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1 A mismatch in *Torymus sinensis* emergence may affect biocontrol at local level?

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7

8 **Abstract:**

9 The biocontrol agent *Torymus sinensis* Kamijo represents one of the most successful examples of classical biocontrol
10 programs. This parasitoid represented a feasible option to obtain sufficient, sustainable, and long-term control of the
11 Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu. Recently, an unusual presence of galls was
12 recorded in a few chestnut orchards of Northern Italy, although *T. sinensis* was released in previous years. We
13 hypothesized that the increase in infestation rate over the years was related to a mismatch in the parasitoid/host
14 phenology, resulting in a loss of biocontrol. Investigations were performed in the five-year period 2018-2022 in six
15 chestnut orchards, selected according to the ACGW infestation level, and divided in two categories: non-critical sites
16 (negligible presence of ACGW galls) and critical sites (high presence of galls with suppression of bud growth). We
17 evaluated *T. sinensis*'s parasitism rate and phenology by dissecting chestnut galls, comparing non-critical sites *versus*
18 critical sites. Temperatures were monitored in all the orchards with data loggers. In non-critical sites the parasitism rate
19 by *T. sinensis* was stable and/or growing in all years, accounting from 77% to 99%. Conversely, the parasitism rate in
20 critical sites experienced a severe decline, reaching values under 50% in all sites in 2021. Specifically, parasitism on
21 average reduced by 44% when comparing 2019 *versus* 2021. The dissection of the galls recorded in non-critical sites in
22 winter (February) highlighted that on average most *T. sinensis* were larvae and immature pupae (about 70%).
23 Conversely, in critical sites most of the individuals were black pupae (76%), with presence of newly formed adults
24 (12%). The mean temperature recorded in critical sites was higher than 2.72°C and 2.34°C in January and February
25 respectively, when compared to non-critical sites in the three-year period. Moreover, in critical sites the early
26 emergence of the biocontrol agent (late February-early March) was recorded when current-year ACGW fresh galls were
27 not available. Our results suggest that the phenological asynchrony between *T. sinensis* and the ACGW heavily affected
28 the role of the released agent in the suppression of the pest's outbreaks. Future studies are needed to clarify whether this
29 event is increasing in relevance, also in a climate change perspective.

30

31 **Keywords:** phenological asynchrony, parasitoid-host interaction, warmer winters, classical biocontrol, chestnut

32 **Introduction**

33 Classical biocontrol (CBC) is considered as a cost-effective tool for managing exotic invasive insect pests, allowing
34 offspring of the released natural enemies build up populations which are large enough for suppression of pest
35 populations during many subsequent years (Van Driesche et al., 2010; Kenis et al., 2017). One of the most successful
36 and recent examples of classical biocontrol programs was targeted to control the Asian chestnut gall wasp (ACGW),
37 *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae). This exotic invasive pest, native to China, was first
38 reported in Europe (Italy) in 2002 and has rapidly spread throughout European countries, seriously affecting sweet
39 chestnut (*Castanea sativa* Miller) in orchards and coppices (Avtzis et al., 2019). The severe reduction in fruiting and the
40 economic impact on chestnut production made it necessary to control the pest by releasing a specific parasitoid,
41 *Torymus sinensis* Kamiyo (Hymenoptera, Torymidae) (Quacchia et al., 2008). This biocontrol agent was able to
42 establish, reproduce and spread, having a self-sustaining effect on ACGW, and significantly reducing the pest
43 outbreaks. Good control (parasitism rates up to 98%), and net economic benefits were achieved, thus preventing the
44 build-up of pest populations before economic damage is done. The synchronization in the parasitoid/host's life cycle
45 allowed a quick establishment and a significative annual increase in *T. sinensis* population, corresponding to a
46 concomitant steady decrease in *D. kuriphilus* infestation (Moriya et al. 2003; Cooper and Rieske 2007; Ferracini et al.
47 2019). The efficacy of *T. sinensis* as a biocontrol agent was investigated and ascertained in several European countries,
48 and highly successful field programs were able to cope with the pest as fully documented in the scientific literature
49 (Borovieck et al., 2014; Matošević et al. 2017; İpekdal et al., 2017; Pérez-Otero et al. 2017; Avtzis et al. 2019; Ferracini
50 et al., 2019). Currently, the release of the parasitoid is still underway in Greece, Portugal, Spain, Turkey, and UK (CF,
51 personal communication).

52 Concerns about the safety of CBC and its possible consequences of non-target effects have been raised, and risk
53 assessments have been documented in Italy. In particular, a post-release risk assessment was carried out to investigate
54 the potential impacts of this BCA on non-target hosts (native cynipid gall inducers) and the likelihood of hybridization
55 with native *Torymus* species phylogenetically closely related to *T. sinensis*. Specifically, parasitism on non-target hosts
56 was recorded on non-target oak galls (mainly *Andricus curvator* Hartig and *Andricus inflator* Hartig), but the incidence
57 was negligible and no changes in the distribution or abundance of non-target hosts is expected (Ferracini et al. 2015,
58 2017). Moreover, while in Japan hybridization was found between *T. sinensis* and the closely related *T. beneficus*
59 Yasumatsu and Kamiyo (Yara et al., 2010), no evidence was ever reported in the area of introduction (Ferracini et al.,
60 2017; Pogolotti et al., 2019).

61 This parasitoid has been proven to effectively control ACGW outbreaks, and its successful use highlights how CBC
62 may represent a cost-effective tool for managing an exotic invasive pest, balancing pest populations below damaging

63 levels. In NW Italy, where *T. sinensis* was first released in 2005, approximately 7–8 years were due to noticeably
64 decrease the ACGW (Ferracini et al., 2019). The pest and its parasitoid started to produce an ever-changing pattern of
65 travelling waves as predicted by a mathematical model (Paparella et al. 2016), and no reported evidence of infestation
66 resurgence was reported after 17 years from first release.

67 Anyhow, although the parasitoid proved to be an effective biocontrol agent in almost all the introduced areas, in recent
68 years chestnut growers reported in a few orchards an increase in the presence of *D. kuriphilus*, thus questioning the
69 effectiveness of biocontrol by *T. sinensis* at local level.

70 Due to these constraints, investigations were carried out in NW Italy in the five-year period 2018-2022 to evaluate
71 parasitism rate in chestnut orchards, comparing sites where biological control had long been achieved and sites with an
72 abnormal presence of galls, although the previous release of the parasitoid.

73 In this study, our aim was to investigate if the presence of higher number of galls were correlated to a mismatch
74 between *T. sinensis* adult emergence and the gall formation on chestnut trees. In particular, we supposed that higher
75 temperatures in January and February when larvae were overwintering inside the galls played a crucial role in
76 determining adults' early emergence and potential changes in densities from one generation to the next, with negative
77 consequence on the control of the ACGW.

78 Specifically, in all the survey sites we recorded: (i) the parasitism rate by *T. sinensis* in dissected galls, (ii) the
79 phenological stage of *T. sinensis* in four different months every year, (iii) the average monthly temperature.

80

81 **Materials and Methods**

82 *Survey sites*

83 Investigations were performed in a five-year period (2018-2022) in sweet chestnut orchards located in three northern
84 Italian regions, namely Liguria, Piedmont, and Tuscany. A total of six sampling sites was chosen in relation to the
85 infestation by the ACGW recorded in 2018 (as described in the following chapter). The sites were located in the
86 municipalities of Buto (44°19'28.6" N, 09°38'38.3" E; 430 m asl), Chiusa di Pesio (44°18'29.0" N, 07°40'54.4" E; 680 m
87 asl), Firenzuola (44°07'03.0" N, 11°24'31.0" E; 716 m asl), Marradi (44°04'13.1" N, 11°36'57.4" E; 550 m asl),
88 Vicchio (43°57'44.7" N, 11°32'46.6" E; 750 m asl), Villar Focchiardo (45°06'25.7" N, 07°13'47.7" E; 600 m asl). All
89 information concerning the surveyed sites is given in the Supplementary Table 1. The survey sites were characterized by
90 managed chestnut orchards. Trees were approximately 80-100 yrs old, 15-20 m in height, planted at 8 m distance along
91 the row and 8 m distance between rows. Tree density was about 120 trees/ha. No insecticide treatment was ever applied
92 in the plots.

93 *Selection of chestnut orchards*

94 Based on the reports received from chestnut growers, six chestnut orchards were visited and chosen in relation to the
 95 infestation level by *D. kuriphilus*. The infestation rate was recorded in late August in all sites in 2018. At each site, 10
 96 chestnut trees were randomly selected, and from each tree ten 1-year old branches were randomly chosen at different
 97 heights of the canopy for a total of 100 branches per site per year. The infestation rate was expressed as the percentage of
 98 total buds infested by the gall wasp, according to Ferracini et al. (2019). The infestation index was assigned based on the
 99 average number of the galls and position along the leaf and/or branch, according to six classes reported in the Table 1.
 100 In all sites the biocontrol agent *T. sinensis* has been released for at least 5 years since the investigations, thus having enough
 101 time to form stable populations.

102
 103 Table 1 – Infestation index recorded in the survey sites according to the average number of galls by *Dryocosmus kuriphilus*
 104 Yasumatsu per branch, gall position, gall size, and average number of larval chambers per gall.

Index	Average no. galls per branch	Position*	Size (width in cm)	Average no. larval chambers per gall
0	0	-	-	0
1	< 2	Leaf midrib, leaf stipules	< 1 cm	<1 (mainly unilocular)
2	2.1-5	Leaf midrib, leaf stipules, and/or branches	1.1-1.5 cm	<15 (mainly multilocular)
3	5.1-9.9	Branches (mainly)	1.6-1.9 cm	16-50 (mainly multilocular)
4	> 10	Branches (mainly)	> 2 cm	>50 (mainly multilocular)

105 * Smallest galls were located along the main leaf midrib and/or leaf stipules, and biggest galls were located mainly on the branches,
 106 thus suppressing the bud growth.
 107

108 *Collection and dissection of the galls*

109 In 2019-2021, ten naturally growing chestnut trees were randomly chosen at each site, and for each tree 100 galls (10
 110 galls x 10 branches) were randomly collected on the crown of the plant, according to the methods described in Ferracini
 111 et al. (2019). Galls were collected each year in four months: summer (June, fresh galls), autumn (November, dry galls),
 112 and winter (January and February, dry galls formed in the previous year). Dissection was performed in laboratory
 113 conditions, according to the methods described in Ferracini et al. (2015), and the parasitism rate and phenological stage
 114 of *T. sinensis* individuals were recorded.

115 *Data logger*

116 Temperatures were monitored in all sites using waterproof battery-powered HOBO data loggers (Climate data logger
 117 BL30, Trotec International GmbH & C. S.a.s., Ora (BZ), Italy). All data loggers, placed on the trunk at a height of 2 m

118 above the ground, were programmed to record the minimum, mean and maximum temperature at 30-min intervals from
119 January 2019 to December 2021.

120 *Statistical analysis*

121 We used a linear regression to investigate the relationship between mean winter temperatures and parasitism rate by *T.*
122 *sinensis*, comparing non-critical and critical sites. After testing for homogeneity of variance (Levene's test), data were
123 analyzed using the Student's t tests ($p < 0.05$) to compare the mean parasitism rate in non-critical and critical sites. All
124 analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

125

126 **Results**

127 The six survey sites were divided in two categories based on the infestation index. The index between 0 and 2, was
128 assigned to chestnut orchards characterized by a low presence of ACGW galls (few and small galls, mainly located on
129 the leaf midrib). Conversely, the index greater than and equal to 3 was assigned to chestnut orchards with a visible high
130 number of ACGW galls (several big galls, mainly located on branches and thus suppressing the bud growth). Hence,
131 Chiusa di Pesio, Marradi, and Villar Focchiardo having an infestation index equal to 1 were considered as sites where
132 the release of the parasitoid *T. sinensis* was highly effective, hereafter referred to as "non-critical sites". The remaining
133 sites (Buto, Firenzuola, and Vicchio) having an infestation index greater than 3, were considered as sites characterized
134 by a failed biocontrol strategy although the release of the biocontrol agent and are hereafter referred to as "critical
135 sites".

136 A total of 72,000 galls (4 collections x 3 years x 6 sites x 10 chestnut trees x 100 galls) were collected in the sampling
137 sites. *T. sinensis* was detected in all chestnut orchards, with different parasitism rate among sites and years (Table 2). In
138 non-critical sites the parasitism rate by *T. sinensis* was on average 84% in the surveyed period, accounting from 76.57%
139 (Villar Focchiardo in 2019-2020) to 99% (Marradi in 2021-2022). Conversely, the parasitism rate in critical sites was
140 on average 52%, experiencing a severe decline in the three years and reaching values under 50% in all sites in 2021-
141 2022. The mean parasitism rate was significantly different when comparing non-critical *versus* critical sites in the three-
142 year period 2019-2021 (t: 5.156, df: 4, $p = 0.007$ in 2019-2020; t: 4.720, df: 4, $p = 0.009$ in 2020-2021; t: 5.422, df: 4, $p =$
143 0.006 in 2021-2022).

144 Parasitism rate by *T. sinensis* and mean temperatures were negatively correlated, comparing non-critical *versus* critical
145 sites ($R^2 = -0.86$) (Figure 1).

146 The dissection of the galls highlighted that 100% of *T. sinensis* was at the larval stage in all sites and years in June and
147 November. Afterwards, a variation in *T. sinensis*'s phenology was recorded comparing the two types of sites. In the
148 non-critical sites, most of the individuals were at the larval stage in January (83% of larvae and 17% of white immature

149 pupae), while in February a higher number of pupae was detected (38% of larvae, 31% immature pupae, and 31% of
150 black pupae). Conversely, the dissection of galls recorded in critical sites revealed that most of the individuals were
151 already at the pupal stage in January (20% of larvae, 45% immature pupae, 33% of mature pupae, and 2% of newly
152 formed adults), and in February almost all individuals were mature pupae close to adult emergence (4% of larvae, 8%
153 immature pupae, 76% of black pupae, and 12% of newly formed adults) (Figure 3). All data concerning dissections are
154 given in the Supplementary Table 2.

155 In critical sites, newly emerged *T. sinensis* adults were observed at the end of February/beginning of March on
156 ornamental flowering plants other than chestnut (*Forsythia*, *Laurus*, *Prunus*) (Supplementary Figure 1).

157 The average monthly temperature recorded with data loggers in the chestnut orchards is shown in the Figure 2 (A-B).

158 In all the three critical sites, temperatures were higher than those recorded in the non-critical sites from December to
159 February in 2019-2021. Temperatures remained markedly high even during March in both Buto and Firenzuola, sites.

160 On average, when comparing non-critical *versus* critical sites, the mean temperature recorded in critical sites was higher
161 than 2.72°C and 2.34°C in January and February, respectively (Figure 2 B).

Table 2 – Average parasitism rate (%) by *Torymus sinensis* Kamijo recorded in the surveyed sites in the three-year period 2019-2021 (non-critical sites, infestation index 0-2; critical sites, infestation index > 3). Each percentage corresponds to the mean parasitism rate recorded in dissected galls in the four sampling months (June, November, January, and February).

		2019-2020	2020-2021	2021-2022	Trend (2019 versus 2022)
Non-critical sites	Chiusa di Pesio (CN)	81.09	89.41	86.77	+ 6%
	Marradi (FI)	77.30	80.30	99	+ 28%
	Villar Focchiardo (TO)	76.57	81.65	86.07	+ 12%
Critical sites	Buto (SP)	68.31	65.03	48.33	- 29%
	Firenzuola (FI)	63.12	56.36	42.25	- 33%
	Vicchio (FI)	59.91	47.38	18.57	- 69%

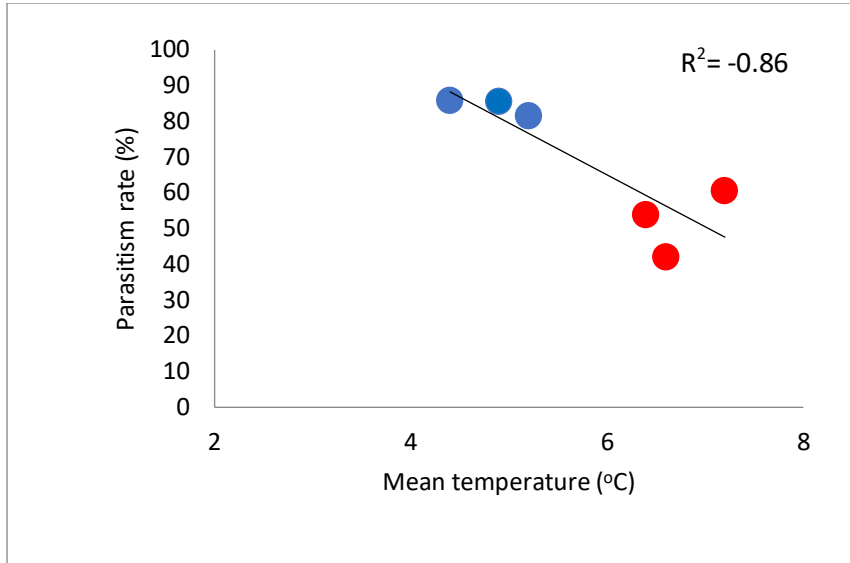


Figure 1 – Correlation between parasitism rate by *Torymus sinensis* (%) and mean winter temperatures (°C) recorded from December to March in 2019-2021 (blue dots refer to non-critical sites, and red dots to critical sites)

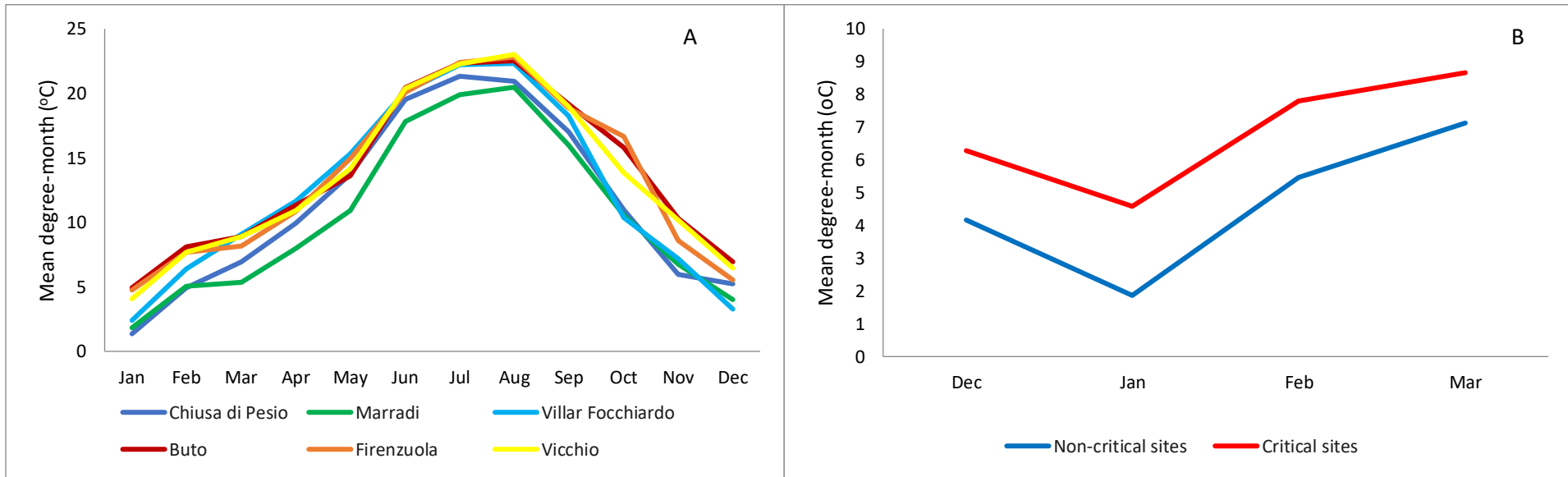


Figure 2 – Mean degree-month recorded from January 2019 to December 2021 in all the surveyed sites (A), and comparison of the mean degree-month recorded from December to March in 2019-2021, distinguishing between non-critical and critical sites (B) (non-critical sites: Chiusa di Pesio, Marradi, Villar Focchiardo; critical sites: Buto, Firenzuola, Vicchio).

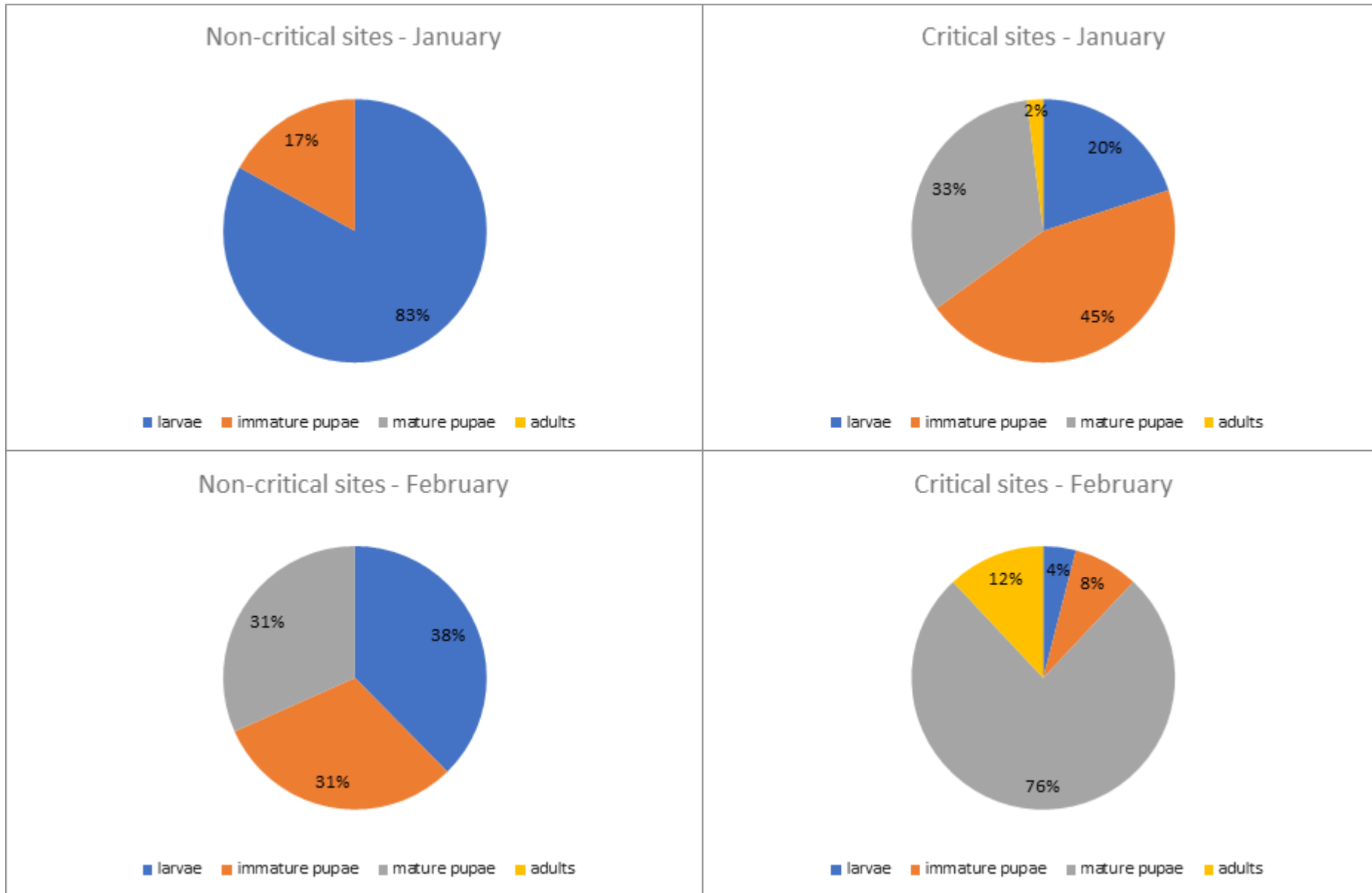


Figure 3 - Phenological stage of the *Torymus sinensis* Kamiyo individuals (%) recorded in the dissected galls, comparing non-critical sites (left) and critical sites (right) in January (top) and February (bottom).

162 **Discussion**

163 The structural dynamics of host–parasitoid populations play a key role in the mechanism of natural community
164 development with invasive species (Choi et al., 2017). In particular, the synchrony between *T. sinensis* emergence and
165 ACGW gall formation has always been considered the crucial point for the effectiveness of CBC programs (Moriya et
166 al., 2003; Quacchia et al., 2008). Generally, hosts and parasitoids synchronize emergence via photoperiod and
167 temperature, and differing responses to variation in one of these cues could desynchronize local host and parasitoid
168 populations (Wetherington et al., 2017).

169 Our results show a phenological asynchrony between *T. sinensis* and the ACGW in sites characterized by warmer
170 temperatures. In non-critical sites (Chiusa di Pesio, Marradi, Villar Focchiardo) about 40% of larvae and 30% of immature
171 pupae were observed overwintering in February. In the same period in the critical sites (Buto, Firenzuola, and Vicchio),
172 most of the individuals recorded were mature black pupa (76%), with some newly formed adults as well (on average
173 12%). In these latter sites, the adult emergence was always detected in late February-early March on ornamental non-
174 target shrubs and trees (*Forsythia*, *Laurus*, *Prunus*) in blossom, as indicating that elevated temperature may favor an
175 anticipated phenology. In non-critical sites the parasitism rate remained stable, with average values of 84% (optimal
176 parasitism rate is considered to be over 75%, CF personal communication). Conversely, in the critical sites a continuous
177 reduction was observed over the years. Specifically, the parasitism rate reduced by 29%, 33%, and 69% when comparing
178 2019 *versus* 2021, in Buto, Firenzuola and Vicchio, respectively.

179 Depending on the geographical and climatic conditions, the ACGW galls usually start to form in mid-/late April, and in
180 case of an early emergence of the biocontrol agent (namely a month and a half in advance in the surveyed critical sites),
181 females cannot lay eggs because current-year fresh galls are not available. Moreover, parasitoid wasps usually feed on
182 various sugar-rich sources in the field, and in case of an early emergence in late winter the risk of mortality may be quite
183 high given the low availability of floral nectar, homopteran honeydew, and/or pollen. In controlled conditions, *T.*
184 *sinensis*'s longevity is known to reach a maximum of 102 days when fed with honey drops (Picciau et al., 2017). However,
185 in field conditions, where a safe source of food is not provided, longevity is significantly lower (e.g., the estimated
186 longevity for female adults in Japan is 37 days in field conditions, Piao and Moriya, 1999). Additionally, in absence of
187 food, since *T. sinensis* is a synovigenic species, the reallocation of resources through egg resorption for somatic
188 maintenance may occur, going to further negatively impact on the female's fitness (Picciau et al., 2019).

189 Both our field and laboratory investigations suggest that temperature may affect phenology and can generate potential
190 cascading effects, as well as loss of biological control. This can be critical for the parasitoid's performance, thus leading
191 to a disruption of the temporal synchronization, a lower parasitism pressure, and an increasing risk of host outbreaks.

192 Although this phenomenon has so far been observed in a few sites, we highlighted how marked was the difference in
193 the mean annual temperatures when comparing non-critical *versus* critical sites (mean annual temperature greater than
194 1.40°C in critical sites *versus* non-critical sites). In particular, the biggest differences were detected in winter with mean
195 temperatures higher than 2.72°C and 2.34°C in January and February respectively, in critical sites.

196 Variation in mean temperature and increased frequency of extreme climate events have already impacted the
197 distribution and phenology of various organisms, including insects (Wetherington et al., 2017). Direct and indirect
198 effects of temperature changes on biotic interactions could thus severely affect population dynamics, community
199 structure and food webs, as well as ecosystem functioning and services (Le Lann et al., 2021). Specifically, the
200 magnitude of winter chilling can influence insect phenology, and warmer conditions experienced during diapause could
201 thus reduce both diapause incidence and duration, exposing insects to unfavourable conditions that further increase
202 mortality (Senior et al., 2020). The diverse ways climate change might impact on natural enemies may result in less
203 control of pest species, affecting the host-enemy synchrony, and emergence time (Thompson et al. 2010).

204 Even if specialist parasitoids often exhibit population dynamic responses that are strongly coupled with those of their
205 hosts, the phenological synchrony between plants and insects and between hosts and parasitoids could become uncoupled
206 if the two processes are temperature driven in different ways (Hance et al., 2007; Evans et al., 2012; Laws, 2017;
207 Urbaneja-Bernat et al., 2019). Our findings confirm that a shift in phenology would increase ACGW survival in chestnut-
208 growing areas. As reported by Hance et al. (2007), when the parasitoids emerge too early, the host population is exposed
209 to a relatively lower number of searching adult parasitoids and may considerably increase. If the advance of emergence
210 is repeated over the years, the parasitoid population may crash to very low levels and eventually becomes extinct. The
211 host population then increases rapidly in the absence of regulation. We reported similar evidence in the critical chestnut
212 orchards. Hence, different environmental conditions can result in relevant spatial and temporal variability in the
213 phenology of both ACGW and its parasitoid *T. sinensis*. Especially, since no native parasitoids were able to adapt and
214 control this invasive species with adequate parasitism rate (Quacchia et al., 2013; Ferracini et al., 2018).

215 In the critical sites characterized by warmer winters, a major presence of galls was detected, but until now no severe
216 reduction in fruiting was highlighted. However, a higher infestation rate by the ACGW may also exacerbate other
217 phytosanitary threats to chestnut production, as the ink disease (*Phytophthora* spp.), the canker blight [*Chryphonectria*
218 *parasitica* (Murrill) M.E. Barr], and the chestnut tortrix moths [*Cydia fagiglandana* (Zeller), and *Cydia splendana*
219 (Hübner)], commonly known in the study area (Vettraino et al., 2005; Ferracini et al., 2020; Lione et al., 2020).

220 However, if warmer winter temperatures may severely affect *T. sinensis*'s emergence, what can be the implications in a
221 climate change scenario? Climate change is already known to affect the abundance, distribution and activity of natural
222 enemies that are important for suppressing herbivore crop pests. Specifically, higher mean temperatures and increased

223 frequency of climatic extremes are expected to reduce parasitoid effectiveness in suppressing hosts (Romo and Tylianakis,
224 2013).

225 **Conclusions**

226 A careful analysis on how host-parasitoid systems react to changes in temperature is needed, to help researchers predict
227 and manage the consequences at the local level. In specific critical situations, when parasitoid/host synchronization does
228 not match, might *T. sinensis* be suitable for augmentative release? In such biocontrol programs, can *T. sinensis* contribute
229 to suppress pest population densities to specified target levels in the short term and compensate for the potential loss of
230 individuals in case of early emergence?

231 Since *T. sinensis* is commercially available, future research should assess if regular large-scale releases of *T. sinensis* will
232 be a feasible option to obtain sufficient, sustainable, and long-term control, allowing to reach an equilibrium in sites
233 characterized by a phenological mismatch due to warmer temperatures. While classical biocontrol programs provide a
234 lasting control of pests but require longer implementation periods (van Lenteren et al., 2018), augmentative releases
235 involve the periodical introduction of natural enemies and are comparatively quicker (van Lenteren and Bueno, 2003).
236 Additionally, since *T. sinensis* cannot be reared on artificial diet, may this approach be cost-effective, especially in a view
237 of large-scale mass production by insectaries or private companies?

238 These results suggest that global warming may increase the magnitude of early emergence of the introduced biocontrol
239 agent. So, this scenario deserves to be better investigated since it might have a greater impact under a warmer climate,
240 especially in the winter season, that may increase this mismatch further. Specific investigations are needed over a longer
241 period to evaluate how local habitat conditions (geographical variability, elevational gradient, exposure) may act as cue
242 for *T. sinensis*'s phenological change, and to clarify whether this event is actually increasing in relevance, also in a climate
243 change perspective.

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248 **Supplementary Materials:** Table S1: Sampling sites monitored in the five-year period 2018–2022. Table S2:
249 Phenological stage of the *Torymus sinensis* Kamijo individuals recorded in the dissected galls. The percentage is
250 calculated on the total number of cells (L:larvae; P1: white immature pupae; P2: black mature pupae; A: newly formed
251 adults). Figure S1: Presence of *Torymus sinensis* Kamijo adults on ornamental *Forsythia* in early March (courtesy of
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255 **Conflicts of Interest:** The authors declare no conflict of interest.

256 **Author Contributions:** AA, and CF conceived research; CP, conducted experiments; CF and CP analyzed data; CF
257 Writing - Original Draft Preparation; AA, CF, CP Writing - Review & Editing; AA Funding Acquisition. All authors read
258 and approved the manuscript.

259 **Ethics approval:** All the insect rearings and experiments were conducted in accordance with the legislation and
260 guidelines of the European Union for the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). All experimental protocols using insects were approved
261 by the *ad hoc* Committee of DISAFA of the University of Torino.
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Supplementary Table 1. Sampling sites monitored in the five-year period 2018–2022.

Site	Region	Geographic coordinates		Altitude (m)	Esposition (m a.s.l.)	<i>Torymus sinensis</i> release (year) *
		N	E			
Buto	Liguria	44°19'28.6"	09°38'38.3"	430	South-West	2014
Chiusa di Pesio	Piedmont	44°18'29.0"	07°40'54.4"	680	East	2005
Firenzuola	Tuscany	44°07'03.0"	11°24'31.0"	450	South	2013
Marradi	Tuscany	44°04'13.1"	11°36'57.4"	360	South-West	2013
Vicchio	Tuscany	43°57'44.7"	11°32'46.6"	750	North-East	2013
Villar Focchiardo	Piedmont	45°06'25.7"	07°13'47.7"	600	Nord-East	2010

*Date of the first official *Torymus sinensis* release reported by the chestnut growers.

Supplementary Table 2 – Phenological stage of the *Torymus sinensis* Kamiyo individuals recorded in the dissected galls. The percentage is calculated on the total number of cells (L:larvae; P1: white immature pupae; P2: black mature pupae; A: newly formed adults).

		2019-2020				2020-2021				2021-2022			
		Jun	Nov	Jan	Feb	Jun	Nov	Jan	Feb	Jun	Nov	Jan	Feb
Non-critical sites	Chiusa di Pesio (CN)	100% L	100% L	81%L 19%P1 25%P2	38%L 37%P1 25%P2	100%L	100%L	78%L 22%P1	34%L 25%P1 41%P2	100% L	100% L	83%L 17%P1	37%L 31%P1 32%P2
	Marradi (FI)	100% L	100% L	79%L 21%P1 26%P2	42%L 32%P1 26%P2	100%L	100%L	86%L 14%P1	39%L 29%P1 31%P2	100% L	100% L	80%L 20%P1	38%L 26%P1 36%P2
	Villar Focchiardo (TO)	100% L	100% L	87%L 12%P1 24%P2	44%L 31%P1 24%P2	100%L	100%L	84%L 16%P1	38%L 39%P1 23%P2	100% L	100% L	85%L 15%P1	29%L 30%P1 41%P2
Critical sites	Buto (SP)	100% L	100% L	36%L 51%P1 11%P2 3%A	8%L 28%P1 56%P2 8%A	100%L	100%L	18%L 81%P1 1%P2	13%L 8%P1 77%P2 2%A	100% L	100% L	2%L 12%P1 83%P2 3%A	3%L 9%P1 85%P2 3%A
	Firenzuola (FI)	100% L	100% L	33%L 51%P1 14%P2 15%A	5%L 27%P1 53%P2 15%A	100%L	100%L	29%L 41%P1 30%P2	7% 11%P1 64%P2 18%A	100% L	100% L	28%L 33%P1 37%P2 2%A	9%L 16%P1 59%P2 16%A
	Vicchio (FI)	100% L	100% L	16%L 44%P1 40%P2 10%A	8%L 34%P1 48%P2 10%A	100%L	100%L	17%L 37%P1 41%P2 5%A	2%L 39%P1 51%P2 8%A	100% L	100% L	5%L 56%P1 38%P2 1%A	82%P2 18%A



Supplementary Figure 1 - Presence of *Torymus sinensis* adults on ornamental *Forsythia* in early March (courtesy of Simone Battistini)