are probably not
535 currently contributing significantly to biological control of *H. halys* in newly invaded areas of
536 British Columbia, either through host egg abortion or offspring development.

537 The susceptibility of *H. halys* to parasitism and egg abortion would depend not only on
538 intraspecific variation in the attacking parasitoid but also potentially on phenotypic variation in
539 hosts that mediates their susceptibility ~~-(Kraaijeveld et al., 1998)Kraaijeveld and Godfray, 1999).~~
540 There is ~~some~~ evidence for genetic differences among geographic populations of *H. halys*,
541 including between ~~e~~Eastern and ~~w~~Western North America (Garipey et al., 2014; Valentin et al.,
542 2017; Abram et al., 2017a8; Cesari et al., 2017), so it is possible that there are differences in the
543 susceptibility of these different populations to parasitoid attack. Variation in host susceptibility is
544 unlikely to be responsible for the significant differences in the traits we measured among lines of
545 parasitoids, as host egg masses were randomly assigned among iso-female line treatments, and

546 experiments were run ~~in parallel~~during the same time period. However, it is possible that some
547 differences between our study (which used wWestern North American *H. halys*) and past studies
548 (Abram et al. 2014, 2016, which used eEastern North American *H. halys*), could be due to the
549 different population of hosts used. The fact that one of the *T. podisi* strains used in our study (Tp_1)
550 is the same as those used in Abram et al. (2014, 2016) allows an informal comparison. In both
551 studies, there was no successful ~~development~~ of this strain in *H. halys* eggs. In the current study,
552 this strain aborted an average of 12% of attacked eggs, which is considerably lower than the 24-
553 30% estimates from past studies. As most aspects of the methodology of the two studies were
554 similar, this comparison could indicate a difference in the susceptibility of wWestern North
555 American *H. halys* to abortion by native parasitoids. However, as some parts of the methodology
556 differed between the two studies – most importantly egg mass size – further research that includes
557 direct comparisons is needed to investigate this matter.

558 Adaptation of native parasitoids to successfully recognize and successfully exploit invasive
559 hosts is typically considered to occur on timescales of multiple decades (Cornell and Hawkins,
560 1993). Given our results as well as those of past studies (e.g., Dieckhoff et al., 2017), it appears that
561 for native scelionid parasitoids exploiting *H. halys* in North America, the variation in traits required
562 for this adaptation is not yet clearly present, as wWe found no evidence of variation in the
563 developmental success of these native parasitoids. Our results do provide some evidence to
564 suggest that variation in behaviours mediating acceptance of *H. halys* may exist, although it is
565 unclear whether avoidance of *H. halys* eggs by these parasitoids could feasibly be selected for given
566 this variation. ~~We found no evidence of variation in the developmental success of the native~~
567 ~~parasitoids.~~ Determining whether these native parasitoids will adapt to the presence of *H. halys* by
568 avoiding its eggs, or alternatively by gaining the capacity to exploit them successfully, will
569 requirecould include long-term studies in several areas invaded by *H. halys*, as well as studies of
570 parasitoid behaviour and development across gradients of time since the invasive stink bug has
571 established.

572

573 **Credit authorship contribution statement**

574 Elena Costi: Validation, Conceptualization, Data curation, Formal analysis, Writing - Original
575 Draft, Writing - Review & Editing

576 Warren Wong: Validation, Data curation, Writing - Review & Editing

577 Joan Cossentine: Review & Editing, Supervision, Resources, Funding acquisition

578 Susanna Acheampong: Review & Editing, Supervision, Resources, Funding acquisition

579 Tim Haye: Writing - Review & Editing, Supervision, Funding acquisition

580 Elijah J. Talamas: Writing - Review & Editing, Data curation, Funding acquisition

581 Lara Maistrello: Writing - Review & Editing, Supervision, Funding acquisition

582 Paul Abram: Conceptualization, Formal analysis, Resources, Writing - Original Draft, Writing -
583 Review & Editing, Visualization, Project administration

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607 **Conflict of Interest**

608 All authors declare that they have no conflict of interest.

609 **Informed consent**

610 Informed consent was obtained from all individual participants included in the study

611

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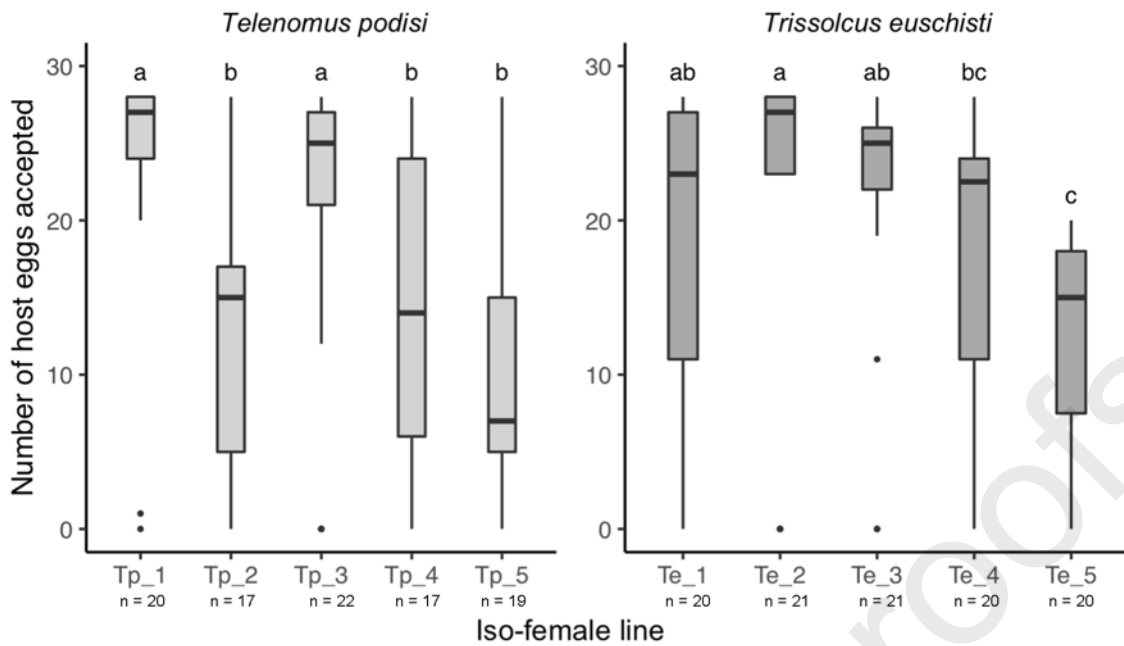
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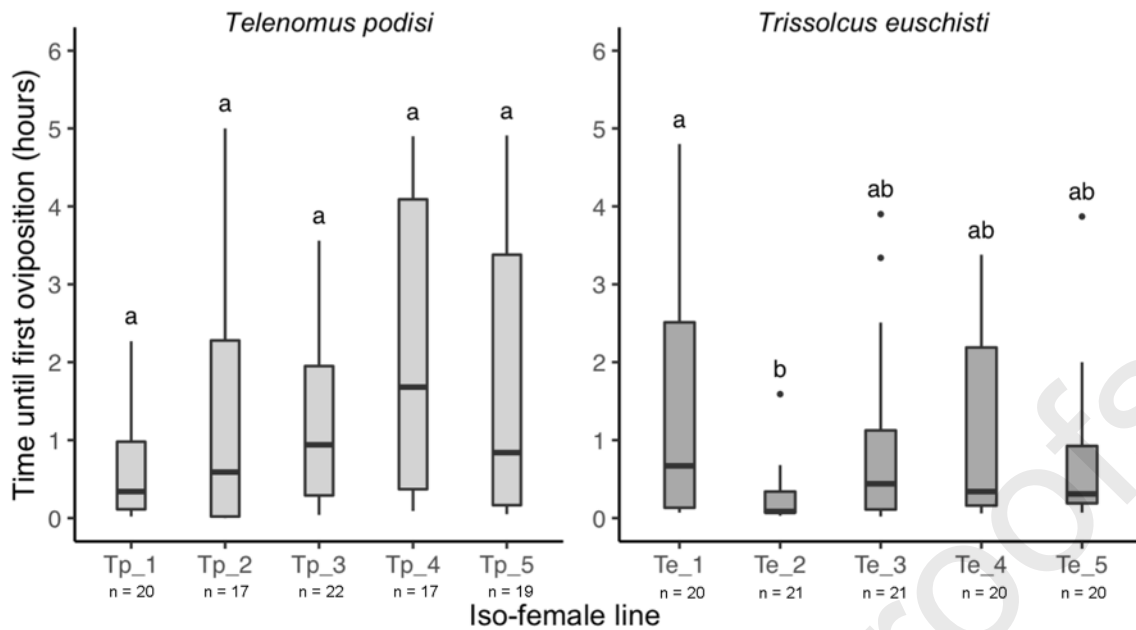


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785 **Figure 1.** The number of *H. halys* eggs in which different iso-female lines of *Trissolcus euschisti*
 786 and *Telenomus podisi* oviposited when observed on *H. halys* egg masses of 28 eggs for 5 h. For
 787 lines within each parasitoid species, boxes not labeled with the same letter are significantly different
 788 ($p < 0.05$; Dunn's Kruskal-Wallis multiple comparison test). In boxplots, horizontal lines show
 789 medians, boxes contain the 25th-75th percentiles, whiskers show the upper and lower deciles, and
 790 points show outliers.

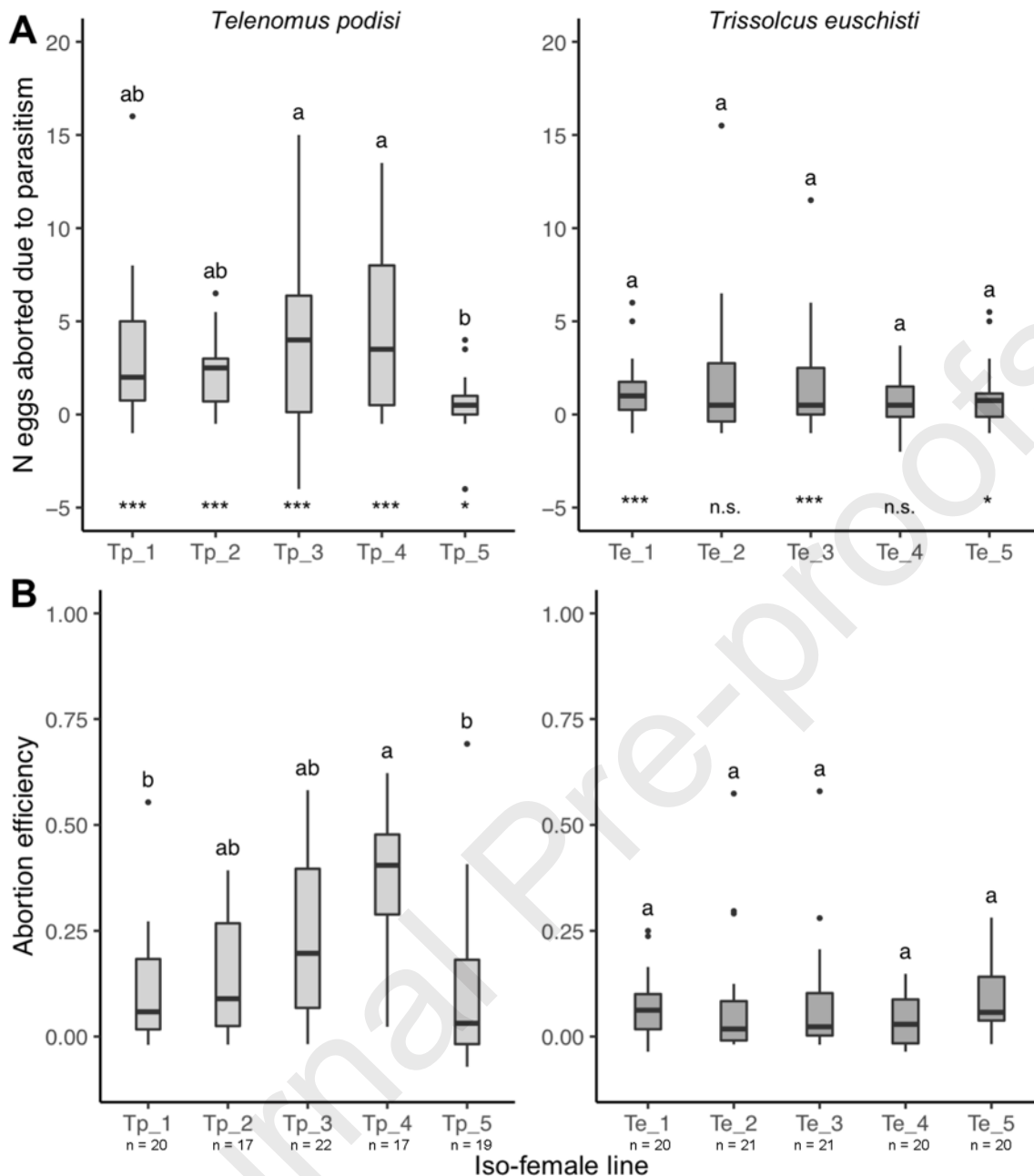
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793 **Figure 2.** Acceptance latency (time until first oviposition) of different iso-female lines of *Trissolcus*
 794 *euschisti* and *Telenomus podisi* on *H. halys* egg masses. For parasitoid lines within each species,
 795 boxes not containing the same letter are significantly different ($p < 0.05$; Dunn's Kruskal-Wallis
 796 multiple comparison test). In boxplots, horizontal lines show medians, boxes contain the 25th-75th
 797 percentiles, whiskers show the upper and lower deciles, and points show outliers.

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Figure 3. (A) ~~The corrected number of eggs aborting~~ [The number of eggs aborting due to](#)

[parasitism](#), and (B) ~~corrected~~ abortion efficiency (number of aborted eggs per parasitoid

oviposition) when *H. halys* egg masses were exposed to different iso-female lines of *Trissolcus*

euschisti and *Telenomus podisi*, after [correcting-adjusting](#) for baseline levels of *H. halys* egg

mortality. Negative values for some observations result from higher mortality in unexposed egg

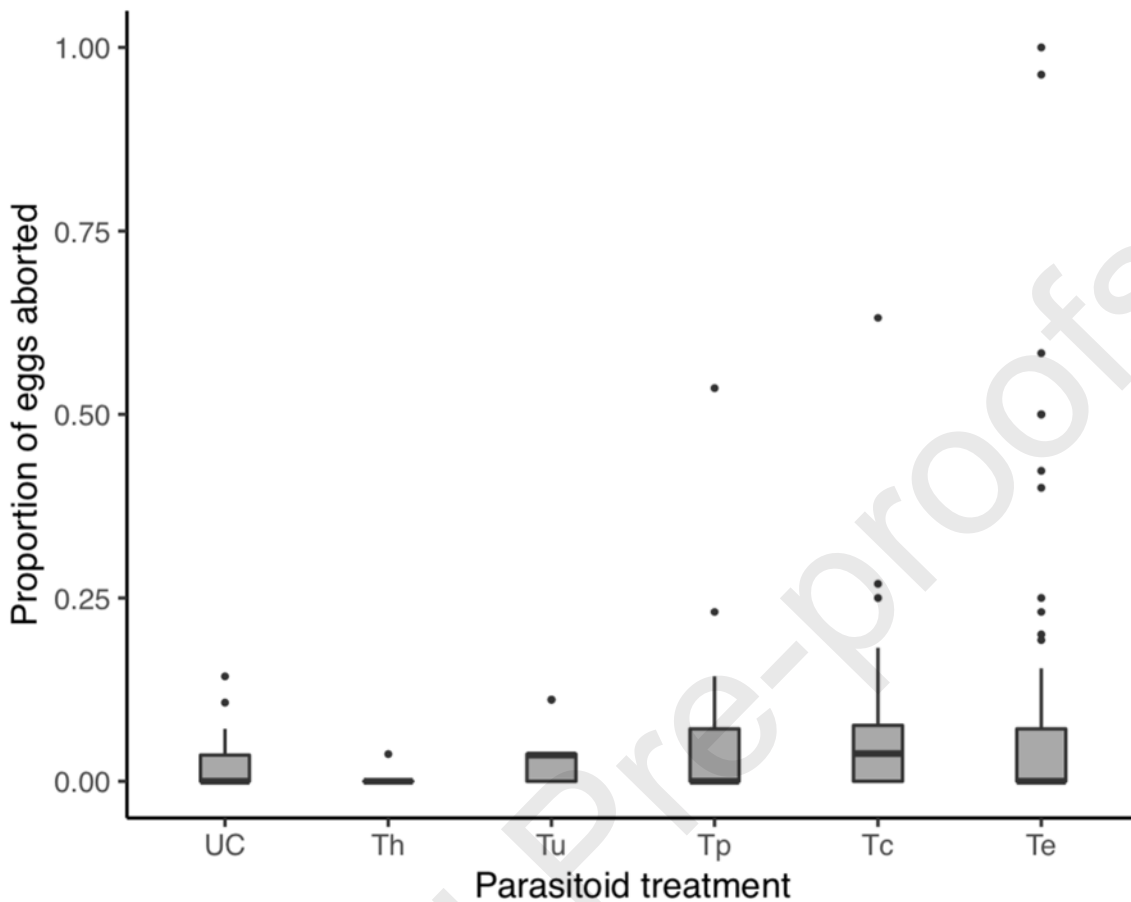
masses than exposed egg masses. In both panels, for lines within each parasitoid species, boxes not

labeled with the same letter are significantly different ($p < 0.05$; Dunn's Kruskal-Wallis multiple

comparison test). In (A), symbols underneath the boxes indicate whether the corrected abortion

levels are significantly different from zero (n.s. – $p > 0.05$; * – $p < 0.05$; *** – $p < 0.001$; Wilcoxon

809 test). In boxplots, horizontal lines show medians, boxes contain the 25th-75th percentiles, whiskers
 810 show the upper and lower deciles, and points show outliers.



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813 **Figure 4.** Percentage of *H. halys* eggs aborting in unexposed control egg masses (UC), compared to
 814 egg masses exposed to one of five parasitoid species in the laboratory for 24 h: Th – *Trissolcus*
 815 *hullensis* (n = 5); Tu – *Trissolcus utahensis* (n = 9); Tp – *Telenomus podisi* (n = 36); Tc – *Trissolcus*
 816 *cosmopeplae* (n = 46); Te – *Trissolcus euschisti* (n = 95). Horizontal lines show medians, boxes
 817 contain the 25th-75th percentiles, whiskers show the upper and lower deciles, and points show
 818 outliers. Abortion levels did not vary significantly among treatments (see the text of Results for
 819 statistical information).

820 **Table 1.** Sources of parasitoid iso-female lines used in experiments.
 821

Species	Line name	State/Province, Country of Collection:	Original	Year of
		GPS	emergence host	collection
<i>Telenomus podisi</i>	Tp-1	Quebec, Canada: 45.556, -73.557	<i>P. maculiventris</i>	2013
	Tp-2	Ontario, Canada: 43.040, -81.219	<i>P. maculiventris</i>	2016
	Tp-3	Ontario, Canada: 43.028, -81.213	<i>P. maculiventris</i>	2016
	Tp-4	North Carolina, USA: 35.428, -82.564	<i>H. halys</i>	2016
	Tp-5	Ontario, Canada: 43.074, -81.337	<i>P. maculiventris</i>	2016
<i>Trissolcus euschisti</i>	Te-1	Ontario, Canada: 43.028, -81.213	<i>P. maculiventris</i>	2016
	Te-2	Ontario, Canada: 43.030, -81.209	<i>P. maculiventris</i>	2016
	Te-3	Ontario, Canada: 43.030, -81.209	<i>P. maculiventris</i>	2016
	Te-4	Ontario, Canada: 43.030, -81.209	<i>P. maculiventris</i>	2016
	Te-5	Quebec, Canada: 45.499, -73.346	<i>P. maculiventris</i>	2016

822

823 **Table 2.** Scelionid parasitoids emerging from *P. maculiventris* egg masses set out and retrieved
 824 from different sites in British Columbia, Canada, and the number of each species from each site that
 825 was subsequently offered *H. halys* eggs in the laboratory: Te – *Trissolcus euschisti*; Tp – *Telenomus*
 826 *podisi*; Tc – *Trissolcus cosmopeplae*; Tu – *Trissolcus utahensis*; Th – *Trissolcus hullensis*.
 827

Region	Site Name (GPS)	# sentinel egg masses set out (total # eggs)	% egg masses parasitized per species ^a [# individuals lab-tested against <i>H. halys</i>]					Total ^b
			Te	Tp	Tc	Tu	Th	
Fraser Valley	Chilliwack #1 (49.158, -122.003)	107 (1,635)	4.67 [1]	3.74 [1]	0.00	0.00	0.00	8.41
	Chilliwack #2 (49.159, -121.997)	55 (807)	14.55 [5]	9.09 [2]	0.00	0.00	0.00	21.82
	Rosedale (49.184, -121.800)	53 (721)	3.77 [0]	7.55 [3]	0.00	0.00	0.00	11.32
	Abbotsford (49.003, -122.263)	25 (364)	20.00 [3]	4.00 [1]	0.00	0.00	0.00	24.00
	Langley (49.122, -122.657)	10 (138)	10.00 [0]	10.00 [0]	0.00	0.00	0.00	20.00
Okanagan Valley	Penticton ^c (49.483, -119.603)	579 (10,208)	4.49 [21]	1.04 [7]	8.29 [46]	2.76[9]	0.69 [5]	14.51
	Kelowna #1 (49.885, -119.484)	115 (1,958)	20.00 [31]	0.00	0.00	0.00	0.00	20.00
	Kelowna #2 (49.884, -119.456)	106 (1,821)	4.72 [5]	15.09 [15]	0.94[0]	0.00	0.00	18.87
	Kelowna #3 (49.880, -119.485)	104 (1,804)	16.35 [20]	2.88 [2]	0.96[0]	0.96[0]	0.00	20.19
	Kelowna #4 (49.871, -119.490)	110 (1,854)	6.36 [9]	2.73 [5]	0.00	0.00	0.00	9.09
Total		1,264 (21,310)	7.75 [95]	3.32 [36]	3.96[46]	1.34[9]	0.32[5]	15.34

828 ^a Percentage of egg masses from which at least one individual of [a given each](#) species emerged

829 ^b Percentage of egg masses parasitized. This value is sometimes lower than row totals due to multiparasitism; i.e.,
 830 multiple parasitoid species emerging from a single egg mass (this occurred in a total of 18 egg masses).

831 ^c One individual of an unidentified Encyrtidae emerged from a single egg mass at this site.

832 Credit authorship contribution statement

833 Elena Costi: Validation, Conceptualization, Data curation, Formal analysis, Writing - Original
 834 Draft, Writing - Review & Editing

835 Warren Wong: Validation, Data curation, Writing - Review & Editing

836 Joan Cossentine: Review & Editing, Supervision, Resources, Funding acquisition

837 Susanna Acheampong: Review & Editing, Supervision, Resources, Funding acquisition

838 Tim Haye: Writing - Review & Editing, Supervision, Funding acquisition
 839 Elijah J. Talamas: Writing - Review & Editing, Data curation, Funding acquisition
 840 Lara Maistrello: Writing - Review & Editing, Supervision, Funding acquisition
 841 Paul Abram: Conceptualization, Formal analysis, Resources, Writing - Original Draft, Writing -
 842 Review & Editing, Visualization, Project administration

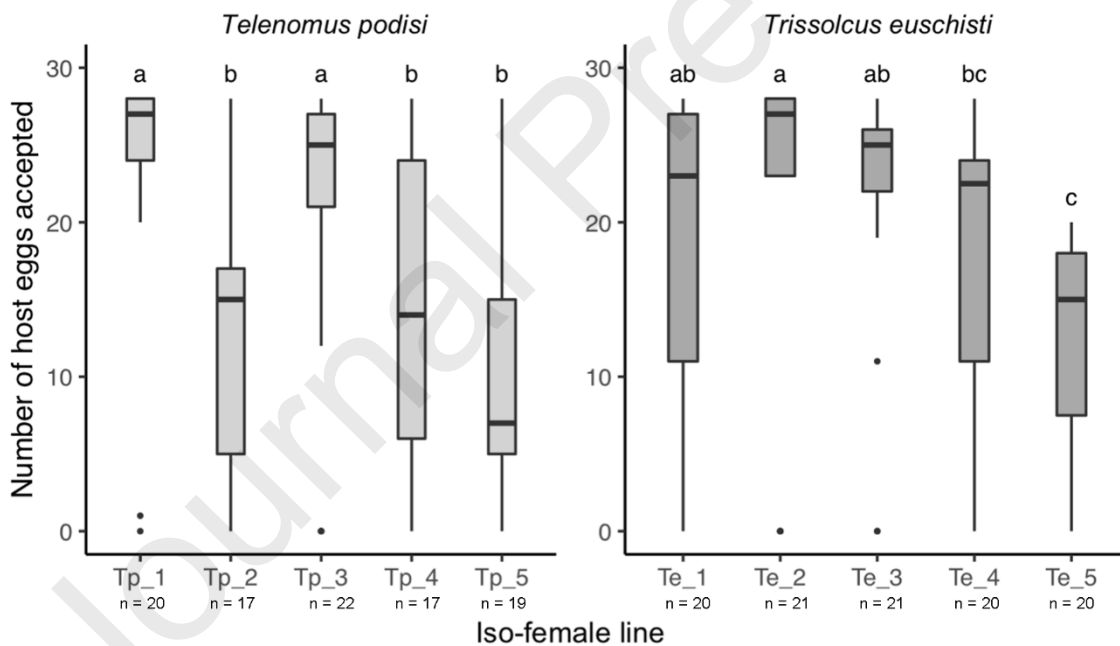
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845 Highlights

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- 847 • Native North American scelionid parasitoids unsuccessfully attack eggs of *Halyomorpha*
- 848 *halys*.
- 849 • Intra-specific variation in parasitoid acceptance behaviours was observed
- 850 • Developmental success of all species and intraspecific strains on *H. halys* eggs was almost
- 851 zero.
- 852 • ~~Variable, but low levels of ariation in~~ parasitoid-induced host egg abortion ~~were~~
 853 ~~observed, but levels were usually low.~~
- 854 •
- 855



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