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Introduction and acclimation of *Torymus sinensis* in the South of Italy

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With 1 figure and 1 table

Abstract: The Asian Gall Wasp *Dryocosmus kuriphilus* was accidentally introduced into Italy in 2004. Since then it has spread all over the peninsula where chestnut represents an economic important fruit crop. In 2005 the pest arrived in Campania, where about 40% of Italian and 25% of European chestnut production is concentrated, with devastating consequences. For its control, the exotic parasitoid *Torymus sinensis* has been introduced from 2009 onward, but so far no survey has been completed to assess its acclimation and, most of all, the level of its activity. In this note data on releasing sites and parasitism rate for the control of *D. kuriphilus* in Campania are reported. We show that the parasitoid well established in a region characterized by mild winters and warm summers, reaching a good level of presence in several sites of the main area of chestnut production which is concentrated in the provinces of Avellino and Salerno. We confirmed the ability of this parasitoid in moving away from releasing sites following its host. In fact, its presence was recorded in an area where only one release was performed. As expected, the larger releases were followed by higher parasitism rates with some exceptions. The presence and activity of *T. sinensis* recorded had played a pivotal role in the drastic reduction of *D. kuriphilus* in Campania within only 5 years from the application of a territorial biocontrol program supported by the local government. Information reported are useful for future AGW management.

Keywords: *Dryocosmus kuriphilus*, biological control, Campania; chestnut

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1 Introduction

The invasive species *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), commonly known as the Asian Gall Wasp (AGW), is a major pest of chestnut worldwide (Abe et al. 2007, Cooper & Rieske 2007, Graziosi & Santi 2008, Quacchia et al. 2008). In Europe the pest adapted to the European chestnut *Castanea sativa* Miller (Fagaceae) with levels of damage depending on the attacked variety (Sartor et al. 2015). Italy is the second largest European producer of chestnut (after Turkey), placing it fourth in the world ranking (Ingino et al. 2006). More than 40% of Italian production, totaling 25% of European one, is located in Campania (Southern Italy) with the province of Avellino reaching nearly 50% of the entire region (Ingino et al. 2006). In 2005, *D. kuriphilus* was accidentally introduced into Campania from Piedmont, on nursery plants that had heavily infested buds (Bernardo et al. 2013) and not surprisingly its effect on the entire production has been devastating. Following the positive experience ripened in the native area of the pest, a biological control approach has been implemented in Italy to reduce the populations of the Asian Gall Wasp (Moriya et al. 2003). This approach is based on the introduction of the exotic parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) characterized by a life cycle strictly linked and synchronized with that of its host. The activity of the exotic parasitoid was complemented by that of native parasitoid. The latter actually quickly adapted to the new host (Aebi et al. 2007, Quacchia et al. 2013, Panzavolta et al. 2013, Palmeri et al. 2014). In Campania, considering the vast extension of cultivated and coppice chestnut woods, governmental and research Institutions have collaborated to coordinating the release of the exotic wasp following a pre-designed grid in order to cover the largest area as possible with the available parasitoid quantities. Eight years after the first release of 10 couples of *T. sinensis* in the province of Avellino, a massive effort has been done to check the presence of the parasitoid and to assess the parasitism rate in the releasing points and in sites close to them. In this communication, we refer about the results of this large survey that constitute the base for planning future interventions in this area and in the entire South of Italy.

2 Materials and Methods

2.1 Parasitoid collection in Piedmont

Every February from 2009 to 2016, 200 galls were collected in several sites of Piedmont (province of Cuneo) and dissected to assess the parasitization level of *T. sinensis*. In those sites were at least 50% of galls were parasitized, between 250.000 and 350.000 galls were collected to obtain the needed couples to be released. Following a well-established procedure (Quacchia et al. 2008), collected galls were cleaned to remove spiders and predator insects from their surface, placed in carton boxes and moved to Montevergine, province of Avellino, at 1270 meters a.s.l. and stored at ambient temperature.

2.2 Parasitoid release

Every March from 2009 to 2016, the galls stored as above described were progressively moved to small carton boxes with two opened 50 ml Falcon tubes inserted on one side to facilitate the collection of emerging parasitoids. The boxes were placed in a climate chamber at 22 °C–25 °C,RU50%, photoperiod 12D:12L and checked daily for parasitoid emergence. At emergence, parasitoids were sexed, grouped in transparent 50 ml Falcon tubes and fed by little drops of honey:water solution (1:1) to favour mating. In the releasing vial, five males, ten females and a few drops of honey solution were placed. In each selected site, the releasing lot was composed by ten releasing vials, totaling 50 males and 100 females of *T. sinensis*. Parasitoid were released in the field by opening the vials in close proximity of chestnut trees. A single releasing point was chosen in each site usually placed in the center of it. As chestnut fruit orchards and coppice woods are present in all provinces of Campania region, the releasing sites were chosen following a theoretical grid (mesh 5 × 5 km). In the provinces of Avellino, Benevento, Caserta and Salerno, which represent the core of Campania chestnut production, more than 90% releasing sites were located in cultivated orchards. Conversely, in the province of Naples, all releasing sites were in coppice woods. Releases in coppice woods had the main aim of creating an undisturbed parasitoid reservoir.

2.3 Parasitoid survey in Campania

In accordance with established protocol (Ghering et al. 2017), on February 2017, in each selected site, 200 galls were collected on 5 different branches of 10 chestnut trees selected randomly. Galls maintenance and parasitoid collection followed the same procedure as described above. In March, to facilitate the parasitoids' emergence galls were moved to small carton boxes with two opened 50 ml Falcon tubes inserted. The boxes were placed in a climate chamber at 22 °C–25 °C, RU 50%, photoperiod 12D:12L and checked daily for parasitoid emergence until the end of June. To avoid the underestimation of parasitoid activity, ten days after the last parasitoid emergence, the boxes were opened to check for died parasitoids and all galls were dissected to count parasitoids not emerged. Kruskal-Wallis test with posthoc Dunn test was used to evaluate differences between province parasitism rates. Linear regression analysis was used to assess the relationship between released and collected *Torymus sinensis*. The sex ratio for each site was analysed by using GLM with binomial distribution.

2.4 Identification of parasitoids

Collected parasitoids were sexed and identified morphologically using key features at a binocular (Otake 1987). From each province, 2 samples each composed of 10 males and 10 females were used for molecular identification (Yara 2006, Yara & Kumini 2009, Matošević et al. 2017) following a non-destructive protocol (see Guerrieri et al.

2016 for details). Median, minimum and maximum presence of *T. sinensis* were calculated for each province. The total number of *T. sinensis* from each site was recorded and related to the total number of galls (200) to calculate the total rate of parasitism.

3 Results

The number of releases in each considered province and the relative presence of *T. sinensis* in terms of average parasitization rate are reported in Table 1 (for details see Supplementary Tables S1–S5). Overall 1326 releases, corresponding to 132600 mated females, were performed in the province of Avellino, 755 in the province of Salerno (totaling 7550 mated females), 417 in the province of Caserta (totaling 41700 mated females), 118 in the province of Benevento (totaling 11800 mated females) and 78 in the province of Naples (totaling 7800 mated females). Recovered parasitoids from collected galls were 4094 in the province of Avellino, 5313 in the province of Salerno, 180 in the province of Caserta, 937 in the province of Benevento and 36 in the province of Naples (Table 1, Tables S1–S5). The sampling effort in 2017 showed the presence of *T. sinensis* in all sampled sites and molecular characterization confirmed the identity of the collected parasitoids. The larger recovery of parasitoids were almost always associated to larger releases. The linear regression between released and collected parasitoids showed a positive correlation ($R^2 = 0.091$; $F_{1–376} = 37.5$; $P < 0.001$; Fig. 1). However, the largest parasitoid collection (329) was recorded in San Cipriano Picentino, Salerno where only one lot of *T. sinensis* was released (i.e. 100 mated females, Tab. S2). Sampling in sites away from releasing points showed that the parasitoid moved following its host for distances as far as 5 km (e.g. Senerchia, Province of Avellino; Perdifumo and Stella Cilento, Province of Salerno) (see Tabs S1 and S2). Parasitism rate ranged from 0.5 % to 164% in relation to 200 galls collected in each site (Tables S1–S5). Considering that in Campania region one gall hosts an average of 2.5 larvae of *D. kuriphilus* (Bernardo et al., 2013), the cited rates can be transformed into individual parasitism rate (i.e. referred to single host larvae) by dividing them for this value. In almost all sites sex ratio was female biased but somewhat close to 1:1 (females to males). Some exceptions were recorded in Salerno Province characterized by strong male biased values (Table S6).

4 Discussion

The effect of the AG Won European production of chestnut has been devastating since its first appearance and Italy is no exception (Gehring et al. 2017). Campania region represents the largest producer of chestnut in Italy and remains second in Europe after Turkey. As a consequence, the arrival of the pest in this region had also consistent social effects. Large releases of the exotic parasitoid *T. sinensis* were programmed but so far no survey has been completed to assess its acclimation and, most of all,

Table 1. Release, acclimation and parasitim rate of *Torymus sinensis* in the provinces of Campania, Italy.

Province[1]	Parasitoids Released[2]								Parasitoids Survey[3]		
	2009	2010	2011	2012	2013	2014	2015	2016	Released[2]	Collected	Median (min/max)	Parasitism Rate ± SE (%)[4]
Avellino (56)	1	3	6	295	262	127	284	348	1326	4094	41 (1/252)	35.7 ± 4.9[ab]
Salerno (61)		1	3	195	116	66	297	77	755	5313	72 (0/329)	47.3 ± 5.4[a]
Caserta (1)		1	1	71	189	60	75	20	417	180	180 (180/180)	90[a]
Benevento (5)				8	4	3	98	5	118	937	212 (38/298)	93.7 ± 22.1[a]
Napoli (4)				3	15	15	45		78	36	9 (2/16)	4.5 ± 1.4[b]

[1] In brackets the number of sites sampled in each province

[2] Number of releasing lots each composed by 100 mated females

[3] Data are referred to 200 galls sampled in each sites. Collected is the total parasitoids obtained per province. Parasitism rate is calculated dividing the number of parasitoids collected for galls

[4] Letters are referred to statistical differences assigned by Kruskal-Wallis test followed by posthoc Dunn tests

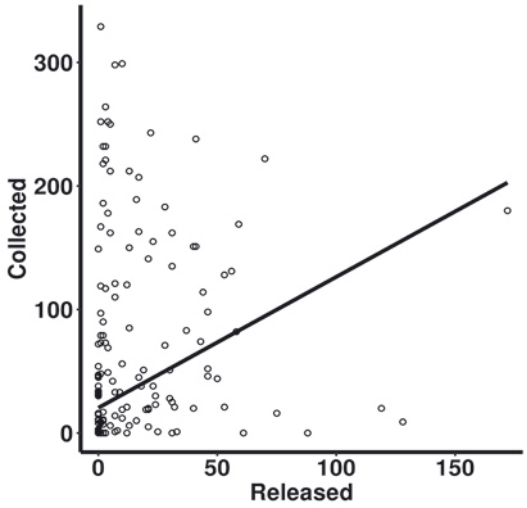

Fig. 1. Released-Collected *Torymus sinensis* relationship. Total number of adults released per sites and adults emerged from galls are presented (regression line: y=1.06x+20.38).

the level of its activity. The data presented here show that the parasitoid established well in the South of Italy reaching a good level of presence in several sites of the main area of chestnut production which is concentrated in the provinces of Avellino and Salerno. This result was accomplished within only 5 years from the first consistent introduction in 2012. Fast acclimation of *T. sinensis* has been observed not only in the North of Italy (Quacchia et al. 2008) but also in other countries (Matošević et al. 2017) characterized by different climatic conditions. In fact, in Campania the release of *T. sinensis* followed an introduction approach closer to inundation than to propagation even though in many sites a single lot composed of 100 mated females was introduced. This choice was suggested by considering two aspects that could have hampered parasitoid performance and diffusion. In Campania, the extension of chestnut woods is by far greater than that present in the North of Italy and in other European countries. Moreover, chestnut woods in Campania are composed by old plants, mainly unmanaged wild coppice and real fruit orchards. The latter are located at lower altitudes and are characterized by younger plants, yearly pruned to keep them low to facilitate the cultural operations and periodically drafted to keep high levels of production. Our data show that the inundative approach was a correct choice in terms of results and time needed to obtain them. As expected, the larger collection of parasitoids were often associated to larger releases (see Tables S1–S5). However, the highest number of parasitoids was collected in a site where only 100 mated females were released (Table S2). This confirms the ability of *T. sinensis* to move following its host covering long distances in line with what was observed in the North of Italy (Quacchia et al. 2008) and in other European countries (Borowiec

et al. 2014, Matošević et al. 2016, 2017). A recent model indicated that the introduction of *T. sinensis* sparks a travelling wave of the parasitoid population that destroys the pest on its passage (Paparella et al. 2016). The pest can later be able to re-colonize the empty area left behind the wave, thus the two populations produce an ever-changing pattern of travelling waves (Paparella et al. 2016). This dynamic provides a good explanation to the low R-squared obtained (Fig. 1). Indeed, in several sites our survey possibly probed the moment between cynipid eradication and its recolonization wave. In these sites, further releases of *T. sinensis* had not proved effective due to the host lacking with negative effects on linear regression. Morover the level of parasitization recorded in our survey well adapt to this theory particularly for the reduced time requested to reach a satisfactory level of parasitization, well shorter than the decade needed in other continents (Japan, Asia) (Moriya et al. 2003). In this view, predicting parasitoid fluctuations is crucial to avoid pest explosions with new devastating effect on production. A periodical survey of pest and parasitoid populations is thus mandatory particularly in territories characterized by intense chestnut cultivation such as Campania region. The data here presented (Tables S1–S5) also indicate to chestnut growers those sites characterized by parasitism rates ≥ 50% suitable to collect parasitized galls to be introduced where the activity of the parasitoid is scarce or null. Despite the total number of parasitoid releases in the province of Salerno was nearly a half in respect to that of the province of Avellino, in the former the average parasitism rate resulted higher. Adverse climatic conditions that hamper parasitoid performance and diffusion, including high temperatures, rainfall and low humidity, could have played a role. However, other causes cannot be excluded at all in an area more intensively cultivated (i.e. the province of Avellino). Among these, the most probable one seems the use of persistent pesticides targeting other pests of chestnut, e.g. fruit borers. Another aspect to be considered when assessing the effectiveness of *T. sinensis* as a biological control agent is represented by sex ratio. Although the overall sex ratio in Campania was slightly male biased (48% females), looking the data aggregated by site, in the majority of them it resulted female biased (42 sites over 82). In the remaining ones, the male biased sex ratio could be partially explained by the lower number of parasitoid releases resulting in smaller population recovered (e.g. San Rufo, Perito, Trentinara in the province of Salerno). These data are in line with what observed so far in Italy and elsewhere and exclude the possibility of the failure of mating that can happen when the density of individuals is low and finding a mate is difficult. The survey of *T. sinensis* in Campania was facilitated by the fact that in this region it is virtually the only "winter" parasitoid. Indeed, autochthonous species are active during spring and summer and usually moves back to their "primary" hosts during autumn to overwinter (Guerrieri et al. in prep). The approach followed to contrast the invasive *D. kuriphilus* in Campania is also a confirmation that only when applied at territorial level, biological control accomplishes the result of keeping pest populations below damage thresholds. The increasing presence and activity of *T. sinensis* in chestnut woods of the South of Italy is demonstrated by the return of canopy development and in turn of chestnut production in all chestnut orchards of Campania after 5 years characterized by a virtually absence of chestnut production as caused by the arrival of the invasive pest (Associazione Castanicoltori Campani, personal communication).

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