Type of the Paper - Article

Short-term thermal changes affect parasitism on Asian chestnut gall wasp

Carmelo Peter Bonsignore[1,*], Giusi Vizzari[1], Gregorio Vono[2,*] and Umberto Bernardo[3]

[1] *Laboratorio di Entomologia ed Ecologia Applicata, Dipartimento PAU. Università Mediterranea di Reggio Calabria, Reggio Calabria, Italy;* cbonsignore@unirc.it
[2] *Università degli Studi Mediterranea di Reggio Calabria, Dipartimento di Agraria;* gregorio.vono@gmail.com
[3] *CNR, Institute for Sustainable Plant Protection, SS of Portici, Portici (NA), Italy;* umberto.bernardo@ipsp.cnr.it
* Correspondence: cbonsignore@unirc.it; Tel.: +39-09651696318; fax: +39 09651696550

Simple Summary: The Asian cynipid gall wasp (ACGW) "*Dryocosmus kuriphilus*" has become widespread in Europe, and in all invaded areas it is parasitized by native parasitoids associated to oak galls, for which ACGW represents a non-saturated adaptation space. Taking into account the last two-decade increase in the frequency of extreme climatic events (i.e. low temperature during the vegetative period of the chestnut tree), this research aimed to elucidate the thermic stress effects (i.e. cold treatment) on both ACGW biology and parasitism by native and introduced parasitoids. The ACGW-parasitoid system represents an ideal subject to evaluate the effect of sudden extreme climatic events due to their biological characteristics that include the ability to complete the development even in galls detached from the plants. We have shown that parasitism and mortality of ACGW in three chestnut fields were affected by the cold treatment. Our results revealed species-specific differences in the abundance and performance of parasitoids associated with ACGW in response to cold stress. For example, the frequency of *Eupelmus* spp. and *Mesopolobus tibialis* doubled in the cold treatment in all three chestnut fields in both years. Therefore, plasticity in response to short-term thermal changes may be associated with individual fitness in some parasitoid species.

Abstract: Thermal variations affect tri-trophic interactions involving plants, herbivores, and parasitoids, causing a mismatch between their phenological cycles. In the current environmental context of climate change, climatic factors can undergo profound and sudden changes, such as sudden hot or cold snaps. Here we show that episodes of short but sustained low temperatures have increased, mainly in the month of May, in the last two decades. We subjected galls induced by Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* to cold stress to assess if and how the pest and its parasitoids were affected. Over the course of two years seasonal parasitism, parasitism rates, the relative abundance of each parasitoid species, and mortality of ACGW were calculated. We found that the cold treatment affected both pest and parasitoids, resulting in a reduction in emerging ACGWs and differing ratios of species within the parasitoid community. The striking example was the change in the relative frequency of three species of *Eupelmus* spp. and of *Mesopolobus tibialis*, which doubled in thermally-stressed galls in all chestnut fields. The thermal effects on the development of the host and the direct effects of cold temperatures on the surface of galls (i.e., humidity or hardness of the galls) warrant further research in this direction.

Keywords: Chestnut; climate thermal fluctuations; Cynipidae; *Dryocosmus kuriphilus*; *Eupelmus* spp.; fitness; host-parasitoid relationship

1. Introduction

In the context of the current global climate change, climatic factors can undergo profound and sudden changes. The increase of the mean temperature is associated with climatic oscillations such as heat and cold waves [1–3]. Modifications in abiotic factors such as thermal variations can act on different biological and evolutionary aspects [4,5], and may disrupt the seasonal phenology patterns of organisms and determine unpredictable changes in ecological niches of different insect groups [6,7], affecting, for example, the tri-trophic interactions involving plants, herbivores, and parasitoids [8]. The role of thermal variations in the trophic mismatch is increasingly understood, although nearly every type of species interaction could be modified by climate changes [6,9,10]. Anomalous and unpredictable cold spells can be observed more and more frequently in a short period of the year [11], thus are among the rapid thermal fluctuations worth investigating to assess possible effects on the abundance, distribution, and function of species in a food web. Temperatures can affect the phenology of both hosts and parasitoids [7,12,13] and asymmetric changes in the seasonal activities of species between closely interacting species are likely responsible for desynchronization in their life cycles [8].

Insects at higher trophic levels are expected to be more strongly affected by environmental changes than organisms from low trophic levels due to cascading effects in the food chain [14]. Parasitism rate can be affected by the phenology of parasitoid species associated with oak gall wasps, which are often multivoltine, as parasitoids must parasitize their hosts during the appropriate "window of vulnerability" [13,15]. Therefore, variation in parasitism rates is an immediate response to environmental factors, including seasonal temperature variations, climate change, and variations in habitat [13,16–18]. However, it is difficult to pinpoint what factors determine variations in the composition of parasitoid species and why some species are more successful after thermal stress.

In this study, we used the Asian cynipid gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu and its parasitoids to investigate their response to thermal stress. The ACGW is a serious pest that affects all chestnut tree species in the genus Castanea (Fagales: Fagaceae) [19–25]. The species also represents a perturbation to the natural trophic relationships between the community of native parasitoids and oak gall wasps [26–28]. ACGW was first detected in the Calabria region (southern Italy) in 2009 [29] about seven years after its first record in Northern Italy (Piedmont), and heavy infestations of chestnut fields in the Aspromonte National Park have since been reported [18]. ACGW is a univoltine species whose females lay their eggs into chestnut buds, which induces the formation of galls on the growing shoots that can inhibit shoot development and flowering [30,31]. Populations are composed entirely of females that reproduce by thelytokous parthenogenesis and can lay more than 100 eggs during their 10 days of life [32,33]. Approximately 30–40 days after oviposition, the first-instar larvae emerge and overwinter in the buds [33,34]. Larval development continues during the following spring with two more instars developing inside the galls [35]. [36] observed that modification of tissues surrounding *D. kuriphilus* eggs that differentiate to form the larval chamber, started approximately one month after oviposition. The larval chambers continued to increase slightly in size throughout the autumn months until January followed by rapid growth from March to May, during the period from bud swelling to bud break [36]. Before adult emergence, between June and August, females remain inside galls in the pharate stage for ovarian maturation so that they are ready to lay eggs in the new buds upon emergence [37]. Recent research on galls morphology has shown that gall mass and volume follow a pattern that can be associated with a climatic cline [38].

In recent years, ACGW has become widespread in Europe [39], and in all invaded areas it is parasitized by native parasitoids [40–43] associated to oak galls, for which ACGW represents a non-saturated adaptation space. A large community of generalist parasitoids related to oak galls rapidly shifted to ACGW [28,41], with local variations in the composition that depends on phenological overlap and habitat features [13,18,44,45]. The composition of this parasitoid community depends on morphological traits of galls (i.e., size, shape, hardness) [30,46], on the developmental stage of the gall (i.e., young or fully formed), cline, and on the number of gall chambers [47]. All newly-associated indigenous parasitoids attack concealed ACWGs through gall tissues, and the larger fully developed galls with a higher number of chambers and thicker sclerenchyma layers surrounding the larval chamber are negatively correlated with parasitoids, indicating that these gall traits are important defense mechanisms. The different lengths of the parasitoids' ovipositors can, therefore, limit or favor some species over others [30].

Other factors that affect the composition of the parasitoid community include the phenology of the host and the parasitoid, the establishment date of the new invasive host species, and the characteristics of the habitat [18,48]. The successful adaptation of a parasitoid to a new host requires synchrony between the presence of adult parasitoids and of suitable host life stages [44].

Since 2005, the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), a generalist parasitoid of galling insect native to China and univoltine, has been released in several European countries to successfully control ACGW populations [49–57]. Early reports relating to the introduction of *T. sinensis* highlighted an altered community structures of parasitoids which recently shifted toward the ACGW in multiple ways: utilizing some oak galls in addition to ACGW galls, and displacing some generalist oak galls parasitoids able to attack ACGW [28,47,58–59] but to date, the magnitude of its impact appears minimal [60] or not well known.

Taking into account the last two-decade increase in frequency of extreme climatic events (i. e. low temperature during the vegetative period of the chestnut tree), this research aimed to elucidate the thermic stress effects (i.e. cold treatment) on both ACGW biology and parasitism by native and introduced parasitoids.

The ACGW-parasitoid system represents an ideal subject to evaluate the effect of sudden extreme climatic events due to their biological characteristics that include the ability to complete the development even in galls detached from the plants. The results obtained can guide further experimental studies on the effects of sudden and extreme temperature events.

2. Materials and Methods

2.1 Study area

Shoots with galls from three chestnut fields were collected weekly between May and July 2017–2018. The chestnut trees were located in an upland within the Aspromonte Mountains (Reggio Calabria, southern Italy) (latitude 38°3', longitude 15°44'; 980 m asl). The fields are adjacent to farmland where other fruit crops (e.g., cherries, apples) and vegetable crops (e.g., cereals and potatoes) are grown. Downy oak (*Quercus pubescens* Willd), European oak (*Quercus robur* L., etc.), and holm oak (*Quercus ilex* L.) are widespread in the study area. The chestnut fields used for data collection included two orchards (with 25 and 50-year old trees, respectively) and one coppice (with 15-year old trees), which were named A, B, and C, respectively. The chestnut orchards are made up of wild plants grafted with the local Nzerta cultivar while the coppice is made up of trees obtained from seedlings of the same cultivar. Fields were managed without pesticides, growth regulators, or fertilizers.

2.2 Study Design

The experimental design took into account both the natural parasitization of different parasitoids species on ACGW and the effects of the thermal stress artificially caused on parasitization and on ACGW emergence. The sampling was weekly with thermal stress tests repeated during the spring-summer period to ensure the presence of all parasitoid species involved in biological control of ACGW.

2.3 Field sampling and seasonal parasitism description

For two years, an observational study design with sequential sampling throughout the larva-to-adult life history period was adopted. Shoots with galls from fields A, B, and C were collected from May until adult ACGWs emerged, which occurs in the study area in July. Approximately 24 shoots were collected weekly from less than 5 m above the ground in each chestnut field (A, B, and C), with one shoot collected from every ordinal direction: NE, SE, SW, and NW, from 6 different trees. Thirty-six galls (12 for each chestnut field) were randomly chosen from the shoots (nine galls in total per ordinal direction) and carefully dissected under a stereomicroscope (SZX9, Olympus®, Tokyo, Japan) within 24 h of collection. All chambers and vital and non-vital stages of gall wasps and parasitoids (i.e., larva, pupae, and pharate adults) were recorded. The parasitism rate was calculated as: number of chambers with living parasitoids (larvae, pupae, adults, emerged) / total number of chambers, excluding chambers damaged, with rot and without an identifiable host.

2.4 Cold stress experiment

To evaluate the effect of thermal stress on parasitoid species composition, 30 galls (from now on referred to as non-thermally-treated, NTT), chosen at random among those present on the collected shoots, were individually placed in closed alveolar containers containing cotton and stored at lab room temperature. NTT was a baseline group that did not receive any treatment, and that provided information on parasitoids that have emerged under natural conditions. Other 15 shoots per field were coated with wax and the leaves were removed to prevent wilting. Each shoot was wrapped in plastic film and stored at a controlled temperature (8°C) in a laboratory fridge for 7 days. This temperature is lower than the theoretical value at which ACGW stops their development but does not die; hence it is a stress factor [13]. These shoots were then repositioned on trees in the field where they were collected to evaluate the response of ACGW and parasitoids to cold stress (lab treatment). After one week, the shoots were collected and carried back to the laboratory, where 30 thermally treated (TT) galls were isolated in alveolar containers to allow for the emergence of ACGW adults and/or parasitoids. NTT and TT galls were kept in a walk-in laboratory room at temperatures ranging from 14º to 25°C. All galls were inspected weekly from May to September and every two weeks from October to April.

2.5 Emergence of the ACGW and its parasitoids

All emerged individuals from NTT and TT galls were separated, identified, and stored in alcohol. Identification was conducted using the following taxonomic keys: [27, 61–65]. Specimens were then compared with individuals identified by the Institute for Sustainable Plant Protection based on molecular protocols (COI, 28S, and ITS2) following [66] and [67].

Due to the difficulty of separating the cryptic species present in the *Torymus flavipes* Walker complex and some species of the genus *Eupelmus* [65,68], some analyses were performed based on aggregated data at the genus level for *Eupelmus* spp. and at the morphospecies level for *T. flavipes*. Voucher specimens have been deposited in the LEEA Laboratory (Laboratorio di Entomologia ed Ecologia Applicata), Università Mediterranea di Reggio Calabria, Italy.

2.6 Historical thermal data

To identify the frequency of the intervals in which thermal stress acts on ACGW and its parasitoids, historical thermal data were collected in the same area and altitude of fields studied for the twenty years (2000-2019) in Arpacal regional environmental monitoring station (see study area).

2.7 Statistical analysis

Data on the number of chambers in galls were tested for normality using Kolmogorov-Smirnov (K-S) tests ($P = 0.05$). The number of chambers in dissected galls in each year was compared using analysis of variance (ANOVA). The data were log transformed to meet the assumption of normality and model included chestnut fields ($n = 3$; A, B, or C), type of gall ($n = 2$; simple or compound, sensu [33], the ordinal direction ($n = 4$; NE, SE, SW, or NW) and chestnut fields - type of gall interaction as variables. We used a generalized linear model of binomial data (0 = chambers not parasitized; 1 = chambers parasitized) to compare differences in parasitism rates of dissected galls collected in each year. The following categorical variables were included in the model: chestnut field (A, B, or C), ordinal direction of galls (NE, SE, SW, or NW), and gall type (simple or compound). Lastly, the date of collection was added as a continuous variable. We restricted our analysis to the distinguishable stages of the gall wasp and its parasitoids.

The number of ACGWs and parasitoids emerging from NTT and TT galls was compared using a chi-squared (χ^2) test of the equality of distributions in each year. For species in the genus *Eupelmus*, another chi-squared (χ^2) test was used to assess the differences between species in the different treatment groups. The -2Log Likelihood value was used to assess the distribution of emerging adults according to the sampling date of the TT and NTT treatments. This last test was performed only for more significant species that emerged in this study.

The historical thermal data for the twenty years (2000-2019) of the monitoring station (n° 2465) was used to find the frequency of periods of at least 4 consecutive days in which the minimum daily temperature was less or equal than 8.5 °C. Historical thermal variations in the cold interval periods from April to August over the twenty years were analysed with the linear regression method.

We used SPSS v.23 [69] for all data analyses and [70] to produce graphs. All data are expressed as untransformed mean values ± standard error (SE).

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1 Field sampling and seasonal parasitism description

Overall, 7,423 chambers were recorded in galls (3,980 in 2017 and 3,443 in 2018). K-S test values were 0.135 in 2017 (P <0.001) and 0.139 in 2018 (P <0.001). The number of chambers per simple gall ranged between 1 and 18 in 2017 and 1 and 24 in 2018. The number of chambers per compound gall ranged between 2 and 37 in 2017 and 2 and 28 in 2018. The mean number of chambers was significantly higher in compound galls in both years (2017: simple =5.73 ± 0.25; n =174; compound = 11.71 ± 0.37, n = 256; 2018 simple = 6.03 ± 0,28; n =238, compound = 10.68 ± 0.39 n = 189) (Table 1). Therefore, in the second year of monitoring simple galls increased while compound galls decreased.

Table 1. Analysis of variance (ANOVA) evaluating the effect of different variables on the number of chambers in galls (Levene test; 2017: F= 1.19; df = 23, 405; P=0.25 - 2018: F= 1.12; df = 23, 404; P=0.32).

	Source	*Df*	*F*	*P*
2017	Intercept	1	5135.96	<0.001
	Gall type (Simple, Compound)	1	172.26	<0.001
	Chestnut field	2	13.55	<0.001
	Ordinal direction	3	0.89	0.45
	Gall type * chestnut field	2	6.30	0.02
2018	Intercept	1	3531.15	<0.001
	Gall type (Simple, Compound)	1	119.92	<0.001
	Chestnut field	2	2.06	0.13
	Ordinal direction	3	0.48	0.70
	Gall type * chestnut field	2	0.885	0.413

The ACGW parasitism rate was affected by several variables (Table 2). The parasitism rate varied between chestnut fields and sampling dates in each year but not between ordinal directions or gall typologies (Table 2). The lowest parasitism rate of galls was recorded during 2017 in all monitored fields (Table 3), with about half of hosts parasitized, whereas ratio was ≃ 0.8 in 2018 (Figure 1).

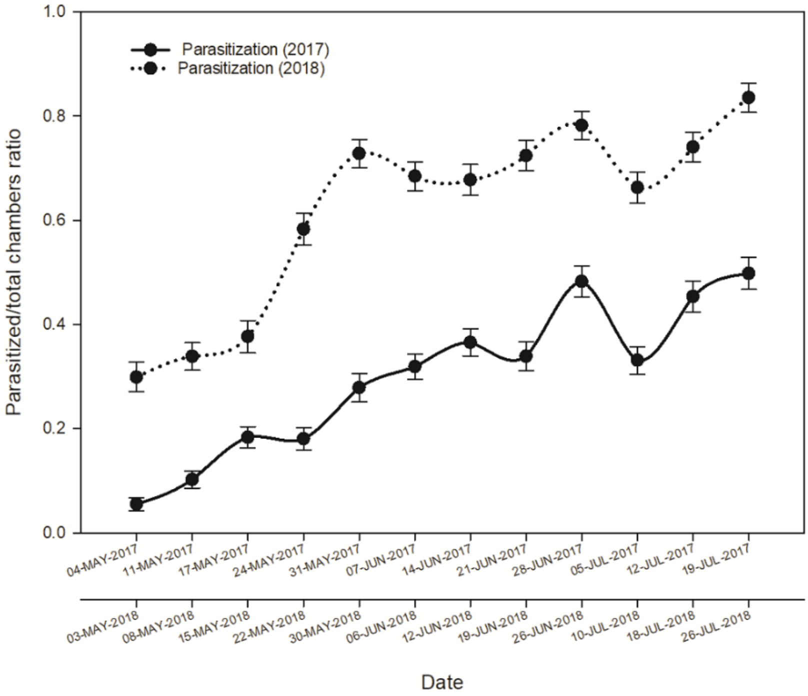


Figure 1. Proportion of chamber parasitism during monitoring periods in 2017 and 2018. The data collected each year in the three chestnut fields were pooled.

Table 2. Binomial generalized linear model (GLM) evaluating the effects of different variables on the parasitism rate (2017: n = 3,784; 2018: n = 3,053).

	Source	*Df*	**Wald chi-square**	*P*
2017	Intercept	1	296.93	<0.001
	Gall type (Simple, Compound)	1	0.88	0.349
	Chestnut field	2	162.75	<0.001
	Date	1	296.78	<0.001
	Collection site	4	2.49	0.647
2018	Intercept	1	244.93	<0.001
	Gall type (Simple, Compound)	1	0.87	0.350
	Chestnut Field	2	21.68	<0.001
	Date	1	244.98	<0.001
	Collection site	3	4.22	0.239

The parasitism rate was significantly higher in field C (coppice chestnut) than in fields A and B (orchard chestnut) in both sampling years (Table 3). Parasitism rates also differed between sampling time points (2017: first week = 0.055, last week = 0.498; 2018: first week = 0.299, last week = 0.835) (Figure 1).

Table 3. Parasitism rate in the chestnut fields during the monitoring period; n = total number of chambers.

	Year			
Chestnut field	2017		2018	
		n		n
A	0.18	1435	0.57	1031
B	0.33	1348	0.59	1048
C	0.40	1001	0.67	974

3.2 Cold stress experiment

The total number of emerging ACGWs and parasitoids differed between years, with a greater number of individuals recovered in 2017 compared to 2018 (2,941 vs 2,230). Chi-square tests revealed significant differences among the adults (ACGW and parasitoids) emerging from the TT and NTT treatment groups in both years (2017: $\chi^2_{(17,\ 2941)} = 598$, $P < 0.001$; 2018: $\chi^2_{(16,\ 2230)} = 234$, $P < 0.001$). The number of ACGW adults that emerged was lower in the TT treatment group (2017: NTT = 845, TT = 168; 2018: NTT = 182, TT = 80) (Figure 2). In 2017, the number of parasitoids was similar between the two groups (NTT = 984, TT = 974) but differed in 2018 (NTT = 1,213, TT = 755) (Figure 2).

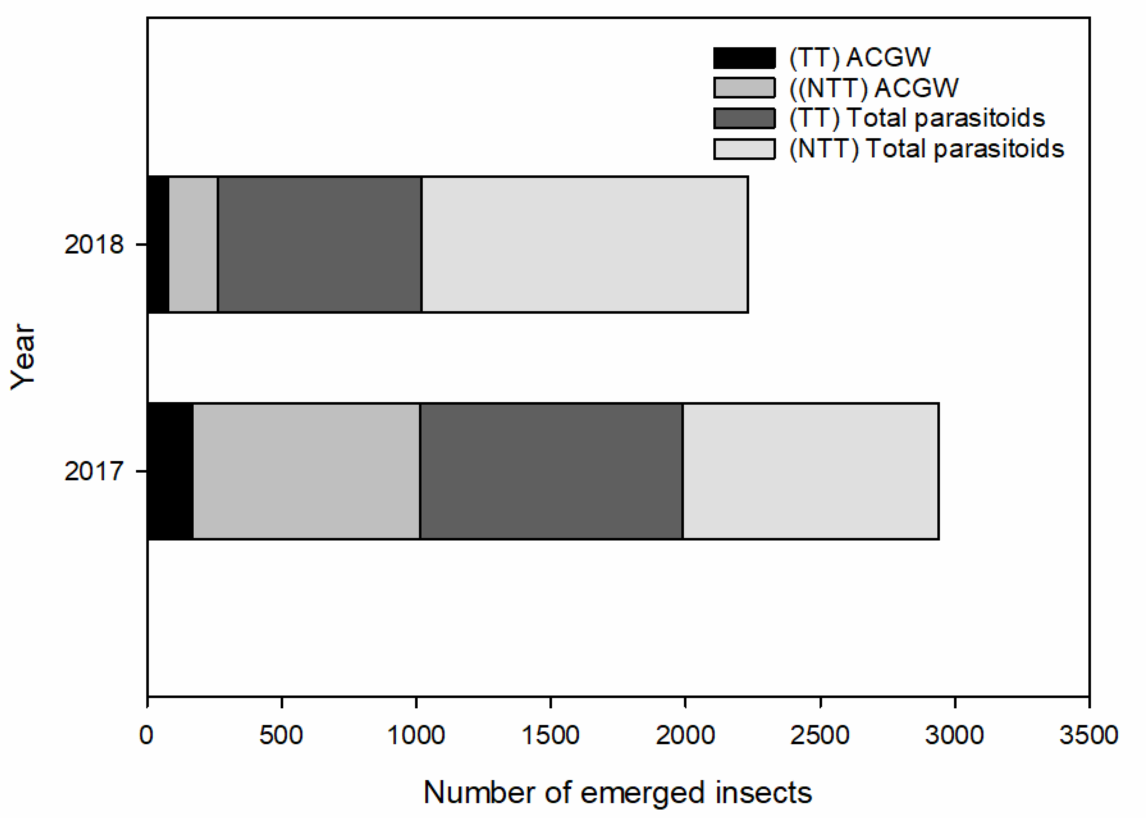


Figure 2. Box plots depicting the number of ACGWs and parasitoids in thermally treated galls (TT) and not thermally treated galls (NTT) collected in different years from different fields. See the text for experimental details. Data from the three examined chestnut fields were pooled.

3.3 Emergence of the ACGW and its parasitoids

A decrease in the number of parasitoids that emerged from TT galls was found for the following species: *Bootanomyia dorsalis* (F), *T. flavipes*, *Sycophila biguttata* (Swederus), and *Sycophila variegata* (Curtis, 1831) (Figure 3). In contrast, the number of emerged specimens belonging to the genus *Eupelmus* and *Mesopolobus tibialis* (Westwood) was two-fold higher in TT than in NT treatment groups (Figure 4), whereas the number of *Torymus auratus* (Geoffroy in Fourcroy, 1785) was similar between the two treatment groups (Figure 3). *Eupelmus* spp. were the most abundant specimens in all chestnut fields. Three species belonging to the genus *Eupelmus* were detected, although they were found to have different relative frequencies. In particular, the most abundant species was *Eupelmus azureus* Ratzeburg (76.4%), followed by *Eupelmus urozonus* Dalman (21.1%) and *Eupelmus kiefferi* De Stefani (2.5%). X^2 tests revealed significant differences in *Eupelmus* spp. between treatment groups only in 2017 (2017: $\chi^2_{(2,\ 433)} = 23.543$, $P < 0.001$; 2018: $\chi^2_{(2,\ 160)} = 2.91$, $P = 0.256$) and between chestnut fields in both years (2017: $\chi^2_{(4,\ 433)} = 14.87.354$, $P = 0.005$; 2018: $\chi^2_{(4,\ 160)} = 9.64$, $P = 0.047$) (Figure A1). The number of observed *E. azureus* adults was higher than expected in the TT treatment group (316 vs 299) and lower in the NTT treatment group (137 vs 154). The comparison between the distributions of adults that emerged in relation to the monitoring date highlighted a difference between TT and NTT ($p < 0.001$) (Figure A2).

Both the increase in the frequency of (*Eupelmus* sp. and *M. tibialis*) and the decrease observed for some species including *B. dorsalis*, were not concentrated on some samples but were observed during the entire sampling period.

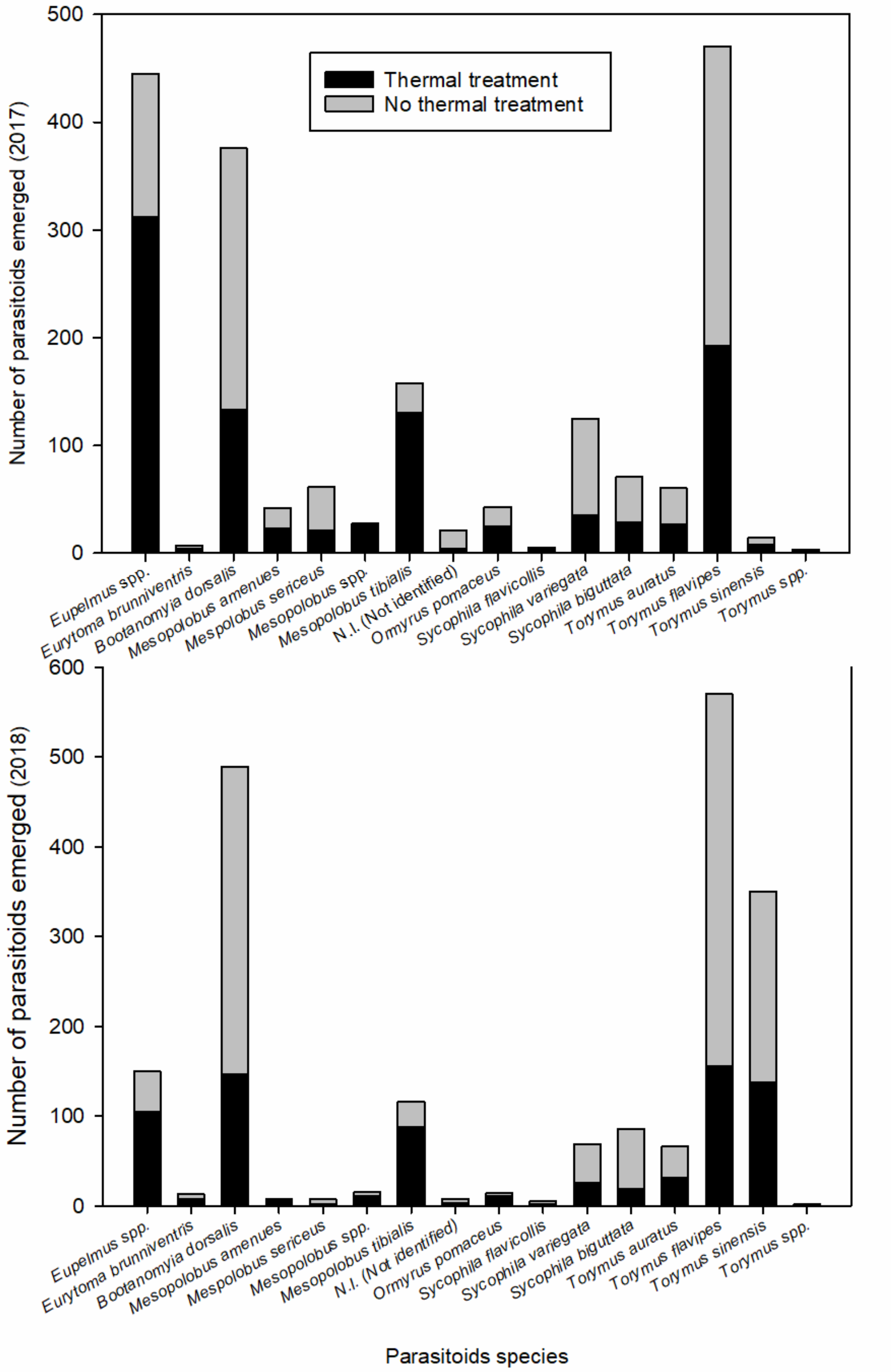


Figure 3. Parasitoid species emerged from galls in different treatment groups from the three chestnut fields (A, B, and C). The data from the three fields were pooled by year.

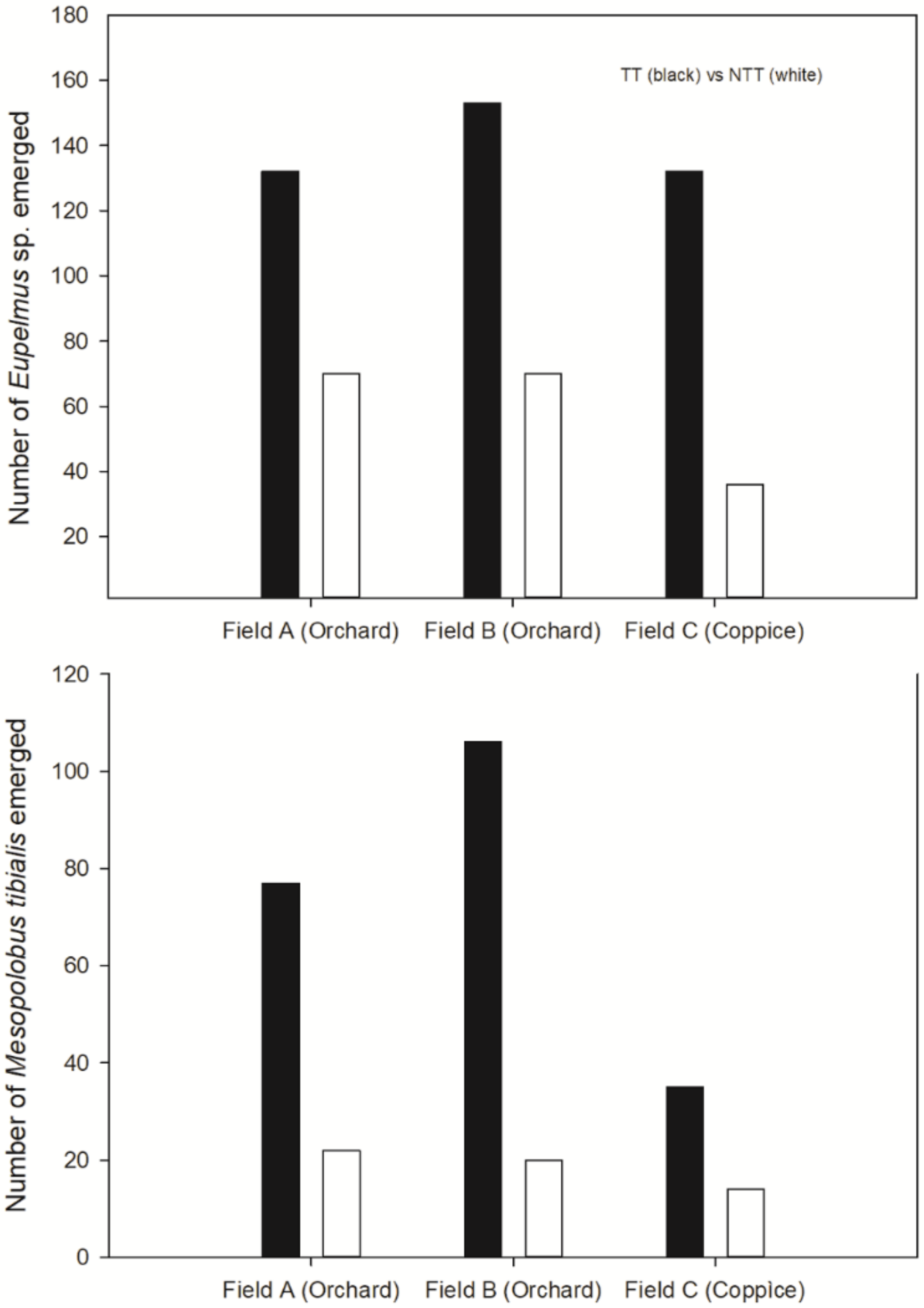


Figure 4. *Eupelmus* spp. and *Mesopolobus tibialis* emerged from galls collected from three chestnut fields in thermally treated galls (TT) and not thermally treated galls (NTT). Data from the two years were pooled.

3.4 Historical thermal data

Historical thermal data of the study area showed that the time intervals of at least 4 days with minimum daily temperatures of 8.5°C or less mainly occurred between the months of May and June (69 and 11 intervals). In the twenty years analyzed, only shorter intervals (1-3 days) were detected in July at temperatures close to 8.5°C (Figure. A3, A4). Time intervals with minimum daily temperatures lower than or equal to 8.5°C for at least 4 consecutive days are increasingly frequent in the month of May (R square 0.569; df = 2.18; P <0.001; Figure S3, S4).

4. Discussion

In our experimental approach, we simulated thermal stress events at low temperatures, which caused a variation in the ratio of parasitoids species. The analysis of temperatures of our study area over the past twenty years highlighted that cold snaps are occurring more frequently in May. Unpredictable cold waves have a direct and more easily verifiable effect on plants. [71] highlighted that, despite an increase in the mean annual temperature, unpredictable cold waves may retard the expansion of plants towards higher altitudes and latitudes.

We have shown that parasitism and mortality of ACGW in three chestnut fields were affected by the cold treatment. The ACGW population was smaller in 2018 than in 2017, and this was linked with higher parasitism rates in galls. The parasitism rate varied throughout single seasons (from May to July), and different mean values of parasitism were found between years and between chestnut fields. In particular, higher parasitism rates were found in the coppice field, which is consistent with previous results from studies in the same chestnut fields (2013–2015) [18].

T. sinensis was introduced to the study area in 2014, but not in the experimental fields, and just recently it is becoming the dominant parasitoid (Bonsignore, unpublished data). The higher parasitism rates recorded in 2018 were associated with a strong increase in *T. sinensis*, which, however, resulted very variable in the different samplings indicating a non-uniform distribution on the trees and hence-that dynamic parasitoid adaptation was still in progress.

This proliferation is a clear indication of adaptation of this species to ACGW in the study area and confirms results recorded in Italy and other European countries [54,56,57,72–74].

The cold treatment did not affect the composition of the parasitoid community in any chestnut field, which is congruent with what reported by [75], who did not found differences in diversity, richness, or evenness of ACGW-associated species despite differences in their habitat. Conversely, [76] showed that native parasitoid communities emerging from galls differed between pure and mixed chestnut stands at the same altitude, even if there was no difference in their abundance.

Our results revealed species-specific differences in the abundance and performance of parasitoids associated with ACGW in response to cold stress. In particular, the frequency of *Eupelmus* spp. and *M. tibialis* doubled in the cold treatment in all three chestnut fields in both years. Typically, *M. tibialis* parasitizes ACGW in early May (see Figure A2) and completes its development quickly. In contrast, *Eupelmus* spp. females are active later in June and are usually the last parasitoids to emerge. The high proportion of specimens belonging to the genus *Eupelmus* recorded at all sample sites (TT) may be related to the ability of *Eupelmus* spp. to develop as hyper-parasitoids [20,77]. However, the higher presence of *Eupelmus* spp. could also be linked to variations in emissions of herbivore-induced plant volatiles in response to thermal stress [78].

The three detected species of *Eupelmus* were present in all fields, but while the frequency of *E. azureus* increased after thermal stress treatment, *E. urozonus* and *E. kiefferi* were less affected. The increase of *E. azureus* can be related either to the greater ability of this species to develop also on decaying hosts (a reduction in the emergence and therefore an increase in ACGW mortality was observed in 2018) or to better resistance to cold stress. The influence of temperature on both ACGW and its parasitoids was also highlighted in a previous study, although in that case, the differences in the temperatures occurred in fields at different altitudes [13].

Thermal stress, although limited to one week, prolonged the development of the juvenile stages of ACGW and its parasitoids inside galls and delayed the emergence of parasitoids. This prolonged phenology may have ensured a wider temporal window for parasitism by some parasitoid species. Furthermore, re-exposure of galls in trees could have caused excessive drying and possibly resulted in the emission of plant volatile substances that are attractive to *Eupelmus* spp. and *M. tibialis*, as well as the variations in the water content of the galls, could have affected parasitoid success.

Thermal stress negatively affected the abundance of *T. flavipes*, *T. sinensis*, *S. biguttata*, and *Ormyrus pomaceus* (Geoffroy). Interestingly, all of these species can develop in the same larval stages of ACGW as *M. tibialis*. Furthermore, our results confirmed that some parasitoids, such as *Torymus auratus* (Geoffroy in Fourcroy, 1785) and *B. dorsalis*, prefer to attack later during pupal formation.

These parasitoid species, along with *S. variegata* (Curtis), were the last to emerge during the last few days of June and throughout July [42] and their number was also reduced by thermal stress.

5. Conclusions

In conclusion, the present study contributes to our understanding of the thermic stress on insect growth and host stage succession, and provides information on parasitoid adaptation to new host phenologies. A new host can share parasitoids that already exist in the area, but the processes that influence their adaptation are far more complex than the simultaneous presence of the adult parasitoid and the susceptible stage of the host. Therefore, this research focused on variations that occur in the parasitoid community due to simple phenological variation and stress conditions. We have shown that exposure of galls to low temperatures affects the structure of the parasitoid community. Understanding which processes affect parasitoid community structure is fundamental, particularly when a phytophagous insect shifts towards a new host, as occurred with ACGW and *C. sativa*.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Graphical abstract; Figure S1, Figure S2, Figure S3, Figure S4.

Author Contributions: CPB conceived and designed the research. CPB, GV1, GV2, UB conducted the experiments. CPB and UB analyzed the data. CPB, UB, GV1, GV2, wrote the manuscript. All authors read and approved the manuscript.

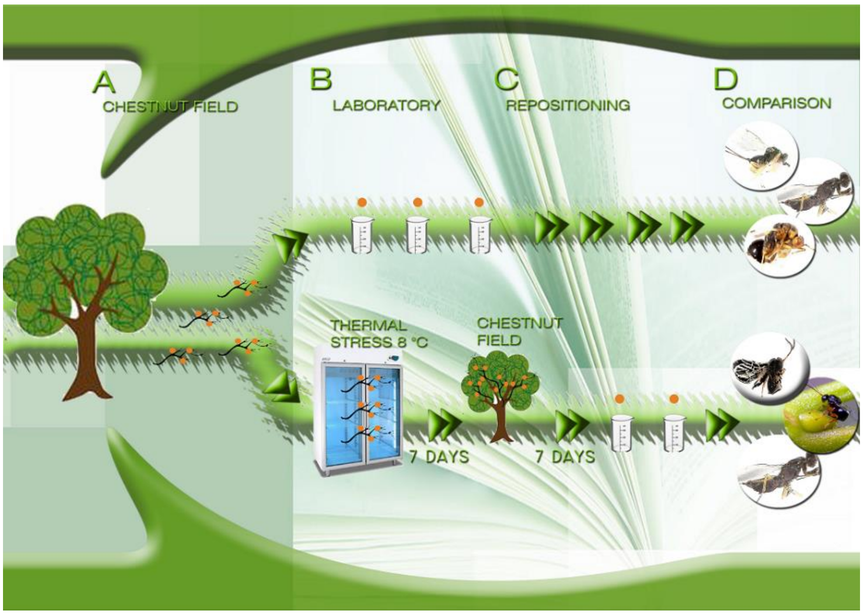
Funding: This research was partly funded by Aspromonte National Park N. 451 (The Asian cynipid gall wasp (*Dryocosmus kuriphilus* Yasumatsu) in the National Park of Aspromonte: Study on adaptation to new environments and ecological response of native natural enemies) assigned to C. Bonsignore (Reggio Calabria).

Acknowledgments: The authors thank Fabio Anello for all drawings in the graphical abstract, Marco Gebiola for critically reviewing the manuscript. We thank Aspromonte National Park and Antonio Siclari for administrative and technical support furnished to research activity.

Conflicts of Interest: There are no conflicts of interest associated with this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results

Appendix A

Data supplemental to "Short-term thermal changes affect parasitism on Asian chestnut gall wasp"



Graphical Abstract. We examined the effect of thermal cold stress on *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) and its parasitoids. We found that cold treatment affected parasitism, resulting in a general reduction in emerging ACGWs and parasitoids under field conditions and differing ratios of parasitoid species compared to untreated galls. A striking example was the relative frequency of *Eupelmus* spp. and *Mesopolobus tibialis*, which doubled in thermally-stressed galls. Experimental design. An overview of the experimental setup is illustrated (not drawn to scale). Galled shoots were stressed at 8°C for 7 days and placed back into their chestnut field of origin (below) to compare insect emergence with that of non-thermally-treated galls (above).

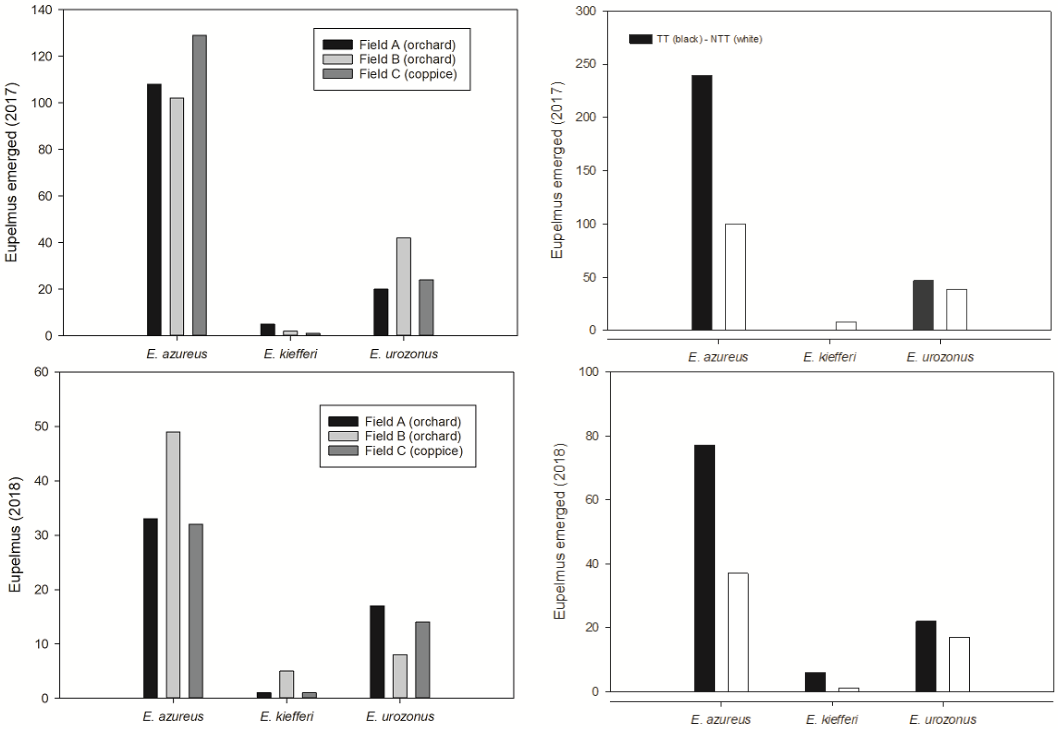
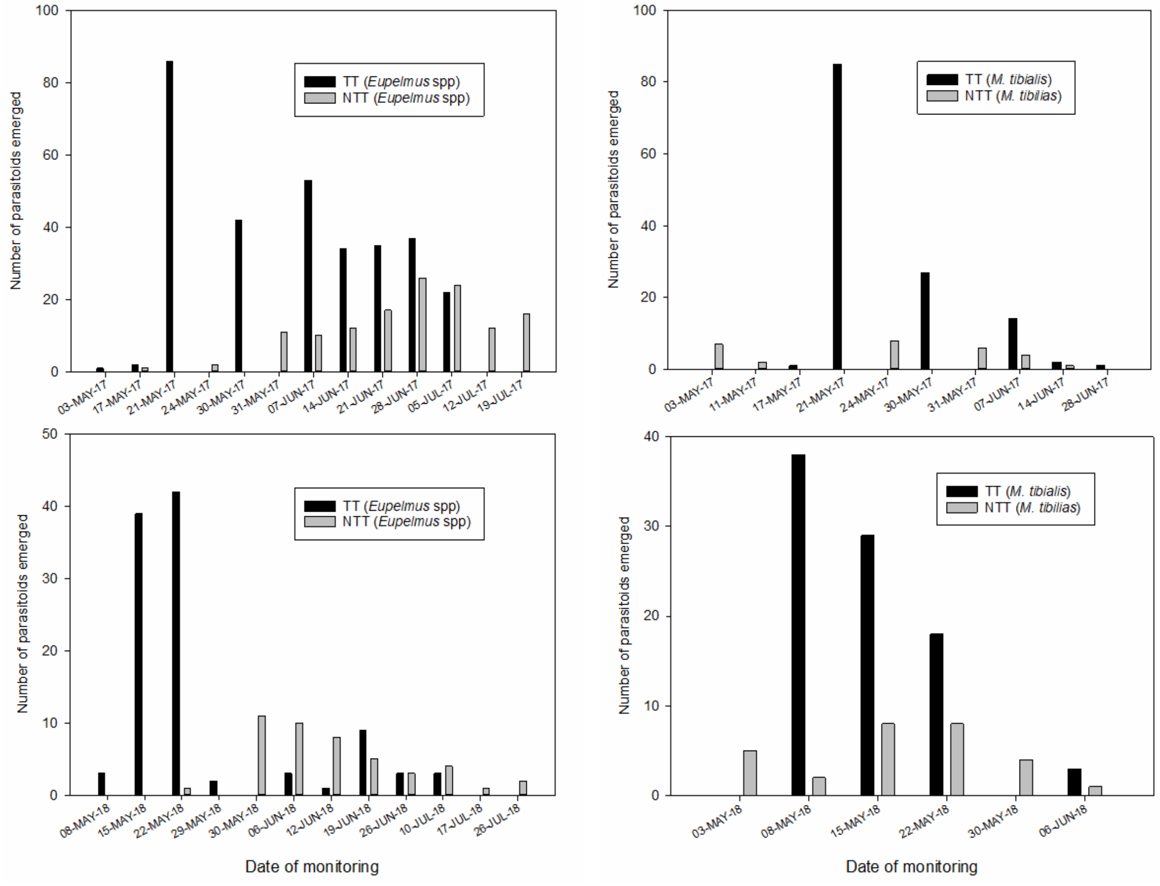


Fig. S1. Relationship among *Eupelmus* species at different chestnut fields (two orchards and one coppice) (left) and at different treatments (NTT = No thermal treatment, TT = thermal treatment).


Number of parasitoids emerged
TT (Eupelmus spp)
NTT (Eupelmus spp)
03-MAY-17
17-MAY-17
21-MAY-17
24-MAY-17
30-MAY-17
31-MAY-17
07-JUN-17
14-JUN-17
21-JUN-17
28-JUN-17
05-JUL-17
12-JUL-17
19-JUL-17
TT (M. tibialis)
NTT (M. tibilias)
03-MAY-17
11-MAY-17
17-MAY-17
21-MAY-17
24-MAY-17
30-MAY-17
31-MAY-17
07-JUN-17
14-JUN-17
28-JUN-17
TT (Eupelmus spp)
NTT (Eupelmus spp)
08-MAY-18
15-MAY-18
22-MAY-18
29-MAY-18
30-MAY-18
06-JUN-18
12-JUN-18
19-JUN-18
26-JUN-18
10-JUL-18
17-JUL-18
26-JUL-18
Date of monitoring
TT (M. tibialis)
NTT (M. tibilias)
03-MAY-18
08-MAY-18
15-MAY-18
22-MAY-18
30-MAY-18
06-JUN-18
Date of monitoring

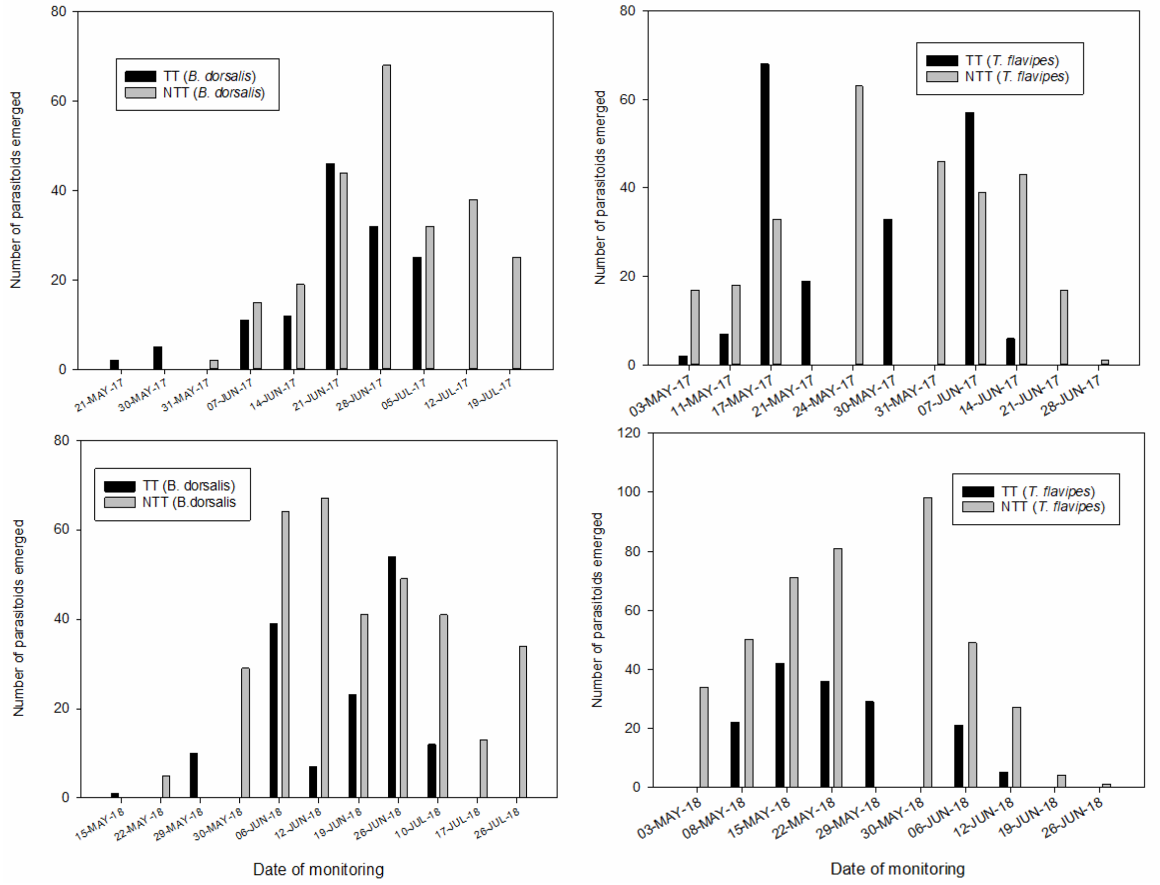

Number of parasitoids emerged
TT (B. dorsalis)
NTT (B. dorsalis)
21-MAY-17
30-MAY-17
31-MAY-17
07-JUN-17
14-JUN-17
21-JUN-17
28-JUN-17
05-JUL-17
12-JUL-17
19-JUL-17
TT (T. flavipes)
NTT (T. flavipes)
03-MAY-17
11-MAY-17
17-MAY-17
21-MAY-17
24-MAY-17
30-MAY-17
31-MAY-17
07-JUN-17
14-JUN-17
21-JUN-17
28-JUN-17
TT (B. dorsalis)
NTT (B.dorsalis
15-MAY-18
22-MAY-18
29-MAY-18
30-MAY-18
06-JUN-18
12-JUN-18
19-JUN-18
26-JUN-18
10-JUL-18
17-JUL-18
26-JUL-18
Date of monitoring
TT (T. flavipes)
NTT (T. flavipes)
03-MAY-18
08-MAY-18
15-MAY-18
22-MAY-18
29-MAY-18
30-MAY-18
06-JUN-18
12-JUN-18
19-JUN-18
26-JUN-18
Date of monitoring

Fig. S2. Relationship among date of monitoring of the galls and some emerged parasitoids. *Eupelmus* sp (2017: log.Lik$_{12,443}$ = 173.44, P < 0.001; 2018: log.Lik $_{11,150}$ = 99.52, P < 0.001); *Mesopolobus tibialis* (2017: log.Lik $_{9,158}$ = 132.10, P < 0.001; 2018: log.Lik $_{5,116}$ = 37.04, P < 0.001); *Bootanomyia dorsalis* (2017: log.Lik $_{9,376}$ = 61.10, P < 0.001; 2018: log.Lik $_{10,489}$ = 105.68, P < 0.001); *Torymus flavipes* (2017: log.Lik $_{9,158}$ = 124.73, P < 0.001; 2018: log.Lik $_{5,116}$ = 37.10, P < 0.001) are compared by way of example (NTT = No thermal treatment, TT = thermal treatment).

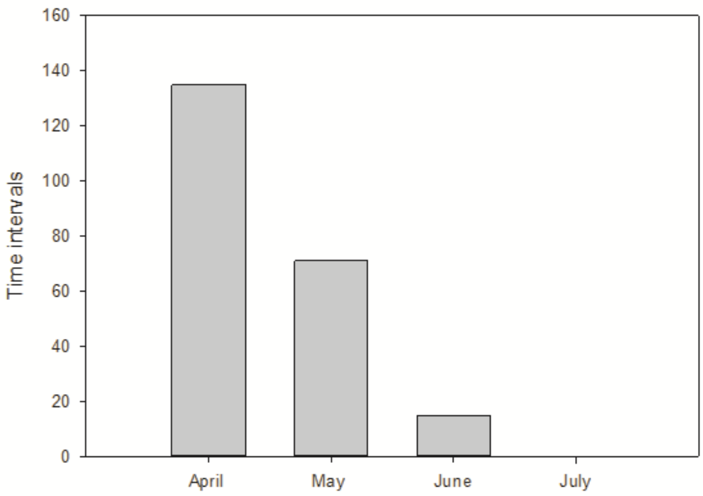


Fig. S3. Relationship among monitoring months and intervals time with daily minimum temperature lower or equal to 8.5 degree for 4 consecutive days (the data refers to twenty years, 2000 - 2019). Historical data was collected at meteorological station of the monitoring

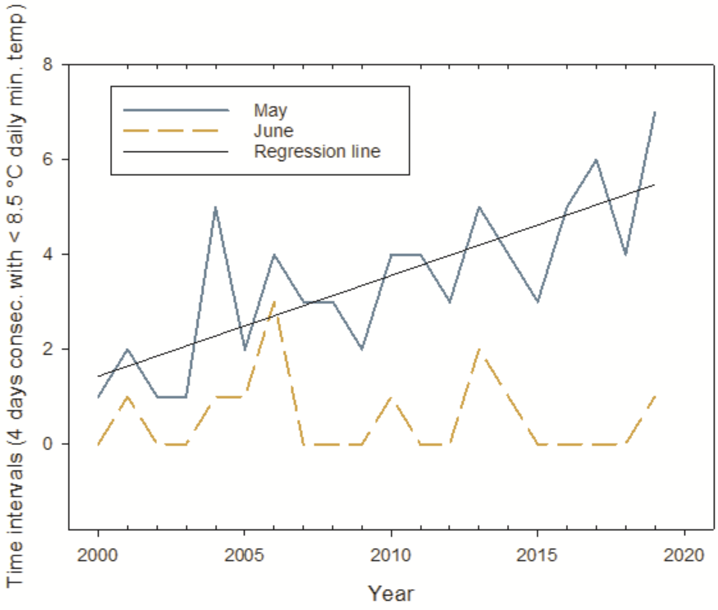


Fig. S4. Relationship among years (May and June) and intervals time with daily minimum temperature lower or equal to 8.5 °C for 4 consecutive days (the data refer to twenty years, 2000 - 2019). Historical data was collected at meteorological station of the monitoring (n° 2465). Regression line on May (f = y0+a*x; R square 0.569; df= 2,18; P <0.001).

References

1. Dietz, H.; von Arx, G. Climatic fluctuation causes large scale synchronous variation in radial root increments of perennial forbs. Ecology **2005**; 86, 327–333. DOI: 10.1890/04-0801
2. Reusch, T.B.H.; Ehlers, A.; Hämmerli, A.; Worm, B. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. P. Natl. Acad. Sci. USA **2005**; 102, 2826–2831. DOI: 10.1073/pnas.0500008102
3. Rigby, J.R.; Porporato, A. Spring frost risk in a changing climate. Geophys. Res. Lett. **2008**; 35, L12703. DOI: 10.1029/2008GL033955
4. Sinclair, B.J.; Addo-Bediako, A.; Chown, S. Climatic variability and the evolution of insect freeze tolerance. Biol. Rev. Camb. Philos. Soc. **2003**; 78, 181–195. DOI: 10.1017/S1464793102006024
5. Blackburn, D.G. Evolution of viviparity in squamate reptiles: Reversibility reconsidered. J. Exp. Zool. Part B **2015**; 324, 473–486. DOI: 10.1002/jez.b.22625
6. Klapwijk, M.J.; Grobler, B.C.; Ward, K.; Wheeler, D.; Lewis, O.T. Influence of experimental warming and shading on host-parasitoid synchrony. Global Change Biol. **2010**; 16, 102–112. DOI: 10.1111/j.1365-2486.2009.01918.x
7. Chen, C.; Gols, R.; Biere, A.; Harve, J.A. Differential effects of climate warming on reproduction and functional responses on insects in the fourth trophic level. Funct. Ecol. **2019**; 33, 693–702. doi.org/10.1111/1365-2435.13277
8. Tougeron, K.; Brodeur, J.; Le Lann, C.; Baaren, J. How climate change affects the seasonal ecology of insect parasitoids. Ecol. Entomol. **2020**; 45, 167–181. DOI: 10.1111/een.12792
9. Tylianakis, J.M.; Didham, R.K.; Bascompte, J.; Wardle, D.A. Global change and species interactions in terrestrial ecosystems. Ecol. Lett. **2008**; 11, 1351–1363. DOI: 10.1111/j.1461-0248.2008.01250.x
10. Renner, S.S.; Zohner, C.M. Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. Annu. Rev. Ecol. Evol. S. **2018**; 49, 165–182. doi: 10.1146/annurev-ecolsys-110617-062535.
11. Zhang, C.; McPhaden, M.J. Intraseasonal surface cooling in the equatorial western Pacific. J Climate **2000**; 13, 2261–2276. DOI: 10.1175/1520-0442(2000)013
12. Abarca, M.; Lill, J.T. Latitudinal variation in the phenological responses of eastern tent caterpillars and their egg parasitoids. Ecol. Entomol. **2019**; 44, 50–61. doi.org/10.1111/een.12673
13. Bonsignore, C.P.; Vono, G.; Bernardo, U. Environmental thermal levels affect the phenological relationships between the chestnut gall wasp and its parasitoids. Physiol. Entomol. **2019**; 44, 87–98. DOI: 10.1111/phen.12280
14. Gilman, S.E.; Urban, M.C.; Tewksbury, J.; Gilchrist, G.W.; Holt, R.D. A framework for community interactions under climate change. Trends in Ecol. Evol. **2010**; 25, 325–331. DOI: 10.1016/j.tree.2010.03.002
15. Plantard, O.; Rasplus, J.W.; Hochberg, M.E. Resource partitioning in the parasitoid assemblage of the oak galler *Neuroterus quercusbaccarum* (L.) (Hymenoptera: Cynipidae). Acta Oecol. **1996**; 17, 1–15.
16. van der Putten, W.H.; Macel, M.; Visser, M. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philos. Trans. R. Soc. London B **2010**; 365, 2025–2034. DOI: 10.1098/rstb.2010.0037
17. Johnson, C.A.; Coutinhoc, R.M.; Berlin, E.; Dolphin, K.E.; Heyer, J.; Kim, B.; Leung, A.; Sabellon, J.L.; Amarasekare, P. Effects of temperature and resource variation on insect population dynamics: the bordered plant bug as a case study. Funct. Ecology **2016**; 30, 1122–1131. DOI: 10.1111/1365-2435.12583
18. Bonsignore, C.P.; Bernardo, U. Effects of environmental parameters on the chestnut gall wasp and its complex of indigenous parasitoids. Sci. Nat. **2018**; 105, 20. doi.org/10.1007/s00114-018-1545-1
19. Torii, T. Studies on the biological control of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hym. Cynipidae), with particular reference to the utilization of its indigenous natural enemies. J. Fac. Agric. Shinshu Univ. **1959**; 2, 71–149.
20. Aebi, A.; Schonrogge, K.; Melika, G.; Quacchia, A.; Alma, A.; Stone, G.N. Native and introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*. EPPO Bulletin **2007**; 37, 166–171.
21. Abe, Y.; Melika, G.; Stone, G.N. The diversity and phylogeography of cynipid gall wasps (Hymenoptera, Cynipidae) of the eastern Palaearctic and their associated communities. Orient. Insects **2007**; 41, 196–212. doi.org/10.1080/00305316.2007.10417504
22. Fan, B.; Zhu, D.H. Molecular phylogenetic relationships of *Dryocosmus kuriphilus* Yasumatsu of different geographical populations based on partial sequences of 16S rRNA gene. J. Hunan Univ. Nat. Sci. **2010**; 33, 78–82.

23. Battisti, A.; Benvegnù, I.; Colombari, F.; Haack, R.A. Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agr. Forest Entomol. **2014**; 16, 75–79. DOI: 10.1111/afe.12036
24. Gehring, E.; Bellosi, B.; Quacchia, A.; Conedera, M. Evaluating *Dryocosmus kuriphilus*-induced damage on *Castanea Sativa*. Jove-J. Vis. Experiments **2018**; 138, e57564. DOI: 10.3791/57564.
25. Yasumatsu, K. A new *Dryocosmus* injurious to chestnut trees in Japan (Hym., Cynipidae). Mushi **1951**; 22, 89–93.
26. Askew, R.R.; Plantard, O.; Gómez, J.F.; Nieves, M.H.; Nieves-Aldrey, J.L. Catalogue of parasitoids and inquilines in galls of Aylacini, Diplolepidini and Pediaspidini (Hym., Cynipidae) in the West Palaearctic, Zootaxa, **2006**; 1301, 60. DOI: 10.11646/zootaxa.1301.1.1
27. Askew, R.R.; Melika, G.; Pujade-Villar, J.; Schoenrogge, K.; Stone, G.N.; Nieves-Aldrey, J.L. Catalogue of parasitoids and inquilines in cynipid oak galls in the West Palaearctic. Zootaxa **2013**; 3643, 1–133. doi.org/10.11646/zootaxa.3643.1.1
28. Ferracini, C.; Bertolino, S.; Bernardo, U.; Bonsignore, C.P.; Faccoli, M.; Ferrari, E.; Lupi, D.; Maini, S.; Mazzone, L.; Nugnes, F.; Rocco, A.; Santi, F.; Tavella, L. Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp? Biol. Control **2018**; 121, 36–43. doi:10.1016/j.biocontrol.2018.01.009
29. Palmeri, V.; Cascone, P.; Campolo, O.; Grande, S.B.; Laudani, F.; Malacrinò, A.; Guerrieri, E. Hymenoptera wasps associated with the Asian gall wasp of chestnut (*Dryocosmus kuriphilus*) in Calabria, Italy. Phytoparasitica **2014**; 42, 699–702. DOI: 10.1007/s12600-014-0411-8
30. Cooper, W.R.; Rieske, L.K. Gall structure affects ecological associations of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). Environ. Entomol. **2010**; 39, 787–797. DOI: 10.1603/EN09382
31. Gehring, E.; Kast, C.; Kilchenmann, V.; Bieri, K.; Gehrig, R.; Pezzatti, G.B.; Conedera, M. Impact of the Asian Chestnut Gall Wasp, *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae), on the chestnut component of honey in the Southern Swiss Alps. J. Econ. Entomol. **2018**; 111, 43–52. DOI: 10.1093/jee/tox338
32. Zhu, D.; He, Y.; Fan, Y.; Ma, M.; Peng, D. Negative evidence of parthenogenesis induction by *Wolbachia* in a gallwasp species, *Dryocosmus kuriphilus*. Entomol. Exp. appl. **2007**; 124, 279–284. DOI: 10.1111/j.1570-7458.2007.00578.x
33. Bernardo, U.; Iodice, L.; Sasso, R.; Tutore, V.A.; Cascone, P.; Guerrieri, E. Biology and monitoring of *Dryocosmus kuriphilus* on *Castanea sativa* in Southern Italy. Agr. Forest Entomol. **2013**; 15, 65–76. DOI: 10.1111/j.1461-9563.2012.00588.x
34. Nugnes, F.; Gualtieri, L.; Bonsignore, C.P.; Parillo, R.; Annarumma, R.; Griffo, R.; Bernardo, U. Resistance of a local ecotype of *Castanea sativa* to *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in Southern Italy. Forests **2018**; 9, 94. DOI: 10.3390/f9020094
35. Viggiani, G.; Nugnes, F. Description of the larval stages of *Dryokosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), with notes on their phenology. J. Entomol. Acarol. Res. **2010**; II 42, 39–45. DOI: 10.4081/jear.2010.39
36. Reale, L.; Tedeschini, E.; Rondoni, G.; Ricci, C.; Bin, F.; Frenguelli, G.; Ferranti, F. Histological investigation on gall development induced by a worldwide invasive pest, *Dryocosmus kuriphilus*, on *Castanea sativa*. Plant Biosyst. **2016**; 150, 35–42. DOI: 10.1080/11263504.2014.918058
37. EPPO *Dryocosmus kuriphilus*. EPPO Bulletin **2005**; 35, 422–424.
38. Gil-Tapetado, D.; Cabrero-Sañudo, F.; Polidori, C.; Gómez, J.; Nieves-Aldrey, J. Climate as a possible driver of gall morphology in the chestnut pest *Dryocosmus kuriphilus* across Spanish invaded areas. B. Entomol. Res. **2020**;1-14. DOI: 10.1017/S0007485320000450
39. EPPO New data on quarantine pests and pests of the EPPO Alert List. EPPO Reporting Service **2018**; 8.
40. Matošević, D.; Melika, G. Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia. B. Insectol. **2013**; 66, 231–238.
41. Quacchia, A.; Ferracini, C.; Nicholls, J.A.; Piazza, E.; Saladini, M.A.; Tota, F.; Melika, G.; Alma, A. Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north-western Italy. Insect Conserv. Diver. **2013**; 6, 114–123. DOI: 10.1111/j.1752-4598.2012.00192.x
42. Panzavolta, T.; Croci, F.; Bracalini, M.; Melika, G.; Benedettelli, S.; Tellini Florenzano, G.; Tiberi, R. Population dynamics of native parasitoids associated with the Asian Chestnut Gall Wasp (*Dryocosmus kuriphilus*) in Italy. Psyche **2018**, 2018, 1–13.
43. Dorado, J.F.; Pujade-Villar, J.; Muñoz-Adalia, E.J.; Vinagrero, J.C.; Diez-Casero, J.J.; Fernández-Fernández, M.M. Characterization of native parasitoid community associated with the invasive pest *Dryocosmus*

kuriphilus (Hymenoptera: Cynipidae) in Cantabria (northern Spain). Scand. J. Forest Res. **2020**; DOI: 10.1080/02827581.2020.1808055

44. Budroni, M.A.; Loru, L.; Pantaleoni, R.A.; Rustici, M. Effects of an asynchronous alien host on a native host-parasitoid system. Ecol. Complexity **2018**; 33, 84–92. doi.org/10.1016/j.ecocom.2018.01.001
45. Gil-Tapetado, D.; Gómez, J.F.; Cabrero-Sañudo, F.J.; Nieves-Aldrey, J.L. Distribution and dispersal of the invasive Asian chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), across the heterogeneous landscape of the Iberian Peninsula. Eur. J. Entomol. **2018**; 115, 575–586. DOI: 10.14411/eje.2018.055
46. Bailey, R.; Schönrogge, K.; Cook, J.M.; Melika, G.; Csóka, G.; Thuróczy, C.; et al. Host niches and defensive extended phenotypes structure parasitoid wasp communities. PLoS Biol. **2019**; 7(8), e1000179. doi.org/10.1371/journal.pbio.1000179
47. Cooper, W.R.; Rieske, L.K. A native and an introduced parasitoid utilize an exotic gall-maker host. BioControl, **2011**; 56, 725–734. DOI: 10.1007/s10526-011-9350-1
48. Cornell, H.V.; Hawkins, B.A. Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders. Am. Nat. **1993**; 141, 847–865. DOI: 10.1086/285512
49. Murakami, Y.; Ao, H.B.; Chang, C.H. Natural enemies of the chestnut gall wasp in Hopei Province, China (Hymenoptera: Chalcidoidea). Appl. Entomol. Zool. **1980**; 15, 184–186. doi.org/10.1303/aez.15.184
50. Murakami, Y.; Toda, S.; Gyoutoku, Y. Colonization of imported *Torymus* (Syntomaspis) *sinensis* Kamijo (Hymenoptera: Torymidae) parasitic on the chestnut gall wasp (Hymenoptera: Cynipidae). Success in the eighteenth year after release in Kumamoto. Proc. Ass. Plant Protec. Kyush **2001**; 47, 132–134. doi.org/10.4241/ kyubyochu.47.132
51. Moriya, S.; Inoue, K.; Ôtake, A.; Shiga, M.; Mabuchi, M. Decline of the chestnut gall wasp population, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) after the establishment of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae). Appl. Entomol. Zool. **1989**; 24, 231–233. doi. org/10.1303/aez.24.231
52. Borowiec, N.; Thaon, M.; Brancaccio, L.; et al. Classical biological control against the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in France. Plant Prot. Q. **2014**; 29, 9–12.
53. Matošević, D.; Lacković, N.; Melika, G. et al. Biological control of invasive *Dryocosmus kuriphilus* with introduced parasitoid *Torymus sinensis* in Croatia, Slovenia and Hungary. Period. Biol. **2015**; 117, 471–477. DOI: 10.18054/pb.2015.117.4.3445
54. Pérez-Otero, R.; Crespo, D.; Mansilla, J.P. *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) in Galicia (NW Spain): pest dispersion, associated parasitoids and first biological control attempts. Arquivos Entomolóxicos **2017**; 17, 439–448.
55. Gehring, E.; Bellosi, B.; Reynaud, N.; Conedera, M. Chestnut tree damage evolution due to *Dryocosmus kuriphilus* attacks. J. Pest Sci. **2019**; doi.org/10.1007/s10340-019-01146-0
56. Avtzis, D.N.; Melika, G.; Matošević, D.; Coyle, D.R. The Asian chestnut gall wasp *Dryocosmus kuriphilus*: a global invader and a successful case of classical biological control. J Pest Sci. **2019**; 92, 107–115. doi.org/10.1007/s10340-018-1046-1
57. Ferracini, C.; Ferrari, E.; Pontini, M.; Saladini, M.A.; Alma, A. Effectiveness of *Torymus sinensis*: a successful long-term control of the Asian chestnut gall wasp in Italy. J. Pest Sci. **2019**; 92, 353–359. doi.org/10.1007/s10340-018-0989-6
58. Yara, K.; Yano, E.; Sasawaki, T.; Shiga, M. Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera: Torymidae) in central Japan, using malic enzyme. Appl. Entomol. Zool. **2000**; 35, 201–206. DOI: 10.1303/aez.2000.201
59. Ferracini, C.; Ferrari, E.; Saladini, M.A.; Pontini, M.; Corradetti, M.; Alma, A. Non-target host risk assessment for the parasitoid *Torymus sinensis*. BioControl **2015**; 60, 583–594. DOI: 10.1007/s10526-015-9676-1
60. Ferracini, C.; Ferrari, E.; Pontini, M.; Hernández Nova, L.K.; Saladini, M.A.; Alma, A. Post-release evaluation of non-target effects of *Torymus sinensis*, the biological control agent of *Dryocosmus kuriphilus* in Italy. BioControl **2017**; 62, 445–456. doi.org/10.1007/s10526017-9803-2
61. Askew, R.R. A study of the biology of species of the genus *Mesopolobus* Westwood (Hymenoptera: Pteromalidae) associated with cynipid galls on oak. T. Roy. Ent. Soc. London **1961**; 113, 155–173.
62. de Vere Graham, M.W.R.; Gijswijt, M.J. Revision of the European species of *Torymus* Dalman (s. Lat.) (Hymenoptera: Torymidae). Zool. Verh. **1998**; 317, 1–202.

63. Askew, R.R.; Nieves-Aldrey, J.L. The genus *Eupelmus* Dalman, 1820 (Hymenoptera, Chalcidoidea, Eupelmidae) in peninsular Spain and the Canary Islands, with taxonomic notes and descriptions of new species. Graellsia **2000**; 56, 49–61. doi.org/10.3989/graellsia.2000.v56.i0.309
64. Roques, A.; Skrzypczyńska, M.) Seed-infesting chalcids of the genus *Megastigmus* Dalman, 1820 (Hymenoptera: Torymidae) native and introduced to the West Palearctic region: taxonomy, host specificity and distribution. J. Nat. Hist. **2003**; 37, 127–238. DOI: 10.1080/713834669.
65. Al Khatib, F.; Fusu, L.; Cruaud, A.; Gibson, G.; Borowiec, N.; Rasplus, J.Y.; Ris, N.; Delvare, G. An integrative approach to species discrimination in the *Eupelmus urozonus* complex (Hymenoptera, Eupelmidae), with the description of 11 new species from the Western Palaearctic. Syst. Entomol. **2014**; 39, 806–862. doi.org/10.1111/syen.12089
66. Gebiola, M.; Bernardo, U.; Burks, R.A. A reevaluation of the generic limits of *Pnigalio* Schrank (Hymenoptera: Eulophidae) based on molecular and morphological evidence. Zootaxa **2010**; 2484, 35-44. DOI: 10.11646/zootaxa.2484.1.3
67. Guerrieri, E.; Bernardo, U.; Iodice, L.; Gebiola, M. Identificazione morfo-bio-molecolare ed interazioni trofiche degli antagonisti autoctoni di *Dryocosmus kuriphilus* Yasumatsu in Campania: metodologia e risultati preliminari. Att. Accad. Naz. Ital. Entomol. **2011**; 58, 115–120.
68. Kaartinen, R.; Stone, G.; Hearn, J.; Lohse, K.; Roslin, T. Revealing secret liaisons: DNA barcoding changes our understanding of food webs. Ecol. Entomology **2010**; 35, 623–638. DOI: 10.1111/j.1365-2311.2010.01224.x
69. SPSS v23 (2015) SPSS Inc., Chicago, IL, USA.
70. Sigmaplot 13.0 (2018) Systat Copyright © Systat Software, USA.
71. Jalili, A.; Jamzad, Z.; Thompson, K.; Araghi, M.K.; Ashrafi, S.; Hasaninejad, M.; Panahi, P.; Hooshang, N.; Azadi, R.; Tavakol, M.S.; Palizdar, M.; Rahmanpour, A.; Farghadan, F.; Mirhossaini, S.G.; Parvaneh, K. Climate change, unpredictable cold waves and possible brakes on plant migration. Global Ecol. Biogeogr. **2010**; 19, 642–648. DOI: 10.1111/j.1466-8238.2010.00553.x
72. Bernardo, U.; Nugnes, F.; Gualtieri, L.; Scarpato, S.; Gargiulo, G.; Griffo, R. Cinipide del castagno, cresce il controllo biologico in Campania. L'Inf. Agr. **2017**; 27, 51–53.
73. Matošević, D.; Lacković, N.; Kos, K.; Kriston, E.; Melika, G.; Rot, M.; Pernek, M. Success of classical biocontrol agent *Torymus sinensis* within its expanding range in Europe. J. Appl. Entomol. **2017**; 141, 758–767. DOI: 10.1046/j.1365-294X.2003.01773.x
74. Nieves-Aldrey, J.-L.; Gil-Tapetado, D.; Gavira, O.N.; Boyero, J.R.; Polidori, C.; Lombardero, M.J.; Blanco, D.; Rey del Castillo, C.; Rodríguez-Rojo, M.P.; Vela, J.M.; Wong, M.E. *Torymus sinensis* Kamijo, a biocontrol agent against the invasive chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu in Spain: its natural dispersal from France and first data on establishment after experimental releases. For. Syst. **2019**, 28, e001. doi.org/10.5424/fs/2019281-14361
75. Cooper, W.R.; Rieske, L.K. Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in eastern North America. Ann. Entomol. Soc. Am. **2007**; 100, 236–244. DOI: 10.1603/0013-8746(2007)100[236:CAOAEG]2.0.CO;2
76. Fernandez-Conradi, P.; Capdevielle, X.; Castagneyrol, B.; Maltoni, A.; Robin, C.; Selvi, F., Van Halder, I.; Vettillard, F.; Jactel, H. Plant neighbour identity and invasive pathogen infection affect associational resistance to an invasive gall wasp. Biol. Invasions **2017**; 20, 1459–1473. DOI: 10.1007/s10530-017-1637-4
77. Gibson, G.A.P.; Gates, M.W.; Buntin, G.D. Parasitoids (Hymenoptera: Chalcidoidea) of the Cabbage Seedpod Weevil (Coleoptera: Curculionidae) in Georgia, USA. J. Hymenopt. Res. **2006**; 15, 187–207.
78. Cusumano, A.; Harvey, J.A.; Dicke, M.; Poelman, E.H. Hyperparasitoids exploit herbivore-induced plant volatiles during host location to assess host quality and non-host identity. Oecologia **2019**; 189, 699–709. DOI: 10.1007/s00442-019-04352-w