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

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

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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.

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Conflict of interest

The authors declare that they have no confict of interest.

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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

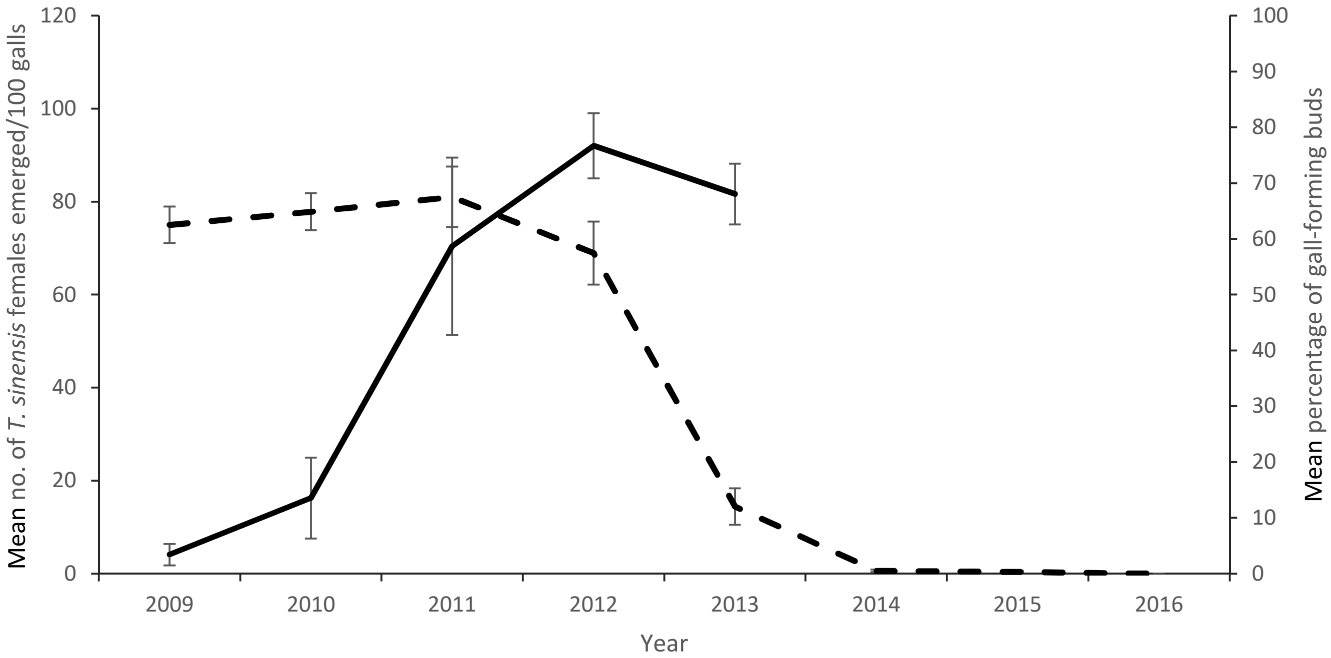

Figure 1 – Mean number of *Torymus sinensis* females emerged per 100 galls (solid line) and mean infestation rate (percentage of infested buds by the total amount of buds; dotted line) recorded in the sampling sites of Piedmont region. Lines represent mean ± SE.

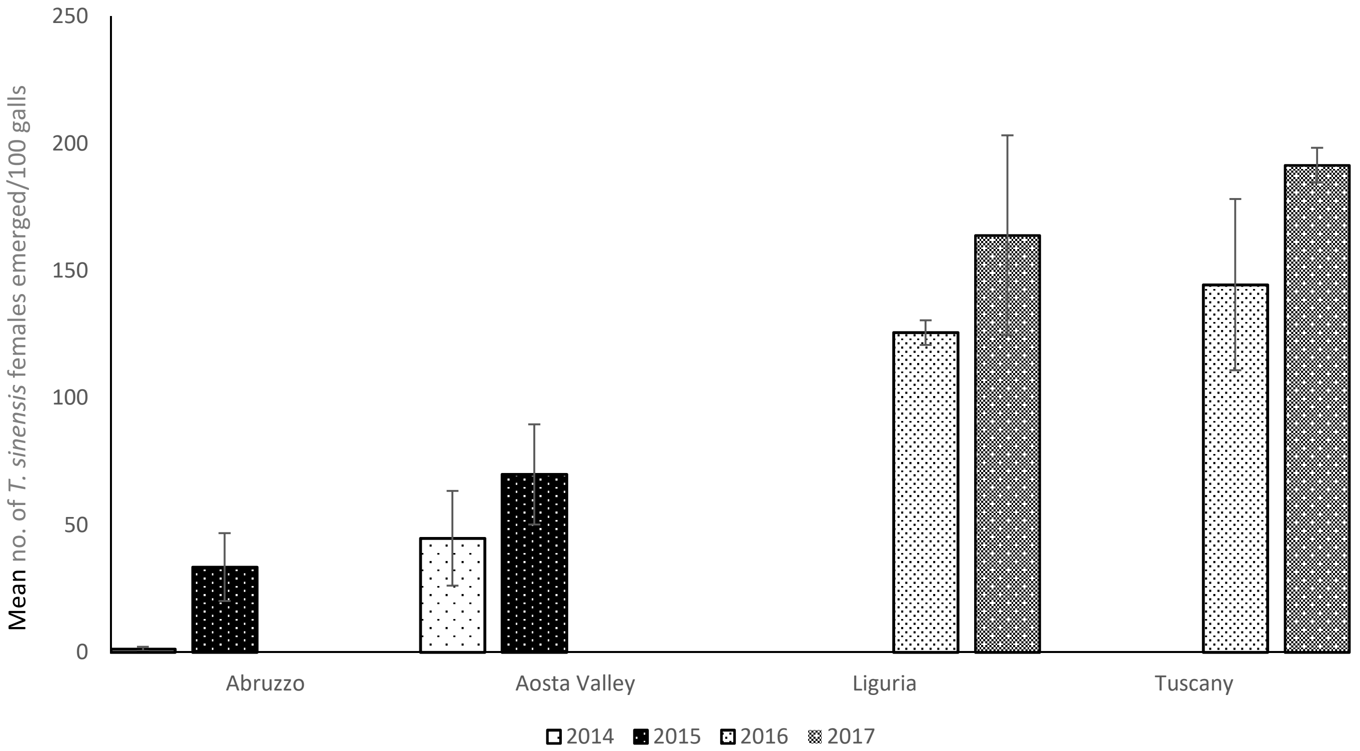

Figure 2 – Mean number of *Torymus sinensis* females emerged per 100 galls recorded in the sampling sites of Abruzzo and Aosta Valley regions in 2014-2015, and Liguria and Tuscany regions in 2016-2017. Bars represent mean ± SE.