



HAL
open science

Sublethal doses of pyriproxyfen stimulate reproduction and aggressive behavior in a non-target parasitoid wasp

Solène Liégeois, Manon Delaunay, Charlotte Lécureuil, Marlène Goubault

► To cite this version:

Solène Liégeois, Manon Delaunay, Charlotte Lécureuil, Marlène Goubault. Sublethal doses of pyriproxyfen stimulate reproduction and aggressive behavior in a non-target parasitoid wasp. *Science of the Total Environment*, 2022, 842, pp.156880. 10.1016/j.scitotenv.2022.156880 . hal-04243895

HAL Id: hal-04243895

<https://hal.science/hal-04243895v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Sublethal doses of pyriproxyfen stimulate reproduction and aggressive behavior in a non-target parasitoid wasp

Author names: Solène Liégeois¹, Manon Delaunay¹, Charlotte Lécureuil^{1#} & Marlène Goubault^{1#}

These authors are co-last authors

Author affiliation:

IRBI UMR 7261 CNRS-Université de Tours

Parc de Grandmont

37200 Tours, France

Corresponding author:

Marlène Goubault

IRBI UMR 7261 CNRS-Université de Tours

Parc de Grandmont

37200 Tours, France

Mail: marlene.goubault@univ-tours.fr

tel: +33 2 47 36 70 87

Author contributions:

Solène Liégeois: Methodology, Investigation, Formal analysis, Writing - Original Draft

Manon Delaunay: Investigation

Charlotte Lécureuil: Conceptualization, Methodology, Writing - Review & Editing

Marlène Goubault: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing

1 **Abstract:**

2 Insecticides are commonly used to control populations of pests and disease vectors. However, they
3 can have multiple unintended effects on non-target species. Assessing their impacts on the physiology
4 and behavior of beneficial insects, such as biological control agents, is thus necessary to gain insight
5 into the diversity and nature of such side effects. Here, we investigated the effect of sublethal doses
6 of the endocrine disrupting insecticide pyriproxyfen, a juvenile hormone agonist, on females of the
7 solitary ectoparasitoid *Eupelmus vuilleti* (Hymenoptera: Eupelmidae). These parasitoid wasps can be
8 used as biological control agents to control the cowpea weevil, *Callosobruchus maculatus*
9 (Coleoptera: Bruchidae), that infests cowpea seeds, *Vigna unguiculata* (Fabacea). To do so, in
10 addition to classical measures on female fecundity and survival, we focused on female behaviors that
11 can have important consequences on female fitness and host exploitation. First, we showed that
12 pyriproxyfen stimulated egg production without affecting female survival. Second, we observed that
13 low doses of this insecticide had no effect on females' exploration and host discrimination ability but
14 stimulated their aggressiveness when fighting for host access. Although a negative impact on other
15 life-history traits cannot be ruled out at this point, these results showed low doses of pyriproxyfen
16 can have unintended positive effects on ectoparasitoid *E. vuilleti* females, by enhancing at least
17 temporarily their reproduction and host access in a situation of competition. Our work thus highlights
18 the importance of studying the diversity of possible unintended sublethal effects of pesticides on
19 beneficial insects.

20

21 **Keywords:** endocrine disruptor, juvenile hormone agonist, fecundity, behavioral strategies, non-
22 intentional effects, *Eupelmus vuilleti*

23

24

25

26 **1. Introduction**

27 It is now well established that beneficial insects play an important role in agro-ecosystem
28 functioning. Agricultural practices, including pesticide use, must therefore take these insects into
29 account to be effective. Beneficial arthropods indeed provide many ecosystem services: in addition
30 to ensuring crop pollination, they also permit natural control of crop pests and maintenance of soil
31 fertility (Haan et al., 2021). They thus have a recognized economic importance and monetary value.
32 While 5-8% of global agricultural production, representing an annual market value of \$235-577
33 billions, is directly dependent on pollination (IPBES, 2016), natural pest control by insects is
34 estimated to be worth \$4.5 billions per year in the United States of America alone (Losey and
35 Vaughan, 2006). These natural enemies are mainly predators or parasitoids. For example, the
36 parasitoid wasp *Lariophagus distinguendus* is used as a biological agent to control the weevil
37 *Sitophilus granarius*, a major grain pest in temperate climates (Niedermayer et al., 2016).
38 Nevertheless, while the effects of pesticides are widely investigated in honeybees, due to their
39 important economic role in crop pollination (as reviewed in Barascou et al., 2021), there are still few
40 studies focusing on the effects of pesticides on parasitoids (Abbes et al., 2015; Desneux et al., 2004;
41 Tappert et al., 2017). However, pesticides can have indirect effects on these beneficial arthropods and
42 decrease their effectiveness in controlling pest populations (Cloyd, 2012). To build integrated pest
43 management (IPM) programs, it is now necessary to select pesticides having the least possible impact
44 on pests' natural enemies.

45 Even if research on pesticides' effects on non-target species has increased since the 1950s
46 (Sánchez-Bayo, 2011; Theiling and Croft, 1988), their sub-lethal effects (i.e. effects measured at
47 doses that do not induce a significant increase in mortality, Desneux et al., 2007) remain to be
48 explored in-depth. Such effects can be observed at the physiological level, like the reduction of queen
49 bees' and drones' fertility, but also at the behavioral level (Barascou et al., 2021). Behavioral changes
50 can further modulate how animals respond to abiotic and biotic environmental changes and impact
51 their fitness (Jacquin et al., 2020; Sievers et al., 2019). Analysis of behavioral responses is

52 increasingly used to measure the impacts of environmental stresses such as pesticide exposure
53 because behavioral alterations tend to be more sensitive than fitness indicators (Montiglio and
54 Royauté, 2014 for a review). In insects, several studies are reporting sublethal effects of insecticides
55 on the behavior of non-target species that may be beneficials. For instance, deltamethrin, used
56 worldwide in many crops but also for public health (Davies et al., 2012; Liao et al., 2018) has a
57 specific effect on maternal egg care behavior of earwigs, which are recognized as a crop protection
58 agent against pests in orchards. (Meunier et al., 2020; Mauduit et al., 2021).

59 Hormones mediate both physiology and behavior of animals. By affecting their sensory
60 systems, central integrators and/or peripheral effectors, hormones allow individuals to adjust their
61 behaviors to the situation in response to specific stimuli (Nelson, 2010). Juvenile hormone (JH) is
62 one of the main hormones in insects. In addition to regulating molting processes in immature
63 individuals, its main known function in adults is to control female reproduction. At the physiological
64 level, JH has a gonadotropic effect: increased JH levels stimulate the production of vitelline protein
65 precursors, vitellogenins, by the fat body and their uptake by developing oocytes (Roy et al., 2018).
66 Thus, high JH titers are generally positively associated with female fertility, such as in the facultative
67 eusocial bee *Megalopta genalis* (Smith et al., 2013), or in primitively eusocial bumblebees and
68 halictine bees (Tibbetts and Crocker, 2014). At the behavioral level, JH is known to play various roles
69 in reproductive behavior, from stimulating sexual pheromone production to regulating female
70 receptivity (Ringo, 2002). Aggressive behaviors during reproduction can also be mediated by JH. In
71 odonates, for instance, territory defense is more intense in males with high JH levels (Córdoba-
72 Aguilar and Munguía-Steyer, 2015). Similarly, in eusocial Hymenoptera, aggressive and dominance
73 behaviors are more frequent in females presenting high JH circulating levels (Tibbetts et al., 2020,
74 2011). Juvenile hormone also mediates competitive behaviors for access to reproduction sites in a
75 parasitoid wasp: JH-supplemented *Eupelmus vuilleti* females are more aggressive when fighting for
76 hosts against intraspecific competitors than control females supplemented with a solvent (Mathiron
77 et al., 2019). Given the important role of these behaviors in driving female reproduction success in

78 such beneficial insects used to control pest populations (here, the cowpea weevil), it is now essential
79 to investigate the effects of exposure to endocrine disrupting pesticides on such behaviors in non-
80 target insects.

81 Insect Growth Regulators (IGR) are insecticides with selective toxicity on biochemical
82 pathways or processes essential for insect growth and development. They consequently affect the
83 development of targeted insects by disrupting their molting process, leading to their death, but also
84 to malformations and reduction in fertility in imagoes. Adults are also subjected to sublethal effects,
85 such as a reduced fertility in females or the developmental inhibition of specific imago organs
86 (Desneux et al., 2007). When they interfere with the action of arthropods' hormones (e.g. ecdysone,
87 juvenile hormone), they can be considered as endocrine disrupting insecticides (Sánchez-Bayo,
88 2012). Among IGRs, the pyriproxyfen has been synthesized to act as a JH agonist, by competing with
89 JH molecules on receptors (Sullivan and Goh, 2008). Since the early 1990s, it is commonly used in
90 agriculture to control insect pests but also for veterinary purposes, as an antiparasitic for domestic
91 animals. It is also increasingly used in a sanitary context, to prevent *Aedes aegypti* mosquito larvae
92 from becoming adults and transmitting viruses (Hustedt et al., 2020). Yet, numerous studies have
93 shown that pyriproxyfen disrupts the development, physiology, and behavior of a variety of taxa,
94 from aquatic species (e.g., fish, amphibian tadpoles, crustaceans, daphneids; see Devillers, 2020;
95 Moura and Souza-Santos, 2020) to terrestrial species, in particular non-target insects (Devillers and
96 Devillers, 2020). For instance, exposure of honeybee larvae to sublethal doses of pyriproxyfen leads
97 to the greater avoidance of young bees by other colony members, and accepted bees exhibit less social
98 behavior and more self-directed behavior than untreated bees. In particular, they spend more time
99 grooming themselves and being immobile (Fourrier et al., 2015). Since the effect of pyriproxyfen on
100 fitness is highly species-dependent, it is not possible to generalize what is observed in one species to
101 others (Cloyd, 2012).

102 In the present study, we investigated the potential effects of pyriproxyfen on a non-target
103 beneficial insect, the parasitoid wasp *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae).

104 Females of this species parasitize and feed on larvae and pupae of the cowpea weevil, *Callosobruchus*
105 *maculatus* (Fab) (Coleoptera: Bruchidae), which infest cowpea seeds, *Vigna unguiculata* (L.) Walp
106 (Fabaceae) (Terrasse and Rojas-Rousse, 1986), both in the field and in storage areas. These parasitoid
107 wasps can be used as biological control agents to control this pest. Nevertheless, IGRs such as
108 methoprene, a juvenile hormone analog, are often used to reduce *C. maculatus* populations in grain
109 stocks (Abo-Elghar et al., 2004). The use of pyriproxyfen is also effective for a long period of time:
110 it reduces *C. maculatus* oviposition rate, egg hatching rate during the month post-treatment, and
111 suppresses adult emergence up to 8 months after treatment (Abo-Elghar et al., 2004). Moreover, the
112 tri-trophic system (*E. vuilleti*, *C. maculatus* and the cowpea seeds) is located in the western part of
113 the Afro-tropical ecozone (i.e Burkina Faso, Mali, Nigeria, Benin and Togo; Gibson and Fusu, 2016).
114 Its range overlaps that of *Aedes aegypti* (Kamal et al., 2018; Thabet et al., 2020), against which
115 pyriproxyfen is used (Hustedt et al., 2020). As a result, *E. vuilleti* females are likely exposed to
116 pyriproxyfen in the field and in grain stocks. Although not targeted by this insecticide, occasional
117 exposure, even at reduced doses, may affect their behaviors and reproductive success, hence their
118 efficiency as biological control agents. Here, we, therefore, measured the effects of sub-lethal doses
119 of pyriproxyfen on *E. vuilleti* females' reproduction (egg maturation and host exploitation) and
120 competitive behaviors for host access.

121

122 **2. Material and methods**

123 **2.1 Laboratory breeding**

124 *Eupelmus vuilleti* and *C. maculatus* individuals were collected in 2007 in Togo, West Africa.
125 Parasitoids were then mass-reared in a climatic chamber, maintained at 28°C, with a photoperiod of
126 12:12LD following the method described by Jaloux et al., (2004). Their hosts were also maintained
127 in the laboratory, on cowpea seeds of the Blackeye California variety, under controlled conditions
128 (12:12 LD, 30°C : 25°C, 30% humidity).

129 *Eupelmus vuilleti* females used in experiments were isolated upon emergence to prevent any
130 experience of oviposition on a host of unknown quality and competition between females before
131 assays. They were then individually placed in a Petri dish (diameter: 9.2 cm, height: 1.4 cm) and
132 provided with a cotton ball soaked with water for hydration. One male was added during 24h for
133 mating and oogenesis stimulation (Terrasse and Rojas-Rousse, 1986), except in the survival
134 experience as explained below. Females were also provided with hosts that were replaced daily until
135 behavioral assays were carried out. In experiments measuring discrimination ability and exploratory
136 activity of females, hosts were provided as one seed containing 2 to 3 larvae of *C. maculatus*. For the
137 other trials, they were presented as individual fourth-instar *C. maculatus* larvae dissected from seeds
138 and located in a transparent gelatin capsule (length: 18mm, diameter: 5mm). This mimics the cowpea
139 seed and allows better experimental control of host larval stage and it also facilitates the observation
140 of oviposition by the parasitoid (the egg can be seen through the gelatin capsule during its deposition
141 on the host; Gauthier and Monge, 1999), without altering female behavior (Gauthier and Monge,
142 1999; Jaloux et al., 2004), despite a slight reduction in the number of eggs laid (Damiens et al., 2001).
143 All behavioral experiments were carried out in a climate room with a LD photoperiod of 12:12,
144 maintained at 28°C and constant 70% relative humidity.

145

146 2.2 Exposure to pyriproxyfen

147 Because *E. vuilleti* juveniles develop (from the host) inside the cowpea seeds, they are unlikely
148 to be exposed to sprayed pesticides. We therefore focused on the effects of pyriproxyfen of free-living
149 adult females, using external application to mimic, in a controlled way, female exposure to pesticide
150 sprayed on treated areas. Females were thus topically applied with pyriproxyfen (Sigma-Aldrich, Inc.
151 #34174) diluted in pure acetone using one of four concentrations: 0 (control), 9.6, 96 or 960 µg/mL.
152 Such doses are consistent with what females can encounter in treated fields, since the Togolese
153 legislation authorizes the use of pyriproxyfen at 30g/ha, which is equivalent to 300ng/cm² (Unité de
154 coordination nationale REDD+, direction nationale ODEF, 2020). Our two lower doses were within

155 that range, as they consisted of applying 9.6 and 96 ng of active substance to females. To apply the
156 solution, females were briefly cold anesthetized at -20°C, and received 1 µL of solution on the ventral
157 side of their thorax, where the presence of numerous spiracles (pores) allow the solution to enter their
158 body. Afterwards, females were individually placed in a 1.5mL tube for 4 or 24 hours before being
159 used in experiments.

160

161 2.3 Female survival

162 To measure the survival of females in dependence of the solution to which they are exposed,
163 females were treated with either acetone (control, N = 24) or pyriproxyfen (N = 23 for each of the 3
164 concentrations) on the day of their emergence. A fifth group of 23 females (i.e. additional control
165 group) underwent the same procedure as for the other treatments (e.g. handling and cold anesthesia)
166 but no solution was applied. Females were then individually placed in a Petri dish with a cotton ball
167 soaked with water (but no male and no host) and checked daily, until their death.

168

169 2.4 Gonadotropic effect

170 The gonadotropic effect of pyriproxyfen was tested by dissecting 3-day old females and
171 counting their mature eggs, 4h or 24h after exposure (N = 20 for each concentration and time tested).
172 Mature (i.e. ready-to-lay) eggs were identified based on their homogeneous content and their long
173 and thin pedicel (Bodin et al., 2009). In the 4h treatment, females were treated on day 3, whereas
174 those in the 24h treatment were treated on day 2 and remained in a tube for 24 hours. These latter
175 females therefore had the same age at the time of the dissection as those of the 4h treatment but had
176 access to one fewer host. The egg load of control females (pure acetone treatment) could therefore
177 not be compared between both time delays because oviposition modifies egg load.

178 In the following behavioral assays, as egg number is known to influence host exploitation
179 behavior including aggressiveness and contest outcome in *E. vuilleti* (Mathiron et al., 2019, 2018;

180 Mohamad et al., 2010), we chose to investigate the effect of pyriproxyfen on behavior at a time where
181 egg load was not modified. Since our results showed the number of mature eggs increased 24h after
182 pyriproxyfen application but not yet 4h after (see Results section), we performed all behavioral
183 observations four hours after pyriproxyfen application.

184

185 2.5 Mobility and exploration

186 The arena used to measure the influence of pyriproxyfen on female mobility and exploration
187 consisted of nine chambers connected by thin corridors and sandwiched between two glass plates
188 (Supplementary Material, Figure S1). The central chamber was connected to four others, each linked
189 to a peripheral zone. A half seed containing a single larva of the beetle *C. maculatus* was placed in
190 one of the four peripheral chambers. The complete device was placed on an infrared table with a
191 BASLER BCA 1300 (Germany) camera fixed above (Media Recorder v4.0, Noldus Information
192 Systems, The Netherlands). Three-day-old females treated with acetone or one of the three
193 concentrations of pyriproxyfen (N = 27, 26, 27 and 27 respectively) were placed, 4 hours after
194 exposure, in the central area of the device, initially closed with a ring blocking the different exits.
195 After a 5-minute acclimation period, the ring was removed, allowing the female to freely move in the
196 entire arena. Their movement were recorded for 60 minutes. The number of eggs laid was
197 subsequently counted by opening the seeds and inspecting the hosts' surface. Female egg load was
198 measured by dissecting females (as previously explained). We then analyzed videos both manually
199 and using ToxTrac software (version 2.92 (2021/03/30)) to measure the distance covered by females,
200 their speed or time spent stationary. The time taken to arrive at the seed and the time spent on it were
201 also noted.

202

203

204

205 2.6 Host discrimination

206 To evaluate the influence of pyriproxyfen on females' host discrimination ability, three-day-
207 old females (N = 20 for each concentration of pyriproxyfen: 0, 9.6, 96 and 960 $\mu\text{g}/\text{mL}$) were
208 individually introduced four hours after treatment, for one hour, into a Petri dish (diameter: 9.2cm,
209 height: 1.4cm) at equidistance between of two seeds. Each seed contained a single fourth-instar larva
210 of *C. maculatus*: one had been parasitized 2 hours before the trial by another 3-day-old *E. vuilleti*
211 female (not used further in experiments) while the other was unparasitized. Using BORIS software
212 (v 7.10.2 – 2021-01-16), we recorded the occurrence and duration of the following behaviors:
213 grooming, resting, antennal exploration and oviposition. The location where these behaviors were
214 displayed was noted: on the unparasitized or parasitized host or on the Petri dish, called 'neutral zone'.
215 At the end of each experiment, cowpea seeds were opened to count the number of eggs present on
216 the host and confirm the oviposition observed during experiments.

217

218 2.7 Female aggressiveness and conflict resolution

219 To identify females individually during contests, we marked them, on the day before the trial,
220 with a yellow or red dot of acrylic paint deposited on the dorsal part of their thorax, avoiding touching
221 the tegula (wing insertion), and after reducing their locomotor activity by cold exposure. Three-day-
222 old females were then either treated with pure acetone or 9.6 $\mu\text{g}/\text{mL}$ pyriproxyfen and maintained 4h
223 without host before running behavioral assays, to increase their motivation to lay eggs. It has to be
224 noted that, due to the complexity of setting up these trials, we tested only one pyriproxyfen
225 concentration which was the lowest dose of pyriproxyfen having a gonadotropic effect (i.e 9.6 $\mu\text{g}/\text{mL}$,
226 see results section) and compared its effect to control (i.e. females treated with acetone).

227 We set up dyadic encounters between either (i) two control females (i.e. both females were
228 exposed to pure acetone; N = 45), or (ii) one control female and one female exposed to pyriproxyfen
229 (N = 36). Each dyad consisted of visually similar-sized wasps of distinct color. No effect of color on

230 contest was found (Logistic analysis, LR $\chi^2 = 0.374$, df = 1, P = 0.541). At the beginning of each
231 observation, both females were simultaneously introduced into an arena consisting of three circular
232 chambers connected by a narrow corridor (following Petersen and Hardy, 1996), with a *C. maculatus*
233 fourth instar larva placed in a gelatin capsule in the central chamber. During each encounter, we
234 recorded which female was (i) first on host, (ii) first to oviposit, and (iii) first to initiate agonistic
235 interactions with her opponent. We also noted the number of the following behaviors: antennal
236 contact, pushing (i.e. a female pushed her opponent with her body), chasing the opponent, climbing
237 to the opponent or kicking the opponent. All these behaviors, apart from antennal contact, were
238 considered aggressive. Contests lasted a maximum of 1 h or were stopped when one of the females
239 exited the central chamber for more than 2 min, or when neither female touched the host or had any
240 agonistic interactions for more than 2 min. A female was designated the winner of the contest when
241 her opponent left the central chamber for more than 2 min. Similarly, when both females in a dyad
242 remained in the central chamber for the entire duration of the observation, or when both females
243 apparently lost interest in the host, the winning female was designated as the one that exhibited
244 oviposition behavior. Contests were considered unresolved, when the interaction was not interrupted
245 and both females exhibited oviposition behavior. This was also the case when none of the protagonists
246 appeared to be interested in the host.

247 After each encounter, females were frozen at -20°C, and the number of eggs laid on the host
248 by the winner counted. Subsequently, females were weighed using an electronic scale (Sartorius
249 Quintix 125 D-15, accuracy 0.01 mg) and dissected to count the number of mature eggs in their
250 abdomens. The initial mature egg load of the winning females was calculated as the number of mature
251 eggs in their ovaries plus the number of eggs laid on the host.

252

253 2.8 Statistical analysis

254 All statistical analyses were performed with R software v4.0.4 and R studio v1.4.1106 with
255 the packages car, DHARMA, emmeans, and using $\alpha = 0.05$. The general approach was to use

256 parametric analyses. However, when this was not possible because the parametric assumptions were
257 not met, nonparametric tests were used. The distribution and homoscedasticity of each model was
258 checked using the 'testResiduals' function of the DHARMA package (Hartig, 2021).

259 To estimate differences in female mortality due to pyriproxyfen treatment, we carried out
260 parametric survival analyses using the 'survreg' function of the Survival package. An initial model
261 comparison showed that a Weibull distribution had a better fit than an exponential distribution ($P <$
262 0.005).

263 To explore the effect of pyriproxyfen exposure on female egg load, 4h and 24 h after
264 application, we ran two linear models, followed by Tukey's 2-to-2 multiple comparisons when
265 appropriate.

266 In the mobility and exploration experiment, we analyzed the effect of pyriproxyfen exposure
267 on several variables. We investigated the effect of pesticide concentrations on the total distance
268 covered by females in the arena, the resting time and the time they spent on the seed using Kruskal-
269 Wallis tests. The effect on the latency before reaching the seed was evaluated using a generalized
270 linear model with a Gamma error distribution, and the effect on the number of eggs laid on a host
271 with a Poisson error structure. It has to be noted that 8 of the 114 females were removed from this
272 later analysis because either the number of eggs laid could not be accurately determined (i.e. the host
273 was damaged during its extraction from the cowpea seed making potential parasitoid eggs impossible
274 to locate) or were sterile (i.e. they were no oocytes in their ovaries).

275 To explore the effect of pyriproxyfen on female host discrimination ability, we tested whether
276 the probability that females laid eggs on unparasitized and parasitized hosts varied with the
277 concentration of pesticide they were exposed to, using a generalized linear mixed model ('glmer'
278 function of the lme4 package) with a Binomial error structure, taking the identity of the female as a
279 random variable. The effect of pyriproxyfen exposure on immobility, grooming and foraging time

280 was evaluated by Kruskal-Wallis tests, and antennal exploration time by a linear regression (with a
281 normal error structure).

282 Among the 81 dyadic encounters set up to explore the effect of pyriproxyfen exposure on
283 female aggressiveness and contest resolution, 65 were clearly resolved: only these were considered
284 in the following analyses. The effect of the treatment on the aggressiveness of the focal females was
285 evaluated using two generalized linear mixed models with the identity of the fight as a random
286 variable ('glmer' function). For the first model, the response variable was whether or not females
287 expressed aggressive behaviors (binomial error structure). The total number of agonistic behaviors
288 displayed by each female was considered in the second model (Poisson error structure). The relative
289 difference in egg load between females was calculated as the result of '((egg load of focal female -
290 egg load of opposing female) / egg load of focal female) x 100)'. Two GLMs were then run with a
291 binomial distribution of errors (logit link function). The first model explored the influence of
292 treatment (acetone or pyriproxyfen), relative egg load difference and their interaction on the
293 probability of the focal female winning the contest. A similar analysis was performed with the relative
294 difference in mass instead of egg load. The differences in mass and egg load were not entered into
295 the same model because these two variables are correlated. Indeed, the greater the mass of a female,
296 the greater her egg load (Linear model, $F_{1,63} = 10.526$, $P = 0.002$). Binomial tests ('binom.test'
297 function) were also performed to explore whether the probability of the focal female exploiting the
298 host first was significantly different from the null expectation at 0.5.

299

300 **3. Results**

301 3.1 Female survival and reproduction

302 Survival did not significantly differ between females manipulated but not exposed to any
303 solution, females treated with acetone and females treated with one of the different concentrations of
304 pyriproxyfen ($\chi^2 = 6.29$, $ddl = 4$, $P = 0.18$). In contrast, egg load was significantly higher in females

305 treated with pyriproxyfen, regardless of the concentration received, than in females treated with
306 acetone, 24h after exposure ($F_{3,77} = 6.720$, $P < 0.001$; Figure 1). Four hours after application, this
307 effect was, however, not yet visible: female egg load did not significantly differ between all
308 treatments ($F_{3,76} = 0.066$, $P = 0.978$).

309

310 3.2 Mobility and exploration

311 Regardless of the pyriproxyfen concentration received, females' exploration ability to explore
312 a complex arena and motivation to find an oviposition site did not differ between treatments (Figure
313 S2). Indeed, the time (Gamma LR- $\chi^2 = 1.787$, $df = 3$, $P = 0.618$) and distance (Kruskal-Wallis: $H =$
314 2.229 , $df = 3$, $P = 0.513$) walked by females to reach the host –containing seed and the time spent on
315 that seed ($H = 2.240$, $df = 3$, $P = 0.524$) did not significantly vary across treatments. All females,
316 regardless of the pyriproxyfen concentration received, spent the same proportion of time resting (H
317 $= 3.880$, $df = 3$, $P = 0.275$) and laid a similar number of eggs (Poisson LR- $\chi^2 = 2.598$, $df = 3$, $P =$
318 0.458).

319

320 3.3 Host discrimination

321 The effect of pyriproxyfen on female host discrimination ability was assessed in a choice test
322 between a parasitized and an unparasitized host. The interaction between host value and the different
323 concentrations of pyriproxyfen tested had no significant effect on whether females laid eggs on
324 unparasitized or parasitized hosts (LR- $\chi^2 = 6.496$, $df = 3$, $P = 0.090$). Females laid eggs on the first
325 host they encountered, regardless of its quality.

326 Female behavior on hosts before laying eggs was not modified by the treatment (Figure S3).
327 Indeed, there was no significant difference in the time spent immobile (Kruskal-wallis: $H = 2.136$, df
328 $= 3$, $P = 0.545$), grooming ($H = 7.268$, $df = 3$, $P = 0.064$), or foraging for the seed ($H = 4.294$, $df = 3$,
329 $P = 0.231$), nor any effect on antennal exploration time ($F_{1,78} = 0.026$, $P = 0.871$).

330

331 3.4 Aggressiveness and conflict resolution

332 The effect of pyriproxyfen exposure (9.6 µg/mL) on female contest behavior and outcome
333 during host access was studied. Females exposed to pyriproxyfen were more likely to display
334 aggressive behavior than those exposed to acetone (logistic analysis, LR- $\chi^2 = 4.146$, df = 1, P = 0.042).
335 They also showed a greater number of aggressive behaviors than did control females (Poisson LR- χ^2
336 = 6.243, df = 1, P = 0.012, Figure 2A). In spite of this aggressiveness, the probability of the focal
337 female winning was not influenced by pyriproxyfen or acetone treatment, the relative difference in
338 mature egg load between contestants, or the interaction between these main effects (Table 1). The
339 difference in reproductive status, i.e. their mature egg load, also had no effect on the probability of
340 winning when only acetone-acetone dyads were considered (logistic analysis, LR- $\chi^2 = 0.714$, df = 1,
341 P = 0.398). Similarly, the probability that the focal female won did not vary with the relative mass
342 difference with the opponent, the treatment received or the interaction between both (Table 1). When
343 considering only acetone-acetone female contests, the effect of mass difference was also not
344 significant, despite a trend in which heavier females were slightly advantaged (LR $\chi^2 = 3.615$, df = 1,
345 P = 0.057). In contrast, focal females treated with pyriproxyfen were more frequently the first of their
346 dyad to exhibit host oviposition behavior (binomial test: P = 0.007, Figure 2B), than focal females
347 exposed to acetone (P = 0.296).

348

349 **4. Discussion**

350 This study aimed to evaluate the potential sublethal effects of pyriproxyfen, an insecticide that
351 regulates growth by mimicking juvenile hormone, in females of *E. vuilleti*, a beneficial parasitoid
352 wasp that attacks the food stock pest, *C. maculatus*. We found that low doses of pyriproxyfen do not
353 affect survival but modulate both reproductive physiology and behavior in this parasitoid.
354 Pyriproxyfen has a gonadotropic effect in *E. vuilleti* females. Additionally, while exploratory and
355 host discrimination behavior did not seem to be modified after pyriproxyfen exposure, agonistic

356 behaviors were affected. Females exposed to pyriproxyfen were more aggressive during female-
357 female contests for hosts and were more likely to be the first of the dyad exhibiting oviposition
358 behavior. Even if many studies focused on the impact of pesticides on egg-laying behavior in insects
359 (see Barascou et al., 2021 for bees), especially parasitoids (effects of various neurotoxic insecticides:
360 Delpuech, 2017; Delpuech and Delahaye, 2013; Desneux et al., 2004; Rafalimanana et al., 2002;
361 Sheng et al., 2021; Wang et al., 2016) due to their economic importance as biological control agents,
362 very few studies explored the effect of juvenile hormone analogues on adults (Beloti et al., 2015;
363 Cloyd and Dickinson, 2006) and, to our knowledge, none during intraspecific competition for
364 oviposition sites. Here, we showed that a pesticide that is an Insect Growth Regulator, IGR, can have
365 sublethal effects on non-target insects by impacting their fecundity and oviposition behavior under
366 intraspecific competition.

367 Regarding the effect of pyriproxyfen on reproductive physiology, we showed that, 24 hours
368 after exposure, *E. vuilleti* females had on average 2 to 3 more mature eggs than control females (i.e.
369 exposed to solvent), regardless of the concentration applied. Four hours after application, this effect
370 was, however, not yet visible. These results confirm pyriproxyfen mimics JH's action on reproduction
371 in *E. vuilleti*. Juvenile hormone, known to be one of the main gonadotropic hormones in insects by
372 stimulating vitellogenesis (Roy et al., 2018), has indeed been demonstrated to enhance egg maturation
373 in *E. vuilleti* (Mathiron et al., 2019): injection of JH in females stimulates the maturity of additional
374 eggs. This gonadotropic effect was observed 24h after exposure but not yet after 4h (Mathiron et al.,
375 2019). Altogether, these results suggest the complete maturation of new eggs following hormonal (JH
376 or pyriproxyfen) signal takes between 4h and 24h in this species. Furthermore, the duration of
377 pyriproxyfen effect on egg maturation remains to be investigated: it can result in a transient increase
378 that does not necessarily modify females' lifetime egg production, or a longer-lasting effect
379 enhancing females' reproductive potential. Whether eggs produced following exposure to this
380 insecticide develop normally should also be analyzed. IGRs can indeed impact egg viability in adult
381 insects as observed in pests, such as *Platynota idaeusalis* and *Anthonomus grandis grandis* (Myers

382 and Hull, 2003; Rolim et al., 2019). Similarly, exposure of female ladybugs *Tenuisvalvae notata* to
383 pyriproxyfen resulted in a temporary sterility (Barbosa et al., 2018): treated females laid a similar
384 number of eggs, but these eggs laid during treatment, and up to 4-5 days after the end of exposure,
385 were not viable and did not result in the development of any offspring.

386 Under field conditions, the consequences on female fitness, hence parasitoid population size,
387 will also depend on the availability of hosts when females show an enhanced number of ready-to-lay
388 eggs. If parasitoid population size increases, the use of low doses of pyriproxyfen would have dual
389 effects on the control of cowpea pests, both by negatively affecting pest survival, and by favoring
390 parasitism rate. On the contrary, if parasitoid population size is negatively impacted by egg quality
391 or asynchrony between mature eggs and available hosts, a negative indirect effect on pest regulation
392 is expected. The potential consequences of low doses of pyriproxyfen on offspring production in *E.*
393 *vuilleti* remains to be further investigated to understand its effect on pest control efficiency.

394 In addition to affecting the insects' physiology, pyriproxyfen could also alter their behavior.
395 Previous experiments carried out in the eusocial bee *Apis mellifera* showed that, despite not being a
396 demonstrated juvenile hormone-controlled behavior, workers' mobility was impacted by exposure to
397 pyriproxyfen. Their flight ability was reduced and they spent more time immobile (Fourrier et al.,
398 2015; Gomes et al., 2020). This pair of studies are among the very few exploring the influence of
399 pyriproxyfen on this type of behavior in insects. Our video tracking data did not indicate similar
400 effects in female *E. vuilleti*. This means either these behaviors are not JH-dependent in *E. vuilleti* or,
401 regardless of the underlying mechanisms regulating them, exploration and mobility were not
402 disrupted by this insecticide at the doses applied. The ability to discriminate host quality, in particular
403 its parasitism status, is common in parasitoid wasps (Godfray, 1994) and is often based on the
404 perception and recognition of chemical cues. After locating their hosts using long-range cues or
405 signals, females detect and recognize chemical information about host quality through the activation
406 of sensory neurons of sensillae mainly located on their antennae and ovipositor, and then adjust their
407 oviposition decisions accordingly (Cury et al., 2019). Neurotoxic pesticides can disrupt the insect

408 olfactory system (Delpuech and Leger, 2011), and smell disruption is a possible effect of endocrine
409 disruptors. In *Spodoptera littoralis*, bis(2-ethylhexyl)phthalate alters male detection of female sex
410 pheromone by down-regulating the transcription level of calmodulin and ecdysteroid nuclear
411 receptors in their antennae (Aviles et al., 2020). We therefore anticipated endocrine disruptors may
412 alter parasitoid females' discrimination ability in species where host quality assessment is based on
413 chemical cues perceived during antennal examination, like in *E. vuilleti* (Jaloux, 2004). In contrast to
414 previous work (Darrouzet et al. 2007), we did not observe host discrimination ability in our
415 experiment. This surprising difference may be due to the time elapsed since the first oviposition,
416 which was shorter in our set-up. Nevertheless, our results did not show any effect of pyriproxyfen in
417 modulating oviposition strategies on parasitized and unparasitized hosts in this parasitoid wasp, at the
418 tested concentrations: parasitized host acceptance rates did not differ between treated and control
419 females.

420 Despite the absence of sublethal effects of pyriproxyfen on exploration and host
421 discrimination behavior in *E. vuilleti*, we observed that female exposure to a low dose of this
422 endocrine disruptor increased the occurrence and number of aggressive behaviors. Interestingly, other
423 insecticides that are not JH analogues have already been shown to increase the aggression of insects.
424 For instance, females of the beetle *Phaedon cochleariae* exposed to pyrethroid lambda-cyhalothrin
425 show more aggressive behavior than untreated females (Müller et al., 2019). Our result is also in
426 agreement with what is known about the hormonal regulation of aggressiveness in many species.
427 Indeed, high levels of JH in adults are often associated with high levels of aggressiveness and elevated
428 dominance rank (Tibbetts et al., 2020). Furthermore, a similar stimulatory effect of aggression after
429 exposure to another JH analogue, fenoxycarb, has already been demonstrated in *Schistocerca*
430 *gregaria* and *Locusta migratoria* (Wiesel et al., 1996). This phenomenon was also found in the
431 necrophagous beetles *Nicrophorus orbicollis* and *N. tomentosus* when exposed to methoprene (Scott,
432 2006). In the absence of a resource to fight over, exposure to methoprene indeed increased
433 individuals' aggressiveness: when a treated female competed with a untreated conspecific, injuries

434 were more frequent than when neither of the opponents was treated (Scott, 2006). Altogether, these
435 studies, including this current study, support the hypothesis that juvenile hormone-like growth
436 regulator insecticides can influence the behavior of non-target insects, especially in competitive
437 situations.

438 Additionally, *E. vuilleti* females treated with pyriproxyfen were more likely to be the first of
439 their dyad exhibiting host exploration compared to control females (exposed to solvent). This could
440 reflect a greater motivation among pyriproxyfen treated females to lay eggs. Indeed, JH is known to
441 stimulate egg maturation within 24h (Mathiron et al., 2019), which in turn increases the value that
442 females place in the host (i.e. higher subjective value, Mohamad et al., 2010), hence their motivation
443 to access hosts. Juvenile hormone would thus be the direct or indirect signal modulating motivation
444 to access the host. Exposure to pyriproxyfen would induce a similar signal and lead to an increase in
445 female motivation to take possession of hosts. It would then be interesting to analyze how long this
446 effect lasts. Nevertheless, this increased probability of being first on host (and being first to exhibit
447 oviposition behavior) did not translate into a higher probability of being first to lay an egg (i.e. a fully
448 realized win of a contest for host resources). The oviposition sequence of females was frequently
449 interrupted by aggressive behavior of their opponent, leading to their departure from the host or even
450 from the central chamber, while the opponent fully parasitized the host. As a result, females exposed
451 to the insecticide were not more likely than control females to win the overall conflict. These
452 surprising results are consistent with previous work showing that, while *E. vuilleti* agonistic behaviors
453 are modulated by JH (Mathiron et al., 2019), contest outcomes are mediated by another hormone,
454 ecdysone (Mathiron et al., 2020). Thus, whereas *in silico* analyses revealed a possible interaction of
455 pyriproxyfen with USP-EcR, the receptor dimer known to mediate the effects of ecdysteroids in bees
456 (Spaggiari et al., 2021), it seems that in *E. vuilleti* there is no cross communication between
457 pyriproxyfen and ecdysteroid activity.

458

459

460 5. Conclusion

461 Our results revealed exposure to sublethal doses of pyriproxyfen enhances, at least
462 temporarily, egg maturation, aggressiveness and motivation to access host during competition in *E.*
463 *vuilleti* females. If these effects lead to a higher fitness in these parasitoids, they could improve the
464 biological control of the cowpea seed pest, *C. maculatus*. Similarly, positive effects of sublethal
465 concentrations of other insecticides on natural enemies of pests have been identified (Müller, 2018;
466 Stothard and Hardy, unpublished). For instance, the frequency of flight and walking, as well as the
467 duration and distance of these movements in ladybugs *Harmonia axyridis* exposed to beta-
468 cypermethrin is increased (Xiao et al., 2017). Female fecundity is also enhanced (Xiao et al., 2016).
469 As flight performance is important for predation but also for dispersal, such changes in a beneficial
470 insect may have important consequences in its biocontrol efficiency. In addition, pesticides can cause
471 transgenerational effects and impact populations over multiple generations even though they are no
472 longer present in the environment (Müller, 2018). However, because a same insecticide can have
473 effects on both pests and beneficial organisms, care must be taken to ensure that the dose used is more
474 beneficial to the latter species than to the former. Furthermore, positive sublethal effects are rare and
475 in most cases insecticides have harmful effects on non-target insects (Desneux et al., 2007). Finally,
476 the influence of pesticides on interactions between sympatric species, whether interspecific or
477 between prey and predators, must be investigated to ensure the maintenance of ecosystem dynamics
478 in IPM programs.

479 Under the experimental conditions of this study, pyriproxyfen modulated upward *E. vuilleti*
480 females' fecundity and aggressiveness during conflict for host access. This highlights that
481 pyriproxyfen may have, though relatively specific, unintended effects on non-target species,
482 including parasitoids used to control cowpea pests. At this point, it remains difficult to anticipate the
483 consequences at the population level of these positive effects on reproduction and behavior and
484 effects on other life-history traits cannot yet be ruled out. This study raises the importance of studying
485 the diversity of unintentional sublethal effects of pesticides that may affect non-target species,

486 including arthropods, to improve knowledge of their potential risks to species used in biological
487 control.

488

489 **Competing interest statement**

490 The authors have no competing interests to declare.

491

492 **Ethical Note**

493 No animal ethics approval was required. Nevertheless, we handled all individuals with care, and we
494 kept handling time to an absolute minimum. Acrylic paint used for marking does not cause injury to
495 wasps. Dissected wasps were freeze-killed prior to decapitation.

496

497 **Acknowledgements**

498 We are grateful to two anonymous reviewers and Ian Hardy for helpful comments, Joel Meunier for
499 his video-tracking recording system and Fabrice Vannier for technical assistance.

500

501 **References**

502 Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G., Chermiti, B., Zappalà, L.,
503 2015. Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon*
504 *nigricans*. PLoS One 10, e0138411. <https://doi.org/10.1371/journal.pone.0138411>

505 Abo-Elghar, G.E., El-Sheikh, A.E., El-Sayed, F.M., El-Maghraby, H.M., El-Zun, H.M., 2004.
506 Persistence and residual activity of an organophosphate, pirimiphos-methyl, and three IGRs,
507 hexaflumuron, teflubenzuron and pyriproxyfen, against the cowpea weevil, *Callosobruchus*

508 *maculatus* (Coleoptera: Bruchidae). Pest Management Science 60, 95–102.
509 <https://doi.org/10.1002/ps.783>

510 Aviles, A., CORDEIRO, A., Maria, A., Bozzolan, F., Boulogne, I., Dacher, M., Goutte, A., Alliot,
511 F., Maibèche-Coisne, M., Massot, M., Siaussat, D., 2020. Effects of DEHP on ecdysteroid pathway,
512 sexual behaviour and offspring of the moth *Spodoptera littoralis*. Hormones and Behavior 125.
513 <https://doi.org/10.1016/j.yhbeh.2020.104808>

514 Barascou, L., Brunet, J.-L., Belzunces, L., Decourtye, A., Henry, M., Fourrier, J., Le Conte, Y.,
515 Alaux, C., 2021. Pesticide risk assessment in honeybees: Toward the use of behavioral and
516 reproductive performances as assessment endpoints. Chemosphere 276, 130134.
517 <https://doi.org/10.1016/j.chemosphere.2021.130134>

518 Barbosa, P., Oliveira, M., Barros, E., Michaud, J.P., 2018. Differential impacts of six insecticides on
519 a mealybug and its coccinellid predator. Ecotoxicology and Environmental Safety 147, 963–971.
520 <https://doi.org/10.1016/j.ecoenv.2017.09.021>

521 Beloti, V.H., Alves, G.R., Araújo, D.F.D., Picoli, M.M., Moral, R. de A., Demétrio, C.G.B.,
522 Yamamoto, P.T., 2015. Lethal and sublethal effects of insecticides used on citrus, on the
523 ectoparasitoid *Tamarixia radiata*. PLOS ONE 10, e0132128.
524 <https://doi.org/10.1371/journal.pone.0132128>

525 Bodin, A., Jaloux, B., Delbecq, J.-P., Vannier, F., Monge, J.-P., Mondy, N., 2009. Reproduction in
526 a variable environment: How does *Eupelmus vuilleti*, a parasitoid wasp, adjust oogenesis to host
527 availability? Journal of Insect Physiology 55, 643–648.
528 <https://doi.org/10.1016/j.jinsphys.2009.04.003>

529 Cloyd, R., 2012. Indirect effects of pesticides on natural enemies, in: Pesticides - Advances in
530 Chemical and Botanical Pesticides. IntechOpen. <https://doi.org/10.5772/48649>

531 Cloyd, R.A., Dickinson, A., 2006. Effect of insecticides on mealybug destroyer (Coleoptera:
532 Coccinellidae) and parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of
533 citrus mealybug (Homoptera: Pseudococcidae). *Journal of Economic Entomology* 99, 1596–1604.
534 <https://doi.org/10.1093/jee/99.5.1596>

535 Córdoba-Aguilar, A., Munguía-Steyer, R., 2015. To be or not to be? Mating success and survival
536 trade-offs when switching between alternative reproductive tactics. *Journal of Evolutionary Biology*
537 28, 2119–2124. <https://doi.org/10.1111/jeb.12721>

538 Cury, K.M., Prud'homme, B., Gompel, N., 2019. A short guide to insect oviposition: when, where
539 and how to lay an egg. *Journal of Neurogenetics* 33, 75–89.
540 <https://doi.org/10.1080/01677063.2019.1586898>

541 Damiens, D., Imbert, E., Bressac, C., Thibeau, C., Chevrier, C., 2001. Egg-laying, pre-imaginal
542 growth dynamics, and mortality in *Eupelmus orientalis* and *Dinarmus basalis*, two solitary
543 ectoparasitoids of *Callosobruchus maculatus*. *Entomologia Experimentalis et Applicata* 99, 97–105.
544 <https://doi.org/10.1046/j.1570-7458.2001.00806.x>

545 Darrouzet, E., Bignon, L., Chevrier, C., 2007. Impact of mating status on egg-laying and
546 superparasitism behaviour in a parasitoid wasp. *Entomologia Experimentalis et Applicata* 123, 279-
547 285. <https://doi.org/10.1111/j.1570-7458.2007.00544.x>

548 Davies, T.G.E., Field, L.M., Williamson, M.S., 2012. The re-emergence of the bed bug as a nuisance
549 pest: implications of resistance to the pyrethroid insecticides. *Medical and Veterinary Entomology*
550 26, 241–254. <https://doi.org/10.1111/j.1365-2915.2011.01006.x>

551 Delpuech, J.-M., 2017. Elicitation of superparasitization behavior from the parasitoid wasp
552 *Leptopilina boulardi* by the organophosphorus insecticide chlorpyrifos. *Science of The Total*
553 *Environment* 580, 907–911. <https://doi.org/10.1016/j.scitotenv.2016.12.037>

554 Delpuech, J.-M., Delahaye, M., 2013. The sublethal effects of deltamethrin on *Trichogramma*
555 behaviors during the exploitation of host patches. *Science of The Total Environment* 447, 274–279.
556 <https://doi.org/10.1016/j.scitotenv.2012.12.096>

557 Delpuech, J.-M., Leger, L., 2011. Modifications of *Trichogramma* behaviors during the exploitation
558 of host patches induced by the insecticide chlorpyrifos. *EcoHealth* 8, 190–198.
559 <https://doi.org/10.1007/s10393-011-0695-9>

560 Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial
561 arthropods. *Annual review of entomology* 52, 81–106.
562 <https://doi.org/10.1146/annurev.ento.52.110405.091440>

563 Desneux, N., Pham-Delègue, M.-H., Kaiser, L., 2004. Effects of sub-lethal and lethal doses of
564 lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp,
565 *Aphidius ervi*. *Pest Management Science* 60, 381–389. <https://doi.org/10.1002/ps.822>

566 Devillers, J., 2020. Fate and ecotoxicological effects of pyriproxyfen in aquatic ecosystems.
567 *Environmental Science and Pollution Research* 27, 16052–16068. [https://doi.org/10.1007/s11356-](https://doi.org/10.1007/s11356-020-08345-8)
568 [020-08345-8](https://doi.org/10.1007/s11356-020-08345-8)

569 Devillers, J., Devillers, H., 2020. Lethal and sublethal effects of pyriproxyfen on *Apis* and Non-*Apis*
570 Bees. *Toxics* 8, 104. <https://doi.org/10.3390/toxics8040104>

571 Fourier, J., Deschamps, M., Droin, L., Alaux, C., Fortini, D., Beslay, D., Conte, Y.L., Devillers, J.,
572 Aupinel, P., Decourtye, A., 2015. Larval exposure to the juvenile hormone analog pyriproxyfen
573 disrupts acceptance of and social behavior performance in adult honeybees. *PLOS ONE* 10.
574 <https://doi.org/10.1371/journal.pone.0132985>

575 Gauthier, N., Monge, J.P., 1999. Could the egg itself be the source of the oviposition deterrent marker
576 in the ectoparasitoid *Dinarmus basalis*? *Journal of Insect Physiology* 45, 393–400.
577 [https://doi.org/10.1016/S0022-1910\(98\)00138-3](https://doi.org/10.1016/S0022-1910(98)00138-3)

578 Gibson, G.A.P., Fusu, L., 2016. Revision of the Palaearctic species of *Eupelmus* (*Eupelmus*) Dalman
579 (Hymenoptera: Chalcidoidea: Eupelmidae). *Zootaxa* 4081, 1.
580 <https://doi.org/10.11646/zootaxa.4081.1.1>

581 Godfray, H.C.J., 1994. Oviposition behavior, in: *Parasitoids*. Princeton University Press (Ed.).
582 Princeton, New Jersey.

583 Gomes, I., Vieira, K., Gontijo, L., Resende, H., 2020. Honeybee survival and flight capacity are
584 compromised by insecticides used for controlling melon pests in Brazil. *Ecotoxicology* 29, 1–11.
585 <https://doi.org/10.1007/s10646-019-02145-8>

586 Haan, N.L., Iuliano, B.G., Gratton, C., Landis, D.A., 2021. Chapter Five - Designing agricultural
587 landscapes for arthropod-based ecosystem services in North America, in: Bohan, D.A., Vanbergen,
588 A.J. (Eds.), *Advances in Ecological Research, The Future of Agricultural Landscapes, Part II*.
589 Academic Press, pp. 191–250. <https://doi.org/10.1016/bs.aecr.2021.01.003>

590 Hartig, F., 2021. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression
591 Models [WWW Document]. R package version 0.4.3. URL <http://florianhartig.github.io/DHARMA/>
592 (accessed 9.23.21).

593 Hustedt, J.C., Boyce, R., Bradley, J., Hii, J., Alexander, N., 2020. Use of pyriproxyfen in control of
594 *Aedes mosquitoes*: A systematic review. *PLoS Neglected Tropical Diseases* 14.
595 <https://doi.org/10.1371/journal.pntd.0008205>

596 IPBES, 2016. The assessment report of the Intergovernmental Science-Policy Platform on
597 Biodiversity and Ecosystem Services on pollinators, pollination and food production. S.G. Potts, V.

598 L. Imperatriz-Fonseca, and H. T. Ngo (eds), Secretariat of the Intergovernmental Science-Policy
599 Platform on Biodiversity and Ecosystem Services, Bonn, Germany. 552 pages.

600 Jacquin, L., Petitjean, Q., Côte, J., Laffaille, P., Jean, S., 2020. Effects of pollution on fish behavior,
601 personality, and cognition: some research perspectives. *Frontiers in Ecology and Evolution* 8, 86.
602 <https://doi.org/10.3389/fevo.2020.00086>

603 Jaloux, B., 2004. La discrimination interspécifique par *Eupelmus vuilleti* (Hymenoptera :
604 Eupelmidae) des hôtes parasites par *Dinarmus basalis* (Hymenoptera : Pteromalidae) (thesis).
605 <http://www.theses.fr>. Tours.

606 Jaloux, B., Sanon, A., Huignard, J., Monge, J., 2004. Interspecific relationships between the solitary
607 ectoparasitoid, *Eupelmus vuilleti* (Crw.) (Eupelmidae), and its sympatric species, *Dinarmus basalis*
608 (Rond.) (Pteromalidae), in the presence of their host, *Callosobruchus maculatus* Pic (Coleoptera
609 Bruchidae). *Journal of Insect Behavior* 17, 793–808.
610 <https://doi.org/10.1023/B:JOIR.0000048989.36181.4d>

611 Kamal, M., Kenawy, M.A., Rady, M.H., Khaled, A.S., Samy, A.M., 2018. Mapping the global
612 potential distributions of two arboviral vectors *Aedes aegypti* and *Ae. albopictus* under changing
613 climate. *PLoS One* 13. <https://doi.org/10.1371/journal.pone.0210122>

614 Liao, C., He, X., Wang, Z., Barron, A.B., Zhang, B., Zeng, Z., Wu, X., 2018. Short-term exposure to
615 lambda-cyhalothrin negatively affects the survival and memory-related characteristics of worker bees
616 *Apis mellifera*. *Archives of Environmental Contamination and Toxicology* 75, 59–65.
617 <https://doi.org/10.1007/s00244-018-0514-1>

618 Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by ynsects.
619 *BioScience* 56, 311–323. [https://doi.org/10.1641/0006-3568\(2006\)56\[311:TEVOES\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2)

620 Mathiron, A., Dixneuf, C., Mondy, N., Lécureuil, C., Earley, R., Goubault, M., 2020. Ecdysteroids
621 affect female reproductive status and outcome of contest over hosts in the parasitoid wasp *Eupelmus*
622 *vuilleti*. *Hormones and behavior* 125, 104819. <https://doi.org/10.1016/j.yhbeh.2020.104819>

623 Mathiron, A., Earley, R., Goubault, M., 2019. Juvenile Hormone manipulation affects female
624 reproductive status and aggressiveness in a non-social parasitoid wasp. *General and Comparative*
625 *Endocrinology* 274, 80–86. <https://doi.org/10.1016/j.ygcen.2019.01.006>

626 Mathiron, A., Pottier, P., Goubault, M., 2018. Let the most motivated win: Resource value
627 components affect contest outcome in a parasitoid wasp. *Behavioral Ecology* 29, 1088–1095.
628 <https://doi.org/10.1093/beheco/ary084>

629 Mauduit, E., Lécureuil, C., Meunier, J., 2021. Sublethal exposure to deltamethrin stimulates
630 reproduction and has limited effects on post-hatching maternal care in the European earwig.
631 *Environmental Science and Pollution Research* 28, 1–12. [https://doi.org/10.1007/s11356-021-13511-](https://doi.org/10.1007/s11356-021-13511-7)
632 [7](https://doi.org/10.1007/s11356-021-13511-7)

633 Meunier, J., Dufour, J., Van Meyel, S., Rault, M., Lécureuil, C., 2020. Sublethal exposure to
634 deltamethrin impairs maternal egg care in the European earwig *Forficula auricularia*. *Chemosphere*
635 258, 127383. <https://doi.org/10.1016/j.chemosphere.2020.127383>

636 Mohamad, R., Monge, J.-P., Goubault, M., 2010. Can subjective resource value affect aggressiveness
637 and contest outcome in parasitoid wasps? *Animal Behaviour* 80, 629–636.
638 <https://doi.org/10.1016/j.anbehav.2010.06.022>

639 Montiglio, P.-O., Royauté, R., 2014. Contaminants as a neglected source of behavioural variation.
640 *Animal Behaviour*. <https://doi.org/10.1016/j.anbehav.2013.11.018>

641 Moura, J.A.S., Souza-Santos, L.P., 2020. Environmental risk assessment (ERA) of pyriproxyfen in
642 non-target aquatic organisms. *Aquatic Toxicology* 222, 105448.
643 <https://doi.org/10.1016/j.aquatox.2020.105448>

644 Müller, C., 2018. Impacts of sublethal insecticide exposure on insects — Facts and knowledge gaps.
645 *Basic and Applied Ecology* 30, 1–10. <https://doi.org/10.1016/j.baae.2018.05.001>

646 Müller, T., Römer, C.I., Müller, C., 2019. Parental sublethal insecticide exposure prolongs mating
647 response and decreases reproductive output in offspring. *Journal of Applied Ecology* 56, 1528–1537.
648 <https://doi.org/10.1111/1365-2664.13398>

649 Myers, C., Hull, L., 2003. Insect growth regulator impact on fecundity and fertility of adult tufted
650 apple bud moth, *Platynota idaeusalis* Walker. *Journal of Entomological Science* 38, 420–430.
651 <https://doi.org/10.18474/0749-8004-38.3.420>

652 Nelson, R.J., 2010. Hormones and behavior: Basic concepts, in: Breed, M.D., Moore, J. (Eds.),
653 *Encyclopedia of Animal Behavior*. Academic Press, Oxford, pp. 97–105.
654 <https://doi.org/10.1016/B978-0-08-045337-8.00236-9>

655 Niedermayer, S., Pollmann, M., Steidle, J.L.M., 2016. *Lariophagus distinguendus* (Hymenoptera:
656 Pteromalidae) (Förster)—Past, present, and future: The history of a biological control method using
657 *L. distinguendus* against different storage pests. *Insects* 7, 39. <https://doi.org/10.3390/insects7030039>

658 Petersen, G., Hardy, I.C.W., 1996. The importance of being larger: parasitoid intruder–owner contests
659 and their implications for clutch size. *Animal Behaviour* 51, 1363–1373.
660 <https://doi.org/10.1006/anbe.1996.0139>

661 Rafalimanana, H., Kaiser, L., Delpuech, J.-M., 2002. Stimulating effects of the insecticide
662 chlorpyrifos on host searching and infestation efficacy of a parasitoid wasp. *Pest Management*
663 *Science* 58, 321–328. <https://doi.org/10.1002/ps.454>

664 Ringo, J.M., 2002. 36 - Hormonal regulation of sexual behavior in insects, in: Pfaff, D.W., Arnold,
665 A.P., Fahrbach, S.E., Etgen, A.M., Rubin, R.T. (Eds.), Hormones, Brain and Behavior. Academic
666 Press, San Diego, pp. 93–114. <https://doi.org/10.1016/B978-012532104-4/50038-X>

667 Rolim, G., Barros, E., Barbosa, P., Arruda, L., 2019. Sublethal effects of insect growth regulators on
668 boll weevil (Coleoptera: Curculionidae). *Journal of economic entomology* 112, 2222–2228.
669 <https://doi.org/10.1093/jee/toz164>

670 Roy, S., Saha, T.T., Zou, Z., Raikhel, A.S., 2018. Regulatory pathways controlling female insect
671 reproduction. *Annual Review of Entomology* 63, 489–511. [https://doi.org/10.1146/annurev-ento-](https://doi.org/10.1146/annurev-ento-020117-043258)
672 [020117-043258](https://doi.org/10.1146/annurev-ento-020117-043258)

673 Sánchez-Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non-target
674 organisms. *Journal of Environmental and Analytical Toxicology* S4, S4-002.
675 <https://doi.org/10.4172/2161-0525.S4-002>

676 Sánchez-Bayo, F., 2011. Impacts of agricultural pesticides on terrestrial ecosystems, in: *Ecological*
677 *Impacts of Toxic Chemicals*. Bentham Science Publishers, pp. 63–87.
678 <https://doi.org/10.2174/978160805121210063>

679 Scott, M.P., 2006. The role of juvenile hormone in competition and cooperation by burying beetles.
680 *Journal of Insect Physiology* 52, 1005–1011. <https://doi.org/10.1016/j.jinsphys.2006.04.006>

681 Sheng, S., Song, Y., Ahmad, S., Wang, J., Shao, Y., Liu, Z., Wu, F., 2021. Sublethal effects of
682 organophosphorus insecticide phoxim on patch time allocation and oviposition behavior in a
683 parasitoid wasp *Meteorus pulchricornis*. *Bulletin of Entomological Research* 1–10.
684 <https://doi.org/10.1017/S0007485321000614>

685 Sievers, M., Hale, R., Parris, K.M., Melvin, S.D., Lanctôt, C.M., Swearer, S.E., 2019. Contaminant-
686 induced behavioural changes in amphibians: A meta-analysis. *Science of The Total Environment* 693,
687 133570. <https://doi.org/10.1016/j.scitotenv.2019.07.376>

688 Smith, A.R., Kapheim, K.M., Pérez-Ortega, B., Brent, C.S., Wcislo, W.T., 2013. Juvenile hormone
689 levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis*
690 (Hymenoptera: Halictidae). *Hormones and Behavior* 63, 1–4.
691 <https://doi.org/10.1016/j.yhbeh.2012.08.012>

692 Spaggiari, G., Iovine, N., Cozzini, P., 2021. In silico prediction of the mechanism of action of
693 pyriproxyfen and 4'-OH-pyriproxyfen against *A. mellifera* and *H. sapiens* receptors. *International*
694 *Journal of Molecular Sciences* 22, 7751. <https://doi.org/10.3390/ijms22147751>

695 Stothard, S., Hardy, I., In prep. The stimulating effect of neonicotinoids: intruder-owner contests and
696 aggression in a parasitoid wasp.

697 Sullivan, J., Goh, K., 2008. Environmental fate and properties of pyriproxyfen. *Journal of Pesticide*
698 *Science* 33, 339–350. <https://doi.org/10.1584/jpestics.R08-02>

699 Tappert, L., Pokorny, T., Hofferberth, J., Ruther, J., 2017. Sublethal doses of imidacloprid disrupt
700 sexual communication and host finding in a parasitoid wasp. *Scientific Reports* 7, 42756.
701 <https://doi.org/10.1038/srep42756>

702 Terrasse, C., Rojas-Rousse, D., 1986. Distribution de la ponte et évitement du superparasitisme chez
703 l'hyménoptère solitaire *Bruchocida vuilleti* Cwf. (Hym., Eupelmidae), parasite des stades larvaires
704 de son nôte, *Callosobmchus maculatus* F. (Col., Bruchidae). *Journal of Applied Entomology* 101,
705 243–256. <https://doi.org/10.1111/j.1439-0418.1986.tb00855.x>

706 Thabet, H.S., Fawaz, E.Y., Badziklou, K., ElDin, R.A.T., Kaldas, R.M., Fahmy, N.T., Tamekloe,
707 T.A., Kere-Banla, A., Diclaro, J.W., 2020. Preliminary screening of mosquito spatial distribution in

708 Togo: With special focus on the *Aedes* (Diptera: Culicidae) species. *Journal of Medical Entomology*
709 56, 1154–1158. <https://doi.org/10.1093/jme/tjz029>

710 Theiling, K.M., Croft, B.A., 1988. Pesticide side-effects on arthropod natural enemies: A database
711 summary. *Agriculture, Ecosystems & Environment* 21, 191–218. <https://doi.org/10.1016/0167->
712 8809(88)90088-6

713 Tibbetts, E.A., Crocker, K.C., 2014. The challenge hypothesis across taxa: social modulation of
714 hormone titres in vertebrates and insects. *Animal Behaviour* 92, 281–290.
715 <https://doi.org/10.1016/j.anbehav.2014.02.015>

716 Tibbetts, E.A., Izzo, A., Huang, Z.Y., 2011. Behavioral and physiological factors associated with
717 juvenile hormone in *Polistes* wasp foundresses. *Behavioral Ecology and Sociobiology* 65, 1123–
718 1131. <https://doi.org/10.1007/s00265-010-1126-6>

719 Tibbetts, E.A., Laub, E.C., Mathiron, A.G.E., Goubault, M., 2020. The challenge hypothesis in
720 insects. *Hormones and Behavior* 123. <https://doi.org/10.1016/j.yhbeh.2019.05.002>

721 Unité de coordination nationale REDD+, direction nationale ODEF, 2020. Evaluation
722 environnementale et sociale stratégique (EESS) de la stratégie nationale REDD+ au Togo, Plan de
723 Gestion des pestes (PGP).

724 Wang, D., Lü, L., He, Y., Shi, Q., Wang, G., 2016. Effects of insecticides on oviposition and host
725 discrimination behavior in *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Journal of*
726 *Economic Entomology* 109, 2380–2387. <https://doi.org/10.1093/jee/tow220>

727 Wiesel, G., Tappermann, S., Dorn, A., 1996. Effects of juvenile hormone and juvenile hormone
728 analogues on the phase behaviour of *Schistocerca gregaria* and *Locusta migratoria*. *Journal of Insect*
729 *Physiology* 42, 385–395. [https://doi.org/10.1016/0022-1910\(95\)00119-0](https://doi.org/10.1016/0022-1910(95)00119-0)

730 Xiao, D., Tan, X., Wang, W., Zhang, F., Desneux, N., Wang, S., 2017. Modification of flight and
731 locomotion performances, respiratory metabolism, and transcriptome expression in the lady beetle
732 *Harmonia axyridis* through sublethal pesticide exposure. *Frontiers in Physiology* 8.
733 <https://doi.org/10.3389/fphys.2017.00033>

734 Xiao, D., Zhao, J., Guo, X., Li, S., Zhang, F., Wang, S., 2016. Sublethal effect of beta-cypermethrin
735 on development and fertility of the Asian multicoloured ladybird beetle *Harmonia axyridis*. *Journal*
736 *of Applied Entomology* 140, 598–608. <https://doi.org/10.1111/jen.12302>

737

738

739 Figure legends

740

741 **Figure 1:** Average number of mature eggs (mean +/- SEM) in *E. vuilleti* females 24h after being
742 treated with pyriproxyfen at four different concentrations. n indicates the sample size per treatment.
743 * indicates a significant difference ($P < 0.05$) in mature eggs between the four treatments. Two
744 different letters indicate a significant difference at $\alpha < 0.05$.

745

746 **Figure 2:** Female behavior during conflicts for access to a host depending on whether *E. vuilleti*
747 females were treated with acetone or pyriproxyfen at $9.6\mu\text{g/mL}$ four hours before. A. Number of
748 aggressive behaviors (mean +/- SEM) displayed by a focal female, treated with pyriproxyfen or
749 acetone, towards its opponent, a non-treated female. B. Proportion of focal females exploiting the
750 host first. Proportions were compared to chance (i.e. 50%). Figures in the bars indicate the number
751 of females observed in each situation. * and ** indicate a significant difference at $\alpha < 0.05$ and $\alpha <$
752 0.001 , respectively. ns: indicates a non-significant difference.

Figure 1

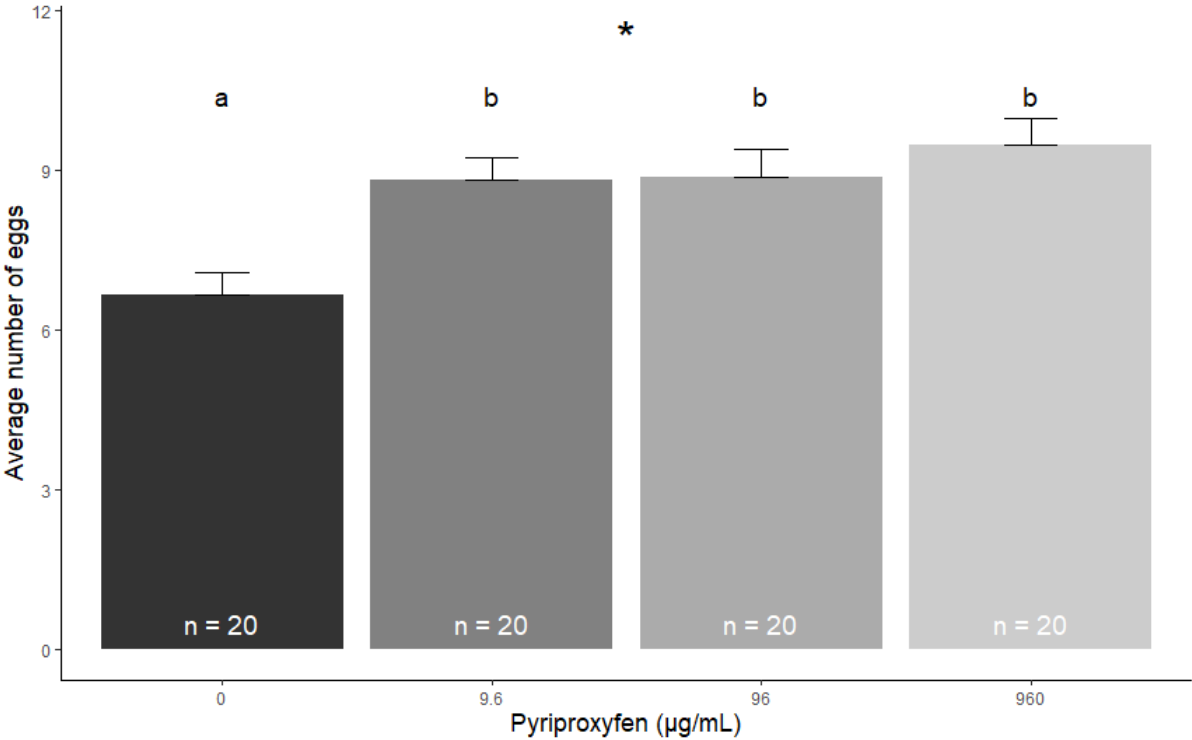


Figure 2

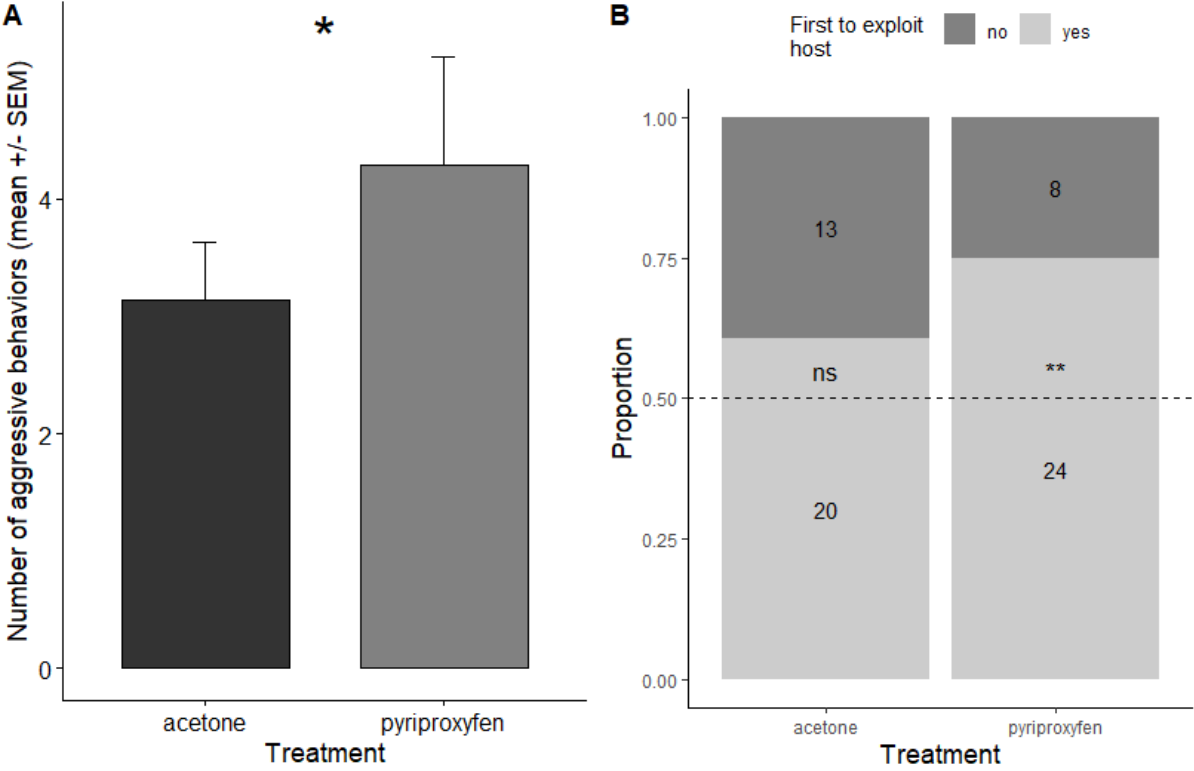


Table 1: Effect of relative egg load or mass difference and treatment on the probability of the focal female winning.

Factors affecting victory	Df	LR χ^2	P
Model 1 : Relative egg load difference			
Relative egg load difference	1	0.101	0.751
Focal female treatment	1	1.514	0.219
Relative egg load difference x Focal female treatment	1	0.727	0.394
Model 2 : Relative mass difference			
Relative mass difference	1	2.470	0.116
Focal female treatment	1	2.680	0.102
Relative mass difference x Focal female treatment	1	1.300	0.254

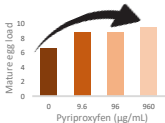
**Side effects of a pesticide on a non-target species:
Does pyriproxyfen, a juvenile hormone agonist, impact physiology and behavior in a beneficial insect?**

External application of
pyriproxyfen

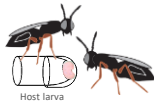


♀ parasitoid of crop pest

Increase in egg production



Increase in female aggressiveness



➔ Sublethal doses of this pesticide has unintended effects on a beneficial insect, what might subsequently disrupt its ecosystem services