Effectiveness of Torymus sinensis: a successful long-term control of the Asian chestnut gall wasp in Italy

DOI:10.1007/s10340-018-0989-6

(Article begins on next page)

[Ferracini, C., Ferrari, E., Pontini, M., Saladini, M.A., Alma, A., 2019. Effectiveness of *Torymus sinensis*: a successful long-term control of the Asian chestnut gall wasp in Italy. Journal of Pest Science 92(1): 353-359, DOI: 10.1007/s10340-018-0989-6]

Effectiveness of *Torymus sinensis*: a successful long-term control of the Asian chestnut gall wasp in Italy

Chiara Ferracini, Ester Ferrari, Marianna Pontini, Matteo A. Saladini, Alberto Alma

Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy

Corresponding author: Chiara Ferracini, e-mail: chiara.ferracini@unito.it

Acknowledgements

The authors are grateful to Elvio Bellini (Chestnut Study and Documentation Centre), Lindsay K. Nova Hernández, Greta Pastorino, Cristina Pogolotti, and Ambra Quacchia for their precious help and cooperation in the laboratory and field activities, and to Daniela Di Silvestro and Giuseppe Siccardi of the Phytosanitary Service of Abruzzo and Liguria, respectively, for their cooperation. The authors would like to thank the referees for their valuable comments which helped to improve the manuscript.

Abstract

The biocontrol agent *Torymus sinensis* has been released into Japan, the USA, and Europe to suppress the Asian chestnut gall wasp, *Dryocosmus kuriphilus*. In this study we provide a quantitative assessment of *T. sinensis* effectiveness for suppressing gall wasp infestations in Northwest Italy by annually evaluating the percentage of chestnuts infested by *D. kuriphilus* (infestation rate) and the number of *T. sinensis* adults that emerged per 100 galls (emergence index) over a 9-year period. We recorded the number of *T. sinensis* adults emerging from a total of 64,000 galls collected from 23 sampling sites. We found that *T. sinensis* strongly reduced the *D. kuriphilus* population, as demonstrated by reduced galls and an increased *T. sinensis* emergence index. Specifically, in Northwest Italy, the infestation rate was nearly zero 9 years after release of the parasitoid with no evidence of resurgence in infestation levels. In 2012, the number of *T. sinensis* females emerging per 100 galls was approximately 20 times higher than in 2009. Overall, *T. sinensis* proved to be an outstanding biocontrol agent, and its success highlights how the classical biological control approach may represent a cost-effective tool for managing an exotic invasive pest.

Keywords

Torymus sinensis, *Dryocosmus kuriphilus*, classical biological control, invasive exotic pests

Key messages

- *Torymus sinensis* is a biocontrol agent used to control outbreaks of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*).
- Long-term monitoring between 2009 and 2017 in Italy was performed to provide a quantitative assessment of the effectiveness of this parasitoid.
- Our data clearly demonstrated that *T. sinensis* effectively reduced the *D. kuriphilus* population, as indicated by a reduced number of galls and by a large increase in the number of *T. sinensis* adults emerging per gall.

Author contribution statement

CF and AA conceived and designed research. CF, EF, MP and MAS carried out field and laboratory assays. All authors contributed to the writing of the manuscript and approved the final manuscript.

Introduction

In recent decades, the number of invasive alien species (IAS) in Europe has increased significantly and is considered to be a major cause of economic and biodiversity loss. The European IAS inventory, reported by the Delivering Alien Invasive Species in Europe (DAISIE) project, clearly showed the exponential growth of exotic species both into and within Europe (Roques et al. 2009). IAS devastate forestry, agriculture, and nurseries (Calabria et al. 2010; EPPO 2014; Haack et al. 2010; Quacchia et al. 2008a), threaten native biodiversity, impact ecosystem services, and cause damage and control costs in excess of €12 billion per year (Shine et al. 2009).

There are several examples of invasive insect pests that have been accidentally introduced through global trade and travel (Gerber and Schaffner 2016), and many of these pests may be controlled by biocontrol agents (BCA) (Cock et al. 2016; DeBach 1964; DiTomaso et al. 2017; Stiling and Cornelissen 2005). The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), was first reported in Italy at the beginning of the 21st century and has rapidly spread throughout Europe (Brussino et al. 2002; EPPO 2016). *D. kuriphilus* is native to China and severely affects chestnut trees; it is responsible for a severe reduction in fruiting and negatively impacts chestnut production (Battisti et al. 2014; Gehring et al. 2018). Chemical control and the use of ACGW-resistant chestnuts proved ineffective to control the impact of ACGW (Moriya et al. 2003). Thus, the BCA parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) was released to suppress gall wasp population growth. *T. sinensis* was introduced into Japan in 1975 and in Georgia (USA) in 1977 (Cooper and Rieske 2007, 2011; Moriya et al. 2003). In Italy, *T. sinensis* was imported from Japan and released in 2005 in chestnut-growing areas as part of a biocontrol program funded by the Piedmont region (Quacchia et al. 2008b). Classical biological control using *T. sinensis* was also performed in Croatia, France, Hungary, Portugal, Slovenia, Spain, and Turkey (Borowiec et al. 2014; İpekdal et al., 2017; Matošević et al. 2014; Pérez-Otero et al. 2017; RefCast 2015).

A major criticism of classical biocontrol is the lack of post-release impact evaluation measures. Indeed, while researchers focus extensively on the identification, safety-testing, and release of control agents, there has been relatively little assessment of post-release control success (Clewley et al. 2012). Recently, the biology (e.g. diapause, reproductive traits, hybridization) and behavior (e.g. host range expansion) of *T. sinensis* have been extensively studied due to the need to provide post-release evaluation to assess the potential impacts of this BCA on non-target hosts (Ferracini et al. 2015a, b, 2017; Montagna et al. 2018; Picciau et al. 2017). The literature has reported a clear decrease in ACGW infestations after *T. sinensis* release (Colombari and Battisti 2016a; Ferracini et al. 2015b; Matošević et al. 2017; Quacchia et al. 2014). However, a quantitative assessment of the effectiveness of *T. sinensis* in the reduction of the ACGW in Europe is still needed. To address this concern, we present the results of long-term monitoring (between 2009 and 2017) of the infestation rate by *D. kuriphilus* in response to *T. sinensis* introduction in different Italian chestnut-growing areas.

Materials and methods

Sampling sites

Investigations were carried out during a 9-year period between 2009 and 2017 in five Italian regions. Surveys started in 2009 at six sampling sites in the Piedmont region (Northwest Italy), where the parasitoid *T. sinensis* was first released and formed stable populations (Quacchia et al. 2008b). From 2014 until 2017, investigations were moved to four other Italian regions where the parasitoid was released. Surveys were carried out in four sampling sites in Abruzzo and Aosta Valley (2014 to 2015), and in three and six sites in Tuscany and Liguria, respectively (2016 to 2017). Table 1 lists the location of the sampling sites.

Infestation rate

The chestnut infestation rate by *D. kuriphilus* was recorded once per year (in late August) from 2009 through 2016 at the six sampling sites in the Piedmont region. At each site 10 chestnut trees were randomly selected, and from each tree 10 one-year old branches were randomly chosen at different heights of the canopy for a total of 100 branches per site per year. For each branch, the infestation rate was recorded on the shoots of the previous vegetative season with respect to the sampling date [we refer to Gehring et al. (2018) for the description of the shoot] and expressed as the percentage of total buds infested by the gall wasp, i.e., affected by the presence of galls.

Gall collection

Ten naturally growing chestnut trees (a new set different from the one used to record the infestation rate) were randomly chosen from each of the surveyed sites, and from each tree, 100 galls that had formed during the previous year were randomly collected (10 galls from each of 10 branches per tree). Once per year (in January), galls were collected by hand from low branches (ground level to 2 m high) and with the aid of lopping shears from the medium–high tree crown (from 2 to 5 m high) in chestnut orchards and/or coppices according to a protocol described by Moriya et al. (2003).

Emergence index

The collected galls were isolated inside cardboard boxes with removable skylights. The boxes were kept outdoors until the emergence of *T. sinensis* adults was complete according to a method described by Ferracini et al. (2015b). The number of *T. sinensis* adults emerging per 100 galls was recorded, and is hereafter referred to as the emergence index**.**

Identification of T. sinensis

T. sinensis adults (5 males and 5 females per site and year; 640 total specimens) were morphologically identified by comparison with voucher specimens deposited at the DISAFA-Entomology laboratory. Additional *T. sinensis* adults (5 males and 5 females per site and year; 640 total specimens) were submitted for DNA extraction and subsequently sequenced for the cytochrome oxidase I (COI) gene to confirm morphological identification following a protocol from Kaartinen et al. (2010).

Results

Chestnut infestation rates by ACGW (that is, percentage of buds with galls) averaged 62.5% during the first year of the survey (2009), and varied little until 2013 (Online Resource 1) when they decreased greatly, especially at Robilante (-95%), Boves (-87%), and Peveragno (-85%). After 2013, the gall wasp infestation levels continued to drastically decline, to 0.500%, 0.300% and, 0.003% in 2014, 2015, and 2016, respectively.

In the 9-year-period of 2009 through 2017, a total of 64,000 galls were collected at the 23 sampling sites, and 93,077 (49,756 females and 43,321 males) *T. sinensis* emerged. The mean sex ratio of *T. sinensis* was 1:1 (53.5% female; Online Resources 2, 3).

In Piedmont, the average number of *T. sinensis* females emerging per 100 galls increased steadily, from 4.08% in 2009, to 16.3%, 70.4%, 92.0%, and 81.6% in the subsequent years from 2010 to 2013. Emergence rate was highest at Robilante in 2011 (249 *T. sinensis* adults per 100 galls) (Online Resource 2). A slight decrease in the number of adults emerging per 100 galls was observed in 2013 at all sites except for Peveragno and Cervasca. The trends in mean parasitism by *T. sinensis* (2009 through 2016) and mean ACGW infestation rates (2009 through 2016) recorded in Piedmont are shown in Figure 1. An increase in the emergence index by *T. sinensis* was also recorded for all the other surveyed regions (Figure 2; Online Resource 3.)

All 1280 collected specimens were confirmed to be *T. sinensis* through morphological characteristics (640 specimens) or by molecular methods, specifically COI gene sequencing and subsequent comparison to the National Center for Biotechnology Information (NCBI) sequence database. A minimum of 99% similarity with *T. sinensis*-related sequences was observed. The phylogenetic analyses revealed the presence of 14 clusters. The COI sequence of a specimen for each cluster was deposited in the European Nucleotide Archive under the accession numbers from MH121609 to MH121622.

Discussion

Our study provides data that annual increases in *T. sinensis* emergence index corresponded to concomitant decreased levels of *D. kuriphilus* infestation and demonstrates the efficacy of *T. sinensis* as a classical biocontrol agent. Previous studies concluded that *T. sinensis* was an effective management option for *D. kuriphilus* in chestnut-growing areas in Italy (Bernardo et al. 2017; Bosio et al. 2013; Colombari and Battisti 2016a) and Europe (Borowiec et al. 2014; Matošević et al. 2017; Quacchia et al. 2014), but provided no specific data on changes in *D. kuriphilu*s infestation levels. In Northwest Italy *T. sinensis* established a presence shortly after its release in Piedmont in 2005 (Quacchia et al. 2008b). Its emergence rate increased exponentially, and by 2012 the number of *T. sinensis* females emerging per 100 galls was approximately 20 times higher than what was recorded in 2009 (exponential growth y=0; R^2=0.9448). In 2011, at Peveragno, the number increased nearly 11-fold compared to the previous year, while the only decline was recorded at Robilante in 2012. This reduction was likely due to a decrease in host density. As of 2014 the number of galls was significantly reduced, and in some sites (Cervasca and Robilante), no galls were found.

Our observations are in agreement with Moriya et al. (2003), who reported that the introduction of *T. sinensis* in Japan was a prominent and successful example of classical biological control. In Japan, the parasitoid kept the population of the ACGW under the damage threshold of 30% shoot infestation (Gyoutoku and Uemura 1985; Moriya et al. 1990). In the USA, Cooper and Rieske (2007) first reported that successful establishment of *T. sinensis* appeared to play a major role in population regulation of *D. kuriphilus*, but did not provide additional details. The time required for *T. sinensis* to establish a population varied by location in Japan. In Central Japan, Moriya et al. (1990) reported a decrease in *D. kuriphilus* chestnut infestation 6 years after *T. sinensis* introduction. Conversely, in Southwest Japan, it took 18 years to establish a *T. sinensis* population. This delay was attributed to a low female sex ratio and high mortality caused by native hyperparasitoids (Murakami and Gyoutoku 1995).

Based on our personal observations, *T. sinensis* required approximately 7 to 8 years to noticeably decrease the *D. kuriphilus* population in Italy. The success of this program was mainly due to a coordinated national and regional effort, where institutions, associations, and private landowners combined their efforts to achieve ACGW population control. Indeed, after being initially released in the Piedmont region, *T. sinensis* was released in several other Italian regions. In 2012, the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) actively pursued the national release of the parasitoid due to the evident impact on the decline in ACGW population. This pursuit led to funding of the Lobiocin and Bioinfocast projects. These programs released a total of 295,220 wasps (approximately 120 females and 60 males per release) at 1,669 sites in 17 regions between 2012 and 2014 (Alma et al. 2014).

Since *T. sinensis* is not native to Europe, several studies have investigated myriad native parasitoids of *D. kuriphilus* in Europe (Aebi et al. 2007; Kos et al. 2015; Matošević and Melika 2013; Palmeri et al. 2014; Panzavolta et al. 2013; Quacchia et al. 2013; Speranza et al. 2009). However, none of these native species effectively controls the ACGW population in the long term, most likely due to incompatible life cycles. As opposed to what occurred in Southwest Japan, no native European parasitoids negatively influenced the establishment of *T. sinensis*. Furthermore, *T. sinensis* so effectively controlled *D. kuriphilus* in Italy that its introduction progressively reduced the number of native parasitoids recruited since the establishment of the ACGW. Specifically, *T. sinensis* has caused the loss of approximately 14% of native parasitoid species, and 32% of the native parasitoid population density associated with the gall wasp, each year since its introduction (Ferracini et al. 2018).

T. sinensis may disperse over long distances through active flight or wind assistance to reach non-release sites (Colombari and Battisti 2016b; Matošević et al. 2017; Moriya et al. 2003). Nevertheless, a few regions in southern Italy exhibit variable *T. sinensis* distribution and/or recurrent ACGW infestation (Armentano 2016). In the 25 years since the initial parasitoid release in Japan, there have been three peaks in the *D. kuriphilus* population that were subsequently followed by peaks in *T. sinensis* (Moriya, personal communication). These observations clearly fit the mathematical model developed by Paparella et al. (2016) that describes the population pattern of *T. sinensis* and its host.

Indeed, according to the model, parasitoid dispersal drastically reduces the ACGW level. The model also predicts that the pest population may increase in parts of the chestnut environment where *T. sinensis* is no longer abundant due to the scarcity of *D. kuriphilus*. These dynamics promote a population wave pattern, where a *D. kuriphilus* population increase will be followed by an increase in *T. sinensis* parasitism.

There has been no reported evidence of ACGW infestation resurgence in North Italy 13 years after release of *T. sinensis*. This parasitoid has been proven to effectively control ACGW outbreaks, and its successful use highlights how classical biological control may represent a cost-effective tool for managing an exotic invasive pest, balancing pest populations below damaging levels. Since population changes and community responses induced by biological control programs often require long periods of time, continuous monitoring is needed to track the host-parasitoid population dynamics, and to verify the efficacy of this biocontrol agent over time.

Compliance with ethical standards

All the insect rearing and experiments were conducted in accordance with the legislation and guidelines of the European Union for the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). All experimental protocols using insects were approved by the *ad-hoc* Committee of DISAFA of the University of Torino.

Funding

This study was partially by the Ministry of Agricultural, Food and Forestry Policies (Lobiocin and Bioinfocast projects).

Conflict of interest

The authors declare that they have no confict of interest.

References

Aebi A, Schönenberger N, Melika G, Quacchia A, Alma A, Stone GN (2007) Native introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*. EPPO Bull 37:166-171

Alma A, Ferracini C, Sartor C, Ferrari E, Botta R (2014) Il cinipide orientale del castagno: lotta biologica e sensibilità varietale. Italus Hortus 21:15-29

Armentano G (2016) Cinipide del castagno: resta critica la situazione in alcune regioni. Inf.tore Agrario 72(33):64-65

Battisti A, Benvegnù I, Colombari F, Haack RA (2014) Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agric For Entomol 16:75-79

Bernardo U, Nugnes F, Gualtieri L, Scarpato S, Gargiulo G, Griffo R (2017) Cinipide del castagno, cresce il controllo biologico in Campania. Inf.tore Agrario 73(27):51-53

Borowiec N, Thaon M, Brancaccio L, Warot S, Vercken E, Fauvergue X, Ris N, Malausa JC (2014) Classical biological control against the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in France. Plant Prot Q 29:7-10

Bosio G, Armando M, Moriya S (2013) Verso il controllo biologico del cinipide del castagno. Inf.tore Agrario 69(14):60-64

Brussino G, Bosio G, Baudino M, Giordano R, Ramello F, Melika G (2002) Pericoloso insetto esotico per il castagno europeo. Inf.tore Agrario 58(37):59-61

Calabria G, Máca J, Bächli G, Serra L, Pascual M (2010) First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J Appl Entomol 136:139-147

Clewley GD, Eschen R., Shaw RH, Wright DJ (2012) The effectiveness of classical biological control of invasive plants. J Appl Ecol 49:1287-1295

Cock MJ, Murphy ST, Kairo MT, Thompson E, Murphy RJ, Francis AW (2016) Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61:349-363

Colombari F, Battisti A (2016a) Native and introduced parasitoids in the biocontrol of *Dryocosmus kuriphilus* in Veneto (Italy). EPPO Bull 46:275-285

Colombari F, Battisti A (2016b) Spread of the introduced biocontrol agent *Torymus sinensis* in north-eastern Italy: dispersal through active flight or assisted by wind? BioControl 61:127-139

Cooper WR, Rieske LK (2007) Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America. Ann Entomol Soc Am 100:236-244

Cooper WR, Rieske LK (2011) A native and an introduced parasitoid utilize an exotic gall-maker host. BioControl 56:725-734

DeBach P (1964) Biological Control of Insect Pests and Weeds. Chapman & Hall, London

DiTomaso JM, Van Steenwyk RA, Nowierski RM, Vollmer JL, Lane E, Chilton E, Burch P, Cowan PE, Zimmerman K, Dionigi CP (2017) Enhancing the effectiveness of biological control programs of invasive species through a more comprehensive pest management approach. Pest Manag Sci 73:9-13

EPPO (2014) First report of *Popillia japonica* in Italy. EPPO Reporting Service 10

EPPO (2016) PQR – EPPO database on quarantine pests (available online). http://www.eppo.int

Ferracini C, Bertolino S, Bernardo U, Bonsignore C, Faccoli M, Ferrari E, Lupi D, Maini S, Mazzon L, Nugnes F, Rocco A, Santi F, Tavella L (2018) Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp? Biol Control 121:36-43

Ferracini C, Ferrari E, Pontini M, Hernández Nova LK, Saladini MA, Alma A (2017) Post-release evaluation of non-target effects of *Torymus sinensis*, the biological control agent of *Dryocosmus kuriphilus* in Italy. BioControl 62:445-456

Ferracini C, Ferrari E, Saladini MA, Pontini M, Corradetti M, Alma A (2015a) Non-target host risk assessment for the parasitoid *Torymus sinensis*. BioControl, 60:583-594

Ferracini C, Gonella E, Ferrari E, Saladini MA, Picciau L, Tota F, Pontini M, Alma A (2015b) Novel insight in the life cycle of *Torymus sinensis*, biocontrol agent of the chestnut gall wasp. BioControl 60:169-177

Gehring E, Bellosi E, Quacchia A, Conedera M (2018) Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters. J Pest Sci 91: 189-202

Gerber E, Schaffner U (2016) Review of invertebrate biological control agents introduced into Europe. CABI Publishing, Wallingford

Gyoutoku Y, Uemura M (1985) Ecology and biological control of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), 1. Damage and parasitization in Kumamoto prefecture [Japan]. Proc Assoc Pl Prot Kyushu 31:213-215

Haack RA, Herard F, Sun JH, Turgeon JJ (2010) Managing invasive populations of Asian longhorned beetle and citrus longhorned beetle: a worldwide perspective. Annu Rev Entomol 55:521-546

İpekdal K, Emin A, Kuzucu AŞ, Karadağ M, Koçluk M, Açıcı Ö, Şah S, Aksu Y, Colombari F (2017) Rearing and releasing *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), larval parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). Turkish Bull Entomol 7:113-129

Kaartinen R, Stone GN, Hearn J, Lohse K, Roslin T (2010) Revealing secret liaisons: DNA barcoding changes our understanding of food webs. Ecol Entomol 35:623-638

Kos K, Kriston E, Melika G (2015) Invasive chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), its native parasitoid community and association with oak gall wasps in Slovenia. Eur J Entomol 112:698

Matošević D, Lacković N, Kos K, Kriston E, Melika G, Rot M, Pernek M (2017) Success of classical biocontrol agent *Torymus sinensis* within its expanding range in Europe. J Appl Entomol 141:758-767

Matošević D, Melika G (2013) Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia. B Insectol 66:231-238

Matošević D, Quacchia A, Kriston É, Melika G (2014) Biological control of the invasive *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)-an overview and the first trials in Croatia. South-east Eur For 5:3-12

Montagna M, Gonella E, Pontini M, Ferrari E, Ferracini C, Alma A (2018) Molecular species delimitation of the Asian chestnut gall wasp biocontrol agent released in Italy. Insect Syst Evol doi: 10.1163/1876312X-00002188

Moriya S, Inoue K, Mabuchi M (1990) The use of *Torymus sinensis* to control chestnut gall wasp, *Dryocosmus kuriphilus*, in Japan. Technical bulletin - Food and Fertilizer Technology Center (FFTC) 118:1-12

Moriya S, Shiga M, Adachi I (2003). Classical biological control of the chestnut gall wasp in Japan, In: Van Driesche RG (Ed) Proceedings of the 1st international symposium on biological control of arthropods. Hawaii, 14-18 January 2002. USDA Forest Service, Washington, DC, USA, pp 407-415

Murakami Y, Gyoutoku Y (1995) A delayed increase in the population of an imported parasitoid, *Torymus* (*Syntomaspis*) *sinensis* (Hymenoptera: Torymidae) in Kumamoto, southwestern Japan. Appl Entomol Zool 30:215-224

Palmeri V, Cascone P, Campolo O, Grande SB, Laudani F, Malacrinò A, Guerrieri E (2014) Hymenoptera wasps associated with the Asian gall wasp of chestnut (*Dryocosmus kuriphilus*) in Calabria, Italy. Phytoparasitica 42:699-702

Panzavolta T, Bernardo U, Bracalini M, Cascone P, Croci F, Gebiola M, Iodice L, Tiberi R, Guerrieri E (2013) Native parasitoids associated with *Dryocosmus kuriphilus* in Tuscany, Italy. B Insectol 66:195-201

Paparella F, Ferracini C, Portaluri A, Manzo A, Alma A (2016) Biological control of the chestnut gall wasp with *T. sinensis*: a mathematical model. Ecol Modell 338:17-36

Pérez-Otero R, Crespo D, Mansilla JP (2017) *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) in Galicia (NW Spain): pest dispersion, associated parasitoids and first biological control attempts. Arquivos Entomolóxicos 17: 439-448

Picciau L, Ferracini C, Alma A (2017) Reproductive traits in *Torymus sinensis*, biocontrol agent of the Asian chestnut gall wasp: implications for biological control success. B Insectol 70:49-55

Quacchia A, Ferracini C, Nicholls JA, Piazza E, Saladini MA, Tota F, Melika G, Alma A (2013) Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north-western Italy. Insect Conserv Divers 6:114-123

Quacchia A, Moriya S, Askew R, Schönrogge K (2014) *Torymus sinensis*: biology, host range and hybridization. Acta Hortic 1043:105-111

Quacchia A, Moriya S, Bosio G, Scapin G, Alma A (2008a) Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. BioControl 53:829-839

Quacchia A, Ferracini C, Bonelli S, Balletto E, Alma A (2008b) Can the Geranium Bronze, *Cacyreus marshalli*, become a threat for European biodiversity? Biodivers Conserv 17:1429-1437

RefCast-Associação Portuguesa da Castanha (2015) Protocolo BioVespa, Luta Biológica contra a Vespa das Galhas do Castanheiro - Uma estratégia global. VI European Chestnut Meeting, Vila Pouca de Aguiar/Valpaços, Portugal, 9-12 September 2015

Roques A, Rabitsch W, Rasplus J-Y, Lopez-Vamonde C, Nentwig W, Kenis M (2009) Alien terrestrial invertebrates of Europe. In: DAISIE (ed.) Handbook of alien species in Europe, Springer, Berlin, pp. 63-79

Shine C, Kettunen M, ten Brink P, Genovesi P, Gollasch S (2009) Technical support to EU strategy on invasive species (IAS) – Recommendations on policy options to control the negative impacts of IAS on biodiversity in Europe and the EU. Final report for the European Commission. Institute for European Environmental Policy (IEEP), Brussels, Belgium. 35 pp.

Speranza S, Stacchiotti M, Paparatti B (2009) Endemic parasitoids of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cinipidae) in Central Italy. In: IV International Chestnut Symposium 844:421-42

Stiling P, Cornelissen T (2005) What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biol Control, 34:236-246

Table 1 – Sampling sites monitored in the present study

Region	Province	Site	Geographic coordinates	
			N	E
Piedmont	Cuneo	Boves	44°19'06.1"	07°33'18.3"
		Caraglio	44°24'31.7"	07°24'05.9"
		Cervasca	44°22'15.3"	07°26'57.2"
		Chiusa di Pesio	44°16'52.2"	07°40'21.8"
		Peveragno	44°18'57.1"	07°35'08.2"
		Robilante	44°18'09.6"	07°31'07.4"
Abruzzo	L'Aquila	Canistro I	41°55'10.2"	13°24'49.8"
		Canistro II	41°55'54.6"	13°24'13.2"
		Civitella Roveto I	41°54'22.2"	13°25'45.0"
		Civitella Roveto II	41°54'19.8"	13°24'55.2"
Aosta Valley	Aosta	Aosta	45°45'23.9"	07°19'59.2"
		Montjovet	45°42'10.4"	07°40'47.7"
		Pondel	45°40'22.1"	07°13'41.4"
		Verres	45°39'06.9"	07°40'35.9"
Liguria	Genova	Masone	44°30'47.0"	08°44'22.0"
		Neirone	44°26'50.1"	09°11'16.4"
		San Colombano Certenoli	44°23'14.1"	09°18'23.0"
	La Spezia	Biassa	44°06'00.6"	09°46'13.3"
		Carro	44°15'43.3"	09°37'18.1"
		Sesta Godano	44°15'25.5"	09°40'56.3"
Tuscany	Firenze	Marradi I	44°04'52.5"	11°35'19.7"
		Marradi II	44°04'42.0"	11°39'39.7"
		Marradi III	44°06'40.6"	11°37'52.5"

Online Resource 1 – Mean infestation rate (percentage of buds infested per tree) of Asian chestnut gall wasp **(**± SE) recorded in the sampling sites of Piedmont region from 2009–2016 (n = 100 branches per site per year).

Site	**Year**															
	2009	SE	2010	SE	2011	SE	2012	SE	2013	SE	2014	SE	2015	SE	2016	SE
Boves	59.85	±2.55	79.56	±2.81	68.97	±5.08	66.65	±6.32	8.34	±2.63	0.92	±0.47	0.50	±0.50	0.00	±0.00
Caraglio	65.50	±3.76	65.67	±4.71	60.08	±4.14	61.02	±4.26	17.16	±4.28	0.20	±0.20	0.00	±0.00	0.00	±0.00
Cervasca	63.09	±3.14	56.53	±4.00	83.80	±3.28	50.55	±7.12	8.28	±3.17	0.00	±0.00	0.00	±0.00	0.00	±0.00
Chiusa di Pesio	59.75	±2.74	59.75	±2.74	45.89	±4.76	51.97	±7.58	24.89	±6.91	0.68	±0.37	0.17	±0.17	0.02	±0.73
Peveragno	75.66	±3.19	66.81	±4.02	69.39	±3.58	77.05	±6.43	11.51	±2.61	1.22	±0.73	1.20	±0.96	0.00	±0.00
Robilante	51.30	±4.79	60.94	±3.85	76.96	±5.08	37.51	±4.85	1.92	±1.28	0.00	±0.00	0.00	±0.00	0.00	±0.00
Mean	62.53	±3.28	64.88	±3.32	67.51	±5.42	57.46	±5.65	12.02	±3.27	0.50	±0.21	0.31	±0.19	0.0033	±0.003

Online Resource 2 – Mean number of *Torymus sinensis* adults emerged from 100 galls recorded in the sampling sites of Piedmont region

Region	**Site**	***T. sinensis* first release (year)**	2009		2010		2011		2012		2013	
			♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Piedmont	Boves	2005	0.3	0.2	3.1	2.7	47.5	41.5	84.1	71.2	80.9	72.7
	Caraglio	2006	3.6	2.7	13.2	12.0	82.3	74.5	99.8	85.6	64.6	93.6
	Cervasca	2006	0.8	0.5	5.6	3.4	9.3	7.6	68.1	66.0	83.5	102.1
	Chiusa di Pesio	2006	1.4	1.6	7.7	5.8	43.7	43.2	80.2	69.4	63.9	73.9
	Peveragno	2005	3.3	2.6	8.7	8.0	98.9	77.7	110.3	99.3	106.0	122.0
	Robilante	2005	15.1	14.2	59.0	53.8	141.0	108.1	109.6	104.5	90.7	114.5

Online Resource 3 - Mean number of *Torymus sinensis* adults emerged from 100 galls recorded in the sampling sites of Abruzzo, Aosta Valley, Liguria, and Tuscany regions

Region	**site**	***T. sinensis* first release (year)**	2014		2015		**Region**	**site**	***T. sinensis* first release (year)**	2016		2017	
			♀♀	♂♂	♀♀	♂♂				♀♀	♂♂	♀♀	♂♂
Abruzzo	Canistro I	2011	0.04	0.09	3.50	2.00	Liguria	Masone	2010	140.80	115.20	80.60	65.90
	Canistro II	2011	1.70	2.00	62.50	81.00		Neirone	2010	131.40	107.50	224.30	195.70
	Civitella Roveto I	2011	3.40	2.50	20.00	20.50		San Colombano Certenoli	2010	128.10	104.80	183.00	142.00
	Civitella Roveto II	2011	0.08	0.30	48.00	43.00		Biassa	2011	120.40	98.50	319.00	261.00
Aosta valley	Aosta	2012	37.20	35.00	42.40	33.70		Carro	2011	127.70	141.30	87.40	100.50
	Montjovet	2012	99.60	95.80	125.30	65.20		Sesta Godano	2011	105.60	86.40	89.10	72.90
	Pondel	2012	20.00	12.40	70.80	37.90	Tuscany	Marradi I	2010	128.70	105.30	203.50	166.50
	Verres	2012	22.40	15.40	41.20	33.80		Marradi II	2010	209.00	171.00	179.70	147.00
								Marradi III	2010	95.70	78.30	191.10	156.40

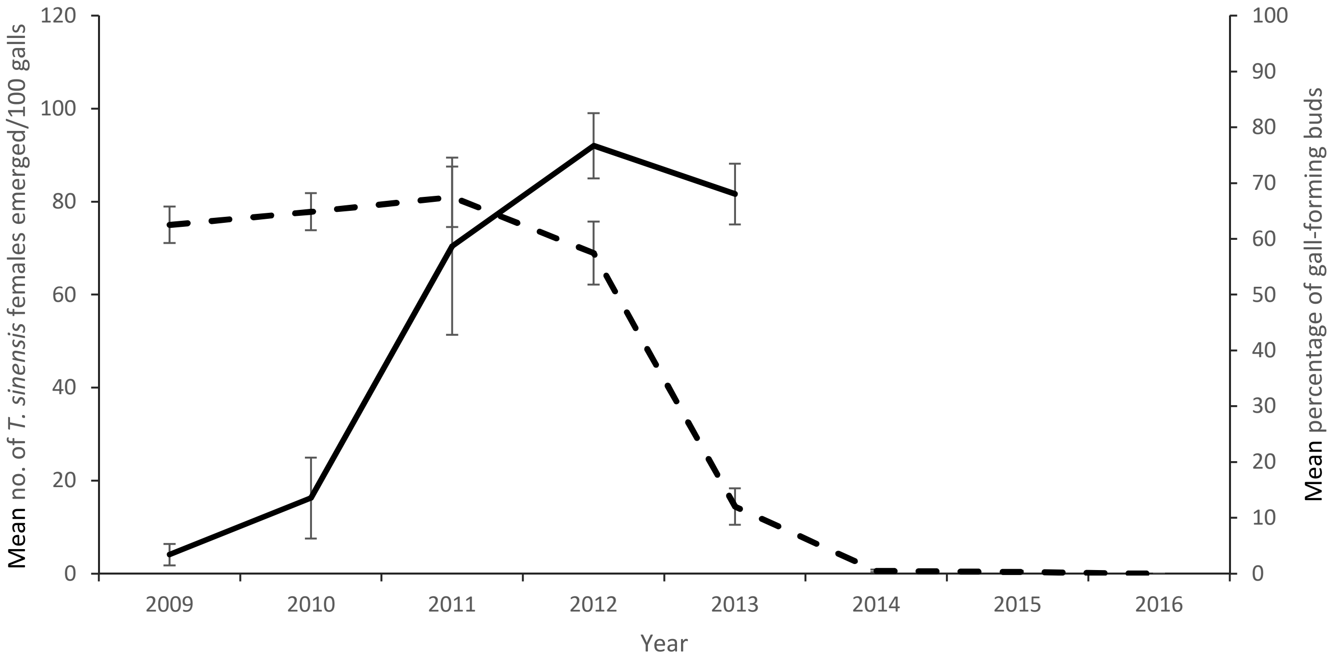

Figure 1 – Mean number of *Torymus sinensis* females emerged per 100 galls (solid line) and mean infestation rate (percentage of infested buds by the total amount of buds; dotted line) recorded in the sampling sites of Piedmont region. Lines represent mean ± SE.

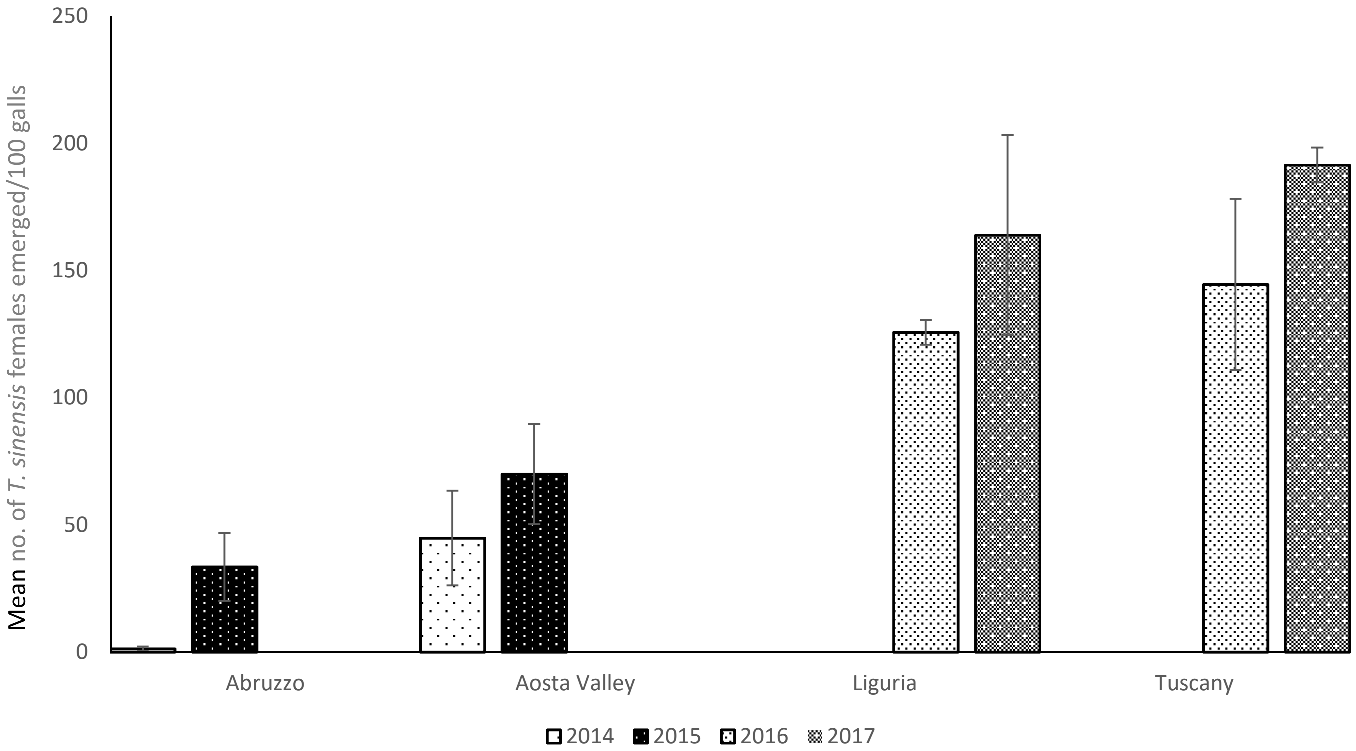

Figure 2 – Mean number of *Torymus sinensis* females emerged per 100 galls recorded in the sampling sites of Abruzzo and Aosta Valley regions in 2014-2015, and Liguria and Tuscany regions in 2016-2017. Bars represent mean ± SE.