16TH INTERNATIONAL SYMPOSIUM ON INSECT-PLANT RELATIONSHIPS

Early population dynamics in classical biological control: establishment of the exotic parasitoid *Torymus sinensis* and control of its target pest, the chestnut gall wasp *Dryocosmus kuriphilus*, in France

Nicolas Borowiec* iD, Marcel Thaon, Lisa Brancaccio, Benoît Cailleret, Nicolas Ris & Elodie Vercken

UMR ISA, INRA-CNRS-University Nice Côte d'Azur, 400 route des Chappes, 06903 Sophia Antipolis, France

Abstract

In response to the introduction and spread of the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) in metropolitan France, including Corsica, the exotic parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) was deliberately introduced in more than 50 locations covering most of the areas of chestnut production. To test the impact of propagule pressure (i.e., the number and size of introductions) on establishment success and post-release dynamics, the introductions were done according to three experimental modalities: (1) a single introduction of 100 females, (2) two sequential introductions of 50 females, or (3) a single introduction of 1 000 females. Post-release surveys demonstrated a very high local establishment of *T. sinensis*, regardless of the introduction modality. Post-introduction dynamics were found to follow a two-phase process. First, early dynamics were characterized by an exponential growth of *T. sinensis* populations without a significant decrease in *D. kuriphilus* populations. Later, middle-term dynamics indicated a global decrease in both *D. kuriphilus* and *T. sinensis* populations. Quite surprisingly, propagule pressure had a limited effect on post-introduction dynamics, with a lasting demographic advantage for the largest introductions, but no influence of the number of introductions. Finally, site connectivity had a positive effect on *T. sinensis* population size, suggesting successful colonization and establishment at a wide spatial scale beyond the introduction sites. In addition, we observed that some *T. sinensis* exhibited a very short developmental time compared to the majority of the emerged individuals, which may be interpreted as a diversified bet hedging strategy against environmental stochasticity. These findings confirm the strong potential of *T. sinensis* for successful establishment and sustainable control of the chestnut gall wasp in Europe.

Introduction

Classical biological control (ClBC) – the planned introduction of an exotic species for its permanent establishment and the long-term control of an agricultural pest (Eilenberg et al., 2001) – is a sustainable alternative to pesticides. In addition to this interest, this method can be used as a 'planned biological invasion' to study the various factors underlying the success or failure of introduced populations in real-life ecosystems (Fauvergue et al., 2012). Indeed, most information on introduced populations is derived from a posteriori analyses of populations that became invasive, which generates major biases for the analysis of the establishment process (Hayes & Barry, 2008; Zenni & Nuñez, 2013). Experimental approaches are thus deeply needed to disentangle the effects of population traits, habitat characteristics, and chance events on the early dynamics of introduced populations, and this is

*Correspondence: Nicolas Borowiec, UMR ISA, INRA-CNRS-University Nice Côte d'Azur, 400 route des Chappes, 06903 Sophia Antipolis, France. E-mail: nicolas.borowiec@inra.fr

where planned introductions of biological control agents can provide valuable insights.

In particular, propagule pressure has been identified as a main determinant of establishment success in comparative studies of invasion success (Simberloff, 2009). Propagule pressure refers to the total number of individuals introduced in a given area, which combines the number of introduction events (propagule number) and the number of individuals for each introduction event (propagule size). Whereas propagule size and number are both expected to positively influence establishment, they involve different demographic or genetic processes (Fauvergue et al., 2012). Propagule size is expected to decrease the influence of demographic stochasticity, Allee effect, or inbreeding depression (Lande, 1988; Reed, 2005; Willi et al., 2006). Propagule number, on the other hand, is expected to decrease environmental stochasticity in space or time and to enhance rescue effects (Brown & Kodric-Brown, 1977; Hanski, 1998). Whereas previous results suggest that propagule size and propagule number have mostly similar influence on establishment in the laboratory (Drake et al., 2005), the relative influence of both processes on post-introduction dynamics has never been investigated in nature.

The chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) (hereafter CGW) originates from China where it is known to develop on several species of *Castanea* (Fagaceae), e.g., *Castanea crenata* Siebold & Zucc. and *Castanea mollissima* Blume (Zhu et al., 2007). In the 1940s, it was reported in Japan (Murakami et al., 1980) and after several other introduction events between 1941 and 1999 (Japan, Korea, USA, and Nepal), it was reported in Europe in 2002 (Brussino et al., 2002), where *Castanea*-based forests covered around 2.5 million ha distributed across 17 countries (Conedera et al., 2016) and where chestnut production and consumption is of notable importance. Following its arrival, CGW colonized almost all of the European area of *Castanea sativa* Mill. in 15 years (for details see Borowiec et al., 2014; CABI/EPPO, 2015). Like other cynipids (except inquilines), CGW develops on its host plant by inducing gall formation using complex physiological mechanisms resulting in a high level of ecological specialization (Shorthouse et al., 2005). CGW thus develops exclusively on *Castanea* species [*C. crenata* and *C. mollissima* in Asia, *Castanea dentata* (Marsh.) Borkh. in North America, and *C. sativa* in Europe; Payne, 1978; Brussino et al., 2002; Zhu et al., 2007]. According to either the tree's vitality or the density of CGW, galls can develop either on the leaf – with quite low impact on the tree's development – or on buds, with severe impact on the development of leaves and twigs, and on the overall tree architecture (Kato & Hijii, 1997; Gehring

et al., 2017). CGW infestation can lead to a decrease of up to 80% of chestnut production (EFSA, 2010). Significant economic impact was also observed in France on honey production and beekeeping (N. Borowiec, unpubl.). Beyond economic impact, high CGW infestations induce deleterious ecological side effects such as the decrease in food sources and refuges for wild animals (Jabr, 2014) or increased susceptibility of chestnut trees to pathogens like chestnut blight, *Cryphonectria parasitica* (Murrill) (Ascomycetes) (Meyer et al., 2015).

In response to these threats, the first attempts to regulate CGW in Japan focused on developing less susceptible chestnut varieties but, after promising initial results, infestations of *D. kuriphilus* increased anew (Moriya et al., 2003). A ClBC programme was then implemented with field explorations in China and the identification of a specific parasitoid, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Murakami et al., 1980). This parasitoid was then successfully introduced in Japan, USA (1977), and Italy (2005) (Murakami et al., 1977; Rieske, 2007; Quacchia et al., 2008). At least in Japan and Italy, *T. sinensis* successfully established and the CGW infestations were significantly reduced after around 8–10 years (Moriya et al., 2003; Quacchia et al., 2014).

We present results collected in the frame of the implementation of a ClBC programme against CGW in France (Borowiec et al., 2014), testing several release strategies, with different levels of propagule size or propagule number, to investigate the influence of propagule pressure on post-introduction dynamics of the exotic parasitoid *T. sinensis*.

Materials and methods

Study area

The study area covers a wide geographical area (920 km from North to South, 1 030 km from East to West) in metropolitan France including Corsica. To select sites, field inspections were carried out in winter, 2–3 months before the releases of *T. sinensis*. During these surveys, 'winter galls' of CGW were collected to assess the presence/absence of the parasitoid (see below). If no *T. sinensis* emerged from collected galls, we considered that the parasitoid was absent from the site and the site was selected either as release site or as control site. To maximize the establishment probability of *T. sinensis*, only sites where chemical treatments were absent or very reduced were selected. All release sites were separated by at least 4 km, whereas most of the control sites were distant from 1 to 2 km from the nearest release site (minimum 500 m, maximum 15 km). Control sites were set up to give information on the natural dispersal of *T. sinensis* as well as to compare the dynamics of the parasitoid between release and control sites. In this

study, we focus on the establishment and the dynamics of *T. sinensis* in release sites only. A total of 58 release sites and 11 control sites were hence selected between 2011 and 2015 (Figure 1, Table 1). Post-release surveys were also extended to the first two sites where *T. sinensis* was first introduced in France (Alcotra project 'Sauvegarde de l'Ecosystème Châtaigne', 2010) (Figure 1). Almost all these sites were classified into three types:

- Productive orchards (n = 26): commercial orchards characterized by the presence of young chestnut trees (<40 years). On these sites, cultivated varieties are based on hybrids (*C. crenata* × *C. sativa*) and mainly cv. 'Marigoule'. Most of the productive orchards are located in agricultural landscapes and are surrounded by other crops (e.g., apple, walnut, and peach). These sites are mostly located in south-western France and at 'low' altitude (mean 306 m, range 21–621 m). Among these sites, 24 were used as release sites and two as control sites.
- Traditional orchards (n = 22): commercial orchards situated in hilly or mountainous areas with old chestnut trees (>40 years). The cultivated varieties are traditional ones (based on *C. sativa*). Most of the traditional orchards are adjacent to or nested in forests with a somewhat difficult access. These sites are mostly located in southeast France (e.g., Ardèche, Corse, and Cévennes). Their mean altitude is 640 m (226–946 m). Among these sites, 17 were used as release sites and five as control sites.
- Forests (n = 22): sites located in natural habitats. Besides *C. sativa*, species composition of these sites varied according to the pedoclimatic conditions [e.g., *Quercus petraea* (Matt.) Liebl., *Quercus robur* L., *Carpinus betulus* L., and *Pinus sylvestris* L.]. The mean
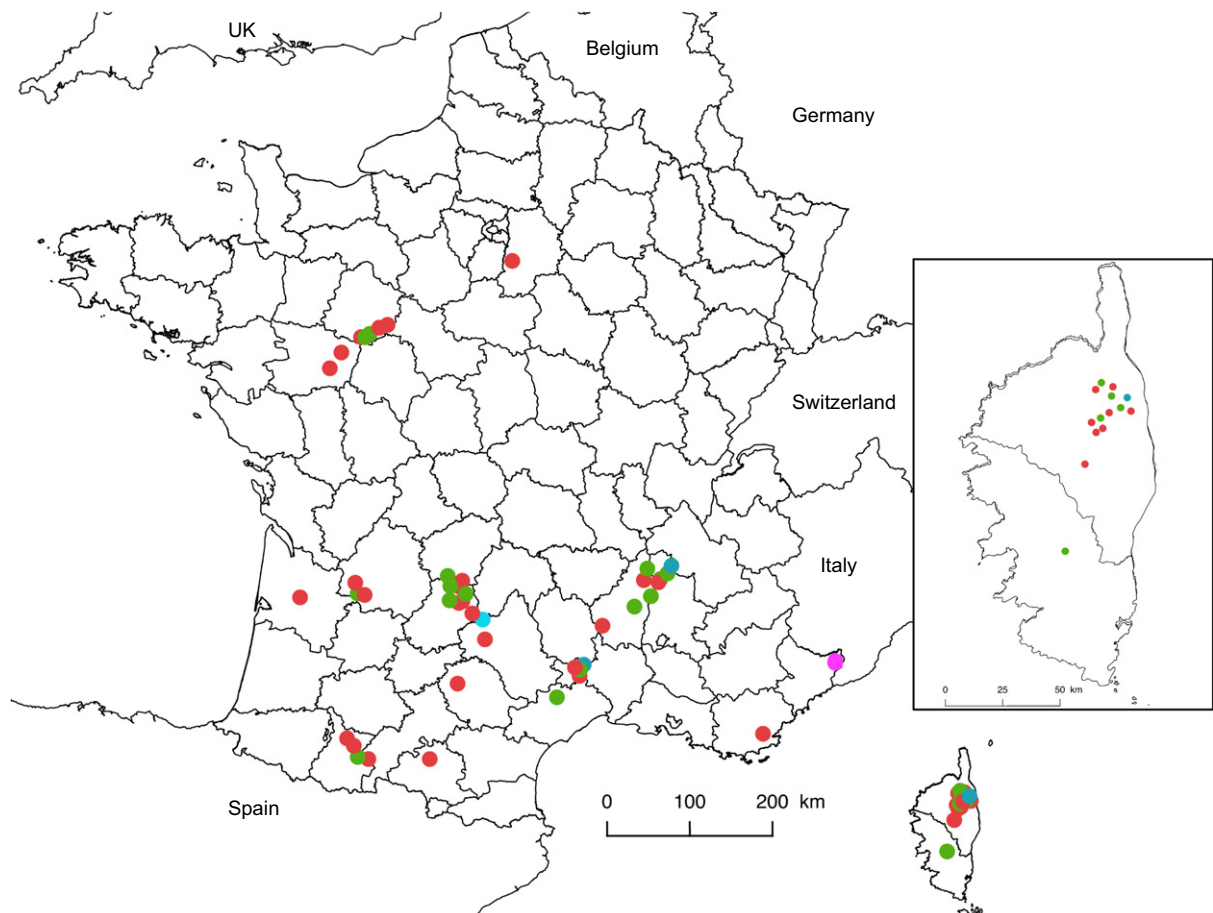

Figure 1 Distribution map of the *Torymus sinensis* releases done between 2011 and 2014 in continental France and Corsica (rescaled in box). Red circles, 1 × 100 females released; green circles, 2 × 50 females released; blue circles, 1 × 1 000 females released; purple circles (extreme southeast of France; Alcotra project), 2 × 110 females released.

Table 1 Detailed information of the sites where *Torymus sinensis* has been introduced in France

Ref	Locality	Altitude (m)	Type[1]	Modality of introduction (no. females)	Year of release	Parasitism rate (%) in 2016[3]	Last parasitism rate (no. emerged from 1000 winter galls) (year)	Last infestation level (% buds with ≥1 gall) (year)
Am1	Tende	771	F	2 × 100	2010–2011	–	4.9 (2015)	3.4 (2015)
Am2	Granile	906	F	2 × 100	2010–2011	–	2.3 (2015)	2.9 (2015)
Ard1	Saint-Georges-les-Bains	222	F	2 × 50	2011–2012	100	1784.6 (2015)	44.6 (2015)
Ard1T	Saint-Georges-les-Bains	145	F	Control[2]	–	–	1409.2 (2015)	47.1 (2015)
Ard2	Colombier-le-Jeune	574	TO	1 × 100	2012	–	2028.2 (2015)	60.6 (2015)
Ard2T	Colombier-le-Jeune	617	F	Control	–	–	1209.1 (2015)	61.7 (2015)
Ard3	Sablières	461	TO	1 × 100	2012	–	1420.8 (2015)	69.9 (2015)
Ard4	Veyras	551	TO	2 × 50	2012–2013	–	3741.8 (2015)	66.5 (2015)
Ard5	Bellevue	409	PO	2 × 50	2013–2014	–	3212.0 (2015)	48.6 (2015)
ArdX6	Désaignes	645	TO	Control	–	–	1427.1 (2015)	68.3 (2015)
Ari1	Montégut-Plantaurel	379	F	1 × 100	2014	–	17.9 (2015)	– (2015)
Avey1	Livinhac-les-Hauts	283	PO	1 × 1000	2013	–	703.7 (2015)	95.5 (2014)
Avey2	Rignac	621	PO	1 × 100	2014	–	7.0 (2014)	65.5 (2014)
Cor1	Campile	466	F	2 × 50	2011–2012	62	1087.3 (2015)	66.9 (2012)
Cor1T	Bisinchi	593	TO	Control	–	–	137.2 (2015)	92.1 (2012)
Cor2	Scolca	684	TO	1 × 100	2011	–	489.9 (2015)	85.8 (2012)
Cor2T	Volpajola	302	TO	Control	–	–	625.5 (2015)	82.1 (2012)
Cor3	Lentu	790	TO	1 × 100	2011	73	739.6 (2015)	84.1 (2012)
Cor3T	Canavaggia	705	TO	Control	–	72	910.4 (2015)	92.9 (2012)
Cor4	Aiti	773	TO	1 × 100	2012	–	11.8 (2013)	68.9 (2012)
Cor5	Muratu	851	TO	2 × 50	2012–2013	–	1.5 (2013)	89.1 (2012)
Cor5T	Muratu	750	TO	Control	–	–	3.3 (2013)	83.9 (2012)
Cor6	San Lurenzu	854	TO	1 × 100	2012	–	517.7 (2015)	76.9 (2012)
Cor7	Gavignanu	807	TO	2 × 50	2012–2013	–	804.0 (2015)	89.9 (2012)
Cor8	Poggiu-Marinacciu	794	TO	1 × 100	2013	–	0.3 (2013)	–
Cor9	Casinca	444	TO	1 × 1000	2013	–	39.8 (2015)	–
Cor10	Prunu	226	TO	1 × 100	2013	–	252.7 (2015)	–
Cor11	Rusiu	946	TO	1 × 100	2013	–	4.8 (2013)	–
Cor12	Pianu	713	TO	2 × 50	2013–2014	–	0.3 (2013)	–
Cor13	Venacu	677	F	1 × 100	2014	–	209.2 (2015)	–
CorS1	Zevacu	870	TO	2 × 50	2013–2014	–	0 (2013)	–

Table 1. Continued

Ref	Locality	Altitude (m)	Type[1]	Modality of introduction (no. females)	Year of release	Parasitism rate (%) in 2016[3]	Last parasitism rate (no. emerged from 1000 winter galls) (year)	Last infestation level (% buds with ≥1 gall) (year)
Corz1	Altillac	328	PO	1 × 100	2012	–	812.3 (2015)	97.6 (2014)
Corz2	Lagleygeolle	402	PO	2 × 50	2013–2014	–	983.5 (2015)	96.2 (2014)
Corz3	Reygades	457	PO	1 × 100	2014	–	39.3 (2015)	–
Dord1	St Pierre d'Eyraud	22	PO	2 × 50	2012–2013	–	12.3 (2014)	83.1 (2014)
Dord1T	Le Fleix	80	F	Control	–	–	0 (2014)	68.2 (2014)
Dord2	Prigonrieux	21	PO	1 × 100	2012	–	144.5 (2015)	95.6 (2015)
Dord3	St Sauveur Lalande	100	PO	1 × 100	2014	–	–	–
Dro1	Châteauneuf sur Isère	146	PO	2 × 50	2011–2012	97	3673.7 (2015)	85.1 (2015)
Dro2	Châteauneuf sur Isère	194	PO	1 × 100	2011	–	3882.9 (2015)	89.8 (2015)
Dro3	Génissieux	267	PO	2 × 50	2011–2012	100	1285.4 (2015)	82.4 (2015)
Dro4	Montmiral	500	F	1 × 1000	2013	–	2592.8 (2015)	62.8 (2015)
Gard1	Le Castanet	375	TO	1 × 100	2012	–	461.3 (2015)	70.5 (2015)
Gard2	Saumane	380	TO	1 × 1000	2013	–	1567.5 (2015)	44 (2015)
Gard3	Puech Sigale	634	F	2 × 50	2013–2014	97	306.4 (2015)	78.5 (2015)
Gard4	Valleraugue	378	PO	1 × 100	2013	–	1607.7 (2015)	76 (2015)
Gir1	Villenave d'Ornon	23	PO	1 × 100	2013	–	0.9 (2015)	15.6 (2013)
Her1	Fozières	351	F	2 × 50	2012–2013	98	744.5 (2015)	65.2 (2015)
Her1T	Fozières	321	F	Control	–	98	520.7 (2015)	41.4 (2015)
Hpyr1	Tibiran Jaunac	488	F	1 × 100	2013	–	532.1 (2015)	74.1 (2014)
Hpyr2	La Barthe de Neste	567	F	2 × 50	2013–2014	–	5.0 (2015)	53.9 (2014)
Hpyr3	Cabanac	258	PO	1 × 100	2014	–	– (2015)	–
Hpyr4	Burg	437	PO	1 × 100	2014	–	22.6 (2015)	33.2 (2014)
Lot1	Aynac	421	PO	1 × 100	2012	92	2259.0 (2015)	99.8 (2013)
Lot2	Bétaille	184	PO	2 × 50	2012–2013	87	1.8 (2015)	67.3 (2014)
Lot3	Molières	434	PO	1 × 100	2012	–	1.5 (2013)	97.3 (2013)
Lot3T	Molières	516	PO	Control	–	–	0 (2013)	71.2 (2012)
Lot4	Cornac	517	PO	2 × 50	2012–2013	–	41.9 (2014)	76.3 (2013)
Lot4T	Cornac	519	PO	Control	–	–	7.5 (2014)	94.5 (2013)
Lot5	Rignac	352	PO	2 × 50	2012–2013	88	1553.5 (2015)	87.8 (2014)
Lot6	Viazac	427	PO	1 × 100	2013	–	226.8 (2015)	73.7 (2014)
Main1	Cuon	80	F	1 × 100	2014	–	0 (2014)	25.9 (2014)
Main2	Louerre	102	F	1 × 100	2014	55	9.2 (2015)	16.3 (2014)
Sart1	Lavernat	110	PO	2 × 50	2013–2014	18	243.5 (2015)	36.3 (2015)
Sart2	Chahaigne	144	F	1 × 100	2013	24	0 (2015)	10.9 (2015)

Table 1. Continued

Ref	Locality	Altitude (m)	Type[1]	Modality of introduction (no. females)	Year of release	Parasitism rate (%) in 2016[3]	Last parasitism rate (no. emerged from 1000 winter galls) (year)	Last infestation level (% buds with ≥1 gall) (year)
Sart3	Le Lude	88	F	1 × 100	2013	–	1.3 (2015)	33.7 (2015)
Sart4	La Cour Denet	69	F	2 × 50	2013–2014	–	662.9 (2015)	36.6 (2015)
Sart5	Ruillé sur Loir	147	F	1 × 100	2014	–	0 (2015)	44.5 (2015)
Sem1	La Rochette	113	Garden	1 × 100	2015	–	62.9 (2015)	92.1 (2015)
Tarn1	Gaillac	145	PO	1 × 100	2014	–	12.0 (2014)	64.5 (2014)
Var1	Garde Freinet	386	F	1 × 100	2011	–	3592.1 (2015)	75 (2014)

[1]F, forests; TO, traditional orchards; PO, productive orchards; Garden, single site located in private garden.
[2]Control, control sites with no release of *T. sinensis*.
[3](no. *T. sinensis* emerged from spring galls/all parasitoids and CGW emerged) × 100%.

altitude is 370 m (69–906 m). Among these sites, 18 were used as release sites and four as control sites.

All of these sites were also categorized according to their connectivity with oak–chestnut natural stands:

- High connectivity for sites (n = 49) nested in a forest or located to less than 500 m from an oak–chestnut natural stand.
- Medium connectivity for sites (n = 16) located between 500 and 1 000 m from an oak–chestnut natural stand.
- Low connectivity for isolated sites (n = 6) or those located at least 1 000 to 2 000 m from an oak–chestnut natural stand.

Torymus sinensis releases

As no laboratory rearing of either CGW or *T. sinensis* was available, parasitoids used for releases were recaptured in the field. During the first 2 years (2011 and 2012), and because populations of *T. sinensis* at that time in France were too small, *T. sinensis* to be released were provided by University of Torino (Italy). In following years (2013–2015), specimens of *T. sinensis* were obtained from 'winter galls' collected in French sites where post-release surveys were realized (see below).

Once identified under a stereomicroscope, *T. sinensis* specimens were sexed and then placed in plastic tubes (11.5 cm long, 2.6 cm diameter) with honey and sealed by a piece of cotton wool. Each tube containing 25 females and 12 males was then put in a climatic chamber at 14 °C, 60% r.h., and L10:D14 photoperiod. Honey within each tube was renewed every Friday. For the releases, only females of less than 3 weeks old were used.

Releases of *T. sinensis* were done according to different modalities of introduction:

- Modality A: one release (year n) of 100 females and 50 males. This modality was set up in 34 sites (14 productive orchards, 10 traditional orchards, and nine forests).
- Modality B: two releases (year n and n + 1) of 50 females and 25 males. These releases were done in 20 sites (nine productive orchards, five traditional orchards, and six forests).
- Modality D: one release (year n) of 1 000 females and 500 males. These large releases were done in four sites (one productive orchard, two traditional orchards, and one forest).

For the two 'Alcotra sites', introductions (modality E, n = 2) were done by University of Torino in 2010 (110 females and 50 males) and 2011 (110 females and 50 males) (Anonymous, 2012).

Each year, releases were positioned according to the phenology of chestnut trees in the sites. Some observations were thus done (for each year and on each site) every 10 days from the last week of March to the releases of *T. sinensis* (mid-April to beginning of May depending of the year and/or of the site considered). Following Bellini et al. (2006), each bud on 20 randomly chosen twigs of ca. 20 cm were categorized and releases were done on a site when ca. 40% of the buds were in the stage 'new leaves appeared and buds perula fell', others still being in previous stages. Releases were done in late morning or early afternoon on several trees of each site. The central release point was located using global positioning system coordinates (Garmin GPSmap 60Cx).

Except for modality D, which was difficult to set up with regard to the large number of *T. sinensis* used, assignation of a release modality to a site was done to have at least 20 sites from each modality of release. Within each modality, we then tried to balance the different types of sites to average any effect related to chestnut variety or cultural practices. Finally, the number of sites within each administrative region was determined by both the geographical coverage of chestnut trees and the economic importance of chestnut.

Estimation of *Torymus sinensis* populations

Winter dry galls. To estimate *T. sinensis* populations, 'winter galls' (i.e., 'spring galls' that withered on trees) were collected each year (from 2011 to 2016) in January–February on each site (initial release point and neighbouring trees). Given the high density of CGW relative to *T. sinensis* at the beginning of the project, 2 000–5 000 galls were collected on each site during the first 2 years of post-release surveys. Then only 500–2 000 galls were collected on each site. In total, around 530 000 'winter galls' were collected during the whole post-release survey.

Once collected, galls were separated from other plant tissues (leaves, twigs) and put in hermetic 34 × 28 × 9 cm black box (500 galls in one box). Each box had two transparent tubes (7 cm long, 3.3 cm diameter) with honey drops to attract, feed, and collect emerging insects. All boxes were kept outside at Sophia Antipolis (France) to maintain a synchronicity with CGW development in the field. However, from 2013, the procedure was improved by first placing all the boxes in controlled conditions (7 °C) during 10 days. This allowed to better synchronize the emergences of *T. sinensis* by reducing the emergence period (N Borowiec, unpubl.). This technique was really helpful to manage emergence of *T. sinensis* according to chestnut phenology in the wide geographical area covered.

Emergence of insects was checked daily between March and June and then 3× a week until September of each year. All the emerged parasitoids were put in 96% ethanol except *Torymus* specimens. These were isolated in small glass vials (five individuals per vial), sexed, and identified under a stereomicroscope. For identification, we used morphological information from Graham & Gijswijt (2001), Grissell (1995), and Kamijo (1982). Doubtful specimens of *Torymus* were put in 96% ethanol for further molecular and morphological characterization. Unambiguous *T. sinensis* were counted and some were re-used for field releases (see previous paragraph). For each site, parasitism rate of *T. sinensis* was calculated as the number of *T. sinensis* emerged divided by the number of galls collected.

Spring fresh galls. As variable number of chambers can be observed within galls (Otake, 1989), the number of *T. sinensis* emerged from galls did not reflect the true parasitism rate. To complete these data, we thus collected 'spring galls' in June 2016 in 15 sites (Table 1). For each site, galls (from 122 to 233) were put in emerging boxes (see above) and were then placed in controlled conditions at 21 °C, 65% r.h., and L14:D10 photoperiod. Emergence was checked 3× a week between June 2016 and June 2017. All emerged specimens were put in 96% ethanol, kept at −20 °C, and then sorted according to morphological characters. For each site, the parasitism rate by *T. sinensis* was calculated a posteriori as the number of emerged *T. sinensis* divided by the total number of *D. kuriphilus* and parasitoids (*T. sinensis* and native ones) emerged from galls.

Estimation of CGW populations

Given the large experimental design, it was not possible to sample 'spring galls' before the emergence of CGW each year and on each site. As a consequence, we used the infestation levels of *D. kuriphilus* as an estimator for CGW populations. For that, 10 trees were randomly selected on each site and, on each tree, we randomly selected 10 twigs from which we counted the buds that gave at least one leaf and the galls associated with each bud. The infestation levels of *D. kuriphilus* were expressed as the percentage of buds with at least one gall, i.e., (total number of buds with at least one gall divided by total number of buds) × 100%.

Statistical analysis

After preliminary observations, the dataset was split into two subsets, one dealing with the first 4 years after *T. sinensis* introductions ('early dynamics') and the second one dealing with the years 5 and 6 ('middle term dynamics'). In both cases and for each site and sampling date, the main variables were as follows: (1) for *T. sinensis* dynamics, the transformed abundance index of the parasitoid. The raw abundance index is calculated as the number of *T. sinensis* emerging from 1 000 galls, with values comprised between 0 and 3 800. The transformation was realized using ln(raw index + 1), rounded to the nearest integer. This quantitative variable followed a Poisson distribution, with values comprised between 0 (absence of *T. sinensis*) and 8 (highest abundance). And (2) for the CGW dynamics, the percentage of infected chestnut tree buds, which followed a Gamma distribution. These two variables were analysed with general linear mixed models (package 'glmmadmb'; Skaug et al., 2016) with a Poisson or a Gamma distribution, respectively, and a log link. Model comparisons were done using AIC and, if relevant, the impacts of fixed effects were interpreted using P-values.

To investigate 'early dynamics' (<4 years after Ts introduction), population was included as a random effect, and fixed effects included site connectivity and the interaction between year (starting at introduction) and introduction modality. We compared this full model with a 'null' model including only year as fixed effects, and all intermediate models in between (Table S1). To investigate 'middle term dynamics' (until years 5 and 6 after *T. sinensis* introductions), population was included as a random effect, and the effect of year was modelled with a second-degree term to allow for non-monotonic dynamics. We compared this full model with a 'null' model including only a linear effect of year as a fixed effect (Table S2).

We also tested the correlation between host and parasitoid dynamics, by running a model with the estimator of *D. kuriphilus* population size as the main variable, and the estimator of *T. sinensis* population size and year as fixed effects. Because of few replicates within this dataset, other possible factors and/or covariates were not taken into account. All the analyses were performed using R v.3.2.2 (R Core Team, 2015). Figure 1 was generated by QGis v.2.18 (Quantum GIS Development Team, 2016).

Results

Releases and establishment of *Torymus sinensis*

Between 2011 and 2015, 14 100 *T. sinensis* were introduced in 58 sites in France as follows: 900 in 2011 (eight sites), 2 250 in 2012 (17 sites), 8 550 in 2013 (21 sites), 2 250 in 2014 (11 sites), and 150 in 2015 (one site; Table 1). According to the year considered, releases were done during a 2-week period between mid-April and early-May. This short release window required a complex logistical organization to cover the large geographical area considered.

Five of these sites for which the monitoring was interrupted quite early were discarded. For the other sites, *T. sinensis* was recaptured on 87% of the sites during the first post-release survey (year n + 1; n = 46) and the remaining 13% from the second field monitoring (year n + 2; n = 7). The rate of establishment of *T. sinensis* within our field design is thus especially high (100%), with no difference between introduction modalities and no significant effects of other factors like altitude or longitude. Populations of *T. sinensis* are now well established in 18 French departments and half of the administrative regions in metropolitan France including Corsica (Table 1).

Among the 11 control sites, one was discarded from the analysis because field monitoring was interrupted after only 1 year. For the 10 remaining sites, three were colonized by *T. sinensis* 2 years after the first introduction in the closest release sites and six were colonized by the parasitoid 3 years after first closest introduction. For one site (Dord1T), *T. sinensis* was not yet detected 3 years after first closest introduction but field monitoring could not be continued further.

Dynamics of *Torymus sinensis* and *Dryocosmus kuriphilus* (winter dry galls)

Dynamics of *T. sinensis* can be divided into two phases. Early dynamics are characterized by an exponential-like growth over the 4-year period following introduction (Figure 2). The mean number of *T. sinensis* per 1 000 galls rapidly increases from year 1 to 4 as follows: 6.1 (range 0–63), 50.6 (0–612), 520.9 (0–3 212), and 1 171 (1.8–3 742), respectively. During this phase, no effect of the modality of introduction was observed on population growth rate, but populations founded from 1 000 introduced females retained a demographic advantage over the period and were larger on average (GLMM, introduction modality: $P = 0.00039$; no interaction with year; Figure 2, Table S1). Interestingly, there was a positive correlation between *T. sinensis* dynamics and site connectivity (GLMM, $P = 0.015$; Table S1). Indeed, populations from sites with a high connectivity level were larger than those from more isolated sites (Figure 3). During this early post-introduction period, the dynamics of *D. kuriphilus* were stable and characterized by very high population densities, with several very highly infested sites (more than 90% of buds with galls) (Figure 4). No significant effect of either the year or *T. sinensis* introduction modality was found on the CGW dynamics.

Middle-term dynamics revealed a decreasing trend in both *T. sinensis* and CGW population size after 5 years (Figures 4 and 5). The dynamics of host and parasitoid were positively correlated (GLMM on CGW abundance: positive effect of *T. sinensis* abundance, $P<10^{-6}$; negative effect of year, $P<10^{-6}$), thus supporting the hypothesis of a significant control of *D. kuriphilus* populations. Between 2011 and 2015, the percentage of highly infested sites (more than 70% of the buds with galls) markedly decreased from 60 to 30% of the total number of sites (Figure 6). On sites where effective control was achieved (less than 5% of buds with galls), *T. sinensis* populations appear to be now stabilized at a low level (on average 3.6 *T. sinensis* per 1 000 galls; Table 1).

Parasitism observed in spring fresh galls

The apparent parasitism rate (ratio between the number of emerged *T. sinensis* and the total number of *D. kuriphilus* and parasitoids) was estimated in 2016 from 15 locations (Table 1). These apparent parasitism rates ranged between 18 and 100% (only *T. sinensis* emerged) with a median of 90%. These estimates were positively correlated with the
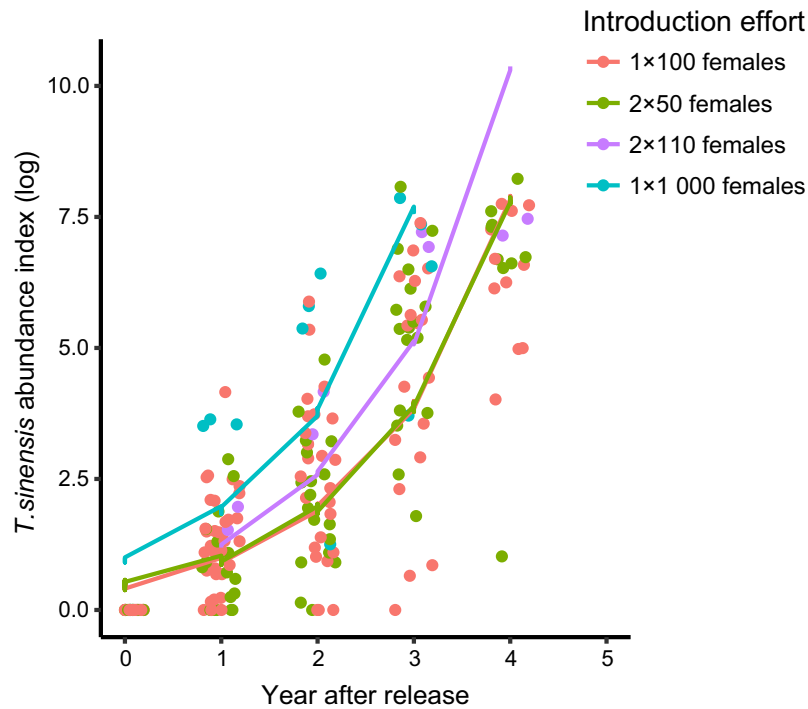
Figure 2 Abundance of *Torymus sinensis* (logarithm of raw data) over time (years since release). Lines represent the model predictions (GLMM with a Poisson distribution). Colours correspond to the various modalities of *T. sinensis* introductions.
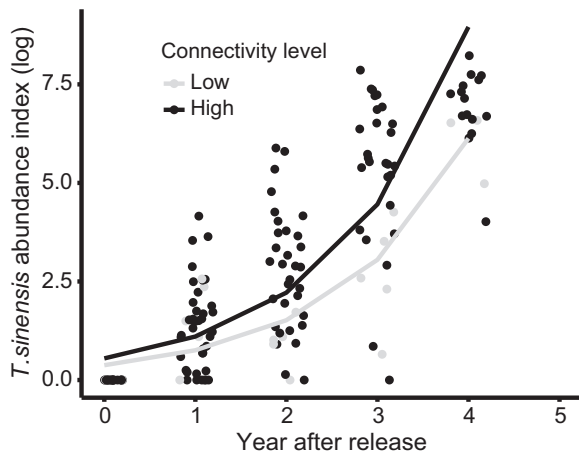

Figure 3 Abundance of *Torymus sinensis* (logarithm of raw data) over time (years since release). Lines represent the model predictions (GLMM with a Poisson distribution). Colours correspond to levels of connectivity of study sites with natural oak–chestnut stands.
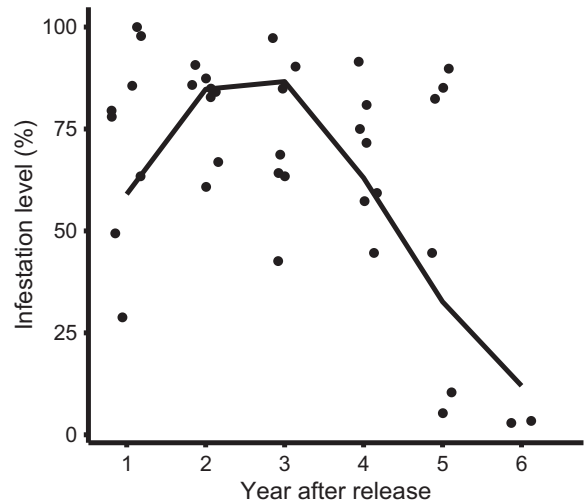
Figure 4 Infestation level (% buds with at least one gall) of *Dryocosmus kuriphilus* over time (years since release of *Torymus sinensis*), based on the dataset including populations monitored over 4. The black line represents the model predictions (GLMM with a binomial distribution).

mean number of *T. sinensis* per 1 000 galls observed in 2016 (Spearman's rho coefficient = 0.55, P = 0.032).

In our conditions, most of the *T. sinensis* adults emerged from mid-February to early-May, but,

interestingly, a small percentage of individuals emerged only few months after female oviposition. More precisely, the percentage of such 'short developments' (emergence
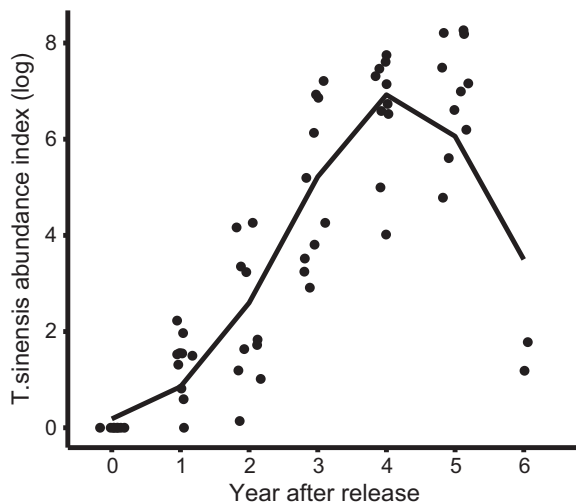
Figure 5 Abundance of *Torymus sinensis* (logarithm of raw data) over time (years since release), based on the dataset including populations monitored over 4 years. The black line represents the model predictions (GLMM with a Poisson distribution).

between June and August 2016) varies from 0 to 17, with a median of 1.2%.

Discussion

Since its first documented translocation from China to Japan, *D. kuriphilus* has markedly extended its area of distribution, most probably because of human activities. According to the economic and/or ecological importance of *Castanea* species in invaded areas, these unintended introductions were apparently more or less discreet and/or documented. Quite surprisingly, for instance, little

information is available about the management of *D. kuriphilus* in the USA during the 1970s (Cooper & Rieske, 2011). The introduction of *D. kuriphilus* in Europe from Italy 15 years ago was quickly perceived as an important issue because of the abundance of *C. sativa* in European forests as well as the chestnut-related economies in various areas. Following the initial success in Japan and the following introductions in other countries, *T. sinensis* was hence introduced in Italy starting in 2005. To some aspects, this primo-introduction in Europe was quite controversial because of possible unintentional effects (lack of host specificity and possible interspecific hybridization; Gibbs et al., 2011). However, the decision was taken to extensively introduce *T. sinensis* in France from 2010, based on (1) the fast expansion of *D. kuriphilus* in France, (2) its local outbreak in areas of chestnut production, and (3) evidence of a high establishment rate of *T. sinensis* and its quick dissemination in Italy.

Besides the agronomic impact on the regulation of *D. kuriphilus* populations, we wanted to use this opportunity to precisely document the post-release dynamics of *T. sinensis* and understand the influence of one experimental and one environmental factor. Propagule pressure was deliberately manipulated because of its pervasive importance on the success/failure of biological control introductions (Hopper & Roush, 1993; Fauvergue et al., 2012). However, we found no effect of either propagule size or number on establishment success, or on subsequent population dynamics. It should be noted nevertheless that initially high populations retained a demographic advantage for several generations, which could have improved their establishment probability in a harsher environment. However, in our case, the absence of effect of propagule pressure on establishment is most likely due to the high
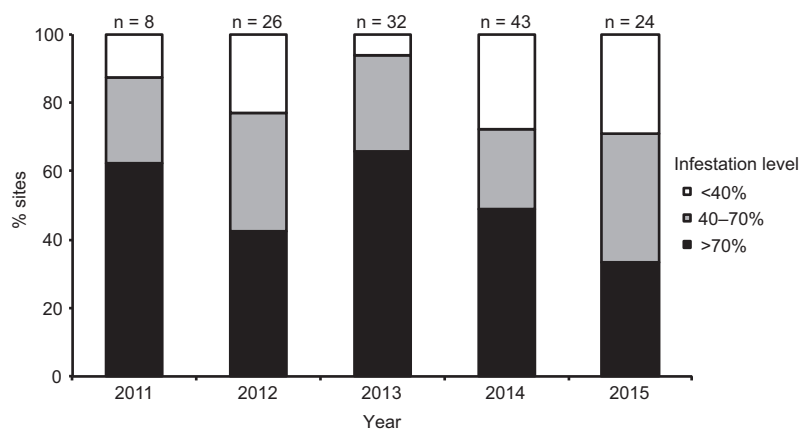

Figure 6 Development of infestation levels of *Dryocosmus kuriphilus* (% sites of a particular class among all sites) observed between 2011 and 2015. Numbers above columns indicate the total number of sites.

colonizing efficiency of *T. sinensis*. Indeed, our results show that even small releases (100 females) are as efficient as large ones to establish and multiply *T. sinensis* populations. Moreover, we find a positive effect of the connectivity of the site on *T. sinensis* population size, suggesting successful colonization and establishment beyond release sites. This hypothesis is further supported by the natural colonization by *T. sinensis* of nine control sites of 11 only 3 years after first releases.

All these results demonstrate the efficiency of *T. sinensis* to locally establish, increase, and spread. Intrinsic properties are probably involved including behavioural adaptations to locate the mates and/or the hosts even at low density (little information available) and strategies to avoid a lack of synchronicity with the host. With regard to this second point, our study demonstrated that the window of emergence of *T. sinensis* is quite wide, covering late winter and spring. Moreover, we recorded that a small number of *T. sinensis* does not overwinter but emerges after 3–4 months. Ferracini et al. (2015a) demonstrated the presence of extended diapause for some individuals. Taken together, these results speak for a diversified bet hedging which could lower the risk of local mismatch with its host's biological cycle. Such strategies may, however, have costs that are currently not understood (Hardy & Cook, 2010).

Concerning the level of control obtained on *D. kuriphilus*, our study indicated a two-phase dynamics after *T. sinensis* introductions. As detailed earlier, the early dynamics were characterized by high abundance of *D. kuriphilus* followed by severe damage on chestnut trees but no limitation of the growth of *T. sinensis*. After 4 years, a significant decrease in *D. kuriphilus* was observed probably as the consequence of the important populations of *T. sinensis*. Where the decrease in *D. kuriphilus* was high, *T. sinensis* populations also tended to decrease, probably as the consequence of the scarcity of hosts. Such a pattern suggests a tight coupling between the host and parasitoid populations (Hassell, 2000), which should allow obtaining sustainable and significant control over the populations of *D. kuriphilus*. Indeed, in some areas of France, it is now difficult to collect galls that are more frequently smaller and mainly found on leaves. However, to date this level of control has only been demonstrated on a few sites for which we have acquired 5–6 years of post-release surveys. This emphasizes the need for biological control programmes to cover sufficient time and geographical scales to be able to evaluate host–parasitoid dynamics beyond the early post-introduction period.

In the same way, implementation of 'middle term' post-release surveys may bring to light unintentional effects of biological control programs. For instance, the decrease in host populations after a few years may result in resource limitation for parasitoids and trigger interspecific competition between exotic and native species (Harvey et al., 2013; Cusumano et al., 2016). Around 30 native Chalcidoidea wasps are reported from *D. kuriphilus* in Europe (Noyes, 2017) and most of them switched from oak gall wasps' communities (Aebi et al., 2006). Next steps will be (1) to analyse the dynamics of these native parasitoids on CGW in association with various ecological factors (e.g., habitat connectivity), and (2) to assess potential interactions of native parasitoids and exotic *T. sinensis*, in particular, as host resource is becoming limited. Although *T. sinensis* is only reported from *D. kuriphilus* in its native area, as well as in Japan, Korea, and USA, it appears important to better evaluate the risk that *T. sinensis* switch from CGW to some oak gall wasps species. As shown by our results, 4 years after the release of *T. sinensis*, the populations of *D. kuriphilus* are still high and the probability for *T. sinensis* to encounter galls induced by other cynipids on oak may be low. However, in areas where densities of *D. kuriphilus* have been greatly reduced, this risk may be increased. To date, the only report of *T. sinensis* on other hosts than *D. kuriphilus* – i.e., three males emerged from galls of *Biorhiza pallida* (Ollivier) – came from an area where *T. sinensis* was first released in Italy (Ferracini et al., 2015b).

References

Aebi A, Schönrogge K, Melika G, Alma A, Bosio G et al. (2006) Parasitoid recruitment to the globally invasive chestnut gall

wasp *Dryocosmus kuriphilus*. Galling Arthropods and Their Associates: Ecology and Evolution (ed. by K Ozaki, J Yukawa, T Ohgushi & PW Price), pp. 103–121. Springer, Tokyo, Japan.

Anonymous (2012) Alcotra Sauvegarde de l'Écosystème Châtaigne. Rapport Final d'Éxecution. European Union, Piemont Region, Chambre d'Agriculture des Alpes-Maritimes.

Bellini E, Giannelli G, Giordania E & Picardi E (2006) Fenofasi del Castagno (*Castanea sativa* Mill.). Atti del 'IV Convegno Nazionale-Castagno 2005', Montella 20-22 October 2005, pp. 138–142.

Borowiec N, Thaon M, Brancaccio L, Warot S, Vercken E et al. (2014) Classical biological control against the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in France. Plant Protection Quarterly 29: 7–10.

Brown JH & Kodric-Brown A (1977) Turnover rates in insular biogeography: effect of immigration on extinction. Ecology 58: 445–449.

Brussino G, Bosio G, Baudino M, Giordano R, Ramello F & Melika G (2002) Dangerous exotic insect for the European chestnut. Informatore Agrario 58: 59–61.

CABI/EPPO (2015) Distribution Maps of Plant Pests, No. 677: *Dryocosmus kuriphilus*. CABI, Wallingford, UK.

Conedera M, Tinner W, Krebs P, De Rigo D & Caudullo G (2016) *Castanea sativa* in Europe: distribution, habitat, usage and threats. European Atlas of Forest Tree Species (ed. by J San-Miguel-Ayanz, D De Rigo, G Caudullo, T Durrant & A Mauri), pp. 78–79. Publications Office of the European Union, Luxembourg.

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

Cusumano A, Peri E & Colazza S (2016) Interspecific competition/facilitation among insect parasitoids. Current Opinion in Insect Science 14: 12–16.

Drake JM, Baggenstos P & Lodge DM (2005) Propagule pressure and persistence in experimental populations. Biology Letters 1: 480–483.

EFSA (European Food Safety Agency) (2010) Risk assessment of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* for the EU territory and identification and evaluation of risk management options. EFSA Journal 8: 1–114.

Eilenberg J, Hajek A & Lomer C (2001) Suggestions for unifying the terminology in biological control. BioControl 46: 387–400.

Fauvergue X, Vercken E, Malausa T & Huftbauer RA (2012) The biology of small, introduced populations, with special reference to biological control. Evolutionary Applications 5: 424–443.

Ferracini C, Gonella E, Ferrari E, Saladini MA, Picciau L et al. (2015a) Novel insight in the life cycle of *Torymus sinensis*, biocontrol agent of the chestnut gall wasp. BioControl 60: 169–177.

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

Gehring E, Bellosi B, Quacchia A & Conedera M (2017) Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters. Journal of Pest Science 91: 189–202.

Gibbs M, Schönrogge K, Alma A, Melika G, Quacchia A et al. (2011) *Torymus sinensis*: a viable management option for the biological control of *Dryocosmus kurihpilus* in Europe? BioControl 56: 527–538.

Graham MWR & Gijswijt MJ (2001) Revision of the European species of *Torymus* Dalman (Hymenoptera: Torymidae). Zoologische Verhandelingen 317: 1–202.

Grissell EE (1995) Toryminae (Hymenoptera: Chalcidoidea: Torymidae). A Redefinition, Generic Classification, and Annotated World Catalog of Species. Memoirs on Entomology, International 2. Associated Publishers, Gainesville, FL, USA.

Hanski I (1998) Metapopulation dynamics. Nature 396: 41–49.

Hardy NB & Cook LG (2010) Gall-induction in insects: evolutionary dead-end or speciation driver? BMC Evolutionary Biology 10: 257.

Harvey JA, Poelman EH & Tanaka T (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. Annual Review of Entomology 58: 331–351.

Hassell MP (2000) Host-parasitoid population dynamics. Journal of Animal Ecology 69: 543–566.

Hayes KR & Barry SC (2008) Is there any consistent predictors of invasion success? Biological Invasions 10: 483–506.

Hopper KR & Roush RT (1993) Mate finding, dispersal, number released, and the success of biological control introductions. Ecological Entomology 18: 321–331.

Jabr F (2014) A new generation of American chestnut trees may redefine America's forests. Scientific American 310: web exclusive. Available at: https://www.scientificamerican.com/article/chestnut-forest-a-new-generation-of-american-chestnut-trees-may-redefine-americas-forests/) (accessed 15 July 2017)

Kamijo K (1982) Two new species of *Torymus* (Hymenoptera, Torymidae) reared from *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in China and Korea. Kontyu 50: 505–510.

Kato K & Hijii N (1997) Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. Journal of Applied Entomology 121: 9–15.

Lande R (1988) Genetics and demography in biological conservation. Science 241: 1455–1460.

Meyer JB, Gallien L & Prospero S (2015) Interaction between two invasive organisms on the European chestnut: does the chestnut blight fungus benefit from the presence of the gall wasp? FEMS Microbiology Ecology 91: 1–10.

Moriya S, Shiga M & Adachi I (2003) Classical biological control of the chestnut gall wasp in Japan. Proceedings of the 1st International Symposium on Biological Control of Arthropods (ed. by RG Van Driesche), pp. 407–415. USDA Forest Service, Honolulu, HI, USA.

Murakami Y, Umeya K & Oho N (1977) A preliminary introduction and release of a parasitoid (Chalcidoidea, Torymidae) of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Cynipidae) from China. Japanese Journal of Applied Entomology and Zoology 21: 197–203.

Murakami Y, Ao HB & Chang CH (1980) Natural enemies of the chestnut gall wasp in Hopei Province, China

(Hymenoptera, Chalcidoidea). Applied Entomology and Zoology 15: 184–186.

Noyes JS (2017) Universal Chalcidoidea Database. World Wide Web electronic publication. Available at: http://www.nhm.ac.uk/chalcidoids (accessed on 5 April 2017)

Otake A (1989) Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) analyses of records on cell contents inside galls and on emergence of wasps and parasitoids outside galls. Applied Entomology and Zoology 24: 193–201.

Payne JA (1978) Oriental chestnut gall wasp: new nut pest in North America. Proceedings of the American Chestnut Symposium (ed. by WL Macdonald, FC Cech, J Luchoc & C Smith), pp. 86–88. West Virginia University Press, Morgantown, WV, USA.

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

Quacchia A, Moriya S & Bosio G (2014) Effectiveness of *Torymus sinensis* in the biological control of *Dryocosmus kuriphilus* in Italy. Acta Horticulturae 1043: 199–204.

Quantum GIS Development Team (2016) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: https://www.qgis.org (accessed on 18 November 2016).

R Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/ (accessed 10 September 2015).

Reed DH (2005) Relationship between population size and fitness. Conservation Biology 19: 563–568.

Rieske LK (2007) Success of an exotic gall maker, *Dryocosmus kuriphilus*, on chestnut in the USA: a historical account. EPPO Bulletin 37: 172–174.

Shorthouse JD, Wool D & Raman A (2005) Gall-inducing insects – Nature's most sophisticated herbivores. Basic and Applied Ecology 6: 407–411.

Simberloff D (2009) The role of propagule pressure in biological invasions. Annual Review of Ecology, Evolution and Systematics 40: 81–102.

Skaug H, Fournier D, Nielsen A, Magnusson A & Bolker B (2016) glmmADMB: Generalized Linear Mixed Models Using 'AD Model Builder'. R Package v.0.8.3.3. Available at: https://rdrr.io/rforge/glmmADMB/ (accessed 30 March 2016).

Willi Y, Van Buskirk J & Hoffmann AA (2006) Limits to the adaptive potential of small populations. Annual Review of Ecology, Evolution, and Systematics 37: 433–458.

Zenni RD & Nuñez MA (2013) The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122: 801–815.

Zhu DH, He YY, Fan YS, Fan YS, Ma MY & Peng DL (2007) Negative evidence of parthenogenesis induction by *Wolbachia* in a gallwasp species, *Dryocosmus kuriphilus*. Entomologia Experimentalis et Applicata 124: 279–284.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of statistical models compared for the analysis of the early dynamics of *Torymus sinensis*.

Table S2. Summary of statistical models compared for the analysis of the middle term.