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

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Abstract:

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

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

Introduction

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

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

This parasitoid has been proven to effectively control ACGW outbreaks, and its successful use highlights how CBC may represent a cost-effective tool for managing an exotic invasive pest, balancing pest populations below damaging levels. In NW Italy, where *T. sinensis* was first released in 2005, approximately 7–8 years were due to noticeably decrease the ACGW (Ferracini et al., 2019). The pest and its parasitoid started to produce an ever-changing pattern of travelling waves as predicted by a mathematical model (Paparella et al. 2016), and no reported evidence of infestation resurgence was reported after 17 years from first release.

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

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

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

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

Materials and Methods

Survey sites

Investigations were performed in a five-year period (2018-2022) in sweet chestnut orchards located in three northern Italian regions, namely Liguria, Piedmont, and Tuscany. A total of six sampling sites was chosen in relation to the infestation by the ACGW recorded in 2018 (as described in the following chapter). The sites were located in the 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 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), 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 information concerning the surveyed sites is given in the Supplementary Table 1. The survey sites were characterized by managed chestnut orchards. Trees were approximately 80-100 yrs old, 15-20 m in height, planted at 8 m distance along the row and 8 m distance between rows. Tree density was about 120 trees/ha. No insecticide treatment was ever applied in the plots.

Selection of chestnut orchards

Based on the reports received from chestnut growers, six chestnut orchards were visited and chosen in relation to the infestation level by *D. kuriphilus*. The infestation rate was recorded in late August in all sites in 2018. At each site, 10 chestnut trees were randomly selected, and from each tree ten 1-year old branches were randomly chosen at different heights of the canopy for a total of 100 branches per site per year. The infestation rate was expressed as the percentage of total buds infested by the gall wasp, according to Ferracini et al. (2019). The infestation index was assigned based on the average number of the galls and position along the leaf and/or branch, according to six classes reported in the Table 1.

In all sites the biocontrol agent *T. sinensis* has been released for at least 5 years since the investigations, thus having enough time to form stable populations.

Table 1 – Infestation index recorded in the survey sites according to the average number of galls by *Dryocosmus kuriphilus* 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)

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

Collection and dissection of the galls

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

Data logger

Temperatures were monitored in all sites using waterproof battery-powered HOBO data loggers (Climate data logger BL30, Trotec International GmbH & C. S.a.s., Ora (BZ), Italy). All data loggers, placed on the trunk at a height of 2 m above the ground, were programmed to record the minimum, mean and maximum temperature at 30-min intervals from January 2019 to December 2021.

Statistical analysis

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

Results

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

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 sites. *T. sinensis* was detected in all chestnut orchards, with different parasitism rate among sites and years (Table 2). In non-critical sites the parasitism rate by *T. sinensis* was on average 84% in the surveyed period, accounting from 76.57% (Villar Focchiardo in 2019-2020) to 99% (Marradi in 2021-2022). Conversely, the parasitism rate in critical sites was on average 52%, experiencing a severe decline in the three years and reaching values under 50% in all sites in 2021-2022. The mean parasitism rate was significantly different when comparing non-critical *versus* critical sites in the three-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= 0.006 in 2021-2022).

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

The dissection of the galls highlighted that 100% of *T. sinensis* was at the larval stage in all sites and years in June and November. Afterwards, a variation in *T. sinensis*'s phenology was recorded comparing the two types of sites. In the non-critical sites, most of the individuals were at the larval stage in January (83% of larvae and 17% of white immature pupae), while in February a higher number of pupae was detected (38% of larvae, 31% immature pupae, and 31% of black pupae). Conversely, the dissection of galls recorded in critical sites revealed that most of the individuals were already at the pupal stage in January (20% of larvae, 45% immature pupae, 33% of mature pupae, and 2% of newly formed adults), and in February almost all individuals were mature pupae close to adult emergence (4% of larvae, 8% immature pupae, 76% of black pupae, and 12% of newly formed adults) (Figure 3). All data concerning dissections are given in the Supplementary Table 2.

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

The average monthly temperature recorded with data loggers in the chestnut orchards is shown in the Figure 2 (A-B). In all the three critical sites, temperatures were higher than those recorded in the non-critical sites from December to February in 2019-2021. Temperatures remained markedly high even during March in both Buto and Firenzuola, sites. On average, when comparing non-critical *versus* critical sites, the mean temperature recorded in critical sites was higher 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%

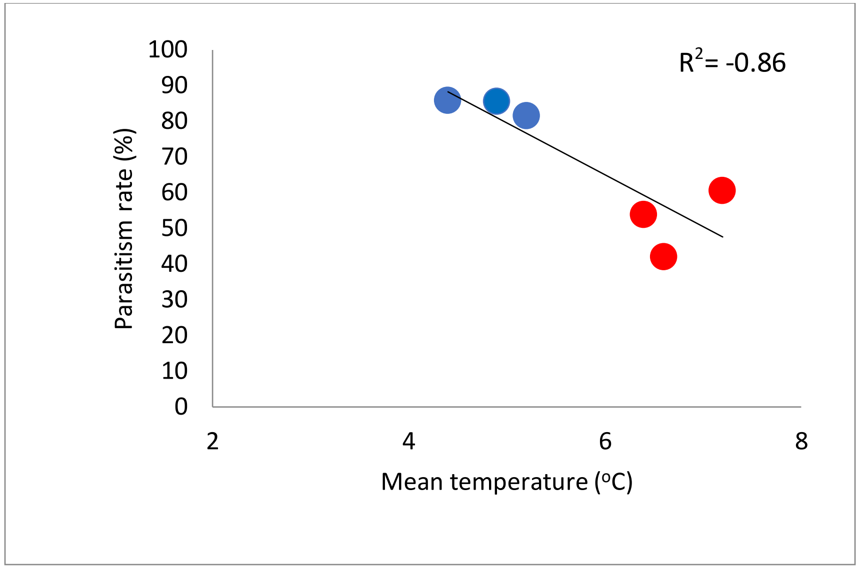

Figure 1 – Correlation between parasitism rate by *Torymus sinensis* (%) and mean winter temperatures (°C) recorded from December to March in 2019-2021 (blue dots refer to non-critical sites, and red dots to critical sites)

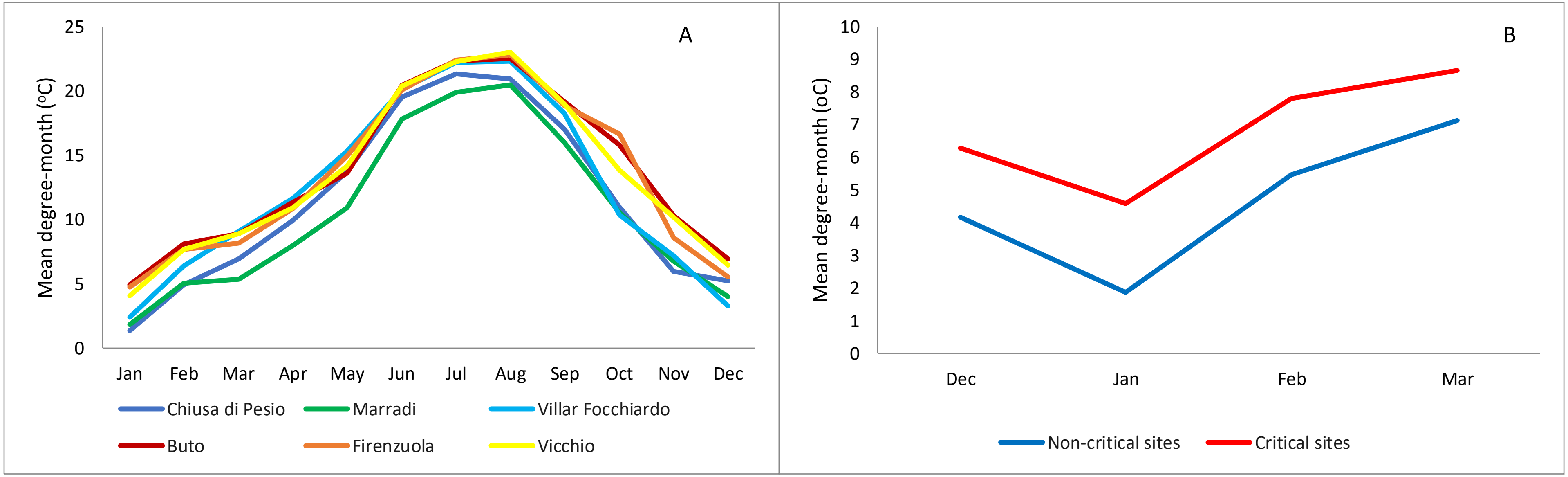

Figure 2 – Mean degree-month recorded from January 2019 to December 2021 in all the surveyed sites (A), and comparison of the mean degree-month recorded from December to March in 2019-2021, distinguishing between non-critical and critical sites (B) (non-critical sites: Chiusa di Pesio, Marradi, Villar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

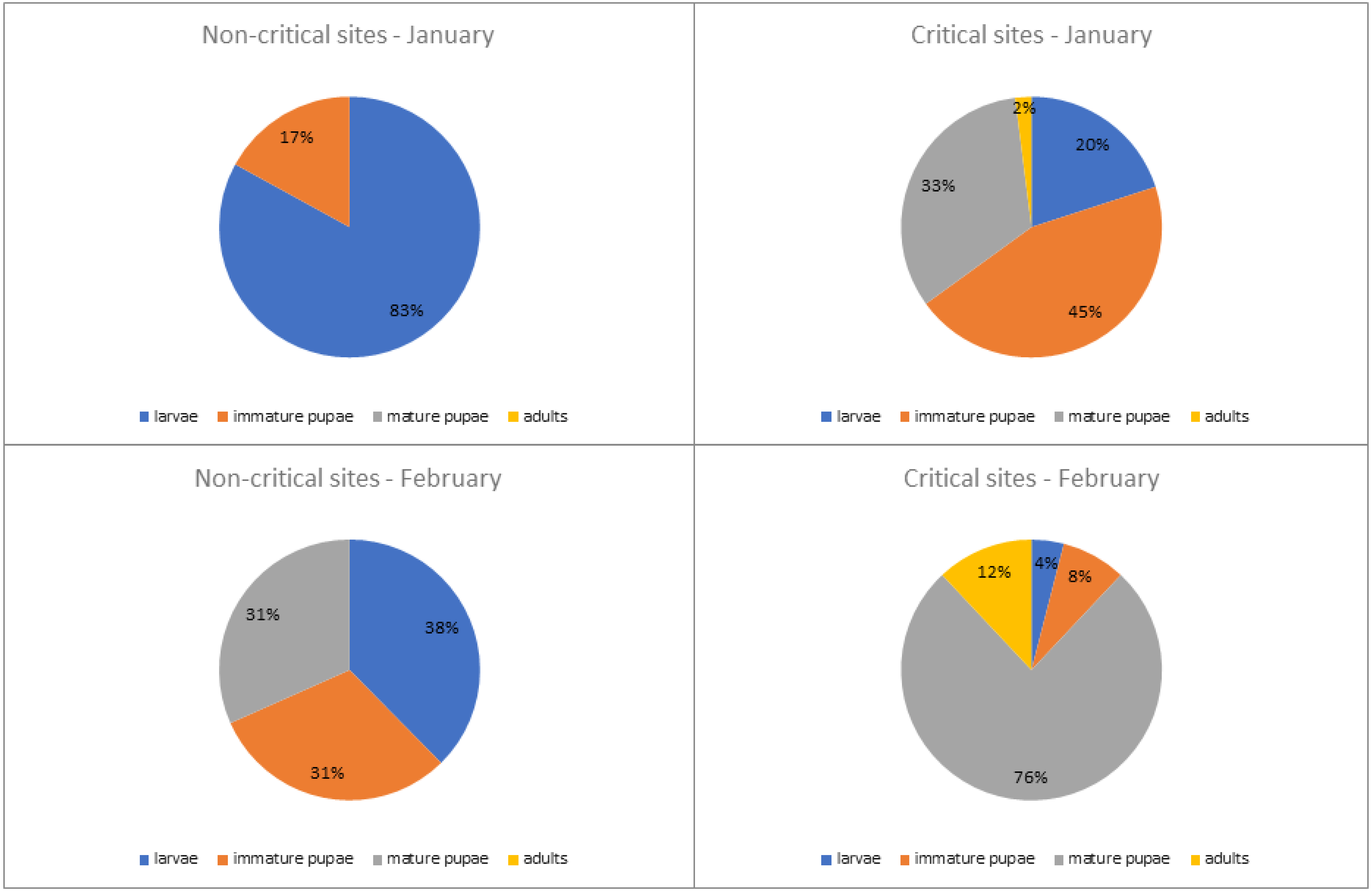

Figure 3 - Phenological stage of the *Torymus sinensis* Kamijo individuals (%) recorded in the dissected galls, comparing non-critical sites (left) and critical sites (right) in January (top) and February (bottom).

Discussion

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

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

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

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

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

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

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

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

However, if warmer winter temperatures may severely affect *T. sinensis*'s emergence, what can be the implications in a climate change scenario? Climate change is already known to affect the abundance, distribution and activity of natural enemies that are important for suppressing herbivore crop pests. Specifically, higher mean temperatures and increased frequency of climatic extremes are expected to reduce parasitoid effectiveness in suppressing hosts (Romo and Tylianakis, 2013).

Conclusions

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

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

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

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Supplementary Materials: Table S1: Sampling sites monitored in the five-year period 2018–2022. Table S2: Phenological stage of the *Torymus sinensis* Kamijo 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). Figure S1: Presence of *Torymus sinensis* Kamijo adults on ornamental *Forsythia* in early March (courtesy of Simone Battistini).

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Conflicts of Interest: The authors declare no conflict of interest.

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

Ethics approval: All the insect rearings and experiments were conducted in accordance with the legislation and guidelines of the European Union for the protection of animals used for scientific purposes (http://ec.europ a.eu/environment/chemi cals/lab_animals/legislation_en.htm). All experimental protocols using insects were approved by the *ad hoc* Committee of DISAFA of the University of Torino.

References

Avtzis, D.N., Melika, G., Matošević, D., Coyle, D.R., 2019. The Asian chestnut gall wasp *Dryocosmus kuriphilus:* a global invader and a successful case of classical biological control. J. Pest Sci. 92, 107–115. https://doi.org/10.1007/s11676-020-01197-5.

Borowiec, N., Thaon, M., Brancaccio, L., Warot, S., Vercken, E., Fauvergue, X., Ris, N., Malausa, J.C., 2014. Classical biological control against the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in France. Plant Prot. Q. 29, 7–10. https://doi.org/10.29352/mill0214.21135.

Choi, W.I., Jeon, M., Park, Y., 2017. Structural dynamics in the host-parasitoid system of the pine needle gall midge (*Thecodiplosis japonensis*) during invasion. PeerJ 5:e3610 https://doi.org/10.7717/peerj.3610.

Cooper, R.W., Rieske, L.K., 2007. Community associated of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America. Ann. Entomol. Soc. Am. 100, 236-244. https://doi.org/10.1603/0013-8746(2007)100.

Evans, E.W., Carlile, N.R., Innes, N.B., Pitigala, N., 2012. Warm springs reduce parasitism of the cereal leaf beetle through phenological mismatch. J. Appl. Entomol. 137, 383-391. https://doi.org/10.1111/jen.12028.

Ferracini, C., Ferrari, E., Saladini, M.A., Pontini, M., Corradetti, M., Alma, A., 2015. Non-target host risk assessment for the parasitoid *Torymus sinensis*. Biocontrol 60, 583–594. https://doi.org/10.1007/s10526-015-9676-1.

Ferracini, C., Ferrari, E., Pontini, M., Hernández Nova, L.K., Saladini, M.A., Alma, A., 2017. Post-release evaluation of non-target effects of *Torymus sinensis,* the biological control agent of *Dryocosmus kuriphilus* in Italy. Biocontrol 62, 445–456. https://doi.org/10.1007/s10526-017-9803-2.

Ferracini, C., Bertolino, S., Bernardo, U., Bonsignore, C.P., Faccoli, M., Ferrari, E., Lupi, D., Maini, S., Mazzon, L., Nugnes, F., Rocco, A., Santi, F., Tavella, L., 2018. Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp? Biol. Control 121, 36-43. https://doi.org/10.1016/j.biocontrol.2018.01.009.

Ferracini, C., Ferrari, C., Pontini, M., Saladini, M.A., Alma, A., 2019. Effectiveness of *Torymus sinensis*: a successful long-term control of the Asian chestnut gall wasp in Italy. J. Pest Sci. 92, 353-359. https://doi.org/10.1007/s10340-018-0989-6.

Ferracini, C., Pogolotti, C., Lentini, G., Saitta, V., Busato, E., Rama, F., Alma, A., 2020. Performance of pheromone-baited traps to monitor the seasonal abundance of tortrix moths in chestnut groves. Insects 11, 807; https://doi.org/10.3390/insects11110807.

Hance, T., van Baaren, J., Vernon, P., Boivin, G., 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annu. Rev. Entomol. 52, 107–126; doi: 10.1146/annurev.ento.52.110405.091333

İpekdal, K., Emin, A., Kuzucu, A.S., Karadağ, M., Koçluk, M., Açici, O., Şah, S., Aksu, Y., Colombari, F., 2017. Rearing and releasing *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), larval parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). Turkish Bull. Entomol. 7, 113–129. http://dx.doi.org/10.16969/teb.96587.

Kenis, M., Hurley, B.P., Hajek, A.E., Cock, M.J.W., 2017. Classical biological control of insect pests of trees: facts and figures. Biol. Inv. 19, 3401–3417. https://doi.org/10.1007/s10530-017-1414-4.

Laws, A.N., 2017. Climate change effects on predator–prey interactions. Curr. Opin. Insect Sci. 23, 28-34. https://doi.org/10.1016/j.cois.2017.06.010.

Le Lann, C., van Baaren, J., Visser, B., 2021. Dealing with predictable and unpredictable temperatures in a climate change context: the case of parasitoids and their hosts. J. Exp. Biol. 224, jeb238626. https://doi.org/10.1242/jeb.238626.

Lione, G., Giordano, L., Turina, M., Gonthier, P., 2020. Hail-induced infections of the chestnut blight pathogen *Cryphonectria parasitica* depend on wound size and may lead to severe diebacks. Phytopathol. 110(7), 1280-1293. https://doi.org/10.1094/phyto-01-20-0006.

Matošević, D., Lacković, N., Kos, K., Kriston, E., Melika, G., Rot, M., Pernek, M., 2017. Success of classical biocontrol agent *Torymus sinensis* within its expanding range in Europe. J. Appl. Entomol. 141, 758–767. https://doi.org/10.1111/jen.12388.

Moriya, S., Shiga, M., Adachi, I., 2003. Classical biological control of the chestnut gall wasp in Japan, in: Van Driesche, R.G. (ed), Proceedings of the 1st international symposium on biological control of arthropods, Hawaii, 14–18 January 2002. USDA Forest Service, Washington, DC, USA, 407–415.

Paparella, F., Ferracini, C., Portaluri, A., Manzo, A., Alma, A., 2016. Biological control of the chestnut gall wasp with *T. sinensis*: a mathematical model. Ecol. Model 338, 17–36.

Pérez-Otero, R., Crespo, D., Mansilla, J.P., 2017. *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) in Galicia (NW Spain): pest dispersion, associated parasitoids and first biological control attempts. Arq. Entomol. 17, 439-448.

Piao, C., Moriya, S., 1999. Oviposition of *Torymus sinensis* (Hymenoptera: Torymidae) under natural conditions. Entomol. Sci. 2, 329-334.

Picciau, L., Ferracini, C., Alma, A., 2017. Reproductive traits in *Torymus sinensis*, biocontrol agent of the Asian chestnut gall wasp: implications for biological control success. Bull. Insectol. 70, 49–55.

Picciau, L., Alma, A., Ferracini, C., 2019. Effect of different feeding sources on lifespan and fecundity in the biocontrol agent *Torymus sinensis*. Biol. Control 134, 45-52. https://doi.org/10.1016/j.biocontrol.2019.04.002.

Pogolotti, C., Cuesta-Porta, V., Pujade.Villar, J., Ferracini, C., 2019. Seasonal flight activity and genetic relatedness of *Torymus* species in Italy. Agric. Forest Entomol. 21, 159-167. https://doi.org/10.1111/afe.12316.

Quacchia, A., Moriya, S., Bosio, G., Scapin, G., Alma, A., 2008. Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. Biocontrol 53, 829–839. https://doi.org/10.1007/s10526-007-9139-4.

Quacchia, A., Ferracini, C., Nicholls, J.A., Piazza, E., Saladini, M.A., Tota, F., Melika, G., Alma, A., 2013. Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in North-Western Italy. Insect Conserv. Divers. 6, 114–123. https://doi.org/10.1111/j.1752-4598.2012.00192.x.

Romo, C.M., Tylianakis, J.M., 2013. Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. PLoS ONE 8(3): e58136. https://doi.org/10.1371/journal.pone.0058136.

Senior, V.L., Evans, L.C., Leather, S.R., Oliver, T.H., Evans, K.L., 2020. Phenological responses in a sycamore–aphid–parasitoid system and consequences for aphid population dynamics: A 20 year case study. Glob. Change Biol. 26, 2814-2828. https://doi.org/10.1111/gcb.15015.

Thomson, L.J., Macfadyen, S., Hoffmann, A.A., 2010. Predicting the effects of climate change on natural enemies of agricultural pests. Biol. Control 52, 296-306. https://doi.org/10.1016/j.biocontrol.2009.01.022.

Urbaneja-Bernat, P., Ibáñez-Gual, V., Montserrat, M., Aguilar-Fenollosa1, E., Jaques, J.A., 2019. Can interactions among predators alter the natural regulation of an herbivore in a climate change scenario? The case of *Tetranychus urticae* and its predators in citrus. J. Pest Sci. 92, 1149-1164. https://doi.org/10.1007/s10340-019-01114-8.

Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D.L., Blossey, B., Brancatini, V., Casagrande, R., Causton, C.E., Coetzee, J.A., Cuda, J., Ding, J., Fowler, S.V., Frank, J.H., Fuester, R., Goolsby, J., Grodowitz, M., Heard, T.A., Hill, M.P., Hoffmann, J.H., Huber, J., Julien, M., Kairo, M.T.K., Kenis, M., Mason, P., Medal, J., Messing, R., Miller, R., Moore, A., Neuenschwander, P., Newman, R., Norambuena, H., Palmer, W.A., Pemberton, R., Perez Panduro, A., Pratt, P.D., Rayamajhi, M., Salom, S., Sands, D., Schooler, S., Schwarzländer, M., Sheppard, A., Shaw, R., Tipping, P.W., van Klinken R.D., 2010. Classical biological control for the protection of natural ecosystems. Biol. Control 54, 2-33. https://doi.org/10.1016/j.biocontrol.2010.03.003.

Van Lenteren, J.C., Bueno, V.H.P., 2003. Augmentative biological control of arthropods in Latin America. Biocontrol 48, 123-139. https://doi.org/10.1023/A:1022645210394.

Van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. Biocontrol 63, 39–59. https://doi.org/10.1007/s10526-017-9801-4.

Vettraino, A. M., Morel, O., Perlerou, C., Robin, C., Diamandis, S., Vannini, A., 2005. Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink Disease and crown decline. Eur. J. Plant Pathol. 111(2), 169–180. https://doi.org/10.1007/s10658-004-1882-0.

Wetherington, M.T., Jennings, D.E., Shrewsbury, P.M., Duan, J.J., 2017. Climate variation alters the synchrony of host–parasitoid interactions. Ecol. Evol. 7, 8578-8587. https://doi.org/10.1002/ece3.3384.

Yara, K., Sasawaki, T., Kunimi, Y., 2010. Hybridization between introduced *Torymus sinensis* (Hymenoptera: Torymidae) and indigenous *T. beneficus* (late spring strain), parasitoids of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). Biol. Control 54, 14-18. https://doi.org/10.1016/j.biocontrol.2010.03.006.

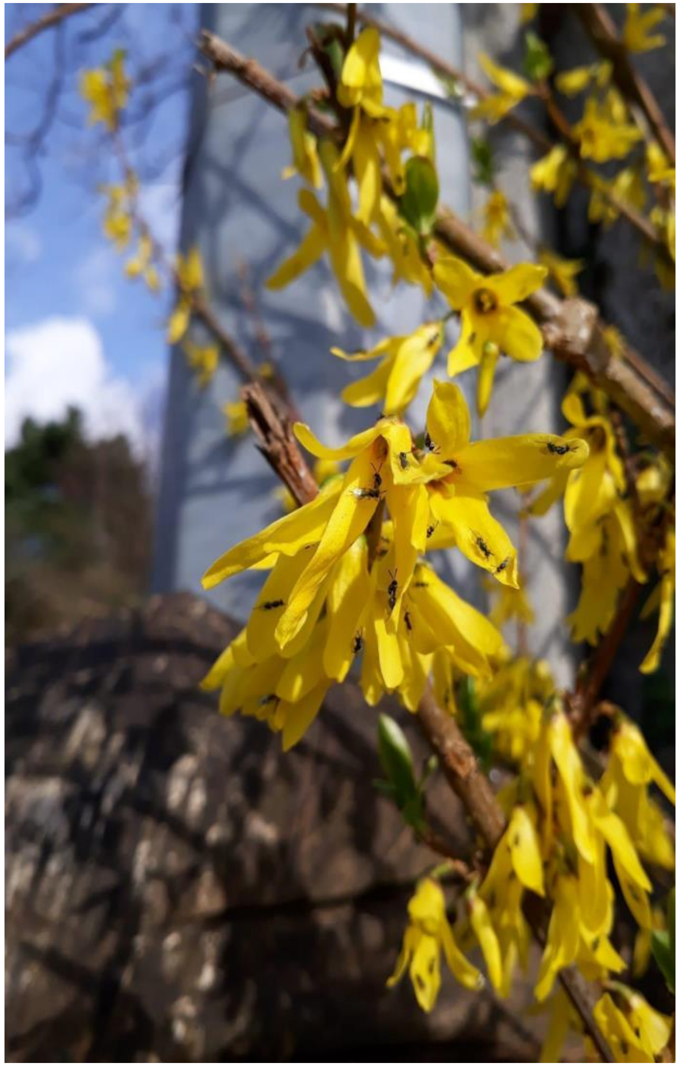
Supplementary Table 1. Sampling sites monitored in the five-year period 2018–2022.

Site	**Region**	**Geographic coordinates**		**Altitude (m)**	**Esposition (m a.s.l.)**	***Torymus sinensi* 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* Kamijo 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	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	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	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	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	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)