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Review paper

Biological Control of the Invasive *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) - an Overview and the First Trials in Croatia

Dinka Matošević [1]✉, Ambra Quacchia [2], Éva Kriston [3], George Melika [3]

[1] Croatian Forest Research Institute, Cvjetno naselje 41, HR-10450 Jastrebarsko, Croatia
[2] GreenWood Service Srl., Corso Palestro, 7 - 10122 Torino, Italy
[3] Plant Health and Molecular Biology Laboratory, National Food Chain Safety Office, Budaörsi út 141-145, H-1118 Budapest, Hungary

✉ Corresponding author: e-mail: dinkam@sumins.hr

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Abstract

Background and Purpose: *Dryocosmus kuriphilus* is a globally invasive insect pest, spreading very quickly in new habitats and making serious damage to sweet chestnut forests in Croatia and in several other European countries. Indigenous parasitoid species trophically associated with oak gallwasps have adapted to this new host but cannot effectively regulate its population density. Classical biological control using parasitoid *Torymus sinensis* has been proven to be the only effective method of controlling the populations of *D. kuriphilus* and has been successfully applied in Japan, South Korea, the USA and Italy. The aim of this review paper is to provide overview and up-to date knowledge about biological control of *D. kurphilus* and to describe first steps of introduction of *T. sinensis* to sweet chestnut forests in Croatia.

Conclusions and Future Prospects: Results presented in this paper show adapted biology and behavioural traits of *T. sinensis* to its host *D. kuriphilus.* The history and results of introductions of *T. sinensis* to Japan, the USA, Italy, France and Hungary are shown. The first report of release of *T. sinensis* to sweet chestnut forests in Croatia is given with discussion on native parasitoids attacking *D. kuriphilus*. Possible negative effects of *T. sinensis* on native parasitoid fauna and risks that could influence the successful establishment of *T. sinensis* in Croatia are discussed. Previous experiences have shown that *T. sinensis* can successfully control the population density of *D. kuriphilus,* slowing down the spread and mitigating negative impact of this invasive chestnut pest and keeping the damage of *D. kuriphilus* at acceptable level. High specificity of *T. sinensis* suggests that it has limited potential of exploiting native hosts but further detailed monitoring of native parasitoid and possible interactions with introduced *T. sinensis* is strongly suggested.

Keywords: *Torymus sinensis, Dryocosmus kuriphilus,* classical biological control, parasitoid, sweet chestnut, host specificity, gallwasp, release

INTRODUCTION

Alien species may become invasive in new environment due to - among other factors - lack of natural enemies [1, 2]. Such an invasive species can spread quickly and have negative influence on native ecosystems. If it attacks economically important plants, the invasion can develop into a serious problem [3]. *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipide) is globally invasive species and considered as one of the most damaging pests of sweet chestnuts [4]. During its expansion into new territories it has shown all the typical traits of an invasive species: easy establishment, quick spread and damage [5, 6]. It was first recorded in Croatia in 2010 [7] in Istria and Zagreb area and it spread very quickly to other parts of Croatia [8]. Currently the infestation rates are very high and the pest is spreading to new uninfected areas in Croatia.

Sweet chestnut (*Castanea sativa* Mill.) plays an important role in Croatian forest ecosystems: this is a multipurpose tree used for timber and tannin production, chestnuts are prized and nutritious food, honey is of high quality and it is a valuable landscape and heritage tree [9]. Sweet chestnut trees and forests in Croatia are threatened by two highly invasive species: chestnut blight (*Cryphonectria parasitica* (Murrill) Barr. and *D. kuriphilus* [10, 11]. Chestnut blight fungus has devastated sweet chestnut forests in Croatia and recent research shows that galls of *D. kuriphilus* are facilitating the infection by chestnut blight [12]. These are new negative developments that could worsen the fragile and already weak health status of sweet chestnut forests.

Although within its native range in China *D. kuriphilus* populations are kept at low densities by natural enemies, in Japan, South Korea, the USA, Italy, Slovenia, Croatia, France and Switzerland the attack rates of indigenous parasitoid species are low (typically less than 2%) [5, 13-19]. The parasitoid complex which trophically associates with oak gallwasps (Hymenoptera, Cynipidae, Cynipini) in Europe adapted to the native hosts and parasitoids' phenology differs from that of *D. kuriphilus* and thus these species cannot effectively regulate the population density of the new invasive cynipid, *D. kuriphilus* [20]. Insect communities associated with oak gallwasps, Cynipini, to which *D. kuriphilus* belongs, also include a large number of so-called obligatory cynipid inquilines, Synergini, some of which are lethal to the gall-inducer larvae. No cynipid inquilines were reared from the galls of *D. kuriphilus* yet [18, 21].

Classical biological control using parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) has been proven to be the only effective method of controlling the populations of *D. kuriphilus* and has been successfully applied in Japan, South Korea, the USA and Italy [6, 16, 22-25].

The aim of this paper is to provide overview and up-to date knowledge about biological control of *D. kurphilus* so the experiences of the methods used and results achieved in other countries, in as well as outside Europe, can be used in Croatia. First steps of introduction of *T. sinensis* to sweet chestnut forests in Croatia are also presented.

BIOLOGY AND SOME BEHAVIOURAL TRAITS OF *TORYMUS SINENSIS*

T. sinensis is native to China and is the only parasitoid of *D. kuriphilus* known to be host specific and phenologically well adapted to the biology of its host [6, 16, 17, 23, 26, 27]. The parasitoid has one generation per year, as its host, which is quite different to other native polyphagous parasitoid species that are present in the new invaded areas of *D. kuriphilus*. They mostly have more than one generation per year and their phenology and biology, particularly those from the genus *Torymus,* are not closely matched with *D. kuriphilus* [28-31]. *T. sinensis* adults emerge from withered galls, mate and females lay eggs in the newly developed galls of *D. kuriphilus* in spring (mostly late April). Each female can lay 70 eggs on average. The emergence is synchronized with the budburst of sweet chestnut and development of new galls [24]. Females locate the host with a combination of visual and olfactory stimuli from fresh galls and chestnut foliage [32] which could also explain its specificity to *D. kuriphilus.* Parasitoid larva feeds ectoparasitically and pupates in late winter the following year. The parasitoid aestivates as larva, and overwinters as last larval or early pupal stage [33]. Females have pre-ovipositon period of 6 days, during which the mating occurs and additional imaginal feeding is necessary for the egg-load development; females lifespan is 37 days in the field [27]. This life longevity makes them suitable for raising and manipulating in the laboratory prior to release in the field. *T. sinensis* selects the body surface of the host larva for oviposition rather than the chamber wall as observed in other *Torymus* species [27]. According to Piao and Moriya [27] this oviposition behaviour in the selection of the host larva is most probably caused by the adapted ovipositor length of *T. sinensis* to the *D. kuriphilus* gall structure and size compared to other species which makes this parasitoid species more able to exploit the available resources of the host. In Asia and USA *T. sinensis* is able to parasitise larger galls than native parasitoids due to its longer ovipositor [17, 27]. However, this is different in Europe where a number of native *Torymus* species (e.g. *T*. *auratus* (Müller), *T*. *cyaneus* Walker, *T*. *flavipes* (Walker), *T*. *notatus* (Walker)) have the same length of the ovipositor as *T*. *sinensis*, thus they can parasitize the same sized *D*. *kuriphilus* galls as *T*. *sinensis*.

One of the adaptive advantages of *T. sinensis* when compared to other univoltine parasitoids present in the invasion range (e.g. *Torymus beneficus* Yasumatsu & Kamijo) of *D. kuriphilus* is only one emergence period without peaks which makes this species better synchronized with its host. This very fine phenological difference together with other morphological features (ovipositor length) makes *T. sinensis* highly efficient as biological agent against *D. kuriphilus* [34, 35]. In case if mating does not occur, females lay unfertilized eggs, parthenogenetically producing only males [27]. These details should be considered when planning rearing and release to new sites and proper male/female ratio. Personal experience of authors of this paper (A. Quacchia and G. Melika) has shown ability of *T. sinensis* to survive at 15° C when fed with honey up to 4 months. *T. sinensis* also has a specific strategy to survive temporary extinction of its host - a prolonged diapause over 2 years [36] which makes it species specific and less likely to parasitize other members of Cynipidae family (e.g. oak gall wasps, Cynipini).

T. sinensis tracks the expanding populations of *D. kuriphilus* [16, 25]. During the early years of the release, population of *T. sinensis* will disperse very slowly and, over the years, the dispersion will be faster and exponential. Two release sites 8 km away from each other will see the merging of the populations of the parasitoid in 5 years, two sites 20 km apart in 7 years [25, 37].

A study on potential of *T. sinensis* as viable management option for biological control of *D. kuriphilus* [4, 6] has raised some important questions to be further studied and answered: (i) the general risk that *T. sinensis* could shift to native gall wasps related to *D. kuriphilus* and (ii) could hybridize with native *Torymus* species. It was suggested that a host specificity test should be performed for evidence of attack of native oak galls [6]. First tests were done with *T. sinensis* females offering them alternative host galls: *Mikiola fagi* Hartig (Diptera: Cecidomyiidae) developing on *Fagus*, galls of asexual generation of oak gall wasps as *Cynips quercusfolii* Linnaeus (Hymenoptera: Cynipidae) and *Andricus kollari* Hartig (Hymenoptera: Cynipidae) and no oviposition was recorded [24]. These tests were considered insufficient [4, 6] so additional host range tests have been performed [36]. Seven species of oak cynipids (Cynipidae: Cynipini) which occur at similar times in the field as *D. kuriphilus* were tested: *Andricus crispator* (Tschek), *A. curvator* (Hartig), *A. cydoniae* (Giraud), *A. grossulariae* (Giraud), *A. multiplicatus* (Giraud), *Biorhiza pallida* (Olivier) and *Dryocosmus cerriphilus* (Giraud). All the seven mentioned oak gallwasp species are known to have two alternate generations per year; in spring the sexual generations are developing. These species were chosen for the host specificity tests, proposed by EFSA [4] according to their ecological similarity, spatial and temporal attributes and accessibility and availability for *T. sinensis* at the period of parasitation. Few and brief ovipositor pricking were observed on *A. cydoniae, B. pallida* and *D. cerriphilus* but no eggs were laid [36]. These results additionally confirmed the host specificity of *T. sinensis*. *Torymus sinensis* was introduced to the USA in late 70s [16], to Japan in 1979 (reviewed in Aebi et al. [5]), to Italy in 2004 [24]. However, no other host than *D. kuriphilus* was ever mentioned in the literature to be parasitized by introduced *T. sinensis* [38]. The risk assessment of possible shift of *T. sinensis* onto other hosts (native oak gallwasps) was discussed in details in Gibbs et al. [6]. Hybridization of a biological control agent with native species is considered as an environmental risk to non-target species [6]. Till now, the only reported case of *T. sinensis* hybridization is with the native *T. beneficus* in Japan: *T. sinensis* and *T. beneficus* were successfully crossed in the laboratory to produce fertile hybrid females [39]. Hybrids were also detected in the field and their hybrid origin proved with molecular markers [40].

The probability of hybridization with native European *Torymus* species (Hymenoptera: Toymiidae) was tested in mating experiments on *Torymus flavipes (*Walker)*, T. auratus* (Muller)*, T. affinis* (Fonscolombe) *and T. geranii* (Walker). No mate recognition and mating were recorded in the laboratory experiments using these native species [36]. However, these species have potential to hybridize with *T. sinensis* as these closely related species overlap geographically (they may even parasitize identical galls on single chestnut trees), and partially also can overlap in their seasonality. Recently the risk of hybridisation between *T. sinensis* and native *Torymus* species was evaluated by molecularly analysing *Torymus* specimens reared from oak and chestnut galls, collected in Switzerland and Italy. Hybridisation between *T. sinensis* and *T. cyaneus* was documented in only one case [38], however, this result must be confirmed.

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN JAPAN

The introduction of *T. sinensis* is regarded as a successful case of classical biological control of invasive species in Japan [23] and was the first introduction of *T. sinensis* as biological control agent outside its native range. *D. kuriphilus* arrived in Japan around 1941 and after long years of research of the host and most suitable control measures, 260 mated females of *T. sinensis* were released in 1982 in Ibaraki prefecture [41]. The parasitoid population grew by factor of 25 times by 1989 [23], the parasitoid expanded its range soon after the release, adults being raised more than 12 km from release point showing good dispersal ability. In the first few years the parasitoid spread at rate less than 1 km/year but the speed increased in the following years to app. 60 km/year. Over the years the parasitoid has dispersed naturally several hundred kilometres from the release point (Figure 2) [23]. The infestation rate of *D. kuriphilus* decreased rapidly to tolerable injury level of 30% (Figure 1) [23].

This classical biological control program succeeded in drastically reducing the damage caused by *D. kuriphilus* in Japan [42]. Japanese experience shows that the tolerable injury level from *D. kuriphilus* is about 30% infestation [23]. After the introduction of *T. sinensis* the infestation rates decreased steadily from 43% to less than 1% [23] which is the result of established population of introduced *T. sinensis.*

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN THE USA

D. kuriphilus was first observed in the USA in 1974 negatively influencing chestnut production and health status of chestnut trees [16]. *T. sinensis* was introduced for biological control, expanding together with its host to

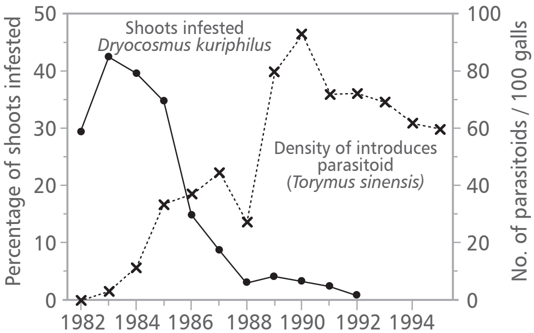

FIGURE 1. Decrease in infestation rate with *Dryocosmus kuriphilus* after the release of *Torymus sinensis* in Japan (source Moriya et al. [42])

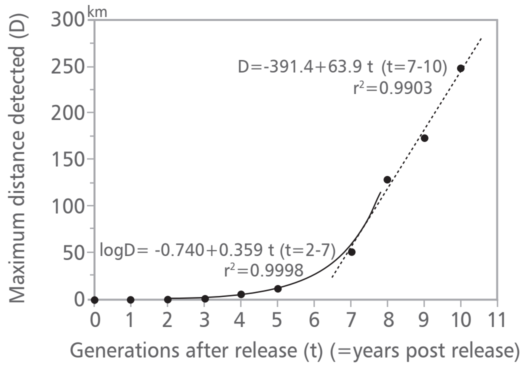

FIGURE 2. Range expansion of *Torymus sinensis*, relationship between the number of generations (t) after the release in Japan (Tsukuba) in 1982 and maximum distance (D) from the release site to the locality where T. sinensis has been detected (source Moriya et al. [22, 23])

new sites [16]. Recent study [17] confirmed that *T. sinensis* is the dominant parasitoid of *D. kuriphilus* in eastern USA. *T. sinensis* was not present in oak galls collected on the sites and it can be considered as specialist on *D. kuriphilus* in North America [17]. Studies [16, 17] have also provided evidence that *T. sinensis* is hyperparasitized by native cynipid parasitoids which could potentially suppress *T. sinensis* populations and influence its potential as biological control agent. At present it is effective control agent of *D. kuriphilus* in the USA [17].

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN ITALY

D. kuriphilus has been introduced to Italy with infested plants around 2002 and quickly spread through Italian peninsula [43]. First releases of *T. sinensis,* raised from galls imported from Japan, were in 2005 (90 females and 80 males) and 2006 (1 058 females and 889 males) [24]. The increase of parasitoid populations has been exponential, surpassing 90% in 5-7 years after release, which is significantly bigger than the parasitism rate of native parasitoids of 3-5% [25]. The biological control of *D. kuriphilus* is giving visible results in Italy, in Cuneo region (the region of first European infestation by *D. kuriphilus*), where first releases began 5-7 years ago. The parasitisation rates nowadays often exceed 85-90% with a significant reduction of number of infested leaves and shoots. The galls are decreasing in numbers and vegetative growth of chestnut trees is recovering [25].

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN FRANCE

Based on previous successful operations in other countries, a classical biological control using the parasitoid *T. sinensis* has been implemented in France since 2011 [44]. During the two first years 42 releases of *T. sinensis* were made in different parts of France (one single introduction of 100♀ and 50♂ versus two introductions of 50♀ and 25♂ with a one-year interval per site). First results indicate that even though very few specimens of this biocontrol agents were released, the rate of establishment of *T. sinensis* is high (app. 80%) [44].

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN HUNGARY

In May 2014, *T. sinensis* was released also in the southwestern part of Hungary, Zala County in two sites (Dobri and Kerkateskánd) where the population of *D. kuriphilus* is rapidly growing [45]. In Dobri were 200 females, while in

Kerkateskánd 100 females previously mated with males in the laboratory conditions were released. The males and females originate from the Cuneo region in Italy (G. Melika, personal comm.).

NATIVE PARASITOIDS OF *D. KURIPHILUS* IN CROATIA AND POSSIBLE NEGATIVE EFFECTS OF *T. SINENSIS* ON NATIVE PARASITOID FAUNA

After the first record of *D. kuriphilus* in Croatia [7] 15 species of native parasitoids have adapted to the new host in few years [18]. The indigenous parasitoids fauna fails to exert sufficient biological control of *D. kuriphilus* and reduction in leaf area and sweet chestnut fruit yield are reported from all attacked areas in Croatia. *Torymus flavipes* was the most abundant species and first to adapt to a new host [18], with two more *Torymus* species (*T. geranii* and *T. auratus*). The biology of native parasitoids differs from that of *T. sinensis*, where native *Torymus* species emerge just before the emergence of *D. kuriphilus* (end of May, early June) [46] and have two generations per year [28-31]. Therefore their development is not entirely synchronized with the phenology of *D. kuriphilus*. Low attack rates of natural parasitoids make therefore *T. sinensis* a viable solution. Visible damage and rapidly expanding pest in Croatian sweet chestnut forests have prompted for initiation of biological control of *D. kuriphilus.* In all invaded sweet chestnut forests in Croatia large and multiple *D. kuriphilus* galls have been found which could also be a reason for low success of native parasitoids but a favourable trait for *T. sinensis* population due to its longer ovipositor and ability to parasitize larger galls [17, 27].

BIOLOGICAL CONTROL OF *D. KURIPHILUS* IN CROATIA

The biological control of *D. kuriphilus* in Croatia has started in spring 2014. Before the release all the necessary aspects and precautions have been considered: host-specificity, effects on non-target species, location of the site of first release, good settlement prospects, gall size (larval stage) and national regulations regarding the introduction of alien bio control species. Approximately 1 300 withered galls have been imported from Italy in March 2014 from two localities i.e. Borgo d'Ale and Torre Canavese in Torino region, where multiple releases of *T. sinensis* occured and high parasitism rates were achieved. The galls were kept at 7°C to delay the emergence of adults and to synchronize it with development of galls in the field. A natural sweet chestnut forest in Pazin (locality Lovrin) (area 12 ha) on Istria Peninsula was chosen as a site of first release of *T. sinensis*. When the *D. kuriphilus* galls started to develop in the field, the withered galls with *T. sinensis* were taken from the fridge and kept at room temperature, until after few days first males and then females started to emerge. They were coupled together (10 females/5 males) in plastic tubes, fed with honey and kept at 15°C in climatic chamber (L:D=12:12) until release. On 11 April 2014, 1 200 females and 600 males were released in Pazin (45.233482N; 13.920008E). This is the first attempt of biological control of a forest pest with introduced bio control agent in Croatia and hopefully the population of *T. sinensis* would be dense enough and parasitism rate high so that Pazin site could be used as a "bank" of *T. sinensis* population for raising adults for the release in other parts in Croatia in the next years.

CONCLUSIONS AND FUTURE PROSPECTS

Release and establishment of *T. sinensis* in Japan, the USA and Italy is an example of successful biological control of an invasive species. However, several risks that could influence the successful establishment of *T. sinensis* should be considered.

Hyperparasitism of *T. sinensis* by native parasitoids could limit the establishment of *T. sinensis* in forest habitats [16, 17].

Throughout Europe, *Eupelmus urozonus* Dalman (Hymenoptera: Eupelmidae) is raised as one of the most common *D. kuriphilus* native parasitoid. This species very often acts as a facultative hyperparasitoid in oak cynipid galls [28]. Thus it is possible that hyperparasitoids can influence the population densities of *T. sinensis* [24], which should be confirmed by further studies.

The sex ratio of first Croatian population of *T. sinensis* should also be monitored as there is a possibility of male-biased sex ratio due to the failure of mating. This can happen when the density of individuals is low and finding a mate is difficult [24].

The failure in establishment due to the mismatch between the emergence of adults and suitable gall development stage can also pose a risk, but this can easily be avoided by regulating the emergence dates of *T. sinensis* by adjusting the emergence and rearing temperature in laboratory. The unavailability of hosts is not expected to be a risk in the establishment *T. sinensis* population as *D. kuriphilus* is present in high densities in Croatia and expanding its range very quickly. Native parasitoids are not able to influence the population density of *D. kuriphilus* [18] in such an extent that it could cause the extinction of *T. sinensis* population.

All previous experiences have shown that *T. sinensis* can successfully control the population density of *D. kuriphilus* (Figure 1) [17, 23, 24]. The synchrony of biology of *T. sinensis* with the biology of its host and ability to disperse into the invading territory with pest population and spread rapidly (Figure 2) make this parasitoid suitable for mitigating negative impact of this invasive chestnut pest as well as keeping the damage of *D. kuriphilus* at acceptable level. High specificity of *T. sinensis* suggests that it has limited potential of exploiting native hosts [36]. However, there is still a possibility of hybridization with native *Torymus* species [6, 38] so we strongly suggest further detailed monitoring of native parasitoids and possible interactions with introduced *T. sinensis* populations and competition of native and introduced parasitoids in Croatia. To assess indirect effects of introduction of *T. sinensis* (i.e. influence on non-target species) molecular methods will be used for identification of potential hybrids of *T. sinensis* with native *Torymus* (if any will be found in Croatia).

There is also a possibility of augmentative biological control [6] using native parasitoids in Croatia. Therefore, the research on native parasitoids and their influence on population densities of *D. kuriphilus* will continue, in first as well as in the last invaded sites so the results and effects can be compared with classical biological control using *T. sinensis.* In conclusion, on the basis of the review of references, an extensive experience of scientists and personal experience of the authors, the use of *T. sinensis* as biological control agent together with the potential of natural parasitoids should be considered the best possible solution to control the invasive pest *D. kuriphilus* in natural sweet chestnut forests in Croatia.

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