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Novel insight in the life cycle of *Torymus sinensis*, biocontrol agent of the chestnut gall wasp

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Torymus sinensis Kamijo (Hymenoptera: Torymidae) is a biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). It is reported in the literature as univoltine, but in NW Italy it exhibits a prolonged diapause mainly as late instar larva. Diapause is extended for 12 months, and adults emerge in April as usual, showing a two-year life cycle. 2nd year emergence individuals are able to mate, and the presence of mature eggs was confirmed in females which parasitised fresh chestnut galls, showing the same parasitism behaviour as 1st year emergence individuals. Both sexes of 2nd year emergence individuals proved to be smaller than the univoltine ones according to ovipositor sheath length, pronotum width, and hind tibia length. Proving evidence of the extended diapause plays an important role for the establishment of *T. sinensis* especially in the first years after its release. Future studies are needed to clarify the factors which trigger off this response.

1 **Novel insight in the life cycle of *Torymus sinensis*, biocontrol agent of the chestnut gall wasp**

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6 **Introduction**

7 The alternation of active and dormant stages is an important trait of many invertebrate animals,
8 including arthropods, affecting several aspects of their life cycles such as duration, phenology, and
9 flexibility (Belozerov 2008). Indeed, many insects undergo diapause periods to get through adverse
10 conditions in seasonal environments. Environmental conditions, principally temperature and
11 photoperiod, activate the different steps of diapause induction, and influence its maintenance and
12 termination; moreover, other aspects like food availability and quality, type and physiological status
13 of the host, population density, are also involved in this event (Leather et al., 1993; Velarde et al.,
14 2002). In fact, diapause can evolve as bet-hedging mechanism, occurring wherever there is a
15 temporal variation in the suitability of the environment; this variation may be caused by temporally
16 varying levels of parasitism, and it usually occurs in populations whose seasonal resources fluctuate
17 unpredictably in abundance and availability (Ringel et al., 1998; Moraiti et al., 2012).

18 In some individuals of an insect population, such dormancy may be extended for more than one
19 year and prolonged during the favourable season; this phenomenon is termed “prolonged diapause”
20 (Waldbauer 1978; Hanski 1988). Prolonged diapause, by spreading adult emergence over time,
21 allows the insect to overcome unpredictable environmental changes, allowing to some progeny to
22 be ready for reproduction under better conditions (Corley et al. 2004). For this reason, for many
23 insect species, this strategy is thought to protect demographic and genetic resources in fluctuating
24 environments (Suez et al. 2013). On the other hand, the extension of the diapause period may be
25 costly in terms of reproductive success, as dormant specimens could die before emergence (Soula
26 and Menu 2003). Moreover, although metabolism is maintained at low rates during diapause (Lees
27 1955), dormant insects undergo continuous resource consumption, which may significantly affect
28 fitness when diapause lasts for a long time (Matsuo 2006).

29 One of the insect models that has been studied for prolonged diapause is that of host-parasitoid
30 systems. Parasitoids may undergo extended diapause in order to stay in synchrony with their hosts,
31 in addition to maintaining the population during unfavourable conditions (Doutt et al. 1976).

32 Although many studies have been conducted, the effect of prolonged parasitoid diapause on the
33 stability of interactions with the host is still unclear (Ringel et al. 1998; Corley et al. 2004).

34 *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), native to China, is an exotic parasitoid of the
35 Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a globally
36 invasive pest of chestnut (*Castanea* spp). It was released as a biocontrol agent in Japan in 1975, in
37 Georgia (USA) in the late 1970s, and in Italy in 2005 (Moriya et al. 2003; Cooper and Rieske 2007,
38 2011; Quacchia et al. 2008). It is phenologically well synchronised with its host and in all cases
39 after its release is able to disperse successfully alongside *D. kuriphilus* by expanding its population,
40 reducing shoot infestation rates below the tolerable damage threshold, and significantly containing
41 gall wasp outbreaks.

42 *T. sinensis* is reported in the literature as univoltine like its host, predominantly reproducing
43 amphigonically. Female lays eggs into newly formed galls, usually one egg per host larva. Under
44 natural conditions, multiple eggs per host larva have been observed in a single chamber, but only
45 one larva could grow up because of cannibalism among hatched young larvae (Piao and Moriya
46 1999). After hatching, the larva feeds externally on the mature host larva until pupation, which
47 occurs during late winter. Adult wasps emerge from the withered galls of the chestnut gall wasp in
48 the spring, synchronous with sprouting of chestnut trees and also with the appearance of *D.*
49 *kuriphilus* galls (Moriya et al., 2003; Quacchia et al., 2008; EFSA Panel on Plant Health 2010;
50 Cooper and Rieske, 2011).

51 In order to monitor the success of *T. sinensis* biocontrol activity in NW Italy, in 2012 withered galls
52 were dissected after *T. sinensis* emergence to evaluate the number of unemerged specimens; during
53 dissection, the presence of live *T. sinensis* larvae was revealed, highlighting a new aspect of the life
54 cycle of this parasitoid. On the basis of this finding, the frequency of prolonged diapause in
55 populations of *T. sinensis* was investigated in this area.

56 **Materials and methods**

57 *Collection and dissection of the galls*

58 In order to study the frequency of prolonged diapause of the parasitoid *T. sinensis*, investigations
59 were carried out in 2013 in Cuneo province (NW Italy) where the parasitoid was first released in
60 2005 and then successfully established, forming stable populations. A total of five sampling sites
61 were chosen. The sites were located in the municipalities of Boves (44°19'06''N, 7°33'18''E; 638
62 m asl), Caraglio (44°24'31,74"N, 7°24'05,98"E; 654 m asl), Cuneo (44°22'18,97"N, 7°33'51,81"E;
63 540 m asl), Peveragno (44°18'57''N, 7°35'08''E; 716 m asl), Robilante (44°18'14''N, 7°31'09''E;
64 775 m asl) (Fig. 1). Five naturally growing chestnut trees were randomly chosen at each site, and
65 for each tree 200 galls that had formed during the previous year were randomly collected (20 galls x
66 10 branches) on the crown of the plant both during winter (February) and summer (June). Half of
67 the winter-collected galls were individually isolated in plastic vials (120 mm in height by 25 mm in
68 diameter) and kept in outdoor conditions until *T. sinensis* emergence. The number of *T. sinensis*
69 adults emerging per gall was recorded, and the galls were then dissected. The remaining galls were
70 stored in rearing cardboard boxes in outdoor conditions until the emergence of the adults. Summer-
71 collected galls were divided in two subsets as well: half were immediately dissected, and half were
72 stored until adult emergence as described above (Fig. 2).

73 Dissection was conducted using a stereomicroscope with the aid of a scalpel. The number of cells
74 per gall was recorded, as well as the number of live larvae, pupae and/or unemerged adults.

75 Five newly emerged diapausing females and males, less than 24 h old, unfed and naïve, were
76 isolated in a Petri dish containing dry filter paper (one female and one male per each dish, 12 mm in
77 diameter) and their behaviour was observed to verify if they were able to mate and lay eggs. All
78 mated females were then individually isolated in a Petri dish as described above containing a fresh
79 unparasitised chestnut gall. Experiments, carried out under laboratory conditions (24±2°C,
80 60%RH), lasted 1 h or terminated when mating or oviposition occurred. Parasitised galls were then
81 dissected using a stereomicroscope and the presence of eggs was recorded.

82 Twenty adults (ten males and ten females) were killed upon emergence with ethyl acetate and the
83 pronotum width (maximum width), the hind tibia length, and the ovipositor sheath length (mm)

84 were recorded, comparing 1st and 2nd year emergence *T. sinensis*. Measurements were taken using a
85 Leica MZ16A stereomicroscope (50x magnification) with the software LAS version 3.7.0.

86 *Identification of Torymus sinensis larvae*

87 All the larvae and pupae found in the dissected galls were morphologically identified by
88 comparison with the voucher specimens deposited at the DISAFA-Entomology laboratory.
89 Furthermore, a sample of *T. sinensis* larvae (five larvae per each site and season), pupae (five pupae
90 per each site and season, or all the pupae when fewer than five were found, with the exception of
91 Robilante), and adults emerging in the second year (five males and five females per each site and
92 season, or all the insects when fewer than five were found) were submitted to DNA extraction and
93 then sequenced for the cytochrome oxidase I (COI) gene following Kaartinen et al. (2010) to
94 confirm their morphological identification.

95 *Statistical analyses*

96 The number of individuals with extended diapause was referred to the total number of gall cells
97 calculated within dissections in each year and site. After testing for homogeneity of variance
98 (Levene test, $P < 0.05$), data were analysed by Student's t-tests ($P < 0.05$) to compare records obtained
99 in different collection periods or by one-way analysis of variance (ANOVA) followed by Tukey test
100 ($P < 0.05$) to compare sites. To assess the sex ratio of emerged adults and diapausing pupae, χ^2 tests
101 were performed ($P < 0.05$). All analyses were performed using the software SPSS version 20.0
102 (SPSS, Chicago, IL).

103

104 **Results**

105 A total of 10,000 galls (2 collections x 5 sites x 5 trees x 200 galls) were collected at all the
106 sampling sites. The number of cells per gall ranged from 3.563 ± 0.069 , recorded at the site of
107 Peveragno, to 4.398 ± 0.085 , observed at the site of Cuneo, with an average of 3.851 ± 0.026 .

108 The average number of 1st year *T. sinensis* emerging per 100 cells in winter-collected galls was
109 85.37. Males were significantly more abundant than females (χ^2 test: df=1; $\chi^2=47.297$; $P<0.05$),
110 representing 54.91% of emerged adults, while females were 45.09%.

111 *Dissected galls*

112 Overall, considering the winter-collected galls, 90.70% of *T. sinensis* emerged in the first year,
113 whereas the 2.56% remained inside the galls and the 6.72% died (Table 1). Gall dissection revealed
114 an extended diapause at the larval and/or pupal stage occurring at all sites. Diapause rates related to
115 such stages had significantly higher levels in galls collected in June than in winter-collected galls
116 (Student's t-test: df=48; $t=5.066$; $P<0.05$). Moreover, the recorded number of dead parasitoids
117 inside the galls was significantly lower in summer-collected galls (Student's t-test: df=48; $t=6.845$;
118 $P<0.05$). Considering the totality of dissected galls (winter and summer collections) in single sites, a
119 variability was observed (ANOVA: df=4, 45 ; $F=6.568$; $P<0.05$). According to the Tukey test,
120 Caraglio showed a higher incidence of diapausing specimens than in all other localities.

121 Both larvae and pupae were found from galls collected at all sampling sites and periods, with the
122 exception of winter-collected galls from Robilante, where only larvae were observed. However,
123 diapausing larvae were always definitely more frequent, representing more than 80% of the
124 individuals detected. Among pupae, a higher number of males than females was reported, with a
125 mean of 0.22 ± 0.05 male pupae and 0.12 ± 0.04 female pupae per gall but no significant differences
126 were detected (χ^2 test: df=1; $\chi^2=0.004$; $P=0.951$). Nonetheless in Peveragno we found more female
127 than male pupae (χ^2 : df=1; $\chi^2=0.03$; $P=0.873$).

128 *Stored galls*

129 From the galls stored until 2014, adult parasitoids emerged in the spring of the second year,
130 simultaneously with the emergence of univoltine adults. The average number of 2nd year *T. sinensis*
131 emerging per 100 cells was 0.37 ± 0.06 for winter-collected galls and 1.05 ± 0.22 for summer-
132 collected galls (Student's t-test: df=48; $t=2.835$; $P<0.05$). Among the different sites, considering the
133 totality of dissected galls (winter and summer collections) differences in parasitoid emergence after

134 two years were observed (ANOVA: $df=4, 45$; $F=7.921$; $P<0.05$). The Tukey test showed that
135 Robilante and Boves had the highest rates. Overall, we found a significantly higher number of
136 males than females (χ^2 test: $df=1$; $\chi^2=53.1$; $P<0.05$); males represented 80.46% of emerged adults,
137 whereas females represented 19.54%.

138 Diapause lasted one year; in fact, from all the stored galls from which *T. sinensis* adults emerged,
139 dissection during summer 2014 did not show any live larvae or pupae continuing their cycle.
140 However, a mean of 7.81 ± 0.05 and 1.07 ± 0.14 dead specimens per 100 cells were found for winter-
141 collected and summer-collected galls, respectively (Student's t-test: $df=48$; $t=12.355$; $P<0.05$).
142 Hence, for winter-collected galls, we observed that 91.34% of *T. sinensis* emerged after one year,
143 whereas 0.39% emerged after two years and 8.27% died (Table 1). Generally the emergence rates of
144 diapausing individuals were lower than the diapause rates that we detected based on gall dissection
145 in 2013; on average, the number of 2nd year emergence adults represented 26.06% of larvae and
146 pupae that we observed in dissected galls. Conversely, considering only summer-collected galls
147 from the sites of Boves and Robilante, the number of emerged adults from stored galls was higher
148 than that recorded for juveniles in dissected galls.

149 All the newly emerged diapausing adults were able to mate and females laid eggs in fresh
150 unparasitised chestnut galls. The average number ($\pm SE$) of eggs recorded per gall was 1.20 ± 0.270 .

151 Measurement of ovipositor sheaths length, pronotum width and hind tibia length (mm) in 1st and 2nd
152 year emergence *T. sinensis* showed striking differences for all values (Table 2). The length of the
153 ovopositor sheath was significantly shorter in 2nd year emergence females, with an average measure
154 of 1.420 ± 0.046 (Student's t-test: $df=8$; $P<0.05$; $t=3.393$). Also, the pronotum width differed
155 significantly between 1st and 2nd year emergence adults in both males (Student's t-test: $df=18$;
156 $P<0.05$; $t=5.915$) and females (Student's t-test: $df=18$; $P<0.05$; $t=9.498$). Similarly, significant
157 differences were observed when measuring the hind tibia length of the two specimen groups, both
158 in males (Student's t-test: $df=18$; $P<0.05$; $t=6.301$) and in females (Student's t-test: $df=18$; $P<0.05$;

159 $t=9.471$). Hence, 2nd year emergence adults were smaller than those that emerged in the first year in
160 both sexes.

161 All the 406 morphologically analysed diapausing specimens were indeed *T. sinensis*. The
162 cytochrome oxidase I gene obtained from a total of 75 specimens submitted to molecular
163 identification was sequenced and sequences were compared with those in the National Center for
164 Biotechnology Information (NCBI) sequence database. In all cases, a minimum of 99% similarity
165 with *T. sinensis*-related sequences was observed. The COI sequence of a specimen was deposited
166 in the European Nucleotide Archive under the following accession numbers: LM651395.

167

168 **Discussion**

169 This study demonstrates that *T. sinensis* can undergo extended diapause, showing a two-year cycle.
170 Second year emergence specimens were detected in the galls collected in all the surveyed sites.
171 Since the time of the first release of *T. sinensis* in Piedmont in 2005, the number of *D. kuriphilus*
172 host in the area has dramatically decreased, similar to the Japanese experience (Moriya et al., 1989),
173 by limiting the food availability for the population of this monophagous wasp. A prolongation of
174 diapause may be a response to food shortage (Hanski 1988). *T. sinensis* is known to be a specialist
175 parasitoid, and the decline of the chestnut gall wasp may be one of the reasons why it exhibited an
176 extended diapause. On the contrary, for the native parasitoid community commonly associated to
177 gall wasps, extended diapause is a rare strategy probably because they are generalists and may shift
178 on other available potential hosts in case of resource less predictable. Although we observed a lower
179 incidence of extended diapause than in previous reports concerning beetles and wasps (Soula and
180 Menu 2003; Mahdjoub and Menu 2008; Geisert and Meinke 2013; Suez et al. 2013), the percentage
181 of individuals which prolong their diapause could grow over time in relation to *D. kuriphilus*
182 availability. Even if extended diapause rates were always low, both gall dissection and 2nd year
183 emergence adults showed a higher incidence of diapause when galls were collected in the summer.
184 Also, mortality rates inside the galls were lower when collection was carried out in June, suggesting

185 an influence of prolonged gall handling and storage on the successful life cycle completion of the
186 insects.

187 Even taking into account the effect of gall collection, our results show that the number of
188 diapausing larvae and pupae found in the galls was generally higher than the number of adults
189 actually emerging in the second year. Furthermore, mortality rates detected by gall dissection in
190 2014 (after the emergence of 2nd year emergence parasitoids) were always higher than those
191 recorded in 2013. Additionally, individuals that emerged after a two-year diapause were
192 significantly smaller than 1st year emergence specimens. Individuals that express prolonged
193 dormancy are in fact exposed to increased mortality and they postpone reproduction, both of which
194 may result in fitness costs. Trade-offs in the allocation in the metabolic reserves between
195 maintenance during dormancy and reproductive activity after dormancy have been reported in other
196 wasp such as *Neodiprion swainei* and *N. sertifer* (Moraiti et al., 2012).

197 Taken together, these evidences highlight the cost of extended diapause, in terms of increased
198 mortality and reduced growth, which is likely to be related to consumption of metabolic resources.
199 Similar disadvantages have been previously reported; nonetheless, they are generally thought to be
200 balanced by an increased chance of survival due to overcoming adverse conditions (Hanski 1988).
201 Although reproductive costs for specimens with prolonged diapause have been reported as well
202 (Soula and Menu 2003), we found that 2nd year emergence *T. sinensis* females were able to mate
203 and lay fertile eggs on chestnut galls.

204 The gall dissection highlighted a male-biased sex ratio among diapausing pupae, although without
205 significant differences, confirming that for *T. sinensis* prolonged diapause is more common in
206 males. These results are in agreement with Kraaijeveld and van Alphen (1995), who reported a
207 similarly unbalanced sex ratio for individuals of the parasitoid *Asobara tabiada* Nees with extended
208 diapause. Menu (1993) also detected a male-biased sex ratio for the chestnut weevil *Curculio*
209 *elephas* Gyllenhaal emerging after three or four years. Although for *A. tiabida* the authors suggested
210 that males underwent extended diapause more frequently than females (Kraaijeveld and van Alphen

211 1995), in the case of *C. elephas*, a higher emergence success in males than in females was observed
212 (Menu 1993).

213 A striking variability in extended diapause rates was observed among different collection sites.
214 According to gall dissection in summer 2013, the sites of Caraglio and Peveragno showed the
215 highest extended diapause rates, whereas Boves and Robilante had the highest adult emergence
216 rates after two years. Such variable results are likely to be due to the general unevenness of the
217 extended diapause phenomenon itself. A detailed survey of microclimatic conditions in different
218 localities could elucidate the possible influence of differences in temperature, relative humidity or
219 rainfall, as commonly observed (Danks 1987). Furthermore, diapause intensity may be a response to
220 winter warming, as suggested for the weevil *Exechesops leucopis* Wolfrum and the fruit fly
221 *Ragoletis cerasi* Loew (Matsuo 2006; Moraiti et al. 2014).

222 This study contributes to the knowledge base needed to develop appropriate *T. sinensis*
223 management strategies. In fact, extended diapause may have an adaptive value in protecting the
224 population against the yearly fluctuation in food supply.

225 The presence of a parasitoid reservoir, consisting of larvae and pupae inside withered galls after the
226 emergence of the univoltine population, is an important aspect for growers. In fact, throwing away
227 or burning pruning discards after spring will eliminate diapausing individuals, reducing the wasp
228 population emerging in the following spring. Therefore, all the plant material bearing galls
229 (branches, suckers) can be cut away but not remove from the orchard at least for two years.

230 Even if the recorded diapause was low, this finding reveals its importance in sites where *T. sinensis*
231 has not been released long or where its population is still at a low rate. Preserving the diapausing
232 population will favour, in fact, the establishment of the parasitoid as well. Hence this novel insight
233 in *T. sinensis*'s life cycle provides a decision-making tool to growers, playing an important role in
234 chestnut orchard management in the first years after its release.

235 Prolonged diapause is a dynamic process and there is no doubt that the regulation of this strategy is
236 extremely complex, making hard to speculate which are the factors that trigger off this response.

237 Unpredictable resources, adverse climatic conditions, and photoperiod induction may deeply
238 influence growth and development in many insects (Schmidt et al., 2005; Moraiti et al., 2012;
239 Ming-Xing et al., 2013).
240 Our investigations highlighted a new aspect of *T. sinensis* life cycle, but future studies are needed,
241 carrying out surveys over a longer period of time at different parasitism rates, in order to clarify
242 whether this event is actually increasing in relevance. Since in many insects geographically
243 separated populations of the same species may show variations in diapause characteristics, it will be
244 interesting to carry out investigations also in other European chestnut growing areas at different
245 latitudes.

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378 **Table 1** Parasitism levels of 1st and 2nd year emergence *T. sinensis* from galls sampled in different sites in NW Italy during 2013. Student's t-tests
379 were performed on the data expressed as a mean of the five sites according to the collection month (Average line); dissected and stored galls were
380 considered separately. In the average line within the same column values followed by the asterisk are significantly different ($P < 0.05$), values
381 followed by NS are not significantly different ($P < 0.05$). ANOVA tests were carried out comparing values from different sites according to the
382 collection month; dissected and stored galls were considered separately. Within the same column letters indicate significantly different values
383 (Tukey test; $P < 0.05$).

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	Month	Site	1 st year	Live larvae / 100 cells \pm SE ^b	Live pupae / 100 cells \pm SE ^b	Total diapausing	Dead	2 nd year	Total
			emergence / 100 cells \pm SE ^a			<i>T. sinensis</i> / 100 cells \pm SE ^b	<i>T. sinensis</i> / 100 cells \pm SE ^b	emergence / 100 cells \pm SE ^c	<i>T. sinensis</i> / 100 cells \pm SE
Dissected galls	February	Boves	82.90 \pm 0.97 A	2.17 \pm 0.60 AB	0.28 \pm 0.18 A	2.45 \pm 0.67 AB	4.63 \pm 0.48 A	-	89.98 \pm 1.12 A
		Peveragno	92.10 \pm 0.69 B	2.17 \pm 0.63 AB	0.21 \pm 0.08 A	2.38 \pm 0.71 AB	4.88 \pm 0.29 A	-	99.35 \pm 0.31 B
		Robilante	82.45 \pm 0.49 A	2.67 \pm 0.75 B	0.00 A	2.67 \pm 0.75 AB	5.31 \pm 0.90 A	-	90.43 \pm 0.97 A
		Cuneo	82.99 \pm 0.38 A	0.29 \pm 0.15 A	0.28 \pm 0.29 A	0.57 \pm 0.43 A	8.97 \pm 2.09 A	-	92.53 \pm 2.11 A
		Caraglio	82.53 \pm 0.81 A	3.36 \pm 0.21 B	0.51 \pm 0.18 A	3.87 \pm 0.36 B	7.58 \pm 0.88 A	-	93.98 \pm 0.93 A
		Average	84.59\pm0.82 ND	2.13\pm0.30 ND	0.26\pm0.08 *	2.39\pm0.33 *	6.27\pm0.57 *	-	93.26\pm0.85 ND
	June	Boves	-	1.10 \pm 0.37 A	0.27 \pm 0.15 A	1.37 \pm 0.52 A	1.26 \pm 0.21 A	-	-
		Peveragno	-	2.01 \pm 0.39 A	1.69 \pm 0.40 B	3.70 \pm 0.50 AB	1.97 \pm 0.66 AB	-	-
		Robilante	-	1.79 \pm 0.72 A	0.37 \pm 0.16 A	2.16 \pm 0.71 A	1.02 \pm 0.27 A	-	-
		Cuneo	-	2.35 \pm 0.39 A	0.30 \pm 0.09 A	2.65 \pm 0.46 A	2.53 \pm 0.50 AB	-	-
		Caraglio	-	4.75 \pm 0.75 B	0.60 \pm 0.27 A	5.35 \pm 0.68 B	3.54 \pm 0.33 B	-	-
		Average	-	2.40\pm0.34 ND	0.65\pm0.15 *	3.05\pm0.37 *	2.07\pm0.26 *	-	-
Stored galls	February	Boves	80.66 \pm 0.44 A	-	-	-	7.52 \pm 0.87 ABC	0.60 \pm 0.17 A	88.78 \pm 1.18 A
		Peveragno	93.75 \pm 0.69 C	-	-	-	4.84 \pm 0.92 A	0.43 \pm 0.11 A	99.03 \pm 0.35 B
		Robilante	77.79 \pm 1.13 A	-	-	-	11.08 \pm 0.96 C	0.45 \pm 0.15 A	89.31 \pm 0.98 A
		Cuneo	87.70 \pm 1.17 B	-	-	-	8.68 \pm 0.57 BC	0.16 \pm 0.05 A	96.54 \pm 1.63 B
		Caraglio	91.30 \pm 0.61 BC	-	-	-	6.94 \pm 0.79 B	0.20 \pm 0.04 A	98.44 \pm 0.53 B
		Average	86.24\pm1.30 ND	-	-	-	7.81\pm0.54 *	0.37\pm0.06*	94.42\pm1.01 ND
	June	Boves	-	-	-	-	0.23 \pm 0.08 A	1.52 \pm 0.36 B	-
		Peveragno	-	-	-	-	1.12 \pm 0.28 AB	0.39 \pm 0.14 A	-
		Robilante	-	-	-	--	1.11 \pm 0.22 AB	2.75 \pm 0.39 B	-
		Cuneo	-	-	-	-	1.87 \pm 0.29 B	0.34 \pm 0.10 A	-
		Caraglio	-	-	-	-	1.04 \pm 0.29 AB	0.24 \pm 0.09 A	-
		Average	-	-	-	-	1.07\pm0.14 *	1.05\pm0.22 *	-

378 ^aAdult emergence was recorded in April 2013.

379 ^bData were obtained by gall dissection carried out in June 2013 (dissected galls group) or in June 2014 (stored galls group)

380 ^cAdult emergence was recorded in April 2014.

381 **Table 2.** Average (\pm SE) ovipositor sheath length, pronotum width, and hind tibia length (mm) of 1st
 382 and 2nd year emergence *T. sinensis* (N=10). Student's t-tests were performed on data referred to
 383 males and females separately; within the same column values followed by the asterisk are
 384 significantly different ($P<0.05$).

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Gender	Emergence year	Average ovipositor sheath length (mm) \pmSE	Average pronotum width (mm) \pmSE	Average hind tibia length (mm) \pmSE
male	1 st year	-	0.360 \pm 0.005 *	0.679 \pm 0.012 *
	2 nd year	-	0.288 \pm 0.010 *	0.532 \pm 0.022 *
female	1 st year	1.735 \pm 0.025 *	0.404 \pm 0.006 *	0.752 \pm 0.012 *
	2 nd year	1.420 \pm 0.046 *	0.328 \pm 0.005 *	0.592 \pm 0.012 *

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405 Figure 1 Location of the sampling sites in the province of Cuneo (black dots). The inset indicates
406 the location of the Piedmont region in Italy

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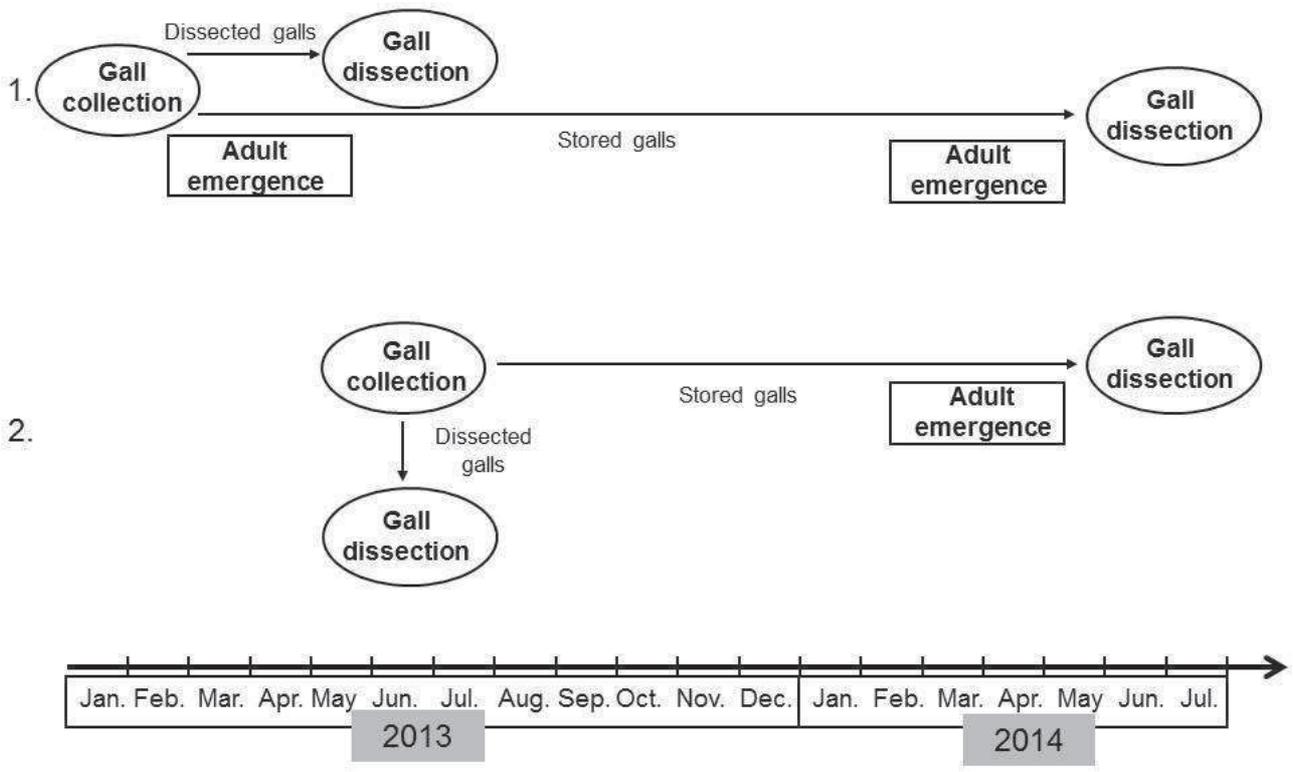
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421 **Figure 2** Experimental chart to evaluate the incidence of extended diapause in *T. sinensis*
 422 population in Piedmont, Italy, from winter-collected (1) and summer-collected (2) galls

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Acknowledgments

We wish to thank Johann Laimer who kindly provided the unparasitised chestnut galls used in the behavioural trials. We are also grateful to Michalis Bourellas, Marida Corradetti, Silvia Di Stefano, Cecilia Ferrara, Federica Fleury, and Valentina Tosi for their technical assistance.

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table 1

	Month	Site	1 st year	Live larvae / 100	Live pupae / 100	Total diapausing	Dead	2 nd year	Total
			emergence / 100						
			cells ±SE ^a			100 cells ±SE ^b	100 cells ±SE ^b	100 cells ±SE ^c	100 cells ±SE
Dissected galls	February	Boves	82.90±0.97 A	2.17±0.60 AB	0.28 ±0.18 A	2.45±0.67 AB	4.63±0.48 A	-	89.98±1.12 A
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		Cuneo	-	2.35±0.39 A	0.30±0.09 A	2.65±0.46 A	2.53±0.50 AB	-	-
		Caraglio	-	4.75±0.75 B	0.60±0.27 A	5.35±0.68 B	3.54±0.33 B	-	-
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		Caraglio	91.30±0.61 BC	-	-	-	6.94±0.79 B	0.20±0.04 A	98.44±0.53 B
		Average	86.24±1.30 ND	-	-	-	7.81±0.54 *	0.37±0.06*	94.42±1.01 ND
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		Peveragno	-	-	-	-	1.12±0.28 AB	0.39±0.14 A	-
		Robilante	-	-	-	--	1.11±0.22 AB	2.75±0.39 B	-
		Cuneo	-	-	-	-	1.87±0.29 B	0.34±0.10 A	-
		Caraglio	-	-	-	-	1.04±0.29 AB	0.24±0.09 A	-
		Average	-	-	-	-	1.07±0.14 *	1.05±0.22 *	-

^aAdult emergence was recorded in April, 2013.

^bData were obtained by gall dissection carried out in June 2013 (dissected galls group) or in June 2014 (stored galls group)

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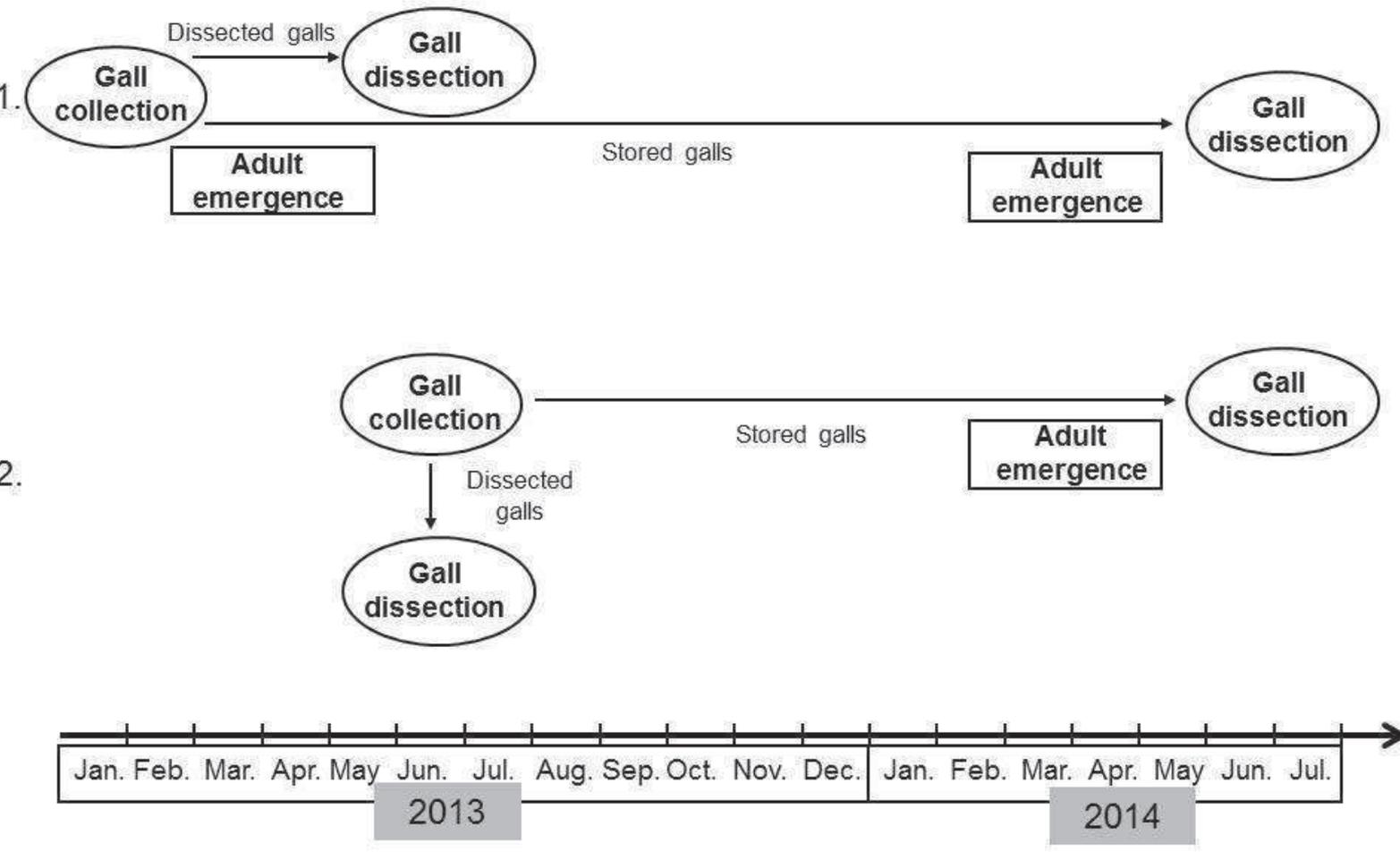
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Figure 1



Figure 2



Chiara Ferracini is an entomologist researcher involved in the integrated management and biological control of native and exotic agricultural and forestry pests.

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