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Indigenous parasitoids associated with *Dryocosmus kuriphilus* in a chestnut production area of Emilia Romagna (Italy)

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Abstract

The associations occurring between the Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) and native parasitoids were investigated over the 3-year period 2010-2012 in a chestnut plantation located in the municipality of Castel del Rio (high Santerno Valley, Bologna province, Emilia Romagna, Italy). The chestnut trees were mixed with other plant species, including oaks, and were heavily infested by *D. kuriphilus* (which was first recorded in Emilia Romagna in 2008). In the survey period, a total of 12,015 spring galls were collected and an overall number of 7,094 insects emerged (5,182 *D. kuriphilus* and 1,912 parasitoids). The parasitoids, belonging to five families (Eupelmidae, Eurytomidae, Ormyridae, Pteromalidae, Torymidae), were presumably recruited from oak gall wasps. They were identified using morphological characters and the most represented genus was *Torymus*. Within this genus, the morphospecies *Torymus flavipes* (Walker) was identified. *Mesopolobus* was the second most abundant genus in 2010 and 2011. The percentages of parasitism (calculated considering an average of 3.5 cells) were 3.06, 10.18 and 0.09 in 2010, 2011 and 2012, respectively. The dramatic reduction of parasitoids found in 2012 might have been a consequence of biocoenotic dynamics, in particular of the instability of the interactions occurring between the ACGW and the generalist native parasitoids.

Key words: Asian chestnut gall wasp, native parasitoid complex, high Santerno Valley, northern Italy.

Introduction

The introduction of exotic insect species is becoming more and more frequent all over the world (Wittenberg and Cock, 2001; Kenis *et al.*, 2009). The Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae), native to China, has spread to many chestnut forests and orchards of the world, and is considered as one of the major global insect pests of *Castanea* trees (Stone *et al.*, 2002; Aebi *et al.*, 2006; Ács *et al.*, 2007; Gibbs *et al.*, 2011). This cynipid is the only known chestnut gall wasp originating from the Palearctic region, apart from *Synergus castaneus* Pujade-Villar, Bernardo et Viggiani n. sp., which was recently found to be associated with an unknown gall on *Castanea* spp. in China and was inferred to be a gall maker (Bernardo *et al.*, 2013).

In Europe, the ACGW was first recorded in Italy (Piedmont) in 2002 (Brussino *et al.*, 2002) and is now distributed throughout Italy and other European countries (EPPO, 2014). A high impact on plant growth and nut production has been reported in chestnut areas severely infested by this pest (EFSA Panel on Plant Health, 2010). In chestnut orchards in north-eastern Italy, nut yield losses were as high as 80% when the number of current-year galls exceeded six per 50-cm twig (Battisti *et al.*, 2014). Owing to the threats for chestnut-growing, the ACGW has been listed on the EPPO A2 list since 2003 (EPPO, 2005; 2014).

Biological control is considered as a potential means of solving the problem of the ACGW in the areas of introduction. Classical programs, based on the importation of the parasitoid *Torymus sinensis* Kamijo (Hymenoptera Torymidae) from the ACGW native areas, have been carried out in different countries, Japan (1975), USA (in the late 1970s), Italy (2005), France (2011), Croatia (2014) and Hungary (2014) (Moriya *et al.*,1989; Cooper and Rieske, 2007; Quacchia *et al.*, 2008; Borowiec *et al.*, 2014; Matošević *et al.*, 2014). This system is regarded as a valid management option of this invasive insect (Moriya *et al.*, 2003; Cooper and Rieske, 2007; Quacchia *et al.*, 2008; Borowiec *et al.*, 2014). Different factors, including the likelihood of hybridization with native *Torymus* and the risks posed to non-target species as recently highlighted by Ferracini *et al.* (2015a), should, however, be taken into account to assess the environmental risks associated with a widespread release of this exotic beneficial insect in the introduction areas. A different strategy, alternative or complementary to classical biocontrol, could be the exploitation of indigenous parasitoids shifting to the ACGW in the areas of introduction, considering that new associations between native parasitoids and novel hosts may even become more effective than the old ones (Hokkanen and Pimentel, 1989). In fact, in old host-parasitoid associations, parasitoids and hosts may develop some degree of balance, whereas in new associations no interspecific balance has been evolved by the host and the parasitoid (Hokkanen and Pimentel, 1984). The parasitism by native natural enemies has also been reported as a possible tool for the management of other exotic insect pests, as recently reported for *Tuta abso-*

luta (Meyrick) (Ferracini *et al*., 2012), and *Cacyreus marshalli* Butler (Dindo *et al*., 2013). New associations between the ACGW and indigenous parasitoids - i.e. *Torymus flavipes* (Walker), *Torymus auratus* (Muller), *Sycophila iracemae* Nieves Aldrey, *Eurytoma brunniventris* Ratzeburg, *Mesopolobus mediterraneus* (Mayr), *Mesopolobus tarsatus* (Nees), *Megastigmus dorsalis* (F.), all antagonists of oak gall wasps - have been recorded in different chestnut areas in Italy (Aebi *et al*., 2006; 2007; Speranza *et al*., 2009; Guerrieri *et al*., 2011; Santi and Maini, 2011; Boriani *et al*., 2013; Matošević and Melika, 2013; Panzavolta *et al*., 2013; Quacchia *et al*., 2013; Palmeri *et al*., 2014). These findings show that, when ACGW becomes established in new environments, native gall parasitoids are rapidly recruited as its enemies (Quacchia *et al*., 2013). The ongoing adaptation of indigenous parasitoids of oak (or other native plant) gall wasps to the ACGW deserves to be monitored in different areas of introduction of the invasive pest and the present study was conducted in this framework.

The high Santerno Valley (Bologna province, Emilia Romagna, northern Italy), which includes the municipalities of Castel del Rio, Borgo Tossignano, Casalfiumanese and Fontanelice, is well-known for the production of high quality marrons from the chestnut ecotype "Marrone di Castel del Rio" P.G.I (Protected Geographical Identification). ACGW was first recorded in Emilia Romagna in 2008 (Graziosi and Santi, 2008) and has now spread throughout the region, including this area (Servizio Fitosanitario Regione Emilia Romagna, 2014). The purpose of our study, carried out in the three-year period 2010-2012, was to investigate the associations occurring between the ACGW and the native parasitoids in a chestnut plantation located in the municipality of Castel del Rio. In detail, our research was aimed at characterizing the native parasitoid complex of ACGW and assessing their relative abundance in the study area. Parasitism rates of the target insect by the native parasitoids were calculated and laboratory data about the timing of emergence of the native parasitoids compared to ACGW were obtained. It has to be stressed that *T. sinensis*, which has been released since 2009 in a wide regional biocontrol programme for the ACGW (Servizio Fitosanitario Regione Emilia Romagna, 2014), had not yet been released in the study area during the surveyed period.

Materials and methods

Study area and sampling time

The study area was a plantation of *Castanea sativa* Miller ecotype "Marrone di Castel del Rio", mixed with other plant species, either spontaneous (in particular *Quercus pubescens* Willd.) or cultivated. It was located at "Località Sestetto" (municipality of Castel del Rio, Bologna province; 44°12'50"N 11°30'15"E; 460 m a.s.l.). The chestnut trees were heavily infested by ACGW.

Collections were performed in the three-year period 2010-2012 in late spring and early summer (table 1). In 2010, samplings were meant to be preliminary, in order to assess the recruitment of native parasitoids from ACGW galls collected in the study area. Since associations were found between the pest and native parasitoids, samplings were continued in 2011 and 2012.

Gall collection and rearing

ACGW galls were collected randomly from chestnut trees at a height < 3 m. For each sampling, the collected galls were transferred to the laboratory, counted and put in 30 × 18 × 10 cm plastic and net boxes (100 galls/box). The boxes were maintained in the laboratory at room temperature (20-25 °C) and checked daily for the emerging insects (either ACGW or parasitoids), which were killed by freezing and counted. The dates of ACGW and parasitoid emergence from the galls were recorded only for the samplings of 2011, because in 2010 the gall collections were meant to be preliminary and too few parasitoids were found in 2012.

Parasitoid identification

The parasitoids were stored in 70% ethanol before identification. They were identified by their morphological characteristics using dichotomous keys (Graham and Gijswijt, 1998; Chinery, 2010) and with the help of experts (R. Askew, U. Bernardo, F. Santi) for dubious specimens. Voucher specimens have been deposited at the University of Bologna, DipSA, Entomology area.

Result evaluation

For each sampling date and year, the parasitism (%) was determined as (ePa/cG × 3.5) × 100, where ePa and cG indicate the number of emerged parasitoid adults and collected galls, respectively. As ACGW galls are multilocular, an average of 3.5 cells/gall was considered following Aebi *et al*. (2011).

Table 1. Chestnut galls collected in the years 2010-2012 at "Località Sestetto" (municipality of Castel del Rio), ACGW and parasitoid adults emerged from the galls in the laboratory and % parasitism per sampling date and per year. The % parasitism was calculated as (number of emerged parasitoid adults/ number of collected galls × 3.5) × 100.

Year	Sampling time	Galls (No.)	Emergence (No.)		% parasitism	
			ACGW	Parasitoids	Per sampling	Per year
2010	June 01	649	409	37	1.63	3.03
	June 16	2,061	927	254	3.52	
2011	May 25	2,287	416	1,272	15.47	10.18
	June 09	2,218	1,221	334	4.30	
2012	May 28	3,500	971	6	0.05	0.09
	June 21	1,300	1,238	9	0.20	

Table 2. Parasitoid taxa attacking ACGW found in the years 2010-2012 at "Località Sestetto" (municipality of Castel del Rio). Identification was performed using morphological characters.

Taxon	Family	2010	2011	2012
Eupelmus spp.	Eupelmidae		x	
Eurytoma brunniventris Ratzeburg	Eurytomidae	x	x	
Sycophila iracemae Nieves Aldrey	Eurytomidae		x	x
Ormyrus pomaceus (Geoffroy)	Ormyridae	x	x	
Mesopolobus spp.	Pteromalidae	x	x	
Mesopolobus amaenus (Walker)	Pteromalidae		x	
Mesopolobus tarsatus (Nees)	Pteromalidae	x	x	
Mesopolobus tibialis (Westwood)	Pteromalidae	x	x	
Megastigmus dorsalis (F.)	Torymidae	x	x	x
Torymus spp.	Torymidae	x	x	x
Torymus flavipes (Walker)	Torymidae	x	x	x
Torymus geranii (Walker)	Torymidae			x

For each year, the relative abundance of parasitoids and of ACGW was calculated as (ePa/ePa+e*D*a) × 100 and (e*D*a/ePa+e*D*a) × 100, respectively, where e*D*a was the number of emerged *Dryocosmus* adults. These parameters were also calculated separately for the two samplings of 2011. By this calculation method, parasitism success may be overestimated compared with the field situation, because laboratory conditions cause a higher adult mortality of ACGW inside galls compared to parasitoids (Ôtake, 1982). Relative abundance however provides an indication of insect emergence.

The relative abundance of identified parasitoid taxa was determined for each year as PT/tIP × 100, where PT and tIP were the number of parasitoids for each taxon and the total number of identified parasitoids, respectively. The relative abundance of *Torymus* spp. was also calculated for each of the first two sampling dates in 2011. Only the emerged parasitoids were considered for this evaluation.

For the year 2011, 2 × 2 contingency tables were used for testing the independence of the sampling date and the relative abundance of 1) parasitoids *vs. D. kuriphilus* and 2) *Torymus* spp. *vs.* other parasitoid taxa. Statistical tests were performed using STATISTICA 10.0 software (StatSoft, 2011).

Results

Emerged parasitoids

In the three years, a total of 12,015 galls were collected (table 1). An overall number of 7,094 insects (5,182 ACGW and 1,912 parasitoids) emerged from the galls. Table 1 shows the parasitism rates (%) found in 2010, 2011, and 2012. The highest percentage occurred in 2011, with a peak in the sampling of May 25. All parasitoids were hymenopterans from the superfamily Chalcidoidea. In 2010, 1,627 insects emerged, the majority of which (1,336) were ACGW while the others (291) were all parasitoids, most belonging to *Torymus* Dalman (Torymidae) (relative abundance = 79.9%). Within this genus, the morphospecies *T. flavipes* was identified. The second most abundant genus (10.3%) was *Mesopolobus* Westwood (Pteromalidae). Individuals were identified as *M. tibialis* (Westwood), and *M. tarsatus*. *Ormyrus pomaceus* (Geoffroy) (Ormyridae) (7.8%), *E. brunniventris* (Eurytomidae) (1.2%), and *M. dorsalis* (Torymidae) (0.8%) were also found.

In 2011, 3,243 insects emerged from the galls and almost half were parasitoids (1,606 *vs*. 1,637 gall wasps). In this year too, the most represented parasitoid genus was *Torymus* (relative abundance = 92.2%) and samples were identified as *T. flavipes*. Other parasitoids belonged to the genus *Mesopolobus* (4.1%) and three species, namely *M. tibialis*, *M. tarsatus*, and *M. amaenus* (Walker), were detected. A very few parasitoids of other taxa were recorded, *i.e. O. pomaceus* (3.1%), *E. brunniventris* (0.1%), *M. dorsalis* (0.3%), *S. iracemae* (Eurytomidae) (0.1%), and *Eupelmus* spp. (0.1%).

Finally, in 2012, 2,224 insects emerged from the galls. Almost all (2,209) were chestnut gall wasps and only 15 were parasitoids. Three parasitoid taxa, namely *Torymus* spp. (relative abundance = 58.3% of all parasitoids), *S. iracemae* (25%) and *M. dorsalis* (16.7%) were detected. Within the genus *Torymus*, samples belonging to *T. flavipes* were found. One specimen was identified as *Torymus geranii* (Walker). Table 2 summarizes the parasitoid taxa attacking ACGW found in the years 2010-2012 in the study area.

Timing of emergence (year 2011)

The data obtained in 2011 give an indication on the timing of insect emergence, though they cannot be considered as fully representative of the field situation, since the galls were all maintained in the laboratory at standard conditions. The highest number of *Torymus* spp. (the most abundant parasitoid taxon) emerged a few weeks after the first field sampling (figure 1A) and a few days after the second (figure 1B). After mid June the number of emerging specimens decreased rapidly. Conversely, ACGW started emerging at the beginning of June and the peak occurred almost one month after the second sampling (figure 1). The ACGW emergence ended in the first decade of July. Of the other taxa (far less abundant than *Torymus*), the genus *Mesopolobus* showed two peaks of emergence, in early and late June. *O. pomaceus* also showed two peaks of emergence, both in mid June (figure 2). The other parasitoid taxa were found too rarely and were thus not considered.

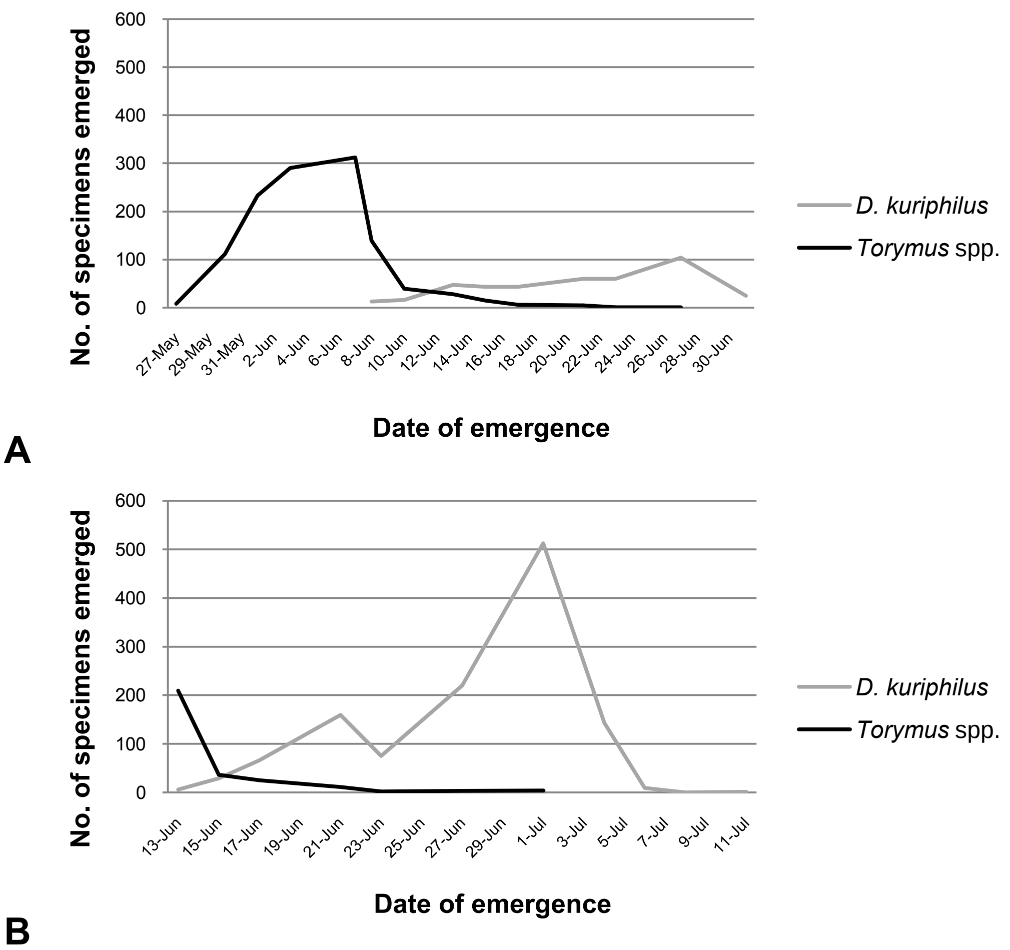

Figure 1. Timing of emergence in the laboratory of ACGW and *Torymus* spp. adults from the galls collected on the first (May 25, A) and second (June 9, B) sampling of the year 2011 at "Località Sestetto" (municipality of Castel del Rio). The graphs show the number of parasitoids found daily in the boxes containing the galls.

Sampling date effect (year 2011)

The sampling date (= date of gall field collection) significantly influenced the relative abundance of parasitoids and ACGW emerged from galls. From the galls collected on June 9, the parasitoid relative abundance (21.49%) was dramatically lower than in the sampling of May 25 (75.36%) (χ^2 = 939.03; df = 1; P = 0.00001). The sampling date also significantly affected the relative abundance of *Torymus* spp. *vs.* the parasitoids belonging to other taxa (χ^2 = 15.57; df = 1; P = 0.0001). In detail, the relative abundance of non-*Torymus* parasitoids increased in the second sampling (13.17%) compared to the first (6.6%).

Discussion

The classical biocontrol approach, based on the importation and release of *T. sinensis*, seems to be a good long-term option to control the ACGW in the areas of introduction, considering the high specificity of interactions occurring between the two wasps (Quacchia *et al*., 2008). In Japan and in North America, *T. sinensis* has shown a great ability to adapt to new environments and to provide effective biological control of the target pest (Gyoutoku and Uemura 1985; Cooper and Rieske, 2007; 2011). Also in Italy, *T. sinensis* is known to have become successfully established in different introduction areas (Gibbs *et al*., 2011), including some sites located in Emilia Romagna (F. Santi, personal communication, May 28, 2014). The efficacy of biological control using this parasitoid for the management of ACGW in Italy aided in restoring a habitat to similar conditions as those observed prior to the pest introduction, even if some potential negative effects on non-target native galls makers present on oaks (*Quercus* spp.) have been recently reported (Bosio *et al*., 2013; Ferracini *et al*., 2015a). Those native parasitoids of gall wasps that are capable of exploiting the ACGW as a host may provide an important contribution to the control of this pest, as emphasised by a number of authors, including recently Matošević and Melika (2013), Panzavolta *et al*. (2013), Quacchia *et al*. (2013) and Palmeri *et al*. (2014).

The present study provides information on the interactions occurring between native parasitoids and ACGW in an area of Emilia Romagna where release of *T. sinensis* had not yet been carried out at the time of our observations. Parasitoids belonging to 7 genera in 5 chalcid families were found to be associated with ACGW galls over the 3-year study period and 9 morphospecies were

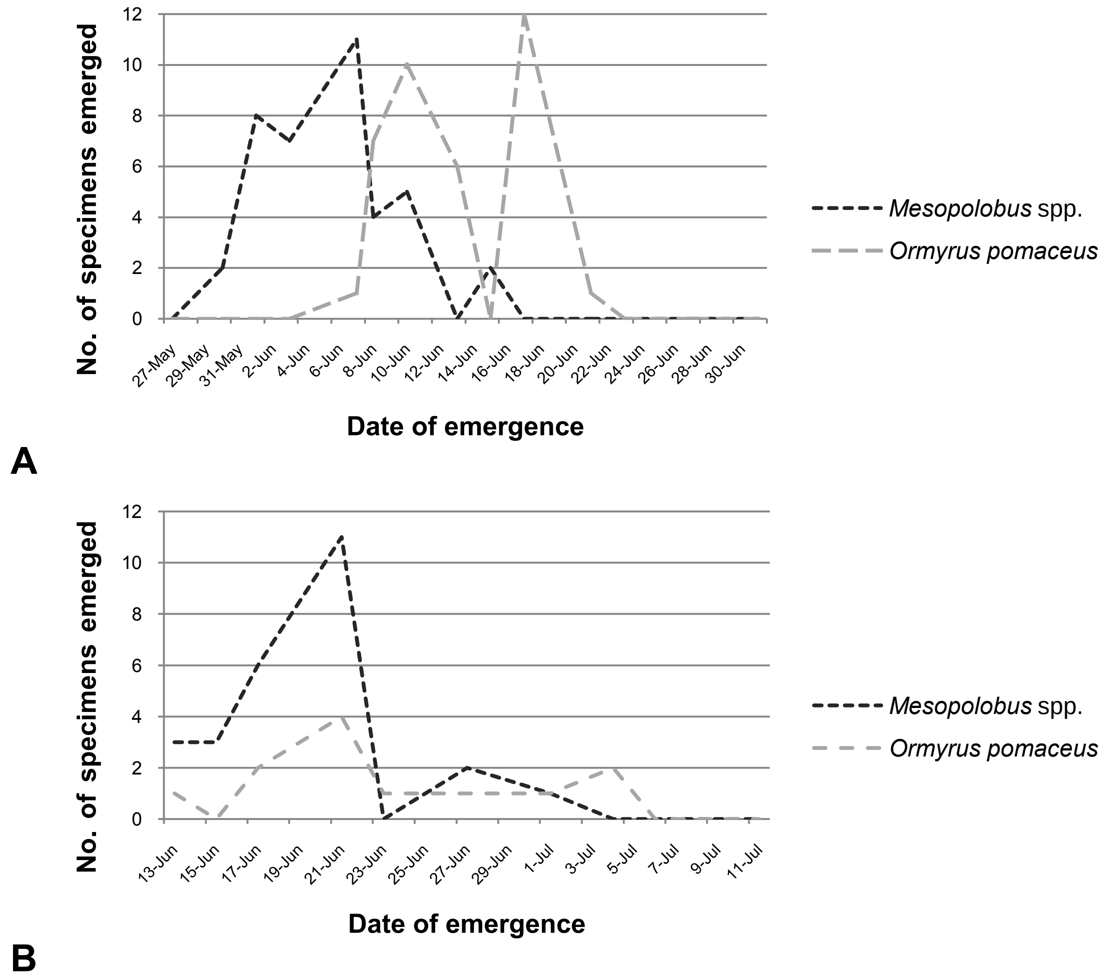

Figure 2. Timing of emergence in the laboratory of *Mesopolobus* spp. and *O. pomaceus* from the galls collected on the first (May 25, A) and second (June 9, B) sampling of the year 2011 at "Località Sestetto" (municipality of Castel del Rio). The graphs show the number of parasitoids found daily in the boxes containing the galls.

identified. All of them are known as parasitoids of oak gall wasps [i.e. *Neuroterus quercusbaccarum* (L.), *Andricus caputmedusae* (Harting) and *Cynips quercusfolii* (L.)] (Noyes, 2014), although members of the genus *Ormyrus* and *Eupelmus* may also be facultative hyperparasitoids (Cooper and Rieske, 2011; Quacchia *et al*., 2013). *E. brunniventris* is known to be polyphagous and to feed also on gall tissues (Askew *et al*., 2013).

All parasitoid species had already been recorded as associated with ACGW in Italy (Santi and Maini, 2012; Quacchia *et al*., 2013). In our study, most species were found in a low number, but *T. flavipes* has shown a great ability to exploit the ACGW, thus confirming the finding of Santi and Maini (2011). Parasitoids were only morphologically identified, but the evidence of cryptic taxa in insect lineages and more specifically within both gall inducing and parasitic members of the oak gall community has been already pointed out in literature (Nicholls *et al*., 2010; Guerrieri *et al*., 2011; Quacchia *et al*., 2013). That is why a comprehensive molecular genetic analysis will help defining the role of cryptic species, in order to avoid potential mis-identications of the parasitoid community emerged.

The low number of non-*Torymus* parasitoids found in the 3-year research may indicate that ACGW is less suitable for them compared to *Torymus* spp. Given the large number of *Torymus* spp. present in the study area (at least in 2011), competition may have occurred among the different parasitoid species. On the other hand, low specificity may enable also the non-*Torymus* parasitoids to better exploit ACGW in the near future (Quacchia *et al*., 2013).

From 2010 to 2011 the number of parasitoids and the percentage of parasitism increased rapidly. In 2011, the second sampling produced a dramatically lower relative abundance of *Torymus* spp. and other parasitoids compared to the first sampling, probably because it was carried out after the emergence of most of these beneficial insects. Conversely, the dramatic reduction of parasitoids found in 2012 is unexplained. It might have been a consequence of biocoenotic dynamics, in particular of the instability of the interactions occurring between the ACGW and the generalist native parasitoids. An insufficient synchronization between the development of the exotic pest and the native parasitoids might also be hypothesized, considering that, as reported by Washburn and Cornell (1981), the generation time of many parasitoids of galls is shorter than the generation time of the host gall wasp. Moreover, the host shift of native parasitoids to a new invasive host may not necessarily result in prompt adaptation. According to the "Adjustment Hypothesis" a lag between introduction of an exotic species and recruitment of native parasitoids can be predicted, as parasitoids adapt to the novel host (Grabenweger *et al*., 2010). It is also possible that the parasitoids were killed by fungi which invaded galls, as observed for other gall wasps (Wilson, 1995) and ACGW itself (Addario and Turchetti, 2011).

The area of our research was a plantation dominated by *C. sativa*, but containing other plants (trees, shrubs and herbaceous plants). Among them oaks are hosts to a complex of gall wasps which can, in turn, be parasitized by the native parasitoid species found, all displaying a wide host range (Noyes, 2014). These parasitoids (including *T. flavipes*) have shown the ability to develop on ACGW larvae when the galls are fresh, but their destiny after emergence is still unknown. They may have a second generation and overwinter in oak (or other plant) galls, but this hypothesis has not been tested yet. Little information exists on the biology of the indigenous parasitoids found. Future research should thus be aimed at improving knowledge on these beneficial insects. The population dynamics of oak gallwasps has however the most important influence on the parasitoid recruitment of ACGW galls (EFSA Panel on Plant Health, 2010). Studies are currently in progress to investigate the relationship occurring between the number of oak galls and the level of parasitoid recruitment, so as to obtain useful information for chestnut plantation management.

Relatively scant information is also available on the exotic parasitoid *T. sinensis* itself. This species was described quite recently (in 1982) and since then its biological cycle (highly synchronized with that of *D. kuriphilus*) has been studied (Alma and Quacchia, 2012). Recently, Ferracini *et al*. (2015b) reported that, since the first release of *T. sinensis* in Piedmont (North Western Italy) in 2005, the presence of ACGW in the area has severely decreased. The authors suggested that host shortage may at least partially explain the prolonged diapause (and the two-year cycle) shown by some *T. sinensis* specimens in the study area, a strategy which rarely occurs for the native, generalist parasitoids associated with gall wasps (Ferracini *et al*., 2015b).

The complex of parasitoids found includes species which have also been detected in other regions of Italy (Quacchia *et al*., 2013; Palmeri *et al*., 2014). In other areas of Italy different parasitoid species may be capable of exploiting this host.

In general, the exploitation of native natural enemies can be complementary to classical biological control. But even if in all the countries invaded by the pest a rich parasitoid community has been reported, the attack rates have remained low (Aebi *et al*., 2007; Gibbs *et al*., 2011; Quacchia *et al*., 2013; Alma *et al*., 2015). Hence, more research on the parasitoids involved in the new association with the chestnut gall wasp is required. In particular, in the sites where *T. sinensis* has been released (now including our study area), the interactions between the exotic and native parasitoid species need to be investigated.

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