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Evolution of the *Castanea sativa* - *Dryocosmus kuriphilus* - *Torymus sinensis* - system in southern Switzerland

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Résumé

L’arrivée de nouveaux organismes qui peuvent s’avérer potentiellement nuisibles à l’égard des personnes et des écosystèmes est devenu un objet de préoccupation quotidienne, à tel point qu’il existe en Suisse environ 800 espèces reconnues d’organismes étrangers, dont au moins 107 sont considérées invasives. Pour maîtriser ce genre d’insectes nuisibles l’une des méthodes possibles consiste à importer et introduire des ennemis naturels provenant de leur pays d’origine en leur permettant d’agir en tant qu’agents de lutte biologique. Un excellent exemple type est le cas récent du cynips du châtaignier originaire d’Asie (*Dryocosmus kuriphilus* Yasumatstu) qui a fait son arrivée en 2002 en Europe et qui a été combattu avec l’importation de son ennemi naturel *Torymus sinensis* Kamijo .Malgré la présence d’un grand nombre d’études menées par les chercheurs japonais, au moment où j’ai commencé cette thèse, nous ne disposions que de connaissances limitées sur l’ensemble des dégâts provoqués par des attaques répétées du *cynips* et sur la capacité de reprise de l’arbre-hôte (*Castanea sativa* Miller) lorsque la lutte biologique atteint son but. L’objectif de cette thèse est donc d’étudier et de présenter l’évolution des forêts de châtaignier, de l’arrivée du ravageur jusqu’au contrôle biologique, en mettant en évidence l’ensemble des dégâts subis par l’arbre, ses modalités de reprise et les effets considérables qu’une lutte biologique tardive peut entraîner.

Nous nous sommes d’abord concentrés (chap.2) sur la partie méthodologique en recherchant les meilleurs indicateurs pour analyser l’évolution épidémique sur le châtaignier, en particulier sur le plan des dégâts et des processus de réparation. Plus précisément, nous avons utilisé la relation aire foliaire-section d’aubier en nous basant sur l’approche du *pipe model* pour faire une estimation quantitative de la biomasse verte d’une branche avant et après l’arrivée de l’insecte nuisible, y compris pendant la période de pic de l’épidémie et la première et deuxième année de rétablissement grâce l’action progressive de *T. sinensis*. Ensuite, la perte de la surface foliaire a été utilisée comme variable réponse dans une analyse de modèle de régression mixte pour choisir les caractéristiques architecturales les plus appropriées (par ex. le type de galle, le type de bourgeon, le type de pousse) permettant d’expliquer l’évolution des dégâts au niveau des branches. Les résultats ont montré que durant le pic de l’épidémie, les pertes de la surface foliaire excédaient 70% avec des altérations significatives de l’architecture des branches en fait de bourgeons dormants (réserves de l’arbre) et d’augmentation de pousses mortes. De plus notre analyse a révélé que les indicateurs traditionnellement utilisés et généralement mentionnés dans la littérature pour mesurer le taux d’infestation du cynips du châtaignier (par ex. le nombre de bourgeons infestés vs le nombre de bourgeons sains) ne parviennent pas à fournir une estimation fiable de la perte effective de surface foliaire, tandis que la combinaison de trois caractéristiques architecturales des branches d’un arbre (galles, pousses mortes, bourgeons dormants) dans un index composé (DCI) permet d’obtenir une évaluation beaucoup plus réaliste des dégâts subis par les branches pendant toute la durée de l’épidémie. Ce chapitre se conclut par une réflexion sur le sens et l’utilité des index disponibles (DCI, taux d’infestation, transparence de la couronne).

Simultanément (chap.3) nous nous sommes penchés sur la procédure habituelle de vérification de la présence du parasite par dissection de galles recueillies sur le terrain; ainsi nous avons optimisé le nombre de galles à récolter pour évaluer avec précision le taux de parasitisme de *T. sinensis* aux dépens de *D. Kuriphilus*. À l’aide d’une approche de substitution spatio-temporelle (space-for-time substitution approach) nous avons utilisé 15 sites pour créer un suivi temporel (*temporal*) qui nous a permis de reconstruire les phases principales du développement de la population de *T. sinensis*, à partir du moment de son arrivée ou du lâcher sur le site jusqu'à son établissement et son contrôle complet sur la population du cynips. À l'aide de simulations de *bootstrap*, nous avons analysé la précision que différents essais d'échantillonnage (par ex. quantité de galles à récolter) pouvaient conférer à l'évaluation du succès des lâchers de *T. sinensis* (détection de l'insecte dans les galles) et sur l'évolution de son taux de parasitisme pendant l'établissement et la croissance de sa population jusqu'à ce que la lutte biologique soit achevée. Les résultats ont démontré qu'après le premier lâcher, 200 galles suffisent pour évaluer en toute confiance sa présence, tandis que lorsque les populations de *T. sinensis* deviennent stables, 100 galles suffisent pour évaluer son taux de parasitisme.

L'évolution du système *Castanea sativa – Dryocosmus kuriphilus – Torymus sinensis*, de l'arrivée de l'insecte nuisible à la situation après quatre ans de lutte biologique (chap.4), a ensuite été analysée en utilisant les index décrits dans les chapitres précédents sur 15 sites choisis sur une grille de 10 x 10 km de manière à couvrir toute la surface du nord au sud de la Suisse méridionale. Les résultats montrent que la dynamique de la population de *T. sinensis* (chap. 4.1) affiche une croissance exponentielle avec différents taux en fonction de la distance du site italien de lâcher le plus proche et, partiellement, de l'abondance du cynips. De ce fait le temps requis pour atteindre le seuil de lutte biologique depuis l'arrivée de *T. sinensis* s'est révélé beaucoup plus court (seulement deux ans) pour les peuplements des régions du nord que pour ceux du sud (sept ans) qui ont donc subi de plus graves dommages. Une fois que la population de *T. sinensis* devient consistante, le contrôle exercé sur le cynips semble garantir un niveau d'infestation bas et stable avec de légères fluctuations. À la longue les deux populations baissent sensiblement en termes de nombres absolus à tel point qu'il devient presqu'impossible de trouver des galles. En ce qui concerne le châtaignier (chap. 4.2) les dégâts varient en fonction du temps écoulé entre l'arrivée du cynips et le contrôle biologique par *T. sinensis*. Plus précisément, les arbres qui se trouvent sur des sites où les attaques incontrôlées du cynips se sont répétées pendant sept ans présentent des portions importantes de couronne mourante et des réactions provoquées par le stress (par ex., rejets le long du tronc) alors que sur les sites où les deux insectes sont arrivés presque simultanément, les dégâts sont inférieurs et presque imperceptibles.

Enfin, les effets importants du contrôle tardif du cynips sur six sites suisses se sont vérifiés par la perte temporaire de produits typiques tel que le miel de châtaignier monofloral (chap.5). Nous avons en particulier déterminé la composante due au châtaignier dans la composition du miel à l'aide d'une approche combinée chimique et sensorielle, et nous avons mis en relation les résultats obtenus avec les index de dégâts. Les statistiques ont montré que la présence de la châtaigne dans la composition du miel diminue de manière significative à partir d'un niveau d'infestation de *D. kuriphilus* de 30%, tandis que la production du typique miel de châtaignier monofloral devient presqu'impossible dans le secteur observé par l'analyse lorsque les dégâts atteignent 40% et plus.

En synthèse cette thèse fournit de nouvelles connaissances sur les dégâts causés par le cynips au châtaignier en présentant les retombées écologiques (et culturelles) lorsque la lutte contre l'insecte advient avec un certain retard. Elle contribue également à éclaircir la méthodologie adoptée pour évaluer correctement l'impact global de l'épidémie causée par le cynips. Enfin et surtout, elle présente un cas de lutte biologique réussie dans un système agroforestier. Pour finir, quelques lignes de recherche future sont formulées dans le chapitre conclusif.

Mots clés : hyménoptère, ravageur parasite, lutte biologique, dégâts aux arbres, processus de reprise

Abstract

The arrival of new organisms that may potentially become harmful for people and ecosystems has become a world-wide daily concern to the point that in Switzerland about 800 established alien species exist, of which at least 107 are considered invasive. A possible way to control such pests is by importing and releasing natural enemies from their countries of origin, letting them to act as so-called biocontrol agents. The discovery in 2002 of the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu) in Europe and the subsequent importation of its natural enemy *Torymus sinensis* Kamijo is a prime example in this sense.

Even though a lot of studies have already been done by Japanese researchers, when I started my thesis, there was poor knowledge on the overall damages due to repeated *D. kuriphilus* attacks as well as on the reactions and recovery patterns of the host tree (*Castanea sativa* Miller) when the biocontrol is reached. Therefore the aim of this thesis is to present the evolution of the chestnut forests from the arrival of the pest to the biological control, highlighting the overall tree damages, its recovery patterns and the far-reaching effects that a delayed pest control can cause.

We first focused (chapter 2) on the methodological part by studying the best indices to analyze the epidemic evolution on the chestnut tree, especially in terms of damages and recovery pattern. Specifically, we used the leaf to sapwood area relationship based on the pipe model approach to quantitatively estimate the green biomass of a branch before the arrival and after the arrival of the pest, including the epidemic peak and the first and second year of recovery due to the progressive biological control by *T. sinensis*. Leaf area loss has then been used as a response variable in a mixed regression model analysis, to select the most suitable explanatory architectural features (e.g. gall types, bud types, shoot types) explaining the damage evolution at branch level. Results showed that during the epidemic peak, leaf area losses exceeded 70%, with significant alterations in branch architecture in terms of reduction of dormant buds (tree reserves) and increase of dead shoots. Moreover, the model analysis revealed that the traditional damage assessment based on the infestation rate (e.g. number of infested buds vs number of buds) usually reported in literature fails to provide a sound estimation of effective leaf area loss, whereas the combination of three branch architectural features (galls, dead shoots, dormant buds) in a damage composite index (DCI) enables a by far more realistic assessment of the branch damages during the entire epidemic process. We concluded discussing the meaning and the usefulness of the available indexes (DCI, infestation rates, Crown transparency).

Simultaneously (chapter 3), the gall collection approach has been tailored to optimize the time-consuming procedure to evaluate *D. kuriphilus* parasitism by *T. sinensis*, which typically consists of dissecting galls collected in the field and checking its presence on them. Using a space-for-time substitution approach we used 15 sites to create a temporal which allowed us to reconstruct the main phases of the *T. sinensis* population development, from its release or arrival in a site to its complete establishment and control of the pest population. By the means of bootstrap simulations, we analyzed the effect of different sampling efforts on the precision of evaluating the success of *T. sinensis* release and the evolution of its parasitism rate towards biocontrol. Results show that after the first release, 200 galls are required to confidently assess its presence whereas once *T. sinensis* is well established 100 galls are sufficient to estimate its parasitism rate.

The evolution of the *Castanea sativa* – *Dryocosmus kuriphilus* – *Torymus sinensis* system from the pest arrival to the situation of four-year biocontrol (chapter 4) has then been analyzed by using the indices described in the previous chapters in 15 sites selected on a grid of 10 x 10 km so as to cover the entire southern Switzerland area from north to south. Results show that the population dynamics of *T. sinensis* (chapter 4.1) displays an initial exponential growth at different rates as a function of the distance from the nearest Italian release site and partially of the *D. kuriphilus* abundance. Consequently, the time needed to reach the biocontrol threshold since the *T. sinensis* arrival was much shorter (only two years) for the northern populations than for the southern one (seven years), which consequently experienced more severe damages. Once the *T. sinensis* population becomes massive and stable, the control exerted on *D. kuriphilus* seems to guarantee a low and stable infestation level with small fluctuations. On the long run both populations become very low in terms of absolute numbers to the point that galls become almost impossible to find. From the chestnut point of view (chapter 4.2) damages vary as a function of the time elapsed between pest arrival and biological control by *T. sinensis*. Specifically, trees in sites experiencing seven years of *D. kuriphilus* uncontrolled attacks show larger dying crown portions and stress-induced reactions (e.g., suckers along the stem) compared to sites where the two insects arrived nearly simultaneously.

Finally, the far-reaching effects of the delayed pest control in six Swiss sites are highlighted in the temporary loss of typical products such as the unifloral chestnut honey (chapter 5). Specifically, we determined the chestnut component in honey via a combined chemical and sensory approach and related the obtained results with the damage indices. Decreases in the chestnut component of the honey were statistically significant starting from a *D. kuriphilus* infestation level of 30%, whereas the production of the typical unifloral chestnut honey became almost impossible in the study area when damages raised to 40% or higher.

Overall, this thesis provides some new knowledge on the damages caused by *D. kuriphilus* on the chestnut tree, highlighting possible ecological (and cultural) far-reaching effects caused by a delayed pest control. It also sheds some light on the methodology to properly evaluate the overall impact of the pest epidemic. Last but not least, it shows a successful biocontrol story in an agroforestry system. Finally, some future research lines are given in a concluding chapter.

Keywords: hymenoptera, invasive pest, classical biological control, tree damage, tree recovery process

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Chapter 1
General introduction

1.1 Exotic pests: a global problem

The arrival of new organisms that can become potentially harmful for people and ecosystems has become a world-wide daily concern (e.g. Kenis et al. 2009; Kenis and Branco 2010; Williams et al. 2010; Aukema et al. 2011) and is considered in the meantime as the most important threat to biodiversity and plant communities after habitat loss (e.g. Mooney and Hobbs 2000; Dirzo et al. 2014). Alien species are exported from their native regions to a new ecosystem outside of their natural potential dispersal through many different human activities; however, the international trade in wood plants is recognized as the main driver for their introduction including the associated arthropod pests and microbial diseases (Brasier 2008; Aukema et al. 2010; Roques 2010; Liebhold et al. 2012; Santini et al. 2013; Eschen et al. 2015; Migliorini et al. 2015). The increasing international and world-wide trade and globalization combined with climate change (Hellman et al. 2008) help overcoming geographic barriers and accelerate the establishment of alien organisms outside their native lands (Hulme et al. 2008; Hulme 2009; Walther et al. 2009; Roques 2010) causing not only biodiversity threat but also imposing severe costs on forestry, agriculture and human health (Williamson and Fitter 1996; Cock 2001; Pimentel et al. 2002, 2005; Vilà et al. 2010).

This is particularly true for Europe which doesn't have a strict regulation in this field and even promoted the free circulation of people and goods since 1992 (European Communities 1992). Probably because of this, the rate of alien insect species establishment has nearly doubled during the last few decades and has been estimated to be around 19.6 species per year in 2008 (Roques 2010). In Europe there are more than 12000 plant and animal alien species and at least 15% of them are known to cause extreme damage to ecosystem, severe costs to the community as well as societal implications (Vilà et al. 2010; Defra 2014; Mitchell et al. 2018). For example, *Fraxinus excelsior* which is threatened by *Hymenoscyphus fraxineus* in the UK, has an estimated commercial value of around £22 million a year (Defra 2014) and the loss of *Buxus spp.*, threatened by *Cydalima perspectalis* and *Calonectria pseudonaviculata*, could cause social, cultural and religious changes (Mitchell et al. 2018).

In order to slow down and prevent such introductions, various tools such as pest risk analysis and cross-border policy combined with phytosanitary border inspections have been implemented (Cardinale et al. 2012; Eschen et al. 2014; Essl et al. 2015; Latombe et al. 2017). However, it is not always clear which pests and pathogens should be targeted during the analysis because of the lack of accurate information on alien species distribution (Eschen et al. 2014; Essl et al. 2015; Latombe et al. 2017) and the difficulty to identify potential pest or to forecast species invasion behavior (Roques et al. 2015; Vettraino et al. 2017; Kenis et al. 2018).

For example, although exotic nematodes are often intercepted during phytosanitary border inspections, rare are the known cases in which they were established and created severe damages (Roques and Auger-Rozenberg 2006; Eschen et al. 2015) compared to arthropods and pathogens, the last having the highest establishment success in a new area. The main reason for the imbalanced number of interceptions is probably that pathogens are difficult to be visually detected if the plants are asymptomatic during the inspection (Eschen et al.

2015), which may lead to very high economic and environmental impacts. For example, *Fusarium circinatum* or *Phytophthora ramorum*, that are known to be serious pests, are still easily spreading throughout Europe (see EPPO Reporting Service https://gd.eppo.int/taxon/PHYTRA/reporting). Arthropods are also sometimes difficult to detect depending on their life cycle at the inspection moment. This has been the case for the Asian chestnut gall wasp (*Dryocosmus kuriphilus*,Yasumatsu) which has been imported from China to Italy probably with nursery material such as twigs or scions (Brussino et al. 2002; Quacchia et al. 2008b). In fact, the only method to detect the presence of the pest in this material would have been by dissecting the buds and looking for their eggs, seeing that the oviposition scars on the buds (that are difficult to see) tend to disappear (Bernardo et al. 2013) with time. Finally, although a quarantine period after phytosanitary inspection would have been useful to detect this hidden pest before releasing the material into the country, it appears difficult to implement such policy in Europe for many genera because of the huge volume of plant imports (Eschen et al. 2015).

Once a pest arrives in a new area, its invasion mainly follows 4 main phases: arrival/introduction, establishment, dispersal, and outbreak (e.g. Blackburn et al. 2011; Lockwood et al. 2013). Different management techniques exist according to the invasion phase going from initially early-warning and prevention technology (introduction), detection, monitoring eradication and spread blocking (establishment and lag phase), biological control and ecological manipulation (dispersal and outbreak; Wan and Yang 2016). Considering the last phase, biological control has been widely applied to control arthropod pests and is still considered nowadays a valuable and effective strategy (Greathead 1995; Bigler et al. 2006; Hajek et al. 2016). According to van Bigler et al. (2006) at least 165 pest species have been successfully controlled worldwide in just more than a century and many successful examples exist (e.g. Sweetman 1935; Shiga 1997; van Lenteren 2000; Hajek 2004; Cock 2015). However, the introduction of a new species in a non-native ecosystem may also lead to unexpected and undesirable consequences such as non-target host population (see Elkinton and Boettner 2012 for the most famous case and Hajek et al. 2016 and Van Driesche and Hoddle 2016 for a comprehensive list), intraguild competition and predation on closely-related species (Evans 2004; Roy et al. 2016), and hybridization with native species (Yara et al. 2010).

Because of these negative effects, many countries have changed their legislation on the release of biological control agent and have developed specific guidelines to limit the risk of non-target effects. Unfortunately, in some cases such legislations became so strict that they turned out to be a double-edged sword (Kenis et al. 2017). This is what recently happened in Switzerland with the case *D. kuriphilus* when trying to create a comprehensive environmental risk assessment (Aebi et al. 2011) before the release of the biological control agent *T. sinensis*. Because of that and the official prohibition to introduce *T. sinensis* (UFAM 2012), the chestnut forest of southern Switzerland experienced up to 7 years of *D. kuriphilus* undisturbed attacks, suffering from major damage.

In this thesis I will use this case to present the evolution of an agroforestry system from the arrival of the pest to the biological control, highlighting the overall damages and the far-reaching effect that a delayed pest control can cause.

1.2 The system Castanea sativa – *Dryocosmus kuriphilus – Torymus sinensis*

In the next three subchapters I'll present the main component of the studied system which are *C. sativa*, the tree acting as a host species, the Asian chestnut gall wasp *D. kuriphilus* that is its most significant insect pest, and *T. sinensis* which represents the specific enemy and main biological control agent.

1.2.1 Castanea sativa: biology, life cycle and history in Ticino

Common name: sweet chestnut
Scientific name: *Castanea sativa* (Miller)
Order: *Fagales*
Family: *Fagaceae*

Biology and life cycle

The Genus *Castanea* is a group of shrub and tree species native to the northern Emisphere belonging to the *Fagaceae* family. Dozens of species are known worldwide but only one species is present in Europe: *Castanea sativa* also called the (European) sweet chestnut.

The species can grow up to 35m and when cultivated, can live up to 1000 years and more.

In young trees the bark is grey and smooth, with age it develops to brown-grayish with deep furrows or fissures. Leaves are dark green with a brighter lower surface. Their shape is oblong-lanceolate with a dentate-crenate margin and a pointed tip. They vary between 8 to 25 cm in length and are between 5 and 9 cm in width.

This species is monoecious (both sexes are found on the same tree) but autopollination hardly occurs. In the Northern Hemisphere flowers develop from late June to July and are mainly pollinated by insects and wind (in dry conditions). Male flowers are gathered in long yellow catkins with female flowers usually positioned at the base of the catkins on the top of the shot of the year. If pollinated, female flowers develop into edible brown nuts grouped by 3-7 wrapped in spiny green cupules that are shed in autumn (Rudow and Conedera 2001; Conedera et al. 2016).

The sweet chestnut history in southern Switzerland

The sweet chestnut cultivation has a long tradition in many areas of Europe. This is prevalently due to its suitability for timber production as well as a basic source of food for local populations (Pitte 1987; Conedera et al. 2000).

The introduction of the species in Ticino (southern Swiss Alps) coincides with the Roman conquest of this region (Conedera et al. 2004). Initially it has been mainly used for wood production in agricultural sectors, military buildings and civilians. Despite the likely pre-eminence of wood production, the species soon demonstrated its double utility with the increase of fruit-based cultivation especially from the High Middle Age (Krebs et al. 2014).

The definitive affirmation and the extension of the chestnut cultivation started only after the year 1000 in concomitance with a better climate and general demographic expansion (Conedera and Krebs 2008). Chestnut has been then intensively cultivated until the mid-eighteenth and the beginning of the nineteenth century when socio-economic developments, such as the introduction of alternative food (e.g. potatoes, corn), the improvement of communication routes (e.g. railway line of the Saint Gotthard 1882) and the related abandonment of the rural areas led to a decrease in the importance of the traditional chestnut products and to a progressive decline in its cultivation (Arnaud et al. 1997; Conedera and Krebs 2008). Finally, the general infection by the chestnut blight (*Cryphonectria parasitica*) started in 1948 (Conedera 1993) definitively ends the chestnut cultivation activity.

Since then natural evolutionary processes began to get back, forcing *C. sativa* to face the competition with other species. The lack of anthropogenic management combined with the scanty potential competition of the species (Conedera et al. 2000; Bacilieri et al. 2006), brought the chestnut belt to be inexorably invaded by other local species and to evolve towards mixed forests (Conedera et al. 2001a). Chestnut trees are however still present nowadays mostly as naturalized stands interrupted by the presence of other broad-leaved species, such as *Tilia cordata*, *Quercus petraea*, *Quercus pubescens*, *Alnus glutinosa*, *Prunus avium*, *Acer* spp., or *Fraxinus* spp. Finally, these chestnut forests shifted their function from timber and food production to protection against natural hazards such as rockfall and shallow landslide.

Consequences of the lack in landscape management

The abandonment of the chestnut cultivation and the lack in landscape management lead to different consequences. In addition to the gradually potential disappearance of the species, *C. sativa* tends to uproot in overaged chestnut coppices (Vogt et al. 2006; Pividori et al. 2008; Conedera et al. 2009). This can create potential risks especially in some specific topographic conditions (e.g. watershed, steep slopes, near waterbed) as obstruction of watercourses bringing to dam-break phenomena and shallow landslide problems causing potential damages (Vogt et al. 2006; Conedera et al. 2009). More generally, the formations of clearings in the forest due to this phenomenon may increase the natural regeneration of the stand on one hand, but also may lead to a decreased hydrological efficiency of the stand and favour the establishment of invasive pioneer such as *Ailanthus altissima* and other neophytes (Wunder et al. 2016).

In the 1990s people recognised the risk of losing the chestnut culture and the related historical, and ecological elements (Conedera et al. 1997). As a consequence, different initiatives started for the restoration of chestnut orchards (Rudow and Borter 2006), the protection of the genetic material linked to the chestnut varieties (Conedera 1994) and the management of coppice stands for quality wood production (Giudici 1995).

These initiatives have been accompanied and linked to various research activities (e.g. Conedera and Giudici 1994) to understand the dynamic of chestnut stands (Conedera et al. 2001b; Muster et al. 2007; Morales-Molino et al. 2015) as well as their biodiversity (e.g. Python et al. 2013), the biology of the species (e.g. ecological plasticity; Gehring et al. 2015, 2016), its competition ability with neophytes (Knüsel et al. 2015, 2017) its reaction to extreme climate events (Conedera et al. 2010; Gehring et al. 2016) and pathogens attacks (Prospero and Forster 2011; Ugolini et al. 2014; Meyer et al. 2015) in order to better plan forestry management and environment safeguard for the future.

Present distribution in southern Switzerland

The chestnut forests of canton Ticino cover ca. 20’000 ha representing ca. 15% of the whole forest area of the canton (Fig. 1). The climate (annual mean precipitation from 1800 to 2100 mm, annual mean temperature ranging from 10.2 to 12.4 °C – MeteoSwiss climate normals for the period 1981-2010; http://www.meteoswiss.admin.ch), the geological substratum (generally classified as haplic podzol on crystalline bedrock) and the morphology contribute to make the forest composition very varied. Chestnut tree represents the most widespread species (20%) followed by *Fagus sylvatica* (18%), *Picea abies* (17%), *Larix decidua* (13%), *Abies alba* (3%), *Quercus* spp. (3%), *Fraxinus* spp. (2%), and other species (Ceschi 2014).

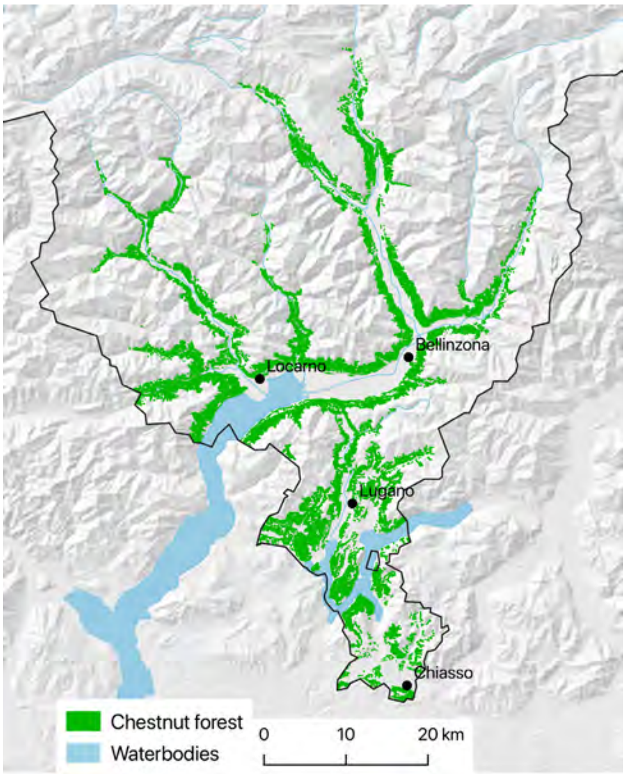

Figure 1 Distribution of the chestnut tree in southern Switzerland.

The chestnut tree is nowadays mainly found at elevation ranging from 200 m a.s.l. (lowest point of the area at Lago Maggiore) up to 900-1100 m a.s.l. that is considered the ecological limits of the species (Krebs et al 2012). As a result, the species forms a chestnut forest belt continuum from south to north (Brändli 1998; Ceschi 2014) with a mix of cultivated and abandoned stand.

1.2.2 *Dryocosmus* kuriphilus: biology and life cycle

Common name: Asian Chestnut Gall Wasp
Scientific name: *Dryocosmus kuriphilus* (Yasumatsu)
Order: *Hymenoptera*
Family: *Cynipidae*

D. kuriphilus is a gall wasp native to China (Murakami 1980) belonging to the *Cynipidae* family. It is the gall wasp species considered as the most significant widespread insect pest for the genus *Castanea* (Aebi et al. 2006; Abe et al. 2007). On the European sweet chestnut tree, it may cause severe damages and severe yield and economic losses to fruit production (e.g. Battisti et al. 2014a).

Biology and life cycle

The adult Asian Chestnut Gall Wasp (*D. kuriphilus*) is 2.5–3 mm long and has a black body with orange/yellow-brown legs, scapus and pedicels of antennae, and mandibles (Fig. 2 A). An inexperienced eye could confuse the adult wasp with the European oak cynipid wasp (*Dryocosmus cerriphilus*) that induces galls on *Quercus cerris* or the recently discovered *Dryocosmus zhuili Liu et Zhu* inducing galls similar to *D. kuriphilus* on *Castanea henryi* in China (Zhu et al. 2015).

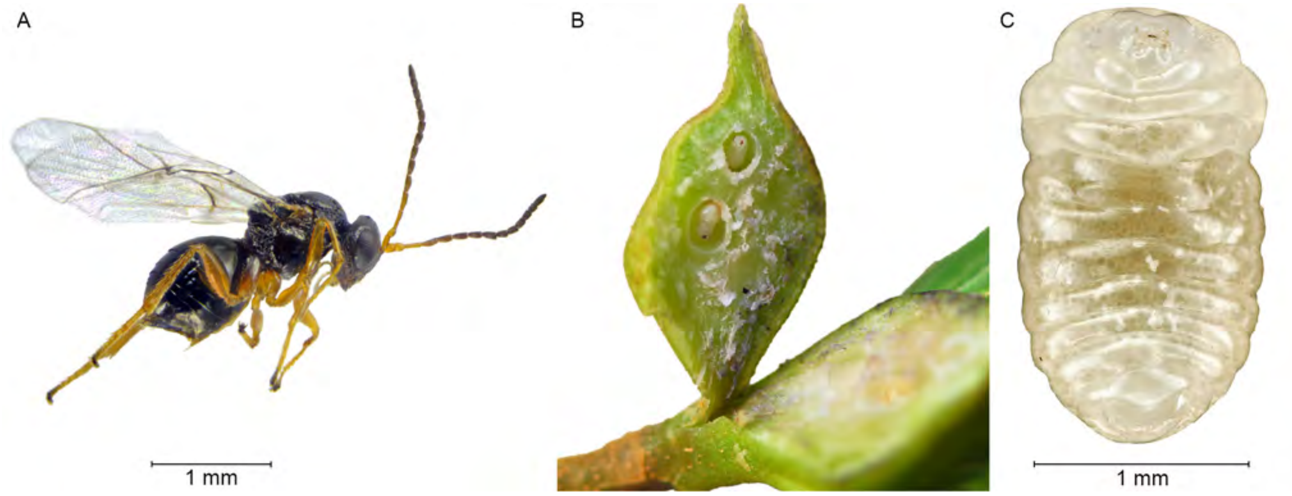

Figure 2 Three different life stages of *Dryocosmus kuriphilus*. A) Female adult, B) two pupae in a gall section, C) Terminal-instar larvae.

D. kuriphilus pupa is on average 2.5 mm long and its colour varies from white to black depending on its life cycle period (Fig. 2B). The terminal-instar is white and 2.5 mm long without legs and eyes (Fig. 2C). Finally, eggs are white, oval and on average 0.1-0.2 mm long with a stalk (CABI 2018; Dixon et al. 1986; Viggiani and Nugnes 2010).

D. kuriphilus attacks all species of Genus *Castanea* except for *C. pumila* and *C. alnifolia (CABI 2018)*. Other Euro-Japanese hybrid-resistant cultivars (*C.* sativa x *C.* crenata) have been identified such as “Bouche de Bétizac”, “Marlhac”, “Maridonne” and “Vignols” where *D. kuriphilus* infects the buds but no galls develop in spring (Dini et al. 2012; Sartor et al. 2015). Recently, two *C. sativa* cultivars resistant to *D. kuriphilus* (no gall development in spring) where found: “Pugnenga”, native from Cuneo Province and “Savoye”, from the Midi-Pyrénées Region (Sartor et al. 2015).

Given that no male has been observed to date, *D. kuriphilus* is considered as a univoltine thelytokous species, meaning that it produces fertile eggs by parthenogenesis (CABI 2018). Its life cycle is closely related to temperature and chestnut phenology which in turn are highly correlated with other environmental variables such as altitude and aspect (Otake 1980; Bosio et al. 2010). The average egg load per female reported in Europe by

Graziosi et al. (2014) is 190 and the total number of laid eggs that can be found in a chestnut bud varies greatly from 1 to 176 (Panzavolta et al. 2012). 30-40 days after oviposition, the first-instar larvae hatch from eggs but do not grow until the following spring when buds begin developing (Bernardo et al. 2013). It is at this time that larvae induce the formation of a variable number of galls differing in size, position (on a leaf, along the main shoot axis, on a stipule, or on a flower), and number of hosted larvae (Fig. 3). After 20-30 days of feeding, the larvae inside the gall pupate (~mid-may / mid-june) and complete their development by July, when they emerge. The adult life outside the gall is short and is recorded to be on average between four days to seven days without feeding (Bosio et al. 2010).

Figure 3 *Dryocosmus kuriphilus* galls on a chestnut branch. The main gall types are present on the branch: galls developed on leaves, along the main shoot axis and on stipule.

Symptoms

D. kuriphilus gall size is variable (5-30mm in diameter, 0.1-2.7 cm^3; Bloch 2013) and depends on many factors such as the habitat (Kato and Hijii 1993), the population density and the tree type (e.g. cultivated or wild; Cooper and Rieske 2010; Panzavolta et al. 2013). They can be unilocular or multilocular and can develop on shoots, leaves, petioles and flower (for a thorough description see Maltoni et al. 2012). Their colour varies from green to red and after adult emergence, they dry out and become wood-like (Fig. 3).

In newly invaded regions of the world where no specific natural enemies are present, *D. kuriphilus* outbreaks over multiple years result in reductions in tree vigour and wood production (Kato and Hijii 1997; Ugolini et al. 2014) and nut yield reductions of up to 80% (Battisti et al. 2014; Sartor et al. 2015). Moreover, when *D. kuriphilus* attacks are uncontrolled for many years, chestnut trees can be more easily affected by different abiotic (drought and hail) and biotic events such as the chestnut blight (*Cryphonectria parasitica*), the penetration of which into the branches is facilitated by the presence of the galls and the flickering holes (Prospero and Forster 2011). Even, when severe damages occur, heavily wakened trees can also die (Miyashita et al. 1965; Dixon et al. 1986).

Dispersal range

The dispersal range by active flight of *D. kuriphilus* is difficult to measure and can vary according to wind speed, host plant distribution, and landscape characteristics (Graziosi and Rieske 2012). Because adult aren't good fliers, wind seems to be the main trigger for dispersal. Low wind speed stimulates the dispersal initiation whereas when blowing above 2.15 m/s the flight is inhibited and the passive transport is enhanced (Oho and Shimura 1970). The rate of dispersal by active and wind-assisted flight has been estimated by various authors and varies from 3 km/year (Graziosi and Rieske 2012) to ca. 25 km/year (Payne 1981; Rieske 2007; Graziosi and Santi 2008). By the mean of a stratified dispersal model that consider different variables such as local population growth, dispersal type (active and passive) and biology, Gilioli et al. (2013) calculated a mean annual dispersal range of ca. 6.6 km with a peak of 11 km. This is more or less in line with the invasion speed that occurred in Ticino where *D. kuriphilus* progressed more or less 90 km in 7 years.

D. kuriphilus can also spread by passive long-distance dispersal mainly due to trade of infested material (trees, twigs or scions) and unintentional human-mediated transport (Aebi et al. 2006; Bernardo et al. 2013). This dispersal mode is considered to be the most important factor determining the worldwide spread of the pest.

Control strategy

Phytosanitary border controls can reduce the long distance spread of *D. kuriphilus* but are ineffective to reduce its natural dispersal across a region once it is established. The use of chemical pesticides has been tested but their efficiency resulted low and their application difficult because the pest is protected within the plant tissues during the majority of its life cycle (Moriya et al. 1989; Cooper and Rieske 2007; EFSA Panel on Plant Health (PLH) 2010). Moreover, depending on the country, the use of chemicals is not allowed in the forests; nevertheless, it would be unthinkable and ecologically harmful to treat an entire forest.

The research of resistant varieties and hybrids produce encouraging results (e.g. Dini et al. 2012; Sartor et al. 2015), but it would be useless for existing chestnut populations.

Numerous indigenous parasitoids attacking *D. kuriphilus* have been found in various countries but none of them were specialized enough to keep the *D. kuriphilus* population below the damage threshold (e.g. Moriya et al. 1989; Murakami and Gyoutoku 1995; Aebi et al. 2006; Cooper and Rieske 2007; Quacchia et al. 2013).

To date, the only known way to control the *D. kuriphilus* population is using the specific Chinese antagonist, the paraitoid: *T. sinensis* (Moriya et al. 2003; Matošević et al. 2017a; Ferracini et al. 2018b).

1.2.3. *Torymus sinensis*: biology and life cycle

Common name: not known
Scientific name: *Torymus sinensis* (Kamijo)
Order: *Hymenoptera*
Family: *Torymidae*

Torymus sinensis Kamijo is native to China and is to date the only natural enemy phenologically well-synchronized with its host *D. kuriphilus* and thus able to efficiently control the *D. kuriphilus* population (Moriya et al. 2003; Gibbs et al. 2011).

Biology and ecology

The adult is 2.5 mm long and has a shiny metallic green coloring with yellow legs (Fig. 4). Sexes are easily distinguishable by the long ovipositor that projects over the female abdomen. Besides the body color, the main morphological details discriminating it from other species of parasitoids are the tarsi (five tarsometers), the large coxae, and the carina on the scutellum (Antipolis 2012).

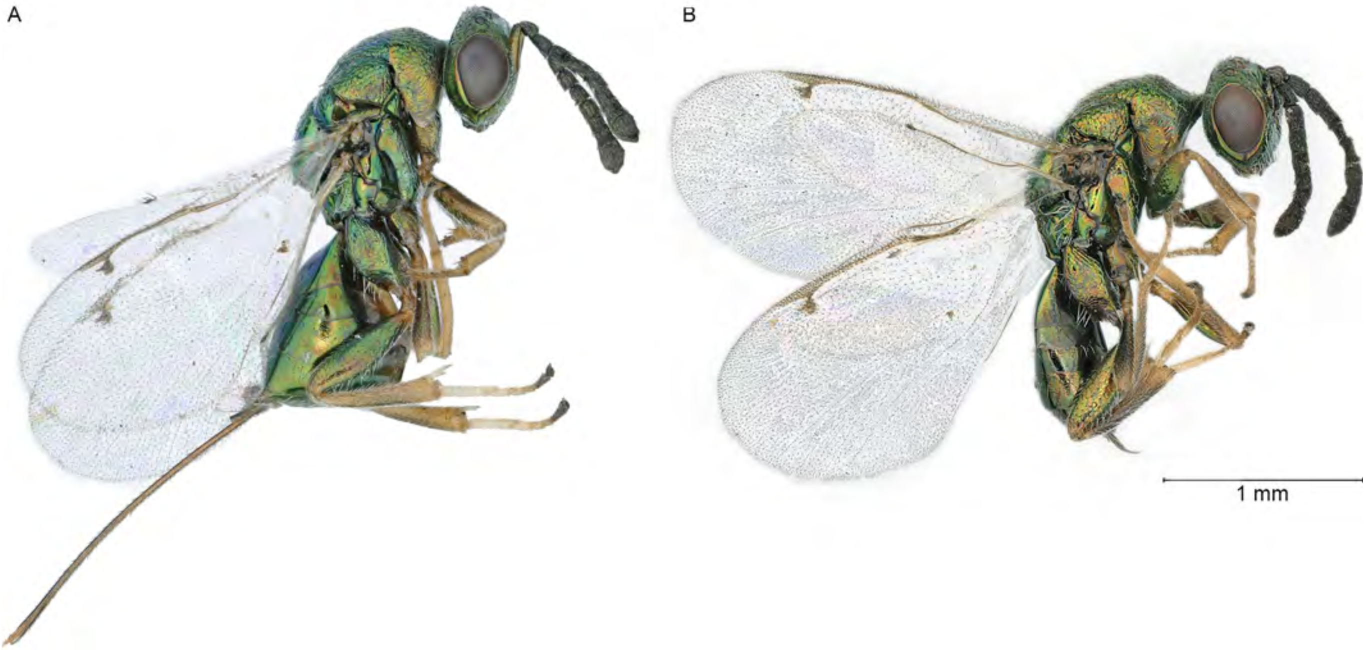

Figure 4 *Torymus sinensis* adult individuals. A) female, B) male.

T. sinensis pupa is on average 2.5 mm long and its color varies from completely white to white with red eye, to shiny metallic green, depending on its life cycle stage (Fig. 5A). The terminal-instar larva is 2.5 mm long, white with brown red stripes, and has very long setae at each segment (Fig. 5B). The strong mandibles have long teeth (Doğanlar and Savaş 2018). Finally, the eggs are on average 0.1-0.2 mm long, white oval-shaped with a small oval nose (Fig. 5C).

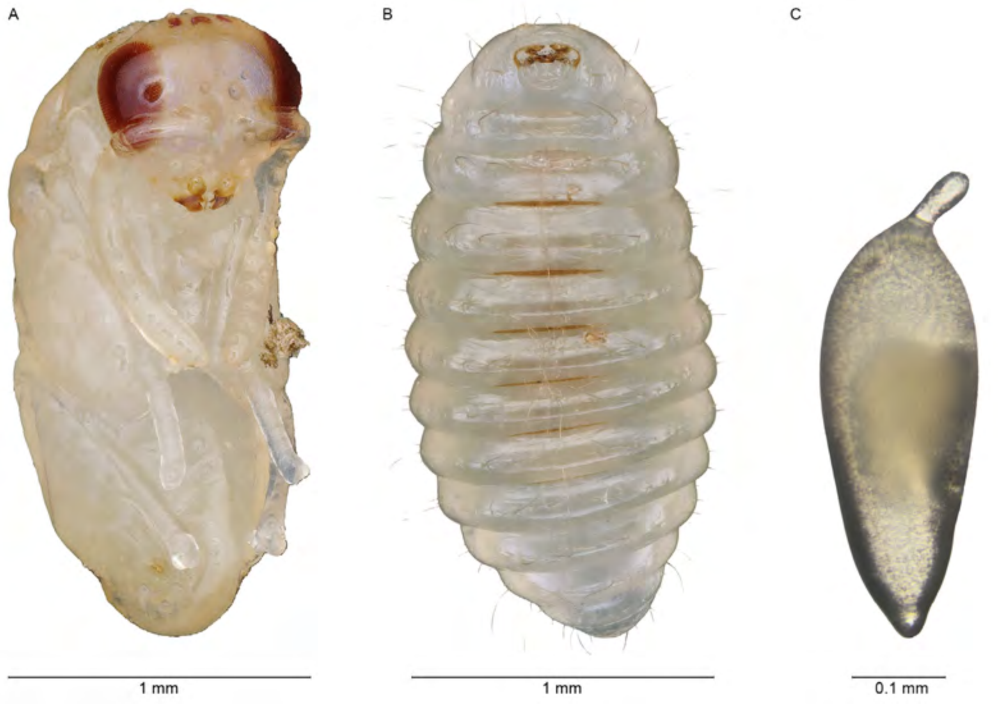

Figure 5 Three different life stages of *Torymus sinensis.* A) White pupa with red eye, B) terminal-instar larva with its characteristics brown-red stripes, C) egg.

T. sinensis is mainly univoltine like its host (Moriya et al. 1990; Quacchia et al. 2014a) but a very small part of the population (around 3%) can emerge the year later after a prolonged diapause (Quacchia et al. 2014a; Ferracini et al. 2015b). Contrary to *D. kuriphilus*, *T. sinensis* adults emerge in spring from the previous year's withered galls and need to mate before (amphigonically reproduction) laying a variable number of fertile eggs into the newly formed galls (Quacchia et al. 2008b). Their female's egg load is around 215 eggs (considering mature and immature oocytes; Picciau et al. 2017). Usually only one *T. sinensis* egg per *D. kuriphilus* larval chamber is laid. A multiple egg oviposition in the same chamber has already been observed in nature, but only one individual completes its life cycle because of cannibalism among young larvae (personal observation; Piao and Moriya 1999). Soon after its oviposition, the egg hatches and the young larva feeds ectoparasitically on the *D. kuriphilus* larvae. Males hatch from unfertilized eggs (haploid) and females from fertilized ones (haplodiploidy). By late spring and the beginning of summer, the larva reaches its adult stage with its characteristic brown stripes on the abdomen. It then pupates in autumn and completes its life cycle by the following spring (Quacchia et al. 2008b).

Average adult survival has been reported to be between 1 and 2 months under field temperature (Piao and Moriya 1992) and depending the resource availability until a maximum of 102 days and 73 for diapausing individuals (Picciau et al. 2017). In Italy *T. sinensis* is well established in regions characterized by mild winters and warm summers (Cascone et al. 2018).

T. sinensis mainly finds its host galls using olfactory and visual cues (Graziosi and Rieske 2013) and it has recently been reported to have a very good dispersal ability depending on resource concentration and wind, although it is difficult to estimate the yearly range distance. Colombari and Battisti (2016) found that by active flight and under no wind condition the minimum known distance is 650 m/year and that in optimal condition (e.g. with wind and a continuum forest with widespread gall presence) the average yearly population spread could be of at least 21 (± 9) km. A similar Japanese experience, although not exactly the same, highlighted an initial and slow spread (< 1 km/year), followed by a more rapid and accelerated spread until a rate of ca. 60 km per year (Moriya et al. 2003). Based on our personal experience and making a fairly conservative estimate, *T. sinensis* spread in southern Switzerland could have been of at least 18 km/year, which is in line with the result found by Colombari and Battisti (2016).

Host range and Hybridization

Since the first introduction in Japan and Europe, *T. sinensis* host range has been studied and has been confirmed to be potentially broader than usually reported in the literature, although up to now it is impossible to evaluate the magnitude of the impact.

No oviposition behaviour has been observed during laboratory experiment on non-target galls, chosen on the basis of the EFSA Panel on Plant Health list for testing the host-specificity of *T. sinensis* under controlled condition (Quacchia et al. 2014a), although very rare cases of *T. sinensis* oviposition on *Andricus curvator* galls have been observed (Ferracini et al. 2015a), without confirming the death of its host.

Considering rearing target gall experiments, three *T. sinensis* female emerge from 856 *Biorhiza pallida* galls collected in the field (Ferracini et al. 2015a) and some recent finding highlights that *T. sinensis* represent 1.3% of the parasitoid population emerged from 14’512 non-target host galls of 15 different species (Ferracini et al. 2017). Although some occasional host range shift has been observed, the impact appears minimal and at the moment does not seem to have any significant impact on the non-target-host population.

The potential hybridization of *T. sinensis* with native species has been tested in laboratory. Japanese researchers successfully crossed *T.sinensis* and *Torymus beneficus* individuals producing fertile hybrid females (Moriya et al. 1992). In contrast, Quacchia et al. (2014) and Ferracini et al. (2015a) didn’t observe mating behaviours between *T. sinensis* and other native species. Nevertheless, hybrids between *T. sinensis* and *T. beneficus* have been reported from Japan with a frequency varying from 8% (Toda et al. 2000) to 22% considering only late-spring strain of *T. beneficus* (Yara et al. 2010).

1.3 *Dryocosmus kuriphilus and Torymus sinensis worldwide* spread history

D. kuriphilus was initially observed in China in 1929 and was known as an unnamed *Biorhiza sp*. Only 22 years later Yasumatsu described the wasp and gave the current scientific name *Dryocosmus kuriphilus* (Yasumatsu 1951; Murakami 1980). Japan has been the first nation where it has been accidentally introduced in the early 1940s, probably when importing from China chestnut cultivars (Oho and Umeya 1975; Moriya et al. 1990). In nearly 20 years the pest spread in the entire Japanese chestnut growing area (Shimura 1972) causing a severe impact to chestnut fruit production (Shirakami 1951; Oho and Shimura 1970). However, this dramatic experience, did not prevent *D. kuriphilus* from spreading worldwide by the end of 2018 where its host plant is present. In fact, *D. kuriphilus* was recorded in Korea in 1958 (Tamura 1962; Cho and Lee 1963) and 16 years later in the USA (Payne et al. 1975). In 1999 *D. kuriphilus* galls where also found on the cultivated Japanese and Chinese chestnut tree in Nepal (Ueno 2006) and in 2012 it entered Canada (Huber and Read 2012). In Europe, it was officially recorded for the first time in 2002 from Piedmont region in Italy (Brussino et al. 2002) although, based on customs' statistics, Quacchia et al. (2008) estimated the first arrival to be 2-3 years before. Moreover, because the galls were already present over an area of 160 square km at that time, Aebi et al. (2006) hypothesize that the arrival time could even date back to 1995—1996, in concomitance with the introduction of eight Chinese chestnut cultivars in this region. Since its discovery in 2002, the pest quickly spread out in every direction reaching southern Italy in 2005 (Paparatti and Speranza 2005) and the Lake Orta region in 2006; then it entered southern Switzerland presumably in 2007 (Forster et al. 2009) from where it moved northwest at a rate of spread up to 25 km/year (Meier et al. 2013), colonizing the whole chestnut area by 2014 (Fig. 6A). In the meantime, the pest has been established in Slovenia (2004; Knapič et al. 2010), France (2005; Aebi et al. 2006) and Netherlands (2008; Eppo 2010) where a promptly eradication program (NPPO 2013) freed the nation from it until its new arrival in 2013 (EPPO 2015). Nowadays, *D. kuriphilus* is widely distributed in Europe and is established in many countries including Hungary, (first found and eradicated in 2009 but present again in some areas since 2013; EPPO 2013) Croatia (2010; Matosevic et al. 2010), Slovakia (2011, Pástor et al. 2017), Czech Republic (2011; EPPO 2012), Germany (2012; EPPO) (2012; EPPO 2012b), Spain (2012; Pujade-Villar and Torrell 2013), Austria (2013; EPPO 2013c), Romania (2013; Radócz et al. 2016), Portugal (2014; EPPO 2014), Turkey (2014; Çetin et al. 2014), Greece (2014; Michaelakis et al. 2016), Bosnia and Herzegovina (2015; Delalic 2016), United Kingdom (2015; Malumphy 2015), Belgium (2015; EPPO 2016) and North Caucasus of Russia (Gninenko and Lyanguzov 2017).

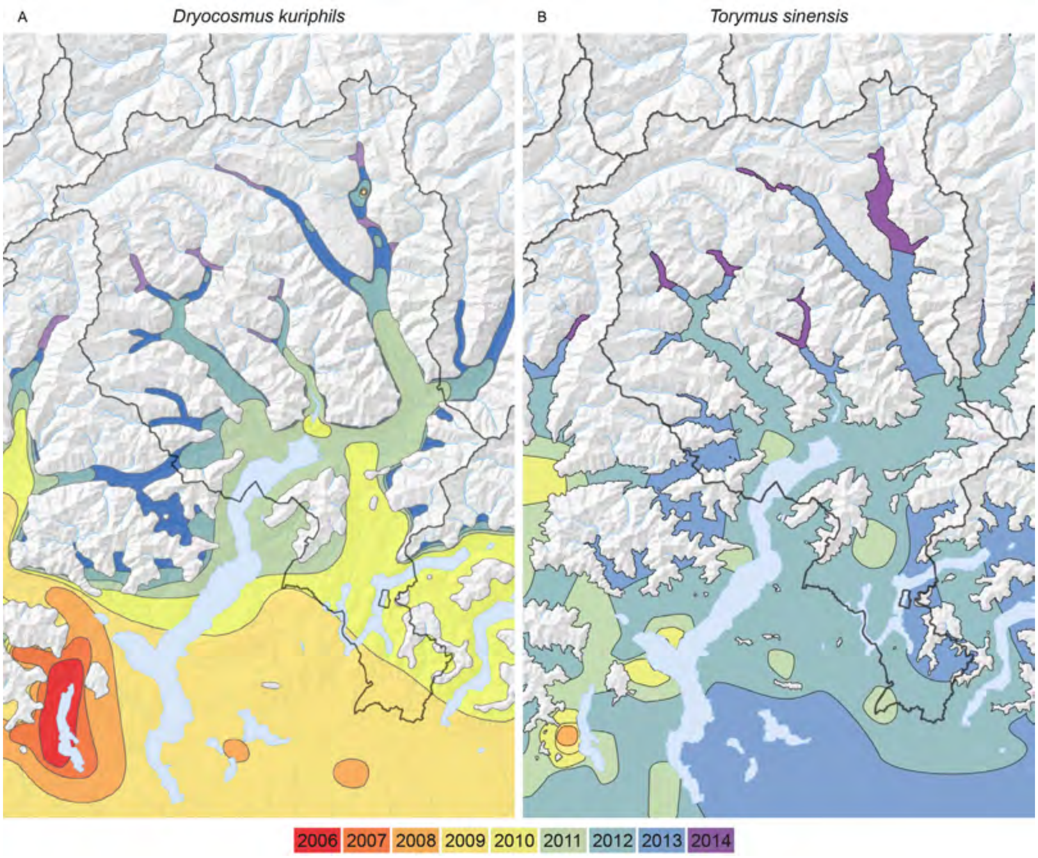

Figure 6 *Dryocosmus kuriphilus* and *Torymus sinensis* arrival in southern Switzerland. Sources: Phytosanitary services of Piedmont and Lombardy region, Forest service of Canton Ticino, WSL.

The introduction of *D. kuriphilus* in Europe is thought to be the result of a single introduction of a Chinese founder population (Martinez-Sañudo et al. 2018) and its successful invasion is probably the result of different factors such as the increasing international and world-wide trade in wood plants combined with the exchange of infested plants at national and local level (e.g. Hulme 2009), the difficulty to detect the insect due to its minute size and cryptic life cycle when it is inside the buds (Bernardo et al. 2013), its fast and effective parthenogenetic reproductive strategy, and the continuity of chestnut forest in certain areas of Europe (Avtzis and Matošević 2013).

Despite the fact that the arrival of *D. kuriphilus* is very well documented, the release of its natural parasitoid *T. sinensis* is sparser. Chinese *T. sinensis* was first recorded as *Torymus* (*Syntomaspis*) sp. by Murakami et al. (1977) and only five years later Kamijo described it as a new species (Kamijo 1982).

Since neither natural enemy native to the area of introduction, nor resistant chestnut varieties were able to keep *D. kuriphilus* population at low level in Japan, different studies have been conducted to find a specific parasitoid native to China to act as a biological control (Murakami et al. 1977). Because *T. sinensis* seemed the most promising specific and well-synchronized parasitoid, many introductions started from 1976 (e.g. Murakami and Kiyota 1983; Otake et al. 1984) giving the first successful results in 1988 when the level of infested shoots decreased far below the tolerable level of 30% (Moriya et al. 1989).

Exception for Korea where *T. sinensis* was already present and has never been imported from China nor Japan (Murakami et al. 1995), several other countries followed the Japanese experience using this parasitoid as a biological control agent such as USA (Rieske 2007), Italy (Quacchia et al. 2008b), France (Borowiec et al. 2014), Croatia (Matošević et al. 2015), Hungary (Matošević et al. 2015), Turkey (Doğanlar and Savaş 2018), Slovenia (Matošević et al. 2015), Spain (Pérez-Otero et al. 2017), Portugal (Personal communication with Ambra Quacchia; Table 1) and Slovakia (Personal communication with Ambra Quacchia). Unlike all these countries, the release of *T. sinensis* in Switzerland was never granted by the federal authorities because of open biosafety questions (Aebi et al. 2011; UFAM 2012). A matter of particular concern was the risk of hybridization between *T. sinensis* and native *Torymus* species. Nevertheless, the parasitoid progressively spread from Italy and, also benefiting from numerous releases near the swiss boarder (chapter 4.1), colonized the entire chestnut area by 2014 although sporadic illegal introduction cannot be excluded (Fig. 6B; chapter 4.1.). Similarly, no official releases have been so far carried out in Bosnia and Herzegovina, but the parasitoid naturally spread from Croatia (Matošević et al. 2017b). Finally, in Greece (Michaelakis et al. 2016) and England (Everatt 2015) its introduction is still under consideration whereas no information was found until now for Nepal, Netherlands, Canada and Czech Republic.

Table 1 First release or finding and first biocontrol year of *Torymus sinensis*

Country	1st release or finding year	1st biocontrol year	Note	Reference
Japan	1975	1988		(Murakami et al. 1977; Moriya et al. 1989)
USA	1977	Not known		(Rieske 2007)
Italy	2005	2012		(Quacchia et al. 2008a, 2014b)
France	2011	2014		(Borowiec et al. 2014, 2018)
Switzerland	2011	2014	*T. sinensis* never imported but spread from Italy	Chapter 4.1.
Croatia	2014	2015		(Matošević et al. 2015, 2017a)
Hungary	2014	2015		(Matošević et al. 2015, 2017a)
Turkey	2014	Not yet		(Doğanlar and Savaş 2018)
Slovenia	2015	2015		(Matošević et al. 2015, 2017a)
Spain	2015	Not yet		Pérez-Otero et al 2017
Portugal	2015	Not yet		Personal communication with Ambra Quacchia
Austria	2016	Not yet		(Ländliches Fortbildungs Institut LFI 2017)
Bosnia-Hercegovina	2016	2017	*T. sinensis* never imported but spread from Croatia	(Matošević et al. 2017b)
Slovakia	2017	Not yet		Personal communication with Ambra Quacchia
Korea			T. sinensis never imported but already present	(Murakami et al. 1995)
Greece			Releases still under consideration	(Michaelakis et al. 2016)
England			Releases still under consideration	(Everatt 2015)
Nepal	NA	NA	No information found	
Netherlands	NA	NA	No information found	
Canada	NA	NA	No information found	
Czech Republic	NA	NA	No information found	
Germany	NA	NA	No information found	
North Caucasus of Russia	NA	NA	No information found	
Romania	NA	NA	No information found	
Belgium	NA	NA	No information found	

1.4. Current research overview and rationale of the thesis

Research history of Dryocosmus kuriphilus in China before its introduction to Japan

To our knowledge, before 1941 research data on *Dryocosmsus kuriphilus* in the presumed native range of China are rare and difficult to access, although damages caused by *D. kuriphilus* on chestnut trees were already reported in 1929 by Gao Zhonglin (Tarcali and Radocz 2009). Furthermore, it is nowadays well known that the pest is widely distributed in many Chinese provinces often causing important yield loss and also tree death (Tarcali and Radocz 2009; Zhi-Yong 2009).

Research history in Japan

Research activity probably actually started when the *D. kuriphilus* has been introduced to Japan and created serious problems to chestnut cultivation that were initially free from gall-inducing insect pests. First published research refers thus to the situation when the damages on the Japanese chestnut *Castanea crenata* were already severe (Oho and Shimura 1970; Otake 1980).

Research started from scratch and focused on the tolerance of *C. creanata* cultivars to the gallwasp attacks (Shiraga 1948). Fukuda and Okudai (1951) described then the resistance mechanism, which led to the beginning of various breeding programs that allowed creating many *D. kuriphilus* resistant varieties, temporarily solving the problem (Kajiura and Machida 1961; Murakami 2010). With time, also the most tolerant *C. creanata* cultivars got attacked by new ecotypes of *D. kuriphilus*, so that the cultivar selection program became ineffective, causing a renewed increase of the *D. kuriphilus* epidemic impact in the 1970s (Shimura 1972; Moriya et al. 2003). In the meantime, the investigations on the effectiveness of the biological control by native parasitoids turned out not to be economically effective, although allowing to partially reduce the *D. kuriphilus* population (e.g. Yasumatsu and Kamijo 1979; Otake et al. 1982).

These problems induced researchers to evaluate a different strategy. The approach changed as soon as the long-term presence of the *D. kuriphilus* in China has been certified as well as the presence of effective natural enemies keeping this pest population at low densities (Murakami 1980).

Although the research on indigenous parasitoids native to Japan never stopped (e.g. Toda et al. 2000), most studies focused since then on the Chinese natural enemies, and on a promising torymid parasitoid in particular (Moriya et al. 2003), later described as *Torymus sinensis* Kamijo. The Japanese *D. kuriphilus* biological control program using *T. sinensis* started in 1975 and consisted of an implementation phase of importing, breeding, rearing, and releasing the parasitoid (Murakami et al. 1977; Otake et al. 1984; Moriya et al. 2003). Then an efficiency control phase begun by monitoring its natural reproduction, population growth, dispersal range and finally its effectiveness in controlling the *D. kuriphilus* population (Moriya et al. 2003) as well as the population dynamics between *D. kuriphilus*, *T. sinensis* and other parasitoids (e.g. Ito 1967; Nakamura et al. 1977; Piao and Moriya 1999). Further accompanying studies concerned the crossing behaviour of Torymids under experimental and natural conditions (Toda et al. 2000; Moriya et al. 2003; Yara et al. 2007), the insect biology (Abe 1994; Izawa et al. 1995; Kato and Hijii 2001) and ecology (Otake 1982) as well as on methods to quantify the *D. kuriphilus* population (Kotobuki et al. 1985; Inoue et al. 1992) and their impact on chestnut trees (Kato and Hijii 1997; Kazutaka 2000).

Although USA were also concerned by the problem since 1974, very little was published, so that to my knowledge, the first published report on the success of *T. sinensis* biological control dates back only to 2007 (Rieske 2007).

Research history in Europe

When the *D. kuriphilus* has been discovered in Europe in 2002 (Brussino et al. 2002), most of the available literature was in Japanese and referring to *Castanea crenata*. Moreover, being on a different continent with another climate, also the general environmental conditions in terms of potential indigenous parasitoid and related host trees changed, claiming for the need to clarify many of the already known aspects of the Japanese experience. As a consequence, a new wave of intense research started.

Due to the importance of the chestnut culture and fruit production in many European countries and in Italy in particular, one of the first priority consisted in replaying the successful biological control approach applied in Japan. The University of Turin soon started the necessary measures to import the parasitoid *T. sinensis* and to optimize the procedures for rearing (feeding and optimal temperature) and releasing (time of the year) *T. sinensis* and for monitoring its establishment success and possible indirect effects on non-target species (Quacchia et al. 2008a). First tests on non-target species were later evaluated as inadequate (EFSA Panel on Plant Health (PLH) 2010; Gibbs et al. 2011) and thus repeated in the following years with other species and modalities (Quacchia et al. 2013; Ferracini et al. 2015a) including the possible hybridization with native *Torymus* species (Quacchia et al. 2014a; Ferracini et al. 2017; Pogolotti et al. 2018) and their displacement (Ferracini et al. 2018a).

The contribution of native parasitoids on the *D. kuriphilus* control have been also studied as a management option in Italy (Aebi et al. 2006; Santi and Maini 2011; Panzavolta et al. 2013; Quacchia et al. 2013; Palmeri et al. 2014; Bonsignore and Bernardo 2018), Croatia (Matošević and Melika 2013), Slovenia (Kos et al. 2015) and Switzerland (Cara et al. 2014, unpublished data). Other authors focused on alternative methods based on fungi (Addario and Turchetti 2011; Gaffuri et al. 2015; Graziosi and Rieske 2015; Tosi et al. 2015; Vannini et al. 2017; Fernandez-Conradi et al. 2018), bacteria (Iskender et al. 2017), tolerance of selected cultivars (Dini et al. 2012; Sartor et al. 2015; Nugnes et al. 2018), and mechanical control (Maltoni et al. 2012a) but without results for practical applications so far.

Methods to quickly detect *D. kuriphilus* in chestnut buds to make the transport of scions and trees more effective and secure have been proposed by Sartor et al. (2012), but unfortunately only when the pest was already widely distributed in Italy and in the neighbouring states. Finally, the effect of tree diversity and species composition on *D. kuriphilus* population has also been investigated showing a decreasing infestation in mixed forest compared to pure chestnut stands (Guyot et al. 2015; Pollastrini et al. 2016; Fernandez-Conradi et al. 2018).

Several efforts were also made to clarify the *T. sinensis* and *D. kuriphilus* biology such as their longevity and reproductive traits (Quacchia et al. 2008a; Picciau et al. 2017), morphology (Viggiani and Nugnes 2010; Doganlar 2017), life history (Bernardo et al. 2013; Quacchia et al. 2014a; Ferracini et al. 2015b), host location (Germinara et al. 2009, 2011; Graziosi and Rieske 2013), genetics (Bonal et al. 2018; Montagna et al. 2018), potential fecundity (Graziosi and Rieske 2014), dispersal range (Graziosi and Santi 2008; Graziosi and Rieske 2012; Gilioli et al. 2013; Gil-Tapetado et al. 2018) and future theoretical population dynamics (Paparella et al. 2016; Budroni et al. 2018). Similarly, gall structure, characteristics (Maltoni et al. 2012b) and physiology (Warmund 2013; Reale et al. 2016) have also been investigated.

Surprisingly, the literature available about *D. kuriphilus* impact on the chestnut tree and its response is sparse so far. Few researches exist focusing on fruit yield reduction (Battisti et al. 2014; Sartor et al. 2015), possible ill-omened indirect interaction between *D. kuriphilus* and fungi such as Cryphonectria parasitica (Prospero and Forster 2011; Meyer et al. 2015) and Gnomoniopsis castaneae (Lione et al. 2016), and shoot vigor reduction (Ugolini et al. 2014). Moreover, attempts at quantifying leaf area loss due to repeated *D. kuriphilus* attacks were few in number (Ugolini et al. 2014; Sartor et al. 2015; Guyot et al. 2015) and often based on the comparison between leaf characteristics (leaf with gall and without gall). Detailed information regarding important tree vitality-related aspects such as reserves (e.g. dormant buds), reaction structures (e.g. reactivated dormant buds and second flushes), previous year damage (e.g. dead shoots) and leaf area loss is still lacking. Finally, the success of the biological control by *T. sinensis* is often measured in terms of the reduction of the galls on shoots, but very little is known about the recovery process of the tree.

Therefore, this thesis focuses on the chestnut tree and will try to fill the described knowledge gap bringing some new ideas on how to assess the pest damage in forestry. Moreover this work intends also showing how harmful a pest can be for the entire forest system, especially when free from any enemy for many years.

1.5 Aim and outline of the thesis

The main aim of this thesis is to investigate how the *Castanea sativa-Dryocosmus kuriphilus-Torymus sinensis* system evolves in space and time and what are the overall damages on the chestnut tree caused by the accidental introduction of an exotic pest into the chestnut forest ecosystems of the Swiss southern Alps. Other secondary but not less important aims are to investigate and define the proper indexes to measure and follow the entire system.

Chapter 2: How can we measure the crown and branch architecture damages on the chestnut tree?

To ensure thorough assessment, it is important to understand the meaning and related damage degree indicated by the index used. We soon realized that all index only based on galls available in literature where not revealing the correct level of damage on the chestnut tree. Using the leaf-to-sapwood area relationship based on the pipe model approach, we quantitatively estimate the damage to chestnut trees caused by *D. kuriphilus* and we created a damage composite index allowing a realistic assessment during the entire epidemic.

2.1 Damage composite index

Original paper: Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters

2.2 Application of the Damage Composite Index

Original paper: Evaluating *Dryocosmus kuriphilus*-induced damage on *Castanea sativa*

Chapter 3: How can we measure the *D. kuriphilus* and *T. sinensis* population dynamics?

In this chapter we present different methods to quantify the population of *D. kuriphilus* and *T. sinensis*. Unfortunately, their application procedure is often time-consuming and for this reason, we initially analysed the effect of different sampling efforts on the precision of evaluating the success of *T. sinensis* release and the evolution of its parasitism rate towards biocontrol which helped us in saving time and money without making compromises on accuracy.

3.1 Indices and their applications and optimization

Original paper: Adapting sampling effort to assess the population establishment of *T. sinensis*, the biocontrol agent of the chestnut gallwasp

Chapter 4: How did the system *Castanea sativa-Dryocosmus kuriphilus-Torymus sinensis* evolve in southern Switzerland between 2013 and 2018?

Using the indexes presented before, we analysed the evolution of the entire system from the pest arrival to the fourth year of *T. sinensis* biocontrol. In the first subchapter (4.1), this system is analysed from the insects point of view specifically highlighting the growth dynamic and the spread capacity of *T. sinensis.* Moreover, we used a space-for-time substitution approach extending the study area to the Piedmont region in northern Italy and in Cuneo region where *T. sinensis* biocontrol has already been present for many years. This allowed us to draw a complete picture including the equilibrium phase usually occurring at least after 5 years from the first biocontrol year. In the second subchapter (4.2), the evolution of the damages and the reactions of chestnut trees are investigating. Specifically, we show how damage varies as a function of the time elapsed between pest arrival and biological control by *T. sinensis*.

4.1 *Torymus sinensis* and *Dryocosmus kuriphilus* population growth and evolution

Torymus sinensis local and regional early population dynamics in the Insubrian and Piedmont region

4.2 Damage and recovery patterns of chestnut trees since *D. kuriphilus* epidemic peak

Original paper: Chestnut tree damage evolution due to *Dryocosmus kuriphilus* attacks

Chapter 5: What are the far-reaching effects of the damage caused by *D. kuriphilus*?

The accidental introduction of an exotic pest directly harms the host species decreasing its vitality and making it more vulnerable to abiotic (e.g. drought and hail) and biotic (e.g. fungi) factors. However, the products provided by the host species can be affected too. In this chapter we will present an example of a far-reaching effect caused by *D. kuriphilus* on the chestnut honey.

5.1 The honey example

Original paper: Impact of the Asian Chestnut Gall Wasp, *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae), on the chestnut component of honey in the southern Swiss Alps.

Chapter 6: General discussion and conclusions

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Chapter 2

How can we measure the crown and branch architecture damages on the chestnut tree?

2.1

Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters

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Nicola Reynaud collecting branches from the crown of a chestnut tree

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Abstract

Dryocosmus kuriphilus has become a major issue for chestnut (*Castanea sativa*) cultivation since its introduction in Europe. Thus far, however, quantitative assessments of the impact of *D. kuriphilus* in terms of green biomass loss are lacking. In the present study we used the leaf area to sapwood area relationship (A_L:A_S) —based on the pipe model approach as a green biomass indicator— to quantitatively estimate the damage to chestnut trees caused by *D. kuriphilus* attacks and its recovery when the pest is controlled by *Torymus sinensis* in the chestnut stands of southern Switzerland. Leaf area loss is used as a response variable to select the most suitable explanatory architectural features at branch level to be combined in a damage index that permits a realistic assessment covering the entire epidemic. In our case study, the peak of the epidemic was reached in the fourth year of repeated *D. kuriphilus* attacks (leaf area losses exceeding 70%) with significant alterations in branch architecture. Although damage symptoms first decreased once biological control was reached (fifth year), branch architecture recovery was still lacking. From a methodological point of view, our A_L:A_S approach highlights how traditional methods of assessing the degree of infestation (i.e., proportion of attacked buds) fail to give sound estimations of resulting damage whereas the proposed composite damage index enables a more realistic assessment of the entire epidemic process, including the recovery phase when the gall wasp is biologically controlled.

Keywords *Castanea sativa,* leaf area- sapwood area ratio, branch architecture, tree damage, *Torymus sinensis.*

Introduction

The chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is native to China and is the only member of the tribe Cynipini that attacks the genus *Castanea*, for which it represents the most significant global insect pest (Stone et al. 2002; Abe et al. 2007; Aebi et al. 2011). *D. kuriphilus* galling activity results in preventing or inhibiting the development of normal shoots and in the production of abnormal plant structures (Maltoni et al. 2012a). This causes a progressive loss of the photosynthetic biomass (galled shoots have a reduced leaf area; Kato and Hijii 1997), a decrease in tree vigour (Kato and Hijii 1997), and an increase in branch mortality due to gall wasp post-emergence fungal attacks (Turchetti et al. 2010; Meyer et al. 2015). Although mortality only occurs in cases of severe damage on young plantlets or weak plants (Kato and Hijii 1997; Cooper and Rieske 2007), *D. kuriphilus* attacks can strongly reduce the quality and quantity of timber (Kato and Hijii 1997; Maltoni et al. 2012b), nuts (Battisti et al. 2014), flowers and chestnut honey production (Conedera and Gehring 2015). The pest can be successfully controlled biologically through its natural enemy, the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), which is also native to mainland China (Moriya et al. 1989; Moriya and Adachi 2003; Quacchia et al. 2014).

Since the detection of the pest in Europe, research on the behaviour and impact of the gall wasp on the European chestnut (*Castanea sativa* Mill.) has focused on various aspects. These include insect biology (Viggiani and Nugnes 2010; Bernardo et al. 2013), host-gall wasp interactions (Germinara et al. 2011; Panzavolta et al. 2012), biological (Quacchia et al. 2008) and mechanical control (Maltoni et al. 2012b; Turchetti et al. 2012), identification of less susceptible *C. sativa* genotypes (Dini et al. 2012; Sartor et al. 2015), visual crown damage assessment (Guyot et al. 2015), damage description and classification (Maltoni et al. 2012a). In this context, and based on the experience of prior Japanese research, a number of different methods for the assessment of infestation degree (MAID) have been implemented and used (Kotobuki et al. 1985; Matošević et al. 2010; Sartor et al. 2015) (Table 1). Even though green biomass is considered a major proxy of tree vitality and vigour (e.g., Johnstone et al. 2013), to date there have been very few attempts at quantifying leaf area loss due to repeated *D. kuriphilus* attacks (e.g. Kato and Hijii 1997; Guyot et al. 2015)—likely because of the difficulty in finding healthy reference trees. Moreover, detailed information regarding important aspects such as tree reserves (e.g., dormant buds), reaction structures (e.g., reactivated dormant buds and second flushes), and previous year damage (e.g., dead shoots) are usually ignored (Appendix – Fig. A1).

Table 1 Methods of assessing the *Dryocosmus kuriphilus* infestation degree (MAID) from the literature

Index	Symbol	Source
No. galls / bud	GB	Sartor et al. 2015
No. galls / shoot*	GS	Matošević et al. 2010
No. attacked buds / no. buds	AB	Kotobuki et al. 1985

* The index formula is derived from the authors' description

The overall aim of this study is to quantitatively estimate the evolution of leaf area and branch architecture during a typical *D. kuriphilus* epidemic that includes an initial infestation phase by the gall wasp and a subsequent progressive control by its natural enemy *T. sinensis,* until effective biological control has occurred. In particular, we test the suitability of a suite of different branch parameters as proxy indicators of the *D. kuriphilus* impact on leaf area. We also test the customary MAID in order to assess the possible relationship between the degree of infestation and leaf area loss. For these purposes, we use the leaf area to sapwood area relationship based on the pipe model approach (Shinozaki et al. 1964a, 1964b) as green biomass indicator. We then take the leaf area loss induced by the *D. kuriphilus* attacks at branch level as the response variable to select the most appropriate set of explanatory architectural features. Finally, we propose an empirical and synoptic damage index (DCI) that permits the realistic assessment of tree damage evolution through all stages of an epidemic, including the recovery phase which occurs when biological control through *T. sinensis* takes effect.

Materials and method

Study area

The study was carried out in the chestnut forests in Canton Ticino (southern Switzerland) and Piedmont (northern Italy) (Fig. 1). The Swiss part of the study area is characterized by a moist-warm temperate climate (annual mean precipitation from 1,800 to 2,100 mm, annual mean temperature ranging from 10.2 to 12.4°C - MeteoSwiss climatic normals 1981-2010; http://www.meteoswiss.admin.ch/home/climate/past/climate-normals.html) and soils are generally classified as haplicpodzol (cryptopodzol) on crystalline bedrock (Blaser et al 2005). The chestnut area of the Piedmont region has rather similar bedrock types but drier climatic conditions with annual mean temperatures ranging from 10.6 (Cuneo) to 12.4 °C (Pallanza) and an annual mean precipitation ranging from 1,066 (Cuneo) to 1,764 mm (Pallanza; ISPRA climatic normals 1961-1990 for temperature and 1951-1980 for precipitation; http://www.scia.isprambiente.it/Documentazione/rapporto_Valori_normali_def.pdf).

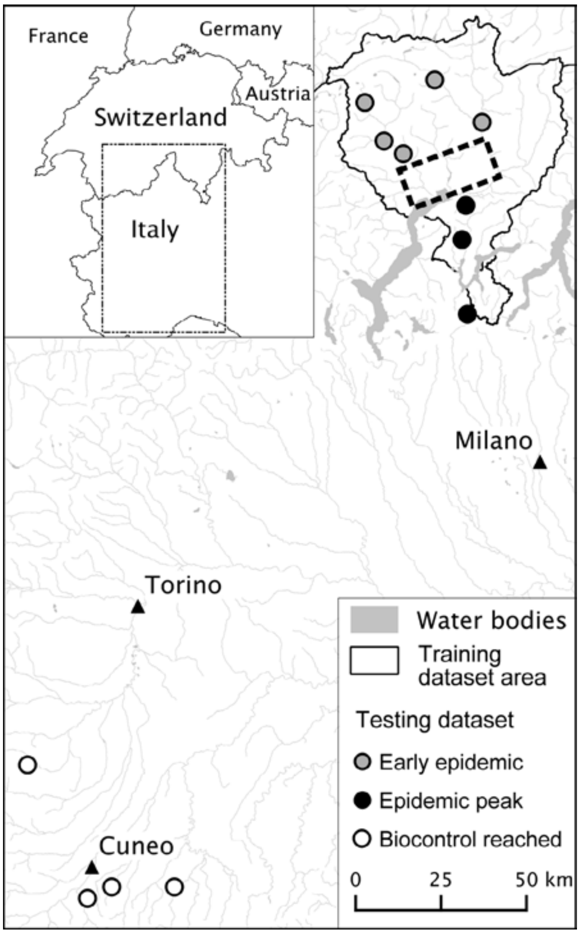

Figure 1 Detailed location of the study sites. The dashed rectangle shows the training dataset area, while circles represent the testing dataset sites in Switzerland and in Italy. Circle colours represent the epidemic stages: grey = early epidemic stage; black = epidemic peak; white = biological control by *Torymus sinensis.*

In both areas, the chestnut cultivation has a long tradition (Conedera et al. 2004; Conedera and Krebs, 2008). As a consequence, it is the dominant tree species over large areas where it plays an important role for wood and fruit production (Bounous, 1999; Conedera et al. 2004) as well as for the stability of the steep slopes (Vogt et al. 2006; Conedera et al. 2010).

Chronology of D. kuriphilus-T. sinensis spread

D. kuriphilus was accidentally introduced into Italy at the end of the late 1990s (Quacchia et al. 2008), although the first record of its occurrence was in 2002 in the Cuneo Province (Brussino et al. 2002), from where it progressively spread throughout the entire chestnut area in Piedmont. It reached southern Switzerland in 2007 (Forster et al. 2009) and colonized the southern Swiss chestnut area in its entirety by 2013.

The parasitoid *T. sinensis* was actively released into chestnut stands in Italy since 2005 (Quacchia et al. 2008) and in France in 2010 (Borowiec et al. 2013). Unlike Italy and France, the release of *T. sinensis* in Switzerland has not been allowed by federal authorities because of open biosafety questions (BAFU 2012, http://www.bafu.admin.

ch/biotechnologie/01760/08944/index.html?lang=it). Nevertheless, the parasitoid was found in 2013 in nearly the entire chestnut area of southern Switzerland (Conedera et al. 2015), which the authors estimate to be the result of a progressive spread from Italy into the area since 2011 (although some cases of active introduction cannot be excluded).

The pipe model approach to C. sativa

The pipe model principle, as postulated by Shinozaki et al. (1964a,b), assumes that the conductive tissue of woody plants (sapwood area – A_S) is equivalent to an assemblage of pipes supporting a corresponding proportion of leaves, and that the ratio between the supported leaf area (A_L) and the supporting sapwood area (A_S) remains constant within branches or any portion of a tree. Gehring et al. (2015) confirmed the general validity of the pipe model and the leaf to sapwood area relationship (A_L:A_S) for chestnut trees in the study area before the gall wasp's arrival (i.e., on fully healthy and developed tree branches). In particular, the A_L:A_S is constant at branch level, varies between different types of branches (architectural crown branches *vs* epicormic shoots) and slightly decreases within a tree as a function of branch height.

Sampling design: training dataset

The leaf area of branches in pre-epidemic conditions refers to a subset of the data collected by Gehring et al. (2016) from 41 mature wild trees in their 2010 survey on the pipe model theory (Fig. 1, dashed rectangle). Sample trees are seed-originated, naturalized and dominant individuals that we selected in unmanaged mature chestnut stands to assure a natural and undisturbed crown development (e.g., no pruning activities). For the assessment of the impact of *D. kuriphilus*, four additional field campaigns took place in the second (2012) and the fourth (2014) year of the (repeated) *D. kuriphilus* attack, as well as in the first (2015) and the second (2016) year of recovery due to the progressive biological control by *T. sinensis*.

For each field campaign, an architectural crown branch from every selected sample tree was cut in July—when all leaves were fully developed—for a total of 205 branches (5 branches from 41 trees over 5 years). In order to avoid any influence from epicormic shoots, abiotic factors (such as degree of shadow) and branch height (Gehring et al. 2015), only crown branches at full light and at the same tree height were collected. Tree-climbing techniques were used to reach crown tops. Cut branches were transported to the laboratory and processed all in the same day in order to preserve the original leaf form and turgor.

Sampling design: independent testing dataset

In order to test the suitability of the selected leaf area proxies (i.e., single MAID and the proposed Damage Composite Index - DCI) to describe the epidemic evolution of the *C. sativa* – *D. kuriphilus* – *T. sinensis* system, we used a space-for-time substitution approach in which a temporal gradient of epidemic stages over the whole study area was created. In total, we retained 12 sites (circles in Fig. 1) at different times since the arrival of *D. kuriphilus* and *T. sinensis*. These roughly represent the early epidemic stage (*D. kuriphilus* arrival and beginning of tree damage, grey circles), the epidemic peak (high level of *D. kuriphilus* attacks, medium to severe damage on trees, black circles) and the recovering system and biological control by *T. sinensis* (decreasing pressure due to *D. kuriphilus* parasitization by *T. sinensis*; progressive tree recovery, white circles).

Sampling took place in the month of July, from 2013 to 2015. At each of the 12 sites, 10 wild trees were randomly selected and one architectural branch was collected from the upper region of the crowns (120 validation branches in total). Branch structures were assessed following the same protocol as for the training data set.

Assessing branch structures

In order to assure a reasonable architectural-feature dataset for modelling purposes, we collected branches of ca. 130 cm in length on average and with at least 50 branching points (shoots). For details on the definitions of the main branch structures considered as explanatory variables, including the gall wasp-induced features, please refer to Table 2 and Figure 2.

Branch analysis started from the base. In order to calculate the sapwood area (A_S), two diameters (minimum and maximum) were measured at 1-3 cm below the first branching point, and the formula for the area of an ellipse area was applied. When heartwood was present, the corresponding area was subtracted from the branch section to calculate the A_S. The age of the branch and the maximum branch length were also recorded for general information.

All shoots (the sprouts formed during the previous vegetative season with respect to the sampling date) of the branch in question were then classified as dead or alive. Of the living shoots, all nodes were numbered (progressively) from the base to the apex and classified into five different categories (developed bud, quiescent bud, dormant bud, inflorescence scar or dead). When a developed bud was found, all the axillary buds on the corresponding newly formed sprout were counted, progressively numbered (starting from the base) and checked for the presence of galls and second flush sprouts (i.e. “Lammas growth” produced in summer from the newly built buds), following a simplified version of the protocol originally proposed by Maltoni et al. (2012a).

When a gall was present on any organ of the sprout, the corresponding generating node was considered “infested” and the gall was further classified by its position (assigned progressive number along the sprout) and by its type (on shoot, on leaf or on stipule). For the purpose of consistency with existing literature, galls growing on sprouts are hereafter identified as galls on shoots (see also Fig. 2). If a developed bud was found on a stem older than a shoot, the bud was labelled separately as reactivated dormant bud and all the related axillary buds on the sprout produced were analysed accordingly.

All the leaves (both living and dead) of the labelled sprouts were detached and classified into five categories (normal, from second flush, from reactivated buds, with gall, on gall) depending on their origin or gall type (if any). After the removal of existing galls with scissors, leaves were processed with a scanning device (Xerox 9001, 200 dpi, +1 contrast, colour photo option) and the corresponding leaf area (A_L) calculated with the Image Pro plus 6.0 (IPP) image processing program.

The leaf to sapwood area ratio (A_L:$A_{Smeasured}$) was calculated by dividing the total measured leaf area (A_L, in m^2) by the sapwood area (A_S, in cm^2).

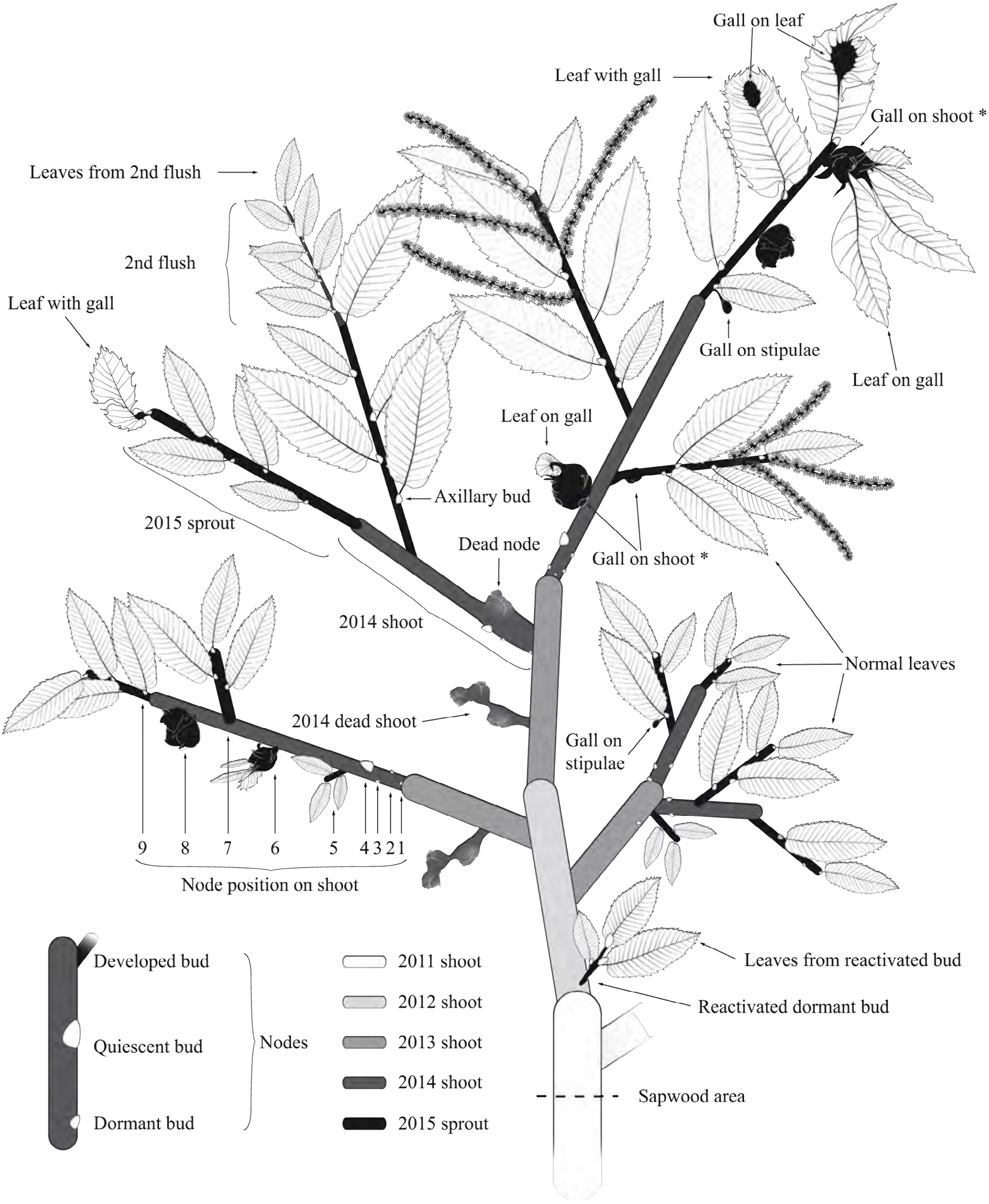

Figure. 2 Schematic structure of a chestnut branch with gall wasp attacks.

Please refer to Table 2 for detailed definitions of branch features.

* Based on our definition, these types of galls should be referred to as “galls on sprouts”. However, we use “galls on shoots” to be consistent with existing literature.

Table 2 Description of the branch parameters considered

Parameter	Symbol	Description/Note	Formula	Unit
Branch	B	Includes the totality of the shoots, sprouts and vegetative organs from the sapwood area measuring point		
Sapwood area	A_S	Calculated using the ellipse formula from the minimum and maximum diameters (a and b) at the base of the branch 1-3 cm from the first branching and subtracting the heartwood part, if any	$(\varnothing a * \varnothing b) / 4 * \pi$	cm^2
Leaf area	A_L	Total area of the leaves on the branch		m^2
Leaf to sapwood area ratio	A_L:A_S	Ratio between A_L and A_S only considering leaves from normally developed shoots and not those from reactivated buds	A_L / A_S	m^2/cm^2
Reactivated leaf to sapwood area ratio	rA_L:A_S	Ratio between A_L and A_S considering leaves from reactivated buds and second flushes only	A_L / A_S	m^2/cm^2
Shoot	S	Sprout from the previous vegetative season with respect to the sampling date. Can be dead or alive. i.e. sampling season = 2015, shoot = sprout that grew in 2014	No. S / A_S	$1/cm^2$
Dead	Sd	Dead shoot after *D. kuriphilus* attack	No. Sd / No. S	proportion
Alive	Sa	Not dead	No. Sa / No. S	proportion
Node		The point on a shoot at which buds are attached		
Developed bud	Bde	Any bud that breaks after a single resting season producing a sprout or a gall on shoot with no stem elongation.	No. Bde / No. Sa	average
Quiescent bud	Bq	Bud remaining quiescent during the current vegetative season	No. Bq / No. Sa	average
Dormant bud	Bdo	Bud remaining dormant during the current vegetative season	No. Bdo / No. Sa	average
Inflorescence scar	Isc	Oval scar due to a previous-year female flower	No. Isc / No. Sa	average
Dead node	Nd	Dead bud or dead gall on shoot	No. Nd / No. Sa	average
Infested bud	Bi	Developed bud with at least one gall	No. Bi / No. Sa	average
Sprout (on a shoot)		Freshly formed sprout grown during the current vegetative season from a developed bud on a shoot		
Axillary bud	An	Freshly formed bud generated during the current vegetative season on a sprout	No. An / No. Sa	average
Second flush	Asf	Freshly formed sprout generated by a freshly formed axillary bud during the current vegetation season.	No. Asf / No. Sa	average
Reactivated dormant bud	BdoR	Freshly formed sprout grown during the current vegetative season from a dormant bud on a multiyear branch part that is older than the shoot	No. BdoR / No. Sa	average
Leaf				
Normal	Ln	Leaves on sprouts from normally developed buds	No. Ln / No. Sa	average
With gall	LwG	Leaves with a gall	No. LwG / No. Sa	average
On Gall	LoG	Leaves growing on a gall	No. LoG / No. Sa	average
Reaction leaves				
From second flush	Lsf	Leaves on second flushes	No. Lsf / No. Sa	average
From reactivated buds	Lr	Leaves on sprouts from reactivated dormant buds	No. Lr / No. Sa	average
Gall		Abnormal outgrowth of plant tissues caused by *D. kuriphilus*		
On shoot	Gons	Gall developed at the base or along the axis of a sprout. Technically these should be called “galls on sprouts” but for consistency purposes with existing literature we refer to them as “galls on shoots”	No. GonS / No. Sa	average
On leaf	Gonl	Gall developed on a leaf	No. GonL / No. Sa	average
On stipulae	Gonst	Gall developed on a stipula	No. GonSt / No. Sa	average

Data processing and analysis

Univariate comparative analysis of the evolution of individual parameters prior to the gall wasp's arrival (2010) and during the epidemic stages (from 2012 to 2016) was performed using different Mann-Whitney U tests (Wilcoxon rank sum tests).

The proportion of leaf area loss (LAL) due to *D. Kuriphilus* attacks was calculated as follows:

$$\text{LAL} = (\text{Al:As}_{\text{measured}} - \text{Al:As}_{\text{refernce}}) / \text{Al:As}_{\text{refernce}}$$

where $\text{Al:As}_{\text{measured}}$ refers to the effective leaf area of a branch in the corresponding years of *D. kuriphilus* attacks (2012 to 2016) and $\text{Al:As}_{\text{refernce}}$ refers to the theoretical leaf area as expected applying the 2010 pipe model formula to the A_S of the branch (Gehring et al. 2015).

A generalized linear mixed model analysis was performed to model the relationship between LAL (response variable) and different sets of explanatory variables. In order to test the explanatory power of the selected MAIDs, four models were run using each individual index as explanatory variable in turn (Table 1). We then ran another set of models that included the architectural branch features as explanatory variables in order to select the most suitable combination to estimate the leaf area loss. Site and tree were set as random factors. The random configuration (random intercept or random intercept and slope) was determined following the procedure described in Zuur et al. (2009).

For the models based on different architectural features, the explanatory variables were first inspected by way of an automatic cluster analysis method using Spearman's correlation coefficients. When two variables were highly correlated (Spearman's $r \geq 0.5$), the one that was more strongly related to the response variable according to the best AICc (Akaike information criterion with a second order correction for small sample size) was selected. We then used a forward model selection procedure starting with no explanatory variable, tested the addition of each variable using the maximum likelihood estimation (ML) and ranked the models with the AICc criterion. The best model estimates were recalculated using restricted maximum likelihood estimation, the distributions of the explanatory variables were checked for normality, and samples of posterior density distribution were generated using the Markov Chain Monte Carlo (MCMC). The diagnostics of the best model were verified analysing residual plots. A Damage Composite Index (DCI) was finally computed using the best model.

The resulting DCI and the best index among the MAID models were then run on the independent testing dataset, and the resulting overall damage evolution scenarios were compared for plausibility with respect to the field observations at different stages of *D. kuriphilus* attack and biological control by *T. sinensis*.

All analyses were performed using R statistical software version 3.2.2 (R Development Core Team, 2015).

Results

Evolution of D. kuriphilus impact

In the training dataset area, *D. kuriphilus* infestation increased from 2011 (*D. kuriphilus* arrival) to 2014 (reaching "number of galls per bud" (GB) = 0.91; "attacked buds" (AB)% = 41%) before decreasing (GB = 0.48; AB% =29%

in 2015; GB = 0.27; AB% = 15%) in 2016 as a consequence of the notable parasitization by *T. sinensis* ($Ts_{\%}$ = no. *T. sinensis* / no. chambers x 100) (Table 3 and Appendix – Table A1). The A_L:$A_{Smeasured}$ shows a significant trend over the years since the arrival of *D. kuriphilus* (Fig. 3), decreasing by a factor of 3.9 (median value of 74% leaf area loss) from 2010 to 2014 before recovering in 2015 by 30% (median value with respect to 2014) and remaining stable between 2015 and 2016. The A_L:$A_{Smeasured}$ recovery (due to reactivated dormant leaves and second flushes) always represents a negligible contribution to leaf area loss compensation, and follows a similar but inverse trend (Fig. 3), reaching the highest median values in 2014 (A_L:$A_{Smeasured}$ = 0.01) and decreasing to 0.007 in 2016. Similarly, the ratio of reactivated dormant buds per branch increased from 2010 to 2014 (from 0 to 0.1 in median) before decreasing to 0.02 (median) in 2016 (Fig. 4). Damage by *D. kuriphilus* with reference to single parameters other than leaf area also peaked in 2014, with a maximal proportion of dead shoots of 37% (median per branch) and a median value of 0.64 of “galls on shoots” (Fig 4.).

Table 3 Evolution of the *Dryocosmus kuriphilus* (Dk) epidemic and the *Torymus sinensis* (Ts) parasitization for the training and testing datasets by sampling year

Training dataset

Year	GB[a]		Ts % [b]	
	mean	*(± sd)*	*mean*	*(± sd)*
2010	0	(± 0)	0	(± 0)
2012	0.28	(± 0.28)	3	(± 1)
2014	0.91	(± 0.58)	58	(± 21)
2015	0.48	(± 0.30)	82	(± 13)
2016	0.27	(± 0.44)	80	(± 18)

Independent testing dataset

Epidemic stage	Yrs. since arrival		GB		Ts %	
	Dk	Ts	*mean*	*(± sd)*	*mean*	*(± sd)*
Early	1	0	0	(± 0)	0	(± 0)
	2	1	0.23	(± 15)	1	(± 2)
	3	2	0.91	(± 15)	5	(± 6)
Peak	4	2	0.74	(± 22)	16	(± 6)
	5	3	0.71	(± 15)	47	(± 14)
	6	3	1.33	(± 24)	85	(± 5)
Recovery	7	4	0.57	(± 13)	75	(± 16)
	8	4	1.05	(± 22)	79	(± 9)
	9	7	0.01	(± 3)	90	(± 14)
Biocontrol	12	8	0.00	(± 0)	89	(± 11)
	15	10	0.00	(± 0)	100	(± 0)
	16	11	0.00	(± 0)	100	(± 0)

[a] GB = (No. galls / bud); [b] $Ts_{\%}$ = (No. *T. sinensis* / No. chambers*100)

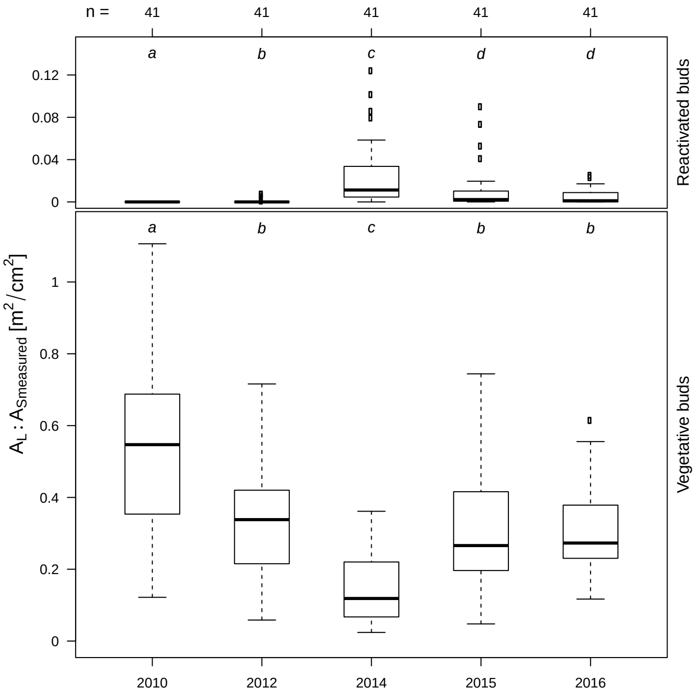

Figure 3 A_L:$A_{Smeasured}$ ratio evolution from 2010 to 2016. Losses in A_L:$A_{Smeasured}$ due to gall wasp attacks (bottom) and gains in A_L:$A_{Smeasured}$ resulting from tree reactions (leaves produced by second flushes and reactivated dormant buds) (top). Labels on top (n) indicate the number of sampled branches per year. Letters represent significantly different distributions ($p < 0.01$) among years based on a non-parametric pairwise Mann Whitney U test with the Holm adjustment for p-values.

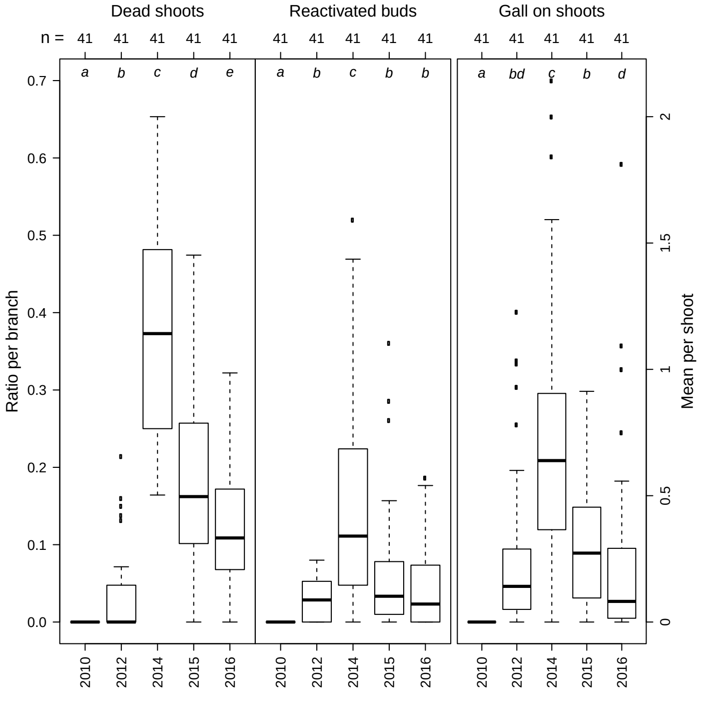

Figure 4 Evolution of the variables retained in the best model from 2010 to 2016. Labels on top (n) indicate the number of sampled branches per year. Letters represent significantly different distributions ($p < 0.01$) among years based on a non-parametric pairwise Mann Whitney U test with the Holm adjustment for p-values. Please refer to Table 2 for more details on variable definitions and units.

At shoot level, tree reactions may be tracked by assessing the evolution of dormant buds, quiescent buds (Fig. 5) and second flushes. Buds remaining dormant significantly decreased from a median value per shoot of 1.6 in 2010 to 0.4 in 2014, before increasing to 1.2 by 2016. Similarly, but in the opposite direction, the median number of quiescent buds gradually increased up to 2015 (from 0 to 0.2) and decreased to 0.07 in 2016. The median number of second flushes increased from 2010 to 2014 (from 0 to 0.02) before decreasing to 0 in 2016. Finally, the number of shoots per cm^2 of sapwood decreased by half between 2010 and 2014 (median from 22 to 11) and did not increase again up to 2016.

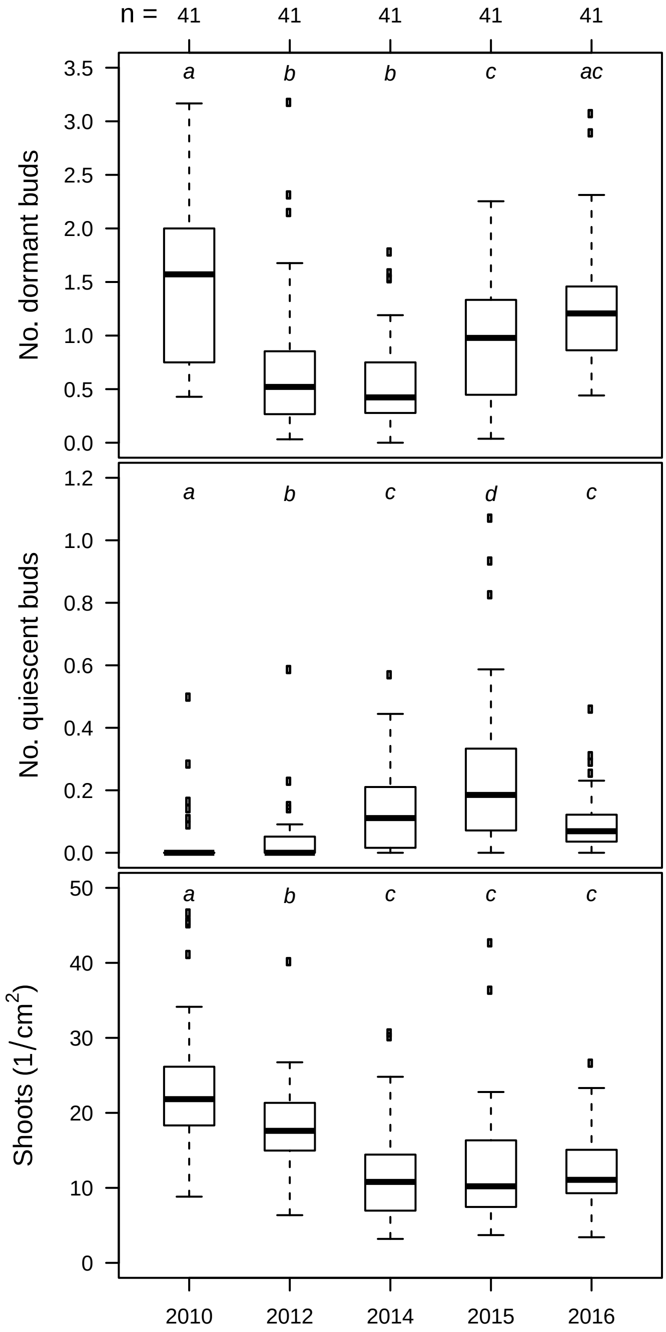

Fig. 5 Evolution of different bud types and shoots from 2010 to 2016. Labels on top (n) indicate the number of sampled branches per year. Letters represent significantly different distributions ($p < 0.05$) among years based on a non-parametric pairwise Mann Whitney U test with the Holm adjustment for p-values. Please refer to Table 2 for more details on variable definitions and units

Model results

The best random configuration factor identified for the mixed modelling approach was an intercept random model. Among MAID models, the GB model performed best (AICc = -54.1, Table 4) and was thus retained for the evaluation on the independent testing dataset. The overall best model in terms of AICc and likelihood estimation was the model including dead shoots, reactivated dormant buds and galls on shoots as explanatory variables (AICc = -95.9,Table 4). In this model, all fixed effects were positively correlated with the loss in leaf area (response variable) with significant p-values. Moreover, none of the HPD intervals crossed 0 (Table 4) and graphical examination of the residuals did not reveal any violation of the modelling assumptions and no patterns were discernible when plotting them against the fitted values (data not shown).

Estimates of the resulting best model were then used to compute a Damage Composite Index (DCI) as follows:

DCI = (dead shoots x 0. 479 + react. dorm. buds x 0. 525 + galls on shoots x 0. 120) x 100.

DCI median values are equal to 6 (with a maximum of 14) for LAL <= 30%, 11 (with a maximum of 32) for LAL > 30 and <= 60% and 23 (with a maximum of 67) for LAL > 60%.

Table 4 Generalized linear mixed models

Model	Variables	Estimate	MCMC$_{mean}$	HPD95$_{L}^{a}$	HPD95$_{U}^{a}$	$Pr(>\lvert t\rvert)^{b}$	pMCMC^{c}	AICc
Branch param.								
Best model	Intercept	0.387	0.378	0.309	0.444	0.000	0.000	- 95.9
	Dead shoots	0.479	0.461	0.262	0.645	0.000	0.000	
	React. dormant buds	0.525	0.540	0.275	0.832	0.000	0.000	
	Galls on shoots	0.120	0.123	0.049	0.190	0.001	0.001	
MAID								
GB	Intercept	0.467	0.459	0.389	0.532	0.000	0.000	- 54.1
	GB	0.191	0.705	0.127	0.252	0.000	0.000	
AB	Intercept	0.447	0.438	0.361	0.516	0.000	0.000	- 51.2
	AB	0.430	0.427	0.269	0.588	0.000	0.000	
GS	Intercept	0.524	0.516	0.444	0.595	0.000	0.000	- 28.0
	GS	0.035	0.037	0.004	0.069	0.036	0.022	

[a] HPD95$_{L/U}$ are the highest posterior density intervals. The last two columns show *p*-values calculated with the posterior distribution (pMCMC) and the *t*-distribution (Pr(>|t|)).
[b,c] *p*-values are calculated using the *t*-distribution Pr(>|t) and with the posterior distribution (pMCMC), respectively.

Testing the obtained models

The temporal evolutions of the DCI and of the best MAID model (GB) as computed using the independent testing dataset are illustrated in Figure 6. DCI values increase gradually after the arrival of *D. kuriphilus* and start decreasing after 4 years of *T. sinensis* presence, progressively returning to a low value (but not to 0) after 10 years. In contrast, the GB values rise quickly reaching over ~ 0.91% on average in the third year, fluctuate up and down until the 8th year after the *D. kuriphilus* arrival, before dropping to 0 in the 7th year after the arrival of *T. sinensis* (see Table 3 for details on GB and $Ts_{\%}$).

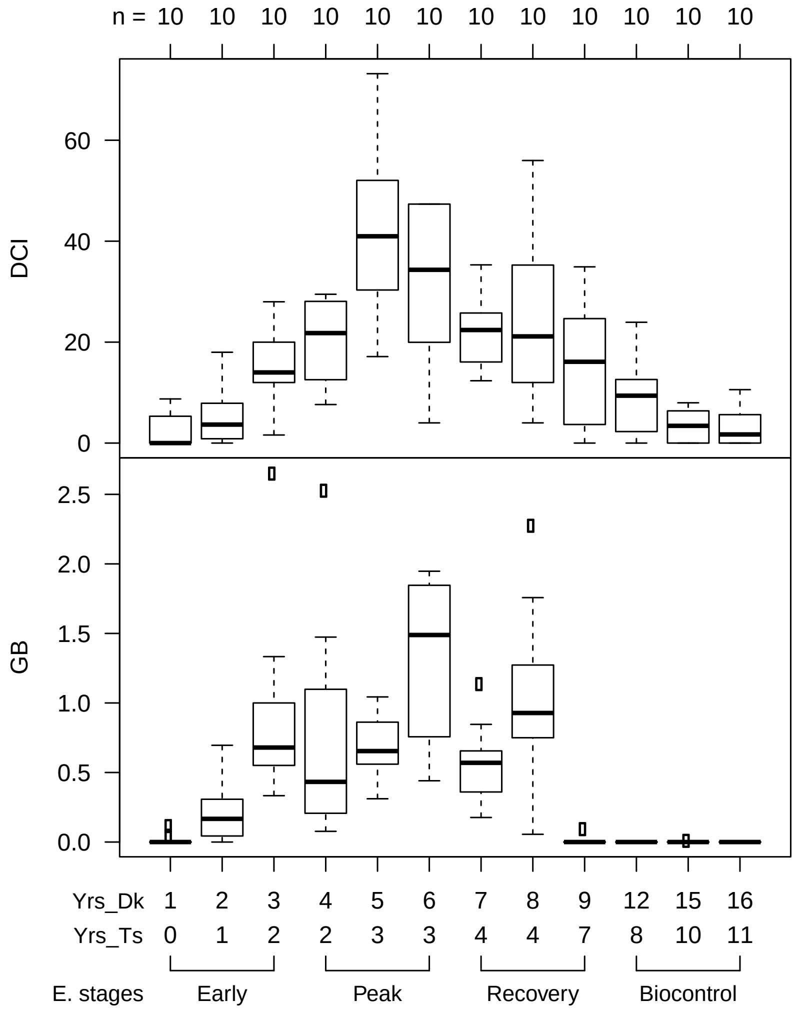

Figure 6 Temporal evolution of the epidemic for the testing dataset. Evolution of DCI (top) and GB (bottom) as a function of years since the arrival of *Dryocosmus kuriphilus* (yrs_Dk) and years since the arrival of *Torymus sinensis* (yrs_Ts). Labels on the top (n) represent the number of sampled branches per yrs_Dk. E. stages = epidemic stages.

Discussion

Gallwasp damage evolution

In this paper, we assess the evolution of the first six years of the gall wasp epidemic in southern Switzerland by tracking the damage caused by *D. kuriphilus* attacks in terms of gall wasp-induced branch features and overall green biomass loss in chestnut trees, including the start of the recovery phase when the pest was controlled by its natural enemy *T. sinensis*. Our results indicate a peak in overall green biomass loss and individual damage features (e.g., galls on shoots, dead shoots, MAID) in the fourth year of the epidemic at which point no significant control by *T. sinensis* had yet occurred. Damage symptoms decreased when a notable parasitization level by *T. sinensis* (Ts% ~ 80%) was reached, which occurred in the Swiss part of the study area towards the fifth year of the epidemic. Tree reactions to severe and repeated *D. kuriphilus* attacks, such as the activation of dormant buds and second flushes, usually began in the second year of the epidemic, but remained far too weak to compensate for the overall leaf area loss. Second flushes and late reacting buds are characterized by a delayed seasonal phenology that may increase the risk of insufficient lignification in case of early or winter frost episodes. The small positive effect of tree reactions may thus simply end in progressive energy consumption and the depletion of tree reserves without any significant tree recovery (Campbell and Valentina 1972; Dobbertin 2005). The activation of tree reactions combined with the loss of leaf area during the first four years of *D. kuriphilus* infestation may, consequently, be detrimental to tree reserves at the shoot level as highlighted by the progressive decrease in dormant buds. It is not clear in this context whether the measured reduction in tree reactions in the fifth year of the epidemic in the training dataset trees is due to the decreasing pressure of *D. kuriphilus* or to the progressive depletion of the reserves of chestnut trees in question.

The negative effects of repeated *D. kuriphilus* attacks are not limited to a gall-related reduction in leaf area (e.g. Kato and Hijii, 1997), but evolve over time to a progressive and significant alteration of branch architecture as a whole. As indicated by the increase in dead shoots when passing from the first to the fourth year of the epidemic under study, possible processes involved in the corruption of branch architecture include the failure of bud production from galled shoots (shoots on galls, Kato and Hijii, 1997) and the dieback of infested branches (Maltoni et al. 2012a), especially in case of subsequent attacks by secondary or endophitic fungi such as chestnut blight (*Cryphonectria parasitica*; Meyer et al. 2015; Turchetti et al. 2010) or chestnut brown rot (*Gnomoniopsis* spp.; Ugolini et al. 2014; Lione et al. 2016).

Seasonal meteorological effects (rainy springs) were not considered in our modelling approach. As reported by Gehring et al. (2015) difference of 22% in spring precipitation between 2009 and 2010 did not result in any statistical variations in leaf area at tree level. Moreover seasonal deviations from normal spring precipitation (calculated as the sum from January to May) during the field campaigns were always positive ranging from +6% in 2015 to +36% in 2014. Any influence of the saisonal precipitation on the presented results is thus very unlikely and in any case by several order of magnitude less than damage by *D. kuriphilus*.

Not considered in our analyses are also possible targeted management options such as manuring or pruning of the trees. According to Maltoni et al. (2012b), applying targeted pruning techniques may contribute to temporary mitigate gallwasp damage by artificially accelerating the development of new shoots. Could manuring of particularly valuable individuals (e.g. chestnut orchard trees) represent a suitable option for accelerating the recovery phase? The question remains open.

Damage Composite Index

Applying the leaf area to sapwood area model to evaluate leaf area loss and chestnut tree recovery following *D. kuriphilus* attacks was found to be a suitable method to provide quantitative estimations of these phenomena. In particular, it was possible to highlight that after four consecutive years of *D. kuriphilus* attacks without any significant control by the specific parasitoid *T. sinensis*, 95% of the trees had a leaf area loss of 50% with nearly half (44%) recording losses of more than 80%. Tree reactions, in contrast, accounted for less than 10% of the leaf area in nearly 90% of the trees in question. Interestingly we noticed that once the recovery phase begins, all main shoot damage indicators such as tree reactions, reserves (dormant buds, quiescent buds), failures (dead shoots) and galls on shoots progressively return to initial values, except for lateral shoots which remain at half their original numbers. As a general rule, mainly lateral buds are attacked by *D. kuriphilus* and are subjected to progressive exhaustion, whereas the number of apical and terminal buds are formed after the oviposition activity of the parasite and thus remain healthy (Maltoni et al. 2012a). Once biological control is reached and most of the formed buds produce normal shoots, the recovery process is initially limited to the apical part of the branches (Appendix – Fig. A1), whereas lateral shoots and buds are still lacking. This results in a delayed and slower reestablishment of pre-epidemic leaf area values.

This deficit in lateral branches caused by the multiannual *D. kuriphilus* epidemic pressure and related decrease in leaf area is perfectly depicted by the DCI, which accurately describes how damage and recovery evolve in the *C. sativa – D. kuriphilus – T. sinensis* system over time, especially during the recovery phase once biological control is reached. MAID, in contrast, fail to provide a sound estimation of effective overall green biomass loss (see also Appendix – Fig. A2). Once biological control by *T. sinensis* is reached (5th year), MAIDs are reduced to 0 (Fig. 6) which may lead to the false assumption that trees had completely recovered, ignoring the still existing deficits at the branch structure level.

The more realistic assessment of tree states provided by the DCI can also be easily understood in light of the individual features the index is composed of. According to Kato and Hijii (1997) and Maltoni et al. (2012a), galls on shoots are responsible for the highest proportion of dead shoots and for the most significant amount of leaf area reduction. This is likely the reason why galls on shoots is the only gall type retained in the best model on which the proposed DCI is based. This type of gall prevents shoot elongation which leads to— in the year subsequent to the appearance of galls on shoots —a reduction in shoots (especially lateral) and related leaf area. This is accounted for and quantified in the DCI by the variable dead shoots. Hence, dead shoots are a proxy of the severity and lethality of the previous year's attack that contributes to a realistic estimation of the current healthy or recovery state of the branch. Finally, the stress caused by the progressive leaf area loss leads to the mobilization of tree reserves, which is represented in the DCI by the number of reactivated dormant buds. More precisely, reactivated dormant buds is the proxy of both the accumulated stress in previous years and the potential bud resources still present in the tree to regenerate new healthy organs to replace completely dead branches.

Conclusion

In this paper we have demonstrated how repeated and uncontrolled attacks by *D. kuriphilus* cause serious damage to European chestnut trees. The related corruption of branch architecture and the significant reduction in the number of lateral shoots represent key factors in the delay of tree recovery once biological control through the natural enemy *T. sinensis* is reached. We therefore strongly support the early introduction of the biocontrol agent once the gall wasp is detected in areas where the chestnut plays an important economic and ecological role.

The proposed Damage Composite Index - DCI (a brief example of how to apply the DCI is provided in Appendix – Fig. A3) helps to determine the stage of gall wasp impact and the degree of branch alteration within chestnut forests. We are aware that it is not always possible or suitable to implement complex and multifactorial surveys of epidemic symptoms when studying gall wasp damage. To address this, we strongly suggest evaluating the overall impact of gall wasp epidemics based on a more holistic approach rather than simply using commonly implemented methods to assess the degree of infestation, especially in case of prolonged attacks. The branch size needed to operationally compute the DCI can be reduced to ~10 shoots. In addition, detailed field observations of the damage and recovery proxies suggested here may be simplified according to needs and integrated with general assessments. These would include the overall degree of crown transparency and additional basic information such as the time since the first arrival of *D. kuriphilus* and the spontaneous arrival or active introduction of the specific parasitoid *T. sinensis*.

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Our heartfelt thanks go to Nicola Reynaud and Franco Fibbioli for their help during data collection.

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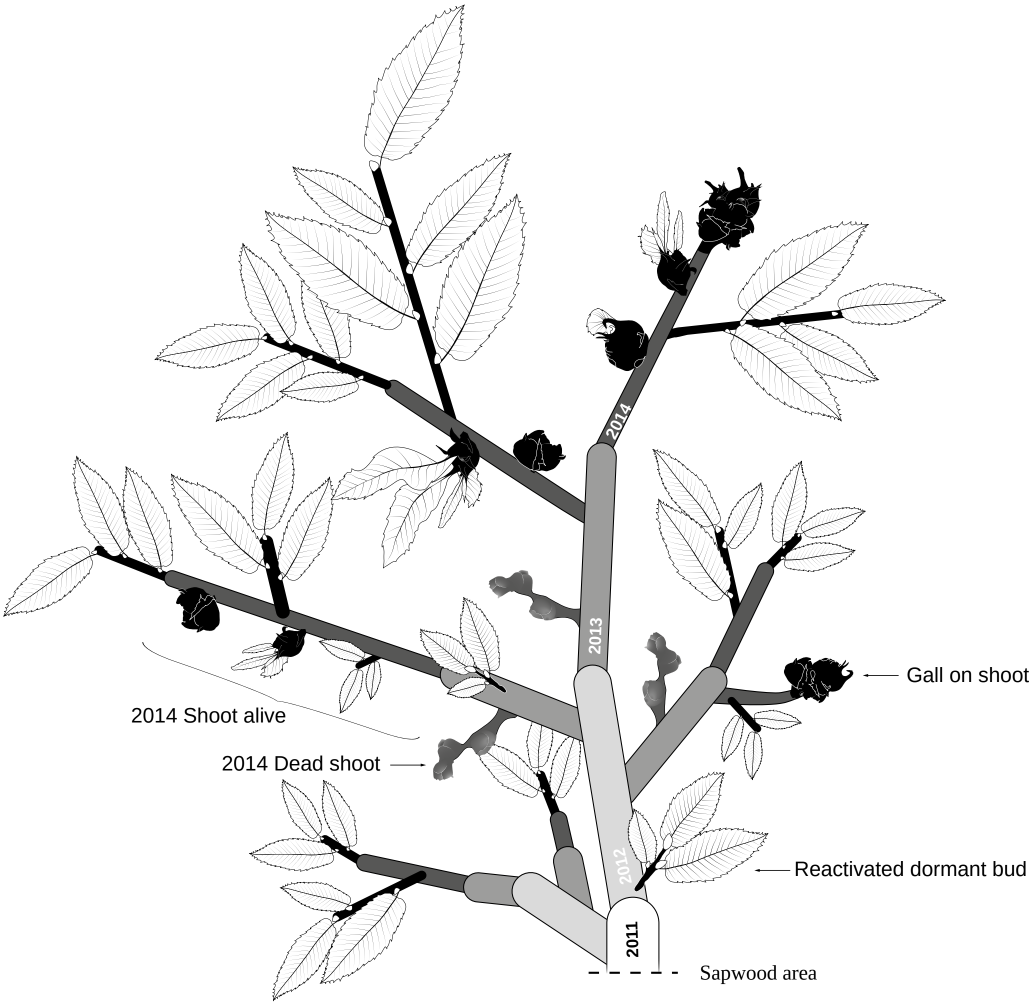
Appendices

Figure A1 Selected examples of branch damage and reactions by *Dryocosmus kuriphilus*. Left: branch significantly recovering on the apex but still lacking basal lateral branches. Tree reactions – Top right: reactivated basal dormant buds on a branch that was severely attacked by the gall wasp. Bottom right: bud from the year that produced a second flush.

Figure A2 Assessing gall wasp damage using the infestation index based on the mean number of galls per bud (GB). The sketch shows two branches with the same level of GB (11 galls / 8 buds) but with very different leaf area losses. Please refer to Figure 2 and Table 2 for more details.

Figure A3 Calculation of the DCI using a branch from the 2015 vegetative season (July). Please note that only the organs and structure considered for the assessment are represented in the sketch. A branch with at least 10 shoots is required to compute the DCI.



Dead shoots = No. dead shoot 2014 / No. shoots 2014

Gall on shoots = No. gall on shoot / No. shoots 2014 alive

React. dorm. bud = No react. dorm. bud / No. shoots 2014 alive

DCI = (Dead shoots * 0.479 + React. dorm. bud * 0.525 + gall on shoot * 0.120) * 100

DCI = (3 / 11 * 0. 479 + 2 / 7 * 0.525 + 8 / 7 * 0.120) * 100 = 41.8

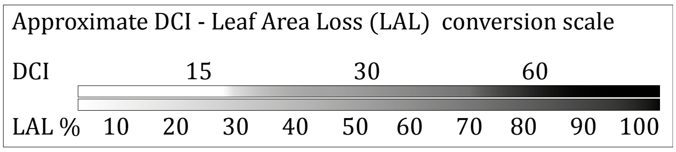


Table A1 Evolution of the methods of assessing the *Dryocosmus kuriphilus* (MAID) infestation degree according to the training dataset and sampling year

Training dataset				
Year	GS[a]		AB[b]	
	mean	*(±sd)*	*mean*	*(±sd)*
2010	0	(± 0)	0	(± 0)
2012	0.7	(±0.86*)*	0.19	(± 0.16)
2014	2.22	(±1.46*)*	0.41	(± 0.21)
2015	1.52	(±1.01*)*	0.29	(± 0.14)
2016	1.01	(±1.49*)*	0.15	(± 0.17)

[a]GS = No. galls / shoot [b]AB = No. attacked buds / No. Buds

2.2

Evaluating *Dryocosmus kuriphilus*-induced damage on *Castanea sativa*

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Branch of a chestnut tree heavily disrupted by repeated *Dryocosmus kuriphilus* attacks

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Abstract

Dryocosmus kuriphilus Yasumatsu has become a major pest for *Castanea sativa* since its arrival in Europe. Its galling activity results in the formation of different gall types and prevents the development of normal shoots. Repeated and uncontrolled attacks cause, besides the production of galls and the attendant gall-related reduction in leaf area, progressive corruption of the branch architecture including the death of branch parts and an increase in dormant bud activation. Thus far, there have been few attempts to quantify branch architecture damage. Further, the different methods for assessing infestation degree (MAID) that have been developed focus only on the presence and abundance of the galls

Using the leaf area to sapwood area relationship as a green biomass indicator, we developed in a previous study a damage composite index (DCI) that considers the most important branch architectural features allowing for realistic damage assessment during the entire epidemic process.

The aim of this study is to present this novel method and highlight differences in the damage description with respect to other broadly used indices. Results show how the DCI depicts branch damage better, especially during the epidemic peak, compared to MAID, which tend to underestimate it. We conclude, suggesting how to properly evaluate the overall impact of the pest by means of our composite damage index, the infestation degree using classic methods, and crown transparency evaluations.

Keywords Pest assessment, branch assessment, tree damage, branch damage, dead branches, tree reaction, *Torymus sinensis*, *Dryocosmus kuriphilus* infestation rate, epidemic evolution.

Introduction

The chestnut gallwasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is the most significant global insect pest of the genus *Castanea* (Stone et al. 2002; Abe et al. 2007; Aebi et al. 2011). Through its repeated galling activity, it prevents and inhibits normal shoot development (Maltoni et al. 2012; Gehring et al. 2018a), causing a progressive reduction of leaf area and a consequent loss of tree green biomass and vigour (Kato and Hijii 1997; Gehring et al. 2018a), dormant bud reactivation (Gehring et al. 2018a) and an increase in gallwasp post-emergence branch mortality (Turchetti et al. 2010; Meyer et al. 2015).

The European experience of the gallwasp epidemic shows that uncontrolled and repeated gallwasp attacks may induce a high level of crown corruption in Sweet chestnut (*Castanea sativa* Mill.). This can result in crown leaf area losses of up to 70% that are neither compensated by substitutive foliage produced by the activation of dormant buds nor by building second flushes during the same vegetation period (Gehring et al. 2018a).

The only successful method to reduce the pest population and allow chestnut trees to recover is biological control through its natural antagonist the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Moriya and Adachi 2003; Quacchia et al. 2014). Once biological control through its natural enemy is achieved, the chestnut trees start to produce new healthy sprouts. If tree damage level is very high, this may occur starting from the terminal bud only, due to the fact that it is usually infestation-free because of its formation after gallwasp oviposition activity (Maltoni et al. 2012). This implies a long recovery process before the whole tree crown is re-established (Gehring et al. 2018a).

In order to check the positive reaction of chestnut trees after biological control by *Torymus sinensis* is reached, and to verify the need for sylvicultural intervention (pruning, thinning), forest managers and chestnut growers need a method for quick and reliable assessment of damage level and related branch architecture and leaf area evolution throughout the gallwasp epidemic from the initial infestation phase by the pest to recovery after biological control by its antagonist. Several methods for assessing gallwasp infestation degree (MAID) have been developed and used worldwide to date, such as measuring the proportion of attacked buds (Kotobuki et al. 1985) or the average number of galls per bud (Sartor et al. 2015) MAID do not directly measure green biomass (e.g., leaf area), reserve structures such as dormant buds, reaction structures (e.g., reactivated dormant buds and second flushes), or previous year damage (e.g., dead shoots) as major proxies of current tree vitality and vigour (Kato and Hijii 1997; Johnstone et al. 2013; Guyot et al. 2015). Moreover, most MAID are only based on the number of galls found on tree branches and underestimate real branch damage, especially during the peak of the pest epidemic (Fig. 1).

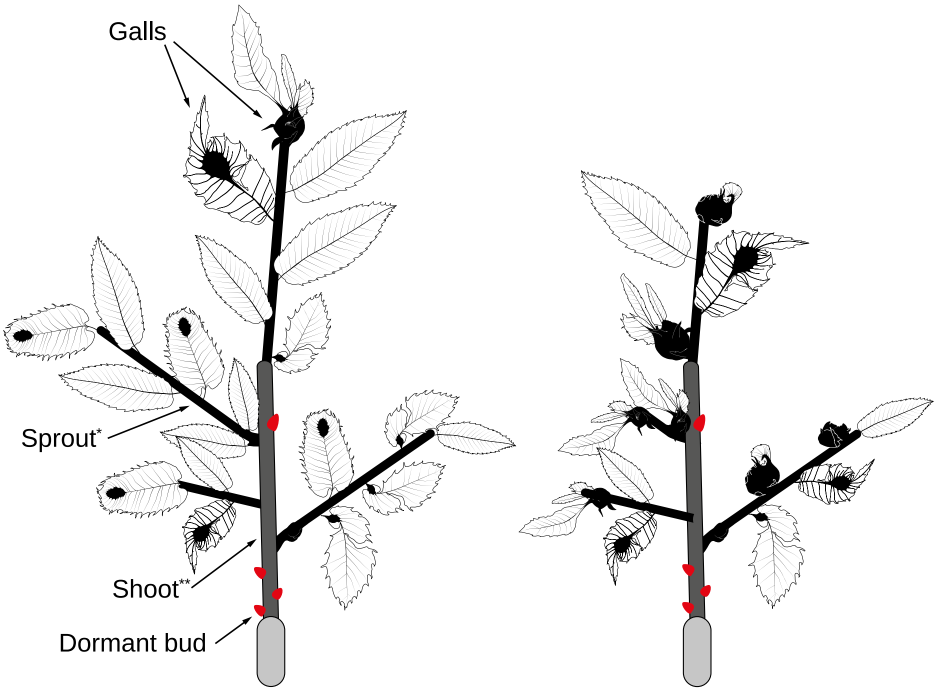


Figure 1 Branch damage evaluation illustrating the limitations of classic methods for assessing gallwasp infestation degree (MAID). The sketches show two branches with the same degree of GB (11 galls / 8 buds) and AB (4 attacked buds / 8 buds) but with very different leaf area losses. *Sprout: grown during the current vegetative season; **Shoot: sprout from the previous vegetative season.

In this paper, we describe the damage composite index (DCI) approach proposed by Gehring et al. (2018a) that considers proxies of green biomass, reserves such as dormant bud, and tree reactions (dormant bud reactivation and second flushes), enabling a realistic, reliable, and reasonably rapid assessment of damage through all stages of an epidemic, especially when combined with the assessment effort optimization proposed by Gehring et al. (2018b).

In particular, the objectives of this paper are: 1) to give a detailed description of the field protocol, including the relevant branch features to be assessed; 2) to present the damage composite index formula; 3) to propose an improved severity scale conversion for the DCI.

Protocol

1. Tree selection and assessment design

1.1. If possible, identify the epidemic stage of the study area by determining the arrival years of *Dryocosmus kuriphilus* and *Torymus sinensis* and the *T. sinensis* parasitization rate by using reliable sources (e.g., scientific publications, forest services, chestnut grove managers' knowledge).

1.1.1. If no reliable sources are available, the four main epidemic stages (Early, Peak, Recovery, Biocontrol) can be identified by computing the *T. sinensis* parasitization rate combined with the field observations described below.

1.1.2. Early epidemic: tree crowns show neither significant damage nor crown transparency; current year galls are rare and *T. sinensis parasitization is very low or negligible*.

1.1.3. Epidemic peak: tree crowns display a high degree of transparency, although dead branches are rare. Current year galls are abundant.

1.1.4. Prolonged epidemic peak: current and previous years' galls are abundant (up to three years previous) and *T. sinensis* parasitization is still very low or absent. Tree crowns still display high transparency and additional damage is represented by the first evidence of dead branches in the crown.

1.1.4. Early recovery stage: *T. sinensis* parasitization rate becomes greater than 75%[10]. Damage is still high but the number of current year galls decreases and some branches produce gall-free shoots, especially from the apical bud.

1.1.5. Recovery stage: *T. sinensis* parasitization rate is permanently greater than 75%. Current year galls are rare and usually limited to single trees only. Most branches produce gall-free shoots. Past years' galls on older branches and dead branches due to *D. kuriphilus* attacks are still visible.

1.1.6. Fully recovered stage: damage and galls (past and current year) are rare or absent. Crowns are fully recovered. In severely damaged trees, some vestiges (e.g., dead shoots or rotten past years' galls) of prior *D. kuriphilus* attacks can still be present inside the crown.

Note: Appendix – Figure A1 shows exemplary tree crown pictures for each epidemic stage.

1.2. Observe chestnut trees in the whole area to visually estimate damage variability among and within trees. Damage variability is usually low during the early epidemic and the recovery stages (crowns are basically healthy) as well as during the epidemic peak (whole crowns are full of galls). In contrast, variability tends to be high in the intermediate epidemic stages, when dead shoots due to past *D. kuriphilus* attacks are still present.

1.3. Based on 1.1 and 1.2, determine the number of trees to analyze. Unfortunately, it is not possible or suitable to give a specific rule regarding sample size which may vary according to the specific epidemic situation in the field and/or the research objectives. Based on our 10 years of experience, for a 10-hectare site we advise the following (also see Table 1 for details):

Table 1 Minimum number of trees and branches required based on epidemic stage and *Torymus sinensis* parasitization rate

Epidemic stage	Years since arrival		Ts%	Tree	Branch
	Dk	Ts	%	n. per site	n. per tree
Early	1-2	0-1	0-5	10	1
Peak	3	2-3	>5 <70	10	1-2
Prolonged peak	4-5	2-3	>5 <30	10	2-3
Early recovery	6-7	4-5	>70	10	2-3
Recovery	8-10	6-8	>70	10	2-3
Fully recovered	>10	>9	>70	10	1

Dk = *Dryocosmsus kuriphilus*; Ts = *Torymus sinensis*; Ts% = *Torymus sinensis* parasitization rate calculated as follows: number of living *T. sinensis* / total number of chambers * 100 (at gall level).

1.3.1. Sample at least ten trees per site, regardless of the epidemic stage. Although during the early epidemic and recovered stage three trees would be enough, increasing the sample size to ten will give more statistical power to the results.

1.3.2. During the early epidemic and recovered stage, sample one branch per tree.

1.3.3. During the epidemic peak, sample one branch per tree if galls are evenly distributed within the tree crown, or two branches per tree if you notice that some crown parts have been attacked more severely.

1.3.4. During the other epidemic stages, increase the number of branches to two (for trees that are recovering well) or three (for more damaged trees) based on the variability of crown damage of every tree.

2. Data collection in the field

2.1. Prepare the appropriate equipment including a clipboard, a camping chair, secateurs, a telescopic tree pruner, a 30m measuring tape, and tree climbing equipment if the top crown above 8 meters requires analysis.

2.2. Select the most representative branches trying to proportionally cover branch diversity within the tree crown (healthy trees usually have similar branches while damaged trees may have branches with different degrees of damage). For example, if you choose to collect three branches per tree, collect the most damaged branch, the healthiest and an intermediate one.

2.3. Whenever possible, select architectural branches only, while avoiding reiterations (trunk suckers or reiterations *sensu* Hallé) (Hallé et al. 1978)

2.4. Branches should be at least 50 centimeters in length and have at least 10 shoots.

2.5. Attach the beginning of the measuring tape near the blade of the telescopic tree pruner in order to measure the height above ground of the branch at the cutting point.

2.6. Cut the branch with the telescopic pruner, record its cutting height, its aspect, its type (architectural or reiteration) and refine the branch selection with secateurs in order to keep only the part for analysis.

2.7. Assign a unique ID to the branch and record its age, its maximum length (from the first branching point to the apex) for general information.

2.8. Take a quick look at the whole branch to obtain a first impression of its history and present status (heavily attacked or not) and identify all the elements and features important for the calculation of the DCI with the help of figures 2 and 3.

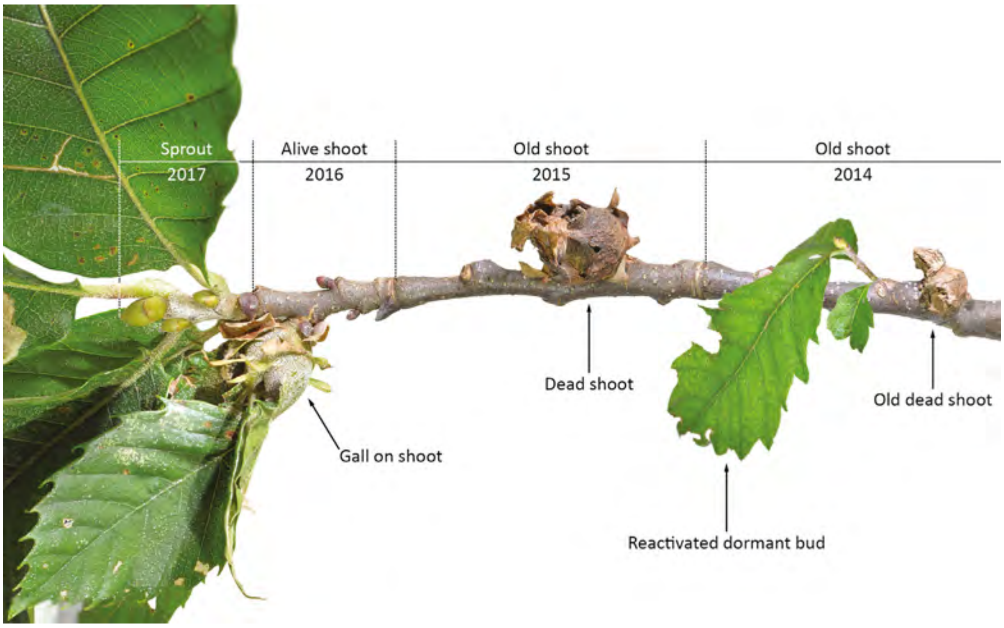
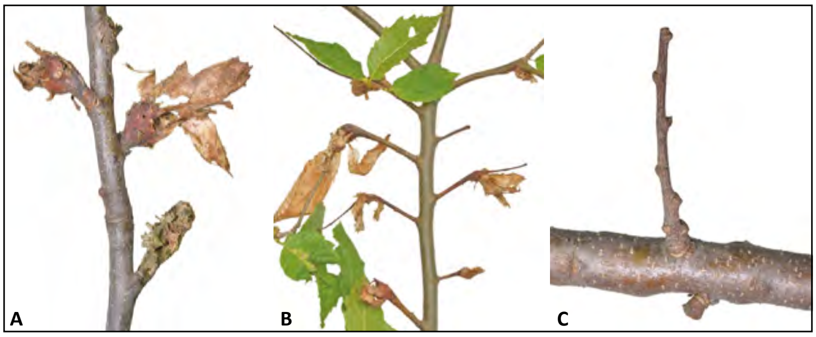
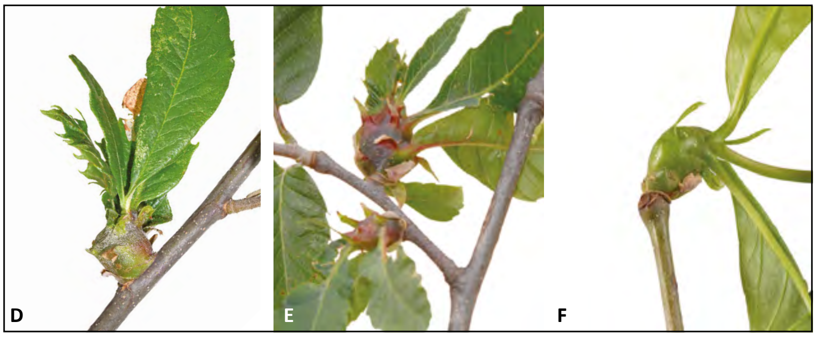


Figure 2 Main features considered when calculating the damage composite index. The dead shoot in the photo is technically a gall on shoot that grew in the previous vegetative season (2016) and completely prevented the elongation of the 2016 shoot. Because the entire gall is dead and no living buds are present on it, it is considered a dead shoot in 2017.

Dead shoot



Gall on shoot



Reactivated dormant bud

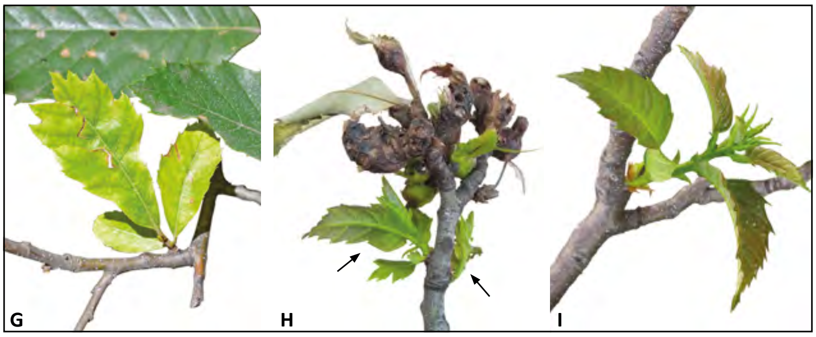


Figure 3 Essential branch features for assessing the damage composite index (DCI). The dead shoots in (A) and (B) are technically galls on shoots that grew during the previous vegetative season, completely prevented the elongation of the shoot, and are now dead. The dead shoot in (C) died for other causes. The rotten shoot at the bottom of (A) was already dead the year before the dead shoot present on that branch, and is consequently not considered in the DCI calculation. (D), (E) and (F) show different examples of galls on shoots, whereas (G), (H) and (I) represent reactivated dormant buds.

3. Branch feature definition

The following definitions are partially or totally reproduced from Gehring et al. (2018a), with the permission of Springer-Verlag Berlin Heidelberg 2017.

3.1. Sprout (on a shoot): Freshly formed sprout that has grown during the current vegetative season from a developed bud on a shoot.

3.2. Shoot: Sprout from the previous vegetative season with respect to the sampling date (i.e., sampling season = 2017, shoot = sprout that grew in 2016). Can be dead or alive.

3.3. Dead shoot (Sd): dead shoot after *D. kuriphilus attack* or due to natural death.

3.4. Alive shoot (As): alive a living shoot. Not to be confused with a reactivated dormant bud.

3.5. Reactivated dormant bud (Bdor): freshly formed sprout that has grown during the current vegetative season from a dormant bud on a multiyear branch part that is older than the shoot.

3.6. Gall on shoot (Gons): gall developed at the base or along the axis of a sprout. Technically, these should be called “galls on sprouts” but for the purpose of consistency with existing literature, we refer to them as “galls on shoots”.

Figures 2 and 3 show examples of selected branch features. More detailed and complete descriptions (which are beyond the scope of this paper) may be found in Gehring et al. (2018a) and Maltoni et al. (2012).

4. Branch analysis

4.1. Count and record all the living shoots (alive shoots).

4.2. Count and record all the dead shoots.

4.3. Count and record all the reactivated dormant buds.

4.4. Count and record all the galls on shoots.

Note: Appendix – Table A1 shows an example of a field sampling form and Appendix – Table A2 shows the sampling form filled out.

5. Calculation of the damage composite index

5.1. Calculate the proportion of dead shoots (Sd) from the number of dead shoots divided by the total number of shoots (dead shoots + alive shoots).

5.2. Calculate the proportion of reactivated dormant buds (BdoR) from the number of reactivated shoots divided by the total number of living shoots (BdoR + alive shoots).

5.3. Calculate the average number of galls on shoots (Gons) from the number of galls on shoots divided by the number of living shoots (BdoR + alive shoots).

5.4. Calculate the DCI using the following formula:

$$\mathrm{DCI} = (\mathrm{Sd} * 0.479 + \mathrm{BdoR} * 0.525 + \mathrm{Gons} * 0.120) * 100$$

5.5. Use Table 2 to evaluate the damage severity.

Note: An R script with the DCI function and its description is available in Appendix - Coding File A1.

Table 2 Conversion scale for the three indices: damage composite index (DCI), number of galls per bud (GB) and attacked buds (AB)

Conversion scale		**DCI**	**GB**	**AB**
Damage severity	*Points*		Average per shoot	Proportion
No damage	1	≤2.5	≤0.1	≤0.1
Very low	2	>2.5 - ≤5	>0.1 - ≤0.2	>0.1 - ≤0.2
Low	3	>5 - ≤7.5	>0.2 - ≤0.3	>0.2 - ≤0.3
Mild	4	>7.5 - ≤10	>0.3 - ≤0.4	>0.3 - ≤0.4
Moderate	5	>10 - ≤15	>0.4 - ≤0.5	>0.4 - ≤0.5
High	6	>15 - ≤20	>0.5 - ≤0.6	>0.5 - ≤0.6
Very High	7	>20 - ≤25	>0.6 - ≤0.7	>0.6 - ≤0.7
Extreme	8	>25 - ≤30	>0.7 - ≤0.8	>0.7 - ≤0.8
	9	>30	>0.8	>0.8

DCI is rescaled according to Gehring et al. (2018), GB according to Sartor et al. (2015), and AB according to Gyoutoku and Uemura (1985).

Results

A total of 25 localities in Ticino, Switzerland were visited between 2013 and 2016 in order to create a temporal gradient covering all gall wasp epidemic stages. In total, we collected and analyzed 94 branches in 5 sites at an early epidemic stage (arrival of the pest and beginning of tree damage), 200 branches in 5 sites at the epidemic peak (medium to severe damage due to high level of *D. kuriphilus* attack), 200 branches in 5 sites at the recovery stage (biocontrol by *T. sinensis* reached and start of progressive tree recovery) 54 branches in 5 sites where the pest returned at a very low level over the past 4-5 years. The DCI and two MAID (i.e., “average number of galls per bud” (GB) (Sartor et al. 2015) and the “proportion of attacked buds” (AB) (Kotobuki et al. 1985) were calculated for each branch. Since the three indices are on different scales, a standardized severity scale from 1 to 9 (1 = very low damage, 9 = extreme damage) is used in order to compare them. DCI is rescaled according to Gehring et al. (2018), GB according to Sartor et al. (2015) and AB according to Gyoutoku and Uemura (1985) (Table 2). Comparisons of DCI and MAID within branches were made using the nonparametric Wilcoxon signed-rank test with the Holm adjustment for *p* values.

At early stages of the epidemic, DCI and MAID are low (median values at 25th and 75th percentiles, DCI = 1 *[1, 1]*, AB = 1 *[1, 2]*, GB = 1 *[1, 2]*) and express the same level of damage ($p > 0.5$; Fig. 4). During the epidemic peak, the DCI indicates very high damage levels (DCI = 8 *[7, 9]*) compared to both MAID that indicate only intermediate branch damage level (AB = 4 *[3, 5]*, GB = 4 *[3, 6]*; Fig. 4). Considering branches with low GB and AB values (<3), 34 out of 71 and 29 out of 59 have more than 30% dead shoots, respectively, whereas from branches with medium GB and AB values (4 and 5), 19 out of 76 and 30 out of 108 have more than 40% dead shoots, respectively. This objectively indicates a high degree of damage. During the recovery phase, differences between DCI and MAID are smaller but still significant ($p < 0.01$), whereas no differences persist when the recovered phase is reached. Figure 5 provides a visual example of the possible causes of these dissimilarities during the different epidemic stages.

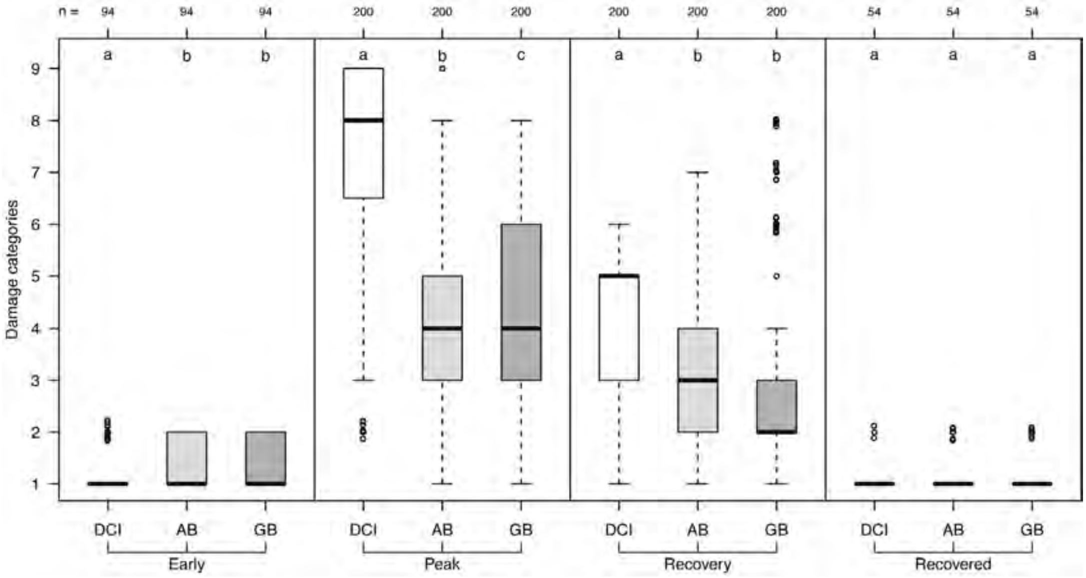


Figure 4 Evolution of the damage composite index (DCI) and two classic methods for assessing gallwasp infestation degree (MAIDs) across the different epidemic stages. Please refer to Table 2 for more details on the damage categories. DCI: damage composite index (see paragraph 5 of the protocol); AB: proportion of attacked buds (no. attacked buds / no. buds); GB: average number of galls per bud (no. galls / no. buds). Labels on top (n) indicate the number of sampled branches per epidemic stage. Different letters indicate significant differences ($p < 0.01$) between DCI and MAID branch values at epidemic stages based on the nonparametric Wilcoxon signed-rank test with the Holm adjustment for *p* values. Outliers are defined as any observation falling outside 1.5 times the interquartile range above or below the upper or lower quartile respectively. Please note that noise has been added to outlier data to avoid excessive overlapping.

Generally speaking, during the epidemic peak, GB and AB expressed the same damage level as DCI in only 5% of the cases, while tending to underestimate it in ca. 85% of the cases. During the recovery phase, correspondence between GB and DCI and between AB and DCI occurred in 12% and 14% of the cases, respectively. Both MAID underestimated damage in 70% of the cases.

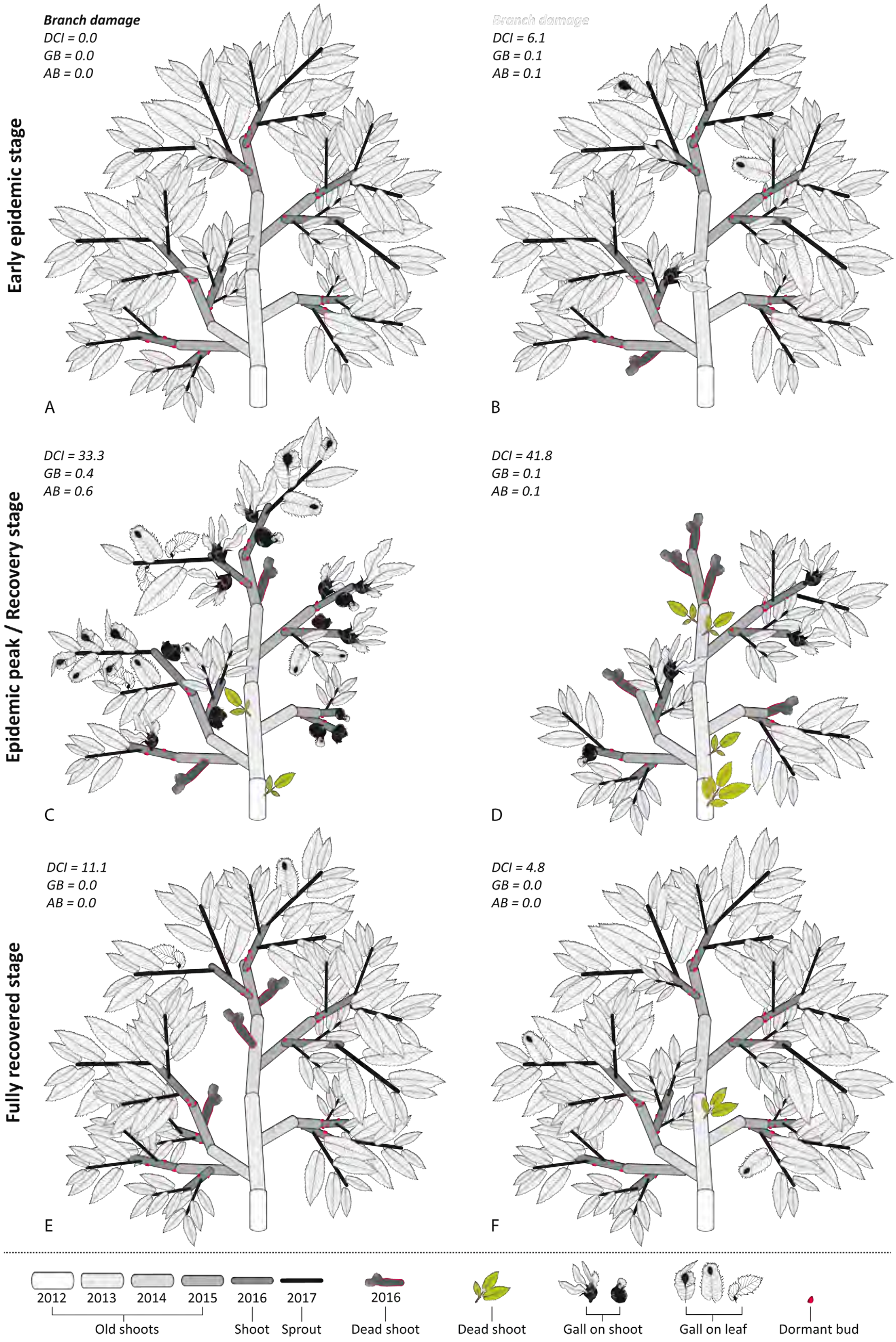


Figure 5 Schematic depictions of chestnut branches at different *Dryocosmus kuriphilus* epidemic stages. At early epidemic stages (A, B), branch architecture is still intact and both the damage composite index (DCI) and MAID (GB and AB) have very low values. Especially during the epidemic peak and recovery stages, branch architecture may become heterogeneously corrupted with different types and degrees of damage. The damage expressed by DCI, GB, and AB can thus be similar (C) or completely different (D) depending on the severity of branch corruption. Finally, at the recovered stage, DCI and MAID again have similarly low values with the DCI being somewhat more sensitive to previous year damage (dead branches).

Discussion

Dryocosmus kuriphilus lay eggs in chestnut tree buds, inducing the formation of galls in spring. Repeated and uncontrolled *D. kuriphilus* attacks cause, in addition to gall formation, general branch corruption including the death of many shoots and a significant loss in green photosynthetic leaf area (Gehring et al. 2018a). Trees usually react by attempting to produce substitutive shoots via the activation of dormant buds. For this reason, especially during the epidemic peak and the recovery stage, classic MAID (based on galls abundance only) tend to underestimate the real damage caused by *D. kuriphilus* while the DCI, which is based not only on gall abundance but also on dead shoots and reactivated dormant buds, better reflect real damage severity and the degree of branch architecture alteration. In fact, MAID focus more on the population level of *D. kuriphilus* rather than on the degree of damage to trees. For example, when the branch damage is low and the presence of galls is insignificant during the early epidemic and the final, recovered stage, both MAID and DCI show very low values. However, during the epidemic peak or when exposed to several years of *D. kuriphilus* attack, a severely damaged branch may display many dead parts but only a few galls, or none at all. Using the criteria for each index, this would result in high DCI values (severe damage) but low MAID values (low damage).

It is thus important to understand the meaning and related damage degree indicated by each index in order to select the most suitable pest assessment approach according to the desired purpose. We therefore suggest evaluating the overall impact of the pest by using the DCI for the assessment of branch architecture damage, especially during the epidemic peak and the recovery stage. To ensure thorough pest assessment, classic MAID may be used to evaluate pest population levels (we suggest Gehring et al. 2018b for advice on how to adapt the sampling effort) whereas DCI may be used for a detailed assessment of branch architecture as well as for the entire tree crown. For a simple general assessment of crown transparency, in contrast, a tree assessment using the SANASILVA crown transparency index (Müller and Stierlin 1990) may be the most suitable in terms of effort-benefit balance.

Once the operator becomes familiar with the main branch structures and features needed, applying the DCI is quite simple and relatively fast. However, in case of highly damaged branches due to repeated *D. kuriphilus* attacks, it could be difficult to properly assess the number of dead shoots because of the presence of older dead branches. It is thus important to try to reconstruct the branch history and to evaluate the degree of rot in the dead branches and shoots. Usually, dead shoots are not rotten or are less so compared to dead branches.

At the tree level, the major difficulty when evaluating the average branch damage of a large tree with heterogeneous crown damage (e.g., a tree displaying healthy, damaged, and dead branches) regards the quantity of branches for analysis and the tree climbing skills and effort needed to reach the top of the crown. Moreover, since the method is destructive, evaluating a damaged tree will inevitably inflict a temporary additional loss of green biomass. It is therefore important to only select the smallest number of most representative branches in order to avoid excessive cutting.

Deciding on the correct sample size can sometimes be tricky. Based on our experience we note that in both the early epidemic and the fully recovered stage, damage on trees is absent and, consequently, its variability is very low. Consequently, sampling 10 trees per site and 1 branch per tree already gives a fair estimate of the damage. In contrast, during the epidemic peak and the recovery stage, trees show different damage levels. A good balance between sampling effort, data accuracy, and tree damage infliction can be reached by collecting 3 branches per tree for a total of 10 trees per site. Please note that this advice is based on our experience and research needs. Others are free to increase or decrease sample sizes according to their specific situation and assessment goals.

Finally, it is possible that at the beginning of the recovered phase, when almost no galls or dead shoots are visible on branches any longer, the DCI (and MAID) underestimate the residual damage present on the branches. This residual damage is represented by the possible lack of lateral shoots and dormant buds that occur especially in severely damaged trees that have suffered from repeated *D. kuriphilus* attacks over the years (Gehring et al. 2018a). The lack of lateral shoots implies that a part of the leaf area is still missing, whereas the lack of dormant buds indicates that the tree hasn't yet fully recovered its reserves.

Future or other applications of the DCI are difficult to imagine because it is species-specific and the constants applied in its calculation are calibrated to the chestnut tree (Gehring et al. 2018a). Nevertheless, the methodology used to create it could be adapted and implemented to any other tree species and related pest.

Acknowledgements

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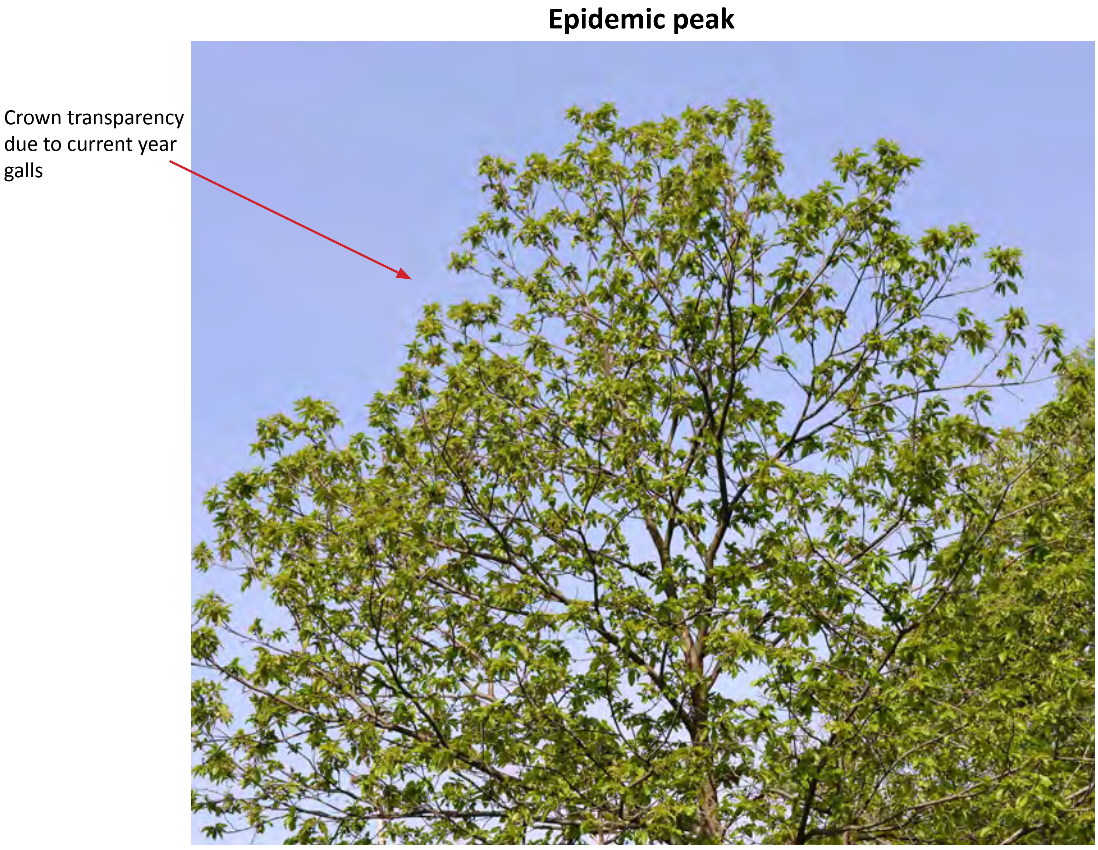
Appendices

Figure A1 Exemplary tree crown pictures for each epidemic stage.

Early epidemic

Figure A1 continued.



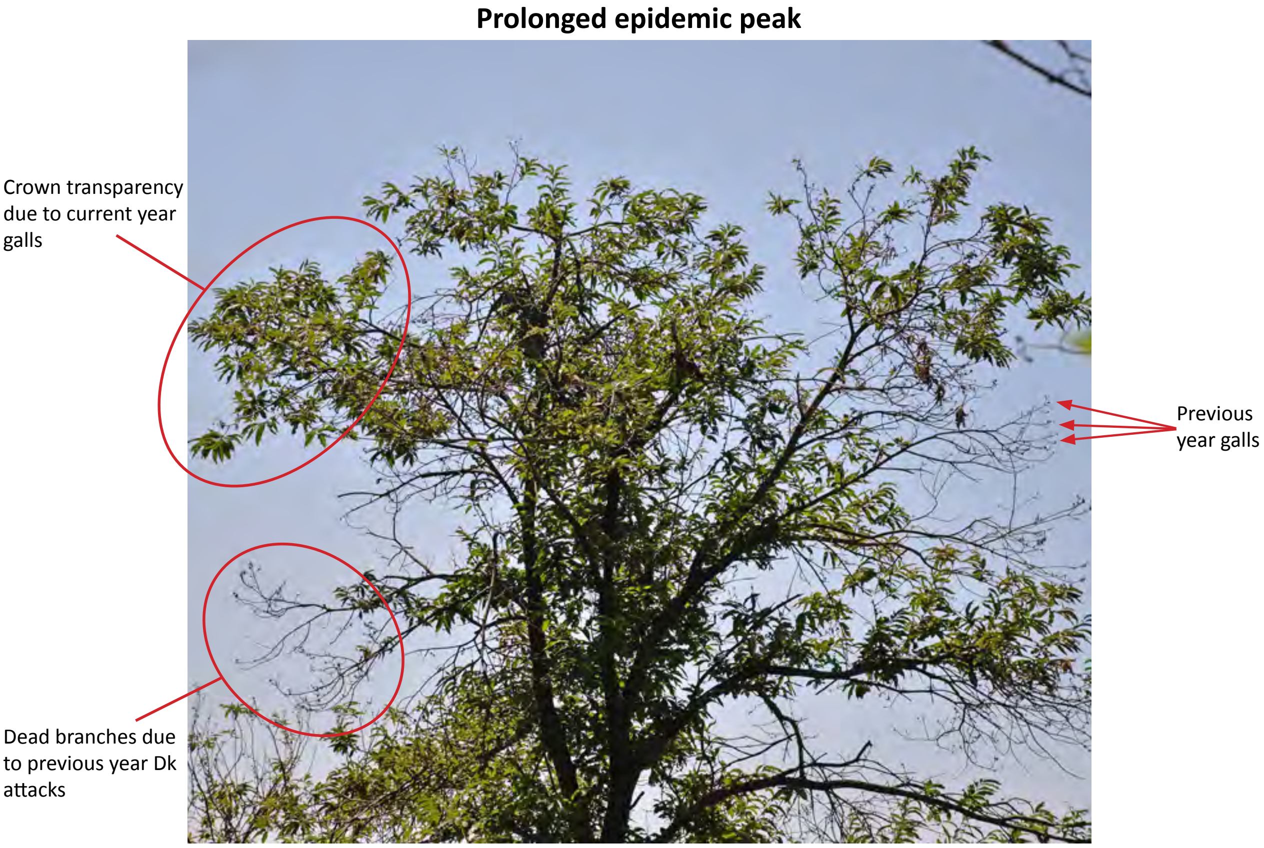
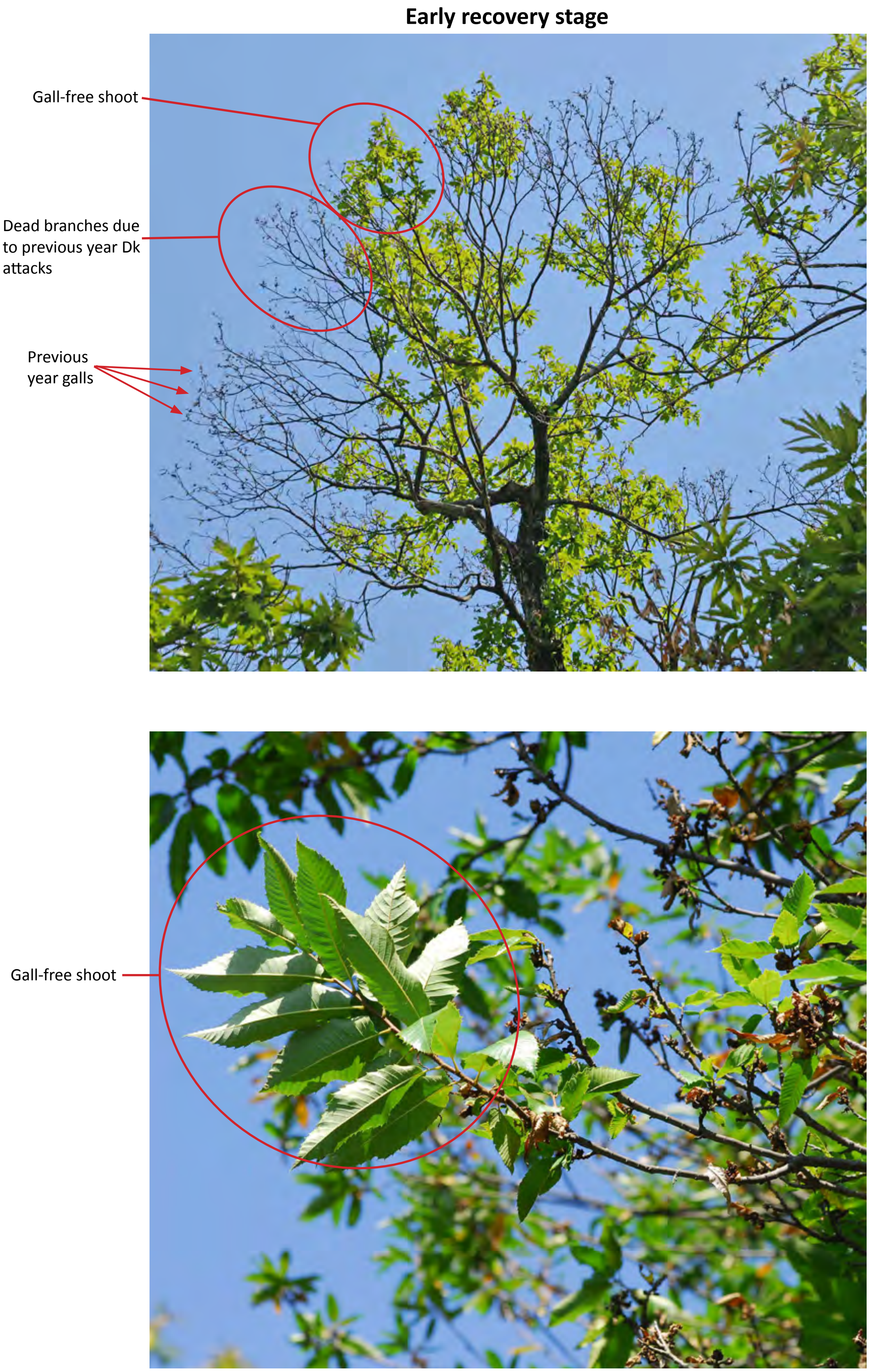


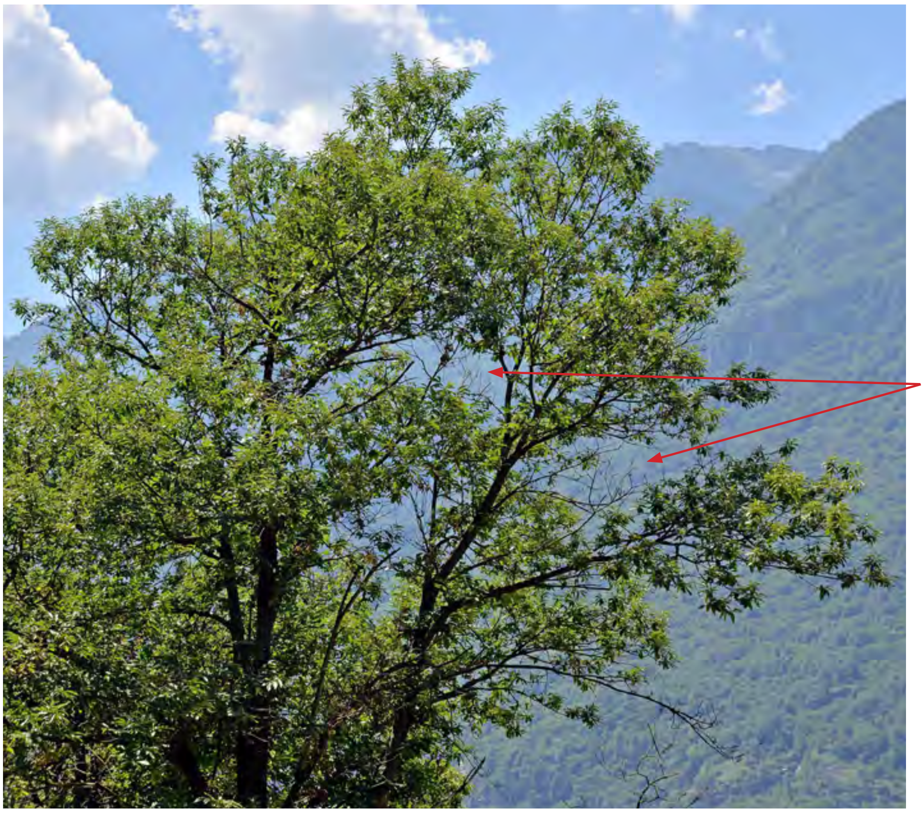
Figure A1 continued.



Early recovery stage

Figure A1 continued.

Recovery stage



Dead branches due to previous year Dk attacks

Dead branches due to previous year Dk attacks

Dead branches due to previous year Dk attacks

Figure A1 continued.

Fully recovered stage

Inside the tree crown

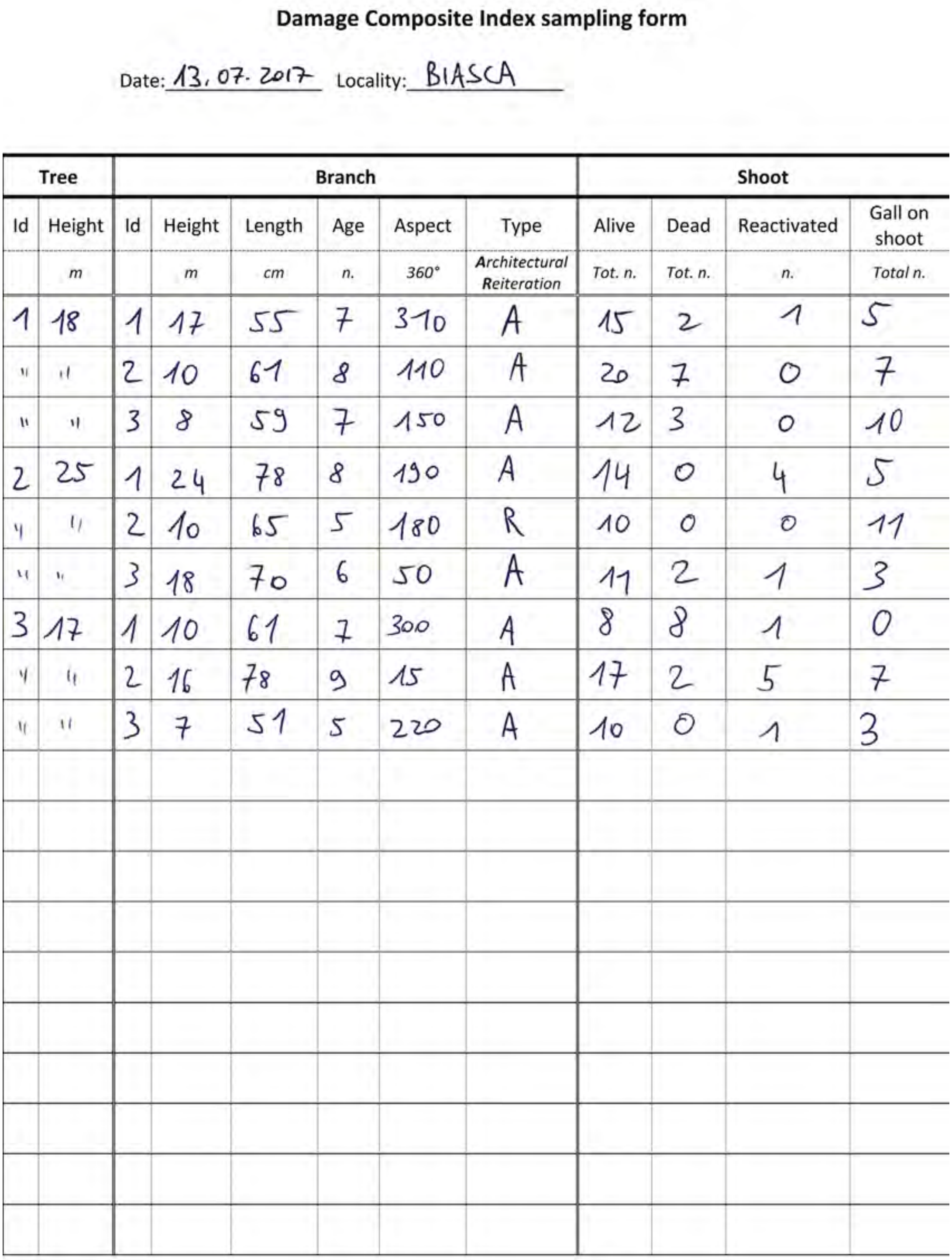
Table A1 Example of a field sampling form.

Damage Composite Index sampling form

Date:________________ Locality:________________

Tree		Branch						Shoot			
Id	Height	Id	Height	Length	Age	Aspect	Type	Alive	Dead	Reactivated	Gall on shoot
	m		*m*	*cm*	*n.*	*360°*	***A**rchitectural* ***R**eiteration*	*Tot. n.*	*Tot. n.*	*n.*	*Total n.*

Table A2 Example of a field sampling form filled out.



Damage Composite Index sampling form

Date: 13. 07. 2017 Locality: BIASCA

Tree		Branch						Shoot			
Id	Height	Id	Height	Length	Age	Aspect	Type	Alive	Dead	Reactivated	Gall on shoot
	m		*m*	*cm*	*n.*	*360°*	*Architectural Reiteration*	*Tot. n.*	*Tot. n.*	*n.*	*Total n.*
1	18	1	17	55	7	310	A	15	2	1	5
"	"	2	10	61	8	110	A	20	7	0	7
"	"	3	8	59	7	150	A	12	3	0	10
2	25	1	24	78	8	190	A	14	0	4	5
"	"	2	10	65	5	180	R	10	0	0	11
"	"	3	18	70	6	50	A	11	2	1	3
3	17	1	10	61	7	300	A	8	8	1	0
"	"	2	16	78	9	15	A	17	2	5	7
"	"	3	7	51	5	220	A	10	0	1	3

Coding File A1 R script with the DCI function and its description.

Description

Calculate the Damage Composite Index (DCI) described in Gehring et. al 2017.

dci() takes four vectors as arguments, calculate the dci, and return the result in a single vector.

dci_level() takes one vector with the dci value as argument and return the correspondent severity level in a single vector.

add_dci() add a column called damage_composite_index to the dataframe with dci values, optional with damage severity level

Usage

dci(alive_shoot, dead_shoot, reactivated_dormant_bud, gall_on_shoot)

dci_level(dci)

add_dci(data, dci_severity_level = FALSE)

Arguments

alive_shoot: numeric vector with the total number of shoots alive per sampled branch. An alive shoot is a sprout alive from the previous vegetative season with respect to the sampling date (i.e., sampling season = 2017, shoot = sprout that grew in 2016).

dead_shoot: numeric vector with the total number of shoots dead per sampled branch. A dead shoot is a sprout from the previous vegetative season with respect to the sampling date (i.e., sampling season = 2017, shoot = sprout that grew in 2016) that died after *Dryocosmus kuriphilus* attack or due to natural death.

reactivated_dormant_bud: numeric vector with the total number of reactivated dormant buds per sampled branch. A reactivated dormant bud is a freshly formed sprout that has grown during the current vegetative season from a dormant bud on a multiyear branch part that is older than the shoot.

gall_on_shoot: numeric vector with the total number of galls on shoot per sampled branch. A gall on shoot is a gall developed at the base or along the axis of a sprout. Technically, these should be called “galls on sprouts” but for consistency purposes with existing literature we refer to them as “galls on shoots”.

dci: numeric vector with damage composite index values.

data: a data.frame including the variables needed to calculate the dci: alive_shoot, dead_shoot, reactivated_dormant_bud, gall_on_shoot. Note: please

dci_severity_level: a logical indicating whether the column dci_severity_level should be added to the data.frame.

Example

```
# Create a dataframe
df <- data.frame(
  locality = c(rep("Lavorgo",9), rep("Stabio", 10), rep("Losone",2)),
  tree_id = c(rep(1, 3), rep(2, 3), rep(3, 3), 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 1:2),
  tree_height = c(18, 18, 18, 22, 22, 22, 25, 25, 25, 22, 22, 20, 20, 19, 19, 25, 25, 24, 24, 22, 28), # metre
  branch_id = c(rep(1:3, 3), rep(1:2, 5), rep(1, 2)),
  branch_height = c(18, 7, 4, 21, 16, 5, 7, 18, 25, 22, 15, 19, 14, 13, 19, 25, 18, 24, 17, 22, 27), # metre
  branch_max_length = c(75, 53, 112, 110, 100, 98, 65, 78, 51, 66, 58, 87, 81, 72, 67, 53, 89, 115, 59, 70, 52),# centimetre
  alive_shoot =c(15,10,17,22,8,12,14,15,9,10, 6, 7, 15, 12, 10, 10, 14, 11, 12, 20, 15),
  dead_shoot = c(1, 0, 5, 10, 2, 1, 1, 5, 1, 1, 7, 4, rep(0,5), 0, 3, 4, 4),
  reactivated_dormant_bud = c(1, 4, 0, 0, 0, 4, 1, 2, 2, 0, 0, 0, 1, 1, 2, 1, 0, 3, 5, 1, 5),
  gall_on_shoot = c(1, 14, 0, 12, 5, 5, 0, 0, 10, 15, 1, 1, 5, 5, 2, 1, 6, 0, 0, 4, 2))

# add two columns to data.frame “df”: damage_composite_index and dci_severity_level
df <- add_dci(data = df, dci_severity_level = T)
```

R-code

```
dci <- function(alive_shoot, dead_shoot, reactivated_dormant_bud, gall_on_shoot) {

        # Calculate DCI variables
        ds_prop <- dead_shoot/(alive_shoot + dead_shoot + reactivated_dormant_bud)
        react_prop <- reactivated_dormant_bud/(alive_shoot + reactivated_dormant_bud)
        gons_me <- gall_on_shoot/(alive_shoot + reactivated_dormant_bud)

        # Compute DCI
        damage_composite_index <- round((gons_me*0.120 + react_prop*0.525 +
        ds_prop*0.479)*100,1)
        return(damage_composite_index)
}

dci_level <- function(dci) {
        lev <-cut(dci,
                breaks = c(0, 2.5, 5, 7.5, 10, 15, 20, 25, 1000),
                labels=c("No damage", "Very low", "Low", "Mild", "Moderate", "High", "Very High",
                "Extreme"),
                ordered=T, include.lowest = T)
        return(lev)
}

add_dci <- function(data, dci_severity_level = FALSE) {

        data$damage_composite_index <- dci(data$alive_shoot, data$dead_shoot,
        data$reactivated_dormant_bud,
        data$gall_on_shoot)

        # Add a column with damage severity level according to DCI value
        if(dci_severity_level) {
        data$dci_severity_level <- dci_level(data$damage_composite_index)
        }
        return(data)
}
```

Chapter 3

How can we measure the *Dryocosmus kuriphilus* and *Torymus sinensis* population dynamics?

3.1

Adapting sampling effort to assess the population establishment of *Torymus sinensis*, the biocontrol agent of the chestnut gallwasp

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Section of a gall with *Dryocosmus kuriphilus* (left) and *Torymus sinensis* (right) larva

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Abstract

Torymus sinensis Kamijo is to date the most effective controlling agent of the chestnut gallwasp *Dryocosmus kuriphilus*. The procedure to evaluate the parasitism rate of *T. sinensis* consists of dissecting *D. kuriphilus* galls collected in the field and checking them for its presence. Such a procedure is, however, time-consuming. Using a space-for-time substitution approach, by creating a temporal gradient within 15 sites and by means of bootstrap simulations, we analysed the effect of different sampling efforts on the precision of evaluating the success of *T. sinensis* release and the evolution of its parasitism rate towards biocontrol. Results show that after the first release, 200 galls are required to assess its presence with a 1% failure rate. Once *T. sinensis* is established, 100 galls are generally sufficient to estimate its parasitism rate by accepting a (median) percentage of error of 14% in the second year and 6% and 3% in the third and fourth year, respectively. Finally, we propose a series of design options to reduce the sampling effort according to the targeted accuracy level and as a function of the obtained parasitism rate.

Keywords Pest invasion, *Dryocosmus kuriphilus*, Effective monitoring, *Torymus sinensis* releases, Parasitism rate, *Castanea sativa.*

Introduction

After its accidental introduction to Piedmont (Italy) in the late 1990s (Quacchia et al. 2008) and its discovery in 2002 (Brussino et al. 2002), the Chinese chestnut gallwasp (*Dryocosmus kuriphilus* Yasumatsu) has become one of the major threats to the chestnut tree in most European chestnut countries (EPPO, 2017). If not controlled, repeated gallwasp attacks may induce over time crown leaf area losses of up to 70% (Gehring et al. 2017), fruit production losses of up to 85% (Bosio et al. 2013), and significant reductions in wood biomass production (Kato and Hijii, 1997). The inefficacy of chemical and mechanical control approaches, as well as the reduced long-term impact of chestnut variety selection programs as reported during the earlier Japanese outbreak in the 1940s (Moriya et al. 2003; Abe et al. 2007) have also been confirmed for the gall wasp epidemic on the European chestnut tree (*Castanea sativa* Mill.) (Maltoni et al. 2012a; Sartor et al. 2015). Similarly, natural biological control through European native parasitoids was found to be highly ineffective in preventing epidemic outbreaks of *D. kuriphilus* (Quacchia, Moriya & Bosio 2014; Francati et al. 2015).

Based on the successful experience of introducing the natural antagonist *Torymus sinensis* Kamijo from China's mainland to biologically control *D. kuriphilus* in Japan (Moriya et al. 2003), *T. sinensis* was introduced in 2005 into Piedmont (Italy) and tested as a biocontrol agent on *Castanea sativa* (Quacchia et al. 2008). The promising results of the Italian campaign (Bosio et al. 2013; Quacchia et al. 2014) soon stimulated similar releases and biocontrol campaigns in other chestnut countries (Borowiec et al. 2014; Matosevic et al. 2015; Matosevic et al. 2017). The use of *T. sinensis* as a controlling agent of the gallwasp epidemic has become standard in most European chestnut countries, despite potential environmental risks (Gibbs et al. 2011; Ferracini et al. 2017). The time required to rear individuals prior to release (Quacchia et al. 2008) and the initial slowness of the antagonist's spread in the field (Boroviec et al. 2013) suggest the need to develop targeted monitoring of the post-release effectiveness of the biological control program in order to optimize any subsequent additional releases and related costs (Colombari et al. 2016). Besides the typical in-field gall collection and the rearing of *T. sinensis* adults in order to check the presence of the antagonist, evaluating the exact parasitism rate of *T. sinensis* is usually performed by dissecting the collected *D. kuriphilus* galls and checking them for the presence of the characteristic *T. sinensis* larvae or pupae. Such a procedure, however, is time-consuming, and tailoring gall collection design for monitoring objectives may result in significant savings in terms of time and money.

In this study, we analyse the effect of different sampling efforts on the scored precision in evaluating the population establishment of *T. sinensis* and the related success of biocontrol treatment in two crucial phases, namely, (i) the establishment of *T. sinensis* at a significant and detectable level (which we have defined as a *T. sinensis* parasitism level (Ts%) of ca. 1%) and (ii) monitoring parasitism rate evolution until biocontrol (parasitism rate above ca. 70-75%, e.g. Quacchia, Moriya & Bosio 2014; Gehring et al. 2017) is achieved. To this purpose, the following specific questions have been tackled:

A) What is the minimum number of galls that require sampling in sites of recent *T. sinensis* release (or arrival) to avoid a false negative (*T. sinensis* in fact present in the site but not found)?

B) What is the minimum number of sampled galls required to confidently estimate the evolution of the parasitism rate at site level where the presence of *T. sinensis* has already been confirmed?

Materials and method

Study area

The study was carried out in an area including Ticino (southern Switzerland) and the eastern part of Piedmont (Italy) (Fig. 1). The area is characterized by homogeneous climate conditions (Ticino: annual mean precipitation from 1,800 to 2,100 mm, annual mean temperature ranging from 10.2 to 12.4°C - MeteoSwiss climatic normals 1981-2010; http://www.meteoswiss.admin.ch/home/climate/past/climate-normals.html; Varallo, eastern Piedmont: annual mean temperature ranging from 9.2 to 11.8 °C and an annual mean precipitation of 1,743 mm - period 1989-2016; http://www.arpa.piemonte.it/rischinaturali/accesso-ai-dati/annali_meteoidrologici/annali-meteo-idro/banca-dati-meteorologica.html) and bedrock (Blaser et al. 2005). In the study area, the chestnut tree has been cultivated for an extensive period of time (Conedera et al. 2004; Conedera & Krebs 2008) and nowadays this region forms a chestnut forest belt continuum with a mix of cultivated and naturalized stands that play an important role in slope stability (Vogt et al. 2006; Conedera et al. 2010).

In the Italian part of the study area (Piedmont), the chestnut gall wasp *Dryocosmsus kuriphilus* was observed in the Lake Orta region in 2006 (Brussino et al. 2002; Quacchia et al. 2008) and rapidly spread along Lake Maggiore, Lake Varese and the province of Como reaching the southern Swiss border in 2007 (Forster et al. 2009) and the western Swiss border (Brissago) in 2011. Subsequently, it spread quickly (within 5 years) throughout the whole chestnut forest in southern Switzerland (Gehring et al. 2017).

The biological control agent *T. sinensis* was released for the first time in the study area at lake Orta in 2008. Since then, many other releases have taken place annually throughout western Piedmont and northern Lombardy (Servizi Fitosanitari Regioni Piemonte e Lombardia, Boriani et al 2012), some of which were very close to the Swiss border. Unlike in Italy, the release of *T. sinensis* was not permitted in Switzerland by federal authorities due to open biosafety questions (BAFU 2012, http://www.bafu.admin.ch/biotechnologie/01760/08944/index.html?lang=it). Nevertheless, *T. sinensis* was officially found in 2013 throughout the entire chestnut belt of Canton Ticino as a consequence of its natural spread from Italy (including some rare cases of active introduction) that was estimated to be in 2011 based on the significant numbers found in some sites.

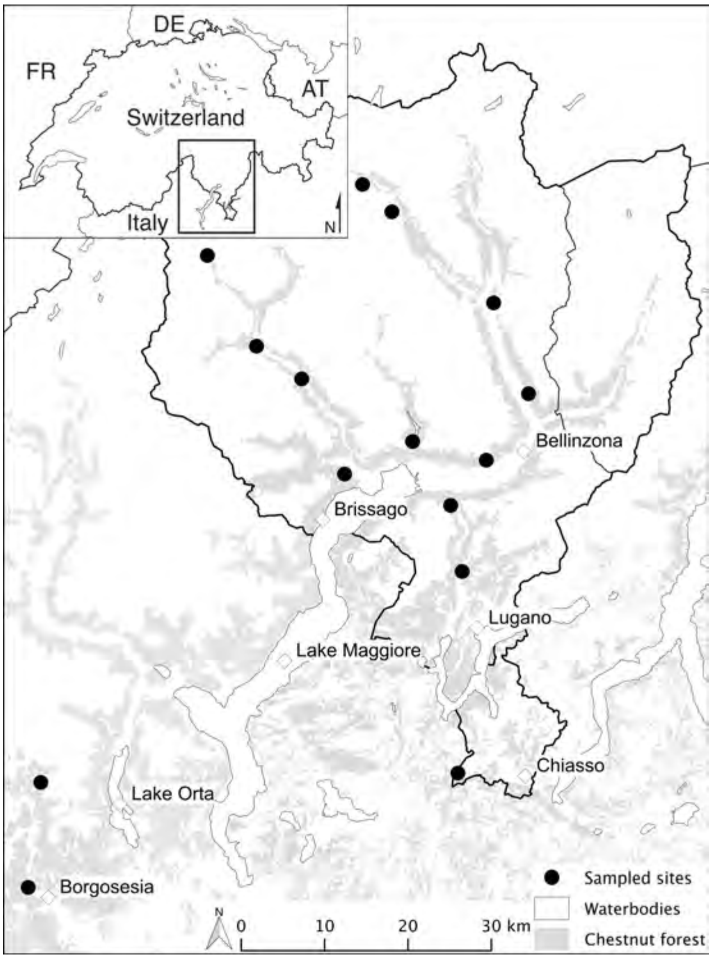

Figure 1 Study area with detailed locations of the study sites. The subfigure (top-left corner) shows the study area (bold rectangle) with respect to Switzerland.

Sampling design and data collection

We used a space-for-time substitution approach creating a temporal gradient within the study area (Fig. 1) from sites where a significant presence of *T. sinensis* had just been recorded (i.e., years since *T.sinensis* presence [TsY] = 1; Ts% = 1% ca.) to sites where biocontrol had already been achieved (i.e., Ts% ≥ 70%). In total, we chose three sites where *T. sinensis* had just reached a significant presence (TsY = 1) and three groups of four sites each where this had occurred two (TsY = 2), three (TsY = 3), and four (TsY = 4) years before, respectively. In all TsY = 4 sites, biocontrol had already been achieved (Table 1).

In order to simulate the working options of a potential operator responsible for assessing the presence of *T. sinensis* and the parasitism rate reached, we randomly selected 10 wild trees at every site. From these, we collected five branches per tree distributed uniformly over the entire crown so as to consider all buds on a tree where *T. sinensis* could potentially oviposit. Branch height was labelled in four categories according to increasing levels of sampling effort, i.e., branch collection from the ground level up to 3 m, 5 m, 8 m, and up to the top of the crown, respectively. In terms of sampling effort, these categories represent different methods by which galls are collected by hand, namely, with a small pole-hook (3 m), with a normal telescopic tree pruner (5 m), with a long telescopic tree pruner (8 m), and by climbing trees to the top. Every effort was made to assure that one to two branches for each height step was collected on each tree sampled.

Table 1 *Torymus sinensis* site characteristics

TsY[a]		1			2				3				4			
Ts%[b]	Mean	1.1	1.4	3.1	7.9	9.8	17.5	22.6	38.1	44.9	48.3	56.6	73.7	77.7	78.9	80.7
	95CI	0.5	0.64	0.89	1.37	1.49	1.7	2.09	2.36	2.49	2.35	2.35	2.26	1.96	1.81	1.98

[a] Years since *Torymus sinensis* arrival. [b] Ts% at gall level = no. *T. sinensis* / no. chambers

Once collected, branches were characterized using different dendrometric parameters (Table 2). On every branch, 20 galls were randomly collected, dissected and examined under a stereo microscope, and the content of every chamber recorded (Table 2).

Table 2 Parameters considered for each site, tree, branch and gall

	Total no.	Parameters	Unit/Values	Note
Sites	15			
Trees	150 (10 per site)	Geographic coordinates	Swiss: CH1903_LV03 Italian: WGS 84 UTM 32N	GPS Trimble, Geoexplorer 2005 series GeoXh
Branch	750 (5 / tree)	Height	m	Measured with a tape measure
		Orientation	0 = southwest facing (more xeric), 2 = northeast facing (more mesic)	According to cardinal points and transformed using Beers et al. (1966) equation, sin(A+45)+1 where A= azimuth of the branch.
		Light	%	Visually estimated in %
		Type	Crown architectural Reiteration	
Galls	15,000 (20 / branch)		Chamber	Empty, Empty with flickering hole, *D. kuriphilus*, *T.sinensis*, local parasitoids

In total, 1,000 galls per site were collected between July and August 2014, on the basis that this number would represent a good approximation of the real *T. sinensis* parasitism situation at site level.

Data analysis: calculating Torymus sinensis presence and parasitism rate

T. sinensis parasitism rate (Ts%) was calculated at gall level as follows: number of living *T. sinensis* / total number of chambers * 100, with mean values calculated at branch and tree levels. Instead, *T. sinensis* presence was defined as a binary variable at gall level with a value of 1 when *T. sinensis* is present in at least one cell of the gall in question.

Data analysis: analysis of Torymus sinensis oviposition distribution

In order to obtain an approximate idea regarding the oviposition preferences of *T. sinensis* within a stand, we performed a preliminary analysis at gall, branch and tree levels. Data were first explored by plotting the distribution of *T. sinensis* presence per gall, branch and tree, and plotting the Ts% at branch level against the branch characteristics (see branch parameters in table 2) for every TsY.

To select branch characteristics that are most closely related to *T. sinensis* presence, a generalized linear mixed model analysis was implemented at gall level, setting the presence/absence of *T. sinensis* as the response variable and the branch parameters (Table 2) as explanatory variables. Four models were run using a subset of data from the first significant presence of *T. sinensis* (TsY = 1) until biocontrol (TsY = 4). Finally, a full model including the entire dataset was run to test overall *T. sinensis* distribution patterns. Sites, trees and branches were set as random factors. The random configuration (random intercept or random intercept and slope) was determined following the procedure described in Zuur et al. (2009). Models were ranked on the basis of the Akaike information criterion and Markov Chain Monte Carlo methods (MCMC) were used to generate samples of posterior density distributions.

Data analysis: bootstrap procedure analysis

The *sample* function from the *Base* package in R was used to generate 5,000 bootstrap replicates for every considered gall quantity. To obtain a more realistic result, we performed the sampling with replacement so as to simulate an infinite population (Baguley 2012). Bootstraps were run with data grouped by site and then an average value for every TsY was computed for plotting. For every bootstrap generated, the average percentage of failure or error (i.e., that *T. sinensis* is, in fact, present in the site but was not found – see section 2.3.4 and 2.3.5 for more details) was computed.

Data analysis: looking for Torymus sinensis presence after first release/arrival

To determine the number of galls needed to obtain a reliable result at 99% (in order to avoid a false negative, i.e., question A: *T. sinensis* present but not detected) in sites of recent (1-2 years before) *T. sinensis* release or arrival, we used the subset of three sites where *T. sinensis* had just arrived (TsY = 1; Table 1) and the binomial *T. sinensis* presence as the response variable (gall with *T. sinensis* = 1, gall without *T. sinensis* = 0). In the first round, bootstraps were performed considering from 10 to 1,000 galls while ignoring branch and tree structure (full design).

In the second step, various less demanding sampling designs were then simulated by sampling 50, 100, 150, 200, 250 and 300 galls selected for different maximum branch heights (up to 3 m, 5 m, 8 m and 25 m) and for different numbers of sampled trees (from 1 to 10), respectively. For each bootstrap generated in this way, the average percentage of failure was calculated as follows:

$$\text{Percentage of failure: } (1 - \Sigma(\text{gall with Ts}) / \text{total galls}) * 100$$

and the respective 95% confidence interval was calculated using the binom.test function from the R package *Stats*.

Data analysis: adapting sampling effort when calculating Torymus sinensis parasitism rate in subsequent years

To determine the most efficient sampling design to confidently estimate Ts% in sites where the *T. sinensis* presence has already been confirmed and Ts% is already above the threshold of 1% for at least a year (question B), we first examined the whole dataset (15 sites) and used different numbers of galls (from 10 to 1,000) when boostrapping, ignoring branch and tree structure (full design). Similar to question A, bootstraps were then performed sampling 50, 100, 150 and 200 galls selected for different maximum branch heights (up to 3 m, 5 m, 8 m and 25 m) and for different numbers of trees (from 1 to 10).

For every bootstrap generated in this manner, an average Ts% was computed and the corresponding percentage of error was calculated as follows:

$$\text{Percentage of error (absolute value): } |PE| = (Ts\%^{site} - Ts\%^{boot}) / Ts\%^{site} * 100$$

where $Ts\%^{site}$ stands for the average Ts% value at site level and $Ts\%^{boot}$ for the average value for each bootstrap resample.

All analyses were done in R (version 3.3.3; R Core Team 2017) and linear mixed-effects models were implemented using the lme4 package (Bates et al. 2015).

Results

Spatial and temporal evolution of Torymus sinensis oviposition patterns

Ts% varied within the sampled sites from 1.1 to 80.7% (Table 1). In sites of fresh release/arrival (TsY = 1), *T. sinensis* parasitism rates ranged from 1.1 to a maximum Ts% of 3.1, increasing 2- to 7-fold the following year (TsY = 2 with mean Ts% ranging from 7.9 to 22.6). Its presence more than doubled from TsY = 2 to TsY = 3 (mean Ts% ranging from 38.1 to 73.7) and stabilized in TsY = 4 in a range between 73.7 and 80.7 Ts% (Table 1).

In sites where *T. sinensis* had been freshly released or had just arrived (TsY = 1), adult larvae were present on most of the trees (60-90%), but only on a subset of branches (24-38%) and a small number of galls (22-25.5%; Fig. 2) per tree. From the second year since its significant presence (Ts% = 1%), *T. sinensis* extends its attack to all trees (100%) and to the majority of the branches (78-98%) ovipositing in 15-42% of the galls. Colonization of all branches and most galls (63-81%) is achieved from the third year on (Fig. 2).

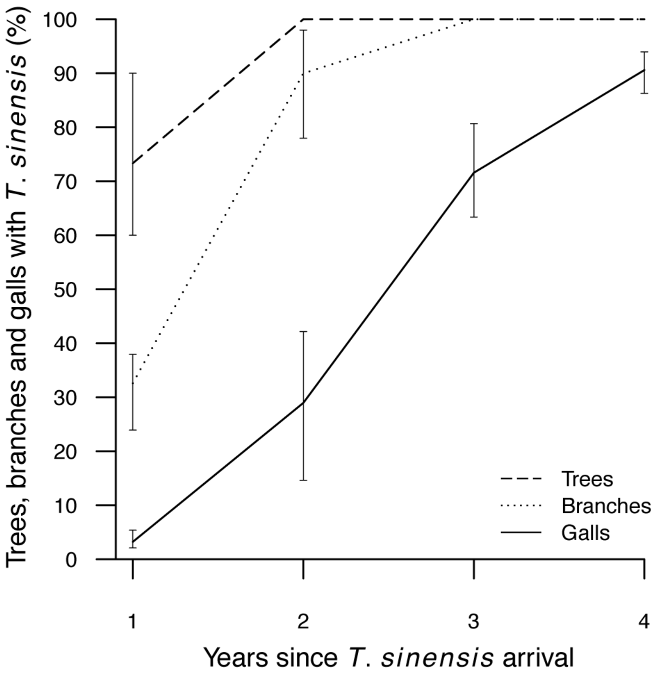

Figure 2 Proportion of trees, branches and galls with *Torymus sinensis* according to years since arrival (TsY). TsY = 1: time of first significant *T. sinensis* presence (mean parasitism rate ≥ 1%). Vertical bars show the minimum and maximum value for each TsY.

The best models that explain the presence of *T. sinensis* between TsY1 and TsY3, as well as the best model considering all sites (TsY1 to TsY4), always include branch height as a unique explanatory variable with a positive and significant relationship. On the other hand, the best model that considers only sites with TsY = 4 included the intercept term only (Table 3).

Table 3 Generalized linear mixed models according to years since *Torymus sinensis* (Ts) arrival

Years since Ts arrival	Fixed effect	Estimate	MCMC mean	Highest posterior density intervals	Pr(>|t|)[a]	pMCMC[b]
1	Intercept	-5.251	-6.142	-7.232 /-5.115	0.001	0.001
	Branch height	0.160	0.197	0.068 / 0.315	0.009	0.001
2	Intercept	-1.600	-1.888	-2.328 / -1.456	0.001	0.001
	Branch height	0.061	0.071	0.031 / 0.112	0.001	0.002
3	Intercept	0.589	0.695	0.328 / 1.046	0.001	0.001
	Branch height	0.087	0.103	0.065 / 0.143	0.001	0.001
4	intercept	2.679	3.090	2.717 / 3.557	0.001	0.001
1+2+3+4	Intercept	-0.565	-0.662	-2.055 / 0.920	0.348	0.336
	Branch height	0.060	0.070	0.047 / 0.095	0.000	0.001

[a,b] *p*-values are calculated using the *t*-distribution Pr(>|t) and with the posterior distribution (pMCMC), respectively. The last two columns show *p*-values calculated with the posterior distribution (pMCMC) and the *t*-distribution (Pr(>|t)).

Assessing the Torymus sinensis presence

To certify the presence of *T. sinensis* in stands where it has been recently released or may have just arrived through natural diffusion (TsY = 1), the collection and analysis of 200 galls is required from the overall crown of ten trees when targeting a failure rate (*T. sinensis* in fact present in the site but not found) of lower than 1% (Fig. 3).

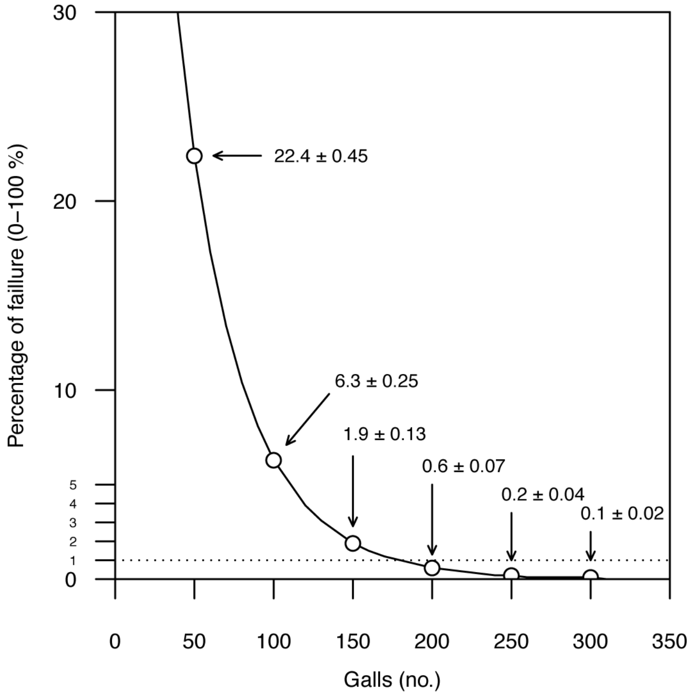

Figure 3 Percentage of failure by sample size at TsY1 sites. *T. sinensis* presence was transformed into a binomial variable (gall with at least 1 *T. sinensis* = 1, gall without *T. sinensis* = 0) and tree/branch structure was ignored. The percentage of failure is defined as the probability of missing *T. sinensis* in a site where the Ts% is at ca. 1%. The black line shows the average value of 5,000 bootstrap resamples with replacement. Average values with 95% confidence intervals are indicated by circles for specific numbers of galls (multiples of 50). Note that the range beyond 350 galls is not plotted because of no resulting additional information.

Accepting a failure rate of approximately 6%, the sampling effort can be reduced in terms of the number of galls (100 galls collected from the overall crown of ten trees; Fig. 3) or in terms of the number of trees or branch heights (200 galls collected from at least 6 different trees and targeting branches up to 8 m; Fig. 4). Appendix Table A1 shows the different percentages of failure according to different possible sampling efforts in terms of the number of trees, galls and branch heights allowing operators to optimize between sampling effort and resulting accuracy.

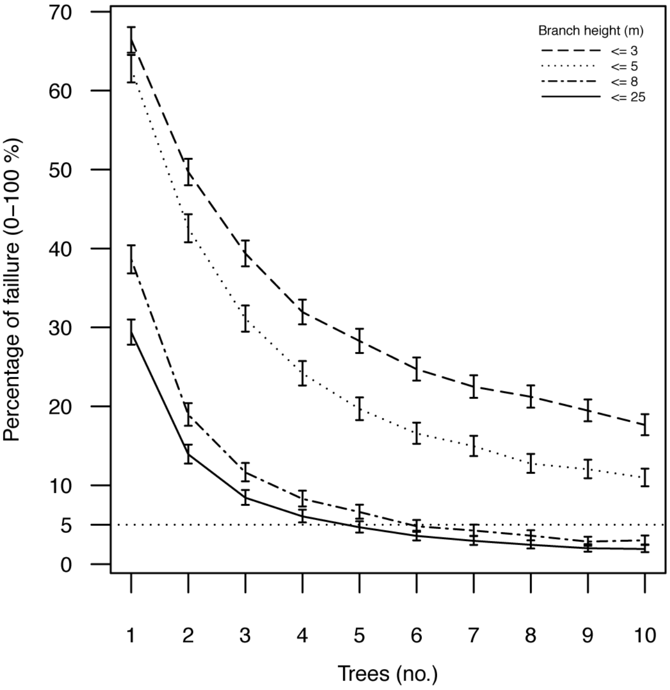

Figure 4 Percentage of failure based on the number of trees and branch heights with a sample size of 200 galls at TsY1 sites. *T. sinensis* presence was transformed into a binomial variable (gall with at least 1 *T. sinensis* = 1, gall without *T. sinensis* = 0) and tree/branch structure was ignored. The percentage of failure is defined as the probability of missing *T. sinensis* in a site where the Ts% is at ca. 1%. The black lines shows the average value of 5,000 bootstrap resamples with replacement and bars show 95% confidence intervals.

Monitoring the evolution of Torymus sinensis parasitism rate

Figure 5 shows the median percentages of error (PE) based on the number of galls and TsY when estimating Ts%. As a general rule, and independently of Ts% (TsY from 1 to 4), a substantial gain in precision can be attained by increasing the sample size (number of galls) up to 50 or 100 galls. After that, the sample size must be at least doubled to further decrease the PE by ca. 25%. (Fig. 5).

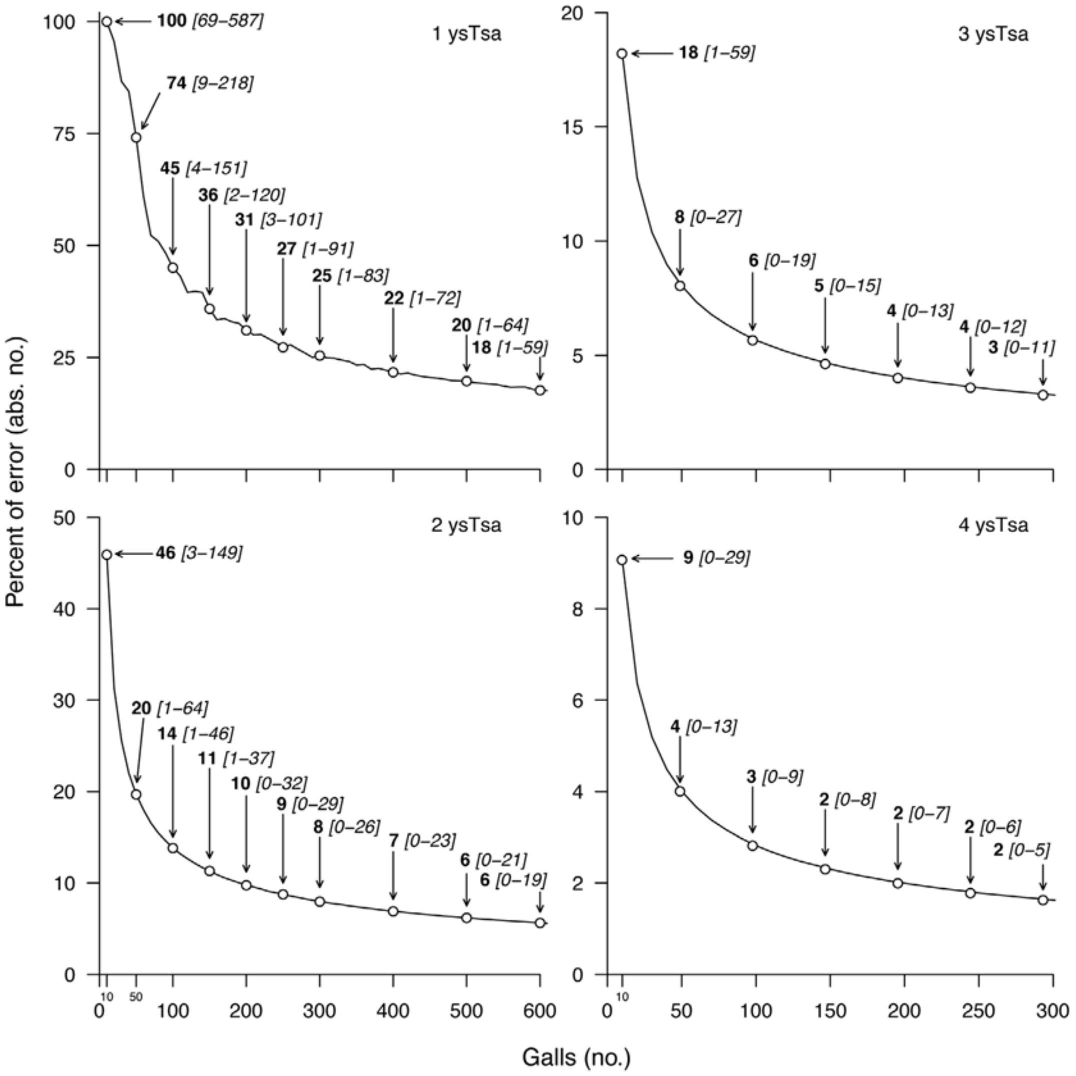

Figure 5 Percentage of error in estimating *Torymus sinensis* parasitism rate by sample size and years since a significant *T. sinensis* presence. Tree and branch data structure has been ignored. The black line shows the median value of 5,000 bootstrap resamples with replacement. Median values with percentiles (0.025 and 0.975) are indicated by circles for specific numbers of galls (multiples of 50). Note that plots have been cut at 300 or 600 galls because of no resulting additional information beyond these values.

Figure 6 shows the median PE when collecting 100 galls from the lower portion of the tree crown and from fewer trees, whereas the Appendix – Table A2 shows different PE values for TsY from 1 to 4 based on different sampling efforts in terms of the number of trees, galls and branch heights. In newly colonized areas (TsY=1), the PE decreases substantially when collecting galls using a long tree pruner reaching 8 m, even when the sampling effort is limited to five different trees (instead of ten). Extending the collection effort to 10 trees further decreases the PE by 8.6 percentage points.

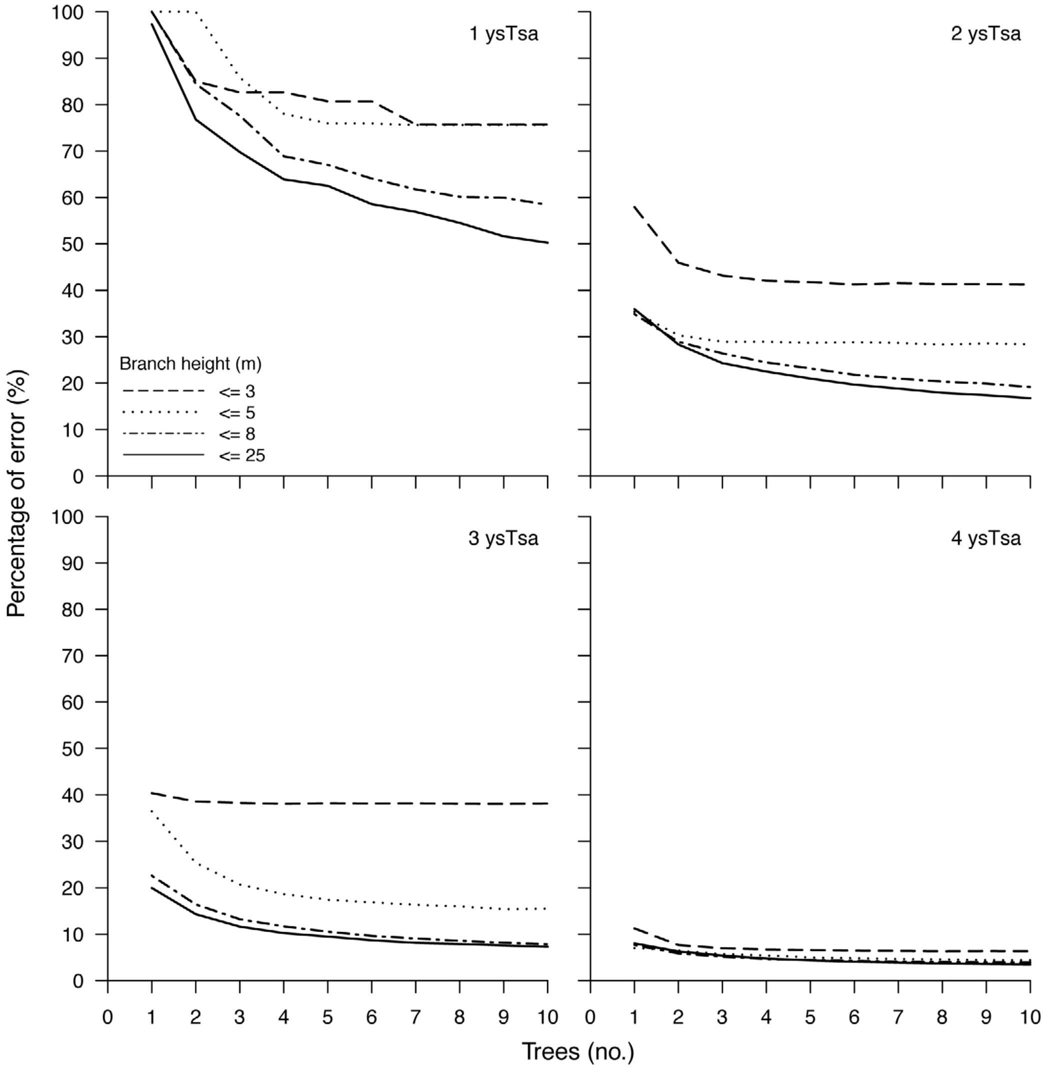

Figure 6 Percentage of error in estimating *Torymus sinensis* parasitism rate as a function of the number of trees, branch height and years since a significant *T. sinensis* presence with a sample size of 100 galls. The black line shows the median value of 5,000 bootstrap resamples with replacement. For the percentiles (0.025 and 0.975) please refer to the tables in the Supplemental Online Material 2.

The sampling effort can be gradually and drastically reduced year by year (Fig. 6). In fact, substantial gains in precision can be obtained by collecting galls distributed on 4 trees and up to 8 m in height where *T. sinensis* has been present for two years (TsY = 2). The number of trees can be further decreased to three in the third year (TsY = 3).

Finally, branch height can be reduced to 3 m in the fourth year (TsY = 4) without significantly increasing the PE if the number of trees is kept constant at three (Figure 6).

Discussion

Gehring et al. (2017) demonstrated how important is to avoid long-lasting, repeated gallwasp attacks on chestnut trees because of the progressive corruption of branch architecture and the significant depletion of tree reserves they cause. Under such conditions, the time needed by a tree from the achievement of biological control to full recovery is significantly longer. When *D. kuriphilus* newly colonizes a chestnut area, the early release of the natural antagonist *T. sinensis* and the monitoring of its establishment and progressive spread towards biological control are thus of paramount importance.

Many different sampling designs and strategies for estimating population densities and pest infestation exist, and choosing the most efficient and appropriate method can often be a demanding task. As a general rule, it is important to define the required degree of accuracy and targeted parameters, so as to select the most suitable sampling design accordingly.

Our results show that *T. sinensis* first target its oviposition in galls within trees. There, it initially colonizes all branches with a slight preference for the upper crown, but without ovipositing in all galls even when very high parasitism rates (Ts% = 70 ca.) have been reached.

Taking into consideration the described oviposition patterns, we propose a simple and practical way to estimate the performance of different sampling designs and efforts to confidently assess the presence of *T. sinensis* after its first release or arrival, and to subsequently monitor the evolution of parasitism rates until biocontrol.

According to the results of the presented study case, in sites where *T. sinensis* has been freshly released and the establishment of the insect is not yet certified, sampling should first target the confirmation of its presence at a significant level (Ts% = 1%). Our simulations show that a sample size of 200 galls is large enough to confidently document the successful establishment of the antagonist. Collecting greater numbers would only slightly increase the assessment precision, causing a disproportionate increase in workload. A recommendation for simplifying the design and reducing the sampling effort while maintaining reasonable accuracy (6% ca. of failure) would be to collect 200 galls up to 8 m in height in the crown from five different trees. Other options to simplify the design according to the desired accuracy are provided in the Appendix – Table A1. As pointed out in various Japanese studies (e.g., Moriya et al 1989, Shiga 1997, Moriya et al. 2003) the dispersal speed of *T. sinensis* just after release is initially low. It is, thus, strongly recommended to plan sampling activities in the proximity of release points, considering that in stands that are heavily infested by *D. kuriphilus*, *T. sinensis* will in all likelihood not move far to oviposit.

Once *T. sinensis* is successfully established, it is important to confidently estimate the evolution of its parasitism rate in order to determine whether additional releases are still needed in order to achieve biocontrol earlier. When the parasitism rate reaches 20% (as was the case at TSY = 2 in our data), the *T. sinensis* population begins to grow exponentially. It is thus reasonable to think that any supplementary release will have little or no effect on the rapidity of achieving biocontrol. From there on, the sampling effort can also be drastically reduced in terms of the number of galls and trees and branch heights, given the more uniformly distributed *T. sinensis* oviposition within the site and trees. Once biocontrol is achieved, we nevertheless recommend maintaining the sample size so that the lower biocontrol threshold of Ts% ~ 70% remains below the lowest level of the confidence interval. In this way, any population fluctuations towards a possible recrudescence phase (e.g. travelling waves of parasitoid populations as postulated by Paparella et al. 2016) can be excluded.

It is important to note that the percentage of error returned by the proposed sampling design should be interpreted in light of the corresponding Ts% level at the site. This is owing to the fact that in sites with Ts% = 1, a PE of 50% would represent an under/overestimation of 0.5%, whereas in sites with Ts% = 70%, it would represent an under/overestimation of 35%.

One final consideration is that the period of gall collection may also influence gathering efforts and related results. Considering data accuracy and ease of collection, gathering galls before *D. kuriphilus* emerges, which usually occurs from the beginning of June until the end of July (EPPO 2005), would ensure that work is done on material that is a) already mature but not excessively lignified, b) not deteriorated by fungal attacks, and c) still in good condition for identification purposes, as is the case with most individuals. On the other hand, assessed larvae are still subject to the *T. sinensis* parasitism rate indirectly via various mortality factors before emergence, such as fungi, hyperparasitism, mites, squashing due to cell growth in the chamber, and possible failure to excavate the emergence tunnel. These factors may lead to an overestimation of the *T. sinensis* parasitism rate. Collecting galls at the end of winter would ensure a more realistic *T. sinensis* parasitism rate estimation on the collected galls. However, available galls on trees at that time would only represent a subset of all galls, principally consisting of galls on shoots that are not subjected to falling with leaves in autumn (Maltoni et al. 2012b). In addition, they would be more degraded by fungi, making the estimation of the *D. kuriphilus* population on the basis of emergence holes far more difficult and imprecise.

Conclusion

Confidently assessing the presence of *T. sinensis* after its first release, or its parasitism rate (Ts%) towards biological control is very important, especially for the planning of future releases, for monitoring its presence over the years and for studying its spread capacity across sites and nations (e.g., Matošević et al. 2017). Howerer, this can quickly become a very time-consuming task especially when considering gall dissection.

Based on the results here presented, we make the following general recommendations.

1. first identify the stage of *T. sinensis* parasitism (from first *T. sinensis* release to biocontrol)
2. set the desired accuracy level *a priori*
3. adapt the quantity of galls to be analysed during the sectioning process according to the Ts% found

Following these three steps using the parameters here described will enable operators to implement the most cost-effective procedures for the purpose of effectively and efficiently monitoring *T. sinensis* parasitism. We further recommend that operators specify the area of the plot under investigation, the number of trees and branches and branch heights from which galls were collected, as well as the number of dissected galls when reporting the obtained Ts%. This will be particularly relevant in case of scientific publications, so that readers are automatically provided with an indication of the estimation accuracy. In addition, such data can be cross-referenced with the findings of the present study for comparison purposes.

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Appendices

(see next pages)

Table A1 Percentage of failure (1 – Σ(galls with Ts) / total galls) * 100) according number of galls, branch height and number of sampled trees

		3 m				5 m				8 m				25 m			
Galls (no.)		50	100	200	300	50	100	200	300	50	100	200	300	50	100	200	300
Trees (no.)	1	73.5 ± 3.1	71.3 ± 3.1	66.4 ± 3.2	62.9 ± 3.2	72.6 ± 3.2	68.8 ± 3.4	62.8 ± 3.5	62.8 ± 3.5	52.4 ± 3.6	44.4 ± 3.6	38.6 ± 3.6	38.2 ± 3.6	45.1 ± 3.6	47.5 ± 3.7	29.4 ± 3.2	24.6 ± 3.0
	2	64.0 ± 3.4	58.0 ± 3.5	49.7 ± 3.3	42.9 ± 3.2	63.7 ± 3.5	54.7 ± 3.6	42.6 ± 3.6	41.2 ± 3.5	41.5 ± 3.6	28.5 ± 3.3	18.9 ± 2.9	16.9 ± 2.7	35.9 ± 3.5	32.9 ± 3.4	13.9 ± 2.4	9.1 ± 2.0
	3	59.7 ± 3.5	50.6 ± 3.6	39.4 ± 3.3	32.4 ± 2.9	60.4 ± 3.5	47.1 ± 3.6	31.1 ± 3.3	28.1 ± 3.2	37.3 ± 3.5	22.5 ± 3.0	11.6 ± 2.3	9.4 ± 2.1	31.5 ± 3.3	25.3 ± 3.2	8.4 ± 1.9	4.4 ± 1.4
	4	57.3 ± 3.5	46.3 ± 3.6	31.9 ± 3.1	25.7 ± 2.6	58.0 ± 3.6	42.0 ± 3.6	24.2 ± 3.1	20.9 ± 2.9	35.2 ± 3.4	18.7 ± 2.8	8.3 ± 2.0	5.3 ± 1.6	29.9 ± 3.3	20.9 ± 3.0	6.1 ± 1.6	2.5 ± 1.0
	5	54.6 ± 3.5	42.5 ± 3.6	28.3 ± 3.1	21.5 ± 2.4	55.9 ± 3.6	39.0 ± 3.5	19.7 ± 2.9	15.0 ± 2.6	32.3 ± 3.3	17.0 ± 2.6	6.6 ± 1.8	3.6 ± 1.3	27.9 ± 3.2	18.8 ± 2.9	4.7 ± 1.4	1.6 ± 0.8
	6	54.0 ± 3.6	41.4 ± 3.6	24.7 ± 2.9	18.2 ± 2.2	55.4 ± 3.6	37.7 ± 3.5	16.6 ± 2.7	11.8 ± 2.3	32.5 ± 3.3	15.4 ± 2.5	4.8 ± 1.5	2.7 ± 1.1	26.8 ± 3.1	18.5 ± 2.8	3.6 ± 1.2	1.1 ± 0.7
	7	54.1 ± 3.5	39.5 ± 3.5	22.5 ± 2.9	14.4 ± 2.0	54.8 ± 3.6	35.2 ± 3.4	15.0 ± 2.6	9.6 ± 2.1	31.6 ± 3.2	14.5 ± 2.4	4.3 ± 1.4	2.0 ± 1.0	25.8 ± 3.1	15.9 ± 2.7	3.0 ± 1.1	0.8 ± 0.6
	8	53.2 ± 3.6	37.5 ± 3.5	21.2 ± 2.8	13.3 ± 2.0	54.2 ± 3.6	34.3 ± 3.4	12.7 ± 2.4	8.1 ± 2.0	30.3 ± 3.2	13.5 ± 2.3	3.6 ± 1.3	1.5 ± 0.8	25.3 ± 3.1	15.4 ± 2.6	2.5 ± 1.0	0.7 ± 0.5
	9	52.2 ± 3.6	36.6 ± 3.5	19.5 ± 2.8	12.1 ± 1.9	53.5 ± 3.6	33.6 ± 3.4	12.0 ± 2.3	7.0 ± 1.9	29.2 ± 3.1	13.3 ± 2.3	2.9 ± 1.1	1.6 ± 0.9	24.8 ± 3.0	13.7 ± 2.5	2.0 ± 0.9	0.5 ± 0.5
	10	52.3 ± 3.6	35.4 ± 3.5	17.6 ± 2.7	10.8 ± 1.8	53.4 ± 3.6	33.2 ± 3.4	11.0 ± 2.2	6.1 ± 1.7	29.8 ± 3.1	12.3 ± 2.2	3.0 ± 1.1	1.2 ± 0.7	24.9 ± 3.0	13.2 ± 2.5	1.9 ± 0.9	0.5 ± 0.4

Numbers with ± show the average probability and CI95 over 5,000 bootstraps with replacement.

Table A2 Different percentage of error values (PE) for TsY from 1 to 4 based on different sampling efforts in terms of the number of trees, galls and branch heights.

TsY = 1. Percentage of error* in estimating *Torymus sinensis* parasitism rates according to number of trees and branch heights for different sample sizes.

		3 m				5 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	100.0 *10.1 - 214.3*	100.0 *7.5 - 176.6*	100.0 *7.0 - 189.5*	100.0 *9.0 - 200.9*	100.0 *14.4 - 214.1*	100.0 *5.1 - 187.6*	100.0 *5.3 - 188.8*	100.0 *6.3 - 193.8*
	2	100.0 *9.8 - 178.2*	85.0 *7.3 - 165.1*	82.4 *7.9 - 166*	84.6 *10.9 - 155.2*	100.0 *9.0 - 153.9*	100.0 *5.7 - 156.8*	93.5 *7.3 - 141.8*	86.7 *6.5 - 145.7*
	3	100.0 *9.8 - 174.1*	82.6 *10.9 - 147.9*	80.8 *12.6 - 148.8*	82.0 *13.2 - 146.1*	100.0 *9.0 - 153.9*	85.8 *5.7 - 132.1*	77.8 *6.1 - 118.8*	76.7 *7.4 - 124.3*
	4	100.0 *9.8 - 166.0*	82.6 *12.3 - 147.9*	80.5 *17.0 - 131.6*	80.0 *17.6 - 132.3*	100.0 *10.4 - 153.9*	78.0 *6.1 - 125.9*	75.6 *6.8 - 112.7*	75.6 *7.5 - 115.2*
	5	100.0 *10.9 - 166.0*	80.7 *15.4 - 137.9*	78.5 *18.7 - 130.9*	77.3 *20.3 - 124.7*	92.9 *10.4 - 153.9*	75.9 *6.5 - 125.9*	72.3 *7.2 - 111.1*	71.7 *8.0 - 112.2*
	6	100.0 *17.1 - 166.0*	80.7 *16.4 - 132.2*	75.2 *19.9 - 122.8*	73.6 *22.9 - 122.7*	92.9 *10.4 - 153.9*	75.9 *6.1 - 120.8*	70.2 *6.5 - 106.2*	70.3 *8.4 - 105.5*
	7	100.0 *16.1 - 154.0*	75.7 *16.9 - 126.5*	73.3 *21.4 - 121.2*	71.4 *23.3 - 117.1*	92.9 *10.4 - 133.8*	75.6 *6.5 - 110.5*	70.8 *8.6 - 100.0*	68.7 *8.4 - 101.5*
	8	100.0 *16.1 - 154.0*	75.7 *18.1 - 128.8*	72.4 *22.2 - 115.3*	70.5 *25.1 - 114.6*	92.9 *10.4 - 133.8*	75.6 *6.2 - 110.5*	70.4 *7.5 - 101.3*	68.1 *11.2 - 100.0*
	9	100.0 *16.9 - 154.0*	75.7 *19.0 - 126.4*	72.4 *22.2 - 116.0*	69.9 *25.1 - 114.6*	92.9 *10.4 - 133.8*	75.6 *6.7 - 105.3*	68.6 *9.7 - 100.0*	67.1 *9.9 - 100.0*
	10	100.0 *17.4 - 154.0*	75.7 *18.7 - 126.4*	72.4 *22.2 - 117.9*	70.0 *26.0 - 113.0*	92.9 *10.4 - 128.8*	75.6 *7.1 – 109.0*	68.6 *10.3 - 100.0*	65.1 *11.1 - 100.0*

		8 m				25 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	100.0 *14.6 - 471.5*	100.0 *5.1 -432.6*	100.0 *3.8 - 411*	100.0 *6.1 - 401.1*	100.0 *14.0 - 511.9*	97.3 *5.7 - 464.6*	90.6 *5.0 - 443.6*	88.8 *7.0 - 441.1*
	2	97.3 *12.8 - 366.8*	84.5 *5.1 - 311.2*	79.7 *4.0 - 285.5*	74.1 *5.2 - 261.5*	94.7 *14.0 - 370.9*	76.8 *5.2 - 306.2*	71.7 *4.7 - 288.7*	73.3 *5.9 - 278.4*
	3	92.9 *12.8 - 300.8*	77.6 *5.1 - 251.1*	68.9 *3.6 - 227.6*	67.5 *5.1 - 213.9*	89.3 *13.7 - 316.8*	69.8 *5.2 - 257.0*	63.3 *4.4 - 230.1*	63.2 *5.2 - 220.3*
	4	89.3 *12.8 - 262.2*	68.9 *5.1 - 221.2*	61.9 *3.2 - 196.7*	60.2 *4.5 - 180.6*	89.3 *13.7 - 286.3*	63.9 *5.2 - 223.8*	58.6 *4.4 - 198.9*	58.0 *4.3 - 190.1*
	5	89.3 *12.8 - 250.1*	67.0 *5.1 - 200.3*	57.8 *2.9 - 173.4*	56.2 *3.9 - 165.9*	89.1 *13.7 - 268.4*	62.5 *5.1 - 211.1*	54.4 *4.3 - 181.6*	54.7 *3.9 - 165.2*
	6	86.6 *11.2 - 243.0*	64.1 *5.1 - 180.1*	54 *2.3 - 152.8*	53.1 *3.6 - 151.0*	86.6 *11.9 - 250.5*	58.5 *4.6 - 190.4*	51.6 *3.6 - 168.2*	49.0 *3.5 - 157.4*
	7	85.8 *12.8 - 234.9*	61.7 *4.5 - 177.5*	52.6 *2.3 - 149.0*	50.2 *3.5 - 141.9*	86.2 *7.2 - 248.5*	56.9 *4.6 - 187.1*	48.4 *3.2 - 162.7*	45.9 *3.1 - 141*
	8	85.8 *8.7 - 232.0*	60.1 *4.5 - 165.6*	*49.1* *2.1 - 142.4*	48.0 *3.0 - 134.9*	76.9 *9.6 - 245.2*	54.5 *4.6 - 180.0*	46.9 *3.1 - 159*	45.0 *3.2 - 135.1*
	9	79.2 *8.7 - 219.2*	60.0 *4.5 - 162.1*	49.1 *2.0 - 139.2*	46.1 *3.0 - 131.5*	76.9 *9.7 - 238.6*	51.6 *4.3 - 173*	45.0 *2.9 - 145.6*	43.3 *3.0 - 131.4*
	10	79.2 *8.7 - 223.5*	58.4 *4.3 - 155.2*	47.1 *2.0 - 134.0*	45.5 *3.0 - 126.4*	79.4 *7.2 - 238.6*	50.2 *4.0 - 172.3*	42.4 *2.4 - 141.1*	42.3 *3.0 - 128.4*

Numbers show the median value with percentiles (0.025, 0.975) in italics.
Percentage of error: $|PE| = Ts\%^{site} - Ts\%^{boot}) / Ts\%^{site} * 100$ where $Ts\%^{site}$ stands for the average Ts% value at site level and $Ts\%^{boot}$ for the average value for each bootstrap resample.

Table A2 continued

TsY = 2. Percentage of error* in estimating *Torymus sinensis* parasitism rates according to number of trees and branch heights for different sample sizes.

		3 m				5 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	58.9 *3.6 - 124.4*	57.9 *4.0 - 123.7*	57.7 *3.5 - 109.6*	58.4 *4.1 - 124.7*	42.2 *2.4 - 114.3*	35.4 *1.6 - 93.9*	35.6 *1.7 - 89.9*	33.7 *1.4 - 87.7*
	2	50.3 *3.1 - 111.7*	45.9 *4.5 - 102.2*	46.2 *5.1 - 99.6*	46.2 *5.2 - 97.4*	35.4 *1.9 - 82.2*	30.3 *1.5 - 75.8*	29.7 *1.3 - 73.2*	28.1 *1.3 - 71.2*
	3	48.1 *4.2 - 103.8*	43.1 *6.9 - 94.2*	41.7 *6.6 - 90.7*	39.7 *6.8 - 87.5*	33.7 *1.7 - 79*	28.9 *1.4 – 71.0*	28.2 *1.5 - 67.9*	27.8 *1.6 - 65.7*
	4	46.5 *5.3 - 100.1*	42.0 *7.5 - 87.4*	41.0 *8.1 - 84.2*	38.6 *8.2 - 81.5*	33.7 *2.1 - 76.8*	28.9 *1.5 - 67.5*	27.9 *1.6 - 64.5*	27.7 *1.7 - 62.2*
	5	45.1 *5.5 - 99.4*	41.7 *8.8 - 85.7*	40.5 *9.2 - 80.0*	38.4 *9.1 - 77.2*	32.6 *1.8 - 75.9*	28.7 *1.7 - 65.3*	28.0 *1.8 - 62.0*	27.6 *2.1 - 59.5*
	6	44.5 *5.4 - 97.9*	41.2 *9.2 - 84*	40.1 *10.1 - 78.2*	38.5 *9.4 - 74.6*	32.6 *1.9 - 75.3*	28.8 *1.8 - 64.5*	27.7 *2.0 - 60.1*	27.8 *2.1 - 57.2*
	7	43.7 *6.0 - 97.3*	41.5 *9.7 - 82.3*	39.9 *10.4 - 76.8*	38.4 *10.5 - 73.0*	32.1 *1.7 - 74.6*	28.7 *1.8 - 63.1*	27.6 *2.0 - 59.5*	27.5 *2.5 - 56.2*
	8	44.6 *6.1 - 96.6*	41.3 *10.6 - 81.5*	39.8 *11.1 - 75.7*	38.0 *10.9 - 72.3*	32.4 *1.8 - 74.1*	28.3 *2.0 - 62.1*	27.4 *2.1 - 57.8*	27.8 *2.7 - 54.8*
	9	43.2 *6.5 - 94.9*	41.3 *10.5 - 80.1*	39.0 *11.4 - 74.5*	38.0 *12.0 - 70.6*	32.0 *1.9 - 73.4*	28.6 *2.0 - 61.6*	27.6 *1.9 - 57.8*	27.3 *2.9 - 54.1*
	10	43.1 *6.5 - 94.8*	41.2 *11.7 - 80.5*	39.2 *12.2 - 73.9*	37.8 *12.4 - 70.1*	32.3 *2.1 - 73.8*	28.4 *2.1 - 60.4*	27.6 *2.4 - 56.9*	27.5 *3.2 - 53.5*

		8 m				25 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	39.5 *1.9 - 149.5*	34.9 *1.6 - 136.3*	36.2 *1.4 - 136.6*	34.0 *1.4 - 128.7*	39.4 *2.1 - 158.5*	36.0 *1.7 - 135.7*	32.2 *1.8 - 129.1*	33.4 *1.8 - 123.0*
	2	33.1 *1.6 - 114.1*	28.9 *1.4 - 101.7*	28.0 *1.2 - 97.4*	26.1 *1.0 - 90.7*	32.4 *1.6 - 112.2*	28.3 *1.4 - 94.5*	26.0 *1.2 - 93.1*	25.8 *1.4 - 88.6*
	3	30.4 *1.5 - 96.1*	26.4 *1.3 - 83.3*	25.1 *1.2 - 79.0*	23.4 *1.1 - 72.9*	29.4 *1.5 - 96.5*	24.3 *1.2 - 80.2*	22.9 *1.1 - 75.5*	21.8 *1.1 - 71.1*
	4	29.1 *1.5 - 86.7*	24.5 *1.3 - 74.4*	22.8 *1.1 - 68.1*	21.0 *1.0 - 63.1*	27.5 *1.5 - 87.1*	22.5 *1.0 - 72*	21.0 *1.0 - 66.3*	19.0 *0.9 - 61.4*
	5	27.1 *1.4 - 80.8*	23.2 *1.2 - 67.3*	21.0 *1.1 - 60.8*	19.6 *1.0 - 56.7*	25.7 *1.2 - 81.4*	21.0 *1.0 - 65.0*	19.2 *0.9 - 59.2*	17.4 *0.8 - 56.0*
	6	26.1 *1.3 - 77.1*	21.8 *1.1 - 62.5*	19.9 *0.9 - 57*	18.6 *0.9 - 52.4*	24.7 *1.2 - 78.8*	19.7 *0.9 - 61.1*	18.1 *1.0 - 56.5*	16.4 *0.8 - 51.9*
	7	25.4 *1.2 - 74.7*	21.0 *0.9 - 58.9*	19.1 *1.0 - 54*	17.7 *0.9 - 49.7*	23.8 *1.2 - 75*	18.8 *0.9 - 59.8*	16.8 *0.8 - 52.5*	15.4 *0.7 - 48.3*
	8	24.7 *1.2 - 74.1*	20.3 *0.9 - 57.8*	18.1 *0.9 - 51.7*	17.0 *0.9 - 47.4*	23.0 *1.1 - 73.7*	17.9 *0.9 - 56.4*	16.2 *0.8 - 50.1*	14.4 *0.7 - 45.6*
	9	24.4 *1.3 - 71.4*	19.9 *1.0 - 56.3*	17.5 *0.8 - 50.3*	16.3 *0.8 - 45.8*	22.8 *1.2 - 72.4*	17.4 *0.8 - 56*	15.4 *0.8 - 48.6*	13.7 *0.6 - 44.4*
	10	24.1 *1.1 - 70.4*	19.1 *0.9 - 54.8*	17.1 *0.8 - 48.5*	15.8 *0.8 - 44.7*	22.2 *1.1 - 70.7*	16.7 *0.8 - 54.1*	14.6 *0.7 - 46.6*	13.2 *0.6 - 42.9*

Numbers show the median value with percentiles (0.025, 0.975) in italics.

Percentage of error: $|PE| = Ts\%^{site} - Ts\%^{boot}) / Ts\%^{site} *100$ where $Ts\%^{site}$ stands for the average Ts% value at site level and $Ts\%^{boot}$ for the average value for each bootstrap resample.

Table A2 continued

TsY = 3. Percentage of error* in estimating *Torymus sinensis* parasitism rates according to number of trees and branch heights for different sample sizes.

		3 m				5 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	40.2 *8.6 - 75.5*	40.4 *12.8 - 71.2*	39.8 *13.8 - 69.5*	39.6 *15.6 - 68.1*	36.0 *3.1 - 78.8*	36.4 *4.0 - 77.1*	35.6 *5.7 - 76.1*	36.3 *5.1 - 76.6*
	2	38.5 *11.2 - 71.8*	38.6 *15.0 - 68.2*	37.4 *16.0 - 67.2*	37.3 *17.4 - 65.8*	25.6 *1.2 - 70.5*	25.4 *1.1 - 68.0*	25.1 *1.1 - 66.6*	24.8 *1.1 - 65.7*
	3	38.2 *12.2 - 68.1*	38.3 *16.6 - 63.4*	37.7 *18.0 - 61.2*	37.3 *19.6 - 60.1*	21.7 *1.0 - 60.9*	20.7 *1.0 - 57.5*	20.7 *0.9 - 57.1*	20.2 *0.9 - 56.5*
	4	38.1 *13.0 - 65.8*	38.1 *17.4 - 61.1*	37.9 *19.2 - 59.3*	37.5 *20.8 - 57.9*	19.5 *1.0 - 56.2*	18.7 *1.1 - 51.8*	18.3 *1.1 - 50.2*	18.3 *1.2 - 50.3*
	5	38.1 *12.8 – 64.0*	38.2 *18.2 - 59.3*	37.8 *20.0 - 57.2*	37.6 *21.6 - 55.9*	18.6 *1.0 - 52.5*	17.4 *1.1 - 48.9*	17.4 *1.4 - 47.2*	17.2 *1.6 - 46.1*
	6	38.0 *13.4 - 63.5*	38.1 *18.7 – 58.0*	37.8 *20.5 - 55.9*	37.6 *22.1 - 53.9*	17.8 *1.1 - 50.2*	16.9 *1.3 - 45.6*	16.6 *1.7 - 44.5*	16.5 *1.9 - 43.4*
	7	37.8 *13.8 – 63.0*	38.2 *19.1 - 57.2*	37.8 *21.0 - 55.1*	37.7 *22.6 - 53.1*	17.2 *1.1 - 47.1*	16.3 *1.6 - 43.5*	16.1 *2.1 - 42.1*	15.9 *2.3 - 41*
	8	37.8 *13.9 - 62.2*	38.1 *19.4 - 56.7*	37.8 *21.6 - 54.5*	37.4 *23.0 - 52.6*	16.9 *1.1 - 45.8*	16.0 *1.9 - 41.9*	15.8 *2.3 - 39.8*	15.6 *2.6 - 38.9*
	9	37.9 *13.6 - 61.6*	38.0 *19.5 - 56.1*	37.7 *21.7 - 53.5*	37.5 *23.6 - 51.9*	16.8 *1.2 - 44.5*	15.4 *2.0 - 39.5*	15.5 *2.5 - 38.1*	15.1 *2.8 - 37.1*
	10	37.8 *13.9 - 61.7*	38.1 *20.0 - 55.7*	37.7 *22.3 - 53.5*	37.5 *23.9 - 51.4*	16.4 *1.2 - 43.4*	15.5 *2.0 - 38.5*	15.2 *2.8 - 36.5*	15.1 *3.2 - 34.8*

		8 m				25 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	23.6 *1.1 - 61.7*	22.6 *1.0 - 59.3*	21.9 *1.0 - 58.0*	21.4 *1.1 - 57.3*	20.7 *1.0 - 62.0*	20.0 *1.0 - 59.4*	19.8 *1.0 - 58.4*	19.9 *0.9 - 58.2*
	2	17.1 *0.8 - 53.0*	16.4 *0.8 - 51.1*	15.8 *0.8 - 49.4*	15.4 *0.7 - 48.9*	15.2 *0.7 - 48.7*	14.3 *0.7 - 44.9*	13.8 *0.7 - 43.4*	13.7 *0.7 - 42.5*
	3	14.3 *0.7 - 46.2*	13.2 *0.6 - 43.5*	12.8 *0.7 - 42.2*	12.7 *0.6 - 41.8*	13.3 *0.7 - 42.3*	11.6 *0.6 - 37.7*	11.3 *0.5 - 36.2*	11.1 *0.5 - 34.8*
	4	12.9 *0.6 - 41.5*	11.7 *0.5 - 37.6*	11.1 *0.5 - 35.9*	10.8 *0.5 - 35.5*	11.9 *0.6 - 38.1*	10.2 *0.5 - 33.2*	9.8 *0.4 - 31.1*	9.7 *0.5 - 30.6*
	5	11.6 *0.6 - 38.6*	10.5 *0.5 - 34.3*	9.9 *0.5 - 32.5*	9.5 *0.5 - 31.5*	11.0 *0.5 - 35.6*	9.5 *0.4 - 30.4*	8.8 *0.4 - 28.4*	8.8 *0.4 - 27.7*
	6	11.1 *0.5 - 36.7*	9.6 *0.4 - 31.9*	9.0 *0.4 - 29.6*	8.8 *0.4 - 28.8*	10.5 *0.5 - 34.1*	8.7 *0.4 - 28.7*	7.9 *0.4 - 26.2*	7.8 *0.4 - 25.4*
	7	10.4 *0.5 - 34.8*	9.1 *0.4 - 29.7*	8.3 *0.4 - 27.1*	8.1 *0.4 - 26.7*	10.0 *0.5 - 33.0*	8.1 *0.3 - 26.9*	7.6 *0.4 - 25.1*	7.2 *0.4 - 23.3*
	8	10.1 *0.5 - 33.4*	8.6 *0.4 - 28.6*	7.7 *0.4 - 26.1*	7.5 *0.4 - 24.6*	9.9 *0.5 - 31.8*	7.9 *0.3 - 25.9*	7.1 *0.4 - 23.8*	6.8 *0.3 – 22.0*
	9	10.0 *0.5 - 32.4*	8.1 *0.4 - 27*	7.4 *0.4 - 24.4*	7.1 *0.4 - 23.3*	9.5 *0.4 - 30.9*	7.6 *0.4 – 25.0*	6.8 *0.3 - 22.4*	6.3 *0.3 - 21.2*
	10	9.8 *0.5 - 32.1*	7.9 *0.4 - 26.2*	7.1 *0.3 - 23.7*	6.7 *0.3 - 22.5*	9.2 *0.4 - 30.9*	7.3 *0.4 - 23.7*	6.4 *0.3 - 21.4*	6.1 *0.3 - 20.2*

Numbers show the median value with percentiles (0.025, 0.975) in italics.

Percentage of error: $|PE| = Ts\%^{site} - Ts\%^{boot}) / Ts\%^{site} *100$ where $Ts\%^{site}$ stands for the average Ts% value at site level and $Ts\%^{boot}$ for the average value for each bootstrap resample.

Table A2 continued

TsY = 4. Percentage of error* in estimating *Torymus sinensis* parasitism rates according to number of trees and branch heights for different sample sizes.

		3 m				5 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	11.5 *0.5 - 27.7*	11.2 *0.4 - 24.7*	11.4 *0.3 - 23.5*	11.5 *0.3 - 22.5*	7.9 *0.3 - 27*	7.0 *0.3 - 25.3*	6.6 *0.3 - 24.2*	6.5 *0.3 - 24.4*
	2	8.5 *0.4 - 25.5*	7.7 *0.4 - 22.7*	7.4 *0.3 - 21.7*	7.4 0.3 - 21.1	7.1 *0.3 - 23.0*	6.5 *0.3 - 21.0*	6.2 *0.3 - 19.9*	6.2 *0.3 - 20.0*
	3	7.7 *0.4 - 23.5*	7.0 *0.4 - 20.6*	6.8 *0.3 - 19.5*	6.7 *0.3 - 18.9*	6.6 *0.3 - 20.4*	5.7 *0.3 - 17.7*	5.7 *0.3 - 17.0*	5.6 *0.3 - 16.8*
	4	7.5 *0.4 - 22.8*	6.7 *0.4 - 19.6*	6.5 *0.4 - 18.3*	6.4 *0.4 - 17.6*	6.0 *0.3 - 18.8*	5.4 *0.3 - 16.3*	5.2 *0.3 - 15.1*	5.2 *0.3 - 15.1*
	5	7.2 *0.4 - 21.5*	6.6 *0.4 - 18.4*	6.5 *0.5 - 17.3*	6.2 *0.4 - 16.6*	5.8 *0.3 - 18.2*	5.0 *0.3 - 15.1*	4.8 *0.2 - 14.0*	4.8 *0.3 - 13.7*
	6	7.1 *0.4 - 20.9*	6.4 *0.4 - 17.5*	6.3 *0.4 - 16.4*	6.2 *0.6 - 15.5*	5.6 *0.3 - 17.5*	4.8 *0.2 - 14.3*	4.6 *0.2 - 13.3*	4.5 *0.3 - 12.7*
	7	7.3 *0.4 - 20.2*	6.4 *0.5 - 17.0*	6.3 *0.6 - 15.6*	6.1 *0.6 - 15.0*	5.4 *0.3 - 17.1*	4.6 *0.2 - 13.9*	4.4 *0.2 - 12.6*	4.4 *0.2 - 12.2*
	8	7.1 *0.4 - 20.1*	6.3 *0.5 - 16.1*	6.3 *0.6 - 15.1*	6.1 *0.9 - 14.4*	5.2 *0.2 - 16.7*	4.6 *0.2 - 13.5*	4.3 *0.2 - 12.0*	4.2 *0.2 - 11.7*
	9	7.1 *0.4 - 19.7*	6.3 *0.6 - 16.0*	6.3 *0.7 - 14.9*	6.1 *1.0 - 13.9*	5.1 *0.2 - 16.4*	4.4 *0.2 - 13.1*	4.2 *0.2 - 11.8*	4.2 *0.2 - 11.3*
	10	7.1 *0.4 - 19.5*	6.3 *0.6 - 15.5*	6.2 *0.7 - 14.4*	6.1 *1.1 - 13.4*	5.1 *0.2 - 16.0*	4.4 *0.2 - 13.1*	4.1 *0.2 - 11.7*	4.0 *0.2 - 10.8*

		8 m				25 m			
Galls (no.)		50	100	150	200	50	100	150	200
Trees (no.)	1	8.4 *0.4 - 40.3*	7.7 *0.4 - 38.6*	7.5 *0.5 - 37.7*	7.3 *0.4 - 37.3*	8.7 *0.4 - 39.8*	8.0 *0.4 - 38.2*	7.7 *0.4 - 37.6*	7.7 *0.5 - 37.3*
	2	6.5 *0.3 - 24.9*	5.8 *0.3 - 24.0*	5.5 *0.3 - 23.9*	5.5 *0.3 - 23.9*	7.0 *0.3 - 27.4*	6.3 *0.3 - 26.5*	6.1 *0.3 - 25.9*	5.9 *0.3 - 25.8*
	3	5.8 *0.3 - 18.6*	5.1 *0.3 - 15.9*	4.8 *0.2 - 15.2*	4.8 *0.2 - 14.5*	6.1 *0.3 - 21.6*	5.4 *0.2 - 19.3*	5.1 *0.2 - 18.4*	5.0 *0.3 - 17.8*
	4	5.5 *0.3 - 16.6*	4.6 *0.2 - 14.0*	4.5 *0.2 - 12.9*	4.3 *0.2 - 12.4*	5.7 *0.3 - 18.8*	4.7 *0.2 - 16.4*	4.5 *0.2 - 15.6*	4.3 *0.2 - 14.9*
	5	5.2 *0.2 - 15.9*	4.4 *0.2 - 13.1*	4.1 *0.2 - 12.0*	4.0 *0.2 - 11.4*	5.2 *0.2 - 17.3*	4.3 *0.2 - 14.2*	4.1 *0.2 - 13.5*	3.9 *0.2 - 12.8*
	6	5.0 *0.2 - 15.7*	4.3 *0.2 - 12.5*	4.0 *0.2 - 11.2*	3.8 *0.2 - 10.7*	5.0 *0.2 - 16.7*	4.1 *0.2 - 13.4*	3.7 *0.2 - 12.3*	3.5 *0.2 - 11.7*
	7	5.0 *0.2 - 15.2*	4.1 *0.2 - 12.1*	3.8 *0.2 - 11.0*	3.7 *0.2 - 10.2*	4.8 *0.2 - 15.8*	3.9 *0.2 - 12.7*	3.5 *0.2 - 11.4*	3.3 *0.2 - 10.9*
	8	4.9 *0.2 - 14.8*	4.0 *0.2 - 11.8*	3.7 *0.2 - 10.6*	3.6 *0.2 - 9.8*	4.7 *0.2 - 15.4*	3.7 *0.2 - 12.2*	3.3 *0.1 - 11.0*	3.1 *0.2 - 10.2*
	9	4.8 *0.2 - 14.8*	3.9 *0.2 - 11.7*	3.5 *0.2 - 10.2*	3.5 *0.2 - 9.6*	4.6 *0.2 - 14.9*	3.6 *0.2 - 11.7*	3.1 *0.1 - 10.4*	3.0 *0.1 - 9.7*
	10	4.8 *0.2 - 14.5*	3.9 *0.2 - 11.5*	3.6 *0.2 - 10.1*	3.4 *0.2 - 9.4*	4.5 *0.2 - 14.8*	3.5 *0.1 - 11.4*	3.0 *0.1 - 10.1*	2.9 *0.1 - 9.3*

Numbers show the median value with percentiles (0.025, 0.975) in italics.
Percentage of error: |PE| = Ts%^{site} – Ts%^{boot}) / Ts%^{site} *100 where Ts%^{site} stands for the average Ts% value at site level and Ts%^{boot} for the average value for each bootstrap resample.

Chapter 4

How did the system *Castanea sativa-Dryocosmus kuriphilus-Torymus sinensis* evolve in southern Switzerland from the arrival of the pest?

4.1

Torymus sinensis local and regional early population dynamics in the Insubrian and Piedmont regions

Gehring Eric, Bellosi Bruno, Quacchia Ambra, Conedera Marco

Torymus sinensis on a *Dryocosmus kuriphilus* gall

Abstract

The effectiveness of the biocontrol agent *Torymus sinensis* for controlling *Dryocosmus kuriphilus* (pest) population has already been shown in various country where it has been released. In this study however we investigated the growth dynamics and the spread capacity of *T. sinensis* when not released by annually sampling the *D. kuriphilus* infestation and its parasitism rate in 15 sites in southern Switzerland (local scale) during six years. We then adopted a space-for-time substitution approach at the regional scale including six Italian sites so as to create a temporal within the entire study area to provide a long-term evolution of this system in terms of absolute numbers. Results show that the population dynamics of *T. sinensis* at local scale displays an exponential growth at different rates as a function of the distance from the nearest Italian release site and partially of the *D. kuriphilus* abundance and that when a long-term equilibrium dynamic is established at the regional scale, both populations are reduced to very low numbers. Overall *T. sinensis* has shown to be very effective in spreading and establishing new populations across long distances despite lack of systematic release campaign in southern Switzerland.

Keywords *Castanea sativa*; *Dryocosmus kuriphilus*; biological control; population evolution; pest invasion.

Introduction

The arrival of new organisms that may potentially become harmful for people and ecosystems has become a world-wide daily concern (Kenis and Branco 2010; Williams et al. 2010; Aukema et al. 2011). Harmful organisms that are able to create large populations and spread through new habitats while incurring severe costs on forestry, agriculture and human health are usually considered as pests (Cock 2001; Pimentel et al. 2004; Vilà et al. 2011). This is likely to happen when no chemical, mechanical or natural (local enemies) method of control exists. Moreover, the small size of some species makes them very difficult to be detected during sanitary border controls, especially when they are hidden in asymptomatic plants (Eschen et al. 2015). One way to control such pests is by importing and releasing natural enemies from their countries of origin, and subsequently allowing them to act as so-called biocontrol agents (Hajek et al. 2016; DiTomaso et al. 2017). The arrival of the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu, Hymenoptera, Cynipidae) in Europe in 2002 (Brussino et al. 2002) is a prime example of this. It literally took Europe by storm (Avtzis and Matošević 2013), colonizing 21 nations in 14 years (Avtzis et al. 2019), causing severe damage to the sweet chestnut tree (*Castanea sativa* Miller) (Ugolini et al. 2014; Sartor et al. 2015; Gehring et al. 2018) and severe fruit yield losses of up to 80% (Battisti et al. 2014; Sartor et al. 2015).

In response, *Torymus sinensis (*Kamijo, Hymenoptera, Torymidae) was imported to Italy from Japan (Quacchia et al. 2008) for biocontrol purposes following the successful Japanese experience (Moriya et al. 2003). Given this course of action was considered to be very promising, if not the only likely solution to control the pest (Moriya et al. 2003) in forest and orchard with very susceptible *C. sativa* cultivars (Dini et al. 2012; Sartor et al. 2015; Nugnes et al. 2018); many other countries followed the Italian example by releasing the biocontrol agent in turn (Borowiec et al. 2014; Matošević et al. 2015; Pérez-Otero et al. 2017; Doğanlar and Savaş 2018). Very good levels of control of *D. kuriphilus* populations were obtained over the short (Quacchia et al. 2014a; Colombari and Battisti 2016a; Matošević et al. 2017a; Borowiec et al. 2018) and long term (Ferracini et al. 2018b). Unlike in these countries, the release of *T. sinensis* in Switzerland was never authorized by federal authorities because of open biosafety questions

(Aebi et al. 2011; UFAM 2012). Nevertheless, the biocontrol agent progressively spread into the country from nearby Italy so as to colonize the entire chestnut growing area of southern Switzerland by 2014 (Gehring et al. 2018). The lack of timely and systematic *T. sinensis* releases since the *D. kuriphilus* arrival in 2007 (Forster et al. 2009), however, caused varying epidemic dynamics in terms of years of undisturbed *D. kuriphilus* population growth (Conedera and Gehring 2015). This situation represents a unique opportunity to study and analyze the natural spread and growth dynamics of the *T. sinensis* population when not directly released. Moreover, in order to have a long-term picture of the epidemic evolution and biocontrol impact, we extend the study area to nearby Italian areas where the biocontrol effects have been in effect at least from 2012 (Quacchia et al. 2014b; Ferracini et al. 2018b). The specific aims of our study are thus defined as follows:

to analyze the natural spread and population growth rate of *T. sinensis* in southern Switzerland;

1. to quantify the long-term evolution of the pest-antagonist system in terms of absolute population numbers;
2. to assess *T. sinensis* efficiency in the biocontrol of the *D. kuriphilus* infestation.

Materials and Methods

Study area and D. kuriphilus-T. sinensis spread history

The extended study area spans from the Cuneo region (Piedmont, Italy) to northern Ticino (southern Switzerland). Both regions have similarly mild climates (http://www.meteoswiss.admin.ch/home/climate/past/climate-normals.html; http://www.scia.isprambiente.it/Documentazione/rapporto_Valori_normali_def.pdf) and both have a long tradition of chestnut cultivation (Bounous 1999; Conedera and Krebs 2008). As a result, *C. sativa* is often the dominant tree species over large areas, characterizing the forest belt continuum (Brändli 1998; Ceschi 2014) with a mix of cultivated and abandoned stands (Figure 1).

The extended study area also includes the Italian municipalities of Boves and Peveragno (Piedmont, Italy; diamond 33 in Figure 1) where the first official *D. kuriphilus* occurrence was recorded in Europe in 2002 (Brussino et al. 2002). From there, the pest spread quickly through chestnut forest stands by natural as well as human-assisted means, reaching the Lake Orta region (northern Italy) in 2006 (diamond 16 in Figure 1). It entered southern Switzerland presumably in 2007 (Forster et al. 2009), from where it moved northwest at a rate of up to 25 km/year (Meier et al. 2013), colonizing the entire chestnut area by 2013.

The release of the parasitoid *T. sinensis* took place for the first time in 2005 in the Cuneo region (Italy; diamonds 18 and 19 in Figure 1) (Quacchia et al. 2008). Many additional releases followed, effectively chasing the spread of *D. kuriphilus* (Figure 1). Despite the prohibition of actively introducing (Aebi et al. 2011; UFAM 2012), the parasitoid entered and spread in Switzerland in 2011, probably benefiting from numerous releases near the Swiss-Italian border (Figure 1). It subsequently spread progressively throughout all over southern Switzerland, colonizing the entire chestnut area by 2014.

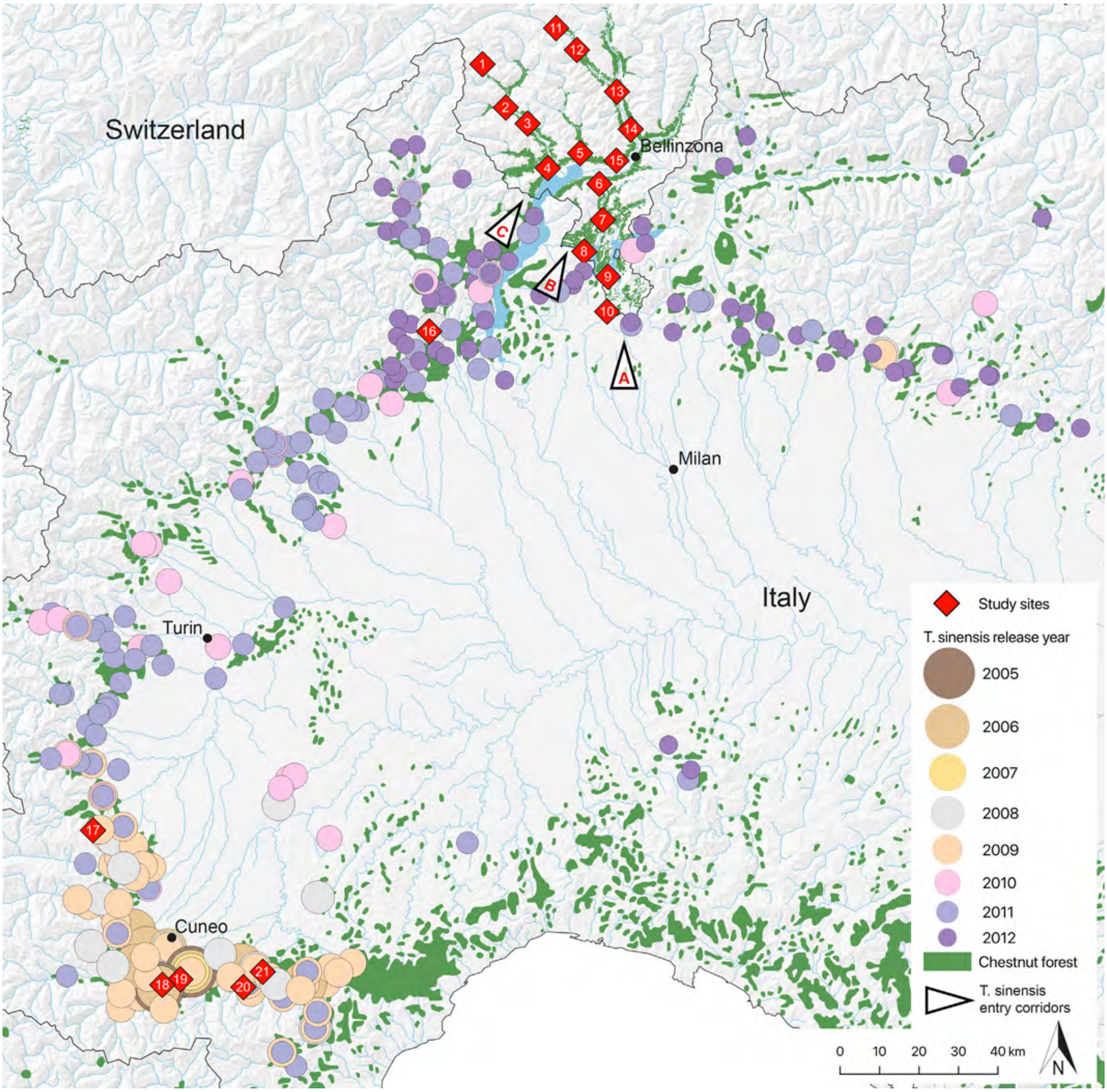

Figure 1 Detailed location of the study sites and *Torymus sinensis* release sites in the Italian area until 2012. Numbers in diamonds represent the study site ID (see Table 1 for details). Triangles with letters represent the three main possible entry corridors in southern Switzerland for *T. sinensis*. Circles represent the release municipality and year of the 271 *T. sinensis* releases retrieved from Boriani et al. 2013 and from personal communication by Giovanni Bosio (Regione Piemonte Settore Fitosanitario).

Sampling design and selection of study sites

In order to provide a complete range of data covering the entire process from the first arrival of *D. kuriphilus* to complete biological control by its natural enemy *T. sinensis*, we adopted a space-for-time substitution approach at the regional scale so as to create a temporal gradient of epidemic stages over the entire study area (Table 1 and A1). In total, 21 sites with different times since the arrival of the pest and its parasitoid were retained. The 15 Swiss sites may roughly represent the arrival and establishment of the pest without the presence of its natural enemy (northern Swiss sites 1, 2, 11 and 12), the early epidemic stage (3, 4, 13 and 14), the epidemic peak (5, 6, 7 and 15) and the end of the epidemic with the beginning of biocontrol (8, 9, and 10). The six Italian sites represent the beginning of the equilibrium between the two populations (sites 16 and 17) as well as the situation with stable *T. sinensis* populations over the medium (20 and 21) and long term (18 and 19).

The 15 sites in southern Switzerland were selected based on a 10 x 10 km grid system covering the whole study area. Final plot locations were moved from the grid point as a function of chestnut presence and site accessibility. In contrast, Italian sites were selected by choosing the most representative cases of the medium- and long-term equilibrium phase. Table 1 and Figure A1 report site characteristics, including years since *D. kuriphilus* and *T. sinensis* arrival. Sampling took place in an area of ca. 12 ha (roughly 350 x 350 m) around the site center.

The arrival time of *D. kuriphilus* and/or release/arrival of *T. sinensis* for dates preceding this study were provided by the Regional Phytosanitary Services of Piedmont and Lombardy and Boriani et al. 2013(Boriani et al. 2013) for Italy and by the Forest Service of Canton Ticino for Switzerland.

Data collection

The field sampling was conducted once a year in summer (from mid-June to mid-august) when *T. sinensis* is present as a terminal instar larva with its characteristic ventral red/brown stripes. The surveys took place from 2011 to 2016 in the Swiss sites and from 2014 to 2016 in the Italian sites (Table 1). In 2011, *D. kuriphilus* presence was checked in every site by searching its galls during half a day. If no galls were found, *D. kuriphilus* was considered absent and the next year this procedure was repeated. Once the galls were found, the presence of *T. sinensis* was checked by dissecting them. The arrival year was set as soon as the insect was found. Then when the parasitoid was found, at each site, we also randomly collected on average 275 shoots (see Gehring et al. 2018 for the definition of "shoot") and 360 galls of any kind (on shoot, on leaf, on stipulae or on flower; see Gehring et al. 2018 for a definition) in total from different trees and crown parts up to 10 meters in height using a telescopic tree pruner. In sites where *D. kuriphilus* had not yet been detected, we only verified its absence by inspecting the whole area as precisely as possible.

On each shoot, the percentage of previous year buds attacked was calculated by counting the number of newly formed shoots displaying *D. kuriphilus* galls with respect to the total number of previous year buds according to the formula:

*D. kuriphilus infestation = (no. of attacked buds / no. of total developed buds) * 100*

Galls were dissected and all their contents identified (Table 2) under a Stereo Microscope (Leica, Wild M3Z). *D. kuriphilus* parasitism rate by *T. sinensis* was calculated at gall level as follows:

D. kuriphilus parasitism by *T. sinensis* = no. of *T. sinensis* / (no. of *D. kuriphilus* chambers) * 100

T. sinensis larvae were identified morphologically by their characteristic red-brownish ventral stripes, which are a quite reliable identification trait for the species at this development stage. In fact, potential confusion with native *Torymus* species exists for *T. cyaneus T. notatus and T. affinis* (Gómez et al. 2008) only, which have however never been reared from *D. kuriphilus* galls (Aebi et al. 2006; Bigler et al. 2006; Aebi et al. 2007; Matošević and

Table 1 Detailed location and survey plan since 2011 for sampling sites with available data

			Areas		Geographic coordinates		Altitude	Slope	Aspect	Arrival year		Distance from nearest Ts release (km)			Sampling year					
			ID	Sites	x	y	(m a.s.l.)	(%)	(°)	*D. kuriphilus*	*T. sinensis*	A	B	C	2011	2012	2013	2014	2015	2016
Dataset 1	Dataset 2	Switzerland	1	Sonlerto	683898	139916	940	14	159	2013	2014	86	47	47	(•)	(•)	(•)	•	•	•
			2	Cevio	689913	128971	469	40	123	2012	2013	73	34	34	(•)	(•)	•	•	•	•
			3	Maggia	695600	125499	424	28	199	2011	2013	66	27	27	(•)	(•)	•	•	•	•
			4	Losone	700893	114040	334	6	91	2011	2012	55	14	14	(•)	•	•	•	•	•
			5	Gordola	709083	117580	546	49	260	2011	2012	47	23	23	(•)	•	•	•	•	•
			6	Rivera	713892	110280	556	15	98	2010	2012	37	23	47	(•)	•	•	•	•	•
			7	Arosio	715210	101423	480	17	220	2009	2011	28	14	36	•	•	•	•	•	•
			8	Pura	710573	92968	455	14	132	2008	2012	23	5	30		•	•		•	•
			9	Melano	716332	86091	636	32	275	2008	2012	14	14	14		•	•		•	•
			10	Stabio	715610	78244	421	18	168	2007	2011	7	7	7	•	•	•	•	•	•
			11	Faido	701825	149674	854	30	177	2012	2014	88	74	78	(•)	(•)	(•)	•	•	•
			12	Lavorgo	708054	143441	738	16	85	2012	2013	80	65	69	(•)	(•)	•	•	•	•
			13	Biasca	718314	134590	386	37	266	2011	2013	65	51	54	(•)	(•)	•	•	•	•
			14	Claro	722203	124162	399	28	234	2011	2013	55	40	44	(•)	(•)	•		•	•
			15	Sementina	718011	116034	646	31	175	2011	2012	44	30	43	(•)	•	•	•	•	•
		Italy	16	Madonna del sasso	450277	5071565	690	11	151	2007	2008								•	•
			17	Sanfront	365113	4945135	787	38	77	2007	2009								•	(•)
			18	Robilante	382694	4906018	752	26	36	2000	2005							•	•	(•)
			19	Peveragno	387318	4907429	729	27	127	2000	2005							•	•	•
			20	Frabosa	403145	4905395	773	9	116	2004	2009								•	(•)
			21	Monastero di Vasco	407977	4909205	750	30	138	2004	2008								•	•

Distances calculated as the crow flies and following the chestnut forest path. Distance A: from entry corridor A only. Distance B: from entry corridor A for sites 9-10; from entry corridor B for sites 6-8 and 11-15; from entry corridor C for sites 1-5. Distance C: as for Distance B but allowing only 2-km transition between interrupted stretches of chestnut forest. Dots indicate when the sites have been visited. Dots in brackets indicate that data concerning *T. sinensis* was not recorded because it was not found or galls were not found. Geographic coordinate system: Italy = *WGS 84 UTM 32*, Switzerland = Swiss grid 1903.

Melika 2013; Quacchia et al. 2013; Palmeri et al. 2014; Francati et al. 2015; Kos et al. 2015; Bernardinelli et al. 2016; Colombari and Battisti 2016a; Panzavolta et al. 2018; Ferracini et al. 2018a; Rondoni et al. 2019; Bonsignore et al. 2019) so far, what drastically decreases the chance of misidentifications.

Table 2 Gall content

Gall content (no.)	**Status/Note**
Chamber	With / without exit hole
Dryocosmus kuriphilus	[a]Alive / Dead
Torymus sinensis	Alive / Dead
Native parasitoids	Alive / Dead
Other	Empty / Fungi / hyperparasitoids / [b]closed / [c]unidentifiable content

[a]An individual is considered dead when it is dry and/or wrapped in a fungus and/or rotten and breaks easily. [b]Proliferation of plant cells filling the chamber. [c]E.g. because too rotten

Data processing and analysis

In order to analyse possible differences in *T. sinensis* population growth rates until the occurrence of biocontrol (defined as 75% *D. kuriphilus* parasitism by *T. sinensis* (Quacchia et al. 2014a)), the relationship between *D. kuriphilus* parasitism (response variable) and years since the arrival of *T. sinensis* (explanatory variable) was analysed for Swiss sites (ID 1-15 in Table 1) using a linear mixed-effects regression approach with the yearly mean values for every site. Since *D. kuriphilus* parasitism is expressed as a percentage and follows an exponential growth pattern (Figure A2), the data were log-transformed. The random effect (site) configuration (random intercept or random intercept and slope) was determined following the procedure described in Zuur et al. (Zuur et al. 2009). Briefly, it consists in creating the so called “beyond optimal model” including all explanatory variables in the fixed component. Then, using the “beyond optimal model”, various random configurations are built. These models are then compared by the means of Akaike information criterion (AIC) and the random structure of the one with the lowest AIC is then used for the subsequent model analysis.

The resulting random effect model estimates (in this case representing the *T. sinensis* population growth rate) were then set as the response variable in multiple linear regression models with the following variables as predictors: distance between Swiss sites and the three nearest possible entry corridors (Figure 1 and Table 1), *D. kuriphilus* infestation at time of *T. sinensis* arrival (representing the host availability for *T. sinensis*) and the number of years from the arrival of *T. sinensis* to biocontrol occurrence. During the model selection procedure, the three distances were always tested separately with the other predictors. Best models were selected on the basis of the AICc coefficient (Akaike information criterion with a second-order correction for small sample size). Then the final model has been checked for possible multicollinearity issues, which imposes variation inflation factors <5 and condition indices <30 (Belsley et al. 1980).

The three distances were calculated “as the crow flies” using Qgis (QGIS Development Team 2018) starting from the nearest Italian *T. sinensis* release points as entry corridors and following the chestnut forest as a spread pathway. Specifically, distance A only took entry corridor A into account. Distance B considered entry corridor A for sites 9-10, B for sites 6-8 and 11-15, and C for sites 1-5. Distance C considered the same entry corridors as B, while only allowing for a 2-km transition between interrupted chestnut forests. *T. sinensis* spread rate was calculated from the selected entry corridors as a spatial baseline and took the oldest Italian release as a distance and time reference point.

Average *D. kuriphilus* infestation and *D. kuriphilus* parasitism values were calculated for each sampling year for all sites in the experimental design, including Italian sites. Means and 95% confidence intervals were grouped based on the number of years since *T. sinensis* arrival or release. Similarly, also mean gall contents (see Table 2 for details) and mean number of galls per developed bud on a shoot were calculated first at site level for each sampling year, and then multiplied by each other in order to obtain a quantitative estimate of the gall content according to the number of years since *T. sinensis* arrival over the whole study area.

All analyses were performed in R (version 3.5.1; R Core Team 2018). Linear mixed-effects models were fit using the lme4 package (Bates et al. 2015) and their goodness of fit evaluated with the r.squaredGLMM function of the MuMIn package (Bartoń 2016).

The dataset used in this analysis has been published in the EnviDat data portal and is available at this link: https://www.envidat.ch/ui/#/metadata/torymus-sinensis-population-evolution-from-arrival-to-biocontrol (Gehring et al. 2019).

Results

Torymus sinensis spread rates and parasitism efficiency

Spread rate estimates for *T. sinensis* in southern Switzerland range from 16 to 26 km/year depending on the entry corridor considered. Based on these corridors, the *D. kuriphilus* parasitism started gradually and then exponentially increased from south to north from 2014 on. The only exception to this general pattern is represented by a single site displaying a higher *D. kuriphilus* parasitism in 2013 already as compared to the surroundings (see site 7 in Figure A3).

For the *T. sinensis* growth rate mixed model analysis, both random intercepts for site and by-site random slopes for years since *T. sinensis* arrival were kept as the best random effects configuration, highlighting variability in *D. kuriphilus* parasitism rates across sites and years. The best model (Table 3) had a marginal R^2 (fixed effect only) of 0.85 and a conditional R^2 (fixed and random effects) of 0.94, emphasizing the importance of the explanatory variable (years since *T. sinensis* arrival). However, random effects estimates (representing *T. sinensis* population growth rate) varied by a factor of approximately 1.6 among sites (Table A1), meaning that for each one-unit increment in years since *T. sinensis* arrival, *D. kuriphilus* parasitism increases 1.6 times as much in certain sites compared to others (Figure 2). These differences are mostly explained by the squared distance A and the *D. kuriphilus* infestation at time of *T. sinensis* arrival (Table 4).

D. kuriphilus and *T. sinensis* interaction and population dynamics

When not controlled by *T. sinensis*, *D. kuriphilus* infestation initially follows an exponential growth pattern reaching its epidemic peak in the second and third year with infestation levels of 36% on average (Figure 3) and highs of up to 84% (Figure A2). *D. kuriphilus* parasitism by *T. sinensis* follows similar growth patterns reaching values of higher than 75% (which is considered the biological control level (Quacchia et al. 2014a)) in three years on average (Figure 3).

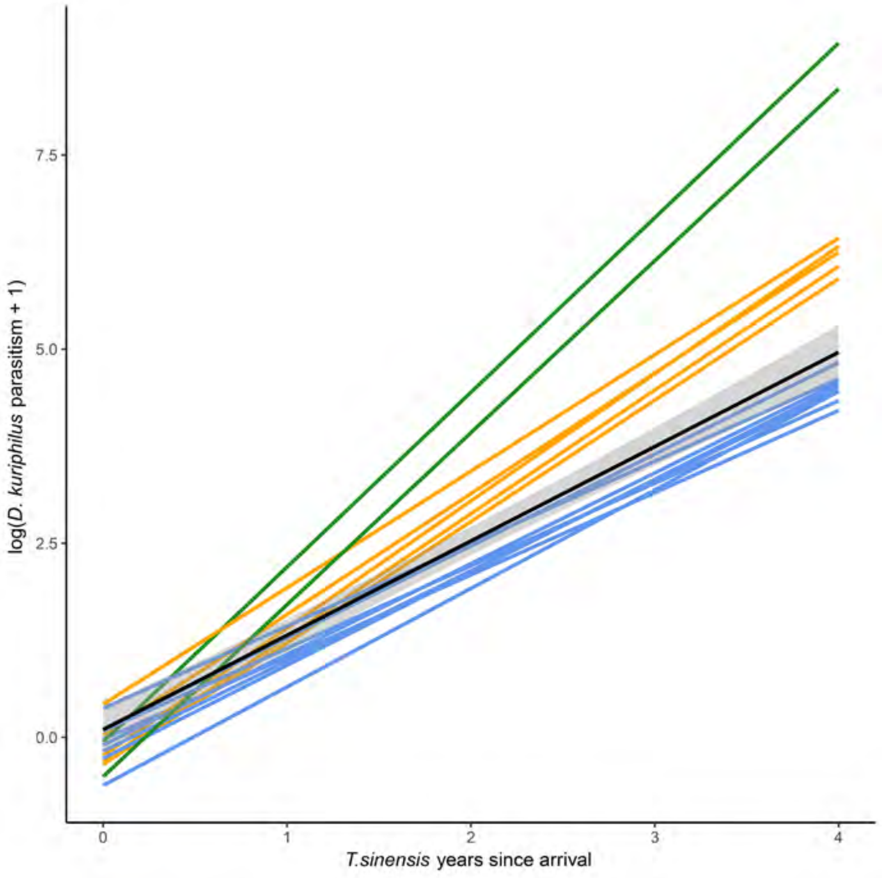

Figure 2 Variation in the *Dryocosmus kuriphilus* parasitism rate as a function of the years since *Torymus sinensis* arrival as predicted by liner mixed-effects models for southern Switzerland. The black line with the grey band (95% confidence interval) shows the fitted values for the overall model (the predicted average variations across sites). Coloured lines represent the effect for each site as obtained by adding intercept and slope adjustments to the overall model fit. Lines are coloured according to three distance categories from the nearest *T. sinensis* release in the Italian region (green: ≥ 75 km; yellow: ≥ 50 & < 75 km; blue: < 50 km).

Table 3 Best linear mixed-effects regression model

Model formula	Fixed effect	Estimate	95% CI	R^2m	R^2c
log(*Dk* parasitism) ~ *Ts.yrs* + *r*	Intercept	-0.057	[-0.281, 0.167]	0.85	0.94
	Ts.yrs	1.362***	[1.198, 1.526]		

N = 65, N(r) = 15; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

95% CI = 95% confidence interval; R^2m = marginal R^2 showing the variance explained by the fixed effects; R^2c = conditional R^2 showing the variance explained by the entire model (fixed and random effects); Dk parasitism = *Dryocosmus kuriphilus* parasitism by *Torymus sinensis. Ts.yrs* = years since *Torymus sinensis* arrival; *r* = random effects configuration: random intercepts for site and by-site random slope for years since *T. sinensis* arrival.

Table 4 Best linear regression model

Response variable	Fixed effect	Estimate	95% CI	Var. Imp.
Ts. growth rate	(Intercept)	0.95498 ***	[0.82759 1.08237]	
	Distance A^2	0.00007 ***	[0.00005 0.00009]	66
	Dk infestation	0.00466 **	[0.00172 0.00761]	34

N = 15; $R^2 = 0.89$, Adj. $R^2 = 0.87$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Ts. growth rate = *Torymus sinensis* population growth rate; 95% CI = 95% confidence interval; Var. Imp. = variable importance in %. Dk infestation = *Dryocosmus kuriphilus* infestation at time of *T. sinensis* arrival.

Subsequently, *D. kuriphilus* infestation progressively decreases until very low values are reached (< 10%) within four years. From then on, both populations appear to be stable over time with minimal fluctuations (Figure 3). In our data, beyond four years after the occurrence of biological control (value 8 on the horizontal axis in Figure 3), *D. kuriphilus* parasitism never decreases below the 75% threshold and *D. kuriphilus* infestation never exceeds 7%.

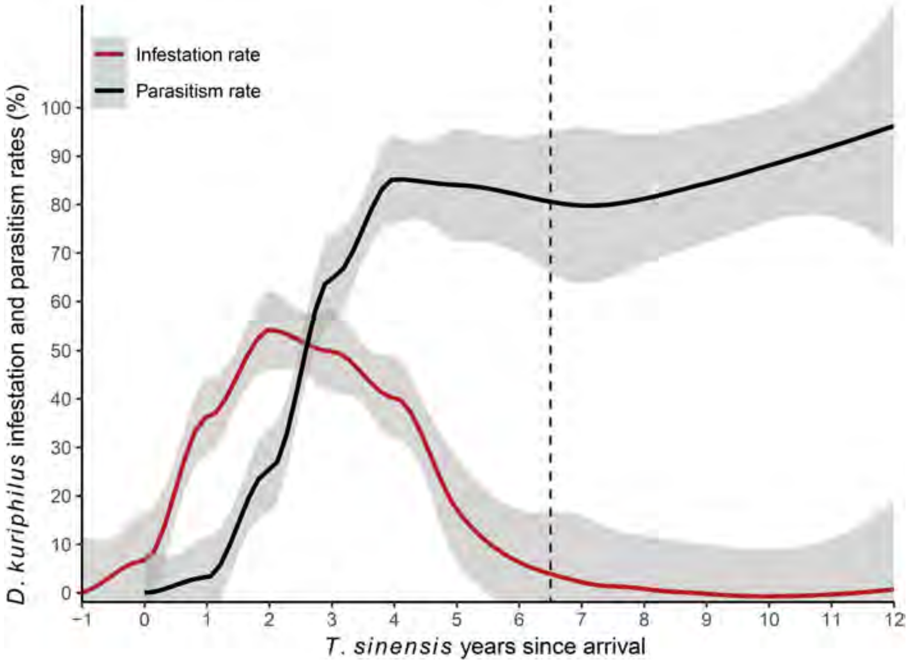

Figure 3 *Dryocosmus kuriphilus* infestation (red) and parasitism rate (black) as a function of years since *T. sinensis* arrival. Grey bands represent the 95% confidence interval. The vertical dashed line on the horizontal axis shows the transition between the Swiss and the Italian data at 6.5.

Considering population absolute numbers, in the initial epidemic phase when *D. kuriphilus* just arrives and *T. sinensis* has not yet arrived (≤ 0 years since *T. sinensis* arrival [Ts.yrs]), galls are rare because of the low population density (< 1 individual per developing bud, see Figure 4). In this phase, galls usually hosts two chambers and are small in size (Figure 4, blue dashed line) and few in number. Being initially not controlled by its natural enemy, the *D. kuriphilus* population grows rapidly and reaches a peak when Ts.yrs = 3 (corresponding to the 5th year from *D. kuriphilus* arrival) with nearly 4 individuals per developing bud (Figure 4, yellow line, Ts.yrs = 2). Similarly, the red line shows the *D. kuriphilus* individuals that successfully terminate their life cycle. This is also the phase with the highest impact in terms of tree damage (ca. 1 gall with 3 to 4 chambers per developed bud; green line and blue dotted line, respectively). Soon after the arrival of *T. sinensis* (Ts.yrs = 2) the number of *D. kuriphilus* that successfully complete their life cycle (red line) begins to decrease. As a consequence, starting from the very next year, the number of galls decreases slightly (green line). As soon as *T. sinensis* parasitism reaches the biocontrol threshold (> 75%, Ts.yrs = 4 in Figure 3 and Figure 4), the *D. kuriphilus* population successfully completing its life cycle (red line) drastically decreases to one individual every ~10 developed buds. Within three years after, galls become sparse and difficult to find in the field (~1 gall per 76 developed buds on average).

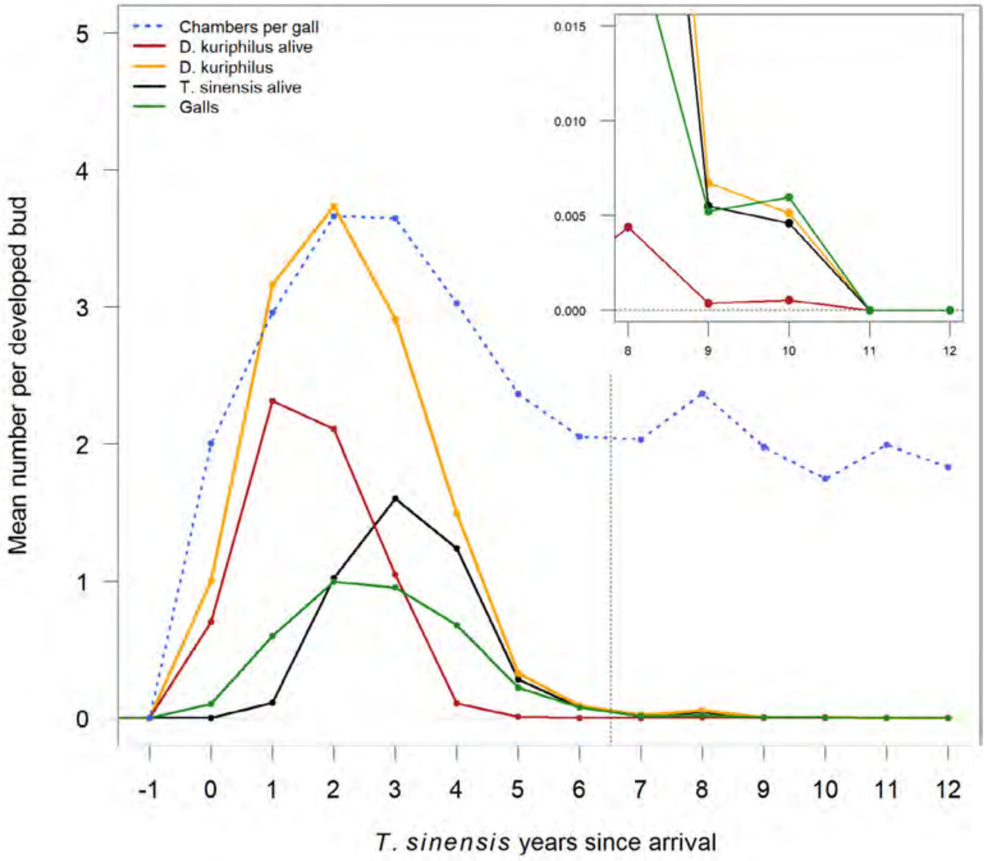

Figure 4 Mean number of galls and gall content per developed bud as a function of years since *Torymus sinensis* arrival. The upper right box shows a close up of the right part of the main figure. "*D. kuriphilus* alive" (red) refers to the individuals that successfully completed their life cycle; "*D. kuriphilus*" (yellow) refers to the number of individuals that were initially present in the galls (beginning of the vegetative season). "*T. sinensis* alive" (black) refers to the number of individuals found alive during the sampling. Please note that the mean chamber number (dashed blue line) is per gall and not per bud. The vertical dashed line on the horizontal axis shows the transition between the Swiss and the Italian data at 6.5.

At the end of the process (~5 years under biological control, roughly corresponding to Ts.yrs = 9 in Figure 4), both populations will be very reduced in terms of absolute numbers (1 *T. sinensis* individual per ca. 182 developed buds and 1 *D. kuriphilus* indivudal per ca. 2665 developed buds, respectively). Most buds produce gall-free shoots, galls on trees are correspondingly rare, mainly localized on leaves (Figure A4), and are relatively small, with a maximum of two chambers per gall. In sites where biological control by *T. sinensis* is achieved after 5 years at least, no large or potentially harmful fluctuations between the two populations can be observed, and the absolute number of *T. sinensis* always remains far greater than the *D. kuriphilus* population (subfigure in Figure 4). Finally (Ts.yrs = 11 to

12) the absolute population becomes even smaller to the point that it was no longer possible to find any galls on more than 850 analysed (or checked) shoots.

Discussion

Our results highlight how *T. sinensis* managed to spread easily from Italy into Switzerland, rapidly colonizing the entire Swiss chestnut grove area within four years. The most probable entry corridor was the southernmost site (Corridor A), very likely as the result of one (or very few) nearby releases in Italy in 2010. Using this corridor as the starting point, the Swiss colonization process spread at a rate of 26 km per year. This spread is likely the result of optimal conditions such as tight chestnut stand connectivity (Borowiec et al. 2018) which manifests itself as an uninterrupted forest belt stretching from south to north (Ceschi 2014) and the widespread presence of *D. kuriphilus* galls acting as concentrated resources (Colombari and Battisti 2016b). Given the very complex geomorphology of the region, it is, in contrast, more difficult to understand the role that winds may have played in such a rapid expansion. Although the dominant winds blow in the opposite directions, their effect is only sporadically significant at the elevation of the chestnut forests, where it is probable that mountain and lake breezes that blow in various directions dominate (Spinedi 1991; Ufficio di statistica del canton Ticino 2004). This very good dispersal ability under optimal conditions has also been noted by other authors in France (Borowiec et al. 2018), Italy (Colombari and Battisti 2016b), Croatia, Hungary and Slovenia (Matošević et al. 2017b) and Japan (Moriya et al. 2003). It is also likely to be enhanced by the lack of limiting factors such as hyperparasitoids and/or competition with native parasitoids. According to Borowiec et al. (Borowiec et al. 2018), in such ideal conditions even a single release of 100 individuals may be as efficient as multiple releases in establishing a large and stable *T. sinensis* population.

In a single case (site 7 in Figure A3), the *D. kuriphilus* parasitism was found to be unexpectedly high compared to site further south, what may be due to an episodic illegal introduction in the area before 2013. In other cases (i.e., sites 3, 4, 5) the *D. kuriphilus* parasitism is only insignificantly higher with respect to the expected values, so that additional illegal introductions cannot be confirmed. Given the high natural rate of spread and growth of the *T. sinensis* population, however, we believe that possible effects of the illegal introductions have been totally masked by the main massive “natural” population wave (Paparella et al. 2016)

The population dynamics of *T. sinensis* in Switzerland are characterized by an initial gradual and subsequent exponential growth at different rates as a function of the distance from the nearest release site and, partially, of *D. kuriphilus* abundance. Consequently, the time needed to reach the biocontrol threshold (Quacchia et al. 2014b) after *T. sinensis* arrival was much shorter (only two years) in the north than in the south (seven years). Such a longer time-lag is similar to what was generally observed in France (Borowiec et al. 2018), Italy (Quacchia et al. 2014b; Colombari and Battisti 2016a; Ferracini et al. 2018b) and Japan (Moriya et al. 2003), although even longer delays may exist in specific situations (Murakami and Gyoutoku 1995). As for the spread, the enhanced rate in *T. sinensis* population growth that occurred in the northern sites could be due to the optimal environmental conditions encountered by the massive travelling wave (Paparella et al. 2016).

A significant decrease in *D. kuriphilus* infestation is noted in all sites as soon as *T. sinensis* parasitism reached levels exceeding 70%, which happened on average three years after the arrival of the antagonist. Once the *T.*

sinensis population becomes massive and stable, the control it exerts on *D. kuriphilus* appears to guarantee a low and stable infestation level with little fluctuation. This level of infestation, in the long run, always remained far below a damage level (30%) which is no longer considered tolerable (Gyoutoku and Uemura 1985; Sartor et al. 2015). Moreover, both populations drastically decreased in terms of absolute numbers, to the point that, in some Italian sites, it was not possible to even find single galls after searching for an entire summer day. Similarly, this level of control is also happening in some specific areas of France, where *T. sinensis* has been released since 6 years (Borowiec et al. 2018).

Several studies have investigated the capacity of indigenous parasitoids to control *D. kuriphilus* populations, showing in specific cases promising initial parasitism levels (Santi and Maini 2011). Unfortunately their ability to exert a significant control on *D. kuriphilus* population turn out to be inadequate to keep an infestation level below the damage threshold on the long term (Aebi et al. 2006; Speranza et al. 2009; Santi and Maini 2011; Matošević and Melika 2013; Quacchia et al. 2013; Palmeri et al. 2014). Although this study did not focus on the role of native parasitoids in controlling the *D. kuriphilus* population, the high values of *D. kuriphilus* infestation in absence of the specific antagonist may suggest a low controlling effect by native parasitoids in the study area.

Finally, this study represents another example of successful classic biological control that does not appear to be confined to European chestnut areas or to the sweet chestnut tree (Matošević et al. 2017a; Ferracini et al. 2018b) but also applies to other continents and other chestnut trees (Moriya et al. 2003; Rieske 2007). Despite this overall positive picture, it is worth noting that in specific cases, the time necessary to reach full biological control may be very long (up to 18 years, (Murakami and Gyoutoku 1995)) because of the negative action of native parasitoid on *T. sinensis*, whereas in other cases, the insect populations may be subjected to such significant oscillation that related damage may exceed the harmful threshold.

Conclusions

T. sinensis has been shown to be effective in controlling *D. kuriphilus* in southern Switzerland, despite the lack of a systematic release campaign in the area. Its ability to migrate and establish from nearby Italy and to spread very quickly across long distances allowed it to significantly decrease the *D. kuriphilus* population in the entire chestnut area of southern Switzerland. The speed with which it achieved biological control, however, differed according to the site. Given these differences, a long-term monitoring program is needed in order to confirm the ubiquity and the stability of the biocontrol. Furthermore, in view of the drastic reduction of its host resources over the long term, possible interactions with native parasitoids and non-target gall-inducing insects should also be investigated.

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Appendices

Figure A1 Data available at site level according to years since *Dryocosmus kuriphilus* and *Torymus sinensis* arrival. Dots represent the Swiss sites (CH) and triangles the Italian sites (I). Size is proportional to the number of sampled sites.

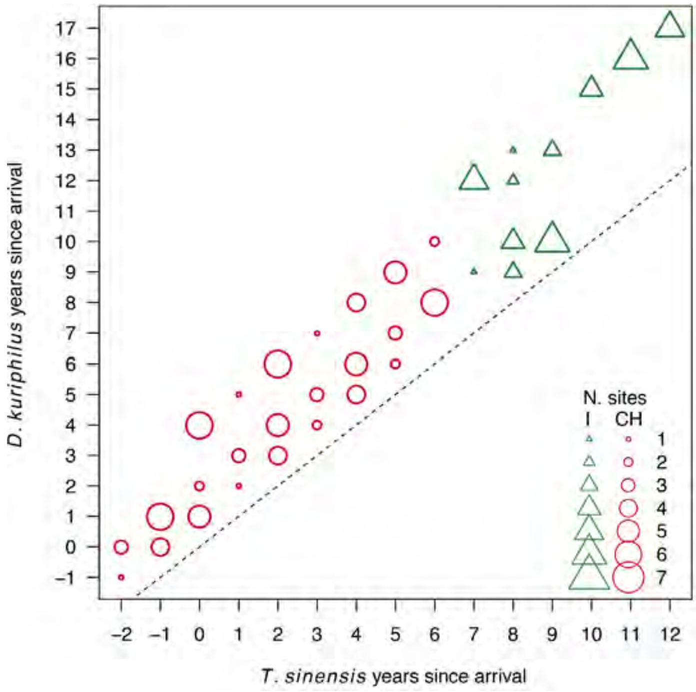

Figure A2 *Dryocosmus kuriphilus* parasitism and infestation rate (%) at each Swiss site as a function of years since *T. sinensis* arrival.

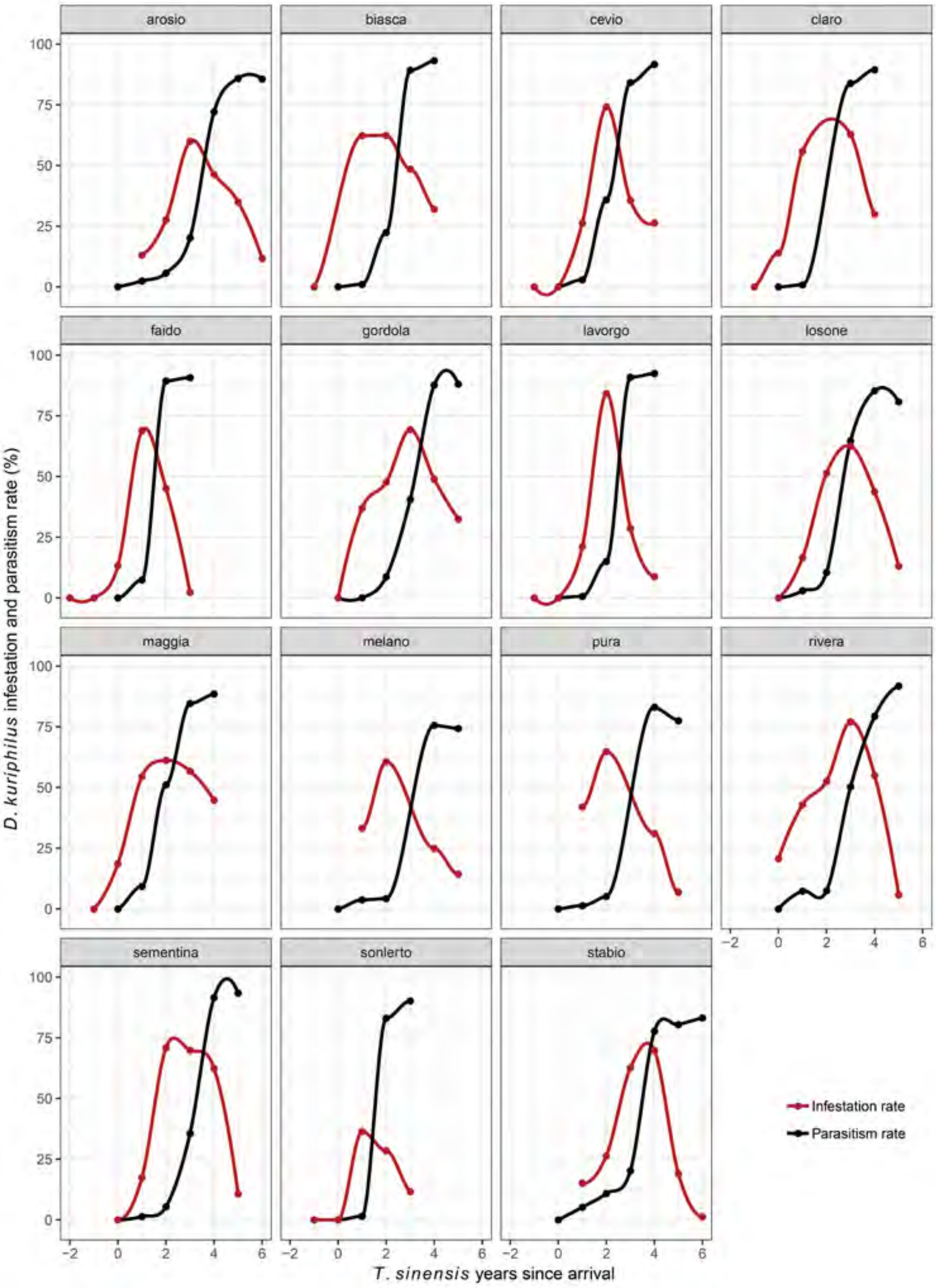

Figure A3 *Dryocosmus kuriphilus* parasitism by *Torymus sinensis* in 2013 according to study sites locations. Numbers in coloured dots represent the area id in Table 1.

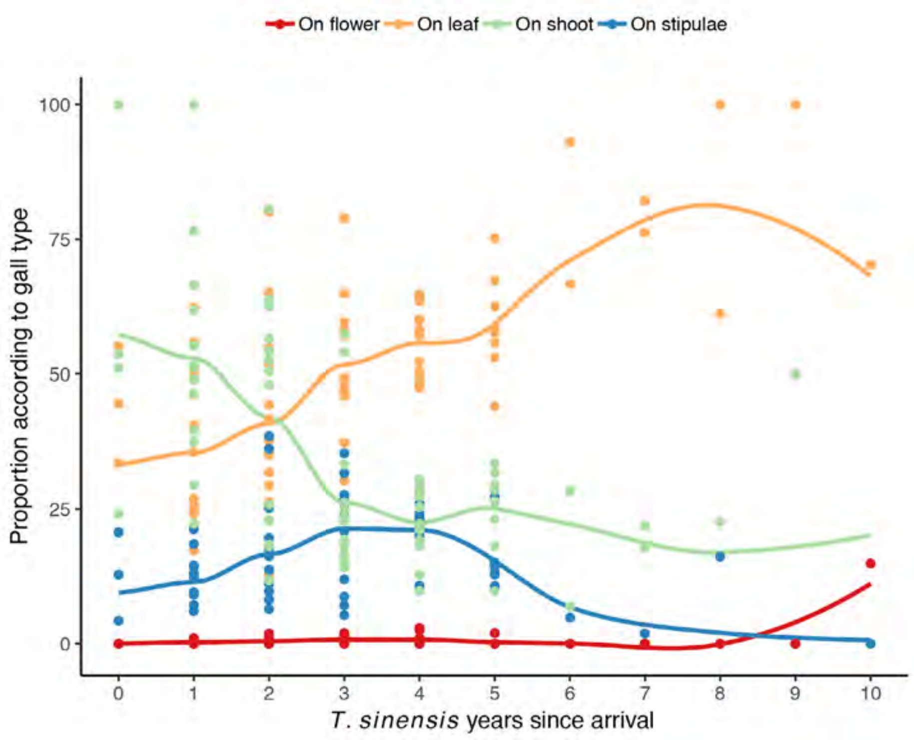

Figure A4 Proportion of gall types according to the years since *Torymus sinensis* arrival.

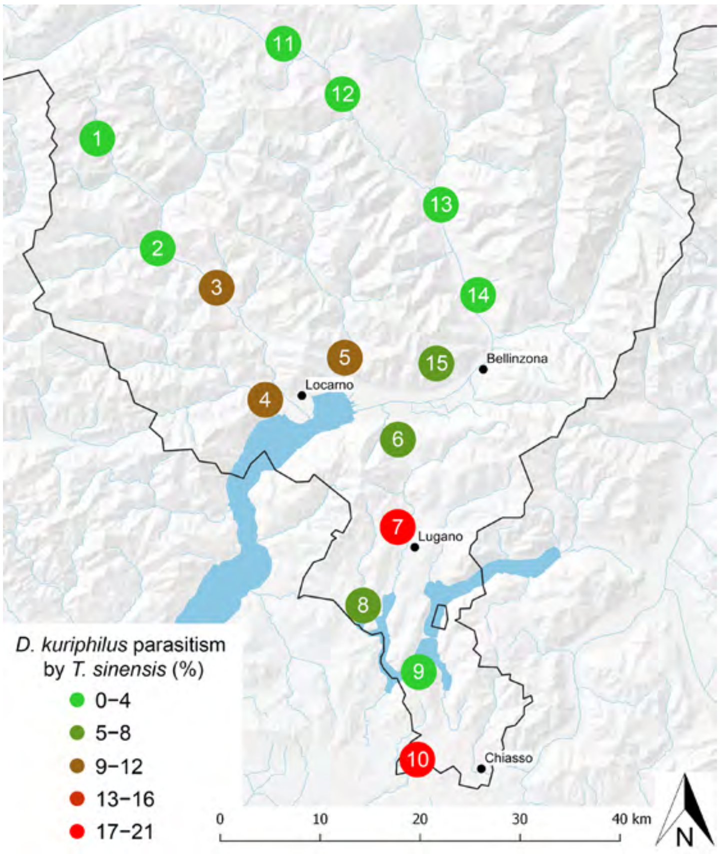

Table A1 Random factor estimates of the best linear mixed-effects regression model

Areas		Estimates	
ID	Sites	Intercept	Ts yrs
1	Sonlerto	0.027	1.650
2	Cevio	-0.009	1.526
3	Maggia	0.018	1.617
4	Losone	-0.088	1.257
5	Gordola	-0.126	1.128
6	Rivera	-0.093	1.240
7	Arosio	-0.127	1.126
8	Pura	-0.107	1.194
9	Melano	-0.109	1.186
10	Stabio	-0.118	1.156
11	Faido	0.078	1.822
12	Lavorgo	-0.043	1.411
13	Biasca	-0.033	1.445
14	Claro	-0.016	1.502
15	Sementina	-0.114	1.169

Ts yrs = years since arrival *Torymus sinensis*

4.2.

Chestnut tree damage evolution due to *Dryocosmus kuriphilus* attacks

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Crown of a chestnut tree heavily disrupted by repeated *Dryocosmus kuriphilus* attacks

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Abstract

Dryocosmus kuriphilus has become a major threat to *Castanea sativa* and chestnut cultivation in Europe since its introduction to Italy in 2002. Thankfully, the biological control agent *Torymus sinensis* has proven to effectively control and reduce the *D. kuriphilus* population. The reaction of chestnut trees to decreasing *D. kuriphilus*-pressure, however, has been sparsely investigated. In this study, we analyze the recovery patterns of chestnut trees since the pest epidemic peak in 15 sites in southern Switzerland that were differently affected by the dephased arrival of *D. kuriphilus* and *T. sinensis.* By using various tree damage and insect population indicators, we show how damage varies as a function of the time elapsed between pest arrival and biological control by *T. sinensis*. Specifically, trees in sites experiencing seven years of *D. kuriphilus* uncontrolled attacks show large dying crown portions and stress-induced reactions (e.g., suckers along the stem) compared to sites where the two insects arrived nearly simultaneously. Moreover, in warm and south-exposed sites, the *D. kuriphilus* population remains significant and damage on chestnut trees still persists suggesting a desynchronisation in the phenology of the antagonist with respect to the pest.

Keywords: *Castanea sativa, Torymus sinensis, Tree damage, Recovery process, Damage recrudescence, Classical biological control*.

Introduction

The Asian Chestnut Gall Wasp (*Dryocosmus kuriphilus* Yasumatsu, Hymenoptera, Cynipidae) is an invasive gall maker that rapidly became epidemic in Europe since its arrival in Italy in 2002 (Brussino et al. 2002). The high invasion potential is characterized by its fast parthenogenetic (thelytokous) reproductive success (Stone et al. 2002; Aebi et al. 2011; Avtzis et al. 2019), its rapid spread, and its asymptomatic conditions, due to its minute size and specific biological cycle, which allow it to remain hidden in chestnut buds during nearly two thirds of its life (Panzavolta et al. 2012; Bernardo et al. 2013). In addition, its dispersion potential and invasive ability is increased by its optimal phenological synchrony with the main European host tree *Castanea sativa* (Miller) (Bernardo et al. 2013), the absence of efficient local natural enemies in the newly invaded areas (Aebi et al. 2006; Cooper and Rieske 2007; Matošević and Melika 2013; Quacchia et al. 2013), the continuity of chestnut groves in many Euopean countries and the lack of efficient mechanical (Maltoni et al. 2012a) or chemical (Cooper and Rieske 2007) control options.

In the case of repeated and uncontrolled attacks by *D. kuriphilus*, overall tree fitness is reduced (Kato and Hijii 1997; Ugolini et al. 2014; Sartor et al. 2015; Gehring et al. 2018a) to the point where chestnut groves experience important reductions in fruit yield (up to 80% - Battisti et al. 2014; Sartor et al. 2015). When the *D. kuriphilus* infestation occur in conjunction with other stresses such as drought and/or weakness pathogens (e.g., the necrophitic *Cryphonectria parasitica* Murrill (Meyer et al. 2015; Rigling and Prospero 2018) and/or *Gnomoniopsis castaneae* G. Tamietti (Lione et al. 2016, 2019)), concerned trees may even die (Tarcali and Radocz 2009; Zhi-Yong 2009).

Due to the importance of the chestnut culture and related fruit production in many European countries and in Italy in particular (Bounous 2006), and inspired by the successful Japanese experience (Moriya et al. 2003), the University of Turin studied and trailed the use of the biocontrol agent *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) which was then first released in 2005 in the province of Cuneo (Quacchia et al. 2008). Following the promising results of the Italian experience, many other release programs followed in France (Borowiec et al. 2014), Croatia, Hungary and Slovenia (Matošević et al. 2015), Turkey (Doğanlar and Savaş 2018), Spain (Pérez-Otero et al. 2017), Portugal and Slovakia (Personal communication by Ambra Quacchia), and Austria (LFI 2017).

Even though the effectiveness of *T. sinensis* in controlling the *D. kuriphilus* population has subsequently been demonstrated in Japan (Moriya et al. 2003), the United States of America (Rieske 2007), Croatia, Slovenia and Hungary (Matošević et al. 2017), and Italy (Ferracini et al. 2018a) information concerning chestnut recovery with respect to decreasing pest-pressure remains sparse. In the present study, we investigate the recovery patterns of chestnut trees in southern Switzerland in 15 sites differently affected by *D. kuriphilus* epidemics in terms of years prior to biological control by *T. sinensis.* Our specific aims are to:

1. investigate chestnut tree reactions to decreasing *D. kuriphilus* pressure as a consequence of *its* parasitism by *T. sinensis.*

2. define the threshold of *D. kuriphilus* parasitism by *T. sinensis* that induces the visible recovery of chestnut trees
3. describe the recovery patterns in different crown sectors (inner and outer crown area) and as function of the time-lag between *D. kuriphilus* and *T. sinensis* arrival.

Materials and methods

Study area

This study was carried out in the chestnut groves of canton Ticino (southern Switzerland). The climate is classified as moist-warm and temperate (annual mean precipitation from 1,318 to 2,134 mm, annual mean temperature ranging from 10.2 to 12.4 °C – MeteoSwiss climate normals for the period 1981-2010 and considering the main chestnut grove areas; http://www.meteoswiss.admin.ch) and soils as haplic podzol on crystalline bedrock (Blaser et al. 2008). The chestnut forests of canton Ticino cover an area of ca. 20,000 ha representing 15% of the entire cantonal forest. *C. sativa* is mainly found at elevations ranging from 200 m a.s.l. (lowest point at Lago Maggiore) up to 900-1,100 m a.s.l. (Krebs et al. 2012). As a result, the species forms a chestnut forest belt continuum across the whole study area with a mix of cultivated, abandoned and naturalized stands (Brändli 1998; Ceschi 2014) (Fig. 1).

D. kuriphilus entered Switzerland from the south most likely in 2007 (Forster et al. 2009) (arrow in Fig. 1). Its population grew rapidly and moved northwest mainly by natural dispersion and wind assisted flight (Oho and Shimura 1970; Graziosi and Rieske 2014), colonizing the entire chestnut area by 2013. The arrival of its specific antagonist, *T. sinensis*, was officially announced in Ticino in 2013, although its presence was recorded by the authors in southern Ticino from 2011 onwards. Because of the prohibition of an active release of the antagonist due to unresolved biosafety issues, its arrival in Switzerland is assumed to be the result of a natural migration process beginning with the Italian releases near the Swiss border combined with some sporadic cases of illegal introductions (Conedera et al. 2015). Similarly to *D. kuriphilus* but much quicker, *T. sinensis* colonized the entire chestnut area by 2014, arriving almost simultaneously with its prey in the northernmost sites. This resulted in very different time-lags between *D. kuriphilus* and *T. sinensis* arrival between southern and northern sites (Fig. 1).

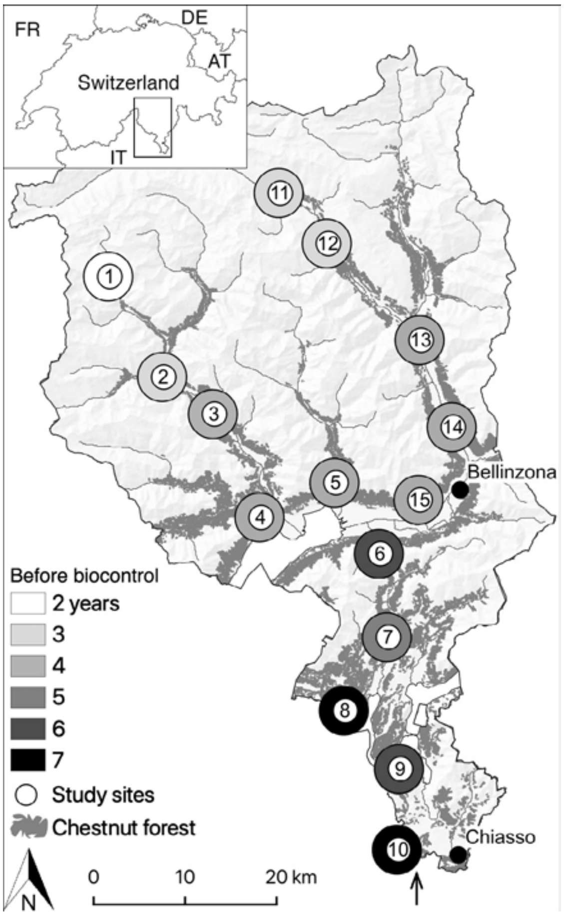

Figure 1 Location of the study sites. The top left subFigureure shows the location of the study area (indicated by the small rectangle) within Switzerland. The numbered circles are colored according to the time-lag between *Dryocosmus kuriphilus* arrival and biological control by *Torymus sinensis*. The arrow in the lower part of the Figureure indicates the main *D. kuriphilus* and *T. sinensis* entry corridor.

Study design and data collection

Study sites were selected in grids measuring 10 x 10 km so as to cover the entire area from north to south and to target heterogeneous situations in terms *of D. kuriphilus* and *T. sinensis* arrival years (Fig. 1, Table 1). General site characteristics such as geographic coordinates, altitude, slope, and aspect were recorded in the field (Table 1). From 2015 on, three temperature data loggers (Thermochron Logger DS1922L, iButton®) were placed in a shaded area in each site and the air temperature was measured at 2-hour intervals.

Within each site, ten mature trees were randomly selected in 2012 within an area of ca. 10 hectares and revisited during each field campaign (from 2013 to 2018). For every sampled tree, an overall visual crown assessment was first performed from four different perspectives for each tree and then averaged to a single value. Specifically, two different indices were recorded: the crown transparency of the living part (Crown transparency) using Müller and Stierlin's (1990) reference photography, and the proportion of the dead crown due to repeated *D. kuriphilus* attacks and *Cryphonectria parasitica* attacks (Dead crown). For the latter, very old dead branches and snags are excluded from the assessment. Recent dieback (ca. ten years) can be identified by the presence of small terminal twigs and/

Table 1 Sampling sites with detailed location and characteristics

Cluster		Site	Geographic coordinates *(Swiss grid CH1903)*		Altitude *(m a.s.l.)*	Slope *(%)*	Aspect *(°)*	Aspect transformed[a]	Temperature *(°C)*[b]		Arrival year		Epidemic peak	Biocontrol[c]
											D. kuriphilus	*T. sinensis*		
Group	ID		X	Y			360°	0-2	Mean	[Min., Max]	Year			
1	3	Maggia	695588	125634	434	28	250	0.09	11.9	*[-4.5, 31.3]*	2011	2013	2013	2015
1	13	Biasca	718372	134801	382	42	200	0.09	11.0	*[-5.2, 33.0]*	2011	2013	2013	2015
1	15	Sementina	717928	115998	646	31	200	0.09	11.3	*[-7.0, 33.7]*	2011	2012	2013	2015
2	2	Cevio	689843	128927	475	41	155	0.66	10.7	*[-5.0, 30.3]*	2012	2013	2014	2015
2	4	Losone	700817	114163	318	4	40	2.00	10.9	*[-5.3, 32.2]*	2011	2012	2014	2015
2	5	Gordola	709094	117459	542	47	290	0.58	9.9	*[-5.7, 31.1]*	2011	2012	2013	2015
2	14	Claro	721970	124254	388	31	240	0.03	11.8	*[-8.2, 34.3]*	2011	2013	2013	2015
3	6	Rivera	713941	110347	552	15	50	2.00	11.5	*[-6.8, 32.9]*	2009	2012	2013	2015
3	7	Arosio	715290	100943	557	12	70	1.91	9.0	*[-7.5, 28.6]*	2009	2011	2013	2014
3	8	Pura	710578	92950	454	19	40	2.00	11.3	*[-6.3, 30.2]*	2008	2012	2013	2015
3	9	Melano	716143	85852	644	34	330	1.26	9.8	*[-7.0, 28.6]*	2008	2012	2013	2014
3	10	Stabio	715265	78329	422	17	110	1.42	11.6	*[-6.7, 30.7]*	2007	2011	2013	2014
4	1	Sonlerto	683916	139793	920	15	60	1.97	8.4	*[-7.3, 28.7]*	2013	2014	2014	2015
4	11	Faido	701571	149740	857	30	115	1.34	9.0	*[-9.8, 28.9]*	2012	2014	2014	2015
4	12	Lavorgo	708157	143244	748	16	135	1.00	9.0	*[-7.3, 30.2]*	2012	2013	2014	2015

[a] Aspect transformed using Beers et al. (1966) equation [sin(azimuth + 45) + 1]. 0 = southwest facing (xeric); 2 = northeast facing (mesic).

[b] Daily mean temperature from March 2015 to March 2018

[c] Biocontrol threshold defined at 75% according to Quacchia et al. 2014

Table 2 Main indicators used for *Torymus sinensis*/*Dryocosmus kuriphilus* population and damage assessments

<table>
<tr><th colspan="3">Indicators</th><th>Short name</th><th>Formula / Description</th><th>Values</th></tr>
<tr><td rowspan="2">Population</td><td></td><td>Dryocosmus kuriphilus parasitism by Torymus sinensis (at gall level)</td><td>D. kuriphilus parasitism</td><td>$D.kuriphilus\ parasitism = \frac{T.sinensis\ alive}{chamber} * 100$</td><td>From 0% (no T. sinensis) to 100% (chambers fully occupied). 75% is considered the biological control threshold level (Quacchia et al. 2014) when D. kuriphilus infestation significantly decreases.</td></tr>
<tr><td rowspan="3">Outer crown</td><td>Number of buds with Dryocosmus kuriphilus galls per available bud (at shoot level)</td><td>D. kuriphilus infestation</td><td>$D.kuriphilus\ infestation = \frac{attacked\ buds}{available\ buds} * 100$</td><td><table><tr><td>0</td><td>≤ 30</td><td>> 30 ≤ 40</td><td>> 40 ≤ 60</td><td>> 60</td></tr><tr><td>No</td><td>Low</td><td>Moderate</td><td>High</td><td>Very high</td></tr><tr><td colspan="5">Damage level</td></tr></table>30% is considered the damage threshold level (Gyoutoku and Uemura 1985, Murakami et al. 2009).</td></tr>
<tr><td rowspan="5">Damage</td><td>Crown transparency assessment of the living part (at tree level)</td><td>Crown transparency</td><td>Visual assessment of crown transparency based on the Sanasilva approach (Müller and Stierlin 1990).</td><td><table><tr><td>0</td><td>≤ 20</td><td>> 20 ≤ 30</td><td>> 30 ≤ 60</td><td>> 60</td></tr><tr><td>No</td><td>Low</td><td>Moderate</td><td>High</td><td>Very high</td></tr><tr><td colspan="5">Damage level</td></tr></table></td></tr>
<tr><td>Crown assessment of the dead part (at tree level)</td><td>Dead crown</td><td>Visual assessment of dead branches from D. kuriphilus and Cryphonectria parasitica attack.</td><td><table><tr><td>0</td><td>≤ 20</td><td>> 20 ≤ 30</td><td>> 30 ≤ 60</td><td>> 60</td></tr><tr><td>No</td><td>Low</td><td>Moderate</td><td>High</td><td>Very high</td></tr><tr><td colspan="5">Damage level</td></tr></table></td></tr>
<tr><td rowspan="2">Inner crown</td><td>Damage Composite Index (at branch level)</td><td>DCI</td><td>$DCI = (dead\ shoots * 0.479 + Reactivated\ dormant\ buds * 0.525 + galls\ on\ shoots * 0.120) * 100$
Detailed assessment of branch architecture. For details, see Gehring et al. 2018a, b.</td><td><table><tr><td>≤ 2.5</td><td>> 2.5 ≤ 7.5</td><td>> 7.5 ≤ 15</td><td>> 15 ≤ 25</td><td>> 25</td></tr><tr><td>No</td><td>Low</td><td>Moderate</td><td>High</td><td>Very high</td></tr><tr><td colspan="5">Damage level</td></tr></table></td></tr>
<tr><td>Number of dormant buds (at shoot level)</td><td>Dormant buds</td><td>Average number of buds remaining dormant during current vegetative season.</td><td></td></tr>
<tr><td>Intense stress</td><td>Suckers per stem-linear- meter (at tree level)</td><td>Suckers</td><td>Visual assessment of stem and branch suckers per linear meter in five categories.</td><td><table><tr><td>0</td><td>1-5</td><td>6-10</td><td>11-20</td><td>> 20</td></tr><tr><td>No</td><td>Low</td><td>Moderate</td><td>High</td><td>Very high</td></tr><tr><td colspan="5">Stress level</td></tr></table></td></tr>
</table>

or lower order branches (Table 2).

Moreover, on each tree, one to three branches, depending on the heterogeneity of the damage, were subsequently sampled every year rotating among different heights and orientations in order to preserve the tree crown. These branches were assessed for *D. kuriphilus* damage and tree reactions using the Damage composite index (DCI - for details see Gehring et al. 2018a, b) which includes parameters such as the number of dead shoots, reactivated dormant buds, developed buds, and the number and position of *D. kuriphilus* galls. In addition, the average number of dormant buds per shoot was recorded (Table 2).

In this manuscript we define the outer crown as the part issued from all the buds that should normally develop and grow (not dormant bud) during a vegetative season and that can be easily assessed with the crown transparency approach and the *D. kuriphilus* infestation indicator. The inner part refers on contrary to the branch-architectural parts of the crown involving dormant buds, reactivated dormant buds and shoots (dead or alive; please refer to Gehring et al. 2018a for the precise definitions). The inner crown are best assessed by the DCI and the dormant buds indicator (Table 2).

D. kuriphilus infestation was computed with the method proposed by Kotobuki et al. (1985) which consists of counting the proportion of attacked previous-year buds (number of attacked buds per available buds) at shoot level on the collected branches (Table 2).

In every site, at least 100 galls were randomly analyzed from the collected branches, additional trees, and crown parts. The galls were then dissected and the presence of *T. sinensis* in the cells recorded in order to calculate the level of *D. kuriphilus* parasitism by *T. sinensis* (hereafter called *D. kuriphilus* parasitism; Table 2). *T. sinensis* larvae were identified morphologically by their characteristic red-brownish ventral stripes, which are a quite reliable identification trait for the species at this development stage. In fact, potential confusion with native *Torymus* species exists for *T. cyaneus*, *T. notatus* and *T. affinis* only (Gómez et al. 2008), which have however to our knowledge never been reared from *D. kuriphilus* galls so far (Aebi et al. 2006, 2007; Bigler et al. 2006; Matošević and Melika 2013; Quacchia et al. 2013; Palmeri et al. 2014; Francati et al. 2015; Kos et al. 2015; Bernardinelli et al. 2016; Colombari and Battisti 2016a; Panzavolta et al. 2018; Ferracini et al. 2018b; Bonsignore et al. 2019), what drastically decreases the chance of misidentification.

During the final field campaign in 2018, additional 50 trees were randomly selected in every site and both the proportion of dead crown and the number of suckers per stem-linear-meter were estimated in order to have a current picture of the still detectable general stress level resulting from the epidemic (Table 2).

Data processing and analysis

The various tree damage and insect population indicators were initially calculated at each sample collection level (gall, shoot, branch) as described in Table 2 and then averaged at tree and site levels. Site aspect was transformed in order to be analyzed using the equation proposed by Beers et al. (1966) which assigns the value of 0 to southwest facing sites (assumed to be more xeric), 2 to the northeast facing sites (more mesic), and an intermediate values for site aspects in between.

A hierarchical cluster analysis (using Euclidean distance with the Ward.d2 clustering method; see Murtagh and Legendre 2014) was performed to group sites based on their geo-physiographic characteristics (see columns “arrival year”, “altitude”, “slope”, “aspect transformed”, and “temperature” in Table 1). The resulting groups were then selected and used as references to analyze possible trends and patterns in the evolution of the *D. kuriphilus* infestation since 2012, the *D. kuriphilus* parasitism, as well as the tree damage indicators.

Daily mean temperatures were first calculated at site level and then summed over three different periods: T1 (yearly site temperature) = yearly (from March 1st to February 28th) average of the daily mean temperature; T2 (*T. sinensis* development period) = average of daily mean temperatures from October 1st (average *T. sinensis* pupation time in our study sites, data not shown) to March 15th (average *T. sinensis* emergence time in our study sites, data not shown); T3 (degree days) = average of the daily mean temperature from January 1st (that is the conventional starting time for calculating degree days for most insect species; Herms 2004) to March 15th.

Descriptive statistics combined with univariate comparative analysis (using Mann-Whitney *U* tests with significant differences at $p < 0.05$) were used in order to quantitatively describe and summarize the evolution of the indicators over the years. Linear regression was used to model the relationship between the 2018 tree damage and insect population indicators and site characteristics (Table 2). Specifically, *D. kuriphilus* infestation, DCI, and crown transparency were separately used as response variables in three different models with all site characteristics as explanatory variables.

All statistical analyses were performed using the R statistical package version 3.5.1 (R Core Team 2018). Figure 1 was generated using the open source geographic information system QGIS (QGIS Development Team 2018). Figure 2 was made using the ggplot2 data visualization package for R (Wickham 2016).

Results

Chestnut trees recovery

The cluster analysis based on site characteristics revealed four major groups (Table 1). Group 1 includes the most sun-exposed sites with high average temperatures at altitudes ranging between 382 and 646 m. a.s.l. At the other extreme, group 4 includes cooler sites at the highest altitudes (857-920 m. a.s.l.) where the time-lag between *D. kuriphilus* arrival and the biocontrol by *T. sinensis* did not exceed 3 years. In between, group 2 includes sites that are similar to those in group 1, but are slightly less sun-exposed and cooler, whereas group 3 includes sites at average altitudes, not particularly sun-exposed and where the time-lag between *D. kuriphilus* arrival and the biocontrol by *T. sinensis* exceeded 4 years (Fig. 1 and Table 1).

D. kuriphilus parasitism by *T. sinensis* reached the biocontrol threshold of 75% in all sites in 2015. In groups 3 and 4, it remained constant until 2018, whereas it decreased to ca. 60% in groups 1 and 2 (Fig. 2). Simultaneously, *D. kuriphilus* infestation drastically decreased after 2014 in all groups and stabilized below the damage threshold level of 30% in groups 2 (~15/20%), 3 and 4 (~5%), whereas it significantly increased again from 2016 onwards in group 1 where it reached an average value of 32% in 2018. Damage indices follow a similar trend with group 1 experiencing

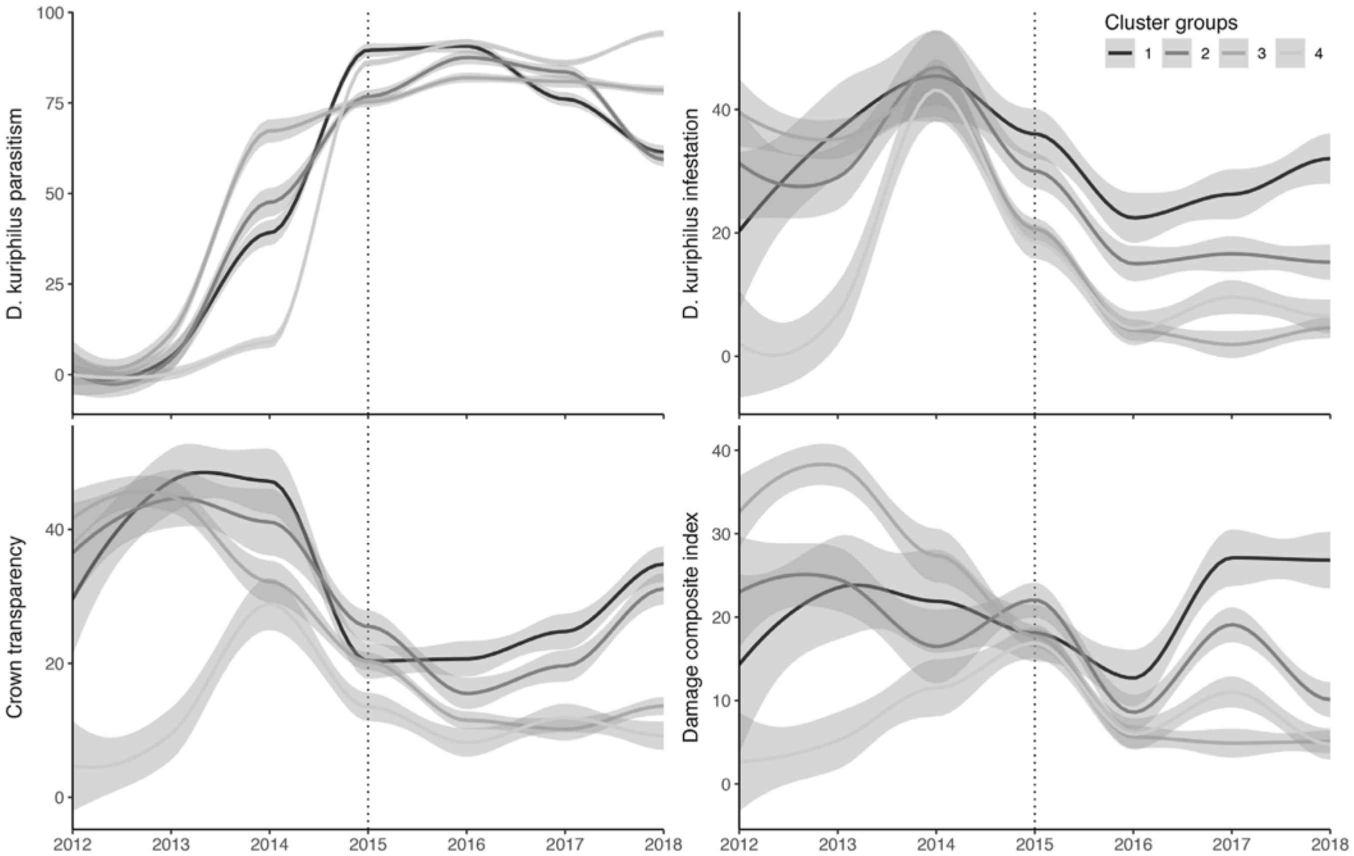

Figure 2 Evolution of selected epidemic and damage indices from 2012 to 2018 by cluster group (see Table 1). The vertical dashed line indicates the average date (between 2014 and 2015) when biocontrol by *Torymus sinensis* was reached in the study area. Grey bands show 95% confidence intervals.

the most severe damage followed by groups 2, 3, and 4, respectively. Specifically, crown transparency decreased from 2015 in all sites and significantly increased again since 2017 in groups 3 and 4. The DCI showed a somewhat similar trend but with much more fluctuation and a significant increase from 2016 on in groups 1 and 2 (Fig. 2). Finally, the later arrival of *D. kuriphilus* in group 4 (Table 1) is clearly visible in Figure 2 as expressed by the delayed damage index increase with respect to the other groups.

Using 2018 data only, the best linear models with DCI and *D. kuriphilus* infestation as the response variables, respectively, included the transformed site aspect (negatively related) and the sum of temperatures from October 2016 to March 2017 (T2; positively related) as the explanatory variables. The two models had adjusted R-squared values of 0.73 and 0.69 (Table 3). The model with crown transparency as the response variable also retained site aspect and a temperature as the best explanatory variables but in this case the yearly sum of temperature (from March 2016 to March 2017) had greater explanatory power. None of the models were affected by multicollinearity problems (VIF < 2) nor did diagnostic plots reveal any violation of model assumptions.

D. kuriphilus parasitism thresholds inducing tree recovery

The first signs of significant tree recovery with respect to all indicators (DCI, number of dormant buds, crown transparency, and *D. kuriphilus* infestation) are registered when *D. kuriphilus* parasitism reaches mean values of 79% (95%CI [74, 84]). Recovery from *D. kuriphilus* infestation is already visible at lower parasitism levels (68% [48, 88]), whereas the number of dormant buds reacts at higher parasitism levels only (86% [83, 89]) (Table 4). The situation is similar, although at much lower *D. kuriphilus* parasitism values, when considering the previous year (Table 4)

Table 3. Best linear models considering various damage index as response variables and only 2018 data

Model variables		Estimate	Confidence Interval		*t* value	Var. imp.[a]	Adj. R^2	R^2
Response[b]	Explanatory		2.50%	97.50%		%		
Crown transparency	Intercept	-22.229	-89.187	44.73	-0.723		0.62	0.68
	Aspect t. [d]	-7.348	-14.923	0.227	-2.114	48.1		
	T1[c]	0.016	0.001	0.031	2.281	51.9		
D. kuriphilus infestation	Intercept	5.303	-22.411	33.016	0.417		0.69	0.74
	Aspect t.	-10.392	-16.12	-4.663	-3.953	68.9		
	T2[c]	0.025	-0.006	0.055	1.78	31.1		
DCI[b]	Intercept	-7.496	-27.468	12.476	-0.818		0.73	0.77
	Aspect .t	-6.053	-10.181	-1.924	-3.195	50.2		
	T2[c]	0.032	0.01	0.054	3.166	49.8		

[a]Var.imp. refers to variable importance as calculated by the function varImp() contained in the R-package caret (Kuhn 2008). [b]See Table 2 for details for the definitions of the response variables. [c]T = sum of daily mean temperature exceeding 0°C; T1 = from 15.03.16 to 14.13.17; T2 = from 15.10.16 to 15.03.17. [d]Aspect t. = Aspect transformed according to the equation proposed by Beers et al. 1966.

Table 4 *Dryocosmus kuriphilus* parasitism thresholds for first signs of recovery.

Crown area	Index	*D. kuriphilus* parasitism rate (%)[a]	
		Recovery year	Previous year
Outer	Dk infestation	68 *[48, 88]*	37 *[18, 55]*
	Crown transparency	75 *[59, 91]*	40 *[20, 60]*
Inner	DCI[b]	81 *[73, 88]*	55 *[34, 77]*
	Dormant buds	86 *[83, 89]*	57 *[38, 76]*
	All[b]	79 *[74, 84]*	47 *[39, 54]*

[a] Values represent means with 95% lower and upper confidence intervals.

[b] All = average value of all indices.

Role of the time-lag between pest and antagonist arrivals

The time-lag between *T. sinensis* arrival and the first sign of tree recovery is longer in sites in groups 1, 2, and 3, compared to that in group 4 (Table 5). For example, the first signs of recovery after the arrival of *T. sinensis* regarding the crown transparency index in group 1 are visible 1.3 years later (on average 3.3 years; 95% CI [2.6, 4.0]) than in group 4 (on average 2.0 years; 95% CI [0.9, 3.1]). This trend is similar when considering the other indices. It is also worth noting that the recovery process began in the outer crown area nearly one year sooner than the inner part (Table 5).

The average damage severity for sites in group 3 experiencing a longer time-lag between the arrival of *D. kuriphilus* and the occurrence of *T. sinensis* biocontrol (~5 - 7 years) is significantly greater in terms of crown transparency (Fig. 3 top) and malformation of the branch architecture (Fig. 3 bottom) compared to those in group 4 where the two insects arrived nearly simultaneously (*T. sinensis* biocontrol within 2-3 years of *D. kuriphilus* arrival). Furthermore, considering 2018 data only (i.e., the present situation where, for every site, at least four years have passed since biocontrol occurrence), 30% of the crown area is still dead in sites that experienced four or more years of uncontrolled *D. kuriphilus* attack. The proportion of dead crown progressively and significantly decreases to 5% in sites with a shorter time-lag (group 4). This is especially pronounced in the site "Sonlerto" (Fig. 4 bottom). Consequently, the stress level endured by trees as indicated by the number of epicormic resprouts ("Suckers" in Fig. 4) follows a similar trend with a significant increase as a function of the time-lag.

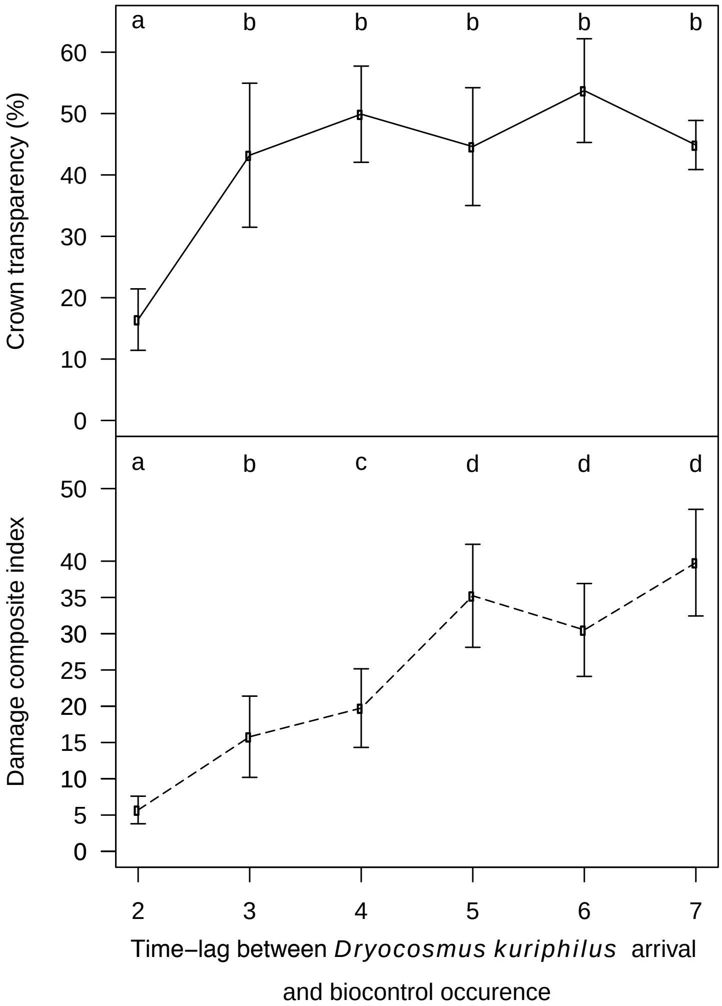

Figure 3 Damage severity during the epidemic peak by time-lag between the *Dryocosmus kuriphilus* arrival and the first biological control year by *Torymus sinensis*. Only data from the epidemic peak to the year before biocontrol occurrence in the sites in question was used to generate this Figure (see Table 1). Error bars show 95% confidence intervals. Letters indicate significant differences with $p < 0.05$ according to the non-parametric Mann-Whitney U test with Holm adjustment.

Table 5 Time lag in years (with 95% confidence intervals) between *Torymus sinensis* arrival and the first signs of tree recovery using various indices and for different cluster groups

Crown area	Index	Cluster groups				All
		1	2	3	4	
Outer	Dk infestation	3.0 *[1.9, 4.1]*	2.8 *[1.3, 4.3]*	3.0 *[2.1, 3.9]*	1.7 *[1.0, 2.4]*	2.7 *[2.1, 3.3]*
	Crown transparency	3.3 *[2.6, 4.0]*	3.2 *[2.3, 4.1]*	3.8 *[3.1, 4.5]*	2.0 *[0.9, 3.1]*	3.2 *[2.7, 3.7]*
Inner	DCI	4.3 *[2.6, 6.0]*	3.8 *[3.3, 4.3]*	3.8 *[3.4, 4.2]*	2.3 *[1.6, 3]*	3.6 *[3.1, 4.1]*
	Dormant buds	4.3 *[2.6, 4.9]*	4.0 *[3.2, 4.8]*	4.4 *[3.9, 4.9]*	2.7 *[1.4, 4]*	3.7 *[3.2, 4.2]*

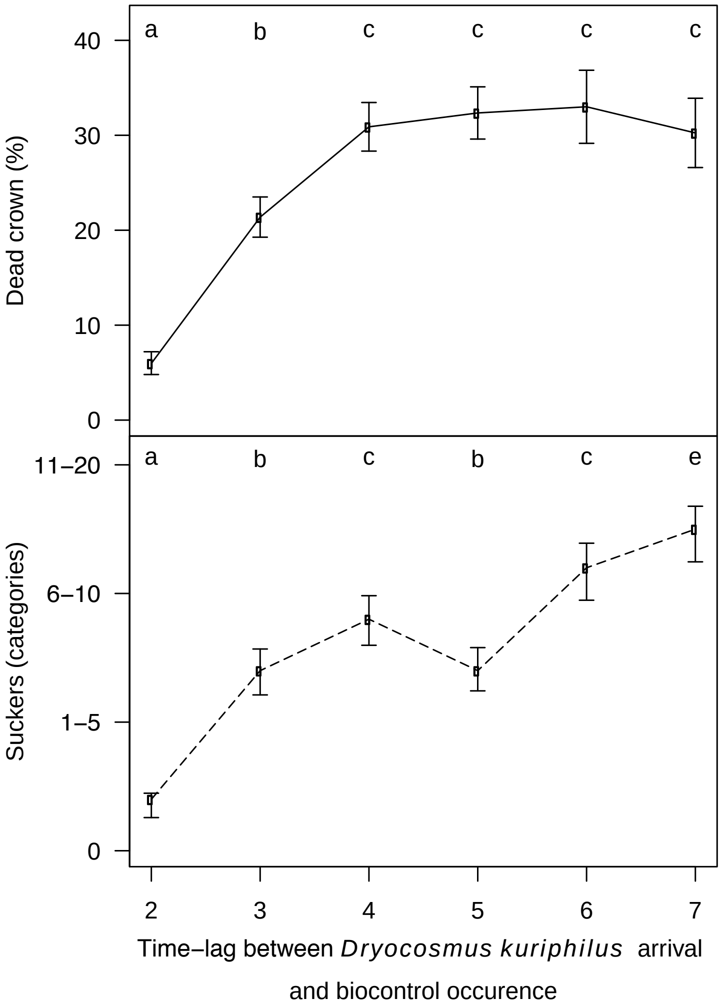

Figure 4 Damage severity and tree reaction in 2018 according to the time-lag between *Dryocosmus kuriphilus* arrival and the first year of biological control by *Torymus sinensis*. Error bars show 95% confidence interval. Letters indicate significant differences with p <0.05 according to the non-parametric Mann-Whitney U test with Holm adjustment.

Discussion

In this study, we assess the evolution of the *D. kuriphilus* epidemic from its arrival to biological control by *T. sinensis*, as well as the resulting damage evolution and recovery patterns, in 15 sites located in southern Switzerland. Generally, damage peaked between 2013 and 2014 and started decreasing as soon as *T. sinensis* reached notable levels of parasitism on *D. kuriphilus*, proving its effectiveness as a biocontrol agent as already shown by other studies (Moriya et al. 2003; Borowiec et al. 2018; Ferracini et al. 2018a; Avtzis et al. 2019). As a general rule, the first signs of chestnut tree recovery are visible as soon as *D. kuriphilus* parasitism reaches values of 80% on average, a parasitism rate that could be considered as the recovery and biocontrol threshold for the chestnut tree. This is in line with other studies that have confirmed a decrease in infestation in terms of gall numbers when the *D. kuriphilus* parasitism reaches the 75% level (Quacchia et al. 2014). By considering previous year parasitism levels, one could also predict recovery to occur one year after *D. kuriphilus* parasitism reaches values of about 50%.

This threshold does not depend on the time-lag between the first *D. kuriphilus* attack and the arrival of *T. sinensis*. In fact, external crown parts react immediately to the achieved biocontrol. This is likely due to the permanent healthy condition of the apical buds which are usually formed after the *D. kuriphilus* oviposition period (Maltoni et al. 2012b). When biocontrol is achieved, the lateral buds of the terminal shoot will also be attacked less such that the external part of the crown will soon consist of normal-shaped branches. This initial recovery process, when the gall-free external crown coat reinvigorates the leaf area, is well reflected in the crown transparency index and the *D. kuriphilus* infestation. The inner crown area, in contrast, experiences a delayed recovery of, on average, one year and varies as a function of the time elapsed between *D. kuriphilus* arrival and the occurrence of *T. sinensis* biocontrol. This is reflected by specific indicators such as DCI and the number of dormant buds. In fact, in sites of severe and repeated *D. kuriphilus* attacks, branch corruption includes the consumption and depletion of tree reserves (dormant buds, Gehring et al. 2018a), the failure of bud production from galled shoots (Kato and Hijii 1997), and the dieback of infested branches (Maltoni et al. 2012b; Gehring et al. 2018a), which all require more time and energy to be restored and renewed.

The speed and completeness of inner crown recovery and eventually of the whole tree depend strongly on the time-lag between the first *D. kuriphilus* attack and the arrival of *T. sinensis*. In sites where the two insects arrived nearly simultaneously (cluster group 4. *D. kuriphilus* and occurrence of *T. sinensis* biocontrol ≤ 3 years), severe damage never occurred and the recovery process was quasi-imminent keeping both the branch and the crown architecture of the affected trees intact. In contrast, sites that experienced more than four years of uncontrolled *D. kuriphilus* attacks suffered overall from more severe crown corruption and tree reserve exhaustion to the point that, even after four years of *T. sinensis* biocontrol (present situation; cluster group 3. *D. kuriphilus* and occurrence of *T. sinensis* biocontrol ≥ 6 years), long-lasting damage is still visible in the form of dead crown parts. This crown deterioration could also be the result of an indirect interaction between *D. kuriphilus* and the chestnut blight (*Cryphonectria parasitica*) that uses the flickering holes of abandoned galls as entry points (Meyer et al. 2015). Moreover, in the locality that suffered from long-lasting uncontrolled attacks, the majority of trees are rebuilding the damaged crown by producing an elevated number of suckers along the trunk and the main branches in reaction to past severe stress. It is therefore reasonable to argue that a proportion of these trees may never fully recover and will be seriously threatened in the immediate future by secondary agents such as hail-induced chestnut blight and drought. Last but not least, possible recrudescence of the *D. kuriphilus* infestation may periodically increase again the pressure on the chestnut trees as it has been already reported for Japan in 2000 and 2007 (Toyama et al. 2009) and as generally predicted by the model developed by Paparella et al. (2016).

In this study we were able to detect different epidemic trajectories based on the geo-physiographic site characteristics and the time-lag between the arrival of the pest and its antagonist. Of particular interest from an epidemiological point of view is the evolution of damage and the *D. kuriphilus* parasitism in cluster 1, where the *Dryocosmus* infestation never reached low levels, and damage increased again in 2017 and 2018 to ca. 35/40% as a consequence of a decrease in *D. kuriphilus* parasitism. One possible explanation for such a fluctuation is the south facing aspect and the related generally high temperatures at these sites. As shown by our model analysis, the damage level in 2018 is positively correlated with the sum of temperature from October 2016 to March 2017 and negatively correlated with aspect. Based on the fact that the time period in question roughly corresponds to development stages of *T. sinensis* from pupation to time of emergence (personal observation of our study sites), we hypothesize a possible earlier emergence of the majority of the antagonist population in such warm sites that causes a desynchronization with gall appearance. This, necessarily follows the leaf unfold phenology of the chestnut tree. In addition, high temperatures combined with arid conditions (southern exposure sites) may also influence the longevity of *T. sinensis* adults as demonstrated by laboratory trials that highlighted a significant decrease in lifespan comparing temperatures of 15 and 25 °C (Quacchia et al 2008) and in the case of food deprivation (Picciau et al. 2019). This may cause a partial failure to control the *D. kuriphilus* population and lead to consequent increased damage the year after.

In optimal condition (forest stand continuum and wind) *T. sinensis* may recolonize the unfavourable sites by wind-aided flight starting from the suitable areas (Colombari and Battisti 2016b). However, in our study area, favourable (e.g., north-facing) and unsuited (south-facing) sites are often at least three kilometres away and not always connected by chestnut forests. Its immediate recolonization success will thus highly depend on the dominant direction of winds and the *T. sinensis* phenology. Late emerging parasitoid may in fact arrive at the unfavourable site when galls are too big and lignified to be parasitized.

Finally, all these possible factors (synchronization, longevity problems and recolonization) are weather-dependent and thus subjected to strong year-to-year variations. We should thus expect important yearly fluctuations of *D. kuriphilus* parasitism at medium or high damage levels without a decrease to undetectable levels of the *D. kuriphilus* population, especially in less favourable sites. If this will reveal to be true, forecasting the future will become very difficult in such a complex geomorphological area with various exposures.

In conclusion *T. sinensis* has shown to be a very effective biological control agent of *D. kuriphilus* on many species of the genus *Castanea* worldwide. The control of the *D. kuriphilus* population by *T. sinensis* displays however also very dynamic and unexpected aspects, especially where or when environmental conditions cause a possible desynchronisation of the phenology of the two insects and a failure of its parasitic action. The history of the *D. kuriphilus* epidemic in southern Switzerland reported here teaches us that the best way to avoid long-lasting gallwasp-induced damage on chestnut trees, resulting in heavily weakened trees with disrupted crowns, is to release the specific antagonist *T. sinensis* as soon as the pest is detected in the area. We are thus convinced that the downside related to the introduction of *T. sinensis* such as the possible hybridization with *T. beneficus* (Yara et al. 2010), the loss of native parasitoid richness (Ferracini et al. 2018b) and the rare host-range expansion (Ferracini et al. 2017) are and will be by far more acceptable than possible consequences on chestnut forests without its introduction or arrival.

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Chapter 5

What are the far-reaching effects of the damage caused by *D. kuriphilus*?

5.1

Impact of the Asian Chestnut Gall Wasp, *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae), on the chestnut component of honey in the southern Swiss Alps

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Torymus sinensis feeding on a honey drop

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Abstract

The Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu*,* Hymenoptera, Cynipidae) is considered as one of the most dangerous pests of the genus *Castanea*. In southern Switzerland repeated heavy Asian chestnut gall wasp attacks prevented chestnut trees from vegetating normally for years before the arrival and spread of the biological control agent *Torymus sinensis* Kamijo (Hymenoptera, Torymidae). This resulted in a greatly reduced green biomass and flower production. In this paper, we analyse the impact of such an ecosystem alteration of the environment on the composition of produced honey. Six beekeepers were chosen from sites with different densities of chestnut trees, each of which providing series of honey samples from 2010 to 2016. We determined the chestnut component in the honeys via a combined chemical and sensory approach, and correlated the obtained results with the degree of yearly Asian chestnut gall wasp-induced crown damage and weather conditions during the period in question in the surrounding chestnut stands. The chestnut component in the analysed honey sample series showed a strong correlation with the degree of Asian chestnut gall wasp-induced crown damage, whereas meteorological conditions of the corresponding year had a very marginal effect. Decreases in the chestnut component of the honey were statistically significant starting from an Asian chestnut gall wasp infestation level of 30%.

Keywords *Castanea sativa*, invasive pest, chestnut honey, honey sensory analysis, tree damage.

Introduction

The Asian chestnut gall wasp (ACGW; *Dryocosmus kuriphilus* Yasumatsu*,* Hymenoptera, Cynipidae) is a univoltine thelytokous (females only) species native to China that attacks different species of the chestnut genus *Castanea* (Moriya *et al.*, 2003). Because of its huge parthenogenetic reproductive capacity and the negative effect on shoot, flower, and fruit production caused by gall formation, *D. kuriphilus* is considered to be one of the most dangerous pests of the genus *Castanea* (Payne *et al.*, 1983; Moriya *et al.*, 1990; Aebi *et al.*, 2006). In newly invaded regions of the world where no specific natural enemies are present, ACGW outbreaks over multiple years result in severely malformed branch architectures including a decrease of dormant buds and an increase of dead shoots with leaf area losses of up to 70% (Gehring *et al.*, 2017), nut yield reductions of up to 80% (Battisti *et al.*, 2014, Sartor *et al.*, 2015) and corresponding reductions in tree vigor and wood production (Ugolini *et al.*, 2014).

In Europe, *D. kuriphilus* was reported for the first time in 2002 in the Cuneo region (Piedmont, Italy; Brussino *et al.*, 2002), from where it progressively spread to the rest of Italy and most European chestnut-growing countries (EPPO, 2017). In Switzerland, the first occurrence of *D. kuriphilus* dates back to 2007 (Forster *et al.*, 2009) when it was found in the southernmost part of Canton Ticino, from where the ACGW spread throughout the whole chestnut area of southern Switzerland by 2013 (Conedera *et al.*, 2016).

Following the successful Japanese experience of biologically controlling *D. kuriphilus* by introducing the chalcid parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) from mainland China, this specific parasitoid was introduced in 2005 into Piedmont (northern Italy), where it was found to be very efficient in controlling the ACGW population (Quacchia et al., 2008; Quacchia et al., 2014) also on *Castanea sativa* Miller (Fagales, Fagaceae) native to Europe and Asia Minor. Unlike Italy and other European countries such as France (Borowiec et al., 2014), Croatia, Slovenia and Hungary (Matosevic et al., 2015), the permission to introduce the biocontrol agent *T. sinensis* to control the ACGW was not granted in Switzerland due to open environmental questions (e.g., potential of hybridization between *T. sinensis* and native *Torymus* species and attacks on non-target native gall wasps, Gibbs *et al.*, 2011). Nevertheless, *T. sinensis* benefited from unauthorized introductions and/or natural cross-border emigration from nearby Italian releases, resulting in its official detection over the entire chestnut area of southern Switzerland by 2013 (Gehring *et al.*, 2014) and on the biological control impact of the ACGW by 2015 (Gehring *et al.*, 2017). Because of the extended time-lag in the arrival of *T. sinensis,* part of the chestnut forests in southern Switzerland experienced up to 7 years of undisturbed and repeated ACGW attacks, preventing chestnut trees from vegetating normally, and causing a progressive malformation of branch architecture and a strong reduction in the production of leaves and flowers (Gehring *et al.*, 2017). This damage may have not only impacted chestnut fruit production, but also the chestnut component in the locally produced honey.

In this study we verify this conjecture by analyzing the impact of ACGW-induced damage on chestnut trees and on the composition of honey produced during the ACGW epidemic phase that started in 2010, became impacting between 2011 and 2012, peaked between 2012 and 2014, and progressively lessened between 2015 and 2016 according to the different sites (Gehring *et al.*, 2017). We in particular hypothesize that the chestnut component in honey undergoes a significant reduction when *D. kuriphilus* damage on tree crowns is high and widespread. The following detailed questions are addressed in this study:

- How can we assess the chestnut component in honey?
- Do changes in chestnut components in honey correlate with tree damage due to *D. kuriphilus* infestation?
- Do meteorological conditions during chestnut flowering have a detectable influence on chestnut component in honey?
- Are there any ACGW damage thresholds beyond which the alteration of the chestnut component in honey is statistically significant?

Materials and methods

Study area

The study area is represented by the chestnut forest of Canton Ticino, in southern Switzerland (Fig. 1). The chestnut tree (*Castanea sativa* Mill.) was introduced to the study area nearly 2,000 years ago (Tinner *et al.*, 1999). Since then, in Canton Ticino, it has spread and been cultivated by man as a monoculture for timber and fruit production (Conedera *et al.*, 2004) at elevations ranging from 200 m (lowest point in the area of Lago Maggiore) up to the ecological limits of the species at 900 to 1,100 m asl. (Conedera *et al.,* 2001). Where the steep mountain slopes restrict alternative agricultural or commercial activities, chestnut cultivation for staple-food production became the main source of subsistence for local populations (Krebs *et al.*, 2012). The decline in the economic importance of the chestnut tree began in the study area in the early 1800s, when alternative crops such as potatoes and maize were introduced and accelerated in the late 1950s coinciding with the rapid post-war socio-economic development of the area (Krebs *et al.,* 2014). As a result, most of the former chestnut stands are now abandoned and subjected to a post-cultural evolution towards mixed broadleaved forests dominated by native tree species such as lime tree

(*Tilia* spp.), deciduous oaks (*Quercus* spp.), ash (*Fraxinus* spp.) and maple (*Acer* spp.) depending on site conditions (Conedera *et al.*, 2001: Pividori *et al.*, 2005). Consequently, the present chestnut tree contribution to forest composition varies strongly as a function of the degree of forest stand abandonment or possible management activities by forest owners, respectively (Krebs *et al.,* 2014).

During its flowering period occurring over about 1 month but stretching from the beginning of June to the end of July depending on the season, the chestnut tree represents one of the best nectar and pollen sources for bees (Maurizio 1941; Persano Oddo & Pirro, 2004; Yang *et al.*, 2012; Aronne *et al.*, 2012). Furthermore, aphids that are often hosted on young chestnut shoots can produce additional honeydew. Accordingly, local honey production during this season ranges from highly sought-after chestnut unifloral (defined in section 2.3. and Table 1) to chestnut-poor honeys (Persano Oddo & Piro, 2004; Apisuisse, 2015) as a function of the density of the chestnut tree and other nectar-providing species at production sites. These include *Rubus* spp., *Rhododendron* spp., and *Tilia* spp., all of which flower in the same period.

Table 1 Beekeepers and beehive site characteristics

						Beehive location					
Beekeeper	Municipality	Samples	*Dryocosmus kuriphilus*	*Torymus sinensis*	Honey[c]	Lat	Long	Altitude	Aspect	Distance from tree damage plot	Distance from meteo station
Code		(no.)	Arrival year		Type	WGS 84 Decimal Degrees		m asl	°	km	km
VM1	Vico Morcote	7	2010	2012	Moderate	45.93130	8.92059	480	S-SE	2.3	4.1
MA1	Manno	7	2010	2012	Moderate	46.03836	8.92156	340	S-E	1.5	3.1
BE1	Bellinzona	5[a]	2011	2012	Unifloral	46.18364	9.03497	500	N	5.0	3.3
VO1	Vogorno	7	2011	2012	Moderate	46.22505	8.85844	610	SW	2.7	5.2
VO2	Vogorno	6[b]	2011	2012	Moderate	46.22613	8.85797	660	SW	2.5	5.0
GO1	Gordevio	7	2012	2013	Poor	46.22943	8.74507	365	SW	4.2	6.3

[a] Honey samples not available for 2010 and 2013; [b] honey samples not available for 2011; [c] Chestnut honey type before or at time of *Dryocosmus kuriphilus* arrival year; *Unifloral* = electrical conductivity > 0.86 mS/cm, free acids < 22.4 meq/kg and sensory level of *Castanea sativa* is maximal and exclusive (RCS = 1); *Moderate* = RCS < 1 and Csc ≥ 0.10; Poor = RCS > 0 and Csc values < 0.10. RCS = chestnut sensory level / cumulated overall sensory levels (chestnut+lime+mixed flowers+honeydew). Csc = Electrical conductivity / free acids * (1+ RCS).

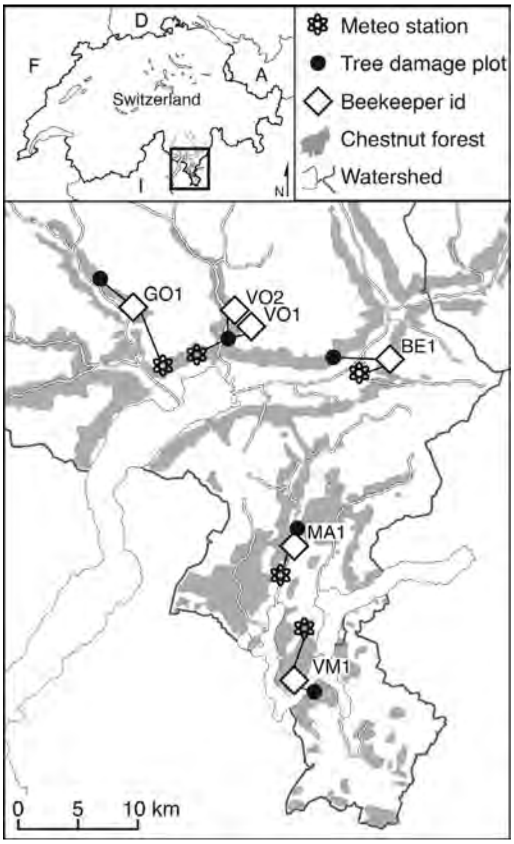

Figure 1 Map of the study area with the location of honey production sites and related chestnut tree damage plots and meteorological stations.sensory level) = chestnut sensory level / cumulated overall sensory levels (chestnut + lime + mixed flowers + honeydew). Dk infestation (*Dryocosmus kuriphilus* infestation) = no. attacked buds / no. total available buds * 100.

Sampling design

In order to minimize potential bias in our experimental design due to honey production techniques (Aronne & de Micco, 2010), we considered only honey samples that fulfilled the following criteria: (1) their production sites (hive location) remained constant for the entire study period (sedentary beekeeping); (2) beekeeping (hive and comb types) and honey production approaches remained unchanged (single honey extraction after chestnut flowering at the beginning of August using the same method) over the whole study period; (3) well-conserved (e.g., no odour or flavour of fermentation or other alterations) honey samples were available under similar storage conditions (e.g., hermetic container, consistent temperatures of approximately 10-15 degree Celsius (°C), no direct sunlight); (4) the selected hive sites were characterized by a nearly synchronic epidemiology of *D. kuriphilus* concerning both the year of arrival of *D. kuriphilus* and *T. sinensis* and subsequent chestnut tree recovery with no late frost events that could potentially destroy chestnut flowering during the study period.

Six beekeepers within a geographic area extending from the Lake of Lugano in the south to the lowlands of the upper valleys (Fig. 1) met all of these conditions over a time span of seven years (2010-2016). One producer (VO2) delivered only six (honey sample not available for 2011) and another (BE1) only five (the 2010 honey sample was not well conserved and fermented, and the 2013 sample was not available) honey samples in the requested quality and quantity, for a total sample size of 39 samples (see details in Table 1).

Assessing the chestnut component in honey

Unifloral chestnut honey is highly characteristic in terms of odour and flavour. Chestnut honey typically has a strong bitter taste and is astringent. The intense flavour can be described as woody, warm, chemical, with a long persistence (Persano Oddo & Piro, 2004). High electrical conductivity (average 1.3 milliSiemens per centimeter (mS/cm); range 0.86 to 1.70 mS/cm; Bogdanov *et al.*, 2008) and low free acidity (average of 11.2 milliequivalents (meq/kg); range 6.7 to 22.4 meq/kg; Bogdanov *et al.*, 2008) are typical physico-chemical characteristics of chestnut honey (Persano Oddo & Piro, 2004; Ruoff *et al.*, 2007). When the chestnut component in the honey is less prominent or low, these physico-chemical characteristics and the corresponding sensory characteristics of the honey vary accordingly.

To determine the relative proportion of the chestnut component in each honey sample, we developed a mathematical equation based on the sensory and physico-chemical characteristics of the various honeys. Electrical conductivity and free acids in the honeys were assessed following the harmonized methods of the International Honey Commission (Bogdanov *et al.*, 1997). The electrical conductivity distinguishes chestnut nectar from floral nectar of other plants, such as *Rubus* spp., *Tilia* spp*.* or *Rhododendron* spp., while the level of free acids enables further discrimination of the chestnut component from honeydew components.

Sensory evaluation followed the standard approach in honey characterization (Gonnet and Vache 1985; 1992; Guyot et al. 1998; Piana et al., 2004; Castro-Vazquez et al., 2010). In particular, we determined the level of the main recognizable tastes such as chestnut, lime (*Tilia* spp.), other botanical sources (mixed flowers, mainly *Rhododendron* and *Rubus* spp.), and honeydew on a seven-point scale: 0 = absent, 1 = weak, 2 = weak-medium, 3 = medium, 4 = medium-strong, 5 = strong, 6 = exclusive. The relative contribution of chestnut to the overall sensory level (for short, RCS = Relative Chesnut Sensory) was then determined according to the following formula:

> RCS = chestnut sensory level / combined overall sensory levels (chestnut + lime + mixed flowers + honeydew).

The *Castanea sativa* component (Csc) of the honey samples was then determined according to the formula:

> Csc = Electrical conductivity / free acids * (1+ RCS)

Chestnut honeys were classified as chestnut unifloral when the sensory level of *Castanea sativa* was maximal (6/6) and exclusive (no other detectable flavours, i.e., RCS = 1, and chemical analysis revealed values meeting the Swiss honey classification standards (electrical conductivity > 0.86 mS/cm; free acids < 22.4 meq/kg; Bogdanov *et al.*, 2008), which, in our case, corresponded to Csc values of ≥ 0.17. Multifloral honeys containing other botanical sources in addition to chestnut were classified as having a moderate chestnut component when RCS < 1 and Csc ≥ 0.10, and a poor chestnut component when RCS > 0 and Csc < 0.10. As reported in Table 1, due to the different forest compositions at the production sites, the beekeepers in the present study already produced honeys with different chestnut components before the ACGW arrival. In order to compare the yearly fluctuation in the Csc among beekeepers, we standardized the Csc values of each beekeeper with respect to its maximal Csc value using the following calculation (example for a single beekeeper):

$$\text{Csc.loss} = 1 - [(\text{Csc}-\text{Csc}_{max})/\text{Csc}_{max} *100],$$

where Csc_{max} stands for the highest Csc value in the beekeeper series under consideration (2010-2016).

Our preliminary tests show that melissopalynology (i.e., the branch of palynology that deals with the pollen contained in honey) is not suitable to discriminate between high and low amounts of chestnut components in honey, likely due to the fact that *Castanea* pollen is strongly over-represented in honey (Persano Oddo & Piro, 2004; Yang et al. 2012).

ACGW and T. sinensis life history

ACGW is a species native to China that accidentally arrived in Europe in 2002 (Brussino *et al.,* 2002) where it became a significant pest for the European Chestnut tree (*Castanea sativa*). The adult wasp is 2.5-3 mm in length and has a black body with orange legs. Given that no male has been observed to date, the species is thelytokous, meaning that it produces fertile eggs by parthenogenesis. Generally in July, females lay between 3 to 5 eggs inside a chestnut bud and can lay up to 100 eggs in total (EPPO 2015). The total number of eggs laid per bud varies greatly from 1 to 176 (Panzavolta *et al.* 2012). Shortly after oviposition, larvae hatch from eggs but do not grow until the following spring when buds begin developing. It is at this time that larvae induce the formation of a variable number of galls differing in size, position (on a leaf, along the main shoot axis, on a stipule, or on a flower), and number of hosted larvae. Development is complete by July upon emergence from galls.

The only natural enemy able to efficiently control its population is *T. sinensis.* Contrary to ACGW, *T. sinensis* adults emerge in spring from the previous year's withered galls and need to mate before laying a variable number of fertile eggs into the newly formed galls (Quacchia *et al.* 2008). Usually only one *T. sinensis* egg per ACGW larval chamber is laid. Soon after its oviposition, the egg hatches and the young larva feeds ectoparasitically on the ACGW larvae. It then pupates in autumn and completes its life cycle by the following spring (Quacchia *et al.* 2008).

ACGW damage assessment

An ACGW monitoring program consisting of 18 sample sites (henceforth referred to as tree damage plots) was designed in 2010 in order to assess ACGW spread and incidence in the area, the year of arrival of *T. sinensis*, its efficiency in terms of parasitism, and related tree damage. During our study period (2010-2016), the overall ACGW damage level varied gradually from south to north as a function of the time of ACGW arrival. Specifically, this means that the damage level was similar within a 5-km radius.

The sampling protocol was exactly the same in every tree damage plot. In each site, the number of mature chestnut trees sampled (mean height: 18 m; standard deviation: 3.5 m) varied depending on the heterogeneity of damage in the stand. Thus, ten trees were sampled in 2010, 2011 (beginning of the epidemic), and 2016 (recovering stage), whereas between 2012 and 2015 (epidemic peak and the beginning of the recovery stage) the number of sampled trees was 15. To randomly select the trees, every year, 10 or 15 points were placed using the QGIS Random points tool (2016) on the corresponding tree damage plot map covering an area of approximately 10 hectares in total. Crown damage due to ACGW attack on the selected sampling trees was assessed during a one-week period in summer between mid-June and mid-July according to three distinct methods (at the same time). The first method consisted of visually assessing tree crown transparency following the Sanasilva approach (hereafter referred to as Sanasilva) as described by Müller & Stierlin (1990). The second method consisted of estimating the severity of ACGW damage by calculating the percentage of attacked buds with respect to available buds (Dk infestation = no. of attacked buds / no. of total available buds) at shoot level (Kotobuki *et al.*, 1985). For this purpose, 10 live shoots distributed over the entire tree crown were collected per sampled tree. These shoots, which correspond to the sprout from the previous vegetative seasons with respect to the sampling date (see Appendix - Figure A1 for branch, shoot, and sprout examples), had average lengths of 5 cm. The number of attacked buds (buds that grew and produced ACGW galls) and the number of total available buds (number of attacked buds plus number of quiescent and dormant buds) were counted for analysis. The last method consisted in applying the damage composite index (DCI) proposed by Gehring *et al.* (2017) on one branch per tree. The DCI enables the assessment of current and past year's damage endured by the tree and is computed on branches of at least half a meter in length that contain a minimum of 10 shoots. Briefly, the branch analysis consisted in counting the average number of galls on shoots (g; representing the current year's damage), the proportion between living and dead shoots (d; representing the previous year's damage) and the proportion between live shoots and reactivated dormant buds (r; buds that started growing mainly because of stress). The DCI is then calculated using the following formula: DCI = (g * 0.120 + d * 0.479 + r * 0.525) * 100 (see Gehring *et al.* (2017) for details). For both methods (Dk infestation and DCI), shoots and branches were collected using a long tree pruner (up to 8 m in length) and by climbing to the top of the tree using tree-climbing techniques.

Finally, the presence of *T. sinensis* and the degree of *D. kuriphilus* parasitism (Ts% = (no. of live *T. sinensis*) / (no. of *D. kuriphilus* chambers) * 100) (Quacchia *et al.*, 2014) were verified at each site by collecting up to 200 galls per site from the previously selected branches and dissecting them.

In order to assess possible relationships between ACGW impact and the chestnut component in the related honey, a tree damage plot from our monitoring program was associated to each of the selected honey production sites based on its geographical area (Fig. 1).

Honeybee foraging behaviour and meteorological data

In our study area, the selected beehives are located inside or close to the forest in which foragers can find all the nectar and pollen of tree species present in the analyzed honey (Table 1). According to Seeley (1986), in honeybee colonies, foragers typically fly distances as short as possible while focusing on abundant, nectar-rich sources. It is known that the chestnut tree represents one of the best nectar and pollen sources for bees (Maurizio 1941; Persano Oddo & Pirro, 2004; Yang *et al.*, 2012; Aronne *et al.*, 2012). It is thus reasonable to assume that in our study area foragers do not fly far away and are able to find all the necessary pollen and nectar within an area of a 5-km radius.

Moreover, the foraging activities of honeybees are also influenced by many weather factors such as temperature, wind velocity, relative humidity and precipitation, as well as the time of the day (see Abou-Shaara, 2014 for a review). In order to consider the possible role of weather conditions during the chestnut tree flowering season in our analysis, we selected the nearest available meteorological station to be associated with each honey production site. These, together with the associated tree damage plots appear linked together in Figure 1.

Weather data from 2010 to 2016 (at a resolution of 10-minute intervals) were downloaded from the OASI website (Osservatorio Ambientale della Svizzera Italiana (http://www.oasi.ti.ch)). We retained only data referring to the flowering period of the considered species in the honey sensory analysis (i.e. *Castanea sativa*, *Tilia* spp., *Rhododendron* spp. and *Rubus* spp.), specifically, from 1st June to 1st August (http://www.meteoswiss.admin.ch) and only for daylight hours which cover potential bee foraging activity. Seasonal mean temperature (°C), seasonal mean wind speed (meters per second; m/s), seasonal mean relative humidity (%), seasonal sum of precipitation (mm) and bee flying time were also retained as potential explanatory variables (see details in Table 2 and Appendix - Figure A2).

Table 2 Main weather parameters considered in the analysis influencing bee foraging activity (BFA) and chestnut growth (CG).

	Parameter	**Unit**	**Notes**
BFA[a]	Precipitation	mm	Sum
	Relative Humidity	%	Average
	Temperature	°C	Average
	Wind Speed	m/s	Average
	Bee fly time	hrs	Sum of hours with dry weather. For calculations, we considered 1 hour with no precipitation as the minimum time for a relevant foraging activity. Because raw data was at 10-minute intervals, we considered 1 hour of dry weather only if it had not rained during 50 consecutive minutes.
	Chestnut bee fly time	prop	Proportion between bee fly time during the peak of chestnut blooming (15th of June to 15th of July) and bee fly time during the whole period considered (1st of June to 1th of August)
CG[b]	Precipitation May	mm	Sum

[a]Weather data considered from the 1st of June to the 1st of August and for daylight hours, i.e., covering potential bee foraging activity.

[b]Weather data considered only during the month of May and over a full 24 hours.

Statistical analysis

The relationship between the *Castanea sativa* component (Csc) with the three different ACGW-induced damage indices (i.e., Dk infestation, Sanasilva and DCI) and weather parameters (Table 2) was first inspected separately by means of a univariate comparative analysis and tested for correlation issues (Pearson R > 0.5) among variables. A linear mixed-effects regression approach was then used to analyze the effects of ACGW damage and weather conditions (explanatory variables) on the Csc variation (response variable) between 2010 and 2016. For the contiguous sites VO1 and VO2, only one meteorological station was available. Therefore, the corresponding Csc and ACGW-induced damage index values were averaged and the resulting mean values were then related to the meteorological data.

The random configuration of the mixed-effects model (between random intercept or random intercept and slope) was set according to the procedure described in Zuur *et al.* (2009). Model selection was made on the basis of the AICc coefficient (Akaike information criterion with a second-order correction for small sample size) and goodness of fit evaluated using pseudo R^2 (Nakagawa & Schielzeth, 2013; Johnson, 2014; Jaeger *et al.*, 2017). Model assumptions were checked with residual plots, and only those that showed no obvious deviations from normality or homoscedasticity were considered.

Table 3 Tree damage, honey composition and chestnut pollen content by beekeeper since 2010

		Tree damage plot				Physico-chemical analysis		Sensory analysis						
Beekeeper Id	Year	Dk infestation %	Sanasilva %	DCI	Ts% %	Electrical conductivity mS/cm	Free acids meq/kg	*Castanea sativa* Score (0-6)[e]	*Tilia* spp.	Mixed flowers[a]	Honeydews	*C. sativa* *component*[b]	Honey[c] Type	Honey composition[d]
VM1	2010	15	10	2	0	1.07	9.99	5	2	0	0	0.18	M	Cs/Ti
	2011	30	35	5	0	1.14	18.62	4	0	2	2	0.09	P	Cs/Mf/Hd
	2012	35	40	15	2	0.87	16.96	2	1	4	2	0.06	P	Mf/Cs/Hd/Ti
	2013	32	46	20	4	0.62	16.59	0	4	2	0	0.04		Ti/Mf
	2014	46	55	19	74	0.78	27.90	1	1	4	0	0.03	P	Mf/Cs/Ti
	2015	14	32	15	48	1.19	16.44	4	0	2	0	0.12	M	Cs/Mf
	2016	7	17	0	55	1.50	16.28	3	0	2	0	0.15	M	Cs/Mf
MA1	2010	10	15	0	0	0.97	11.70	4	2	2	0	0.12	M	Cs/Ti/Mf
	2011	38	30	2	0	1.39	16.82	6	0	0	0	0.17	U	Cs
	2012	45	45	10	1	0.97	9.65	4	4	2	0	0.14	M	Cs/Ti/Mf
	2013	42	51	22	20	0.77	11.20	0	4	2	0	0.07		Ti/Mf
	2014	26	68	7	69	1.36	21.95	6	0	0	4	0.10	M	Cs/Hd
	2015	21	39	10	85	1.26	11.63	4	0	3	0	0.17	M	Cs/Mf
	2016	8	22	0	64	1.64	9.12	6	0	0	0	0.36	U	Cs
BE1	2010	0	0	0	0									
	2011	15	20	1	0	1.43	14.71	6	0	0	0	0.19	U	Cs
	2012	52	55	1	2	0.72	9.78	0	4	2	0	0.07		Ti/Mf
	2013	53	57	22	2									
	2014	55	55	18	36	0.89	19.45	0	4	4	0	0.05		Ti/Mf
	2015	48	33	10	88	0.87	15.30	1	2	4	0	0.06	P	Mf/Ti/Cs
	2016	9	32	0	93	0.92	13.86	0	4	3	0	0.07		Ti/Mf
VO1	2010	0	0	0	0	0.86	13.27	1	0	4	0	0.08	P	Mf/Cs
	2011	7	10	0	0	1.38	11.12	4	0	4	0	0.19	M	Cs/Mf
	2012	36	35	3	3	0.75	10.13	0	6	0	0	0.07		Ti
	2013	30	45	25	3	0.96	14.96	1	2	4	0	0.07	P	Mf/Ti/Cs
	2014	44	44	35	38	1.06	19.28	1	0	2	4	0.06	P	Hd/Mf/Cs
	2015	33	58	19	85	0.76	9.95	2	6	2	0	0.09	P	Ti/Cs/Mf
	2016	21	35	0	83	1.17	10.22	4	0	4	0	0.17	M	Cs/Mf
VO2	2010	0	0	0	0	1.44	11.55	4	0	2	2	0.25	M	Cs/Mf/Hd
	2011	7	10	0	0									
	2012	36	35	3	3	0.75	9.04	1	4	2	0	0.09	P	Ti/Mf/Cs
	2013	30	45	25	3	0.92	13.21	1	6	0	0	0.08	P	Cs/Ti
	2014	44	44	35	38	1.25	22.14	2	0	4	2	0.07	P	Mf/Cs/Hd
	2015	33	58	19	85	0.94	10.68	3	4	4	0	0.11	M	Ti/Mf/Cs
	2016	21	35	0	83	1.10	12.36	1	0	5	0	0.10	M	Mf/Cs
GO1	2010	0	0	0	0	0.79	17.49	2	2	2	0	0.06	P	Cs/Ti/Mf
	2011	0	0	0	0	0.83	19.68	2	0	2	2	0.06	P	Cs/Mf/Hd
	2012	20	20	1	3	0.66	14.01	0	4	2	0	0.05		Ti/Mf
	2013	35	41	22	10	0.61	17.83	0	0	4	0	0.03		Mf
	2014	45	62	5	61	0.69	17.00	0	2	2	0	0.04		Ti/Mf
	2015	29	38	4	87	0.75	24.4	0	1	6	0	0.03		Mf/Ti
	2016	14	38	0	81	0.85	18.00	0	4	2	0	0.05		Ti/Mf

[a] mixed flowers (*Mf)* = other botanical origin, mainly *Rhododendron* spp. *and Rubus* spp.; [b] *C. sativa* component (Csc) = Electrical conductivity/ free acids * (1+ RCS). RCS = chestnut sensory level/cumulated overall sensory levels (chestnut + lime + mixed flowers + honeydew)*;* [c] *Unifloral* (U) = electrical conductivity > 0.86 mS/cm, free acids < 22.4 meq/kg and sensory level of *Castanea sativa* is maximal and exclusive (RCS = 1); *Moderate* (M) = RCS < 1 and Csc ≥ 0.10; *Poor* (P) = RCS > 0 and Csc values < 0.10*;* [d] Cs = *Castanea sativa*, Ti = *Tilia* spp., Mf = Mixed flowers, Hd = Honeydews. [e] Score: 0 = absent, 1 = weak, 2 = weak-medium, 3 = medium, 4 = medium-strong, 5 = strong, 6 = exclusive.

Specifically, two different sets of models were run. The first set included 3 different models, with every single ACGW-induced damage index as an explanatory variable, each run in turn in order to select the one most related to Csc. As random effects, we had intercepts for beekeeper as well as by-beekeeper random slopes for the effect of the different ACGW-induced damage indices.

Forward model selection was then used to test the additional explanatory power provided by each weather parameter with respect to the previous best model with a single damage index, i.e., Csc ~ best ACGW-induced damage index + weather parameters + random terms.

In the second step, possible ACGW-induced damage index (Dk infestation, Sanasilva or DCI) thresholds causing significant Csc.loss that were retained in the best model were tested using Mann-Whitney U tests (Wilcoxon rank sum tests) in a univariate comparative analysis (with significant differences at $p < 0.05$).

All analyses were performed in R (version 3.3.3; R Core Team 2017). Linear mixed-effects models were fit using the lme4 package (Bates *et al.*, 2015) and their goodness of fit evaluated with the r.squaredGLMM function of the MuMIn package (Bartoń 2016).

Results

The sampled honeys ranged from unifloral to chestnut-poor honey. Only two beekeepers (MA1 and BE1) had a chestnut-rich environment enabling them to potentially produce unifloral chestnut honey. MA1 produced unifloral chestnut honey again in 2016, that is, in the second year after biological control was achieved by *T. sinensis* (Table 3). GO1, in contrast, is located in a marginal chestnut site and has only the potential to produce honey with a rather low chestnut component, as was the case even before the ACGW epidemic. In contrast, all other considered beekeepers were located in an intermediate environment that allowed the potential production of honey with a moderate chestnut component (Table 3). During the peak of the epidemic occurring between 2012 and 2014 (see Dk infestation in Table 3), the chestnut component in honey reached minimum values within each beekeeper series.

The best random factor configuration for the modelling approach was represented by random intercepts for beekeeper as well as by-beekeeper random slopes for the Dk infestation effect.The best linear mixed-effects model considering only the ACGW-induced damage index retained Dk infestation as the best explanatory variable (negatively related to the Csc – see Table 4) with a conditional R^2 (R^2c) of 0.80. Since the addition of weather variables resulted in minimal gains in R^2c and did not significantly improve the ACGW-induced damage index model (Table 4), we identified the model including Dk infestation only as the best parsimonious model (Fig. 2).

Figure 3 shows the relationship between the relative decrease in the chestnut component in honey within each beekeeper series (Csc.loss = *Castanea sativa* component loss) and the Dk infestation. Significant increases in Csc.loss are detectable starting from an average Dk infestation of 30%, whereas more than half of the chestnut component is lost in nearly all the honey samples when the Dk infestation exceeds 40% (Fig. 3). Appendix - Figure A3 gives a visual impression of the tree crown habit of the damage categories represented in Figure 3.

Table 4 Best linear mixed-effects regression models according to AIC_c ranking considering all variables, or separately with tree damage due to *Dryocosmus kuriphilus* attack and weather variables

	Model formula	**Fixed effect**	**Estimate *[95% CI]***	S_{resid}	***t* value**	AIC_C	R^2c
Tree damage only	[a]Csc ~ Dk inf + *r*	Intercept	0.165 *[0.102, 0.229]*	0.027	6.22	-115.53	0.80
		Dk inf	-0.002 *[-0.003, -0.001]*	0.000	-5.12		
	Csc ~ Sanasilva + *r*	Intercept	0.174 *[0.104, 0.249]*	0.030	5.78	-104.26	0.73
		Sanasilva	-0.002 *[-0.003, -0.001]*	0.001	-3.78		
	Csc ~ DCI + *r*	Intercept	0.131 *[0.083, 0.180]*	0.020	6.53	-100.01	0.63
		DCI	-0.003 *[-0.006, -0.001]*	0.001	-3.39		
With weather variables	Csc ~ Dk inf + P + *r*	Intercept	0.157 *[0.092, 0.223]*	0.028	5.52	-112.88	0.80
		Dk inf	-0.002 *[-0.003, -0.001]*	0.000	-4.89		
		P	0.000 *[0.000, 0. 000]*	0.000	0.78		
	Csc ~ Dk inf + T + *r*	Intercept	0.244 *[0.089, 0.406]*	0.076	3.21	-113.45	0.82
		Dk inf	-0.002 *[-0.003, -0.001]*	0.000	-5.09		
		T	-0.003 *[-0.010, -0.003]*	0.003	-1.12		
	Csc ~ Dk inf + WS + *r*	Intercept	0.246 *[0.114, 0.380]*	0.065	3.82	-114.06	0.82
		Dk inf	-0.002 *[-0.003, -0.001]*	0.000	-4.98		
		WS	-0.044 *[-0.108, -0.022]*	0.031	-1.4		
	Csc ~ Dk inf + RH + *r*	Intercept	0.146 *[-0.048, 0.318]*	0.084	1.74	-112.34	0.80
		Dk inf	-0.002 *[-0.003, -0.001]*	0.000	-5.18		
		RH	0.000 *[-0.002, 0.003]*	0.001	0.24		

[a]Best model considering all variables (tree damage and weather variables). Please note that because it retained only Dk infestation, the model has been placed in the table under the "Tree damage only" section. 95% CI = Confidence Interval; AIC_c = Akaike Information Criterion with a second-order correction for small sample size; R^2c = conditional R^2 showing the variance explained by the entire model (fixed and random factors); Csc = *Castanea sativa* component; Dk inf = Dk infestation (%); P = precipitation (mm); T = temperature (°C); WS = wind speed (m/s); RH = relative humidity (%); r = random factors

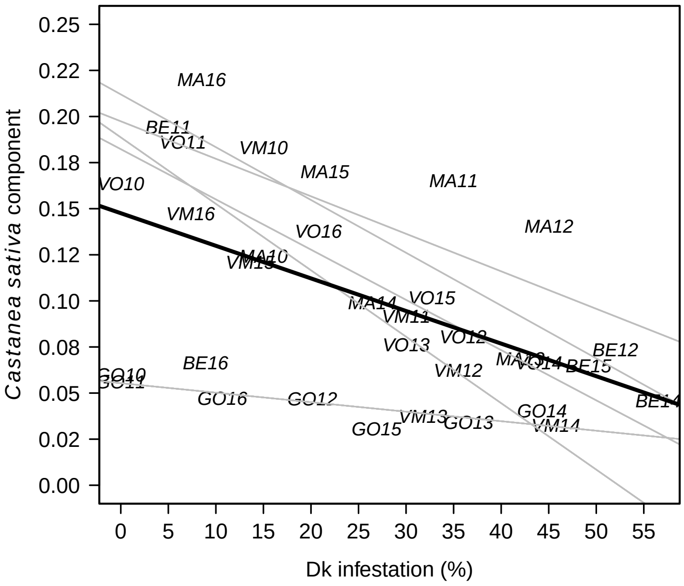

Figure 2 Variation in the *Castanea sativa* component in honey due to *Dryocosmus kuriphilus* infestation as predicted by linear mixed-effects models. The Black line shows the fitted values for the overall model (the predicted average variations across beekeeper) whereas grey lines represent the effect for each beekeeper as obtained by adding intercept and slope adjustments to the overall model fit. Codes are composed of the Beekeeper-ID and the abbreviated year. For the contiguous sites VO1 and VO2 only one meteorological station was available. Consequently, mean values were calculated at the VO site for the *Castanea sativa* component and the ACGW-induced damage.Csc (*Castanea sativa* component) = electric conductivity / free acid * (1 + RCS). RCS (relative contribution of chestnut to the overall sensory level) = chestnut sensory level / cumulated overall sensory levels (chestnut + lime + mixed flowers + honeydew). Dk infestation (*Dryocosmus kuriphilus* infestation) = no. attacked buds / no. total available buds * 100.

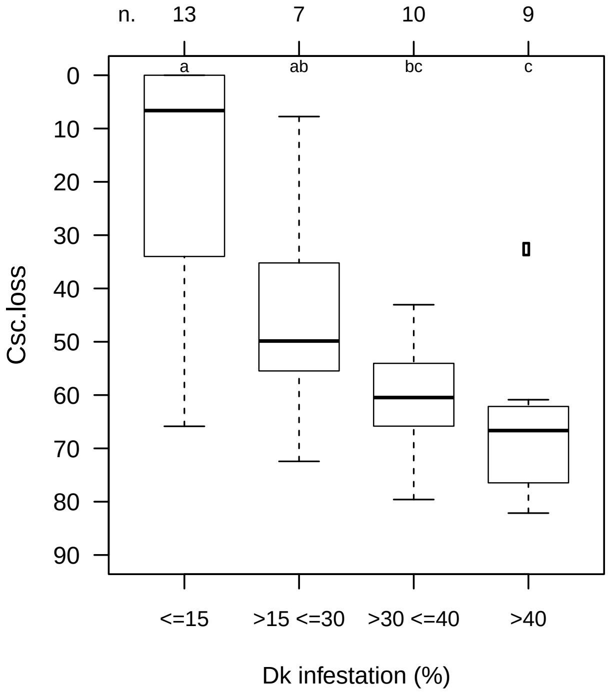

Figure 3 *Castanea sativa* component loss in honey as a function *Dryocosmus kuriphilus* infestation. Different letters indicate significant differences at $p < 0.05$ (non-parametric Mann-Whitney U test with Holm's adjustment). Csc.loss (*Castanea sativa* component loss) = 1 - $((Csc-Csc_{max})/Csc_{max} *100)$, where Csc_{max} stands for the highest Csc value within the beekeeper series under consideration 2010-2016. Dk infestation (*Dryocosmus kuriphilus* infestation) = no. attacked buds / no. total available buds * 100.

Considering the average value of all beekeepers by year, the evolution of the Csc.loss as well as the relative chestnut contribution to the overall sensory-level analysis (for short, RCS = Relative Chestnut Sensory) follows the ACGW epidemic trend (Fig. 4). This is shown in the progressive decrease in the chestnut component in honey (Csc. loss) until the Dk infestation peaked in 2014 and its recovery to almost the same average values as in 2010 during the subsequent chestnut resurgence phase (Fig. 4).

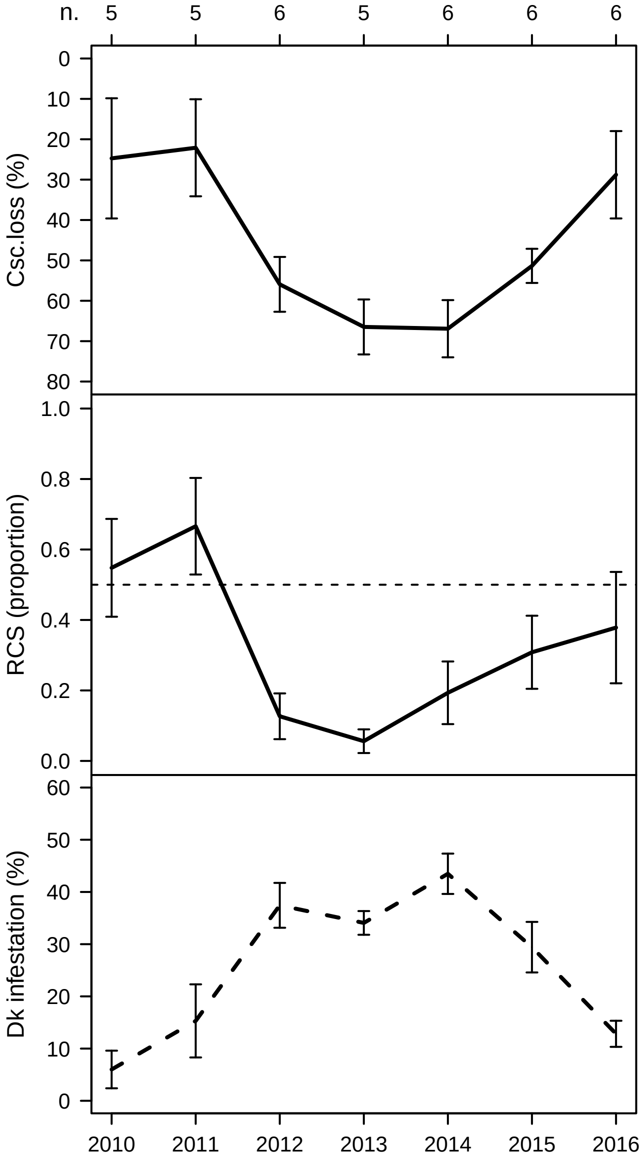

Figure 4 Yearly evolution of *Castanea sativa* component loss in honey, average relative chestnut contribution to the overall sensory analysis, and Dk infestation. Average *Castanea sativa* component loss (Csc.loss; solid line, top plot), average relative chestnut contribution to the overall sensory level analysis (RCS; solid line, center plot) and *Dryocosmus kuriphilus* infestation (dashed line, bottom plot). Vertical bars = 95% confidence interval. Csc.loss (*Castanea sativa* component loss) = 1 - ((Csc-Csc$_{max}$)/Csc$_{max}$ *100), where Csc$_{max}$ stands for the highest Csc value in the beekeeper series under consideration 2010-2016. RCS (relative contribution of chestnut to the overall sensory level) = chestnut sensory level / cumulated overall sensory levels (chestnut + lime + mixed flowers + honeydew). Dk infestation (*Dryocosmus kuriphilus* infestation) = no. attacked buds / no. total available buds * 100.

Discussion

Honey characteristics strongly depend on vegetation and on the abundance and flowering phenology of the polleniferous and nectariferous plants surrounding the apiaries. Several studies document this for different ecosystems and at different spatial scales (Odoux et al., 2012; Bagella et al., 2013) and for chestnut ecosystems in different geographic contexts (Castro-Vázquez et al., 2010; Yang et al., 2012). Flowering phenology and honeybee activities in flower visiting, pollinating and food-storing may also vary as a function of weather conditions (Gerlach, 1985; Crailsheim et al. 1999; Blaschon et al., 1999; Tuell and Isaacs, 2010). In this study we show how honey composition may also become altered as a consequence of a pest-induced multi-annual impact on a widely present pollen-producing forest tree species.

Our results highlight how severe and repeated ACGW damage to chestnut trees has induced a corresponding loss of the chestnut component in honey, especially during the peak of the epidemic that clearly overwhelmed and masked any possible effect of changing weather conditions during the chestnut flowering periods. Among chestnut damage indices, Dk infestation was found to be the most suitable for predicting variation in Csc. The Sanasilva index behaves similarly to the Dk infestation whereas the DCI was found to be less suitable although following similar trends. As described in Gehring et al. (2017), when biological control through *T. sinensis* takes effect and chestnut tree recovery begins, the process starts from the branch apex producing ACGW-free shoots from healthy, flower-producing terminal buds. Such a recovery process is best described by the Dk infestation and the Sanasilva indices. Both proxies were found to be very sensitive to the damage and recovery of the external, light-exposed crown parts that bear the nectariferous catkins on sprout (new shoot growth). The Sanasilva index, however, places more emphasis on existing gaps in the external crown due to dead shoots, whereas the DCI considers the entire branch architecture, thus exaggerating the importance of missing internal lateral branches with respect to the potential flower production of trees.

Decreases in the chestnut component in honey became statistically significant starting from an infestation of 30% (Dk infestation). When Dk infestation reaches 40%, the production of unifloral chestnut honey or honey with a moderate chestnut component becomes difficult in the study area. As soon as chestnut tree damage decreased, within two years the chestnut component in honey returned to almost the same values as before the epidemic (2010). The only exception was at site BE1, which experienced the opposite behaviour with a relatively low chestnut component despite a strong recovery in the damage indices. This may be due to possible additional noise in the data as a consequence of local and episodic disturbances, such as hale events followed by heavy chestnut blight attacks in the tree crowns (Rigling et al., 2014).

ACGW-induced temporary reduction in chestnut pollen and nectar availability resulted in a shift in honeybee foraging to the few phenologically synchronous nectariferous species that were available, such as *Tilia* spp., *Rhododendron* spp., *Rubus* spp., as well as honeydew. Such a shift, which is occasional and fluctuating according to the timing and weather conditions during a normal season of chestnut flowering, may become practically a requirement when chestnut flowering is lacking as a consequence of the severe ACGW attacks.

Meteorological variables showed very low correlations with the decrease in the chestnut component in honey during our study period. Although some fluctuations occurred during the period in question, they did not appear to be directly related to the Csc trend. Moreover, meteorological variables did not significantly improve the best model in terms of AICc or explained variance.

From a methodological point of view, the proposed combined chemical-analytical and sensory approach and the related mathematical equations were found to be a highly suitable method for assessing the chestnut component in honey samples and its evolution over time. In our specific case, a palynological approach alone based on pollen count would likely be insufficient, due to the general overrepresentation of *Castanea* pollen in honey with a traceable chestnut component (Persano Oddo et al, 1995) induced by the conspicuous pollen production of male catkins where nectar is also located. When looking for nectar, bees easily take large amounts of the small pollen grains together with the nectar, which always makes it strongly over-represented in honey.

Conclusions

The present study highlights the possible far-reaching effects of the damage caused by the accidental introduction of an exotic pest into the chestnut forest ecosystems of the southern Alps. As postulated by Quacchia et al. (2014) for Piedmont, the successful and timely control of the ACGW by its specific natural enemy *Torymus sinensis* appears to enable, with time, the almost full recovery of tree crowns. It seems, therefore, reasonable to predict for the near future the normalization of chestnut honey production in the study area, following the trend registered in our study starting in 2015.

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Appendices

Figure A1 Example of a branch, a shoot and a sprout. **Branch:** includes the totality of the shoots, sprouts and vegetative organs. **Shoot:** sprout from the previous vegetative season with respect to the sampling date. **Sprout:** freshly formed sprout grown during the current vegetative season from a developed bud on a shoot.

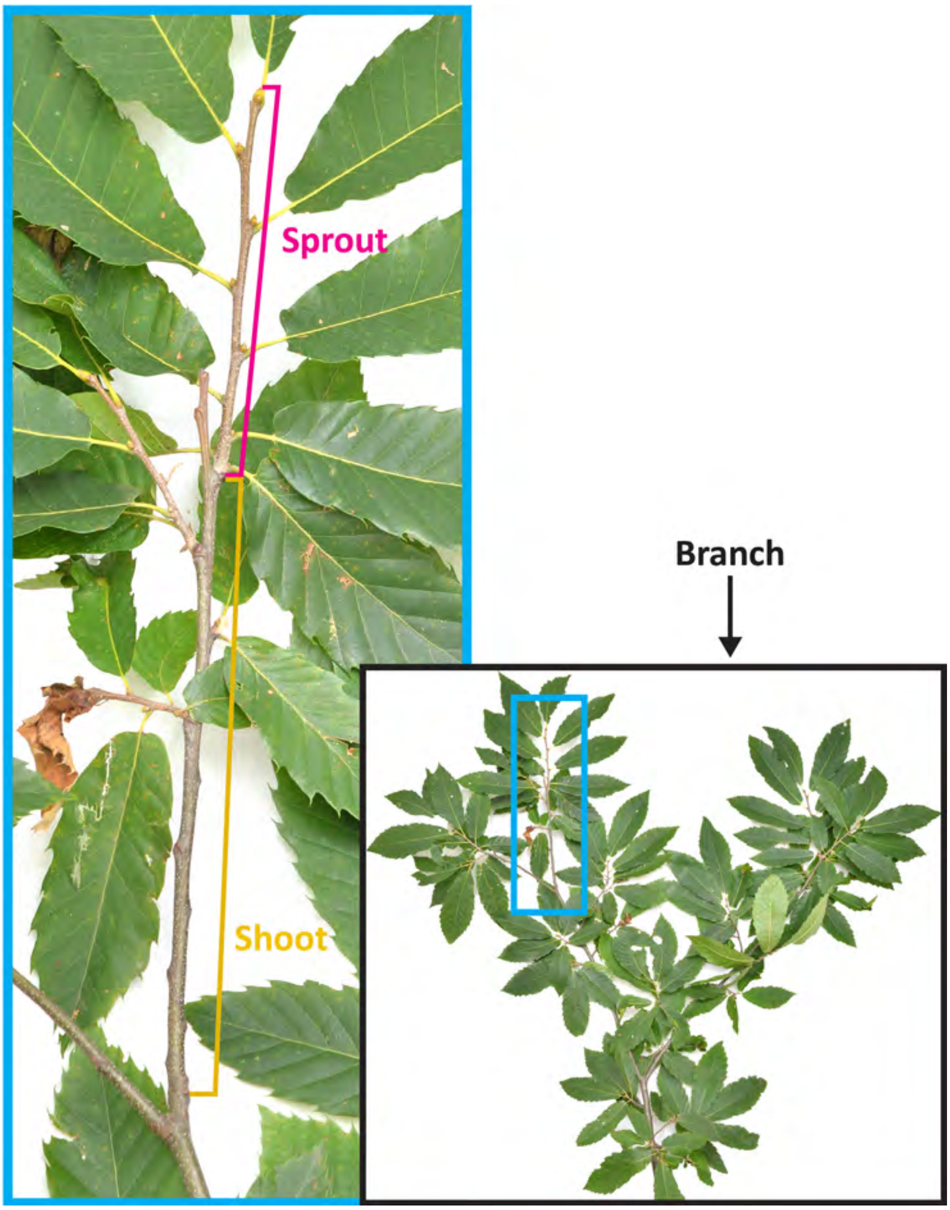


Figure A2 Mean weather data for the study area (Canton Ticino, southern Switzerland) according to year. Lines show the average values and vertical bars the minimum and maximum values respectively. For details, see Table 2.

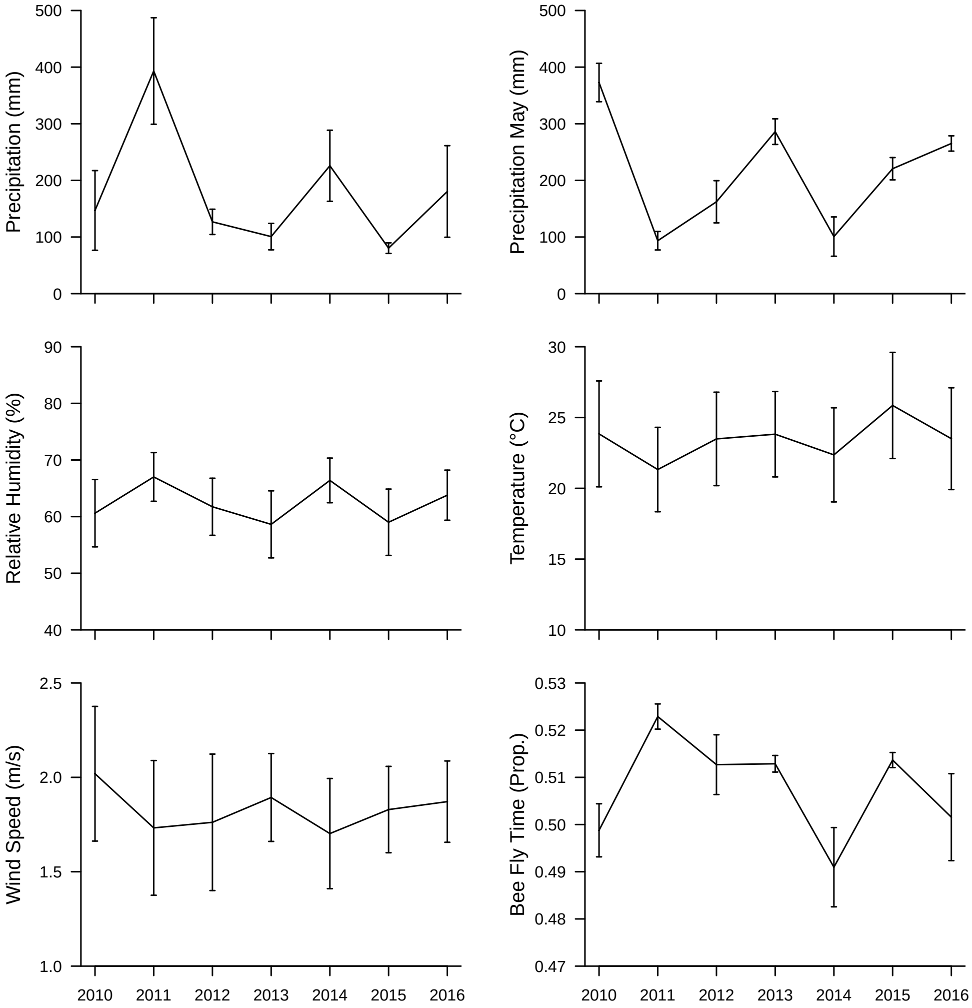
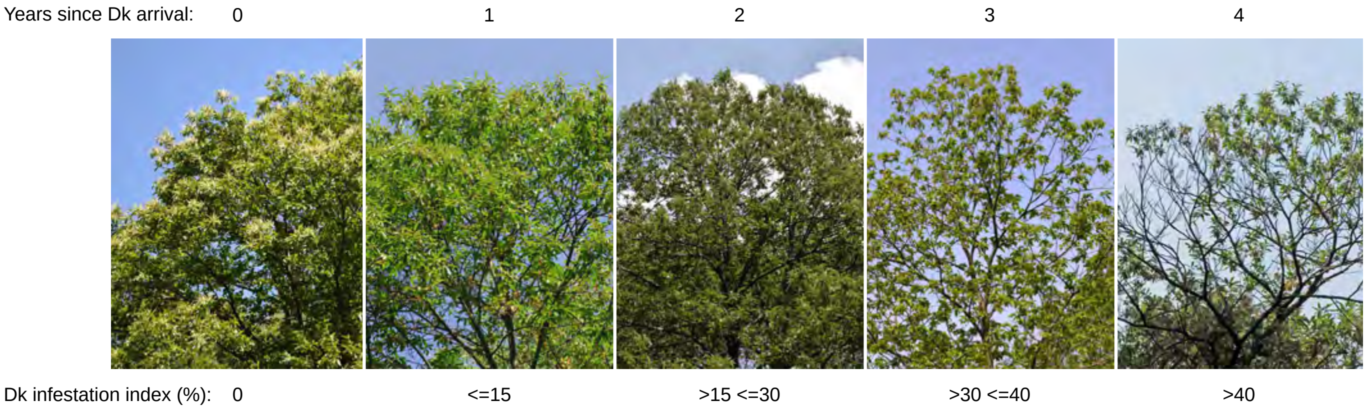


Figure A3 Selected examples of chestnut crowns damaged by repeated attacks of *Dryocosmus kuriphilus* Dk infestation index = *D. kuriphilus* infestation; expressed as percentage of infested buds; Years since *D. kuriphilus* arrival refers to the number of years of *D. kuriphilus* attacks under undisturbed epidemic conditions.



Chapter 6
General discussion and conclusions

My thesis is based on the study case of Asian chestnut gall wasp (*Dryocosmus kuriphilus*) invasion in southern Switzerland to present the evolution of an agroforestry system from the arrival of the pest to the biological control by its natural enemy *Torymus sinensis* by highlighting the dynamics of both insect populations and the progression of the related tree damages and reactions. Specifically, the main objectives are to define the best indexes to characterize both the insect populations dynamics and the tree damages and reactions. In a second step, I use the developed indexes to investigate how the *Castanea sativa* (host)-*Dryocosmus kuriphilus* (pest)-*Torymus sinensis* (biocontrol agent) system evolved in space and time.

When *D. kuriphilus* has been officially discovered by the authorities of Canton Ticino in 2009, the knowledge concerning this pest was sparse and was mainly acquired from the Japanese experience (for details on the Japanese experience see chapter 1.4. “Current research overview and rationale of the thesis”). As a consequence, available literature in Web of Science (https://apps.webofknowledge.com) for Europe was reduced to a handful of paper (less than ten), mainly focusing on the pest arrival and diffusion (Brussino et al. 2002; Graziosi and Santi 2008), on the control efficiency of local parasitoids (Aebi et al. 2006; Speranza et al. 2009) and on the rearing and settlement prospect of the biocontrol agent *T. sinensis* (Quacchia et al. 2008). Similarly, knowledge on the impact on chestnut trees (damages and reactions) was very scanty (Kato and Hijii 1997) and mainly limited to counts of the number of galls with respect to the available buds or shoots (Kotobuki et al. 1985). This lack of knowledge combined with the importance of the chestnut belt in southern Switzerland in protecting the landscape and the villages against natural hazards such as rockfall and shallow landslides, induced the Forest Service of Canton Ticino to immediately start a monitoring program of the pest evolution. The Federal Research Institute WSL soon followed by integrating the monitoring with the research activities in order to better plan forestry management and environment safeguard measures for the future. This thesis encloses the main findings of 7 years of research in southern Switzerland, providing new aspects on the damages caused by *D. kuriphilus* on the chestnut tree and its reaction and recovery pattern.

Thesis highlights

The methodological part

We first focused on the methods to accurately measure the leaf area of a branch (see Annexes). To this purpose we selected some sites still devoid of the pest to have a healthy reference in term of branch leaf area expected on healthy chestnut trees and planning to use this model to quantitatively estimate the loss due to the repeated *D. kuriphilus* galling activity. Inspired by the available literature, we developed a protocol to characterize the branch architecture in terms of leaf area, bud and shoot types and on reaction structures (see chapter 2.1). This allowed us to tightly follow and quantify the evolution of leaf area and branch architecture structure during a typical *D. kuriphilus* epidemic that includes an initial invasion phase by the gall wasp with low impacts and a population and damage peak during the third or fourth year with a subsequent progressive control by its natural enemy *T.*

sinensis until effective biological control has occurred. We in particular demonstrated that the *D. kuriphilus* infestation rate usually reported in the specific literature (Kotobuki et al. 1985; Matosevic et al. 2010; Sartor et al. 2015) fails to provide a sound estimation of the effective overall leaf area loss. On the contrary, we proved that the leaf area evolution is better described by combining multiple parameters such as the number of dead shoots, of reactivated dormant buds and of galls on shoots to a unique index that we called Damage Composite Index (DCI). When compared to other classical indices (i.e., based on gall abundance only) the DCI better depicts the real branch alteration, especially when repeated and uncontrolled D. kuriphilus attacks cause a severe branch corruption, the death of many shoots, as well as the activation of dormant buds. In fact, after multiannual attacks by D. kuriphilus, chestnut tree branches may display many dead parts but only a few galls or none at all, what erroneously results in low *D. kuriphilus* infestation values (low damage) according to the traditional indexes but high DCI values (severe damage, see chapter 2.2)

In order to optimize the investment in the time-consuming procedure for evaluating the parasitism rate of *D. kuriphilus* by *T. sinensis*, we tailored the gall collection design so as to zoom on the two most crucial phases which are the establishment of *T. sinensis* and the parasitism rate evolution until biocontrol. This allowed us to confidently estimate the evolution of the parasitism rate by collecting a targeted amount of galls allowing the suited result accuracy during the entire study period (see chapter 3.2).

Concerning the assessment of the tree crown damages, we opted for applying also a standard crown transparency estimation approach as proposed in the frame of the Swiss Sanasilva survey. Specifically, we separately assessed the crown transparency of the living part using the reference photography of Müller and Stierlin (1990) by separately estimating the proportion of the dead crown due to repeated *D. kuriphilus* attack, *Cryphonectria parasitica* attacks, and other (indefinable) causes (see chapter 2.3).

With all that in mind and considering all the collected parameters, we could tightly follow and describe the evolution of the *Castanea sativa -Dryocosmus kuriphilus - Torymus sinensis* system in southern Switzerland, not only from the pest-parasitoid point of view but also including the host response to the pest attack and its recovery patterns when the biocontrol agent becomes effective.

Evolution of the Castanea sativa -Dryocosmus kuriphilus - Torymus sinensis system

Our monitoring program revealed a huge variation in the time lag between the arrival *of D. kuriphilus and T. sinensis* in Ticino: the southern localities experienced up to seven years of uncontrolled attacks whereas the northern ones only two (see chapter 4.1), with significant consequences for the chestnut trees. In fact, repeated attacks do not limit the negative effects to gall-related reductions in leaf area (e.g. (Kato and Hijii 1997), but generate over time progressive and significant alterations of the branch architecture as a whole, including the failure of bud production from galled shoots (shoots on galls) and the dieback of infested branches, especially in case of subsequent attacks by secondary or endophytic fungi such as chestnut blight (*Cryphonectria parasitica*; (Turchetti et al. 2010; Meyer et al. 2015)) or chestnut brown rot (*Gnomoniopsis* spp.; Ugolini *et al.*, 2014; Lione *et al.*, 2016). Moreover, the small positive effect of tree reactions represented by the activation of dormant buds and second flushes, simply end in progressive energy consumption and the depletion of tree reserves (dormant bud) without any significant recovery for the tree (see chapter 2 and 4.2).

When the population of *T. sinensis* reaches notable levels of parasitization on *D. kuriphilus* (i.e., ~75%) the tree recovery process begins and is usually faster for the outer crown coat compared to the inner one (dormant buds and lateral shoots), because of the developing effect of terminal buds that are usually formed after the flight of the *D. kuriphilus* and remain thus gall-free. Interestingly, the recovery speed and completeness of the whole tree strongly depends on the time lag between the first *D. kuriphilus* attack and the arrival of *T. sinensis*. In fact, trees experiencing more than four years of uncontrolled *D. kuriphilus* attacks displayed more overall severe crown corruption and tree reserve exhaustion to the point that, even after four years of *T. sinensis* biocontrol, long-lasting damage is still visible in the form of dead crown parts. Moreover, the majority of severely attacked and stressed trees tend to rebuild the lost crown by producing an elevated number of suckers along trunk and main branches. It is therefore reasonable to argue that a proportion of these trees may never fully recover and will be seriously threatened in the immediate future by secondary agents such as hail-induced chestnut blight or drought. This is in contrast with the healthy situation in sites where the two insects arrived nearly simultaneously and severe damage never occurred. Here the recovery process was quasi-imminent, keeping both branch and crown architectures of the affected trees intact (see chapter 4). Last but not least, also direct economic effect is caused by this pest epidemic: the reduction of the flowering and the drop-in chestnuts production under repeated *D. kuriphilus* attacks do not only hamper the chestnut fruit market (Battisti et al. 2014; Sartor et al. 2015), but represents also a problem for the local honey producers. In our study we could demonstrate how almost impossible it was to produce the typical, unifloral and very requested honey (Apisuisse 2015) during the epidemic peak due to the significant reduction of the chestnut component in the honey (see chapter 5).

From the biological control point of view, *T. sinensis* confirmed to be a very effective biological control agent of *D. kuriphilus* on many species of the genus *Castanea* worldwide (Moriya et al. 2003; Rieske 2007; Matošević et al. 2017; Ferracini et al. 2018b) and thus also on *C. sativa* in southern Switzerland (see chapter 4). *T. sinensis* easily managed to spread from Italy into Switzerland, rapidly colonizing the entire Swiss chestnut groves within four years from the nearest Italian release points and despite the lack of official releases in the area. Furthermore, it established a large population within maximum four years managing to consequently decrease the pest pressure.

As a general rule (although with few exceptions), where the biocontrol has been achieved 4-5 years ago allowing the epidemic to fluctuate at dynamic equilibrium state for years now, finding *D. kuriphilus* galls in the forest has become increasingly difficult over time, as was the case in 2016 in the Cuneo region (Italy) and in 2018 in the far northern and southern sites in southern Switzerland.

Area for further development and research

To date (winter 2018) only part of the story reached a happy end, even after four years since the first biocontrol. In fact, two clearly trends emerged in southern Switzerland: the first, which includes 2/3 of the investigated sites, shows the very effective action of the biocontrol agent in controlling the pest population. The second, on the contrary, highlights a surprisingly epidemic wavering characterized by an incomplete pest control and chestnut tree recovery, respectively. As a consequence, in the concerned spots *D. kuriphilus* damages are still visible and do significantly affect the chestnut tree vitality. Two possible hypotheses have been formulated in this regard. The first is that in case of sun-exposed sites, high spring temperatures may cause an anticipated *T. sinensis* phenology that cause an asynchronous emergence with respect to the gallwasp-induced gall-formation by the chestnut trees. As a consequence, the pest population never reaches an undetectably low level and some damaged chestnut trees persist. In addition, high temperatures may also influence the longevity of *T. sinensis* adults as demonstrated by laboratory trials that highlighted a significant decrease in its lifespan when increasing the temperatures from 15 to 25 °C and in the case of food deprivation (Quacchia et al. 2008; Picciau et al. 2019). The second hypothesis is totally different and is based on the observation made by other studies. At the community level, it is commonly assumed that ecosystem invasibility (susceptibility to be invaded by neobiota) decreases with increasing species diversity (Kennedy et al. 2002; Richardson et al. 2007). In particular there is an increasing evidence that more diverse forest stand compositions are less prone to insect damage (Jactel and Brockerhoff 2007; Castagneyrol et al. 2013), including the chestnut gall wasp (e.g. Guyot *et al.*, 2015). Because of the great variability in the forest composition in southern Switzerland and in our study sites (that extend from pure chestnut stands to mixed forests), this hypothesis should be also tested and verified to get a thorough understanding of the system, to properly plan future silvicultural measures to restore forest stands from the persistent damages caused by *D. kuriphilus* and to make them resilient to climate change and potential future pest invasions.

Another completely different and generally less explored area of research is the indirect impacts that invasive alien species may have on social and cultural aspect (for a review: Marzano et al. 2017). For example, Mitchell et al. (2018) found that *Buxus* tree, which is seriously threatened by the box tree moth (*Cydalima perspectalis*), is still very important for the Caucasians whose folklore and sacred rites as well as their historical and cultural values mention it as venerable; whereas the devastating damages created by the emerald ash borer (*Agrilus* planipennis) on *Fraxinus* tree indirectly modified the day-to-day human activity in US counties in terms of time spent in outdoor leisure activities versus labour activities (Jones 2016). In southern Switzerland, the chestnut culture and the related historical and ecological elements is still considered important by people (Conedera et al. 1997) and the loss of *C. sativa* could threaten not only local products (see chapter 5 of this thesis) but also the chestnut harvesting, which today has become a leisure activity much appreciated by the local population (Tio 2018a, b). Thus, it would be interesting to understand if outdoor activities such as chestnut harvesting may have been altered when *D. kuriphilus* infestation significantly affected the chestnut production.

On the success of classical biological control of insect tree pests

The success of classical biological control (CBC) programs is usually measured in terms of pest control (drastic reduction of its population, e.g. Bedding 2009) and economic outturn (e.g. Stiling and Cornelissen 2005; Cock 2015; Kenis et al. 2017) whereas the possible benefits that the host tree can undergo is often perceived as a “side effect” and was thus initially poorly documented. One possible reason could be that in the general procedure to implement a CBC program (e.g. Kenis et al. 2017), evaluating its success in the broad terms comes at the last step… often when funds become sparse. In fact, evaluating the success also in terms of ecological benefits (such as the host tree response) isn’t easy nor cheap and funders (for example government) may prioritize emergency situations (new problematic invasions) rather than impact of successful programs (Cock 2015). Moreover, when an invasive alien pest arrives in a new area and starts creating damages, the first impulse is surely to contain it and eliminate it (see for example the case of the Asian longhorn beetle (*Anoplophora glabripennis*) in Switzerland; WSL Swiss federal institute for forest 2017), and if it is no longer possible, to control it. Unfortunately, when not controlled, invasive insects can rapidly kill their host as it is the case for the box tree moth (*Cydalima perspectalis*) defoliating to death *Buxus sempervirens* (Kenis et al. 2013) or the Asian longhorn beetle lethally disrupting the sap flow of different species such as maples, birches, willows, horse chestnuts and poplars, and similarly for emerald ash borer (*Agrilus planipennis*) on *Fraxinus excelsior* (Gibb and Gibb 2015). As a consequence, the benefits of CBC sometimes are only measured with the decreasing death or defoliation of the hots rather than its specific response (e.g. Roland 1995; Collett and Elms 2009).

One of the purposes of my thesis is to try to dig a little deeper and tightly measure the possible biocontrol success not only in terms of pest reduction but also in tree response. Although not frequent in literature, other *host-pest-biocontrol* forest systems have been well described and it seems to me that this goes along with their global importance. For example, although not tightly related to the positive biocontrol effect, the pine response to the invasive *Sirex noctilio* attacks have been extensively well documented, particularly from the biochemical point of view (Bordeaux and Dean 2012) probably because it has become a serious global problem for pine plantations (Fischbein and Corley 2015). Another well studied infamous invader which recently arrived in Europe is the emerald ash borer, which killed and still kills North America's ash tree species to the point that their persistence is threatened (Valenta et al. 2016; Kashian et al. 2018; Kolka et al. 2018). A CBC program including three specialized parasitoids started in 2007 in Michigan and its efficiency has been checked monitoring the parasitism rate and the tree response in term of health changes (crown conditions) and ash regeneration but with little positive effect until now (Duan et al. 2017; Kashian et al. 2018). Moreover, other tree responses were studied such as the contribution of epicormic basal sprouting to ash tree (Aubin et al. 2015; Kashian 2016) as well as the broad ecological impacts that emerald ash borer could have in North American forests (for a review: klooster et al 2018; Kolka et al 2018) (Klooster et al. 2018; Kolka et al. 2018). This is the only case that I could find where they tried to link the success of a biocontrol program with the tree response.

Dryocosmus kuriphilus: a super pest?

The invasion process of a species usually follows four distinct phases: arrival (e.g., introduction into new areas outside its native range), establishment (population becoming abundant enough, so that extinction is highly unlikely), spread (the expansion of the species in uninfested areas), and impact (negative effect on human health, economic interests, biodiversity, ecological integrity or natural environment) (e.g. Hulme 2006; Alvarez 2016). Consequently, different management approaches have been developed as function of the invasion phase: prevention (border controls and quarantine measures), early detection (monitoring programs, remote sensing, aerial photography), rapid response and eradication (removal of the whole initial population), control and containment (poisons, pesticides herbicides, biocontrol, …), restoration and mitigation (re-establishment of native species, restoring original ecosystem functions; Hulme 2006).

Avtzizs and Matosevic (2013) described the *D. kuriphilus* invasion history in Europe as "taking Europe by a storm". In fact, this pest was able to colonize more than 20 European countries in less than 15 years. The difficulty to detect the insect due to its minute size and cryptic life cycle inside the buds (Bernardo et al. 2013) was probably the crucial strength for his global invasion. Then, as it is the case for many potential alien species with initial low-density in populations, the establishment phase represents a critical period affected by stochastic factors such as abiotic influences (e.g. adverse weather), demography (random variation in birth and death processes; Engen et al. 1998) and by the Allee effect (Allee 1931) which includes possible different causes such as the failure to locate mates (Roush and Hopper 1993; Sharov et al. 1995; Berec et al. 2001), inbreeding depression (Lande 1998), predators (Gascoigne and Lipcius 2004), lack of cooperative feeding (Clark and Faeth 2004), and poor genetic architecture of a founding population (Lee 2002; Frankham 2005; Roman and Darling 2007). These factors have brought many invasion attempts to failure (Williamson and Fitter 1996; Simberloff and Gibbons 2004), but did not stop *D. kuriphilus*. On the contrary the pest seems to be immune to almost everything, despite the fact that its arrival in Europe is thought to be the result of a single introduction of a Chinese founder population of very limited size and/or with low genetic diversity, that probably also encountered severe bottleneck during the establishment of its initial population in Italy (Avtzis and Matošević 2013; Martinez-Sañudo et al. 2018). As a matter of fact, *D. kuriphilus* has been able to settle and rapidly grow, similarly to other species such as *Ceratitis capitata* (medfly), *Leptinotarsa decemlineata* (Colorado potato beetle) and *Quadrastichus erythrinae* (Erythrina gall wasp), in other continents (e.g. Bonizzoni et al. 2004; Grapputo et al. 2005; Rubinoff et al. 2010). In this context, factors such as the parthenogenetic reproduction and a general-purpose genotype adapted to various environmental conditions have probably played a major role in the *D. kuriphilus* invasion success (Avtzis and Matošević 2013).

D. kuriphilus seems also to have encompassed potential buffers of the spreading phase, which remains critical because of the initial low abundance of the population at the expanding front (Liebhold and Tobin 2008). Moreover, spread rates may be affected by the habitat variability and its fragmentations in particular (Neubert et al. 2000; With 2002; Kinezaki et al. 2006). However, in the specific case of the chestnut gallwasp the importance of the chestnut culture in Italy caused frequent movements of scions and trees from nurseries to the chestnut growing areas (e.g. Bounous 1999; Carpenito et al. 2016) and man-induced infestations in different regions of Italy may have likely occurred (Bosio et al. 2010; Bernardo et al. 2013). As a result, the pest has not been limited by habitat fragmentations and was enabled to reach Switzerland (Forster et al. 2009), Slovenia (Knapič et al. 2010) and France (Aebi et al. 2006) in only five years since its official discovery in Italy (Brussino et al. 2002) thanks to anthropogenic-induced long-distance dispersal (Liebhold and Tobin 2008). Natural spread involving population growth with diffusive random movement has been estimated to be up to ca. 25 km/year for this pest if wind-assisted (Cooper and Rieske 2007; Graziosi and Santi 2008, and chapter 4). The combination of both processes (the so called stratified dispersal) is the major drive of the spread process for many successful alien species as for example the gypsy moth (*Lymantria dispar*; Liebhold et al. 1992; Whitmire and Tobin 2006), the Hemlock woolly adelgid (*Adelges tsugae*; McClure 1990), emerald ash borer (*Agrilus planipennis*; BenDor et al. 2006) and horse chestnut leafminer (*Cameraria ohridella*; Gilbert et al. 2004).

In some biological systems, a delay in population expansion occurs before growing to levels sufficient enough to be noticed. This was the case for *Popillia japonica* slowly spreading when just arrived into North America, but then gradually increasing its rate of invasion (Shigesada and Kawasaki 1997) as it is probably happening now after its arrival in southern Switzerland (Marazzi 2019). A delay of approximately 12 years has also been reported in North

America from the initial introduction of the gypsy moth before populations were noticed (Liebhold and Tobin 2006). This may also occur for biological control agents as it has been noticed for *Arytainilla spartiophila* (Memmott et al. 2005) or even in Japan for *T. sinensis* (Murakami and Gyoutoku 1995). When *D. kuriphilus* has been officially announced in 2002 in Europe, the Italian chestnut orchards were already experiencing heavy attacks and the pest was already present in at least five chestnut areas of five municipal districts south of the city of Cuneo (Bosio et al. 2010). Based on customs statistics, Quacchia et al. (2008) and Aebi et al. (2006) dated back its physical arrival between three to 6 years before. Its potential latency time-lag has thus been very short. In fact, based on our experience (see chapter 2,3 and 4), *D. kuriphilus* damages peak after three/four years from its arrival, which is typically the time when also non-expert people notice the large amount of galls on twigs. Then, once established and when not controlled for multiple years (impact phase), *D. kuriphilus*'s *galling* activity results in a reduction of the tree vigour (Kato and Hijii 1997; Ugolini et al. 2014, and chapter 2 and 4), combined with a significant alteration of the branch architecture (chapter 2), and a nut yield reduction of up to 80% (Battisti et al. 2014; Sartor et al. 2015).

Viewing this experience in a retrospect, encompassing all the possible problems soaked with its fresh arrival, *D. kuriphilus* has definitely deserved the title of (super) “global invader” (Avtzis et al. 2019). Although various management options exist (e.g. Hulme 2006, Andrew 2008) according to each invasion phase, I have the impression that in this case the time windows to take efficient measures before the pest arrival (prevention) and after (management of spread) were very restricted. As described above, once arrived in a new area, *D. kuriphilus* is so effective in settling down and in establishing new viable populations that any management option during the establishment phase such as eradication (Knapič et al. 2010) or driving the invading population to extinction (e.g. Hulme 2006; Liebhold and Tobin 2008) becomes futile, although these techniques may have proved to be effective for other species (e.g. Myers et al. 1998; Sarty 2007)

Considering the prevention phase, various tools such as pest risk analysis and cross-border policy combined with phytosanitary border inspections have been implemented (e.g. Cardinale et al. 2012; Essl et al. 2015). However, one of the key challenges remains the choice of pests and pathogens that should be targeted during the analysis. The lack of accurate information on alien species distribution (Eschen et al. 2015; Essl et al. 2015) and the difficulty to identify potential pest or to forecast species invasion behaviour (Roques et al. 2015; Vettraino et al. 2017; Kenis et al. 2018) remain the main limitations to make this management option effective. A promising novel method to identify potential pest invaders consists in the use of sentinel nurseries where the woody plants and the associated organisms are analyzed in their region of origin (Roques et al. 2015; Vettraino et al. 2017; Kenis et al. 2018). First experiences in Asia revealed new insect species and host associations not available in literature that could potentially switch on European trees once introduced to Europe. Whether the above problems are true for certain species, I don’t think that at the end of the 20th century *D. kuriphilus* was not yet labelled as a potential invader, having regard to the Japanese (Moriya et al. 2003) and American (Rieske 2007) experience. The problems with this pest were probably more related to its detection during border controls. In fact the only rapid method known to quickly detect its presence during phytosanitary border inspections is destructive (Dini et al. 2012) and therefore not implementable. Also keeping in a quarantine period the enormous volume of plants imported into Europe would run in heavy logistic problems (Eschen et al. 2015).

Research is always improving in this respect (see for example the Qdetect project https://cordis.europa.eu/project/

rcn/94552/reporting/en) but unfortunately human intentional or unintentional introductions are nearly impossible to control and invasive alien species will always manage to slip through to become established, no matter how effective future prevention measures will be. Although this is a very delicate and complex subject, it is thus possible that some so-called “science denialist” may not be totally wrong with their controversial vision which claims for a rethink of our general “a priori” dislike for all invading species (e.g. Thomas 2013). The unstoppable increasing worldwide trade and the money-time constrains will probably force us to make some choices regarding the organisms to target and chase. If we make these choices based on the available scientific evidences, we will probably focus on organisms causing economic loss or that are able to harm humans’ goods, health and safety rather than those endangering biodiversity since it still is not clear who the latter is harming (Sagoff 2018) … but this is another story.

Dryocosmus kuriphilus in southern Switzerland: where will we be in ten years?

Forecasting the development of an ecological system is always very complex because of the countless number of variables involved (species behaviour, weather, new invasion, …), some of which bearing far-reaching effects that are impossible to predict. Nevertheless, although some *D. kuriphilus* populations are still significantly affecting the chestnut tree vitality (to date winter 2018), we are confident that with time its biocontrol agent *T. sinensis* will control the majority of its population in the whole chestnut area. Because *D. kuriphilus* and *T. sinensis* demonstrated a tight coupling between their population, both will probably decrease to very low level as it already happened in some regions of France (Borowiec et al. 2018) and Italy (Ferracini et al. 2018c; and chapter 4). Therefore, if all goes on well, in ten years’ time we’re going to end up with imperceptible *D. kuriphilus* and *T. sinensis* populations that do not significantly impact the chestnut trees anymore. Maybe some trees will perish in the meantime, but their majority will have partially or fully recovered and galls will be very difficult to find. Nevertheless, we have to remember that being a predator-prey system, we have to expect some recrudescence from time to time, as it already happened in Japan in 2000 and 2007 (Toyama et al. 2009) and as generally predicted by the model developed by Paparella et al. (2016).

The great unknown remains the potential non-target impacts of *T. sinensis* on native cynipid gall inducers, which is already happening in Italy and probably also in Switzerland. Evaluating and predicting the magnitude of this adverse effect on the long run seems very difficult at the moment because the amount of this shift may be proportional to the primary host availability which may periodically become available as it has been the case in Japan over 25 years (Ferracini et al. 2017). Constant fluctuation between *D. kuriphilus* and *T. sinensis* population (as predicted by Paparella et al. 2016) may thus limit the undesirable non-target action of *T. sinensis* to harmless levels.

Looking the whole European story from the bright side, we’ll end up with two new species in the Insubrian ecosystem “pacifically” coexisting without significantly harming native species (although some rare case of host shift have been observed; Ferracini et al. 2017), and an increase knowledge on parasitoids of cynipid galls (e.g. Matošević and Melika 2013; Quacchia et al. 2013; Ferracini et al. 2017) and on the chestnut resilience (see this thesis), a successful story of biological control (Matošević et al. 2017; Ferracini et al. 2018c, this thesis) and more than 130 scientific publications (result obtained using *D. kuriphilus* as a keyword in web of science https://apps.webofknowledge.com) increasing the global knowledge on pest and biocontrol agents.

Conclusions

The history of the *D. kuriphilus* invasion in southern Switzerland teaches us so far that *T. sinensis* should be released as soon as the gallwasp is detected in a new area in order to avoid long-lasting damages (e.g., disrupted crowns, reduced tree growth, and lack of flowering and seed production) on the chestnut trees. In fact, the longer the time lag between the pest arrival and the biocontrol agent release/arrival, the more severe and long-lasting tree damages are.

The results of this thesis and the recent scientific publications on the effectiveness of *T. sinensis* (Quacchia, Moriya and Bosio, 2014; Matošević *et al.*, 2017; Ferracini *et al.*, 2018; this thesis), its potential host range and hybridization (Bernardo et al. 2013; Quacchia et al. 2014a; Ferracini et al. 2015, 2017, 2018a), and the economic damages (Battisti et al. 2014; Sartor et al. 2015) contribute to fill the missing aspects highlighted by Aebi et al. (2011) in their evaluation of the use of *T. sinensis* as a biocontrol agent. Unfortunately, such preventive biological risk evaluation is and will always be very difficult to perform efficiently because of the forced general lack of important information at time of the biological invasion. Despite the research is making giant strides in the risk-assessment approach (e.g. Wright et al. 2005; Barratt et al. 2010; Kaufman and Wright 2017), established invasive neobiota will always be one step ahead because of the time needed to perform the related studies on the candidate biological control agent and because the funds will rarely be available before the pest arrival (since we usually react only when the problem is on the spot).

The scenario of a non-introduction in Europe of *T. sinensis* would however have probably led to enormous damages on the whole chestnut groves. For example, in Italy the chestnut fruit has still a huge economic importance with around 4’000 metric tons of fruit collected per year in the Piedmont region alone (Aebi et al. 2011) which has been seriously threatened by *D. kuriphilus* (Sartor et al. 2015). In Switzerland the fruit production has only nowadays a very limited economic importance, although there is an increasing revival of its derived products such as sweets, flour and honey (Associazione ETC 2016). In mountainous areas this would have been combined with a significant loss of the protective function of the chestnut forests (Dazio et al. 2018) and a contemporaneous increase of the colonisation of the affected forest areas by alien tree species such as *Ailanthus altissima* (Knüsel et al. 2018). Finally, although not yet scientifically quantified, social activities such as popular chestnut roast events and chestnut harvesting in the forest would have probably disappeared with time. We are thus convinced that the damage-benefit ratio bound to this biocontrol agent are and will be far more acceptable than possible related downsides.

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Curriculum vitae

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Professional and Research interests

Je m'intéresse à tous les aspects de la dynamique de la forêt et des perturbations, en particulier celles dues à des événements extrêmes comme la sécheresse, les ravageurs et les incendies. Les interactions et les effets à long terme de ces événements extrêmes sont d'une importance capitale pour prédire le changement dans la composition des espèces d'arbres sous l'effet du changement climatique. L'arrivée et la propagation de nouveaux organismes tels que les ravageurs, les maladies et les espèces végétales susceptibles d'avoir un impact significatif sur les écosystèmes indigènes constituent une priorité importante : comment les gérer et les contrôler, comment gérer les écosystèmes afin d'atténuer les impacts directs et indirects possibles ou comment intégrer les nouvelles espèces dans le paysage futur sont actuellement mes principaux intérêts.

Dans mon projet de doctorat, je me suis concentré sur l'interaction entre *Castanaea sativa* (l'espèce hôte), le cynips du châtaignier (*Dryocosmus kuriphilus* ; le ravageur) et *Torymus sinensis* (son ennemi naturel et agent de lutte biologique) et sur leur évolution à court et long termes. En particulier, les principaux objectifs étaient de déterminer comment les châtaigniers réagissent à la diminution de la pression du cynips suite au parasitisme de *Torymus sinensis* et de comprendre la vulnérabilité des arbres affaiblis par les attaques en cas de perturbations ou stress supplémentaires (par exemple les extrêmes climatiques comme la sécheresse). Cette thèse qui s'est déroulé principalement au Tessin et en cotutelle avec le WSL de Cadenazzo et qui va bientôt se terminer représente d'une part une période d'apprentissage nécessaire pour devenir un chercheur, et d'autre part un vrai chapitre de ma vie. Les joies et les émotions que remplissent ce chapitre et qui sont liés directement où pas à ma thèse sont indescriptibles en quelques lignes étant donné que mes deux magnifiques enfants sont nés dans cette période et que le verdict de ma défense privée de doctorat m'a permis d'obtenir un emploi permanent au WSL de Cadenazzo.

Education

From 2015	PhD student at University of Neuchâtel
2008-2010	University of Lausanne, *Master of Sciences in Biology (Behaviour, Evolution and Conservation)*
2004-2008	University of Lausanne, *Bachelor of Sciences in Biology*
2002-2004	Spai Trevano (professional school), *Landscape Gardener* (place of apprenticeship: Beckmann and Puenter, 6672 Gordevio)
1998-2002	Secondary School of Locarno, *Maturità liceale (Economy)*

Ancyllary education

2012	Modul E28 Grundlagen der Holzhauerei
2019	Parent Effectiveness Training (communication skills based on Thomas Gordon Model)

Work Experience

From 2019	Scientific staff member at WSL Cadenazzo (level of employment: 50%)
From 2017	Fellowship at Bern University of Applied Sciences (level of employment: 20%)
2015-2016	Fellowship at University of Naples Federico II
2012-2019	Fellowship at WSL Cadenazzo (average level of employment: 50%)
05.2011-05.2012	Landscaper to Beckmann and Puentener
04.2010-09.2010	Temporary Fellowship at WSL Bellinzona
July 2009	Internship Chinnor Garden Center (Oxford, ENG)

Awards

2010	Interacademic Commission for Alpine Studies 2010: best Master Thesis
2004	Honor Award at the end of the apprenticeship of landscape gardener: best diploma

Supervision of theses

2013-2018	Co-supervision of five Master thesis and one Bachelor thesis with Marco Conedera or Boris Pezzatti

Selected professional activities

External Reviewer for ISI-Journals	Folia Oecologica European Journal of Entomology
Scientific congress	Gehring E., Pezzatti G.B., Krebs P., Conedera M. 24 settembre 2014. Impact of *Dryocosmus kuriphilus* Yasumatsu on the leaf area of *Castanea sativa*. Castanea 2014 (Convegno nazionale sul Castagno), Viterbo, Italy.

	Gehring E., Pezzatti G.B., Krebs P., Conedera M. October 2013. Impact of *Dryocosmus kuriphilus* Yasumatsu on the leaf area of *Castanea sativa*. ISHS II. European Congress on Chestnut. Debrecen, Baia Mare, Modry Kamen.
Invited scientific congress	Gehring E., Pezzatti G.B., Krebs P., Conedera M. 13 november 2014. Assessing the chestnut tree reaction patterns due to the gall wasp attack. Biocastanea 2014 (Jornadas técnico-cientificas. Il monografico internacional sobre la avispilla del castaño), Ponferrada, El Bierzo, Spain.
Divulgation presentations	Gehring E., Bellosi B., Cara C., Reynaud N., Fraia F., Quacchia A., Bosio G., Pezzatti G.B., Conedera M. 27 March 2015. Evolution of the Castanea sativa - *Dryocosmus kuriphilus* - *Torymus sinensis* system in Southern Switzerland: first results. Cs_Dk_Ts project annual meeting, Bellinzona, Switzerland. Gehring E., Bellosi B., Quacchia A., Cara C., Bosio G., Reynaud N., Pezzatti G.B., Krebs P., Conedera M. 19 novembre 2014. Situazione attuale dei castagni a 7 anni dell'arrivo del cinipide. Conferenza per la Società Ticinese di Apicoltura STA sezione di Lugano e Capriasca Ambiente. Cadro, Svizzera. Gehring E., Pezzatti G.B., Krebs P., Conedera M. 28 November 2013. Impact of *Dryocosmus kuriphilus* Yasumatsu on the leaf area of Castanea sativa. WSL, Birmensdorf. Gehring E., Conedera M., Bloch M. Marzo 2013. WSL Research of the dynamic of the *Dryocosmus kuriphilus* infestation in Switzerland. Incontro internazionale sul cinipide del castagno. Bellinzona.
Media, Tv	RSI LA 1. Il Quotidiano. 18.08.2015 RSI LA 1. Falò. 20.08.2015
Media, Radio	RSI Rete 1. L'ora della terra. 30.08.2015 Radiobus Pestalozzi. 20.10.2017

Informatics skills

Good skills	R (Statistical computing) QGIS (Geographic information system) Microsoft Office (including Access) Adobe Photoshop, Illustrator, Premiere, Dreamweaver
Basic skills	HTML, CSS and Java language

Language

Mother tongue	Italian French
Good level	English (speaking, writing and oral comprehension)

Publications

ISI

Gehring, E.; Bellosi, B.; Reynaud, N.; Conedera, M., 2019: Chestnut tree damage evolution due to *Dryocosmus kuriphilus* attacks. Journal of Pest Science, doi: 10.1007/s10340-019-01146-0

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Gehring, E.; Pezzatti, G.B.; Krebs, P.; Mazzoleni, S.; Conedera, M., 2015: On the applicability of the pipe model theory on the chestnut tree (*Castanea sativa* Mill.). Trees: Structure and Function, 29, 2: 321-332. doi: 10.1007/s00468-014-1093-z

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Pezzatti, G.B.; Krebs, P.; Gehring, E.; Fedele, G.; Conedera, M.; Mazzoleni, S.; Monaco, E.; Giannino, F., 2010: Using the leaf area/sapwood area (LA/SA) relationship to assess the ecological plasticity of the chestnut tree (*Castanea sativa* Mill.). In: 2010: I European Congress on Chestnut - Castanea 2009, Cuneo - Torino, Italy, June 2010. 103-109. doi: 10.17660/ActaHortic.2010.866.10

Conedera M., Pividori M., Pezzatti G.B., Gehring E. 2010. Il ceduo come opera di sistemazione idraulica - la stabilità dei cedui invecchiati.In: Carraro, V.; Anfodillo, T. (eds) Atti del 46° Corso di Cultura in Ecologia: "Gestione multifunzionale e sostenibile dei boschi cedui: criticità e prospettive". San Vito, 7-10 giugno 2010. 85-91.

Books / Monographs

Gehring E., Pantellini S. 2013. Avegno Boulder (Rock climbing guidebook). First Edition.

Other publications

Conedera, M.; Pezzatti, G.B.; Krebs, P.; Gehring, E.; Bertogliati, M., 2019: Resoconto delle attività di ricerca del WSL Cadenazzo – anno 2018. Forestaviva, 24-25.

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Conedera, M.; Gehring, E., 2015: Danni da cinipide e miele di castagno. L'Ape: Rivista Svizzera di Apicoltura – Anno 98, 6-8.

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