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Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*

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Abstract *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), is one of the most serious pests attacking chestnut trees. Recently it was incidentally introduced into Northwest Italy and it is now spreading throughout Europe. This pest was successfully controlled in Japan by introducing a parasitoid, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), from China's mainland. Following this successful experience, the parasitoid was introduced into Italy from Japan. One year of preliminary studies led to a successful method of rearing imported galls with a synchronization between the parasitoid's emergence and the presence of the target galls in the field. In two consecutive years, a total of 2,117 individuals were released in several sites covering most of the infested area. There are encouraging data about the settlement of the parasitoid and its synchrony with the host's cycle: from about 64,000 host galls collected in the field over 200 *T. sinensis* were reared. The role of native parasitoids associated with the chestnut gall pest in its Italian distribution range is also discussed.

Keywords Biological control · Chestnut gall wasp · Cynipidae · *Dryocosmus kuriphilus* · Exotic pest · Parasitoid · Torymidae · *Torymus sinensis*

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Introduction

When an exotic pest is introduced into a new area it can have very strong effects on native flora and fauna, and spread quickly. Often the factors naturally controlling its population in its native range are not present. In the pest's new habitat there may be fewer predators or diseases, so its populations grow out of control. This has been the case with the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), one of the most dangerous pests attacking chestnut trees (*Castanea* spp.). It is native of China (Oho and Shimura 1970) and in the past it was accidentally introduced into Japan (1941), Korea (1958), USA (1974), and Nepal (1999) (Abe et al. 2007). In Europe, it was recorded for the first time in Italy (Brussino et al. 2002). The first report was in 2002 from Piedmont (Northwest Italy) but, from customs' statistics, its introduction can be traced back to 2–3 years before with the importation of nursery material from China. Because of its dangerousness it was added to the European Plant Protection Organization (EPPO) A2 Action list (EPPO 2005) in 2003. The pest quickly spread throughout the Italian peninsula; it was found in central and south Italy in 2005 (Paparatti and Speranza 2005) and also abroad, in Slovenia (Jaksa 2006) and France (2005) (Malausa personal communication). The reason for such a rapid spread is explained by the sale of young infested plants from nurseries in the infested area, since the presence of the pest inside the buds is not obvious.

D. kuriphilus is univoltine and thelytokous, and causes the formation of galls on buds in early summer. These galls reduce the total photosynthetic area and can stop the growth of the shoots. In the case of a heavy attack, the plant declines gradually (Kato and Hijii 1997) and the production of fruits can be drastically reduced (Moriya et al. 1990). The plant is more vulnerable to infections, such as the chestnut blight, and can die.

Japanese researchers, who faced this problem for the first time in the 1940s, began controlling the pest by means of insecticides and natural enemies without any result, so they focussed their efforts on breeding and propagating resistant varieties, and the gall wasp became a less worrying problem. Later, however, probably due to the selection and development of a new biotype of *D. kuriphilus*, it become again a serious pest (Murakami 1981). Researches carried out in China, where the gall wasp is present but not harmful, prospected the chance of a biological control attempt. A parasitoid wasp, reared from Chinese galls, was introduced into Japan in the late 1970s. Although the time needed to establish the populations varied in different localities (Murakami and Gyoutoku 1995), after about 6–18 years the parasitoid effectively controlled the pest, keeping its population under the damage threshold (Moriya et al. 1989; Murakami et al. 2001), fixed at a 30% shoot infestation (Gyoutoku and Uemura 1985). This parasitoid, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), is univoltine like its host; adults emerge from withered galls in early spring and, after mating, the female lays eggs into newly formed galls, either onto the body surface of the host larva or on the wall of the larval chamber. The parasitoid's larva feeds ectoparasitically on the pest's mature larva and pupates during late winter.

Chestnut is economically and environmentally important in Italy. Chestnut orchards are part of traditional farming in many Italian regions and provide a non-negligible additional income to farmers. The widespread distribution of chestnuts, cultivated or naturalized, is crucial for the maintenance of a local biodiversity but also to maintain steep slopes in mountain areas. The severity of the pest problem in relation to environmental damage (interpreted as a loss of landscape appearance and quality) and economic loss in the infested area urged a quick action to control the possible damage. The need for control measures was underlined by a Ministerial Decree (Ministero delle Politiche Agricole e Forestali 2006) issued by the Italian Government in 2006 outlining compulsory measures for the pest's control. Also in 2006, an EPPO workshop (EPPO 2006) was held in Cuneo to prepare local standards of selected phytosanitary measures that could be implemented while methods were developed for biological control, i.e. the most promising method of pest control recognized by the Organization. Given the success of biological control in Japan, measures to support the introduction of the parasitoid were started in Italy in 2003 with a survey of the relevant literature and contacts with Japanese researchers. This paper reports on the subsequent stages of the biological control of the chestnut gall wasp using *T. sinensis* and details how the parasitoid population was introduced and how its establishment has progressed.

Materials and methods

Rearing and release

In order to obtain the parasitoid, potentially parasitized galls were provided by The National Agricultural Research Center in Tsukuba (Japan). A data logger recorded the temperature during the gall shipments to Italy. All the packages received were checked and any extra material, such as unwanted arthropods and leaves, were destroyed.

In February 2004, 2,546 withered galls were received from Japan (collected in Ibaraki and Chiba prefectures). These galls were kept refrigerated at 5°C until the beginning of March, then they were individually placed in glass test tubes and kept indoors at room temperature. They were checked every day and any *T. sinensis* that emerged was collected and isolated in glass test tubes so to check its biology and host specificity. Longevity was investigated with relation to two variables: temperature and feeding frequency. Three constant temperatures (15, 20, and 25°C) were tested; at each temperature 50 specimens were reared in a climatic chamber, providing fresh food every day. Four different feeding frequencies were tested: 50 specimens per tested frequency were reared in climatic chamber at 15°C, food was provided every 1, 2, 4, and 6 days. In the trials RH was 80–85% and feeding consisted in one small drop of clear honey on a small card. Mortality was checked daily at the same time. The specificity was checked according to the indication proposed by van Lenteren et al. (2006). The choice of non-target species was made among the galls present in the field in a stage suitable for oviposition. The only prone galls found (newly formed and big enough to let the parasitoid grow) were those of *Mikiola fagi* (Hartig). Due to their relative abundance, also galls of the agamic generation of *Cynips quercusfolii* (L.) and *Andrycus kollari* (Hartig) were chosen. Five galls of each species were tested, a small portion of branch bearing the gall was placed in a small net cage. Mated females were used ten to twenty days after emergence. The behavioural components looked for were: encountering the host (i.e. making contact with the host by means of the antennae), drumming on the host (i.e. inspecting the host with the antennae) and, drilling the host (i.e. adopting the oviposition posture and penetrating the host with the ovipositor). These behavioural components were observed continuously, starting when the female was introduced into the observation cage. The observations lasted 2 h, every day and for 10 consecutive days. After the observation period each female was isolated from the gall until the following trial. A positive control was performed using five galls of the target species *D. kuriphilus* obtained by potted chestnuts reared in glasshouse (in order to have fresh galls earlier than in the field). Females supporting and trials were performed at 15°C.

In 2005, 3,519 galls were sent from Japan (collected in the Ibaraki prefecture) and 21,945 were sent in 2006 (collected in eight different prefectures). They were isolated into small cages and kept in a climatic chamber. The temperature inside the chamber was initially set to 1°C and then increased as chestnuts in the field developed leaves, although with temperatures 5–6 below those measured in the field. The photoperiod was set to 12 h of light and 12 of dark during the storage. Galls were checked every day for emerging insects.

When the formation of galls in the field was first observed, the small cages were taken out of the climatic chamber and kept outdoors in the Faculty of Agriculture of the University of Turin, under a double-net cage. *T. sinensis* emerging every day were kept together inside glass test tubes and fed with small drops of clear honey on a small card; humidity was provided by few drops of water on the cotton plugs of the tubes. The tubes were kept in the climatic chamber at 15°C. Not later than 48 h after emergence, *T. sinensis* adults were moved into the infested area and released in the field by opening the tubes and letting them fly away voluntarily. The release sites were chosen to facilitate the natural dispersal of the parasitoid (e.g. in orchards adjacent to other orchards or mixed woods where chestnut trees formed a continuous distribution, on the top of slopes and in areas with a high gall infestation rate. Three release sites were chosen in 2005 and 10 in 2006 (including the three of 2005). An 11th site was chosen in 2006 as a possible rearing area, as it was an isolated orchard several kilometres far from other chestnut trees, with a high gall infestation rate and a small tree size. Here the parasitoid population was expected to potentially increase rapidly as the isolation could increase the density of the insects by restricting dispersal (Moriya et al. 1990; Aoto and Murakami 1992). This area was established as a source of parasitized galls to obtain specimens for the release in other sites (the small size of the trees making collection easy).

Any parasitoids different from *T. sinensis* and any inquilines that emerged from the Japanese galls were collected, killed with ether and stored in alcohol. Samples of the parasitoids were sent for identification to Dr. George Melika of the Systematic Parasitoid Laboratory, Plant Protection and Soil Conservation Service of County Vas, Koszeg, Hungary.

Settlement prospects

The effectiveness of the releases was assessed by examining the emergence of *T. sinensis* from galls collected in the 2006/2007 winter (galls that formed during the previous spring). The galls were collected in the three sites where *T. sinensis* had been released in 2005 and 2006 and in the rearing area, where the parasitoid had been released for the first time in 2006. Galls from different heights were collected using lopping shears.

These galls were isolated inside cardboard boxes provided with extractable skylights. The boxes were kept inside the infested area, in ambient conditions, so to check whether the emergence of *T. sinensis* would match gall formation during the subsequent spring. The boxes were opened weekly to remove any spiders that could predate the emerging parasitoids. A data logger was placed close to the boxes in order to record the temperature. Until the emergence of the first *T. sinensis*, the boxes were checked weekly; after that they were checked every day. Emerged insects were collected under the skylight with an entomological pooter. Emerged *T. sinensis* were kept in glass test tubes and fed to keep them alive until their release in the field; 30 of them were used to check the longevity at different temperatures as performed in 2004 with the *T. sinensis* received from Japan. Other insects were collected and stored in alcohol for further identification and biocoenosis surveys. The number of emergences was compared with the temperature recorded by the data logger and with the formation of galls on the surrounding chestnut trees.

Data analyses

Correlation between the number of emergences and the temperature recorded by the data logger was assessed using Spearman's Correlation method. Non-parametric Kruskal–Wallis test was used to analyse data of longevity, the values were separated by the post hoc Mann–Whitney. The test was repeated both for the Italian and the Japanese population. The analyses were performed using the software SPSS® 13.0.

Results

Rearing and release

A total of 509 *T. sinensis* adults emerged from the Japanese galls in 2004 (260 females and 249 males). They started emerging three weeks after the galls were placed indoors at room temperature, nearly one month before the appearance of galls in the field. This mismatch with gall appearance meant that the parasitoids could not be released into the field; instead they were used for behavioural trials, that helped to improve later efforts in maintaining adults, and then stored in ethanol for future analyses. Results of these trials (Tables 1 and 2) show how the adult longevity increased at low temperatures, and with reduced time of food deprivation.

None of the females in contact with the galls of *M. fagi*, *C. quercusfolii*, and *A. kollari* showed the behavioural components looked for and no oviposition was registered; the positive control showed behaviours approaching those in the field, so trials were stopped and no direct effects on these non-target species are expected.

In both 2005 and 2006 *T. sinensis* emerged during a 1-month period, 218 specimens (111 females and 107 males) in the first year and 2,238 (1,219 females and 1,019 males) in the second. Thanks to the use of the climatic chamber, the emergence of parasitoids in 2005 and 2006 was well synchronized with the beginning of gall formation in the field (Fig. 1).

A total of 170 *T. sinensis* (90 females and 80 males) in 2005 and 1,947 *T. sinensis* (1,058 females and 889 males) in 2006 were released in the infested area (Fig. 2). Some matings were observed in the tubes before the release. Most of the parasitoids flew away right after the release although some ovipositions were observed in the field.

More than 3,000 parasitoids of species other than *T. sinensis* emerged from the Japanese galls received from 2004 to 2006. Their identification showed the predominance of

Table 1 Longevity of *Torymus sinensis*, reared from Japanese and Italian galls, at different temperatures

Temperature (°C)	No. of tested		Longevity (days)			
			Mean		SD	
	Japanese source	Italian source	Japanese source	Italian source	Japanese source	Italian source
15	50	10	38.6a	44.3a	13.9	15.7
20	50	10	29.2b	31.5b	14.6	13.2
25	50	10	10.9c	13.8c	6.3	10.8

Different letters in the same columns show difference at $P < 0.001$

Table 2 Longevity of *Torymus sinensis* at different feeding frequency periods

Feeding frequency (days)	No. of tested	Longevity (days)	
		Mean	SD
1	50	38.9a	12.6
2	50	37.9a	15.4
4	50	10.3b	3.3
6	50	5.6c	3.4

Different letters show differences at $P < 0.05$ (Mann–Whitney U test)

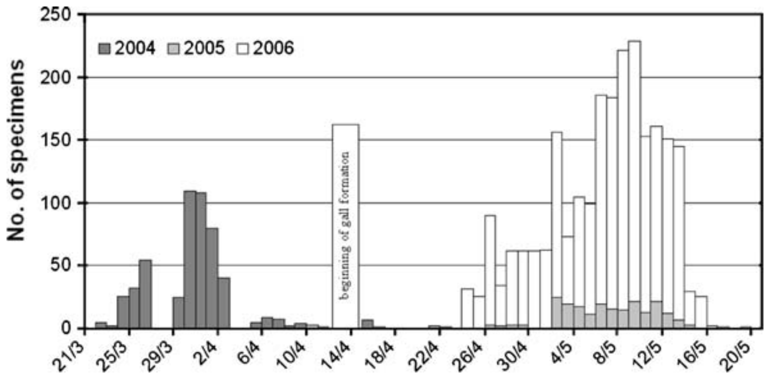

Fig. 1 Comparison between the emergence of Japanese-sourced *T. sinensis* in 2004 (reared in indoor conditions) and in 2005–2006 (reared in a climate chamber at low temperature until the beginning of *D. kuriphilus*'s gall formation, then in outdoor conditions). "Beginning of gall formation" is a mean of the 3 years

Eurytoma brunniventris Ratzeburg 908 specimens, *Eupelmus urozonus* Dalman 790 specimens, *Ormyrus* spp. 756 specimens, and *Torymus geranii* (Walker) 352 specimens.

Settlement prospects

About 64,000 galls were collected from the four sampling sites (including about 28,000 from the breeding area). A total of 30 (14 females and 16 males) *T. sinensis* emerged from galls from the three locations where parasitoids had been released for two consecutive years and a total of 174 (80 females and 94 males) emerged from the galls collected in the breeding area (Table 3). The sex ratio of *T. sinensis* reared from Italian galls (46% females) was similar to the sex ratios of samples reared from Japanese galls (51% in 2004 and 2005, 54% in 2006).

Emergences of *T. sinensis* started on 2nd April when the daily mean temperature first rose above 7°C with regular emergences once the temperature rose above 13°C (Fig. 3). The statistical analysis shows a positive correlation ($r_s = 0.751$; $P < 0.001$) between the number of emergences and the temperature recorded. Males tended to emerge before females, although the peak of the female emergences occurred only 3 days after the male's one. The onset of emergence corresponded with the first observations of gall formation in the field.

The statistical analyses show that the longevity is significantly influenced by the rearing temperature. As far as the food frequency is concerned, significant reduction of longevity

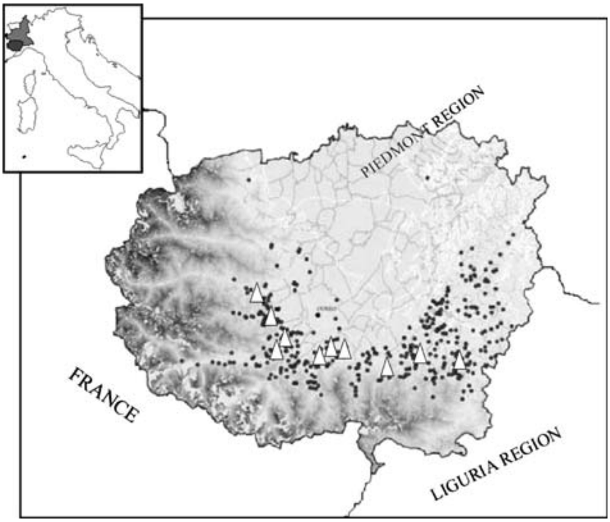

Fig. 2 Location of *D. kuriphilus* infestation records in the province of Cuneo in 2006 (circles) and the release sites of the biological control agent *T. sinensis* (triangles). The inset indicates the location of the province of Cuneo and the Piedmont region in Italy

Table 3 Japanese-sourced *T. sinensis* released, galls collected and *T. sinensis* reared in four release sites in the province of Cuneo, Italy

	Torymus sinensis released (females)		Settlement	
	2005	2006	Galls collected in 2007	*Torymus sinensis* emerged (♀+♂)
Robilante	28	123	12,000	5
Peveragno	35	91	12,000	20
Boves	27	84	12,000	5
Rearing area	_	80	28,000	174
Total	468		64,000	204

was observed when the parasitoids were food deprived longer than 2 days, while no differences were found between intervals of 1 and 2 days.

Discussion

About 28,010 galls were received from Japan in 3 years; 2,117 *T. sinensis* reared from these galls were released into the field, in 11 sites, during 2 years. The parasitoids laid eggs successfully in Italian galls, with 204 *T. sinensis* reared from a collection of 64,000 galls obtained in the year following the final release.

The number of parasitoids obtained from the rearing of Italian galls is promising considering the limited release effort and monitoring. In each monitored site, only 12,000 galls were collected in chestnut woods where *D. kuriphilus* galls were estimated to be millions and the initial release of Japanese-sourced parasitoids was only about a hundred of females in 2 years, so the probability of finding parasitized galls was expected to be lower

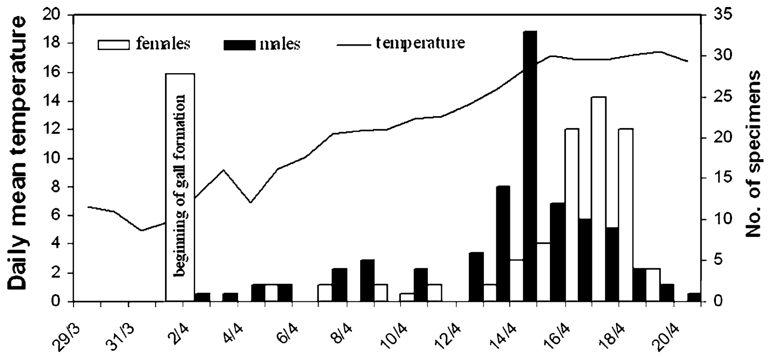

Fig. 3 Emergence of *T. sinensis* from *D. kuriphilus*'s galls collected in Italy in 2007. Time of gall formation is earlier compared with that of the previous years due to an unusually warm winter

than that observed. In addition, the breeding area provided a good indication that the parasitoids were reproducing successfully after only a single native generation. Although further monitoring is required before anything definite can be said, the results obtained to date suggest that the settlement of *T. sinensis* in Italy is quite likely.

Despite this, it is important to consider that the settlement in Italy of *T. sinensis* may not be easily accomplished. Some important aspects, as mentioned by Murakami and Gyoutoku (1995), have to be considered; for instance, the presence in the field of insects that can act as hyperparasitoids (such as *E. urozonus* and *E. brunniventris*, both reared from Italian and Japanese galls, Aebi et al. 2007) and attack the biological control agent, or the possibility of a male-biased sex ratio due to the failure of mating that can happen when the density of individuals is low and finding a mate is difficult. However, it is encouraging that the sex ratio of the first Italian generation is close to that usually observed in China (Murakami and Gyoutoku 1995) and in the galls received from Japan.

Another aspect to be considered when assessing the effectiveness of *T. sinensis* as a biological control agent is its uncertain systematic status. In Japan it hybridizes with a closely related indigenous species, *T. beneficus* Yatsumastu and Kamijo (Yara 2006). In addition to the populations introduced from China, populations of native *T. sinensis* have been found in three localities in Japan (Moriya et al. 2003; Ohkubo 1992; Toda et al. 2000) and also in South Korea (Murakami et al. 1995). It is not possible to distinguish the males morphologically, and the ovipositor sheath length / thorax length ratio, used in the past as a marker for the discrimination of the females, is not always reliable (Yara 2004). This means that the population released in Italy could be made of specimens belonging to both species and their hybrids. However, due to the displacement of *T. beneficus* by *T. sinensis* in Japan (Yara et al. 2007), it is reasonable to infer that the population released in Italy should belong mostly to *T. sinensis* and therefore should not affect the control effectiveness.

Research on the effectiveness of pesticides and resistant varieties were made in Italy since the gall wasp was reported. All the pesticides tested were inefficient and in any case the natural distribution of chestnut trees in Italy did not make their use practicable. Research on resistant varieties are in progress in the Department of Arboriculture of the University of Turin and their first results show that the variety Bouche de Bétizac is the only one resistant to the gall wasp (Sartor et al. 2007); but, because of the importance and richness of the Italian variety heritage the massive diffusion of this single variety would be unadvisable, therefore biological control remains the only suitable option. Due to the severity of the damage caused by the rapid spread of the chestnut gall wasp through Europe (Aebi et al. 2007), the inefficacy of pesticides, the impracticability of using resistant varieties, and the encouraging results of using *T. sinensis*, proved over a long time in Japan (Moriya et al. 1989; Murakami et al. 2001) and more recently in the USA (Rieske 2007), a quick and concrete response was made, keeping in consideration the environmental risk associated with the introduction of an exotic natural enemy (van Lenteren et al. 2003; EPPO 1997; Kimberling 2004). First of all the host range of the parasitoid was considered: Murakami et al. (1977) based on Askew (1975) described *T. sinensis* as host-specific but in practice its host range has not been clarified yet by complete scientific trials. The results obtained on *M. fagi*, *C. quercusfolii* and *A. kollari*, and the personal experience of S. Moriya (one of the authors) who never reared, in Japan, *T. sinensis* from other galls than *D. kuriphilus* support the specificity thesis.

To assess any indirect effects on non-target species, such as possible hybridization with native Torymid parasitoids and, to clarify the identity of the population released in Italy, molecular research is in progress using specimens collected from Japanese and Italian galls. In insects, all the reported cases of hybridization are between species in a single genus or in a species complex within a genus (Hopper et al. 2006) so a collection of native Torymids is ongoing in order to generate a data set of phylogenetic relatedness.

Alongside the control of *D. kuriphilus* by the introduction of the exotic parasitoid, recent investigations into the community dynamics of chestnut galls conducted after the gall wasp was found in Europe have opened the possibility of an augmentative biological control programme using native species. Since 2002, 16 native parasitoid species have already been collected from Italian galls; they are all chalcidid wasps belonging to six different families and are known to be parasitoids of oak cynipid galls. The rapid recruitment of oak cynipid parasitoids of *D. kuriphilus* (Aebi et al. 2006) suggests that despite the current low attack rates there may be some value in evaluating the control potential of native parasitoids.

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