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Research Article

Interactions between invasive pests and pathogens in a native chestnut forest

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Abstract

The introduction in the same area of different invasive species can result in novel interactions, with unpredictable consequences. We carried out a study in Galicia (northwestern Iberian Peninsula) with the aim of clarifying the interactions between two invasive species *Cryphonectria parasitica* and *Dryocosmus kuriphilus*. In 2018, we selected five chestnut plots affected by both species. We compared trees affected only by the insect and trees affected by both the insect and the fungus with respect to attack level, gall characteristics, female size and fecundity, and concentrations of nitrogen, water and secondary metabolites. We also evaluated female preferences in a greenhouse assay. There were higher levels of attack in trees affected by both invaders. However, the greenhouse assay showed that ovipositing females do not preferentially choose trees attacked by the fungus. The presence of the fungus had no effect on the size, wall thickness, or hardness of *D. kuriphilus* galls, but larvae were smaller in trees also affected by the fungus. The fecundity of females was strongly related to the presence of chestnut blight; the number of eggs per female was almost double in trees affected by the fungus. There were no relations between blight and the nitrogen or water content in the galls, where the insects feed. There were also no effects of chestnut blight on the concentration of terpenes or phenols, but condensed tannins were higher in trees with chestnut blight. The higher tannins induced by chestnut blight may directly or indirectly benefit gall wasps. Positive relationships between condensed tannin concentration and reproductive performance of other gall makers were previously reported. Tannins can also improve the negative effects of environmental conditions inside the gall. Our results indicate that the presence of chestnut blight can increase the suitability of chestnut trees for the invasive insect, *D. kuriphilus*, through the increase in tannins due to the presence of the fungus.

Key words: Asian chestnut gall wasp, *Castanea sativa*, chestnut blight, interactions, invasive species

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Introduction

The number of invasive pathogens and insect pests in forest ecosystems has increased dramatically in the last century, mainly due to the growth of international trade and the associated increase in the movement of plants, wood and wood products (Roques et al. 2009; Walther et al. 2009; Santini et al. 2013; Freer-Smith and Webber 2017). Once established in the new environment, nonnative pests

can have negative economic and ecological consequences for forest ecosystems. Nonnative pests can cause growth losses and tree mortality (Aukema et al. 2011; Bonello et al. 2020), act as vectors or facilitate the entry in the plant of other damaging organisms (Meyer et al. 2015) and interfere with ecosystem services by reducing biodiversity and wildlife habitats, or by altering natural landscapes and diminishing their cultural value (Liebhold et al. 1995; Boyd et al. 2013). Also, the invasive forest pests and diseases may affect the ability of forests to sequester carbon, protect watersheds or combat desertification (Boyd et al. 2013; Seidl et al. 2018). In the most dramatic cases, these invasives can lead to functional extinction of the afflicted tree species (e.g., chestnut blight, Dutch elm disease, and emerald ash borer). The introduction in the same area of different invasive species can result in novel interactions, with unpredictable consequences such as the emergence of new associations among plant enemies (Santini and Battisti 2019) and new hybrid enemies (Brasier 2001; Brasier et al. 2004).

The European chestnut, *Castanea sativa* Mill., is widely distributed in Europe and Western Asia in natural and semi-natural forests, as well as in plantations and has been cultivated to produce fruit and wood since ancient times (Conedera et al. 2004). In Spain, chestnut occurs mainly in the north, occupying an area of more than 100,000 ha, of which some 45,000 ha are in Galicia (MARM 2011) where this study was conducted. Global chestnut production has increased continuously over the last 40 years (Freitas et al. 2021) and Spain is among the top three chestnut producers in the world (Freitas et al. 2021; Fernandes et al. 2022).

Chestnut blight (*Cryphonectria parasitica* (Murr.) Barr.) originally from Asia, has been present in southern Europe since the mid-20th century, probably introduced from North America (Dutech et al. 2012). It was detected in Italy in 1938 (Biraghi 1946) and in Spain in 1947 (Elorrieta 1949). Symptoms of chestnut blight involve swelling and cracking of the bark at the infection point on the trunk and branches, which often becomes populated with yellow-orange fruiting bodies. The fungus grows in the inner bark (phloem) and cambium tissue and progresses rapidly from initial infection to large sunken or swollen stem cankers that lead to complete girdling of the twigs, branches or the stems causing them to wilt (Prospero and Rigling 2016; Kolp et al. 2018). However, not all cankers girdle infected stems, canker expansion depends on tree resistance (Hebard et al. 1984), pathogen virulence (Griffin et al. 1983; Enebak et al. 1994), and other abiotic and biotic factors such as associated fungi (Kolp et al. 2018; Kolp et al. 2020). Susceptibility to this pathogen varies widely among *Castanea* species (Graves 1950; Dane et al. 2003; Mellano et al. 2012). Asian species are tolerant to *C. parasitica*, possibly because of a shared evolutionary history, but the European and North American chestnut species are highly susceptible to the pathogen, and infection normally results in tree mortality (reviewed in Griffin et al. 1983; Lovat and Donnelly 2019). The fungus functionally eliminated chestnut trees from the forest canopy in North America, and American chestnut exists today primarily as an under-story shrub emanating from preexisting root systems (Paillet, 2002). In the European chestnut, the fungus might persist for years in the same tree before the tree dies.

Dryocosmus kuriphilus Yasumatsu (Hymenoptera, Cynipidae) is a gall maker wasp, considered one of the most important chestnut pests worldwide (EPPO 2005). The insect, native to China, spread to other parts of the world includ-

ing Europe, where it was detected in 2002 in northwestern Italy (Brussino et al. 2002). From there it expanded rapidly throughout Europe, reaching Spain in 2012 (DOGC 2012; Pujade-Villar et al. 2013). The formation of the galls interferes with the normal development of the vegetative and reproductive structures of the chestnut tree, reducing wood and fruit production (Kato and Hijii 1997; Battisti et al. 2014; Sartor et al. 2015; Marcolin et al. 2021), although damage may vary depending on the forest structure (Castedo-Dorado et al. 2023a).

Galling insects modify plant physiology by inducing the development of complex gall structures in the plant tissue (Harper et al. 2004). Host chemistry can be important both positively and negatively for larval performance of galling insects (Rehill and Schultz 2012; Kot et al. 2018). Numerous reports show that galling insects can manipulate plant defenses to their own advantage (Schultz 1988; Hartley 1998; Oliveira et al. 2016; Lombardero et al. 2022). Increases in terpenes due to gall makers attack might have direct toxic effects on the attacking insect (Naidoo et al. 2018) or attract parasitoids (Borges 2018). Increases in terpenes were reported after *D. kuriphilus* attack in *C. mollissima* (Wang et al. 2024). Phenolics include many compounds with toxic or repellent effects towards insects and microorganisms (Lindroth and Hwang 1996). Nevertheless, the role of phenols for gall makers is ambiguous. Galls may contain lower (Nyman and Julkunen-Tiitto 2000; Allison and Schultz 2005) or higher (Hartley 1998) phenolic concentration compared with ungalled tissue. Some phenols are expressed as tannins, which are antibiotic and antixenotic, and have long been associated with pathogen and herbivore defense (Feeny and Bostock 1968; Griffin and Elkins 1986). Tannins can be dramatically elevated in galled plant tissue (Cornell 1983) including galls of our study system (Lombardero et al. 2022). High tannin concentrations in galls have been interpreted as a protection to the larvae against damaging agents (Cornell 1983; Taper et al. 1986; Schultz 1992).

Terpenes might also function in defense against fungal pathogens in conifers (Zeneli et al. 2006). Changes in phenolic composition in response to injury or fungal infection have been considered an active defense response (Brignolas et al. 1995; Evensen et al. 2000; Viiri et al. 2001), including in *Castanea sativa* (Dinis et al. 2011; Camisón et al. 2019). Tannin deposition has been interpreted as a localized host defense against advancing mycelia during active chestnut blight infection (Lovat and Donnelly 2019). Tannin profile differences between Asian *Castanea* species and *C. dentata* and *C. sativa* may explain some differences in blight tolerance between these two species groups (Elkins et al. 1979; Griffin and Elkins 1986; Cooper and Rieske 2008). However, Cook and Wilson (1915) suggested that tannins were used by *C. parasitica* as a source of nutrition.

Both invasive species, *D. kuriphilus* and *C. parasitica*, can colonize the same trees. Since the entrance of *D. kuriphilus* into Europe, numerous associations have been described between this species and native or introduced pathogenic fungi (Seddaiu et al. 2017; Morales-Rodríguez al. 2019), including reports of higher incidence of *C. parasitica* (Meyer et al. 2015; Vannini et al. 2018). We conducted a study in Galicia (northwestern Iberian Peninsula), one of the most important areas for chestnut production in southern Europe, with the aim of testing for interactions between the two invasive species since it could have implications for chestnut management.

Materials and methods

We conducted studies in the field and in greenhouses to assess potential interactions between *D. kuriphilus* and *C. parasitica* living on the European chestnut.

Field sampling

From January to March of 2017, we established 16 study plots to follow the damage caused by *D. kuriphilus* on *C. sativa* in Galicia (see more details in Lombardero et al. 2021). Five of the plots studied by Lombardero et al. (2021) also had trees attacked by *C. parasitica*; these plots were used for the present study. The number of trees per plot that were affected by both the pathogen and the gall wasp ranged from 5 to 9 and the number of study trees attacked only by *D. kuriphilus* ranged from 12 to 18. The plots were in the inner part of Galicia (Fig. 1) and share similar climatic conditions (mean annual temperature 10–12 °C, total annual precipitation 1100–1350 mm; more details in Castedo-Dorado et al. 2023a). The distance from one plot to the next nearest plot ranged from 1.2 to 19 km. The plots were in private chestnut plantations intended for wood production or both wood and nut production. Trees were 21–22 years old and spaced evenly at 4 × 4 m to 8 × 8 m.

Greenhouse assay

In 2018, we used a greenhouse assay to test whether *D. kuriphilus* female adults tend to avoid or prefer plants affected previously by *C. parasitica*. Study plants were obtained from a nursery with a known outbreak of *C. parasitica* in their seedlings. The infection occurred in the same year of the study (due to contamination during the common practice of grafting seedlings onto rootstocks resistant to *Phytophthora cinnamomic*). We selected 54 seedlings (2 years old) of similar size, half of which were infected by the fungus and half were not infected. We ensured that the uninfected plants had no fresh wounds or growth cracks that might have made them susceptible to cross-infection. The 54 plants were divided in three treatments: 10 plants with no chestnut blight and no exposure to *D. kuriphilus* (Control); 17 healthy plants exposed only to attack of *D. kuriphilus* (DK); and 26 plants infected with chestnut blight and exposed to *D. kuriphilus* attack (DK + CP). One of the fungal-infected plants was harvested to isolate and confirm the fungal identification. Plants were kept in 38-liter pots with similar commercial substrate and irrigation system in the experimental greenhouse of the University of Santiago de Compostela on the Campus of Lugo. Control plants remained inside the greenhouse (temperature night-day 18–24 °C and 80% moisture) during the wasp flight season and covered with anti-thrips mesh. The two groups of experimental plants were moved to an open area next to the greenhouse where they were exposed to wild populations of *D. kuriphilus* that had been well-established in the area since at least 2012 (Pérez-Otero and Mansilla 2012). Trees that were- and were not infected with chestnut blight were interspersed in a grid of approximately 1.5 × 1.5 m. In 2018, all the plants (except the controls) were outside during the flight season of *D. kuriphilus* (from late June to middle August) to test if the ovipositing insects preferred or avoided trees infected by *C. parasitica*. In 2019 and 2020, we repeated the study with the same plants (except 8 affected by chestnut blight that died in the second year) but including controls, outside, to also test if the insect preferred plants that had been attacked the previous year or non- attacked plants (the controls from previous year).

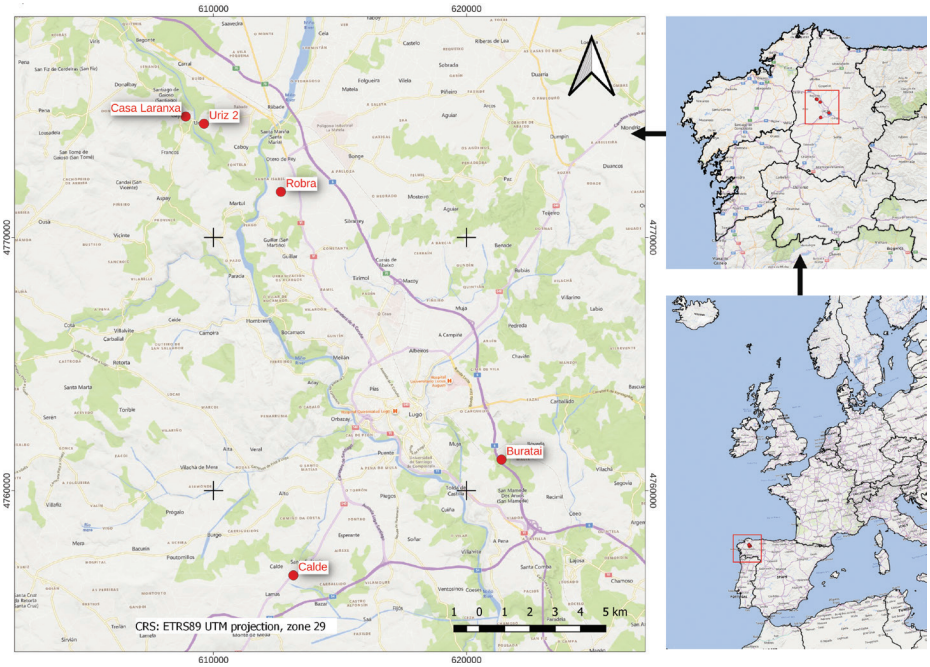

Figure 1. Location of the study plots. Plots are in Central Galicia, in the northwestern Iberian Peninsula.

Attack level

In summer 2018, we selected two branches at random in 17–27 study trees in each of the five field study plots (12–18 trees per plot without *C. parasitica* and 5–9 trees with *C. parasitica* (total of 81 and 36 trees without and with *C. parasitica*, respectively). We did our best to match infected and uninfected trees with respect to size and location in the plot. The different number of trees selected was due to the different availability of trees among plots.

In each branch, we located and examined the portion of the shoot that grew in the previous summer (2017). Within that length of shoot, we counted the number of buds that were present at the end of previous year's growing season (2017) as well as the galls produced in the current year (2018) from these buds. The resulting data allowed us to estimate galls per shoot (adjusted for number of buds per shoot) for each tree. We did not use the shoot of the current year because it was still growing after the insect flight ceased, and it is possible that new buds appeared that were not exposed to the attack. In the greenhouse study, we assessed attack level by counting total galls per tree in the study plants growing in pots.

Larval weight and adult fecundity

In June of 2018, to test if the presence of the pathogenic fungus influenced the growth of *D. kuriphilus* larvae, we measured the dry mass of individual late-instar larvae feeding on trees attacked only by the insect and on trees attacked by both invasive species, respectively. We were able to measure 3–9 larvae from each of 48 trees (8–10 trees per plot, half with and without *C. parasitica*).

We assessed female fecundity by counting the number of eggs produced by 1–10 emerging female adults captured from each of 43 trees (5–12 trees per study plot, 16 with *C. parasitica* and 27 without). We also counted the number of eggs from 10 females that emerged from greenhouse plants affected by chestnut blight and 10 from plants attacked only by *D. kuriphilus*.

Gall characteristics

In June 2018, at each of the five field study plots, we collected and measured 1–4 leaf galls from each of 5–11 trees (total of 76 trees; 34 with the fungus and 42 without). In the laboratory, we measured three perpendicular axes of each gall with digital calipers and averaged them to estimate gall diameter. We also measured the toughness of galls with a penetrometer of small fruits (FT02; Oremor). All galls were subsequently dissected to measure gall wall thickness (with a caliper) and count the number of feeding chambers (each representing one gall wasp progeny).

Effect of the presence of chestnut blight on tree nutritional quality and palatability

In summer of 2018, from each of five field study plots, we collected 2 leaves (one galled and other ungalled) from 6–18 trees (total of 69 trees, 34 with *C. parasitica* and 35 without). In the lab, we then measured total phenols, condensed tannins, and terpenes separately in ungalled leaves (ungalled leaf), in the gall itself (gall), and in the leaf tissue surrounding the gall (galled leaf). In the same sampling, we also collected another five trees per treatment and per plot to analyze water and nitrogen content. Again, we analyzed separately the gall itself and the leaf tissue surrounding the gall and control leaves (ungalled).

Similar measurements were carried out in the greenhouse plants. We sampled one leaf from five control trees (control), and two leaves (one galled and one ungalled) from 20 trees, 10 trees affected only by *D. kuriphilus* alone, and 10 trees affected by both insect and fungus. We also analyzed nitrogen and water content from five leaves from control trees, five from trees attacked by *D. kuriphilus* and five from trees attacked by both insects and fungi. For galled leaves, we analyzed separately gall tissue and the leaf tissue surrounding the gall.

We analyzed concentrations of total terpenes following Wainhouse et al. (1998). Terpene compounds were quantitatively extracted twice with n-hexane (with each extraction including 25 minutes in an ultrasonic bath) from 1 g of leaf or gall from each sample after cutting it into very small sections. Then, the plant material was recovered by filtration, the solvent was evaporated, and the mass of the non-volatile terpene residue was measured with a precision scale.

Phenolics were extracted from 0.5 g of plant tissue with aqueous methanol (1:1 vol:vol) in an ultrasonic bath for 15 min, followed by centrifugation and subsequent dilution of the methanolic extract (Sampedro et al. 2011). Total phenolic content was determined colorimetrically using Folin-Ciocalteu (Pérez et al. 2023) in a BioTek Elx 850 microplate reader at 740 nm quantified with a standard curve of tannic acid and expressed as mg tannic acid equivalent per g dry mass of plant tissue.

We analyzed condensed tannins following the protocol of Waterman and Mole (1994). The same extract used for phenolic analysis was assayed with butanol – hydrochloric acid reagent (0.7 g ferrous sulphate heptahydrate in 50 mL concentrated HCl and n-butanol added to make 1 L), and absorbance was measured at 550 nm with the same microplate reader, using as standard purified condensed tannins of quebracho (*Schinopsis balansae* Engl., Unitan Saica, Buenos Aires).

To analyze water and N content, samples were weighed fresh and then oven-dried at 60 °C for 48 hours. The dried samples were milled to a fine powder and submitted to instant oxidation (as 0.1 g tissue samples); the gases released were identified with a conductimeter. Analyses were performed by the analytical unit of the University of Santiago de Compostela (RIAIDT).

Statistical analysis

Statistical analyses of the field plots followed earlier studies of this system (Lombardero et al. 2021, 2022). Trees were regarded as the experimental units (Neuvonen and Haukioja 1985). The five study plots, which each contained replicate study trees with and without *C. parasitica*, were treated as a fixed effect because we were specifically interested in these plots with known histories (i.e., our study plots were not a random sample from a large population). Population abundance, measured as galls per shoot, was analyzed with a general linear model (Gaussian distribution) that included plot, presence of blight, and their interaction as fixed effects, tree within plot as a random effect, the number of buds per shoot (potential sites for galls) as a continuous variable (covariate), and no intercept (Lombardero et al. 2021). We did not include an intercept in our models because, based on biological knowledge, we know that when there are zero buds, there are also zero galls.

For the remaining variables, where there were multiple measurements per tree, we calculated an average for each tree and used the tree averages for statistical analyses (Neuvonen and Haukioja 1985); this avoided the hazards of pseudoreplication (Hurlbert 1984) and the complication of unequal sample sizes in nested models. Larval size, adult fecundity, size of galls, toughness, wall thickness and number of cells per gall were analyzed with an ANOVA that included plot, the presence of blight, and their interaction as fixed effects. Prior to analyses, to improve normality and homoscedasticity, larval cells per gall were log-transformed.

Water and nitrogen content, terpenes, phenols and condensed tannins, were analyzed with an ANOVA that included plot, the presence of blight, the type of leaf tissue analyzed (ungalled leaf, ungalled portion of galled leaf, or gall), and their interactions as fixed effects and tree nested with plot and the presence of *C. parasitica* as random effect. Prior to analyses, to improve normality and homoscedasticity, terpenes, phenols, and condensed tannins were square root-transformed. Each replicate sample represented a different tree.

Statistical analyses were performed with the package JMP (SAS Institute Inc.).

Results

Field plots

Attack level in field plots, measured as number of galls per shoot, was about 50% higher in trees with chestnut blight (Fig. 2). There were also differences among plots in galls per shoot, and no blight × locality interaction (Table 1).

The presence of chestnut blight had no effects on the size, wall thickness, or toughness of *D. kuriphilus* galls (grand means ± SD = 25 ± 6 mm^3, 3.1 ± 0.9 cell layers, and 1272 ± 498 g × 10 g, respectively; N = 103 galls). Larval mass was 22%

lower in plants affected by chestnut blight ($F_{1,38}$ = 58.11, p < 0.0001; Fig. 3A), and there were not differences among plots (Fig. 3A). Female fecundity was strongly related to the presence of chestnut blight, the number of eggs per female was almost double in trees affected by the fungi ($F_{1,33}$ = 11.02; p = 0.002; Fig. 3B). Effects of blight varied somewhat among plots (main effect of plot: $F_{3,33}$ = 2.26, p = 0.084 plot × blight: $F_{3,33}$ = 2.32, p < 0.077; Fig. 3B).

The presence of chestnut blight was unrelated to the nitrogen content of any of the tissues analyzed (Table 2, Fig. 4A), but there were differences among tissues and plots (Table 2, Fig. 4A). Water content was higher in galls than in leaves (Table 2, Fig. 4B), but there were no differences between trees attacked only by the insects versus trees also infected with chestnut blight (Table 2, Fig. 4B).

Secondary metabolites in field plots showed different results depending on the class of compounds. Total terpenes varied depending on the tissue (Table 3, Fig. 5A) with higher concentration in the leaf tissue surrounding the gall. There were also differences in terpene content among plots, but there were no differences between trees with- *vs.* without chestnut blight (Table 3, Fig. 5A). Differences among trees were also significant and explained about 24% of the variation. Total phenols showed similar results with more dramatic differences among the tissue analyzed (Table 3, Fig. 5B): higher phenols in ungalled leaves followed by the leaf tissue surrounding the galls, and dramatically less in the galls themselves, especially on trees affected by chestnut blight (Table 3, Fig. 5B). There were also differences among plots but no effects of the presence of chestnut blight. Concentration of condensed tannins was significantly affected by the presence of blight, tissue type, plot, and the interaction between presence of the fungus and tissue type (Table 3) with highest concentrations in the gall tissue of fungal-infected trees (Fig. 5C).

Greenhouse study

In the greenhouse study, there was a significant effect of treatment on total galls per tree ($F_{2,126}$ = 3.47; p < 0.03; Fig. 6), but this was because control trees were protected from *D. kuriphilus* attack in the first year. If we only consider plants exposed to the insect, there were no differences in galls / tree in treatments with and without chestnut blight (DK+CP and DK, Fig. 6).

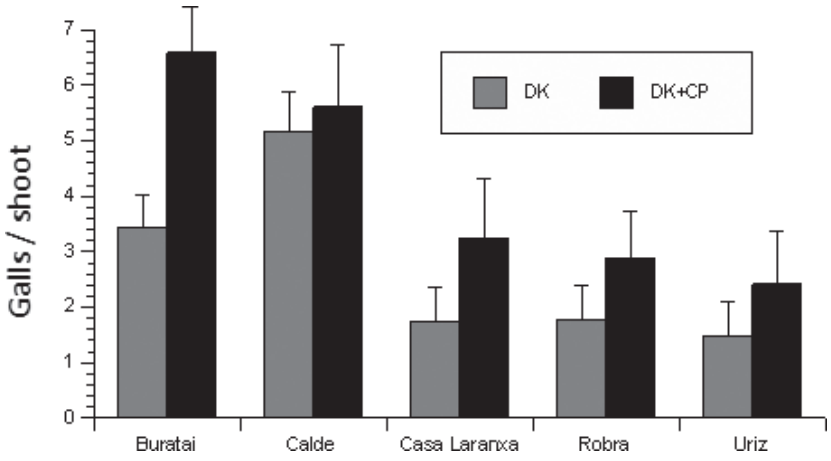

Figure 2. Attack level in trees with (DK + CP) and without infection by *Cryphonectria parasitica* (DK). From five field plots of chestnut trees. Figure shows galls per shoot (least square means ± SE).

Table 1. ANOVA results comparing attack levels (galls / shoot) in trees with and without chestnut blight, in each of five study plots. Corresponds to data in Fig. 2.

Source	df	F
Blight	1, 114	7.40**
Locality	4, 107	7.59***
Locality × blight	4, 107	0.86
Total buds	1, 163	238.04***
Percent random variance attributable to tree within locality × blight		51***

* p < 0.05; ** p < 0.01; *** p < 0.001.

Table 2. ANOVA results comparing percent of nitrogen and percent of water of study trees with and without chestnut blight. Table shows results for three tissue types (ungalled leaves, galled leaves, and galls) from a total of 46 study trees within 5 plots that were affected by *D. kuriphilus* alone and those that were affected also by chestnut blight. Corresponds to data in Fig. 4.

Source	F-statistics for nitrogen and water content		
	df	%Nitrogen	%Water
Blight	1, 36	2.60	0.27
TissueType	2, 88	18.71***	406.81***
Blight × Tissue	2, 88	0.84	1.24
Plot	4, 36	3.14*	2.31
Plot × Blight	4, 36	2.57	3.46*
Percent random variance attributable to tree within blight		43**	19

* p < 0.05; ** p < 0.01; *** p < 0.001.

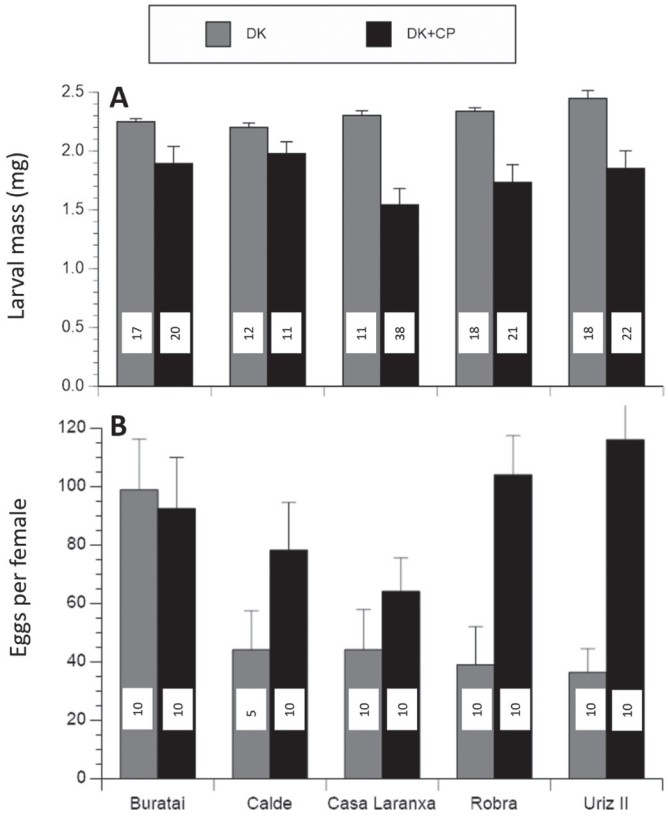

Figure 3. Mass and fecundity of *Dryocosmus kuriphilus*. From five field plots of chestnut trees, mass of late larvae and eggs / female (± SE) in trees with (DK + CP) and without (DK) infection by *Cryphonectria parasitica*. Buratai was the only plot unaffected by the late freeze of 2017. The bar labels show the number of individuals used for measurements.

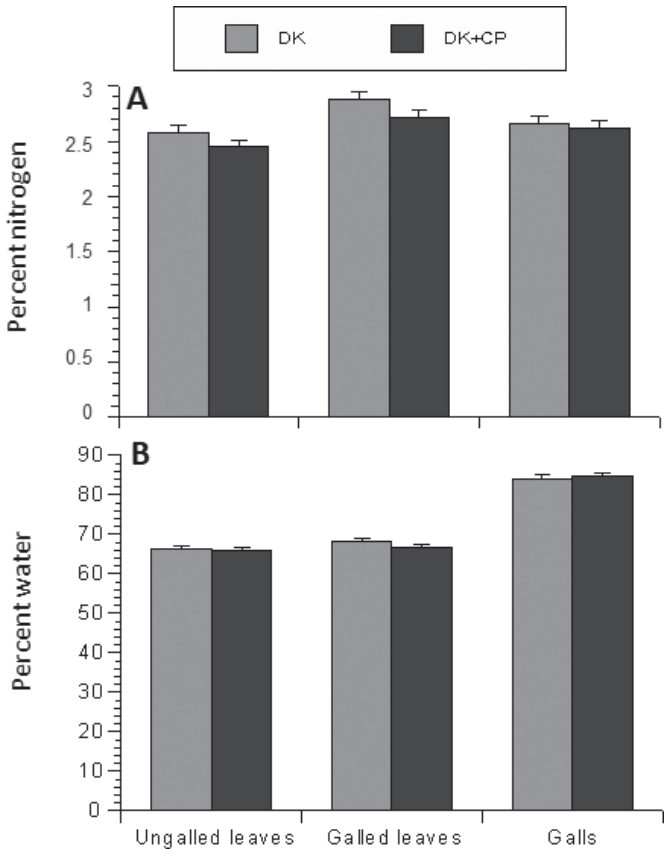

Figure 4. Nitrogen and water content of leaves and galls. From five field plots of chestnut trees, percent nitrogen (**A**) and water (**B**) (± SE) in ungalled leaves, galled leaves, and galls of trees with and without infection by *Cryphonectria parasitica*.

Table 3. ANOVA results comparing chemical attributes of study trees with and without chestnut blight. Table shows measurements of three tissue types (ungalled leaves, galled leaves, and galls) from a total of 35 study trees and 34 study trees that were affected by *D. kuriphilus* alone, or also by chestnut blight, respectively. Corresponds to data in Fig. 5.

Source	df	F-statistics for three measures of phytochemistry		
		Terpenes (sqrt)	Phenols (sqrt)	Tannins (sqrt)
Blight	1, 59	0.46	1.81	61.35***
TissueType	2, 134	4.19*	226.56***	12.20***
Blight × Tissue	2, 134	0.51	9.81***	9.23***
Plot	4, 59	5.59***	21.37***	8.41***
Plot × Blight	4, 59	1.37	1.2	4.64**
Percent random variance attributable to tree within blight		24*	6	34**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The differences between treatments disappeared when all plants were exposed to the insect in 2019 and 2020 regardless of whether the trees were previously attacked or not (Fig. 6).

There was no difference in the number of eggs from females emerging from greenhouse plants attacked only by the insect or by both species (mean ± SE = 131 ± 12 and 142 ± 15 for females from DK and DK + CP respectively).

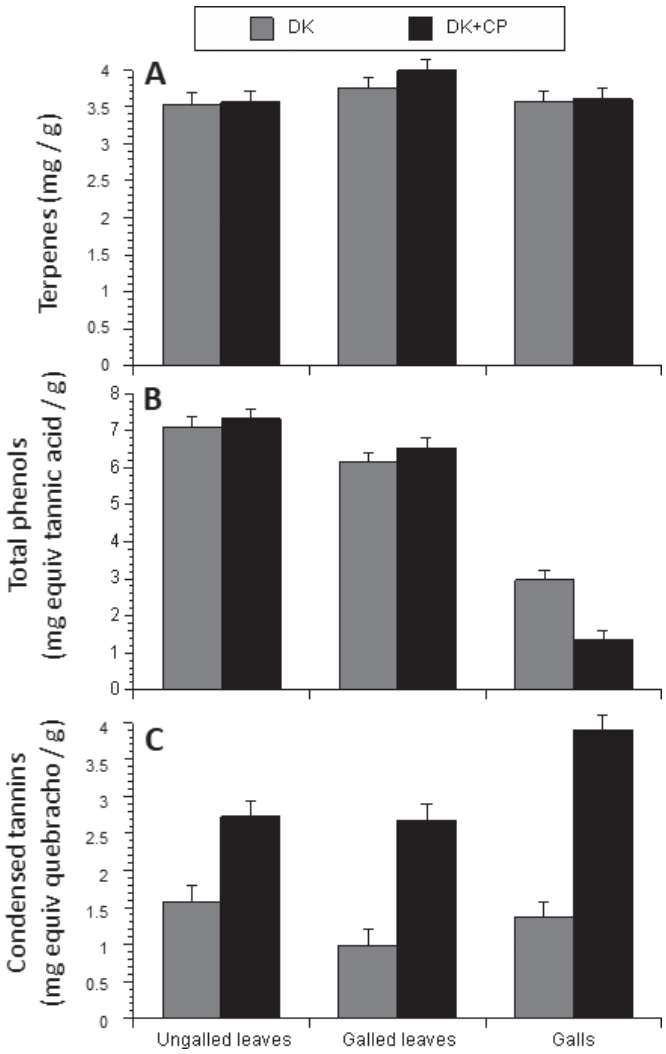

Figure 5. Concentrations of secondary metabolites in the foliage of trees with and without chestnut blight. From five field plots of chestnut trees, concentrations of terpenes (**A**) phenolics (**B**) and tannins (**C**) in ungalled leaves, galled leaves, and galls of trees with (DK + CP) and without (DK) infection by *Cryphonectria parasitica*. Figures show means ± SE of trees in each treatment group (square root transformed data).

There were no differences in nitrogen content due to the presence of chestnut blight or type of tissue analyzed (data not shown). Water content was also not affected by the presence of the fungus, but as in the field plots, water content was significantly higher in the galls compared with the other tissue analyzed ($F_{2,24}$ = 121.98, $p < 0.0001$).

The concentration of secondary metabolites in the greenhouse study differed from that measured in adult plants in the field. Terpenes were overall significantly higher in plants attacked by DK compared with control plants or plants with both invaders, although these differences disappear in the galls (Table 4, Fig. 7A). However, the presence of the fungus did not affect concentrations of phenols or tannins (Table 4, Fig. 7B, C). As in the trees of the field plots, phenol concentrations in seedlings were significantly lower in gall tissue compared with ungalled leaves or the ungalled portion of galled leaves (Table 4, Fig. 7B) and there was no effect of chestnut blight. Unlike in mature trees, there was no significant increase of tannins in seedlings infected with chestnut blight (Table 4, Fig. 7C).

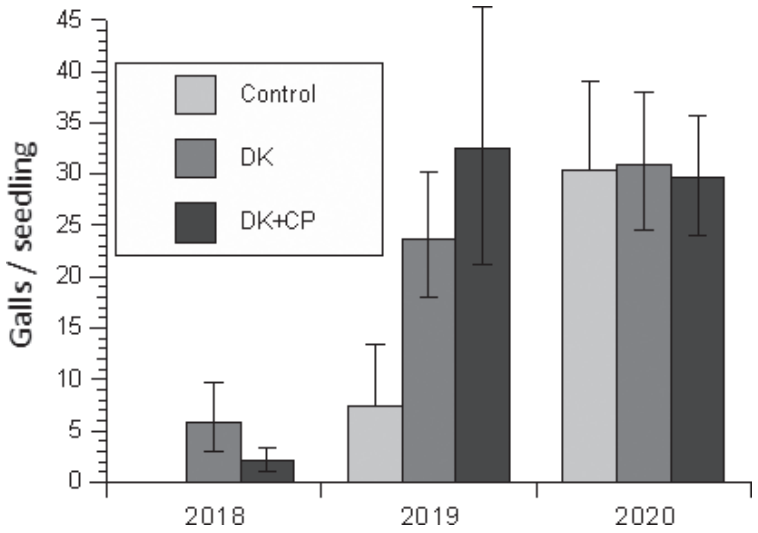

Figure 6. Attacks by *Dryocosmus kuriphilus*. From potted chestnut seedlings, attacks (± SE) in plants that were, and were not, infected by chestnut blight (DK+CP and DK, respectively), and in plants that were not infected by *Cryphonectria parasitica* and were not exposed to *Dryocosmus kuriphilus* in 2018 (Control).

Discussion

Since the entry of *D. kuriphilus* into Europe, numerous associations have been described between this species and native or introduced pathogenic fungi (Seddaiu et al. 2017; Morales-Rodríguez et al. 2019). *D. kuriphilus* has been associated with a higher incidence of *C.parasitica* (Meyer et al. 2015; Vannini et al. 2018) and implicated as a potential vector of numerous other fungi (Meyer et al. 2015; Yang et al. 2021). We add to this knowledge evidence that attack rates of *D. kuriphilus* were greater in chestnut trees infected with *C. parasitica* compared with uninfected trees (Fig. 2). In the field, in plots that had been infested by *D. kuriphilus*, attack rates were about 30 – 60% higher in trees afflicted with chestnut blight compared to nearby trees with no chestnut blight (Fig. 2).

There are several potential explanations for higher attack rates in trees with chestnut blight. It might be that (1) chestnut blight promotes higher attack rates by the gall wasp. However, the greenhouse study showed that the insect does not preferentially oviposit in trees affected by the fungus. (2) It is possible that chestnut trees suffering from higher attack densities by the gall wasp are made more susceptible to chestnut blight. Some previous studies show that *C. parasitica* may benefit from *D. kuriphilus*, since galls are a potential source of fungal inoculum (Meyer et al. 2015). Vannini et al. (2018) also described an increase of fungal infection in the crown of chestnuts related with *D. kuriphilus* attack. However, the symptoms of *C. parasitica* in our study trees were large cankers in the stems that almost certainly preceded the arrival of *D. kuriphilus*. (3) There were more attacks on trees with blight because the female adults emerging from these trees had twice the fecundity of those emerging from uninfected trees (Fig. 3B) and females frequently oviposit in the same tree from which they emerged (Castedo-Dorado et al. 2023b). This hypothesis predicts high philopatry in *D. kuriphilus*. (4) It is possible that there is a genetic association between susceptibility to blight and oviposition preferences of *D. kuriphilus*. Such an association was not evident in our greenhouse studies with saplings, but the expression of chestnut blight in adult trees in nature is more likely to reflect genetic susceptibility than occurrence of blight in

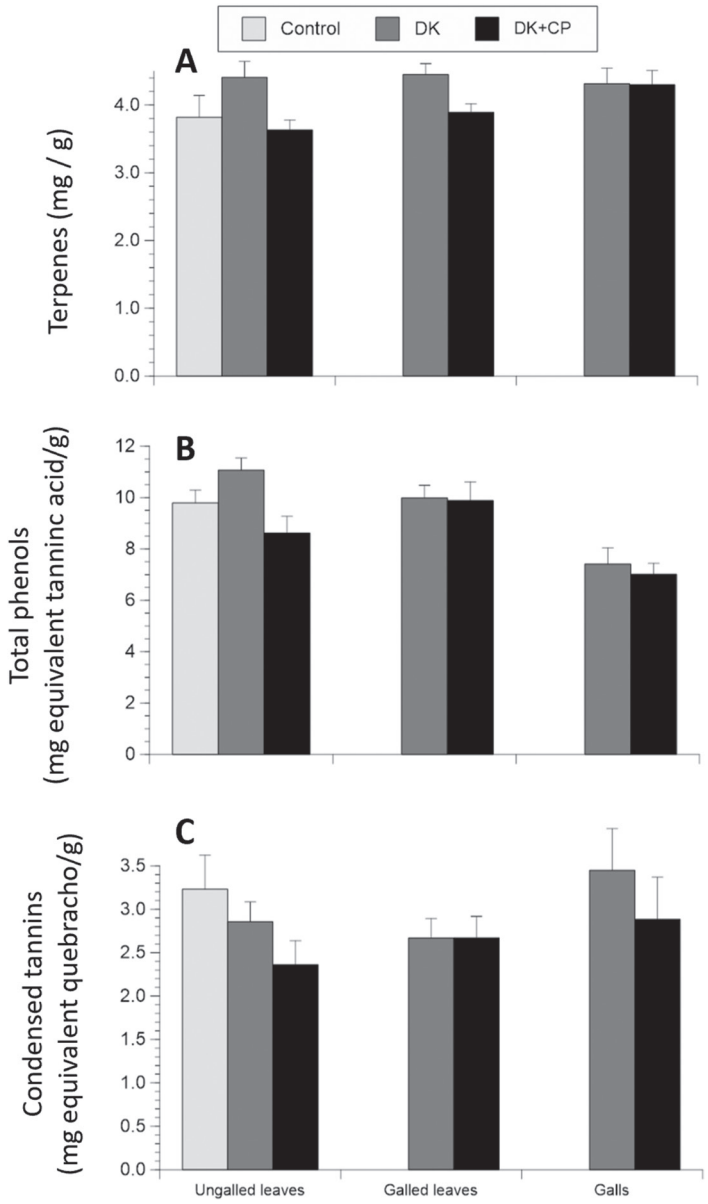

Figure 7. Concentration of secondary metabolites in potted chestnut seedlings. Concentrations of terpenes (**A**), phenolics (**B**), and tannins (**C**) in the ungalled leaves, galled leaves, and galls of plants that were and were not infected by chestnut blight (DK + CP and DK, respectively). Figures show means ± SE of trees in each treatment group (square root transformed data).

Table 4. ANOVA results comparing chemical attributes of study trees from the greenhouse seedlings. Table shows results of three tissue types (ungalled leaves, galled leaves, and galls) from a total of 43 study trees that were also affected by *D. kuriphilus* and those that were affected also by chestnut blight. Corresponds to data in Fig. 7.

Source	df	F-statistics for three measures of phytochemistry		
		Terpenes (sqrt)	Phenolics (sqrt)	Tannins (sqrt)
Blight	1, 17	9.42**	2.9	1.44
TissueType	2, 34	1.02	19.47***	1.68
Blight × Tissue	2, 34	1.9	3.34*	0.43
Percent random variance attributable to trees within blight		0	26	5

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

saplings. Hypotheses 3 and 4 are not mutually exclusive. Further studies will be necessary to discriminate among these hypotheses.

Larval mass was lower in trees affected by chestnut blight (Fig. 3A). However, these differences were apparently not due to nutritional quality because there were no differences in nitrogen or water content of gall tissue, where the larvae develop (Fig. 4). The effects of chestnut blight on *D. kuriphilus* fecundity were also dramatic but in the opposite direction (> 2-fold higher in some the plots; Fig. 3B). This surprising result suggests that larvae were growing at similar rates in both tree types, but that larvae grew for a longer time, and therefore became bigger adults, in trees with chestnut blight.

Plant chemistry offers potential explanations for the higher fecundity of *D. kuriphilus* in trees with chestnut blight. Higher fecundity in trees with chestnut blight was not due to nutritional quality because there were no differences N concentration in galls (Fig. 4A). Higher fecundity in trees with chestnut blight could be due to reduced chemical defenses in the plant tissue (Abrahamson et al. 2003; Naidoo et al. 2018), but the evidence argues against this hypothesis. Neither terpenes nor phenols were in general lower in trees afflicted with chestnut blight compared to those that did not have chestnut blight (Fig. 5). Phenolics were lower in the galls of trees with chestnut blight (Fig. 5B), but this could be a result of concomitant increases of tannins (Fig. 5C).

The most remarkable phytochemical difference between trees with and without chestnut blight was the high concentration of condensed tannins in galls (Fig. 5C). Virtually, all trees affected by *C. parasitica* (97%) showed measurable amounts of tannins within galls, while less than half (37%) of trees affected only by the insect showed measurable tannins within galls. Tannins are part of the antifungal and antimicrobial defense systems in many plant species (Uchida 1977; Griffin and Elkins 1986). Increased tannin concentration in the host can often be seen directly surrounding mycelial mats (Lovat and Donnelly 2019). Some studies have suggested that accumulation of tannins is related to resistance to chestnut blight (Nienstaedt 1953; McCarroll and Thor 1985; Gao and Shain 1995; but see Anagnostakis 1992). However, other studies have suggested that host tannins are used as a carbon source by the fungus through the activity of tannase produced by *C. parasitica* (Cook and Wilson 1915; Elkins et al. 1979). The role of tannins and tannase in chestnut blight pathology remains to be defined (Lovat and Donnelly 2019).

A higher concentration of tannins might influence wasp abundance and attack rates in the field. Castedo-Dorado et al. (2023b) suggested that, in the early stage of invasion, a substantial proportion of adults re-infect the same tree where they developed. Cornell (1983) suggested that high tannin concentrations in galls serve as a protective barrier for larvae developing inside the galls against fungi and other herbivores that are unable to induce galls, but feed on them. Tannins may protect galls from fungal infestation (Taper and Case 1987). Fungal damage is common in galls of *D. kuriphilus* especially caused by *Gnomoniopsis castaneae* (Magro et al. 2010; Maresi et al. 2013; Lione et al. 2015; Muñoz-Adalia et al. 2019). The pathogen has been associated with increased mortality of emerging adults (Magro et al. 2010; Vannini et al. 2014, 2017). The protection generated by tannins could help reduce the impact of this pathogen on *D. kuriphilus* populations developing in trees attacked by chestnut blight. The protection generated by tannins could be an adaptive explanation for why larvae in trees with chestnut blight grew for a longer time and thereby attained higher adult fecundity.

The association between chestnut blight and elevated tannins was not evident in the greenhouse study with seedlings (Fig. 7C) and there was no effect of chestnut blight presence in female fecundity. This may be attributed to the recent occurrence of fungal attack since these seedlings were infected by the disease in the same spring, from contamination during grafting. It could also be related to differences in physiology between seedlings and trees. In any case, trees afflicted with chestnut blight over some years, as in the typical course of chestnut blight infection, had elevated tannin levels in the galls, and the insects that developed within those galls had notably higher fecundity.

Tannins have commonly been regarded as anti-herbivore defenses (Donaldson and Lindroth 2004; Barbehenn and Constabel 2011) which may affect insect fecundity. But if this were the case in our system, we would have expected lower fecundity, rather than higher, in trees that had chestnut blight and displayed higher tannin concentrations in gall tissue (Fig. 5C). Adapted herbivorous insects may benefit from the presence of tannins in their food plants (Karowe 1989) and a variety of tree-feeding Lepidoptera are stimulated to feed by tannic acid (Bernays 1981). Rehill and Schultz (2012) found a positive relationship between condensed tannin concentration and the reproductive performance of fundatrices in another gall maker.

The higher fecundity of *D. kuriphilus* in trees with chestnut blight could also be related to the environment provided by the galls themselves. Arriola et al. (2018), working with another gall maker, showed that galls provided protection but did not enrich nutrition. Galls may protect the insect within from unfavorable abiotic conditions, particularly desiccation (Microenviroment Hypothesis; Price et al. 1987; Miller et al. 2009). However, the presence of chestnut blight had no effects on the size, wall thickness, or toughness of *D. kuriphilus* galls. Nor were there effects of chestnut blight on the water content of tissue within galls (Fig. 4). Still, Lombardero et al. (2021), working in the same plots with trees attacked by *D. kuriphilus* but not by chestnut blight, found greatly reduced fecundity in 2018 in populations exposed to a late freeze in the spring of 2017. All plots in the current study (except Buratai) were affected by the same freezing event, and in the next year (2018), all plots except Buratai, showed much higher fecundity in galls that developed in trees with chestnut blight compared to trees without chestnut blight (Fig. 3B). This could be understood if higher concentration of tannins in gall tissue helped to protect part of the insect population from low temperatures. Tannins can have quite general effects in protecting plant tissue from abiotic stress (Dehghanian et al. 2022) and may contribute to a physical barrier that isolates the gall insect from external environmental conditions. Uhler (1951) suggested that galls may protect against sudden changes in temperature. One prediction is that the thermal insulation within tannin-rich galls is greater when the galls are particularly rich in tannins, as in chestnut trees afflicted with chestnut blight.

Conclusion

The introduction of invasive species can give rise to novel community interactions, and sometimes new positive associations among plant enemies. Our results indicate that the presence of chestnut blight increases the suitability of chestnut trees for the invasive galling insect, *D. kuriphilu*s. Potential explanations include ameliorating the negative effects of environmental conditions. In any case, the positive association between chestnut blight and chestnut gall wasps suggests that management efforts to limit the incidence of chestnut blight may have additional benefits in reducing damage from the chestnut gall wasp.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: MJL. Data curation: MJL, MFRR. Formal analysis: MFRR, MPA, MJL. Funding acquisition: FCD, MPA, MJL. Methodology: FCD, MPA, ANP, MFRR, MJL. Visualization: MJL, FCD. Writing - original draft: MFRR. Writing - review and editing: MPA, MJL, ANP, FCD.

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Data availability

All of the data that support the findings of this study are available in the main text.

References

Abrahamson WG, Hunter MD, Melika G, Price PW (2003) Cynipid gall-wasp communities correlate with oak chemistry. Journal of Chemical Ecology 29(1): 208–223. https://doi.org/10.1023/A:1021993017237

Allison SD, Schultz JC (2005) Biochemical responses of chestnut oak to a galling cynipid. Journal of Chemical Ecology 31(1): 151–166. https://doi.org/10.1007/s10886-005-0981-5

Anagnostakis SL (1992) Chestnut bark tannin assays and growth of chestnut blight fungus on extracted tannin. Journal of Chemical Ecology 18(8): 1365–1373. https://doi.org/10.1007/BF00994362

Arriola IA, Melo JCF, Ferreira BG, Isaias RMS (2018) Galls on *Smilax campestris* Griseb. (Smilacaceae) protect the insects against *restinga* constraints, but do not provide enriched nutrition. Revista Brasileira de Botânica. Brazilian Journal of Botany 41(1): 145–153. https://doi.org/10.1007/s40415-017-0423-y

Aukema JE, Leung B, Kovacs K, Chivers C, Britton KO, Englin J, Frankel SJ, Haight RG, Holes TP, Liebhold AM, McCullough DG, Von Holle B (2011) Economic impacts of nonnative forest insects in the United States. PLoS ONE 6(9): e24587. https://doi.org/10.1371/journal.pone.0024587

Barbehenn RV, Constabel CP (2011) Tannins in plant-herbivore interactions. Phytochemistry 72(13): 1551–1565. https://doi.org/10.1016/j.phytochem.2011.01.040

Battisti A, Benvegnù I, Colombari F, Haack RA (2014) Invasion by the chestnut gall wasp in Italy causes significant yield loss in Castanea sativa nut production. Agricultural and Forest Entomology 16(1): 75–79. https://doi.org/10.1111/afe.12036

Bernays EA (1981) Plant tannins and insect herbivores, an appraisal. Ecological Entomology 6(4): 353–360. https://doi.org/10.1111/j.1365-2311.1981.tb00625.x

Biraghi A (1946) Il cancro del castagno causato da *Endothia parasitica*. L'ItaliaAgricola 7: 406.

Bonello P, Campbell FT, Cipollini D, Conrad AO, Farinas C, Gandhi KJK, Hain FP, Parry D, Showalter DN, Villari C, Wallin KF (2020) Invasive Tree Pests Devastate Ecosystems—A Proposed New Response Framework. Frontiers in Forests and Global Change 3: 2. https://doi.org/10.3389/ffgc.2020.00002

Borges RM (2018) The Galling Truth: Limited Knowledge of Gall-Associated Volatiles in Multitrophic Interactions. Frontiers in Plant Science 9: 1139. https://doi.org/10.3389/fpls.2018.01139

Boyd I, Freer-Smith P, Gilligan C, Godfray C (2013) The consequences of tree pests and diseases for ecosystem services. Science 342(6160): 823–840. https://doi.org/10.1126/science.1235773

Brasier CM (2001) Rapid evolution of introduced plant pathogens via interspecific hybridization. Bioscience 51(2): 123–133. https://doi.org/10.1641/0006-3568(2001)051[0123:REOIPP]2.0.CO;2

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung T, Man Int't Veld WA (2004) *Phytophthora alni* sp. nov. and its variants: Designation of emerging heteroploidy hybrid pathogens spreading on *Alnus* trees. Mycological Research 108(10): 1172–1184. https://doi.org/10.1017/S0953756204001005

Brignolas F, Lacroix B, Lieutier F, Sauvard D, Drouet A, Claudot AC, Yart A, Berryman AA, Christiansen E (1995) Induced responses in phenolic metabolism in two Norway spruce clones after wounding and inoculations with *Ophiostoma polonicum*, a bark beetle-associated fungus. Plant Physiology 109(3): 821–827. https://doi.org/10.1104/pp.109.3.821

Brussino G, Bosio G, Baudino M, Giordano R, Ramello F, Melika G (2002) Pericoloso insetto esotico per il castagno europeo. L'Informatore Agrario 58(37): 59–62.

Camisón A, Martín MA, Sánchez-Belc P, Florsc V, Alcaidea F, Morcuended D, Pintoe G, Solla A (2019) Hormone and secondary metabolite profiling in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomic.* Journal of Plant Physiology 241: 153030. https://doi.org/10.1016/j.jplph.2019.153030

Castedo-Dorado F, Álvarez-Álvarez P, Lombardero MJ (2023a) The impact of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) on chestnut tree growth may be mediated by site resources. Frontiers in Forests and Global Change 5: 1095185. https://doi.org/10.3389/ffgc.2022.1095185

Castedo-Dorado F, Alvarez-Alvarez P, Cuenca Valera B, Lombardero MJ (2023b) Local-scale dispersal patterns and susceptibility to *Dryocosmus kuriphilus* in different *Castanea* species and hybrid clones: Insights from a field trial. New Forests 54(1): 9–28. https://doi.org/10.1007/s11056-021-09893-8

Conedera M, Krebs P, Tinner W, Pradella M, Torriani D (2004) The cultivation of *Castanea sativa* (Mill.) in Europe, from its origin to its diffusion on a continental scale. Vegetation History and Archaeobotany 13(3): 161–179. https://doi.org/10.1007/s00334-004-0038-7

Cook MT, Wilson GW (1915) The influence of the tannin content of the Host plant on *Endothia parasitica* and related species. Botanical Gazette (Chicago, Ill.) 60(5): 346–361. https://doi.org/10.1086/331673

Cooper WR, Rieske LK (2008) Chestnut species and jasmonic acid treatment influence development and community interactions of galls produced by the Asian chestnut gall wasp, *Dryocosmus kuriphilus.* Journal of Insect Science 11(140): 140. https://doi.org/10.1673/031.011.14001

Cornell HV (1983) The secondary chemistry and complex morphology of galls formed by the Cynipidae (Hymenoptera): Why and how? American Midland Naturalist 110(2): 225–234. https://doi.org/10.2307/2425263

Dane F, Lang P, Huang H, Fu Y (2003) Intercontinental genetic divergence of *Castanea* species in eastern Asia and eastern North America. Heredity 91(3): 314–321. https://doi.org/10.1038/sj.hdy.6800300

Dehghanian Z, Habibi K, Dehghanian M, Aliyar S, Lajayer BA, Astatkie T, Minkina T, Keswani C (2022) Reinforcing the bulwark: Unravelling the efficient applications of plant phenolics and tannins against environmental stresses. Heliyon 8(3): e09094. https://doi.org/10.1016/j.heliyon.2022.e09094

Dinis LT, Peixoto F, Zhang C, Martins L, Costa R, Gomes-Laranjo J (2011) Physiological and biochemical changes in resistant and sensitive chestnut (*Castanea*) plantlets after inoculation with *Phytophthora cinnamomic.* Physiological and Molecular Plant Pathology 75: 146–156. https://doi.org/10.1016/j.pmpp.2011.04.003

DOGC (Diari Oficial de la Generalitat de Catalunya) (2012) ORDRE AAM/355/2012, de 2 de novembre, per la qual s'estableixen les zones delimitades per la plaga *Dryocosmus kuriphilus*. DOGC 6253: 56194- 56196.

Donaldson JR, Lindroth RL (2004) Cottonwood leaf beetle (Coleoptera: Chrysomelidae) performance in relation to variable phytochemistry in juvenile aspen (*Populus tremuloides* Michx.). Environmental Entomology 33(5): 1505–1511. https://doi.org/10.1603/0046-225X-33.5.1505

Dutech C, Barres B, Bridier J, Robin C, Milgroom MG, Ravigne V (2012) The chestnut blight fungus world tour: Successive introduction events from diverse origins in an invasive plant fungal pathogen. Molecular Ecology 21(16): 3931–3946. https://doi.org/10.1111/j.1365-294X.2012.05575.x

Elkins JR, Pate W, Hicks S (1979) Evidence for a role of hamamelitannin in the pathogenicity of *Endothia-parasitica.* Phytopathology 69: 1027–1027.

Elorrieta J (1949) El castaño en España. Ministerio de Agricultura, Madrid, 1–319.

Enebak SA, MacDonald WL, Hillman BI (1994) Effect of dsRNA associated with isolates of *Cryphonectria parasitica* from the central Appalachians and their relatedness to other dsRNAs from North America and Europe. Phytopathology 84(5): 528–534. https://doi.org/10.1094/Phyto-84-528

EPPO (2005) *Dryocosmus kuriphilus*. Datasheets on quarantine pests. Bulletin OEPP. EPPO Bulletin. European and Mediterranean Plant Protection Organisation 35(3): 422–424. https://doi.org/10.1111/j.1365-2338.2005.00849.x

Evensen PC, Solheim H, Hoiland K, Stenersen J (2000) Induced resistance of Norway spruce, variation of phenolic compounds and their effects on fungal pathogens. Forest Pathology 30(2): 97–108. https://doi.org/10.1046/j.1439-0329.2000.00189.x

Feeny PP, Bostock H (1968) Seasonal changes in tannin content of oak leaves. Phytochemistry 7(5): 871–880. https://doi.org/10.1016/S0031-9422(00)84845-1

Fernandes P, Colavolpe MB, Serrazina S, Costa RL (2022) European and American chestnuts: An overview of the main threats and control efforts. Frontiers in Plant Science 13: 951844. https://doi.org/10.3389/fpls.2022.951844

Freer-Smith P, Webber J (2017) Tree pests and diseases: The threat to biodiversity and the delivery of ecosystem services. Biodiversity and Conservation 26(13): 3167–3181. https://doi.org/10.1007/s10531-015-1019-0

Freitas TR, Santos JA, Silva AP, Fraga H (2021) Influence of climate change on chestnut trees: A Review. Plants 10(7): 1463. https://doi.org/10.3390/plants10071463

Gao S, Shain L (1995) Activity of polygalacturonase produced by *Cryphonectria parasitica* in chestnut bark and its inhibition by extracts from American and Chinese chestnut. Physiological and Molecular Plant Pathology 46(3): 199–213. https://doi.org/10.1006/pmpp.1995.1016

Graves AH (1950) Relative blight resistance in species and hybrids of Castanea. Phytopathology 40: 1125–1131.

Griffin GJ, Elkins JR (1986) Chestnut blight. In: Roane MK, Griffin GJ, Elkins JR (Eds) Chestnut Blight, other *Endothia* diseases, and the genus *Endothia*. APS Press, St. Paul, MN, 1–26.

Griffin GJ, Hebard FW, Wendt RW, Elkins JR (1983) Survival of American chestnut trees: Evaluation of blight resistance and virulence of *Endothia parasitica.* Phytopathology 73(7): 1084–1092. https://doi.org/10.1094/Phyto-73-1084

Harper LJ, Schonrogge K, Lim KY, Francis P, Lichtenstein CP (2004) Cynipid galls: Insect-induced modifications of plant development create novel plant organs. Plant, Cell & Environment 27(3): 327–335. https://doi.org/10.1046/j.1365-3040.2004.01145.x

Hartley SE (1998) The chemical composition of plant galls: Are levels of nutrients and secondary compounds controlled by the gall-former? Oecologia 113(4): 492–501. https://doi.org/10.1007/s004420050401

Hebard FV, Griffin GJ, Elkins JR (1984) Developmental histopathology of cankers incited by hypovirulent and virulent isolates of *Endothia parasitica* on susceptible and resistant chestnut trees. Phytopathology 74(2): 140–149. https://doi.org/10.1094/Phyto-74-140

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54(2): 187–211. https://doi.org/10.2307/1942661

Karowe DN (1989) Differential effect of tannic acids on two tree-feeding Lepidoptera, implications for theories of plant anti-herbivore chemistry. Oecologia 80(4): 507–512. https://doi.org/10.1007/BF00380074

Kato K, Hijii N (1997) Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym, Cynipidae) on the growth of chestnut trees. Journal of Applied Entomology 121(1–5): 9–15. https://doi.org/10.1111/j.1439-0418.1997.tb01363.x

Kolp M, Fulbright DW, Jarosz AM (2018) Inhibition of virulent and hypovirulent *Cryphonectria parasitica* growth in dual culture by fungi commonly isolated from chestnut blight cankers. Fungal Biology 122(10): 935–942. https://doi.org/10.1016/j.funbio.2018.05.007

Kolp M, Double ML, Fulbright DW, MacDonald WL, Jarosz A (2020) Spatial and temporal dynamics of the fungal community of chestnut blight cankers on American chestnut (*Castanea dentata*) in Michigan and Wisconsin. Fungal Ecology 45: 10092. https://doi.org/10.1016/j.funeco.2020.100925

Kot II, Jakubczyk A, Karas M, Zlotek U (2018) Biochemical responses induced in galls of three Cynipidae species in oak trees. Bulletin of Entomological Research 108(4): 494–500. https://doi.org/10.1017/S0007485317001055

Liebhold AM, Macdonald WL, Bergdahl D, Mastro VC (1995) Invasion by Exotic Forest Pests: A Threat to Forest Ecosystems. Forest Science 41 (Issue suppl. 1): a0001–z0001. https://doi.org/10.1093/forestscience/41.s1.a0001

Lindroth RL, Hwang SY (1996) Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). Biochemical Systematics and Ecology 24(5): 357–364. https://doi.org/10.1016/0305-1978(96)00043-9

Lione G, Giordano L, Sillo F, Gonthier P (2015) Testing and modelling the effects of climate on the incidence of the emergent nut rot agent of chestnut *Gnomoniopsis castaneae.* Plant Pathology 64(4): 852–863. https://doi.org/10.1111/ppa.12319

Lombardero MJ, Castedo-Dorado F, Ayres MP (2021) Extreme climatic events affect populations of Asian chestnut gall wasps, *Dryocosmus kuriphilus*, but do not stop the spread. Agricultural and Forest Entomology 23(4): 473–488. https://doi.org/10.1111/afe.12448

Lombardero MJ, Ayres MP, Álvarez-Álvarez P, Castedo-Dorado F (2022) Defensive patterns of chestnut genotypes (*Castanea* spp.) against the gall wasp, *Dryocosmus kuriphilus.* Frontiers in Forests and Global Change 5: 1046606. https://doi.org/10.3389/ffgc.2022.1046606

Lovat CA, Donnelly DJ (2019) Mechanisms and metabolomics of the host–pathogen interactions between Chestnut (*Castanea* species) and Chestnut blight (*Cryphonectria parasitica*). Forest Pathology 49(6): e12562. https://doi.org/10.1111/efp.12562

Magro P, Speranza S, Stacchiotti M, Martignoni D, Paparatti B (2010) *Gnomoniopsis* associated with necrosis of leaves and chestnut galls induced by *Dryocosmus kuriphilus.* Plant Pathology 59(6): 1171. https://doi.org/10.1111/j.1365-3059.2010.02336.x

Marcolin E, Pividori M, Colombari F, Manetti MC, Peller F, Conedera M, et al. (2021) Impact of the Asian gall wasp (*Dryocosmus kuriphilus*) on the radial growth of the European chestnut (Castanea sativa). Journal of Applied Ecology 58(6): 1212–1124. https://doi.org/10.1111/1365-2664.13861

Maresi G, Longa CMO, Turchetti T (2013) Brown rot on nuts of *Castanea sativa* Mill.: An emerging disease and its causal agent. IForest (Viterbo) 6(5): 294–301. https://doi.org/10.3832/ifor0952-006

MARM (2011). Cuarto Inventario Forestal Nacional. Dirección General del Medio Natural y Política Forestal, Comunidad Autónoma de Galicia, Madrid, 1–52.

McCarroll DR, Thor E (1985) Pectolytic, cellulytic and proteolytic activities expressed by cultures of *Endothia parasitica*, and inhibition of these activities by components extracted from Chinese and American chestnut inner bark. Physiological Plant Pathology 26(3): 367–378. https://doi.org/10.1016/0048-4059(85)90011-6

Mellano MG, Beccaro GL, Donno D, Marinoni DT, Boccacci P, Canterino S, Cerutti AK, Bounous G (2012) Castanea spp. biodiversity conservation: Collection and characterization of the genetic diversity of an endangered species. Genetic Resources and Crop Evolution 59(8): 1727–1741. https://doi.org/10.1007/s10722-012-9794-x

Meyer JB, Gallien L, Prospero S (2015) Interaction between two invasive organisms on the European chestnut: Does the chestnut blight fungus benefit from the presence of the gall wasp? FEMS Microbiology Ecology 91(11): fiv122. https://doi.org/10.1093/femsec/fiv122

Miller DGIII III, Ivey CT, Shedd JD (2009) Support for the microenvironment hypothesis for adaptive value of gall induction in the California gall wasp, *Andricus quercuscalifornicus*. Entomologia Experimentalis et Applicata 132(2): 126–133. https://doi.org/10.1111/j.1570-7458.2009.00880.x

Morales-Rodríguez C, Sferrazza I, Aleandri M, Dalla Valle M, Mazzatto T, Speranza S, Contarino M, Vannini A (2019) Fungal community associated with adults of the chesnut gall wasp *Dryocosmus kuriphilus* after emergence from galls: Taxonomy and functional ecology. Fungal Biology 123(12): 905–912. https://doi.org/10.1016/j.funbio.2019.09.009

Muñoz-Adalia EJ, Rodriguez D, Casado M, Diez J, Fernández M (2019) Fungal community of necrotic and healthy galls in chestnut trees colonized by *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae). IForest (Viterbo) 12(4): 411–417. https://doi.org/10.3832/ifor3014-012

Naidoo S, Christie N, Acosta JJ, Mphahlele MM, Payn KG, Myburg AA, Kulheim C (2018) Terpenes associated with resistance against the gall wasp, *Leptocybe invasa*, in *Eucalyptus grandis.* Plant, Cell & Environment 41(8): 1840–1851. https://doi.org/10.1111/pce.13323

Neuvonen S, Haukioja E (1985) How to study induced plant resistance? Oecologia 66(3): 456–457. https://doi.org/10.1007/BF00378314

Nienstaedt H (1953) Tannin as a factor in the resistance of chestnut, *Castanea* spp., to the chestnut blight fungus, *Endothia parasitica.* Phytopathology 43: 32–38.

Nyman T, Julkunen-Tiitto R (2000) Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. Proceedings of the National Academy of Sciences of the United States of America 97(24): 13184–13187. https://doi.org/10.1073/pnas.230294097

Oliveira DC, Isaias RMS, Fernandes GW, Ferreira BG, Carneiro RGS, Fuzaro L (2016) Manipulation of host plant cells and tissues by gall-inducing insects and adaptive strategies used by different feeding guilds. Journal of Insect Physiology 84: 103–113. https://doi.org/10.1016/j.jinsphys.2015.11.012

Paillet FL (2002) Chestnut: History and ecology of a transformed species. Journal of Biogeography 29(10–11): 1517–1530. https://doi.org/10.1046/j.1365-2699.2002.00767.x

Pérez M, Dominguez-López I, Lamuela-Raventós RM (2023) The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. Journal of Agricultural and Food Chemistry 71(46): 17543–17553. https://doi.org/10.1021/acs.jafc.3c04022

Pérez-Otero R, Mansilla JP (2014) El cinípido del castaño *Dryocosmus kuriphilus* Yasumatsu, 1951 llega a Galicia (NO de la Península Ibérica). Arquivos Entomolóxicos 12: 33–36.

Price PW, Fernandes GW, Waring GL (1987) Adaptive nature of insect gall. Environmental Entomology 16(1): 15–24. https://doi.org/10.1093/ee/16.1.15

Prospero S, Rigling D (2016) Using molecular markers to assess the establishment and spread of a mycovirus applied as a biological control agent against chestnut blight. BioControl 61(3): 313–323. https://doi.org/10.1007/s10526-015-9713-0

Pujade-Villar J, Torrel A, Rojo M (2013) Primeres troballes a la península Iberica de *Dryocosmus kuriphilus* (Hym., Cynipidae), una especie de cinipid d'origen asiatic altament perillosa per al castanyer (Fagaceae). Orsis 27: 295–301.

Rehill BJ, Schultz JC (2012) *Hormaphis hamamelidis* fundatrices benefit by manipulating phenolic metabolism of their host. Journal of Chemical Ecology 38(5): 496–498. https://doi.org/10.1007/s10886-012-0115-9

Roques A, Rabitsch W, Rasplus JY, Lopez-Vaamonde C, Nentwig W, Kenis M (2009) Alien terrestrial invertebrates of Europe. In: DAISIE (Eds) Handbook of Alien Species in Europe. Invading Nature - Springer Series in Invasion Ecology (INNA, volume 3). Springer, Dordrecht, 63–79. https://doi.org/10.1007/978-1-4020-8280-1_5

Sampedro L, Moreira X, Zas R (2011) Resistance and response of Pinus pinaster seedlings to *Hylobius abietis* after induction with methyl jasmonate. Plant Ecology 212(3): 397–401. https://doi.org/10.1007/s11258-010-9830-x

Santini A, Battisti A (2019) Complex Insect–Pathogen Interactions in Tree Pandemics. Frontiers in Physiology 10: 550. https://doi.org/10.3389/fphys.2019.00550

Santini A, Ghelardini L, De Pace C, Desprez-Loustau ML, Capretti P, Chandelier A, Cech T, Chira D, Diamandis S, Gaitniekis T, Hantula J, Holdenrieder O, Jankovsky L, Jung T, Jurc D, Kirisits T, Kunca A, Lygis V, Malecka M, Marcais B, Schmitz S, Schumacher J, Solheim H, Solla A, Szabo I, Tsopelas P, Vannini V, Vettraino AM, Webber J, Woodward S, Stenlid J (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. The New Phytologist 197(1): 238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Sartor C, Dini F, Torello Marinoni D, Mellano MG, Beccaro GL, Alma A, Quacchia A, Botta R (2015) Impact of the Asian wasp *Dryocosmus kuriphilus* (Yasumatsu) on cultivated chestnut: Yield loss and cultivar susceptibility. Scientia Horticulturae 197: 454–460. https://doi.org/10.1016/j.scienta.2015.10.004

Schultz JC (1988) Many Factors Influence the Evolution of Herbivore Diets, but Plant Chemistry is Central. Ecology 69(4): 896–897. https://doi.org/10.2307/1941239

Schultz BB (1992) Insect Herbivores as Potential Causes of Mortality and Adaptation in Gallforming Insects. Oecologia 90(2): 297–299. https://doi.org/10.1007/BF00317190

Seddaiu S, Cerboneschi A, Sechi C, Mello A (2017) *Gnomoniopsis castaneae* asociada con agallas de *Dryocosmus kuriphilus* en castañares en Cerdeña (Italia). IForest (Viterbo) 10: 440–445. https://doi.org/10.3832/ifor2064-009

Seidl R, Klonner G, Rammer W, Essl F, Moreno A, Neumann M, Dullinger S (2018) Invasive alien pests threaten the carbon stored in Europe's forests. Nature Communications 9(1): 1626. https://doi.org/10.1038/s41467-018-04096-w

Taper ML, Case TJ (1987) Interactions between oak tannins and parasite community structure - Unexpected benefits of tannins to cynipid gall-wasps. Oecologia 71(2): 254–261. https://doi.org/10.1007/BF00377292

Taper ML, Zimmerman EM, Case TJ (1986) Sources of mortality for a Cynipid Gall-Wasp (*Dryocosmus dubiosus* (Hymenoptera, Cynipidae)) - the importance of the tannin fungus interaction. Oecologia 68(3): 437–445. https://doi.org/10.1007/BF01036752

Uchida K (1977) Studies on the *Endothia* canker of Japanese chestnut trees caused by *Endothia parasitica* (Murrill) P.J. et H.W. Anderson. Bulletin of Ibaraki-ken Horticultural Experiment Station 4(Special Issue): 1–65.

Uhler LD (1951) Biology and ecology of the goldenrod gall fly, *Eurosta solidaginis* (Fitch). Cornell University Agricultural Experiment Station Memoir 300: 1–51.

Vannini A, Martignoni D, Bruni N, Tomassini A, Aleandri MP, Vettraino AM, Caccia R, Speranza S, Paparatti B (2014) New notes on the biology of the chestnut fungus *Gnomoniopsis* sp. and its possible use as a biocontrol agent of oriental chestnut gall wasp. Acta Horticulturae (235–238): 235–238. https://doi.org/10.17660/ActaHortic.2014.1019.35

Vannini A, Vettraino A, Martignoni D, Morales-Rodriguez C, Contarini M, Caccia M, Paparatti B, Speranza S (2017) Does *Gnomoniopsis castanea* contribute to the natural biological control of chestnut gall wasp? Fungal Biology 121(1): 44–52. https://doi.org/10.1016/j.funbio.2016.08.013

Vannini A, Morales Rodriguez C, Aleandri MP, Bruni N, Valle M, Mazzetto M, Martignoni D, Vettraino AM (2018) Emerging new crown symptoms on *Castanea sativa* (Mill.): Attempting to model interactions among pests and fungal pathogens. Fungal Biology 122(9): 911–917. https://doi.org/10.1016/j.funbio.2018.05.006

Viiri H, Annila E, Kitunen V, Niemela P (2001) Induced responses in stilbenes and terpenes in fertilized Norway spruce after inoculation with blue-stain fungus, *Ceratocystis polonica*. Trees (Berlin) 15(2): 112–122. https://doi.org/10.1007/s004680000082

Wainhouse D, Ashburner R, Ward E, Rose J (1998) The effect of variation in light and nitrogen on growth and defence in young Sitka Spruce. Functional Ecology 12(4): 561–572. https://doi.org/10.1046/j.1365-2435.1998.00232.x

Walther G-R, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D, Moora M, Nentwig W, Ott J, et al. (2009) Alien species in a warmer world: Risks and opportunities. Trends in Ecology & Evolution 24(12): 686–693. https://doi.org/10.1016/j.tree.2009.06.008

Wang W, Wang M, Feng J, Zhang S, Chen Y, Zhao Y, Tian R, Zhu C, Nieuwenhuizen NJ (2024) Terpene Synthase Gene Family in Chinese Chestnut (*Castanea mollissima* BL.) Harbors Two Sesquiterpene Synthase Genes Implicated in Defense against Gall Wasp *Dryocosmus kuriphilus*. Journal of Agricultural and Food Chemistry 72(3): 1571–1581. https://doi.org/10.1021/acs.jafc.3c07086

Waterman PG, Mole S (1994) Method in Ecology. Analysis of Phenolic Plant Metabolites. Wiley-Blackwell, London, UK, 1–248.

Yang XH, Li XM, Zhu DH, Zeng Y, Zhao LQ (2021) The Diversity and Dynamics of Fungi in *Dryocosmus kuriphilus* Community. Insects 12(5): 26. https://doi.org/10.3390/insects12050426

Zeneli G, Krokene P, Christiansen E, Krekling T, Gershenzo J (2006) Methyl jasmonate treatment of mature Norway spruce (*Picea abies*) trees increase the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. Tree Physiology 26(8): 977–988. https://doi.org/10.1093/treephys/26.8.977