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# Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp?

Running title **Native parasitoids of *D. kuriphilus***

Chiara Ferracini[1], Sandro Bertolino[2], Umberto Bernardo[3], Carmelo P. Bonsignore[4], Massimo Faccoli[5], Ester Ferrari[1], Daniela Lupi[6], Stefano Maini[7], Luca Mazzon[5], Francesco Nugnes[3], Anna Rocco[6], Fabrizio Santi[7], Luciana Tavella[1]

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy

[2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, University of Torino, via Accademia Albertina 13, 10123 Torino, Italy

[3]CNR, Institute for Sustainable Plant Protection (IPSP), SS of Portici, Portici (NA), Italy

[4]Laboratorio di Entomologia ed Ecologia Applicata, Dipartimento Patrimonio, Architettura, Urbanistica. Università Mediterranea di Reggio Calabria, Salita Melissari, Reggio Calabria, Italy

[5]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[6]Department of Food, Environmental and Nutritional Sciences, University of Milan, via Celoria 2, 20133 Milan, Italy

[7]Department of Agricultural Sciences, Alma Mater Studiorum University of Bologna, Viale Fanin 44, 40127 Bologna, Italy

**Corresponding author: Chiara Ferracini,** Largo P. Braccini 2, 10095 Grugliasco (TO), Italy phone number 011/6708700, fax 011/6708535, E-mail chiara.ferrracini@unito.it

## Abstract

The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, is one of the most serious pests of *Castanea* spp. worldwide. Since local parasitoids did not effectively control this exotic pest, the Chinese parasitoid *Torymus sinensis* Kamijo was released in Japan and Europe resulting in a successful control of the invader. A survey of native parasitoids usually infesting oak gall wasps but associated with *D. kuriphilus* was carried out, sampling chestnut galls in Italy in 2013. The effect of predictive variables (years since *T. sinensis* release, oak occurrence, type of chestnut formation, latitude) on composition and density of native parasitoid populations was modelled with Generalized Linear Models. In total, 4,410 chalcid parasitoids were recorded, and the most abundant species were *T. flavipes* Walker, *Eupelmus* spp., and *Bootanomyia* (= *Megastigmus*) *dorsalis* (F.). Introduction of *T. sinensis* and oak occurrence influenced native parasitoid richness, conversely to the latitude of the study area. In particular, every additional year of presence of *T. sinensis* was proved to determine the loss of about 14% of native species, and 32% of their population density. A positive correlation between the oak density occurring in the infested chestnut forests and the richness and abundance of native parasitoid species was recorded as well, highlighting as generalist parasitoids may switch hosts easily. A positive effect of increasing densities of oak trees was recorded on most of the taxa, except for *T. geranii* (Walker), *Eupelmus* spp., and *Eurytoma pistaciae* Rondani.

Our results provide insight that both *T. sinensis* and oak trees affect native parasitoids, shedding light on the possible implications related to the release of exotic biological control agents on the recruitment and accumulation of native natural enemies.

**Keywords:** native parasitoid community, *Dryocosmus kuriphilus*, displacement, *Torymus sinensis* release, environmental factors, Generalized Linear Models

## 1. Introduction

The introduction of invasive alien species (IAS) may lead to changes in the structure and composition of ecosystems with consequent significant detrimental impacts to biodiversity, even if for the vast majority of exotic species no quantitative information is available to assess the magnitude of the effects of such introductions (Ehrenfeld, 2010; Jeschke et al., 2014). On one hand, invasive alien pests can pose major threats both to the host species causing large scale die out (Lovett et al., 2006; Mack et al., 2000), and to the biodiversity of native fauna due to competition, predation, or facilitation as well (Kenis et al., 2009; Konopka et al., 2017). On the other hand, the establishment of an IAS may benefit a native species by providing a new exploitable resource at a higher trophic level, leading to the assembly of a complex community structure (Cornell and Hawkins, 1993; Konopka et al., 2017). In fact, when an IAS establishes into a new area, native natural enemies find a new largely available resource to adapt. In some cases, native parasitoids are able to limit the development of their new hosts (Duan et al., 2015), but more often they fail to control the new invader which may become a pest (Abram et al., 2017; Ferracini and Alma, 2007; Mazzetto et al., 2016). In such a case, the most common approach to control the exotic pest is classical biological control through the importation of effective biological control agents (BCAs) from the invader's native range (Hajek et al., 2016; van Driesche and Hoddle, 2017).

One recent example of IAS accidentally introduced into Europe is represented by the Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), which was first reported in Europe (Italy) in 2002 (Graziosi and Santi, 2008). This is one of the major pests attacking chestnut trees (*Castanea* spp.) worldwide, and its infestations may cause a strong reduction of the photosynthetic activity and nut production, with yield losses as high as 80% (Battisti et al., 2014). From literature, ACGW galls are known to support species-richness, closed communities of inquilines and parasitoids that have become a model system in community ecology (Stone et al., 2002). In China, its native range, ACGW is limited by a complex of natural enemies. By contrast, in the countries where ACGW was accidentally introduced, the native parasitoids did not effectively control the pest, and the Chinese parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) was released (Moriya et al., 2003; Cooper and Rieske, 2007; Quacchia et al., 2008). It is phenologically well synchronised with its host and in all cases after its release it was able to disperse successfully alongside *D. kuriphilus* by expanding its populations, reducing shoot infestation rates below the tolerable damage threshold, and significantly containing gall wasp outbreaks. The exponential growth reported in a 5-7-year period both for Japan and Italy make this parasitoid one of the most recent successful examples of classical biological control programmes (Alma et al., 2014; Matošević et al., 2017; Moriya et al., 2003).

Although not effective as BCAs, several native parasitoids have been reported on ACGW in its introduced range, and specific surveys have been carried out to assess their recruitment. Few studies on native ACGW parasitoids are available for Japan and the USA, stating a rate of parasitism ranging from <1% to 8.5%, and around 1%, respectively (Cooper and Rieske, 2007, 2011; Murakami and Gyoutoku, 1995). In Europe, instead, a more abundant literature reports parasitism rates up to 13% for Croatia, Slovenia, and Spain (Jara-Chiquito et al., 2016; Kos et al., 2015; Matošević and Melika, 2013). In Italy, the community of native parasitoids recruited on the ACGW was evaluated in several regions, finding parasitism values quite similar to those reported for other European countries (Bernardinelli et al., 2016; Colombari and Battisti, 2016; Francati et al., 2015; Panzavolta et al., 2013; Quacchia et al., 2013), except for the Emilia Romagna region where percentages as high as 32% for *Torymus flavipes* Walker were recorded in 2011 (Santi and Maini, 2011).

The community of native parasitoids recorded on invading ACGW populations is mainly composed of chalcid species (Hymenoptera: Chalcidoidea), commonly known to be parasitoids of oak cynipid gall wasps (Askew et al., 2006, 2013; Ferracini et al., 2017; Stone et al., 2002). Although parasitoid communities attacking oak gall wasps include specialist parasitoid species, related to a small subset of host gall types, most of them are generalists, and thus able to promptly switch hosts (Bailey et al., 2009). The process of the ACGW invasion, the local adaptation of the native parasitoids, and the effect of *T. sinensis* introduced to control the exotic pest represent a perturbation of the natural trophic relationships. Simplifying, the invader ACGW represents a new resource, i.e. a new host, for the community of native parasitoids while the introduction of the exotic *T. sinensis* may, if effective, remove this resource. In this respect, the main aims of this study were to investigate the composition of native parasitoids associated with to ACGW in Italy and to assess its variations according to the introduction of *T. sinensis*. Agroforestry system (e.g. oak density and type of chestnut formation), and latitude as possible determinants of richness and density of the native parasitoid populations were also evaluated. Our working hypothesis was that *T. sinensis* had a negative effect on the richness and/or abundance of the native community of parasitoids, and this effect should increase with its local abundance.

## 2. Materials and methods

### 2.1 Experimental sites

2.1.1 *Site characteristics*

The study was carried out in Italy on chestnut trees growing in both orchards and mixed forests, in 2013. The main characteristics of the 34 surveyed sites are provided in supplementary Table 1. The investigated chestnut orchards were managed in a similar way, with productive chestnut trees generally 80-120 years old, with a high susceptibility to ACGW. The chestnut trees growing in mixed forests consisted of coppice producing small nuts and usually managed for wood production (firewood, poles, timber), surrounded by various woody broadleaf species (oaks, hophornbeam, wild cherries, maples, and ashes).

2.1.2 *Assessment of the oak density*

To assess the possible effect of oak trees on the population density of ACGW parasitoids, in each experimental site the number of oak trees, their size, and their density were measured. Measurements were performed in a 50×50 m surface per site, where most ACGW galls were collected (see next section 2.2.1). All oaks growing within the sampling surface taller than 1.5 m were counted, and the diameter at breast height (1.3 m) measured. The mean oak density, calculated as surface ($m^2$) occupied by the oak steams, was then referred to the hectare.

### 2.1.3 *Other variables*

Sampled chestnut trees were in orchards (no. 15) or in mixed forests (no. 19), therefore this variable was dichotomous. Italy is a country with a north-south orientation, which corresponds to a cline in weather condition. Between the North and the South, there can be a considerable difference in temperature, mainly during winter, while temperature differences are less marked in summer. The latitude of the experimental site was therefore included in the analyses as a proxy of temperature, while the province where the site occurred was a measure of the local condition.

To evaluate the effect of *T. sinensis* on the richness and/or density of the native community of parasitoids, experimental sites where *T. sinensis* was released since different years (from 1 to 8) were chosen, as well as areas where the species was not present yet. Considering that after the release a species needs several generations to adapt to the new local conditions to increase and spread, the years of *T. sinensis* presence in an area are an indirect measure of its numerical presence (population density). In this respect, the populations of *T. sinensis* were reported to grow and to expand exponentially over time both in Japan and Europe, as well as in our surveyed sites (Colombari and Battisti, 2016; Matošević et al., 2017; Moriya et al., 2003; Paparella et al., 2016).

## 2.2 Parasitoid collection and identification

### 2.2.1 *Gall sampling and storage*

Gall sampling was carried out considering four different agroforestry conditions: chestnuts in orchard and mixed forest, with a high or low presence of oak trees. Between 31 May and 10 August 2013, each site was visited every 15 days, for a total of 6 times. During each survey, 500 ACGW galls were collected randomly from several lateral branches approximately 50 cm long and 2/3-year-old pruned from different chestnut trees. The branches were clipped with a telescopic shear from all sides of the trees up to a maximum height of 7 m. The collected galls were stored separately for each sampling and site in rearing boxes in outdoor conditions until the emergence of the adults, according to the method described by Ferracini et al. (2015a).

### 2.2.2 *Insect collection and identification*

Native parasitoids emerged from the collected galls were individually stored in 99% alcohol. All the species were morphologically identified using dichotomous keys [Al khatib et al., 2014; Askew, 1961; Askew and Thúroczy (unpublished work); de Vere Graham and Gijswijt, 1998; Roques and Skrzypczyńska, 2003], and by comparison with voucher specimens deposited at DISAFA-University of Torino and at IPSP-CNR, previously identified by molecular protocols (COI, 28S, and ITS2). Parasitoids developed upon inquilines or other insects living in the galls, such as aculeate Hymenoptera, Coleoptera and Neuroptera, were discarded. Because of the possible existence of cryptic species, and in the light of the recent revision of the Palaearctic species by Al khatib et al. (2014), the genus *Eupelmus* was considered as a complex to avoid any misidentification. Moreover, *T. flavipes* was considered as a single morphospecies, due to the lack of a dichotomous key separating two cryptic taxa (Guerrieri et al., 2011; Kaartinen et al., 2010)

## 2.3 Statistical analyses

The effect of predictive variables on the number of parasitoid species and their density (number of individuals per species), and community diversity measured through the Shannon Index (Shannon, 1948) was modelled with Generalized Linear Models (GLMs). Before fitting the models, the spatial dependence of observations was explored by calculating Moran's I and the associated p-value by means of the software SAM on model residuals (Rangel et al., 2010). Moran's I tests were all non-significant, therefore spatial-explicit modelling was discarded.

Since parasitoids species and specimens were based on count data, we first assessed the assumption that these data were generated from a Poisson process. The residuals of the full model for influential points and outliers were assessed, and the overdispersion parameter as the scaled Pearson's $\chi^2$ estimated. The number of parasitoid species was then modelled with a GLM with Poisson error distribution and a log link function model, while for the number of parasitoid individuals per each species a negative binomial model to correct for overdispersion was used. Latitude, oak densities, and *T. sinensis* were considered as covariables while Chestnut and Province as factors, analysing main effects as well as first order interactions. To control for heterogeneous sampling schemes, Province was considered as a fixed effect. It was not possible to perform a Generalized Linear Mixed Models (GLMM) considering the variable as a random effect because for GLMM a minimum of 15-20 samples for each block are required (Bolker et al., 2009). Explanatory variables were not correlated ($r < 0.7$), therefore were all retained for the analyses.

Our dataset missed areas where *T. sinensis* releases occurred between four and seven years; the parasitoid was in fact released first in Piedmont, and later in other regions where its effectiveness was confirmed. Therefore, on one hand we were not able to find areas with *T. sinensis* released between four and seven years, and on the other hand areas with the oldest releases occurred all in Piedmont. To test the robustness of our results, we repeated the GLMs removing the four oldest areas in Piedmont with 8-year-old releases.

A multi-model inference was applied, and an information-theoretic approach was used to select models that were the most informative (Burnham and Anderson, 2002). All candidate models were ranked based on the AICc score for small sample sizes and delta AICc ($\Delta_i$) and the Akaike weights ($w_i$) were used to assess the strength of evidence that a particular model was the best within the candidate set.

The $\Delta_i$ is the difference between the AICc of a given model and the AICc of the highest ranked model (i.e. with the lowest AICc); a $\Delta_i < 2$ suggests substantial evidence for the model. The $w_i$ indicates the probability that the model is the best among the whole set of candidate models.

To better evaluate the influence of *T. sinensis* on the parasitoid community, a Canonical Correspondence Analysis (CCA) was used to study the relationship between parasitoid species abundance and environmental variables, including the years since *T. sinensis* release. CCA is a direct constrained ordination technique that simultaneously relates a set of taxa to a set of environmental variables. The eigenvalues associated with each axis give a relative indication of the ability of the axis to separate or order the species distribution. The significance of the overall ordination and each of the first two axes were determined with Monte Carlo permutation tests. The analysis was conducted with the software PAST 3.15 (Hammer et al., 2001).

## 3. Results

### 3.1 Parasitoid species emerged from ACGW galls

Oak density and number of native parasitoid species collected, according to the site and chestnut formation, are reported in Table 1. In total, from an overall amount of 102,000 collected galls, 4,410 parasitoids belonging to the superfamily Chalcidoidea emerged. Five families were recorded (Eupelmidae, Eurytomidae, Ormyridae, Pteromalidae, and Torymidae), and 11 species were identified. The most abundant species were *T. flavipes* (1,829), *Eupelmus* spp. (675), and *Bootanomyia* (= *Megastigmus*) *dorsalis* (F.) (580). A total of 2,715 native torymids emerged, belonging to the genera *Bootanomyia* (= *Megastigmus*) and *Torymus*, accounting for 61% of the total specimens.

All the species occurred in both types of chestnut stands (orchard and mixed forest), but their abundance in terms of number of specimens was higher in mixed forests (62%), than orchards. Their presence was also related to the sampling site. In fact, unlike the *Eupelmus* complex and *B. dorsalis*, some species exhibited an outstanding abundance only in a few regions [e.g. *Eurytoma brunniventris* Ratzeburg in Aosta Valley, *Eurytoma pistaciae* Rondani in Veneto, *Mesopolobus* spp., and *Sycophila* spp. in Calabria, *T. flavipes* in Emilia Romagna and Calabria, and *Torymus geranii* (Walker) in Emilia Romagna]. *Ormyrus pomaceus* (Geoffroy) was the least representative species, with a relative abundance of only 2% (Table 1).

### 3.2 Variables affecting parasitoid species richness in ACGW galls

The best two models explaining the number of species present in ACGW galls contained Oak and *T. sinensis* as nested explanatory variables (Table 2, see also supplementary Table 2 for a list of the first ten models); the two models accounted for 52% of the total weight. Oak showed a positive but limited effect (B = 0.059 ± 0.120), and *T. sinensis* a more pronounced negative effect (B = -0.155 ± 0.043) (Table 3). According to the exponentiated values of the coefficients [Exp(B) = 1.061 for Oak and 0.86 for *T. sinensis*] an increase of a year of *T. sinensis* presence caused a decrease of 14% in the number of species present. Considering a compound effect, this corresponded to the loss of 50% of the species in five years since *T. sinensis* release (Fig. 1, trendline $R^2 = 0.62$).

### 3.3 Variables affecting parasitoid density in ACGW galls

The best two models explaining the parasitoid density in the ACGW galls contained Oak, *T. sinensis*, and chestnut stand type as nested explanatory variables (Table 2, see also supplementary Table 3 for a list of models); the two models accounted for 77% of the total weight. Oak showed a positive but negligible effect (B = 0.002 ± 0.001), *T. sinensis* a negative effect (B = -0.365 ± 0.068) and mixed forest a negative effect in respect to orchard (B = -0.682 ± 0.357) (Table 3). According to the exponentiated values of the coefficients, the number of parasitoids decreased by 58% in mixed forest in respect to orchard [(Exp(B) = 0.506], and by 32% for every year since the introduction of *T. sinensis* [(Exp(B) = 0.694].

### 3.4 Variables affecting the diversity of the parasitoid community

The best models explaining the diversity of the parasitoid community in the ACGW galls contained all *T. sinensis* as explanatory variable, alone or together with chestnut stand type, with a possible effect connected to the latitude (Table 2, see also supplementary Table 4 for a list of models); the two models accounted for 59% of the total weight. *T. sinensis* always showed a negative effect (B = -0.125 ± 0.168 when considered alone). Mixed forest had a negative effect in respect to orchard when included in the model (B = 0.157 ± 0.112), while the effect of latitude was negligible (B = -1.37 $*10^{-7}$). According to the exponentiated values of the coefficients, the diversity of the parasitoid community measured through the Shannon Index decreased by 11.8% for every year since the introduction of *T. sinensis* [(Exp(B) = 0.882]. The results of the GLMs conducted excluding the four oldest areas in Piedmont with 8-year-old releases are reported in supplementary Tables 5-7, and basically confirmed a negative effect of *T. sinensis*.

### 3.5 The CCA analysis

The CCA of parasitoid species is reported as a biplot of the species scores along the first two axes of the ordination (Fig. 2). The overall ordination and the first and second axes were significant at $p < 0.05$. Eigenvalues of the first two axes were relatively low (0.29 and 0.08), indicating a little variance in the species data. The percentage of the canonical variance was 78% and 22%, respectively.

The CCA analysis showed a positive effect of an increasing density of oak on most of the taxa. A presence of *T. sinensis* from a greater number of years had a negative effect on most of the taxa, except for *E. pistaciae*, *Eupelmus* spp. and *T. geranii*.

## 4. Discussion

Expanding populations of exotic invasive species are considered to be main direct drivers of biodiversity loss across the globe, disrupting ecosystems directly through displacement and extinction of native species (Jucker and Lupi, 2011; Wilson, 1997). In addition IAS may also elicit the recruitment of native species which can use these new hosts as potential trophic and reproductive resources, as recently pointed out for several emerging exotic pests (Haye et al., 2015; Lupi et al., 2017). With regard to the ACGW, generalist native parasitoid species quickly recruited to this novel gall wasp host, but they have not provided effective control of this pest in the areas where it was accidentally introduced, with negligible parasitism rate (Quacchia et al., 2013).

Our study provides further evidence of such a recruitment of native parasitoids, as the recorded parasitoid community includes species that commonly parasitize oak- and rose-galling insects (Askew et al., 2013; Ferracini et al., 2015b; 2017). All the parasitoid species found in this study were previously reported for ACGW galls in Europe (Alma et al., 2014; Jara-Chiquito et al., 2016; Kos et al., 2015; Quacchia et al., 2013), but to our knowledge this is the first paper bringing out the influence of the introduced *T. sinensis* and the agroforestry system on the richness and abundance of the native community of parasitoids. Among the nested explanatory variables that can be related to parasitoid richness (e.g. number of species, and number of specimens), years of *T. sinensis* presence, oak density, and type of chestnut formation seem to be more important than latitude and geographical localization. In particular, variability in species composition, and abundance occurring in the native community associated with ACGW galls has been demonstrated, showing that the introduction of the exotic BCA *T. sinensis* may reduce the amount of native parasitoids emerging from chestnut galls. This change has been highlighted both in terms of number of species and specimens, showing that an additional year of presence of *T. sinensis* may determine the loss of 14% of native species, and of 32% of their individuals. In particular, all the native parasitoid species were negative affected by the presence of *T. sinensis*, except for *Eupelmus* spp., *T. geranii* and *E. pistaciae*. These species are known to be important facultative hyperparasitoids of *T. sinensis* in Japan, and this might explain why they have not significantly been affected by the presence of the exotic BCA (Murakami et al., 1995; Daneshvar et al., 2009).

This finding is particularly evident in the Piedmont region, where in a nine-year period (2002-2010), 32 native parasitoid species emerging from chestnut galls were collected in the same area (Cuneo province) (Quacchia et al., 2013). Specifically, the community richness rose from 4 species in 2002, 7 in 2003, 10 in 2004, and to 18 species in 2009. Since 2010 an inverted trend was highlighted and, as reported in this survey, after the next 3 years the native community decreased being composed essentially of the *Eupelmus* complex and – in a minor degree – by few individuals of three other species, namely, *E. pistaciae*, *M. sericeus*, and *T. flavipes*. Adult competition for oviposition sites, larval competition for suitable hosts, or hyperparasitism may have played a role in driving this trend. Since the low native parasitoid biodiversity might be specific to this region, and thus not representing an overall pattern, we repeated the GLMs removing the oldest areas in Piedmont. And even without considering the four areas with 8-year-old releases, the results did not differ, proving the robustness of the analysis. A negative correlation between the introduced *T. sinensis* and the native parasitoids associated with oak-galling wasps was reported in the USA by Cooper and Rieske (2007), as well. Even in Japan, the displacement of *T. beneficus* Yasumatsu *et* Kamijo by the congeneric *T. sinensis* was recorded, being hybridization, reproductive ability, and inter-specific larval competition the main factors in the observed reduction (Yara et al., 2007).

After its introduction, *T. sinensis* caused a density-dependent mortality on *D. kuriphilus*, and in a few years reduced considerably the population density of its superabundant host, both in Japan and Italy. The high pressure by *T. sinensis* (in some NW Italian areas with parasitism rate above 90%, data not shown) reflected on the native parasitoid assemblage, and involved an expansion of its host-range with an occasional feeding also on non-target oak galls (Ferracini et al., 2017). Moreover, parasitoid communities and parasitism level have been shown to be more sensitive to habitat fragmentation than their hosts, and to be especially affected by the habitat features, such as the richness and variability of host plants (Maldonado-López et al., 2015; Stone et al., 2002). Fragmentation of habitats has a greater effect on the abundance and species richness of higher trophic levels, and parasitoids in general are known to be particularly susceptible (Wilby and Thomas, 2002). In particular, the community associated to the exotic ACGW has developed rapidly, involving species shared with local populations of oak and rose gall wasps, typically those with broad host ranges.

All the recorded parasitoid species are commonly associated with the main oak galls (namely *Biorhiza pallida* Olivier, *Andricus quercustozae* Bosc, *A. lignicolus* Hartig, *A. curvator* Hartig, and *A. lucidus* Hartig) in many Italian regions (Askew et al., 2013; Ferracini et al., 2017). Thus, it is reasonable to expect that the oak distributions have probably played a major role in the distribution and species richness of oak cynipids, and consequently in the composition of cynipid gall communities, as already stated by Stone et al. (2002). In the surveyed sites, four main oak species were extensively present, namely *Q. cerris* L., *Q. petraea* (Matt.), *Q. pubescens* Willd., and *Q. robur* (L.), and a positive correlation between the number of oak trees and the richness and abundance of native parasitoid species has been recorded. Deciduous oaks are keystone taxa across the Western Palearctic, supporting more associated insects than any other forest tree (Stone et al., 2012). The CCA analysis showed a positive effect of an increasing density of oak on most of the taxa, except for *Torymus* spp., *Eupelmus* spp. and *E. pistaciae*.

The most common genera able to exploit *D. kuriphilus* proved to be *Eupelmus*, *Bootanomyia* (=*Megastigmus*) and *Torymus*, already known to attack the ACGW in China, Japan, and Korea as well (Murakami et al., 1980, 1995). *B. dorsalis*, *Eupelmus urozonus* Dalman, *E. annulatus* Nees, *E. pistaciae*, and *T. flavipes* seem to attack regularly ACGW galls, while for the others *D. kuriphilus* represents an occasional host (Alma et al., 2014). In particular, *T. flavipes* was the most abundant species, especially in Emilia-Romagna and Calabria regions. The activity of *Eupelmus* spp. as parasitoids of ACGW should be confirmed because in Campania region they resulted absent in galls collected during the winter up to *T. sinensis* was released. Instead, just after *T. sinensis* releases they become frequent. This let us to think their predominant behaviour is as hyperparasitoids. Moreover, specimens of *Eupelmus* spp. are often the last to emerge and this is congruent with their behaviour as hyperparasitoids.

Chestnut growing in mixed forest revealed a number of associated specimens higher than chestnut cultivated in orchard, but no correlation with the number of species was found. Similar evidence was reported by Cooper and Rieske (2007) for the emergence of the parasitoid *Ormyrus labotus* Walker in relation to habitat variability. In fact, emergence of this parasitoid from oak-galling insects differed among the investigated sites, due to the abundance or lack of *Quercus* species.

The results support our working hypothesis, shedding light on the implication of release of *T. sinensis* as BCA on the recruitment and accumulation of native natural enemies. The exotic parasitoid has a negative effect on the richness and abundance of the native community of parasitoids, and this effect increases with its local abundance. Furthermore, while oak density and type of chestnut formation affect this assemblage, latitude did not prove to exert any influence probably due the presence of oak species and galling-former insects in all the experimental sites.

Hence, further research would be needed to evaluate the interspecific relation among *T. sinensis* and the native parasitoid community with a more complete dataset, e.g. over multiple years, investigating in-depth the underlying mechanisms of the potential displacement of the native natural enemies and change in the community pattern, in consideration of other possible causative factors as well.

In the literature there is evidence that invasive hosts tend to accumulate additional parasitoid species with increasing time since the invasion (Cornell and Hawkins 1993; Vindstad et al., 2013), but the speed of this process is not well known. The data available for Italy clearly show this trend. *D. kuriphilus* rapidly recruited an enemy community in its novel environment, as confirmed by the 40 native species reported (Alma et al., 2014), but after 15 years since the first report of the invader in Italy they decreased considerably, and they do not play any effective role in the control of the pest. As already stated by Cornell and Hawkins (1993), even if the species assemblages on introduced hosts may require time (hundreds of years) to physiologically, behaviourally, or phenologically adapt to the novel host, the generally low parasitism reported for *D. kuriphilus* suggests that many of the native parasitoid species represent incidental attacks due to the coexistence of the exotic and native host populations.

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Table 1 Oak density and numbers of native parasitoid species collected, according to the surveyed sites and the chestnut formation (MF=mixed forest; O=orchard)

| **Region** | **Chestnut formation** | **Oak density** | ***T. sinensis* presence (year)**[1] | *Bootanomyia* (=*Megastigmus*) *dorsalis* | *Eupelmus* spp. | *Eurytoma brunniventris* | *Eurytoma pistaciae* | *Mesopolobus sericeus* | *Mesopolobus tibialis* | *Ormyrus pomaceus* | *Sycophila biguttata* | *Sycophila variegata* | *Torymus auratus* | *Torymus flavipes* | *Torymus geranii* | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aosta Valley | MF | 552.37009 | 1 | 133 | 42 | 0 | 4 | 0 | 0 | 13 | 0 | 8 | 0 | 15 | 0 | 215 |
| | MF | 288.98540 | 1 | 34 | 56 | 0 | 3 | 3 | 1 | 0 | 7 | 2 | 0 | 38 | 0 | 144 |
| | MF | 245.01062 | 1 | 24 | 35 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 1 | 51 | 0 | 122 |
| | MF | 173.15892 | 1 | 21 | 64 | 64 | 11 | 8 | 1 | 35 | 17 | 0 | 1 | 190 | 0 | 412 |
| Calabria | MF | 50.95541 | 1 | 19 | 9 | 1 | 0 | 48 | 12 | 0 | 1 | 17 | 5 | 3 | 0 | 115 |
| | MF | 63.69427 | 0 | 2 | 0 | 5 | 0 | 3 | 11 | 0 | 0 | 16 | 0 | 23 | 0 | 60 |
| | O | 6.36943 | 0 | 5 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 17 | 0 | 2 | 0 | 30 |
| | MF | 17.83439 | 1 | 5 | 4 | 0 | 0 | 9 | 14 | 0 | 2 | 5 | 9 | 23 | 0 | 71 |
| | MF | 17.83439 | 0 | 2 | 3 | 0 | 0 | 74 | 35 | 9 | 0 | 30 | 12 | 108 | 0 | 273 |
| | MF | 137.57962 | 0 | 3 | 3 | 4 | 0 | 101 | 25 | 0 | 2 | 15 | 11 | 58 | 0 | 222 |
| | O | 10.19108 | 0 | 31 | 3 | 2 | 0 | 33 | 35 | 0 | 71 | 16 | 3 | 194 | 0 | 388 |
| Emilia Romagna | MF | 15.84004 | 1 | 4 | 55 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 22 | 279 | 81 | 445 |
| | O | 9.50666 | 1 | 0 | 23 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 6 | 262 | 7 | 302 |
| | O | 0 | 1 | 24 | 45 | 0 | 0 | 8 | 0 | 19 | 0 | 0 | 0 | 306 | 5 | 407 |
| | MF | ND[2] | 2 | 18 | 56 | 0 | 0 | 1 | 0 | 6 | 0 | 0 | 30 | 171 | 88 | 370 |
| Lombardy | MF | 1.15420 | 2 | 21 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 39 |
| | O | 0 | 2 | 4 | 4 | 0 | 5 | 0 | 0 | 0 | 2 | 0 | 0 | 11 | 4 | 30 |
| | O | 0 | 3 | 15 | 36 | 4 | 6 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 64 |
| | MF | 1.55330 | 1 | 8 | 8 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 21 |
| | O | 0.89950 | 1 | 15 | 32 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 55 |
| | MF | 0.08200 | 1 | 4 | 4 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 7 | 1 | 21 |
| | MF | 0.70530 | 2 | 4 | 17 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 62 | 4 | 91 |
| Piedmont | O | 0 | 8 | 0 | 23 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 |
| | O | 0 | 8 | 0 | 23 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 29 |
| | O | 0 | 8 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | O | 0 | 8 | 0 | 20 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| Veneto | MF | 7.140 | 3 | 4 | 9 | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 11 | 36 |
| | O | 0 | 3 | 78 | 10 | 1 | 14 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 105 |
| | MF | 1.798 | 3 | 12 | 12 | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 |
| | O | 0 | 3 | 29 | 13 | 3 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 |
| | MF | 2.344 | 3 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| | O | 0 | 3 | 18 | 6 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 |
| | MF | 11.16 | 3 | 5 | 11 | 1 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 24 |
| | O | 0 | 3 | 33 | 44 | 11 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 144 |
| **Total** | | | | 580 | 675 | 102 | 143 | 309 | 136 | 86 | 117 | 127 | 102 | 1,829 | 204 | **4,410** |

[1] This column reports the surface area ($m^2$) occupied by the oak steams, measuring the diameter at breast height (1.3 m) in a 50×50 m sampling square, then referred to the hectare. Only oak plants taller than 1.5 m were considered.

[2] ND = Not determined

Table 2. Parameters of the models selected with the lowest AICc score for number of parasitoid species, number of parasitoid specimens and Shannon Index of parasitoid communities in ACGW galls.

| Parameter | B | 95% confidence interval | | Exp(B) | 95% confidence interval | |
|---|---|---|---|---|---|---|
| | | Lower | Upper | | Lower | Upper |
| *Number of parasitoid species* | | | | | | |
| Intercept | 1.987 | 1.765 | 2.209 | 7.294 | 5.843 | 9.105 |
| *T. sinensis* | -0.155 | -0.240 | -0.070 | 0.857 | 0.787 | 0.933 |
| Oak | 0.059 | -0.177 | 0.294 | 1.061 | 0.838 | 1.342 |
| | | | | | | |
| *Number of parasitoid specimens* | | | | | | |
| Intercept | 5.664 | 5.014 | 6.314 | 288.310 | 150.563 | 552.081 |
| Mixed forest | -0.682 | -1.382 | 0.018 | 0.506 | 0.251 | 1.018 |
| Chestnut orchard | 0 | - | - | 1 | - | - |
| Oak | 0.002 | -0.001 | 0.005 | 1.002 | 0.999 | 1.005 |
| *T. sinensis* | -0.365 | -0.499 | -0.231 | 0.694 | 0.607 | 0.794 |
| | | | | | | |
| *Shannon Index* | | | | | | |
| Intercept | 1.462 | 1.323 | 1.601 | 4.315 | 3.756 | 4.957 |
| *T. sinensis* | -0.125 | -0.168 | -0.083 | 0.882 | .846 | .920 |

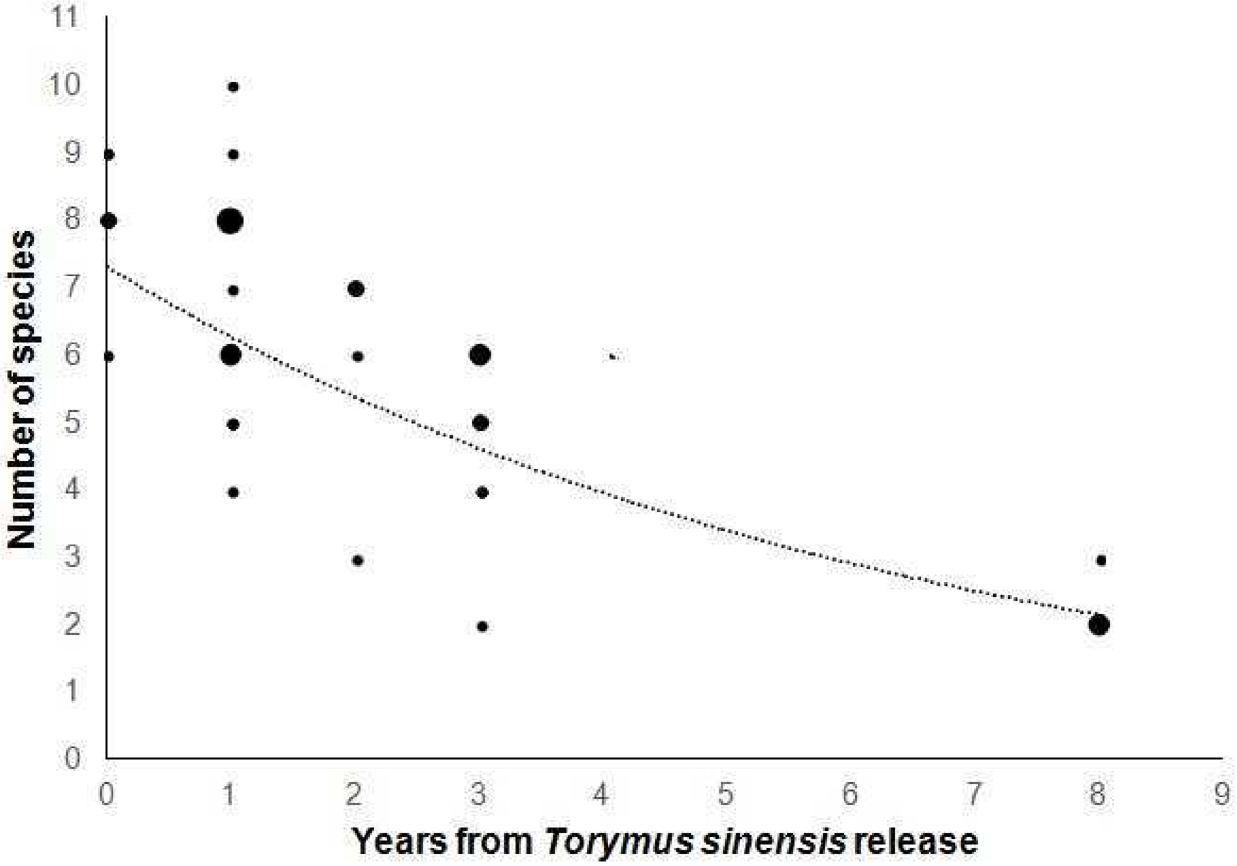

Figure 1. Number of parasitoids species recorded in ACGW galls according to the years since first *T. sinensis* release. The size of dots is proportional to the number of areas with the same value.

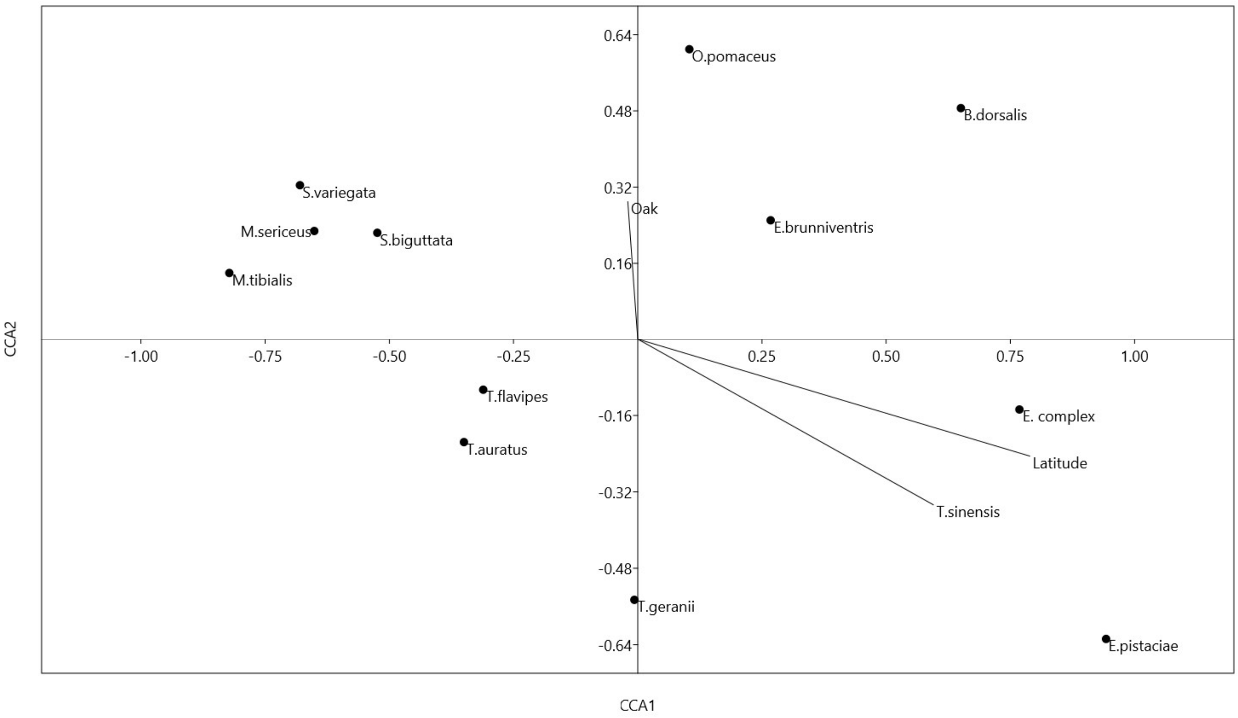

Figure 2. Plot of environmental variables from CCA ordination with parasitoid species abundance. *T. sinensis* refers to the number of years since its first release in the environment and is measure of its establishment.

Supplementary Table 1 Main features of the sampling surveyed sites

| **Region** | **Province** | **Geographic coordinates (WGS84)** | | **Zone** | **Chestnut formation**[1] | ***T. sinensis* presence (year)**[2] |
|---|---|---|---|---|---|---|
| | | **N** | **E** | | | |
| Aosta Valley | Aosta | 5056424 | 396972 | 32T | MF | 1 |
| | Aosta | 5062084 | 397320 | 32T | MF | 1 |
| | Aosta | 5059420 | 362079 | 32T | MF | 1 |
| | Aosta | 5068559 | 370446 | 32T | MF | 1 |
| Calabria | Reggio Calabria | 4245327 | 595178 | 33N | MF | 0 |
| | Reggio Calabria | 4230399 | 579806 | 33N | MF | 0 |
| | Reggio Calabria | 4236312 | 579824 | 33N | O | 1 |
| | Reggio Calabria | 4232070 | 565145 | 33N | MF | 0 |
| | Reggio Calabria | 4248713 | 597224 | 33N | MF | 0 |
| | Reggio Calabria | 4221080 | 567259 | 33N | MF | 0 |
| | Reggio Calabria | 4212350 | 565548 | 33N | O | 1 |
| Emilia Romagna | Bologna | 4910184 | 690200 | 32T | MF | 1 |
| | Bologna | 4905838 | 681141 | 32T | O | 1 |
| | Bologna | 4914186 | 682238 | 32T | O | 1 |
| | Bologna | 4904057 | 689038 | 32T | MF | 2 |
| Lombardy | Brescia | 5044183 | 598551 | 32T | MF | 2 |
| | Brescia | 5049883 | 597802 | 32T | O | 2 |
| | Brescia | 5078001 | 590864 | 32T | O | 3 |
| | Varese | 5069155 | 484233 | 32T | MF | 1 |
| | Varese | 5081227 | 483801 | 32T | O | 1 |
| | Bergamo | 5059910 | 570532 | 32T | MF | 1 |
| | Pavia | 4966527 | 522166 | 32T | MF | 2 |
| Piedmont | Cuneo | 4908015 | 387400 | 32T | O | 8 |
| | Cuneo | 4906691 | 383494 | 32T | O | 8 |
| | Cuneo | 4909442 | 395310 | 32T | O | 8 |
| | Cuneo | 4913980 | 385540 | 32T | O | 8 |
| Veneto | Treviso | 5081636 | 728452 | 32T | MF | 3 |
| | Treviso | 5081636 | 728452 | 32T | O | 3 |
| | Treviso | 5079899 | 722668 | 32T | MF | 3 |
| | Treviso | 5079899 | 722668 | 32T | O | 3 |
| | Treviso | 5081692 | 725811 | 32T | MF | 3 |
| | Treviso | 5081692 | 725811 | 32T | O | 3 |
| | Treviso | 5081438 | 728096 | 32T | MF | 3 |
| | Treviso | 5081438 | 728096 | 32T | O | 3 |

[1] MF = mixed forest; O = orchard.

[2] This column refers to the number of years since the first *T. sinensis* release.

Supplementary Table 2. The first ten selected models explaining the number of species present in ACGW galls with lower AICc values and their Akaike weights ($w_i$); all models include also the intercept. The best two models with $\Delta_i < 2$ are in bold.

| **ID** | **Model** | **K** | **AICc** | **$\Delta_i$** | **$w_i$** |
|---|---|---|---|---|---|
| **1** | **Oak + *T. sinensis*** | **4** | **133.984** | **0.000** | **0.374** |
| **2** | ***T. sinensis*** | **3** | **135.866** | **1.882** | **0.146** |
| 3 | Oak + *T. sinensis* + Latitude | 5 | 136.262 | 2.278 | 0.120 |
| 4 | Oak + *T. sinensis* + Oak * *T. sinensis* | 6 | 136.469 | 2.485 | 0.108 |
| 5 | Oak + *T. sinensis* + Chestnut | 5 | 136.583 | 2.599 | 0.102 |
| 6 | *T. sinensis* + Latitude | 4 | 138.095 | 4.111 | 0.048 |
| 7 | *T. sinensis* + Chestnut | 4 | 138.215 | 4.231 | 0.045 |
| 8 | Oak + *T. sinensis* + Chestnut + Latitude | 6 | 139.054 | 5.070 | 0.030 |
| 9 | *T. sinensis* + Chestnut + *T. sinensis* * Chestnut | 6 | 140.59 | 6.606 | 0.014 |
| 10 | *T. sinensis* + Latitude + *T. sinensis* * Latitude | 6 | 140.674 | 6.690 | 0.013 |

Supplementary Table 3. The first ten selected models explaining the number of specimens with lower AICc values and their Akaike weights (wi); all models include also the intercept. The best two models with Δi < 2 are in bold.

| **ID** | **Model** | **K** | **AICc** | **$\Delta_i$** | **$w_i$** |
|---|---|---|---|---|---|
| **1** | **Oak + *T. sinensis* + Chestnut** | **5** | **375.857** | **0.000** | **0.457** |
| **2** | **Oak + *T. sinensis*** | **4** | **376.608** | **0.751** | **0.314** |
| 3 | Oak + *T. sinensis* + Oak * *T. sinensis* | 6 | 379.157 | 3.300 | 0.088 |
| 4 | Oak + *T. sinensis* + Latitude | 5 | 379.219 | 3.362 | 0.085 |
| 5 | Oak + *T. sinensis* + Chestnut + Oak * *T. sinensis* + Oak * Chestnut | 9 | 381.295 | 5.438 | 0.030 |
| 6 | Oak + *T. sinensis* + Chestnut + Oak * *T. sinensis* * Chestnut | 8 | 381.716 | 5.859 | 0.024 |
| 7 | Oak + Latitude | 4 | 388.169 | 12.312 | 0.001 |
| 8 | Oak | 3 | 388.327 | 12.470 | 0.001 |
| 9 | *T. sinensis* | 3 | 390.946 | 15.089 | 0.000 |
| 10 | Oak + Chestnut | 4 | 390.849 | 14.992 | 0.000 |

Supplementary Table 4. The first ten selected models explaining the Shannon Index of parasitoid communities with lower AICc values and their Akaike weights (wi); all models include also the intercept. The best two models with $\Delta i < 2$ are in bold.

| **ID** | **Model** | **K** | **AICc** | **$\Delta_i$** | **$w_i$** |
|---|---|---|---|---|---|
| **1** | ***T. sinensis*** | **3** | **19.321** | **0.000** | **0.278** |
| **2** | ***T. sinensis* + Chestnut** | **4** | **19.991** | **0.670** | **0.199** |
| **3** | ***T. sinensis* + Latitude** | **4** | **21.212** | **1.891** | **0.108** |
| 4 | *T. sinensis* + Latitude + *T. sinensis* + Latitude | 6 | 21.318 | 1.997 | 0.103 |
| 5 | *T. sinensis* + Chestnut + Latitude | 5 | 21.796 | 2.475 | 0.081 |
| 6 | *T. sinensis* + Chestnut + *T. sinensis* * Chestnut | 6 | 22.007 | 2.686 | 0.073 |
| 7 | *T. sinensis* + Oak | 4 | 22.035 | 2.714 | 0.072 |
| 8 | *T. sinensis* + Oak + Chestnut | 5 | 23.174 | 3.853 | 0.041 |
| 9 | Oak + *T. sinensis* + Latitude | 5 | 24.041 | 4.720 | 0.026 |
| 10 | Oak + *T. sinensis* + Oak * *T. sinensis* | 6 | 24.644 | 5.323 | 0.019 |

Supplementary Table 5. The first ten selected models with lower AICc values and their Akaike weights ($w_i$) for number of parasitoid species, excluding the four oldest areas in Piedmont with 8-year-old releases; all models include also the intercept. The best models with $\Delta_i < 2$ are in bold.

| **ID** | **Model** | **K** | **AICc** | **$\Delta_i$** | **$w_i$** |
|---|---|---|---|---|---|
| **1** | **Oak + *T. sinensis*** | **4** | **122.917** | **0** | **0.285** |
| **2** | **Oak + Latitude** | **4** | **123.782** | **0.865** | **0.185** |
| **3** | ***T. sinensis*** | **3** | **124.731** | **1.814** | **0.115** |
| **4** | Oak + *T. sinensis* + Latitude | 5 | 125.321 | 2.404 | 0.086 |
| **5** | Oak + *T. sinensis* + Oak * *T. sinensis* | 6 | 125.555 | 2.638 | 0.076 |
| **6** | Oak + *T. sinensis* + Chestnut | 5 | 125.623 | 2.706 | 0.074 |
| **7** | Oak | 3 | 125.757 | 2.84 | 0.069 |
| **8** | Latitude | 3 | 126.754 | 3.837 | 0.042 |
| **9** | *T. sinensis* + Latitude | 4 | 127.071 | 4.154 | 0.036 |
| **10** | *T. sinensis* + Chestnut | 4 | 127.154 | 4.237 | 0.034 |

Supplementary Table 6. The first ten selected models with lower AICc values and their Akaike weights ($w_i$) for number of parasitoid specimens, excluding the four oldest areas in Piedmont with 8-year-old releases; all models include also the intercept. The best models with $\Delta_i < 2$ are in bold.

| ID | Model | K | AICc | $\Delta_i$ | $w_i$ |
|---|---|---|---|---|---|
| **1** | **Oak + *T. sinensis* + Chestnut** | **5** | **342.731** | **0** | **0.359** |
| **2** | **Oak + *T. sinensis*** | **4** | **344.154** | **1.423** | **0.176** |
| **3** | Oak + *T. sinensis* + Latitude + Chestnut | 6 | 345.278 | 2.547 | 0.100 |
| **4** | Oak + *T. sinensis* + Latitude * Chestnut | 6 | 345.495 | 2.764 | 0.090 |
| **5** | Oak + *T. sinensis* + Oak * *T. sinensis* | 6 | 345.710 | 2.979 | 0.081 |
| **6** | Oak + *T. sinensis* + Chestnut + Oak * *T. sinensis* + Oak * Chestnut | 9 | 346.386 | 3.655 | 0.058 |
| **7** | Oak + *T. sinensis* + Chestnut + Oak * *T. sinensis* * Chestnut | 8 | 346.871 | 4.140 | 0.045 |
| **8** | Oak + *T. sinensis* + Latitude | 5 | 346.965 | 4.234 | 0.043 |
| **9** | Oak + Latitude + Chestnut | 5 | 348.073 | 5.342 | 0.025 |
| **10** | Oak + *T. sinensis* + Chestnut + Oak * *T. sinensis* + T. sinensis * Chestnut | 9 | 348.22 | 5.489 | 0.023 |

Supplementary Table 7. The first ten selected models explaining the Shannon Index of parasitoid communities with lower AICc values and their Akaike weights (wi), excluding the four oldest areas in Piedmont with 8-year-old releases; all models include also the intercept. The best two models with $\Delta i < 2$ are in bold.

| ID | Model | K | AICc | $\Delta_i$ | $w_i$ |
|---|---|---|---|---|---|
| **1** | **Latitude + Chestnut** | **4** | **18.314** | **0.000** | **0.236** |
| **2** | **Latitude** | **3** | **19.034** | **0.720** | **0.165** |
| **3** | ***T. sinensis*** | **3** | **19.647** | **1.333** | **0.121** |
| **4** | ***T. sinensis* + Chestnut + *T. sinensis* * Chestnut** | **6** | **20.184** | **1.870** | **0.093** |
| 5 | *T. sinensis* + Chestnut | 4 | 20.467 | 2.153 | 0.080 |
| 6 | Latitude + Chestnut + Latitude * Chestnut | 6 | 20.584 | 2.270 | 0.076 |
| 7 | *T. sinensis* + Latitude | 4 | 20.914 | 2.600 | 0.064 |
| 8 | Latitude + Oak | 4 | 20.97 | 2.656 | 0.063 |
| 9 | *T. sinensis* + Chestnut + Latitude | 5 | 21.111 | 2.797 | 0.058 |
| 10 | Latitude + Chestnut + oak | 5 | 21.671 | 3.357 | 0.044 |

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