Native parasitoids associated with Dryocosmus kuriphilus in Tuscany, Italy

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Abstract

The invasive Dryocosmus kuriphilus Yasumatsu (Hymenoptera Cynipidae), the Asian chestnut gall wasp (ACGW), is a major pest of Castanea spp. in Asia, the USA and Europe. The aim of this study is to characterize the parasitoid community associated to D. kuriphilus in Tuscany and determine their relative occurrence, before the introduction of the exotic parasitoid Torymus sinensis Kamijo. Ten hymenopteran parasitoids were collected from D. kuriphilus galls. Mesopolobus fasciventris Westwood is reported for the first time as a D. kuriphilus associate in Italy. Torymus flavipes (Walker) specimens accounted for more than 50% of all collected parasitoids. Facultative hyperparasitoid species were also collected. Furthermore, a higher overall parasitism rate was found on wild chestnut galls (with respect to cultivated ones) and where oak trees were more abundant.

Key words: Asian chestnut gall wasp, cynipid, parasitism, hyperparasitoids, Mesopolobus fasciventris, Torymus flavipes.

Introduction

The invasive Dryocosmus kuriphilus Yasumatsu (Hymenoptera Cynipidae), the Asian chestnut gall wasp (ACGW), is a major pest of chestnuts, Castanea spp. (Fagaceae), in its area of origin (China) (Zhang et al., 2009), as well as in areas of distribution. Since 1941 it has been reported in neighbouring Countries, first in Japan and later in Korea, where it has caused significant reductions in chestnut yields (Payne et al., 1983; Zhang et al., 2009). The ACGW was recorded in the USA in 1974, first on Chinese chestnuts and, later, also on American chestnuts (Rieske, 2007); in Europe, it was found on the European chestnut Castanea sativa Miller in 2002 (Brussino et al., 2002).

Torymus sinensis Kamijo (Hymenoptera Torymidae), ACGW’s parasitoid native to China like its host, was introduced into several Countries to control the invasive pest. Following the experience matured in Japan (Moriya et al., 2003) and in the USA (Rieske, 2007), the introduction of this parasitoid has been considered as a sustainable and effective approach for controlling ACGW. Indeed, this parasitoid has been highly successful in reducing the pest population below the economic threshold in Japan (Murakami et al., 2001; Moriya et al., 2003), and may have also affected ACGW populations in the USA (Rieske, 2007). In 2005 T. sinensis was introduced in Italy, first in Piedmont (Aebi et al., 2007; Quacchia et al., 2008), and, later, in many other Italian Regions, including Tuscany in 2010.

Some doubts have recently been raised about continuing biological control using T. sinensis in Europe (EFSA Panel on Plant Health, 2010; Aebi et al., 2011; Gibbs et al., 2011), because of potential adverse effects. For example, negative interactions might evolve with native parasitoids, leading to their displacement, as reported by Yara et al. (2007). Moreover, T. sinensis might hybridize with native European species of Torymus, as has occurred in Japan with Torymus beneficus Yasumatsu et Kamijo (Moriya et al., 1992; Moriya et al., 2003; Yara et al., 2000; Yara et al., 2010). Finally, few studies focusing on the actual host range of T. sinensis in the new introduction areas have been carried out (EFSA Panel on Plant Health, 2010; Aebi et al., 2011). Although T. sinensis is reported as a generalist parasitoid in China, it seemed to be host specific in Japan (Murakami et al., 1977). However, its host specificity was probably due to the high ACGW population density in the introduction areas, while the same parasitoid could behave differently in ecosystems where several other hosts are largely available (Stone et al., 2002).

Soon after its introduction in Italy, ACGW was used as a suitable host by several native parasitoids that promptly initiated new tritrophic interactions (Speranza et al., 2009; Benvenüti, 2011; Guerrieri et al., 2011; Santi and Maini, 2011; Pollini, 2012; Quacchia et al., 2013). Native parasitoids could be used in biological control to reduce ACGW population density below the economic damage threshold (Aebi et al., 2007), particularly where biotic and abiotic factors make T. sinensis ineffective. For example, both Japanese and North American authors have showed how facultative hyperparasitoids negatively affect T. sinensis population density (Murakami and Gyoutoku, 1995; Cooper and Rieske, 2007, 2011a).

As highlighted by the European Food Safety Authority (EFSA Panel on Plant Health, 2010), more studies are needed to assess the possible negative effects of ACGW biological control based on the introduction of T. sinensis. Similarly, the role and possible use of native parasitoids for ACGW control still need a proper definition (Aebi et al., 2011). The aim of our research was to characterize the complex of native parasitoids associating to the ACGW in areas of Tuscany where T. sinensis had not yet been released and to determine their relative abundance. This information is crucial to assess the im-
pact of *T. sinensis* on ACGW native parasitoid communities once the exotic parasitoid colonizes these areas, either naturally or by future introductions.

Materials and methods

Study sites

Three chestnut forests were surveyed in Tuscany one year after the first record of ACGW in those sites. Castelpoggio (province of Carrara, Italy; 44°07′04″N, 10°03′59″E; 665 m a.s.l.): ACGW was first recorded in 2008. A survey of natural enemies was carried out in 2009. The site was a wild *C. sativa* forest (coppice and high-forest) mixed with sporadic oaks (mainly *Quercus cerris* L.), with a western exposure. Marradi A (province of Florence, Italy; 44°04′23″N, 11°38′00″E; 478 m a.s.l.): ACGW was first recorded in 2009. A survey of natural enemies was carried out in 2010. The site was a plantation of *C. sativa* cultivar “Marron Buono di Marradi” with a northern exposure, close to annual crop fields and to woodlands of coppice and high-forest wild chestnuts, with sporadic European hornbeams and oaks. Marradi B (province of Florence, Italy; 44°03′35″N, 11°38′54″E; 613 m a.s.l.): ACGW was first recorded in 2009. A survey of natural enemies was carried out in 2010. The site was a plantation of *C. sativa* cultivar “Marron Buono di Marradi” mixed with coppice and high-forest wild chestnuts with a western exposure, surrounded by wide mixed forests dominated by oaks (*Quercus pubescens* Willd. and *Q. cerris*).

*T. sinensis* was first released in spring 2010 in chestnut forests of Castelpoggio and Marradi. In both areas, 165 specimens were released (Tuscany Regional Committee Resolution n. 646 of 5 July 2010) in sites located at least 1 km from our study sites. Marradi A and Marradi B were also separated from the release sites by mountain ridges.

Gall collection and rearing

Castelpoggio: a total of 150 chestnut trees were selected along three 500-m-long transects at intervals of 10 m. Current-generation galls were collected on 5 and 19 June 2009. On each sampling date, three galls per tree were collected (900 galls in total); galls were picked randomly at a height < 3 m. After collection each gall was dissected in the laboratory to record the number of cells and the content of each cell. Adult parasitoids found in the cells were promptly placed in absolute ethanol and stored at −20 °C. After the end of the emergences of both ACGWs and parasitoids, each gall was dissected to record the number of cells and the content of each cell.

Parasitoid identification

Castelpoggio: Dr. George Melika (Pest Diagnostic Department, Plant Protection & Soil Conservation Directorate of County Vas Tanakajd, Hungary) identified the parasitoid specimens.

Marradi: Parasitoid specimens were identified using available dichotomous keys (Askew, 1961; Graham, 1969; Bouček, 1970; Pujade-Villar, 1993, Graham and Gijswijt, 1998; Askew and Thuroczy, personal communication) and by comparison with type material and museum specimens. DNA was extracted from single specimens by a non-destructive method (Gebiola et al., 2009) and three genes were sequenced (COI, 28S, ITS2) following Gebiola et al. (2010). Molecular and morphological data were combined to obtain the final identification. The sex-ratio (expressed as females/males) was calculated for each species.

*D. kuriphilus* flight activity

On 10 June 2010, four adhesive yellow traps were placed at Marradi B and were replaced weekly until 20 August 2010. Traps were examined in the laboratory to record the number of ACGW captures.

Statistical analyses

The mean number of cells per gall between the three collection sites were compared by one-way ANOVA using Bonferroni post-hoc comparison. Two-way ANOVA was used to compare the mean number of cells per gall between cultivated and wild chestnuts from the two Marradi sites. The number of cells per gall was log-transformed to meet requirements of homogeneity and homoscedasticity. Proportions of parasitized cells between collection sites were compared by chi-square test, applying an orthogonal chi-decomposition according to Kimball’s (1954) guidelines. Proportions of parasitized cells between cultivated and wild chestnuts from the two Marradi sites were compared by G test (Sokal and Rohlf, 1995).

Results

The mean number of cells per gall was significantly different among the three study sites (one-way ANOVA, df = 2, *F* = 21.18, *P* < 0.001). The value was higher at Castelpoggio (4.12 ± 0.11 SE) than at either Marradi A (2.91 ± 0.10 SE; Bonferroni test, *P* < 0.001) or Marradi B (3.14 ± 0.10 SE; Bonferroni test, *P* < 0.001). In contrast, no differences were recorded between the two sites at Marradi (Bonferroni test, *P* = 0.396). A higher mean number of cells was recorded on cultivated chestnuts (3.29 ± 0.11 SE) in respect to wild chestnuts (2.99 ± 0.10 SE) from both sites at Marradi (two-way ANOVA, df = 1, *F* = 4.31, *P* = 0.039).

Living ACGW larvae were found in 93.14% of the cells at Castelpoggio. The total ACGW mortality within the galls was 6.86%; empty cells were 6.35% and over-
all parasitism rate (parasitized cells / total number of cells × 100) was 0.51% (table 1). Nineteen parasitoid specimens were recorded, 11 of which were adults belonging to six species of three Hymenoptera families (Torymidae, Pteromalidae and Eupelmidae) (table 1). Parasitoid identification was not possible for the preimaginal stages (eight specimens).

From galls collected at Marradi A and B in 2010, a total of 977 ACGW adults and 59 adult parasitoids emerged. ACGW adults emerged from 45.25% of the cells counted during gall dissection, whereas dead ACGW adults were recorded in 43.26% of the cells. Dead ACGW adults inside the cells were not considered in the mortality assessment because laboratory conditions may have affected galls, causing a higher adult mortality during emergence (Ötake, 1982). Total ACGW mortality was 11.49%; empty cells were 8.11% and overall parasitism rate was 3.38%. The parasitism rate at Marradi was calculated by taking into account the number of cells containing parasitoid exuviae, easily distinguishable from those of ACGW.

The total parasitism rate recorded at Marradi B (5.74%) was significantly higher than those at Marradi A (0.60%) and Castelpoggio (0.51%) (table 1). Fifty-nine parasitoids emerged from Marradi A and B galls, for a total of six parasitoid species belonging to five different Hymenoptera families (Torymidae, Eurytomidae, Pteromalidae, Eupelmidae and Ormyridae) (table 1). Differences emerged between the two sites: at Marradi A, only two parasitoid species were collected reflecting a low number of community associates; conversely, at Marradi B, five parasitoid species were collected (table 1). *Torymus flavipes* (Walker) was the only species collected in all three study sites (table 1). *T. flavipes* sex ratio at Marradi (A and B together) was 5.67. *Eupelmus urozonus* Dalman was collected in two out of three sites; its occurrence was higher at Marradi B than at Castelpoggio (table 1). Its sex ratio was 2. All other identified species were collected each from one site only. A single male of the genus *Ormyrus* was collected at Marradi A (table 1). Adult occurrence of *T. flavipes* at Marradi B was the highest recorded in all study sites, followed by that of *Mesopolobus tibialis* (Westwood), also at Marradi B. Among the parasitoids recorded at Castelpoggio, *Mesopolobus tarsatus* (Nees) was the most abundant (table 1).

In the laboratory, ACGW adult emergence started on 28 June 2010 and lasted until the end of July (figure 1), virtually overlapping with field emergences. This result is in line with what observed in other Regions of Italy (Bernardo et al., 2013). Indeed, most of ACGW emergences were recorded from 7 to 22 July 2010, both in laboratory and field conditions (96.59% and 94.50% respectively). Adult parasitoids emerged in the laboratory from 21 June to 14 July 2010, a week before ACGW (figure 1). *Ormyrus* sp., *M. tibialis* and *T. flavipes* were the first parasitoids to emerge in the laboratory. *T. flavipes* emergence reached its peak on 28 June 2010 and lasted until 14 July (figure 1). The first *Eupelmus* emerged on 30 June 2010, and more specifically *E. urozonus* emergence occurred until 12 July.

Finally, a significantly greater number of parasitoids were recorded in galls collected at Marradi on wild chestnuts than on cultivated ones (table 2). Here differences emerged between the two Marradi sites, in that parasitoids at Marradi A were lower than those recorded at Marradi B both considering wild chestnuts or cultivated chestnuts (table 2).

### Discussion

Our study extends our knowledge about the parasitoid community associated to *D. kuriphilus* in Tuscany before

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**Table 1.** Native parasitoids from *D. kuriphilus* galls collected in Tuscany (Italy) at Castelpoggio (Carrara) and Marradi (A and B) (Florence). n = total number of cells examined.

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Family</th>
<th>Castelpoggio</th>
<th>Marradi A</th>
<th>Marradi B</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Torymus flavipes</em> (Walker)</td>
<td>Torymidae</td>
<td>0.05</td>
<td>0.30</td>
<td>3.17</td>
</tr>
<tr>
<td><em>Torymus auratus</em> (Muller)</td>
<td>Torymidae</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Megastigmus dorsalis</em> (F.)</td>
<td>Torymidae</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Eurytoma bruniventris</em> Ratzeburg</td>
<td>Eurytomidae</td>
<td>-</td>
<td>-</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Mesopolobus tarsatus</em> (Nees)</td>
<td>Pteromalidae</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Mesopolobus tibialis</em> (Westwood)</td>
<td>Pteromalidae</td>
<td>-</td>
<td>-</td>
<td>0.69</td>
</tr>
<tr>
<td><em>Mesopolobus fasciiventris</em> Westwood</td>
<td>Pteromalidae</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Eupelmus urozonus</em> Dalman</td>
<td>Eupelmidae</td>
<td>0.05</td>
<td>-</td>
<td>0.51</td>
</tr>
<tr>
<td><em>Eupelmus annulatus</em> (Nees)</td>
<td>Eupelmidae</td>
<td>-</td>
<td>-</td>
<td>0.09</td>
</tr>
<tr>
<td><em>Ormyrus</em> sp.</td>
<td>Ormyridae</td>
<td>-</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Undetermined</td>
<td></td>
<td>0.22</td>
<td>0.10</td>
<td>1.03</td>
</tr>
<tr>
<td>Total parasitism rates</td>
<td></td>
<td>0.51</td>
<td>0.60</td>
<td>5.74</td>
</tr>
</tbody>
</table>

$\chi^2$ and Kimball’s tests results

<table>
<thead>
<tr>
<th>Comparisons of total parasitism rates</th>
<th>$\chi^2$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marradi A vs Marradi B</td>
<td>71.758</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Castelpoggio vs (Marradi A + B)</td>
<td>72.941</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>164.699</td>
<td>2</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Figure 1. Number of *D. kuriphilus* native parasitoids emerged in the laboratory from galls collected in Tuscany (Italy) at Marradi A and B (Florence).

Table 2. Overall parasitism rates of *D. kuriphilus* by native parasitoids in cultivated and wild chestnut galls collected in Tuscany (Italy) at Marradi A and B (Florence).

<table>
<thead>
<tr>
<th>Parasitism rates (%)</th>
<th>Marradi A</th>
<th>Marradi B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivated chestnut galls (CCG)</td>
<td>0.32</td>
<td>3.97</td>
<td>2.12</td>
</tr>
<tr>
<td>Wild chestnut galls (WCG)</td>
<td>1.08</td>
<td>7.65</td>
<td>5.04</td>
</tr>
</tbody>
</table>

G test results

Comparisons of parasitism rates

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCG versus WCG</td>
<td>13.747</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CCG: Marradi A versus Marradi B</td>
<td>22.973</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WCG: Marradi A versus Marradi B</td>
<td>24.542</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>61.262</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

the introduction of the exotic parasitoid *T. sinensis*. Indeed, the parasitoid had not yet been introduced at Castelpoggio and it was released at the time of our samplings (2010) in chestnut forests located at least 1 km from our study sites at Marradi. It is unlikely that the parasitoid had already reached Marradi sites at the time of gall sampling. Indeed, the natural spread of *T. sinensis* is very low during the first three years after release (less than 1 km / year) (Moriya *et al.*, 2003). Furthermore, mountain ridges separated release sites from our study sites at Marradi, and a very low number of specimens were released. Our observations may help in assessing the interactions among native parasitoids of ACGW and *T. sinensis*, when the latter will reach our study sites.

We were able to identify most of the parasitoid-abandoned cells. Ôtake *et al.* (1982) stated that parasitoid- and ACGW-abandoned cells were indistinguishable.
parasitoids have been observed in Japan (Ôtake 1982; Ôtake, 1989). Because high annual fluctuations in parasitism by native parasitoids will be able to control ACGW population density, further surveys are needed at the same study site to verify if native parasitoids were associated with ACGW in Italy (Speranza et al., 2009; Santi and Maini, 2011; Pollini, 2012; Quacchia et al., 2013), whereas Mesopolobus fasciventris Westwood is reported from the first time as an ACGW associate in Italy. More than 50% of the adult parasitoids collected were identified as T. flavipes. This is a common species attacking several oak gall wasps (Noyes, 2012). The second-most-abundant species was M. tibialis, known to develop on numerous gall wasps on different plant species (Noyes, 2012), followed by E. urozonus, both from Castelpoggio and Marradi galls. E. urozonus can develop either as a primary parasitoid or as a hyperparasitoid (Murakami et al., 1995) and has a wide host range (Yasumatsu and Kamijo, 1979). The occurrence of other species was sporadic, including that of M. fasciventris, frequently reported as a parasitoid of oak gall wasps (Askew, 1961). Within just one year from the first ACGW record, in each of the three study sites, up to six native parasitoid species generally associated with oak gall wasps had already adapted to this new host on chestnut trees. The recruitment of several parasitoid species in such a short period of time is in agreement with other European studies, which showed a similar time lag between the introduction of D. kuriphilus and the development of a native parasitoid community (Aebi et al., 2006; Mateščič and Melika, 2013).

The higher overall parasitism rate we recorded at Marradi B could be related to the larger presence of oak trees in this site. Except Eupelmus species, all parasitoids recorded on ACGW are known to associate mainly with oak gall wasps (Aebi et al., 2006). The parasitism rate observed at Marradi B was not high, but encouraging. Whereas in some areas of Italy the native parasitoid emergence rate is typically 2-3 specimens per 100 galls (Quacchia et al., 2013), in Marradi B the rate was 16 specimens per 100 galls. However, further surveys are needed at the same study site to verify if native parasitoids will be able to control ACGW population density, because high annual fluctuations in parasitism by native parasitoids have been observed in Japan (Ôtake et al., 1982; Ôtake, 1989).

The overall parasitism rate on wild chestnut galls was higher than on cultivated chestnut galls. This could be due to the higher number of oaks present near the wild chestnuts in both Marradi A and Marradi B. However, it could be also due to the lower gall size recorded on wild chestnuts, in agreement with other authors (Ôtake et al., 1982; Cooper and Rieske, 2010; 2011a; Bernardo et al., 2013) who stated that parasitism is negatively correlated with the gall size. However, multiple-year studies are needed because the gall size can vary annually at the same site (Panzavolta et al., 2012; Bernardo et al., 2013). Furthermore, during the gall samplings, differences in tree phenology were observed, inasmuch as budburst in wild and in cultivated chestnut trees occurs in different times and this may have affected gall parasitism, as suggested by Cooper and Rieske (2011b) for Chinese and American chestnuts. The infection by parasitic fungi could be a limiting factor on ACGW population and its parasitoid complex (Addario and Turchetti, 2011).

In our study, the presence of native insects reported as facultative hyperparasitoids, such as Eurytoma bruniventris Ratzburg, E. urozonus and Eupelmus annulatus (Nees) (Murakami and Gyoukoto, 1995; Murakami et al., 1995), was recorded. Specifically, E. urozonus and E. bruniventris had an important role in the delay in the population increment of the introduced T. sinensis in some areas of Japan (Murakami and Gyoukoto, 1995). Therefore, investigating the presence and density of these hyperparasitoids could help in predicting the results of biological control with T. sinensis (Murakami et al., 1994).

Among the species collected, T. flavipes was not only the most abundant but also the only one collected in all three study sites. This species has recently been recorded, from the same host, in the nearby Emilia-Romagna region (Santi and Maini, 2011); moreover, it is apparently the dominant species in other regions (personal observations by Guerrieri E.). In our laboratory rearing (2010) T. flavipes emerged from galls about one week before ACGW adults. This result is concordant with that of Santi and Maini (2011), though they recorded insect emergences in the field. Laboratory conditions seemed not to affect ACGW emergence period which virtually overlapped with that recorded in the field. It is possible to speculate that the same could apply to parasitoid emergence as well. The role of T. flavipes in ACGW control needs to be assessed carefully. Further research should focus on trophic relationships of T. flavipes both with oak gall wasps and ACGW.

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