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New association between *Dryocosmus kuriphilus* and *Torymus flavipes* in chestnut trees in the Bologna area (Italy): first results

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Abstract

Investigations into the new association between the recently introduced insect pest *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae), the Asian chestnut gall wasp (ACGW), and the native parasitoid *Torymus flavipes* (Walker) (Hymenoptera Torymidae) were carried out in the Bologna district (Italy). The parasitoid attacks several species of gall-maker host larvae living on plants and trees, particularly oaks. In 2009, *T. flavipes* was already found to be parasitising the new host ACGW on chestnut trees. During 2010 and 2011 the *T. flavipes* parasitisation percentages, its behaviour on host larvae, its emergence trend, and its sex ratio on the non-native ACGW host were analysed.

Key words: natural control, *Torymus flavipes*, *Dryocosmus kuriphilus*, *Castanea sativa*, Asian chestnut gall wasp.

Introduction

The Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) attacks plants of the *Castanea* genus. The insect pest, considered native to China (Oho and Shimura, 1970; Oho and Umeya, 1975), was introduced and described for the first time in Japan (Yasumatsu, 1951) and is now present in other Asian countries. It was accidentally introduced into the USA (Payne *et al*., 1975) and in 2002 was also recorded in Piedmont near Cuneo (Northern Italy) (Brussino *et al*., 2002). ACGW is now established and is spreading throughout Italy and other European Countries (Bosio *et al*., 2010; Graziosi and Santi, 2008). To try to reduce the rapid colonisation of ACGW on all the areas of Italian chestnut production a classical biological control was initiated following a program co-ordinated by entomologists of Turin University. The candidate, as a beneficial insect to be inoculated, reared and, possibly established, was *Torymus sinensis* Kamijo (Hymenoptera Torymidae), known as a polyphagous parasitoid of gall-maker insects in China but considered specific to ACGW in the cultivated chestnut areas of release (Stone *et al*., 2002; Aebi *et al*., 2007; Quacchia *et al*., 2008; 2010). The success of the biological control and the possibility to rear *T. sinensis* (a strain obtained from Japan) and release it in selected chestnut areas in Italy was accompanied by investigations to find possible new associations between ACGW and polyphagous or olygophagous wild native parasitoids. In particular the parasitoids of gall wasp species attacking oaks were linked to a new association with the accidentally introduced ACGW (Aebi *et al.*, 2007; Speranza *et al*., 2009). From 2002 till now, 30 species of Hymenoptera Chalcidoidea (from 6 different families) parasitising ACGW have been identified in Italy (Quacchia *et al*., 2011).

Among these species the Torymidae *Torymus flavipes* (Walker) has, since 2009 (as soon as ACGW had arrived at a significant population in our region), appeared to be the parasitoid most frequently emerging from chestnut galls. Therefore during the 2010 and 2011 seasons, a series of observations were conducted in order to obtain data regarding the biology, behaviour and parasitisation of this native parasitoid on the new insect pest ACGW.

Materials and methods

The main investigations were carried out in a chestnut field located at Monte delle Formiche (44°19'16,02"N 11°23'10,83"E 595 m above sea level), municipality of Pianoro near Bologna (Italy), an area far from the inoculation points of *T. sinensis* strain reared by colleagues from Turin University and released by technicians of the Servizio Fitopatologico Regionale in accordance with our group, the colleagues from Turin University and other groups from a national project regarding the biocontrol against ACGW. The chestnut trees (*Castanea sativa* Miller) in which the galls were sampled were cultivated for both timber and fruit production. In the surrounding area no crops were present and the vegetation complex mainly consisted of oaks, alders, maples, beech and ash trees, and shrubs.

A second area of collection was near Paderno (a locality a few kms away from Monte delle Formiche, Bologna) (44°27'05.0"N 11°19'37.6"E 250 m above sea level) in an abandoned chestnut stand.

The biodiversity of these two ecosystems, composed of a wide variety of botanical species, could possibly represent a rich environment for numerous species of gall wasps and relative primary and secondary parasitoids. It was therefore hypothesized that these gall wasp parasitoids could set up a new association with ACGW. In April 2009 a preliminary sample of 100 galls was collected from the chestnut located in Paderno.

Then, at Monte delle Formiche, starting from the first day of April 2010, 20 ACGW galls were collected weekly for dissection in order to follow the ACGW larvae development and the parasitisation activity of

T. flavipes. So due to these first observation the date of gall collection was planned.

On May 19, 2010 and May 18, 2011, 4 replicates of 25 galls were collected respectively. Each sample was taken at random from different points of the chestnut field (from 0.00 to 2.00 m above the ground). These galls and gall chambers were then analysed internally to find and observe ACGW larvae and to identify those possibly parasitised by *T. flavipes* (other parasitoid species may have been present, but in numbers quite insignificant with respect to the ensuing data on *T. flavipes* emergence in the period considered – April and May). The eggs or larvae of *T. flavipes* (a typical idiobiont ectoparasitoid) were observed inside ACGW gall chambers and gall tissue. In each replicate the 25 galls were counted and examined up to a total of 100 larvae (considered as representative sample).

To obtain more data on *T. flavipes* emergence, a further sample of 500 galls was collected, on June 8, 2010, in the same manner from a chestnut stand in Paderno. The galls were maintained in cardboard boxes with extractable skylights. The boxes were placed in an open environment at the border of the chestnut field protected from direct sunlight by a simple wooden roof. Every day the boxes and transparent vials were examined counting the emergence of parasitoid adults. One week after the last observed *T. flavipes* emergence, the sampling was stopped. An identical methodology was followed during the second year of observations. The 500 gall sample was collected at Monte delle Formiche on May 20, 2011. The only new data considered was the count of the parasitoid sex ratio and the number of galls in which the parasitoid was found inside. Data were analysed by chi square test.

Results and discussion

The first record of *T. flavipes* emergence from AGGW in the Bologna area was in spring 2009.

In 2010 the parasitisation of ACGW larvae was only 3% compared to the data from 2011 where the parasitisation was much higher, reaching 31.75 % (table 1). The increase may be explained by a good capability of *T. flavipes* to find the new host in chestnut galls. However, we also suspect that the increase in parasitisation by *T. flavipes* may be related not only to the increase in the ACGW population but also to the good possibility to attack ACGW larvae at a susceptible stage. In several cases more than one egg or larva of the parasitoid were found inside the gall chamber made by a single host ACGW larva. When the parasitoid larvae were near pupation they were observed boring a tunnel and preparing their own pupation chamber (figure 1). Although native parasitoids of gall-maker cynipids may feed on oak gall tissue (Gómez *et al*., 2011), this behaviour inside the chestnut gall seems to be typical of *T. flavipes* and therefore during the dissection of chestnut galls this characteristic trait could represent a diagnostic feature.

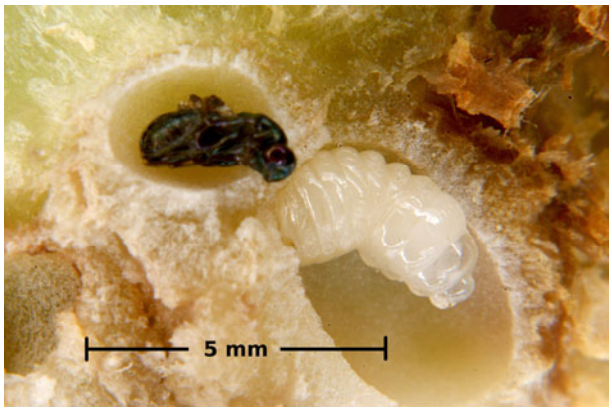

Figure 1. *T. flavipes* pupa in its own constructed pupal chamber (left). Un-parasitised mature ACGW larva inside its chamber in the gall (right).
(In colour at www.bulletinofinsectology.org)

The total number of adults emerging from the Paderno locality in 2010 was 177 from 500 galls. The total number of adults emerging from the 500 galls collected in 2011 at Monte delle Formiche was 397 (102 males and 295 females, sex ratio, female/males 2.89).

The trend of adult emergence of *T. flavipes* was similar in 2010 and 2011. The emergence started at the end of May, then the maximum peak was registered in the first 10 days of June and ended in the third week of June. In figure 2 the slight proterandry can be observed.

In the chestnut field the first *T. flavipes* females parasitising ACGW were observed on April 26, 2011. The parasitisation activity continued till the middle of May. The start of ACGW adult emergence was in the middle of June in 2010 and on June 8 in 2011.

Other parasitoid species were found: 5 specimens of *Mesopolobus* sp. and another 2 not yet identified. However, the difficulty in defining the correct percentage of parasitisation when counting parasitoid adult vs ACGW adult emergence should be taken into account and we can therefore simply report that increasing levels of parasitism were observed in 2011 with respect to 2010. In fact the parasitism percentage calculated in the boxes was different compared to that obtained from the direct check of ACGW host larvae and ectophagous parasitoid larvae found on dissecting the galls. It was noticed, for instance, that some *T. flavipes* died because they were not able to emerge from the pupation chamber.

Table 1. ACGW larvae parasitised by *T. flavipes* in galls collected at Monte delle Formiche. Number 100 larvae per replicate each year ($P < 0.01$; chi square test).

Date	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
19 May 2010	4	2	4	2	3.00%
18 May 2011	36	30	39	22	31.75%

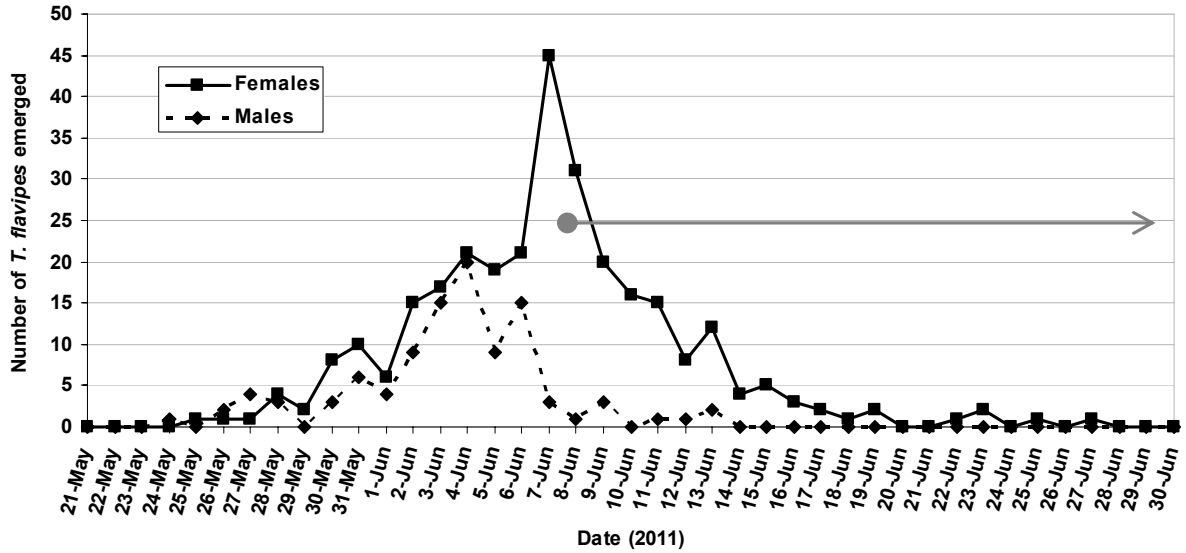

Figure 2. Emergence dates of *T. flavipes* at Monte delle Formiche (year 2011). The first appearance of ACGW adults (June 8) and subsequent days of emergence are represented by arrow.

Furthermore the mortality could also be attributed to the presence of fungi or other factors affecting the vitality of both the host and parasitoid larvae (Cooper and Rieske, 2007; 2010; Magro *et al*., 2010; Addario and Turchetti, 2011).

Conclusions

Many new associations between ACGW and indigenous parasitoids have been observed in Italy (Aebi *et al*., 2006; 2007; 2011). However the majority of the parasitoid species investigated seem to arrive late in the spring, probably encountering the host at an unsuitable stage for parasitisation. Conversely *T. flavipes* adults colonize the chestnut trees and start to find and parasitise the host earlier - for instance the timing seems to coincide with the date for the inoculation releases of *T. sinensis* in Northern Italy just after the gall formation (Quacchia *et al*., 2008). The females of *T. flavipes* were in fact observed (in both years of investigation) in the middle of the spring when the ACGW galls were reddish and probably the parasitisation of host larvae was more successful compared to the appearance later in the season of other species of native gall wasp parasitoids (Aebi *et al.*, 2011). The increase in the *T. flavipes* parasitisation observed from 2010 to 2011 demonstrated the potentially good stage of susceptibility of AGCW and effective host finding by the parasitoid females. However the complete biological cycle of *T. flavipes* and its native gall wasp hosts are still not clear. We sporadically found *T. flavipes* emerging from a gall made by the sexual generation of the cynipid *Neuroterus quercusbaccarum* (L.).

T. flavipes was first recorded in 2007 emerging from chestnut galls in northern Latium (Speranza *et al*., 2009). In 2011, in the same localities, it was still active as a parasitoid of ACGW (Paparatti *in litteris*). In Marche region too, *T. flavipes* was found in 2010 and 2011 on ACGW and *N. quercusbaccarum* (Riolo *in litteris*). Despite the evident asynchrony *T. flavipes* seems to represent a truly promising natural enemy of ACGW. Anyway further investigations are therefore needed to determine and understand in which summer and winter host species *T. flavipes* are able to survive in our country. These first findings indicate the necessity to obtain more data on native ACGW parasitoids in association with the continued release of *T. sinensis* in order to bring about an increase in the areas of sustainable biological control as well as a greater establishment in Italy of the exotic parasitoid *T. sinensis*. The knowledge of native gall-maker hosts of *T. flavipes* is of fundamental importance for further management of the pest insects of chestnut agroecosystems.

As suggested by the EFSA Panel on Plant Health (2010) regarding the *T. sinensis*: "further research is needed, particularly on a) the host range of the parasitoid to determine the direct and indirect non-target effects on closely related oak gall wasps of the Cynipidae; b) the taxonomy and phylogenetic analysis of *T. sinensis* and closely related species and c) the potential of *T. sinensis* for hybridisation with other *Torymus* species".

Favouring the passage of beneficial insects from the native gall-maker hosts to the exotic ACGW could prove to be a promising agroecological technique for the near future. Conservation biological control, as reported also in the USA referring to the case of ACGW limited by the natives and the introduced parasitoid *T. sinensis* (Cooper and Rieske, 2011), instead of control using pesticides, seems the correct approach to mitigate the damage provoked by ACGW and to avoid the increase of other insect pests due to the reduction of their natural enemies.

Moreover we hope that ACGW damage to chestnut trees in Italy will be less severe particularly if the complex of limiting factors and interactions such as diseases, predators and primary and secondary parasitoids, is able to play a positive role of control on ACGW populations.

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