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Abstract

Considered as a major pest of *Castanea* species worldwide, the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) can induce fruit production losses of 60-80%. Reported for the first time in Italy in 2002, this pest is now widespread across the French distribution of chestnut trees.

Based on successful attempts in other countries, a classical biological control program using the parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) has been implemented in France since 2011. Three main objectives were delineated for the program. The first is long-term control of chestnut gall wasp populations, be it in commercial orchards or in forests. The second objective is to take advantage of planned introductions of *T. sinensis* to better understand factors that underpin the establishment and spread of exotic species. The third objective is to describe the recruitment of native parasitoids by invasive *D. kuriphilus* and to assess the impacts of *T. sinensis* on native communities.

During the two first years of this project, 42 releases of *T. sinensis* were achieved with an experimental manipulation of propagule pressure. Initial results indicate that: (i) *T. sinensis* has successfully established in at least 20 of the release sites; (ii) *T. sinensis* populations, as well as populations of native parasitoids, have a positive inter-annual growth rate; and (iii) no significant impacts of parasitoid introductions on the chestnut gall wasp have been observed yet.

Introduction

The superfamily of Cynipoidea contains almost 3 000 species belonging to seven families. All are parasitoids except the Cynipidae and one genus of Figitidae (Csoka *et al.* 2005). The oak gall wasps (Cynipini tribe) is a group of almost 1 000 species worldwide, among which around 140 are reported from the west Palaearctic region (Stone *et al.* 2002). Only four species of oak gall wasps are reported on hosts other than *Quercus* spp. (Fagaceae) (Buffington and Morita 2009). *Dryocosmus kuriphilus* Yasumatsu is one of these exceptions and is the only Palaearctic species developing on *Castanea* spp. (Acs *et al.* 2007).

Unlike most of the species of Cynipini, which reproduce via heterogony (i.e. a strict alternation of sexual and asexual generations) (Stone *et al.* 2002), the chestnut gall wasp is univoltine and purely asexual (thelytokous) (Moriya *et al.* 2003). Females lay eggs in early summer inside chestnut buds where the larvae overwinter and remain immature until the next spring. At bud burst in spring, larval feeding induces the formation of green or rose-coloured galls (Otake 1980, 1989). *Dryocosmus kuriphilus* is native to China where it is widely distributed in the main chestnut producing areas (Zhang *et al.* 2009). Its presence in neighbouring countries was reported in Japan in 1941 (Murakami *et al.* 1980) and in Korea in 1958 (Cho and Lee 1963), and then in Nepal in 1999 (Abe *et al.* 2007). It was detected in United States of America in 1974 (Payne *et al.* 1975), and for the first time in Europe in 2002 (Brussino *et al.* 2002). In Europe, *D. kuriphilus* spread from Italy throughout Europe: Slovenia in 2005 (Knapic *et al.* 2009); France in 2005 (Aebi *et al.* 2006); Hungary in 2009 (Csoka *et al.* 2009); Switzerland in 2009 (Forster *et al.* 2009); Croatia in 2010 (Matosevic *et al.* 2010); Netherlands in 2010 (EPPO 2010); the Czech Republic in 2012 (EPPO 2012); and Spain in 2010 (J. H. Delader, personal communication). Similar to other invasive organisms, the exchange of infested plants by humans is probably the main dispersal pathway (Hulme 2009).

Dryocosmus kuriphilus is considered one of the major pests of *Castanea* (Payne *et al.* 1983, Moriya *et al.* 1989, Brussino *et al.* 2002). It develops on *Castanea crenata* Sieb. & Zucc. (Fagaceae), *C. mollissima* Blume and *C. henryi* (Skan.) Rehder & E.H. Wilson in China, Japan and Korea (Zhu *et al.* 2007), *C. dentata* (Marsh.) Borkh. in United States of America (Payne 1978) and *C. sativa* Mill. in Europe (Brussino *et al.* 2002). In China, yield damage was reported as early as 1929 and since, has ranged from 15% to 30% annually (Zhang *et al.* 2009). In its introduced range, chestnut production losses sometimes reach 80% (EFSA 2010).

Almost 20 years after the accidental introduction of *D. kuriphilus* in Japan, its harmful impacts motivated the identification and the use of resistant chestnut varieties. Results were promising, but the pest populations increased again in the 1970s (Moriya *et al.* 2003). The absence of efficient natural enemies lead to the introduction of a promising and seemingly specific parasitoid, *Torymus sinensis* Kamijo (Hymenoptera, Torymidae), native to China, being planned at the end of the 1970s (Murakami *et al.* 1977). A successful introduction in 1989 reduced *D. kuriphilus* populations far below the tolerable threshold of 30% of infested shoots (Moriya *et al.* 1989). Similarly, invasive *D. kuriphilus* was controlled in the United States of America subsequent to the introduction of *T. sinensis* in 1977 (Rieske 2007). In Europe, this parasitoid was first introduced in Italy in 2005 (Quacchia *et al.* 2008).

Following the arrival of *Drycocosmus kuriphilus* in the main French regions of chestnut production, a biological control program using *Torymus sinensis* was implemented in 2011. The first objective of this project was the permanent establishment of *T. sinensis* in France (continental and Corsica), both in commercial orchards and in natural ecosystems.

Classical biological control operations can be considered "planned biological invasions" as they provide a unique opportunity to study the biology of introduced populations (Fauvergue *et al.* 2012). Among the different factors explaining the demographic success of small introduced populations, propagule pressure is one of the most pervasive. Propagule pressure combines both the number of individuals introduced in each introduction event (i.e. propagule size) and the number of introduction events (i.e. propagule number) (Simberloff 2009). Thus, a second objective of the study was to use the introduction of *T. sinensis* in France as an opportunity to test the effect of different propagule pressures on post-release population dynamics. These results should also serve as an empirical basis to optimise the geographic redistribution of introduced populations.

A third long-term objective of our study dealt with the ecological impacts of *D. kuriphilus* and, possibly, *T. sinensis* on

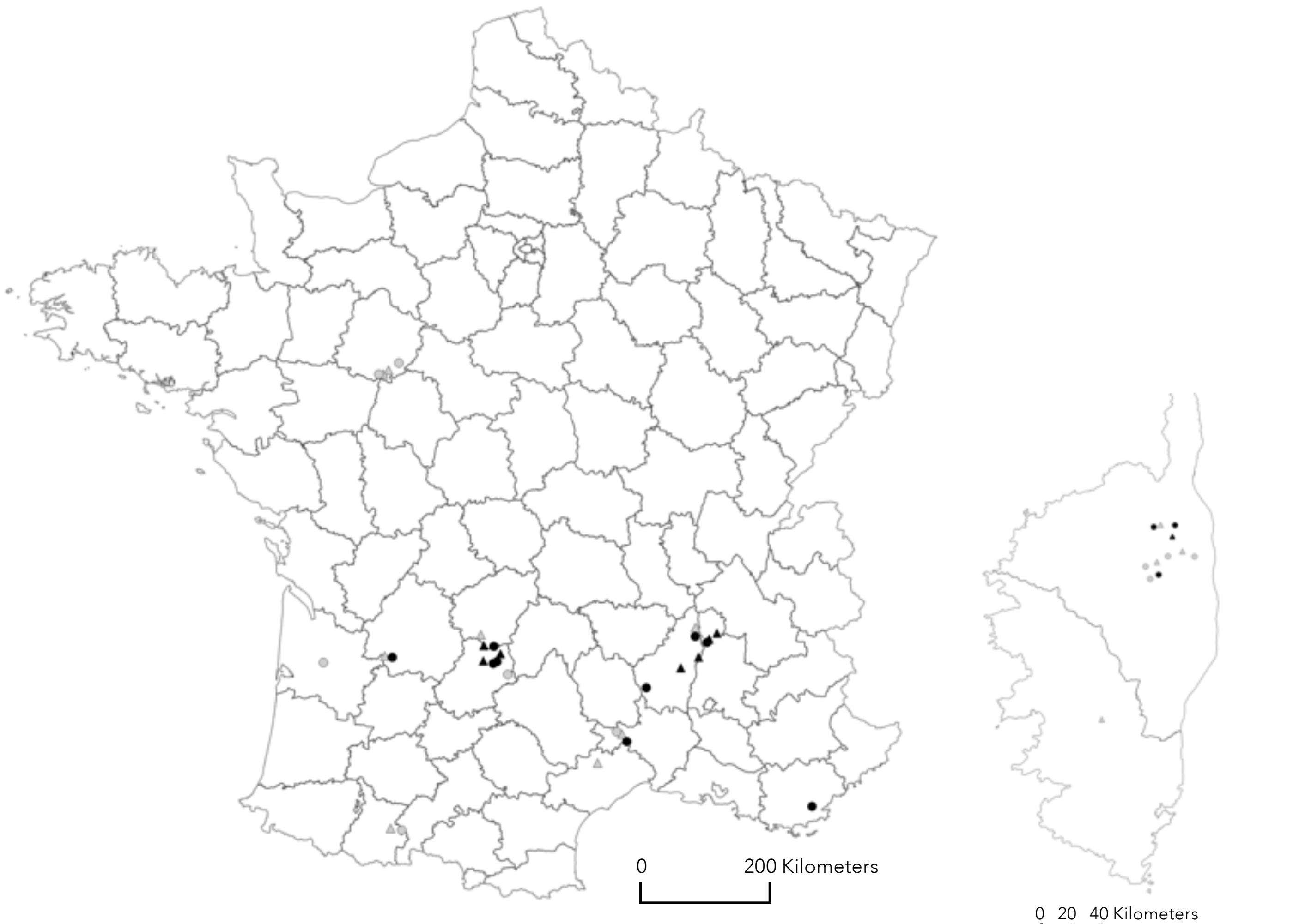

Figure 1. Release sites (2011-2013) and establishment of *Torymus sinensis* in France (continental and Corsica, inset). Circles - 1x100 females released, triangles - 2x50 females released. Black - establisment confirmed. Grey - establishment not confirmed.

native and ecologically-related communities. This facet falls within a "controversial" debate regarding the introduction of exotic biological control agents.

Here, we present the first results concerning the two first objectives.

Material and methods

Insect rearing

No efficient rearing method has been developed for *T. sinensis*, so the released parasitoids were first collected in Italy by the University of Torino (2011 and 2012) and then obtained by collecting galls on French sites where the establishment of *T. sinensis* was confirmed (2013). For this last year, more than 250,000 galls were kept in emergence boxes (1 000 galls per box). Boxes were placed outdoors at Sophia Antipolis to limit a mismatch with favourable stages of *D. kuriphilus* in the field that may limit the establishment of *T. sinensis*. Emergences of *T. sinensis* were checked daily between the end of March and mid-May. All *Torymus* were isolated in glass vials (5 individuals per vial) and identified under a stereo microscope. Doubtful specimens were discarded. Living *T. sinensis* were conditioned in plastic centrifuge tubes (10 females and 5 males to allow mating) and kept in climatic chambers (14°C, photoperiod 14/10 h) until field releases. Since the mean longevity of adults is about 45 days under laboratory conditions (N. Borowiec, unpublished data), all released *T. sinensis* were less than 3 weeks old. Other indigenous parasitoids were kept in absolute alcohol for morphological and molecular identification.

Field releases and surveys

Between 2011 and 2013, two experimental levels of *T. sinensis* releases were compared in continental France and in Corsica:

Level A: a single introduction of 100 females and 50 males (at 4 sites in 2011, 9 sites in 2012 and 9 sites in 2013);

Level B: two introductions of 50 females and 25 males with a one year interval (at 4 sites in 2011-2012, 8 sites in 2012-2013, and 8 sites in 2013-2014).

Field releases were done from 15-30 April 2011, from 25 April to 3 May 2012 and from 25 April to 8 May 2013. At each site, pre- and post-release surveys were achieved by, respectively, collecting 2 000 and 5 000 withered galls of *D. kuriphilus* during the parasitoid's overwintering season (between January and the beginning of March).

Statistical analysis

Analyses were performed with the statistical package "R", version 3.0.1 (R Core Team 2013). We firstly used a Fisher's exact test to test the independence between 3 qualitative factors: the detection of *T. sinensis* one year after the releases (Yes/No); the experimental level of introduction (levels A and B); and the infestation rate of *D. kuriphilus* ("Low", i.e. less than 60% of infested buds and "High", i.e. more than 60% of infested buds).

For the sites where *T. sinensis* was detected, we then tested the influence on the number of *T. sinensis* of: (1) the effect of the number of wasps released (level A:

100♀+50♂ versus level B: 50♀+25♂), one year after first release; and (2) the effect of the number of introduction events (level A: 1 versus level B: 2), two years after first release.

After an explorative step, we decided to fit linear models using: (i) the natural logarithm of the number of *T. sinensis* emerged from 1 000 galls as the dependent variable; and (ii) the experimental level of release (A and B), the year of the first release (2011 or 2012) and the abundance of *D. kuriphilus*, as possible explanatory variables. Explanatory variables were conserved or removed according to the AIC (Akaike Information Criteria) using a backward procedure. The best models were then tested using an ANOVA.

Results and Discussion

Establishment of Torymus sinensis *in France*

A total of 5 700 *T. sinensis* (3 800 females and 1 900 males) were released across 42 sites covering an area of 492 km from north to south and 560 km from west to east in continental France and the island of Corsica (Figure 1). Although the releases covered a large area, they did not encompass the whole distribution area of *Castanea* in France. In particular, more northern release sites have to be included in the future. The commercial importance of chestnut is nevertheless lower there.

At 17 sites for which the first releases were made in 2013, the establishment of *T. sinensis* cannot yet be evaluated as post-release surveys are pending next winter. Two of these sites were also discarded; at one site (released in 2012) where a late frost induced a high mortality of insects such that we postponed the gall sampling in order to preserve a possibly low number of living parasitoids, and at a second site (released in 2011) where observations indicated that the establishment of *T. sinensis* was due to dispersal from neighbouring sites rather than from the locally released *T. sinensis*.

Among the 23 remaining sites (released in 2011 and 2012), the establishment of *T. sinensis* was observed at 18 sites, one year after its introduction. Even though few biocontrol agents were released, this rate of establishment is high when compared to the average establishment success observed in classical biological control (from 10% to 30%) (Hopper and Roush 1993, Bellows 2001). This confirms both the intrinsic efficiency of *T. sinensis* that was previously observed in other countries (e.g. Japan and Italy), as well as our own ability to provide healthy individuals in time. The establishment rate was independent from both the number of introduced individuals as well as from the abundance of *D. kuriphilus* (Fisher test on 3-way table of contingency, P=0.14). A pluri-annual (i.e. lasting several years) post-release survey will be necessary to take into account possible cryptic establishments linked to bias sampling and local extinctions.

Population dynamics of Torymus sinensis

Population dynamic analyses were carried out only from sites where *T. sinensis* was established.

One year after the first releases, parasitoid abundance was very low ranging from 0.2 to 11.9 *T. sinensis* per 1 000 galls. The best model according to the AIC criterion only involves the "experimental level of release" as an explanatory variable. Although its effect was not significant ($F_{1,15}$=0.87, P=0.37; one outlier removed), the tendency is that the mean number of *T. sinensis* is slightly higher in level A (100♀+50♂ than in level B (50♀+25♂, Figure 2).

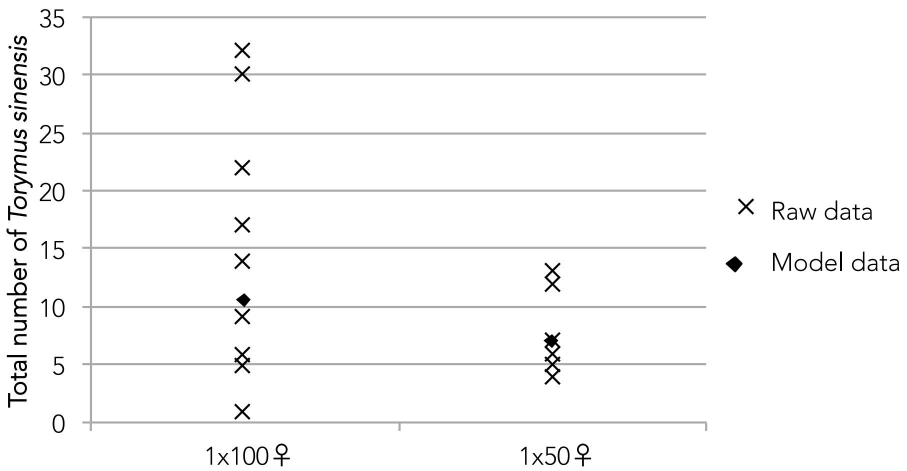

Figure 2. Total number of *Torymus sinensis* recovered as a function of the number of individuals released.

Two years after the first releases, parasitoid abundance was still low, ranging from 0.15 to 69.9 *T. sinensis* per 1 000 galls. The backward procedure only identified the abundance of *D. kuriphilus* as a relevant explanatory variable, but its effect was not significant ($F_{1,15}$=0.41, P=0.55). The tendency suggests a negative correlation between the number of *T. sinensis* and the number of *D. kuriphilus* (data not shown). This may be due to a "dilution" and thus less "detectability" of the *T. sinensis* in locations where *D. kuriphilus* is abundant. In both cases (tests of "propagule size" and "propagule number"), the number of replicates was very limited at that stage of the experiment leading to weak statistical power. These analyses will be repeated when more data becomes available.

Conclusion

The chestnut gall wasp, *D. kuriphilus*, is a "serial invader" that can severely impact agricultural production and natural ecosystems worldwide. Previous studies have clearly demonstrated that classical biocontrol using *T. sinensis* could offer an opportunity to regulate this pest (Moriya *et al.* 1989, Rieske 2007, Quacchia *et al.* 2008). Despite this potential, little data is available to optimise the distribution of *T. sinensis* and the control of *D. kuriphilus* in a recently invaded area. This lack of data motivated us to more precisely investigate the importance of the propagule pressure (Simberloff 2009). For a fixed number of biocontrol agents, favouring the number of individuals per release and per site to the detriment of the number of releases in the same site or different sites (or vice versa) is an important issue. These investigations were implemented under stringent constraints, in particular: (i) the production of very large numbers of the biocontrol agent; (ii) the synchronisation of the *T. sinensis* releases with the (spatially or temporally) heterogeneous phenologies of the chestnuts in the field; (iii) the limitations associated with surveying large numbers of sites, and (iv) meeting the expectations of chestnut producers for a rapid control method. Our investigations are still to be completed in order to provide relevant guidelines for the optimum deployment of *T. sinensis* in the field.

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