Final Scientific Report (2012-2016)

OTKA K101192: Parasitoid assemblages of chestnut gallwasp, *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in Europe

Dryocosmus kuriphilus (Yasumatsu, 1951) Hymenoptera: Cynipidae) (DK), emerged as a pest in the mid-twentieth century and is now one of the most important global insect pests of chestnut (*Castenea* spp. Fagaceae) (Brussino et al. 2002, Melika et al. 2003, Aebi et al. 2006, 2007). This pest species disrupts growth by inducing gall formation on new shoots and leaves, eliminating nut production and causing a gradual decline in the vigor of these long-lived slow-growing chestnut trees (Payne 1978, Payne et al. 1975, 1983, Dixon et al. 1986, Kato & Hijii 1997, 1999). Commonly DK reduces wood production and fruit yield by 50–80% (Melika et al. 2003, Quacchia et al. 2012). The damage may continue for many years. Additionally, severe consecutive attacks may result in the death of the tree, probably in combination with other detrimental factors such as fungal infection, drought or severe attack by other herbivores (Dixon et al. 1986, Quacchia et al. 2012, Szabó et al. 2014). *Dryocosmus kuriphilus* is native to China, and from there this species was accidentally introduced to Japan in 1941 (Murakami et al. 1995). In 1958 was recorded in Korea (Cho & Lee 1963, Murakami et al. 1995), colonized USA in 1974 (Payne et al 1976, 1983, Rieske 2007), Nepal in 1999 (Abe et al. 2007). It was accidentally introduced to Europe in 2002 (Brussino et al. 2002, Aebi et al. 2006, Gibbs et al. 2011). Since 2002, DK has established and spread throughout Italy, and has also been reported in 2005 from France and Corsica (Aebi et al. 2006), Slovenia (Knapič et al. 2009), in 2009 from Hungary (Csóka et al. 2009) and Switzerland (Forster et al. 2009), in 2010 from Croatia (Matošević et al. 2010), in 2011 from Slovakia and Czech Republic, in 2012 from Spain and Canada (Szabó et al 2013, Huber & Read 2012); in 2014 from Greece (Michaelakis et al. 2016), Portugal and Turkey (Çetġn et al. 2014), in 2015 in the United Kingdom (England) (Malumphy 2015) and Romania (Radócz et al. 2015), in 2016 in Bosnia and Herzegovina (Delalić 2016) and the Russian Federation (North Caucasus) (Gninenko & Melika, unpublished data). Currently DK spread across all areas of Europe where there is chestnut timber and nut production (Matošević et al. 2015).

The first record of DK from Hungary is dated by May 2009 when an infested tree was detected near Budapest in Üröm (Csóka et al. 2009, Csóka & Melika 2009). In 2010 an infested tree was found in Pécs; in May 2013 in Budapest also a single DK infested tree was was found (Szabó et al. 2013). In all three cases the infested trees were imported from the infested zone in north Italy and were eradicated by the NÉBIH plant health authority (Szabó et al. 2014). In 2013 DK galls were found in large numbers in the most southwest part of Hungary, in Zala County in 12 localities and no doubts it was a natural spread from Slovenia (Szabó et al. 2014). Systematic surveys evaluated by our group in 2013-2016 in different parts of the country showed that by the end of 2016 DK have had spread across all areas of Hungary where chestnut stands are present, particularly it was found all over Zala and Somogy Counties, in greater part of Vas County, in Budapest, Pilis Mountains, Visegrád, Nagymaros, Sopron and Pécs vicinities (Kriston et al. 2015, Melika et al. 2015). By now DK spreaded all over the territory of Hungary where *Castanea* is growing.

The human exchange of infected cultivars and material for grafting among chestnut growers, the natural range expansion within nearby natural and planted stands are the main factors facilitating the rapid spread of ACGW (EPPO 2005, EFSA 2010, Radócz et al. 2015). Strict control of the movement of infested plant material can reduce long-distance dispersal of ACGW to new areas within Europe. However, there are limited options available for managing existing ACGW populations and to reduce their impact and spread on European chestnut plantations. Mechanical removal of infested twigs (pruning) and the protection of seedlings with nets, although effective, do not represent practical solutions because of their labour intensiveness and high expenses. Since the larval and pupal stages are protected within the galls formed by this species, conventional chemical control is regarded as largely ineffective (EFSA 2010, Quacchia et al. 2012). Developing resistant varieties of *Castanea* spp., for example a japanese-european hybrid Boche de Bètizac, could potentially be a viable management option, but this will only be beneficial for new planting and will not help existing chestnut plantations (EFSA 2010).

Native Parasitoids

The invasive DK, like other exotic species, undergo population explosions and spread rapidly since they are released from their normal controls of disease and natural enemies (van Lenteren et al. 2006). Expanding populations of exotic species can disrupt ecosystems directly through displacement and extinction of native species (Wilson 1997), causing both economic and environmental damage. However, when trophic links are established between invasive species and native communities, invaders can impose indirect effects on communities through perturbed trophic structures (Vitousek et al. 1996, Hennemann & Memmot 2001).

DK is univoltine and solely parthenogenetic (thelytokous), thus only a single female is necessary to establish a population, and these populations can grow rapidly (Askew 1984, Nohara 1956, Miyashita et al. 1965). Females laying eggs in the buds of *Castanea* spp. during summer which then hatch in 30-40 days. Overwintering took place by the first instar larva that grows slowly until the following spring when it start to grow faster leading to the induction of galls inside which the gallwasp larvae develop (Brussino et al. 2002, Melika et al. 2003). It was showed that conventional pest control measurements (pesticides, agrotechnical and plant health measures, resistant varieties) are not enough effective against the invasive DK. Native parasitoids that normally attack galls induced by related cynipid gallwasps on oaks (Fagaceae, *Quercus* spp.) have colonised DK galls everywhere throughout their introduced range (Aebi et al. 2006, 2007). Given the high abundance of DK galls, the use of this species as a host by oak gall parasitoids may have massive, but to date unstudied, impacts on the oak gall community through the process of apparent competition (Holt 1977). However, to understand the impacts of this invading species we first need to know basic information on the identity and behaviour of native parasitoids colonising DK in Europe.

Oak cynipid galls (Hymenoptera, Cynipidae, Cynipini) support species-rich and ecologically closed communities of parasitoids (predominantly chalcid parasitoid wasps) and inquilines (including cynipids) that have become important model systems in the study of community structure. The best-studied cynipid communities and the most species-rich of any cynipid gallwasp group are those associated with oak cynipid gallwasps, particularly in Europe, which support at least 120 species of chalcids in six families – Pteromalidae (27 species), Eurytomidae (10 species), Torymidae (18 species), Ormyridae (2 species), Eupelmidae (8 species), and Eulophidae (30 species) (summarized in Askew 2013, Bailey et al. 2009). We study the parasitoid communities of the Western Palaearctic oak cynipid galls since 2002 (e.g. Ács et al. 2002, Melika et al. 2002a, b, c, Azizkhani et al. 2005, Nazemi et al. 2007, 2008 and others).

In its native range in China and in Japan *D. kuriphilus* populations are also kept at low densities by a well-studied complex of around parasitoid species (reviewed in Aebi et al. 2006; Schönrogge et al. 2006; Abe et al. 2007). A total of 11 species in 5 chalcid families (Torymidae, Ormyridae, Eurytomidae, Eupelmidae and Eulophidae) are known to attack *D. kuriphilus* in its native range in China (Murakami *et al*., 1980; Kamijo 1981; Murakami, 1981; Luo & Huang, 1993). Except TS, which shows high host specificity and a life cycle matching that of its host, all the remaining species also attack related cynipid hosts galling oaks. In Japan introduced *D. kuriphilus* is now attacked by a rich parasitoid wasp assemblage of 24 chalcid species from 7 families and 1 braconid (*Aspilota yasumatsui* Watanabe) (Aebi *et al*., 2006; Abe *et al*., 2007). After its arrival in Korea, *D. kuriphilus* recruited a parasitoid assemblage of 17 chalcid species over a period of only several decades (Ko, 1971; Yasumatsu & Kamijo, 1979; Kamijo, 1981, 1982; Murakami *et al*., 1985, 1994, 1995; Ôtake, 1989; Ôtake *et al*., 1982; Kim, 1998). The number of native parasitoids which attack DK in the USA is much lower

Preliminary surveys of the natural enemies attacking DK were done in Italy in which 27 species belonging to 6 Chalcidoidea families (Eurytomidae, Pteromalidae, Torymidae, Eupelmidae, Ormyridae, Eulophidae) were listed ((Aebi et al. 2006, 2007, Quacchia et al. 2012). All the parasitoids reared from DK galls in Italy (IT) since 2005, Slovenia (SLO) since 2009, Croatia (CRO) since 2011, were send to and identified by the principal investigator of this grant (GM).

During 2012-2016 new species of parasitoids, not listed in earlier works, were shifting on the new invasive species, DK. In order to study the affect of indigenous European parasitoid species on DK we put the next questions to be answered:

(1) Do parasitoids attacking DK show strong overlap with those attacking oak cynipid gallwasps locally?

(2) Are the parasitoids that attack both chestnut and oak cynipids dominated by species with a broad host range?

(3) What is the taxonomic spectrum of parasitoids recruiting to chestnut gallwasp? Is this community variable across the geographic range of DK in Europe?

(4) How good is the phenological match between development of DK galls and emergence of native oak gall parasitoid species?

(5) What is the time lag between the arrival of the invader and the development of a parasitoid community? Which parasitoids recruit first and why?

(6) Are parasitoid assemblages in pure chestnut stands less diverse and less effective in biological control than those in mixed oak/chestnut woodlands?

(7) Are TS parasitoids released in Italy tracking range expansion by DK? Is there any evidence that this parasitoid is attacking native hosts?

Research that was done by us during 2013-2016 showed that all together **43** native European parasitoid species from six Chalcidoidea families shifted onto the new host, DK (Table 1), however, with a very low parasitization rate, 2.0–4.5 % (Matošević & Melika 2013, 2014, 2015, Kos et al. 2015), which is unable to keep DK under economic threshold. Also it is known that in some parts of Italy the native *Torymus flavipes* can control up to 32% of DK population (Santi & Maini 2011). In our samples collected during 2013-2016 on different sites in IT, SLO, CRO and HU, *T. flavipes* was also one of the dominant species within the local parasitoid complexes.

During 2013-2016 we collected DK galls for rearing native parasitoids in different localities in IT, SLO, CRO and HU. Annually two collecting trips were done: (i) March-beginning of April, overwintered galls were collected; late May-June, freshly developed galls were collected. Further collecting trips to IT, SLO and CRO were done in 2013-2015 in order to collect DK galls for rearing TS with their further release in HU.

In addition to our collecting trips, our cooperators also collected DK galls for rearing parasitoids: G. Bosio in Italy, Dr. D. Matošević in Croatia, Dr. K. Kos in Slovenia; all reared parasitoids were sent to us for species identification.

Table 1. European indigenous chalcidoid parasitoid species associate with DK across Italy, Slovenia, Croatia and Hungary (for 2016)

#	**Parasitoid Species Name**	**IT**	**SLO**	**CRO**	**HU**	**NEW***
	Eulophidae					
	Aprostocetus sp., b	+			+	
1.	Aprostocetus biorrhizae (Szelényi), a		+		+	+
2.	Aprostocetus aethiops (Zetterstedt), a		+			+
3.	Aprostocetus glandicola Graham, a		+			+
4.	Aulogymnus arsames (Walker), a,b	+				
5.	Aulogymnus skianeuros (Ratzeburg), a		+	+	+	+
	Aulogymnus sp., b	+				
6.	Baryscapus pallidae Graham, b	+				
	Baryscapus sp., a	+	+			
7.	Pediobius chilaspidis Bouček, b	+				
8.	Pediobius saulius (Walker), b	+	+	+	+	
	Pediobius sp., b	+				
	Eupelmidae					
9.	Eupelmus annulatus Nees, a,b	+	+	+	+	
10.	Eupelmus rostratus Ruschka, b			+	+	+
11.	Eupelmus spongipartus Foerster, a,b	+	+	+	+	
12.	Eupelmus splendens Giraud, a	+	+			
13.	Eupelmus urozonus Dalman, a,b	+	+	+	+	
14.	Eupelmus (= Macroneura) vesicularis (Retzius), b		+	+	+	+
	Eurytomidae					
15.	Eurytoma brunniventris Ratzeburg, a,b	+	+	+	+	
16.	Eurytoma adleriae Zerova, b	+				
17.	Eurytoma pistaciae Rondani, a,b	+	+	+	+	
18.	Sycophila variegata (Curtis), b	+	+	+	+	
19.	Sycophila biguttata (Swederus), b	+	+	+	+	
20.	Sycophila flavicollis (Walker), b	+N	+	+	+	+
21.	Sycophila iracemae Nieves Aldrey, b	+	+	+		
	Ormyridae					
22.	Ormyrus nitidulus (Fabricius), b	+	+	+	+	
23.	Ormyrus pomaceus (Geoffroy), b	+	+	+	+	
	Pteromalidae					
24.	Cecidostiba fungosa Geoffroy in Fourcroy, a	+N		+		
25.	Cecidostiba semifascia (Walker), a		+			+
	Cecidostiba sp., a	+				
26.	Mesopolobus albitarsus (Walker), a		+	+		+
27.	Mesopolobus amaenus (Walker), b	+	+			
28.	Mesopolobus dubius (Walker), b			+		+
29.	Mesopolobus fasciiventris Westwood, b	+N	+	+		+
30.	Mesopolobus mediterraneus (Mayr), b	+				
31.	Mesopolobus sericeus (Foerster), b	+	+	+	+	
32.	Mesopolobus tarsatus (Nees), b	+	+	+		
33.	Mesopolobus tibialis Westwood, b	+	+	+		

	Torymidae					
34.	Megastigmus dorsalis (Fabricius) (sp1), a,b	+	+	+	+	
35.	Megastigmus dorsalis (sp2)	+				
36.	Torymus auratus (Geoffroy in Fourcroy), b	+	+	+	+	
37.	Torymus fastuosus Boheman, b				+	+
38.	Torymus flavipes (Walker), a,b	+	+	+	+	
39.	Torymus formosus (Walker), b		+		+	+
40.	Torymus geranii (Walker), b	+N	+	+	+	+
41.	Torymus nobilis Boheman, b				+	+
42.	Torymus notatus (Walker), a			+	+	+
43.	Torymus scutellaris (Walker), b	+			+	
		31(+3)	31	27	26	16

N, parasitoid species which was not mentioned in the literature earlier; a, species emerged from overwintering galls; b, species emerged from newly formed galls; ab, species emerged from both types of galls.

Our research showed that all the parasitoid species attacking DK are generalists and coming from the local populations of oak gallwasps. Majority of species (26) were reared only from fresh, developing DK galls in early summer, 9 species only from overwintered galls and 8 species from both, fresh and overwintered galls. Sixteen species of parasitoids were found on DK for the first time by our research. The number of parasitoid species shifted on DK across IT (34 species), SLO (31), CRO (27) and HU (26) nearly the same (Table 1). The most common and abundant parasitoid species across Italy- Slovenia-Croatia-Hungary are the same prolong years: *Megastigmus dorsalis*, *Torymus flavipes* and *T. geranii* (Torymidae), *Eupelmus urozonus* and *E. annulatus* (Euplemidae), *Eurytoma brunniventris* and *E. pistaciae* (Eurytomidae), *Ormyrus pomaceus* (Ormyridae).The time lag between the introduction of the new host, DK, and the recruitment of native parasitoid community is short and depends on the longevity of pest’s presence on a particular site. Two scenarios for recruiting new native parasitoids are possible. Specifically, (i) are parasitoids conservative in their choice of host, jump onto the new invader rarely and track the host after a suitable match between parasitoid and host is established (the Host Tracking hypothesis) or (ii) are parasitoids opportunistic and readily jump onto a new host from their local population when it becomes available (the Local Recruiment hypothesis). We believe that Local Recruitment is the more likely hypothesis to explain interactions between native parasitoids and DK. Around half of parasitoid species associated with DK across its expanding range, were not observing in the original site of invasion, thus the Host Tracking is unlikely to be happened. There is no significant difference in species richness between collection sites in the north of Italy and collection sites in SLO, CRO and HU as well as no significant difference in species richness between years was found. So, the Local Recruitment is indicated, also by the observation of up to half of the parasitoids found on DK not being observed in the original site of invasion (the most likely source of Host Tracking because this is where the invader has been established the longest and had the most time for parasitoids to form associations). Another indicator might be that regions that are separated from each other share parasitoids that are missing in regions that would represent logical intermediate steps for a Host Tracking scenario.

There is a mismatch between the phenology of gall development of DK and emergence times of native natural enemies (Quacchia et al. 2013). *T. flavipes* has shown better timing for parasitizing the novel host and could be a good potential in biological control of DK. Native *Torymus* parasitoids have very fine phenological differences, compared to other native polyphagous parasitoids, which could make them more efficient in DK control.

The analyses of genetic variability of parasitoid species was carried out by means of DNA barcoding. A selection of species, which were most likely to be affected by morphological mis-identification and lumping of cryptic taxa were sequenced for DNA barcodes in a mitochondrial DNA region. The outcomes of DNA barcoding included spliting of the 4 morphospecies, *Eupelmus urozonus*, *Eurytoma brunniventris*, *Megastigmus dorsalis* and *Torymus flavipes* into 5, 8, 3 and 8 MOTUs respectively. The investigation of MOTU-specific interactions with oak and chestnut in the different regions reveals that a relatively high diversity of haplotypes can be found on both the oak and chestnut gall assemblages. This suggests that parasitoids will have made the jump from oak to chestnut many times over.

Two papers in co-authoring with our collaborators from Croatia, Slovenia and UK (Edinburgh University) are under preparation: (i) analyses of the native parasitoid complexes, and (ii) genetic variability of parasitoids across IT-SLO-CRO-HU.

Classical biological control of DK with introduced parasitoid *Torymus sinensis*

Torymus sinensis Kamijo, 1982 (Hymenoptera, Torymidae) (TS) is a parasitoid coming from China like its host and has been proven to be the only effective method of controlling the populations of DK. This specific parasitoid has been successfully introduced to Japan, the USA, Italy and France and used in classical biological control, has reduced the populations of DK to the tolerable levels (Matošević et al. 2014, 2015, Moriya et al. 2003, Quacchia et al. 2008, Cooper & Rieske, 2011, Bosio et al. 2013, Borowiec et al. 2014). Adults of TS emerge from withered galls in early spring and, after mating, 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 larva feeds ectoparasitically on the pest's mature larva and pupates during late winter (Matošević et al. 2014, 2015). TS is univoltine, host specific parasitoid, phenologically synchronized and morphologically adapted to DK, it has good dispersal abilities, builds populations quickly and effectively controls the pest already few years after release (Bosio et al. 2013, Matošević et al. 2014, 2015). On sites with releases of TS in Italy in 2005 and 2006, in 6-7 years, the parasitism rates have reached on average 67% (up to 95% in some sites (Bosio et al. 2013).

Same positive results of TS introduction were observed in Slovenia (SLO), Croatia (CRO) and Hungary (HU) (Matošević et al. 2014, Melika et al. 2015).

TS release in Hungary

In Hungary the first release of TS was done in 2014 in three localities: Dobri, Kerkateskand and Tornyiszentmiklos, in Zala County, the most south-western corner of Hungary, not far from the Slovenian border, on 9th April. In the first experiments isolators from fine mesh were used, a branch with 50-80 buds was enclosed by 1st April. DK galls started to form in the first week of April. In Dobri three isolators were put onto two chestnut trees, 20 females and 10 males were released into each of isolators on 9th April. In Kerkateskand 2 isolators were put out with the same number of TS females and males per isolator on the same day as in Dobri. Isolators were removed from the trees on 23rd April, after all TS females died. In autumn, 10 galls were collected from each branch, which were earlier covered with isolators, and the dissection of collected galls showed the presence of TS larvae. In each of dissected galls at least one TS larvae was found. Later DNA analysis showed that the larvae belong to TS. On 26th May 2014 TS females were released onto two trees in Kerkateskand (50 females) and Tornyiszentmiklos (200 females). In 2015, TS was released in Zala and Somogy counties. In Zala, 100 females in Tornyiszentmiklos and 150 females in Dobri were released. In Somogy County, around Iharosbereny 1,950 mated females were released on 15 points. In 2016 TS was released in Hungary again: in Nagymaros (2016 May 13, 580 TS females on 9 sites) and Díiósjenő (2016 May 13, 120 TS females on 1 sites) Nógrád Co.; Pilismarót, Pest Co. (2016 May 13, 480 TS females on 6 sites), and Iharosberény, Somogy Co. (2016 April 22 20, 2000 TS matted females on 20 trees, 100 females on each tree). The parasitization rates were calculated from 100 collected galls of DK on each site where TS was released in 2015 and 2016 (galls of DK were collected on 2015 August 18 and 2016 September 20 and 22). Results are given below:

Date of release	Site	GPS	# released females	*paras. rate, %
2015				
28.04.15	Tornyiszentmiklós	46.53336; 16.54597	100	65,1-88,5
12.05.15	Dobri	46.52166; 16.59260	150	67,6-90,8
25.05.15	**Iharosberény**			
	Iharosberény 1	46,37635; 17,098482	120	37,2
	Iharosberény 2	46,375116; 17,09945	90	40,4
	Iharosberény 3	46,374077; 17,099476	90	29,2
	Iharosberény 4	46,371802; 17,100203	120	68,0
	Iharosberény 5	46,369162; 17,105867	120	44,9
	Iharosberény 6	46,37174; 17,095775	120	34,5
	Iharosberény 7	46,376146; 17,095272	120	22,8
	Iharosberény 8	46,368283; 17,09341	120	42,8
	Iharosberény 9	46,366801; 17,093904	120	40,8
	Iharosberény 10	46,365536; 17,094352	120	31,5
	Iharosberény 11	46,361252; 17,099197	150	65,4
	Iharosberény 12	46,360118; 17,109912	120	55,3
	Iharosberény 13	46,359914; 17,107868	150	49,7
	Iharosberény 14	46,367768; 17,109163	210	66,2
	Iharosberény 15	46,365331; 17,117496	180	31,5
	TOTAL		**1300**	
2016				
03.05.16	Nagymaros	47,788237; 18,946689	5x100	84,3-92,9
03.05.16	Nagymaros, NAIK	47,828478; 18,968491	4x20	59,7
03.05.16	Diósjenő	47,949627; 19,038353	120	77,0
03.05.16	Pilismarót	47,764485; 18,875306	6x80	74,6
22.04.16	Iharosberény	46,365219; 17,117527	100	88,5
	Iharosberény	46,367392; 17,117754	100	93,5
	Iharosberény	46,359795; 17,110308	100	87,8
	Iharosberény	46,360312; 17,107352	100	
	Iharosberény	46,365654; 17,104859	100	93,7
	Iharosberény	46,368640; 17,106389	100	
	Iharosberény	46,370570; 17,103729	100	84,0
	Iharosberény	46,376322; 17,098896	100	
	Iharosberény	46,376246; 17,099671	100	83,8
	Iharosberény	46,374812; 17,098887	100	
	Iharosberény	46,373485; 17,099352	100	84,9
	Iharosberény	46,372523; 17,098683	100	
	Iharosberény	46,370756; 17,101073	100	82,1
	Iharosberény	46,370306; 17,098352	100	
	Iharosberény	46,369690; 17,094653	100	76,9
	Iharosberény	46,368259; 17,094045	100	
	Iharosberény	46,367238; 17,094088	100	89,9

	Iharosberény	46,365724; 17,094239	100	
	Iharosberény	46,364656; 17,095631	100	81,8
	Iharosberény	46,361156; 17,099160	100	79,8

Results of TS introduction to HU, SLO and CRO (we were involved into TS introductions and the evaluation of results in all three countries) are partially published (Csóka & Melika 2016, Kriston et al. 2014, 2015a,b, 2016, Matošević et al. 2014, 2015, Melika et al. 2015).

Success of TS establishment and population genetics of TS

The genetic diversity plays an important role in the success or failure of a classical biological control (Roderick & Navajas 2003, Fauvergue et al. 2012). Newly established populations of a biocontrol agent could suffer from genetic bottleneck due to relatively small number of individuals used as founders of new populations, the phenomenon known as founder effect (Fauvergue et al. 2007; Franks et al. 2011). Following the introduction of TS in Croatia, Slovenia and Hungary in 2015, the research of parasitism rates and population genetic indices on 40 different sites was performed in order to monitor and evaluate the success of establishment. Observed parasitism rates were unexpectedly high and negatively correlated with distance from westernmost locality, while population genetic indices showed that populations of TS did not suffer from bottleneck-induced founder effect phenomenon. Moreover, lack of genetic differentiation demonstrates that all populations share similar genetic structure, which could be shaped only by high levels of gene flow. We conclude that TS established viable and genetically diverse populations and successful spread naturally from Italy across Slovenia to Croatia and Hungary (Matošević et al. 2016, submitted).

Our results have shown that TS has rapidly spread over SLO to CRO and HU. The parasitoid has been confirmed on release and non-release sites in all three countries with unexpectedly high parasitism rates. As TS appeared simultaneously in SLO, CRO and HU in spring 2015 prior to intensive releases (Matošević et al. 2015), it is not possible that the recorded parasitism rates are result of our release efforts. Higher parasitism rates on non-release sites further support this conclusion. TS colonizes the range of its host by stratified dispersal i.e. combination of short and long distance dispersal affected by resource concentration and wind, enabling it to expand quickly (Colombari & Battisti 2015). High parasitism rates observed across SLO and western parts of CRO, accompanied with the negative trend of linear regression and negative correlation to distance from westernmost locality in Italy supports the hypothesis that TS was dispersing eastward from Italy. Intensive releases were made in Veneto region, Italy since 2010 (Colombari & Battisti 2015) and TS has covered the distance crossing SLO to CRO and HU in 4 years. This rapid dispersal of TS was aided by high concentration of the host, as well as lack of competition of native parasitoids (Matošević & Melika 2013; Kos et al. 2015). With the DK present in abundance, successful mate finding and probably no major limiting factors (i.e. hyperparasitoids) affecting new populations, there was no difficulty for TS to exponentially grow in numbers and expand the population. Lack of genetic differentiation between populations was shown in our recent research proves that all populations share common genetic structure (Matošević et al. 2016, submitted). Lack of genetic differentiation shown by AMOVA indicates that both release and non-release sites share common gen pool, adding to the hypothesis that spread already occurred naturally from Italy, probably even before official releases in SLO, CRO and HU.

TS: host acceptance behavior and response to olfactory and visual cues

Several unforeseen gaps in TS biology, systematic, host preference, and life cycle were identified and very little is known on the potential environmental risks of using TS (Aebi *et al*. 2007, 2011, Gibbs *et al*. 2011, Matošević et al. 2014, 2015, Ferracini *et al*. 2015a, b). The introduction of TS is widely known as one of the typical successful cases of classical biological control; however, the risks of the agent concerning potential negative effects on non-target native oak gall inducers have never been evaluated thoroughly. Relatively little is known about its host location and host acceptance behavior that was investigated by using a Y-tube olfactometer (Cooper & Rieske 2007, Ferracini et al. 2015a, Kriston et al. 2016).

In spring 2014-2015 on different HU sites (Dobri, Tornyiszentmiklós, Iharosberény, Pilismarót, Nagymaros, Diósjenő) simultaneously with DK galls collected for the estimation of TS parasitization rates, we collected sexual generation galls of different Cynipini species on admixed oak trees, *Andricus crispator* Tschek, *A.curvator* Hartig, *A. cydoniae G*iraud, *A. grossulariae* Giraud, *A. multiplicatus* Giraud, *Biorhiza pallida* Olivier and *Dryocosmus cerriphilus* Giraud. TS never was reared by us from oak cynipid gallwasps. The same negative results were obtained also in Italy (Quacchia et al. 2008, 2014; Ferracini et al. 2015a, b).

We used six days old, matted and unmated females which were tested for their response to olfactory and visual cues associated with DK galls versus cynipid oak galls (*Biorhiza pallida* and *Andricus curvator*) and chestnut foliage versus oak foliage. Before the trials, the wasps were individually kept at room temperature without any host in a glass tube for 18 h with micro drops of honey to prepare the wasps to the experimental conditions. The null hypothesis was that parasitoid females had a 50:50 distribution across the two odour sources. Higher numbers of TS females were attracted to the chestnut galls compared to non-target cynipid oak gall hosts. The same result was obtained in the case of different proposed foliage: the chestnut foliage appeared to be more attractive for TS females than the oak foliage (*Quercus robur*). The combination of olfactory and visual stimuli provided by a fresh gall coupled with chestnut foliage elicited the strongest positive response. Our results indicate that both visual and olfactory cues are required to enable TS to successfully find suitable hosts. These findings may enhance our ability to manipulate TS for gallwasp management. In the olfactory bioassays the responses of independent parasitoid females will be analyzed by binomial tests and glm-s in R software. Results of bioassays may enhance our ability to manipulate TS for DK management, e.g. effect of neighboring sites of the plantation. Results will be published soon.

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