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Changes in host basal area explain associational resistance of mixed forests to primary pests

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Abstract

Tree species diversity generally has positive effects on forest primary productivity and resistance to natural perturbations, but diversity-function relationships can vary with site conditions. Recently, studies in forest diversity experiments have shown that tree diversity and local climate, in particular drought intensity, interactively affect insect herbivory. On the other hand, many studies focused on the response of forests to drought in terms of tree growth but without analysing the concomitant effects on susceptibility to pests. It is of particular interest to understand the combined effects of drought and tree diversity on the growth of the host tree, since host resource concentration is a determining factor of a pest's host choice.

We used a tree diversity experiment where tree species diversity and drought conditions were both manipulated to evaluate their interactive effects on the susceptibility of maritime pine (*Pinus pinaster* Aït.) forests to two primary pests (i.e. infesting healthy trees): the pine stem borer (PSB) *Dioryctria sylvestrella*, and the pine processionary moth (PPM; a leaf chewer), *Thaumetopoea pityocampa*. Using structural equation models, we investigated the direct and indirect effects (i.e. mediated by host resources) of the presence of birch and drought on the total number of attacks of PPM and PSB, in the same plots and in the same year.

We showed that pine-birch plots were more resistant to both PPM and PSB attacks than pine monocultures. Furthermore, we found that this associational resistance pattern was due to direct effects of birch trees on attacks, possibly related to disrupting non-host volatiles (NHVs), but also to indirect, resource-mediated effects whereby the presence of birch trees reduced the amount of host pine resources available to the pests. Drought conditions modulated birch mediated effects on resistance of maritime pine forests only for PSB attacks. Overall, our work improves our understanding of tree diversity-herbivory relationships and helps explain how climate might modulate such relationships.

Keywords

Betula pendula, biodiversity*, Dioryctria sylvestrella*, host concentration, non-host volatiles, *Pinus pinaster*, *Thaumetopoea pityocampa*, tree growth

Introduction

A consensus is arising about the relevance of tree species diversity for forest ecosystem functioning (Pretzsch et al., 2017). Observational and experimental evidence are accumulating on positive effects of forest compositional diversity on primary productivity (e.g. Zhang et al., 2012; Liang et al., 2016) and resistance to natural perturbations (Jactel et al., 2017). In particular, it has been shown that insect herbivore damage is often less severe on a tree species grown in a mixed stand than in a monoculture, a phenomenon known as associational resistance (Jactel et al. 2021). However, another general finding is that relationships between tree diversity and ecosystem functioning show wide variations at the regional or continental scale, being largely dependent on site conditions (Ratcliffe et al., 2017). For example, Jactel et al. (2018) and Steckel et al. (2019) showed that overyielding in mixed species forests decreases with dryer conditions. This is important because the frequency and severity of droughts are expected to increase under the current climate change (IPCC, 2014; Lehner et al., 2017) with consequences for the long-term stability of mixed forest productivity (Jucker et al., 2014; Morin et al. 2018). Much less is known on how local climatic conditions modulate the effect of tree species diversity on forest resistance to insect herbivores. Only recently, studies in forest diversity experiments have shown that local climate, in particular drought intensity, affected diversity–herbivory relationships in tree stands (Castagneyrol et al., 2018; Jactel et al., 2019; Field et al., 2020; Poeydebat et al., 2020). Conversely, a number of recent studies have investigated the effect of tree species mixing on forest resistance to drought, showing positive, neutral and negative effects depending on forest biomes (Grossiord et al. 2014) and forest species (Forrester et al. 2016). However, these studies mostly focused on the response of forests to drought in terms of tree growth but without analysing the concomitant effects on susceptibility to pests.

One main mechanism to explain associational resistance to insect herbivory in mixed forests is that the presence of heterospecific neighbours around a tree of a focal species leads to a lower probability of host tree finding by insect herbivores (Jactel et al. 2021) due i) to lower host abundance or frequency (resource concentration hypothesis, Root 1973) or ii) to the disrupting effect resulting from the visual or olfactory cues emitted by non-host trees (host apparency hypothesis, Castagneyrol et al. 2013). The positive effects of species mixing on tree growth (Jactel et al. 2018) may affect both mechanisms, as larger host trees provide more food resources but may also release more attractive signals. However, there is a general trade-off between growth and differentiation (Herms and Mattson 1992), so that any effect of tree diversity on growth may alter the ability of trees to produce secondary metabolites (Moreira et al., 2014; Muiruri et al., 2019).

Drought, by causing stomatal closure, and reduced photosynthesis and carbon fixation, can affect host tree growth and secondary metabolism as well (Koricheva et al., 1998: Peñuelas & Staudt, 2010), and thus may interact with species diversity effects on resistance to herbivory (Castagneyrol et al., 2018a; b). Similarly, drought conditions can also affect the growth of heterospecific neighbours and the amount of non-host volatiles (NHVs) they release, thus modulating their role in preventing insect pests from finding their host trees. There is growing evidence that drought conditions and heterospecific neighbours jointly determine the visual and olfactory apparency of host trees, as well as their suitability and nutritional quality to herbivores (Castagneyrol et al., 2018; Muiruri et al., 2020).

In this study, we used a tree diversity experiment where tree species richness and drought conditions were both manipulated to evaluate their interactive effects on the susceptibility of maritime pine (*Pinus pinaster* Aït.) forests to two moths that are both primary pests (i.e. infesting healthy trees): the pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller) with larvae feeding on the needles and the pine stem borer (*Dioryctria sylvestrella* Ratz.) with larvae feeding on the trunk phloem. Although several studies have demonstrated that processionary moth infestations are significantly lower in mixed stands than in pure stands of maritime pine, particularly in the presence of the non-host species *Betula pendula* (Castagneyrol et al., 2013; Damien et al., 2016; Castagneyrol et al., 2020), none have yet investigated whether this associational resistance pattern would change with drought conditions. The pine stem borer preferentially attacks fast-growing trees with more bark cracks that release attractive chemicals (terpenes) in greater quantity (Jactel et al. 1996, 2019). In a previous study, we found that the number of stem borer attacks per plot decreased with the presence of birch, but only in non-water stressed plots, suggesting an interaction between drought and tree species diversity (Jactel et al. 2019).

However, we did not specifically evaluate the respective direct and indirect effects of the presence of birch on associational resistance, i.e. via a direct repellence effect of non-host volatiles (NHVs) or indirectly via a reduction of pine growth due to competition with fast-growing birch neighbours, particularly under water stress conditions. In this study, we particularly focused on the plot scale because it better allowed us to test the resource concentration hypothesis to explain associational resistance in mixed forests (here of pine and birch). Patrolling insects may already come into contact with specific host tree cues, i.e. visual or olfactory signals, outside the forest stand (Hambäck et al., 2010). Once in a suitable habitat, the ‘’resource concentration hypothesis” (Hambäck and Englund, 2005) predicts that insect specialists are less likely to emigrate from habitat patches where host plants are more abundant (Hambäck et al., 2000; Underwood et al., 2014), which is the case in pure, high density stands. In mixed forests, resource abundance would instead decrease in relative (due to the presence of non-host trees) and absolute (depending on host tree density and host tree size) terms, making them less susceptible to pest attack.

Here, using structural equation models, we aimed at disentangling the direct and indirect associational effects of the presence of birch and moderate drought on the number of processionary moth and stem borer attacks on maritime pines, in the same plots and in the same year. By direct effects we mean the effects of the presence of birch trees that disturb the insects' recognition of the favourable habitat (plot) by their olfactory or visual signals. By indirect effects we mean the effects of the proximity of birches on the growth of maritime pine and therefore the quantity of substrate favourable to insect feeding (at the plot level), as well as the amount of visual and olfactory host cues. As this amount of substrate also depends on the density of host trees per plot, we also took this explicitly into account in our models.

Materials & Methods

Model species

The maritime pine (*Pinus pinaster)* is a coniferous species that originates from the western Mediterranean basin (Abad Viñas et al., 2016). Naturally, it grows in warm temperate regions with an oceanic influence, mainly in humid and sub-humid areas, with a preference for poor siliceous soils with a coarse texture (sandy soils, dunes and other poor substrates). Maritime pine is a fast-growing species that cannot tolerate shade but exhibits good tolerance to drought (embolism resistance Ψ_{50} =-3.6 MPa, Corcuera et al., 2011).

The silver birch (*Betula pendula*) is a deciduous tree native to most of Europe (Beck et al., 2016) that tolerates a wide range of climatic and edaphic conditions, but shows lower tolerance to drought than *P. pinaster* (Ψ_{50} =-2.3 MPa, Cochard et al., 2005). It is a pioneer, fast-growing species, with low tolerance to shading.

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Notodontidae) and the pine stem borer (PSB) *Dioryctria sylvestrella* (Pyralidae) are two lepidopteran pests of pines. Both are oligophagous pests that can attack several species of pines (*e.g. Pinus pinaster*, *Pinus nigra*, *Pinus sylvestris*). They can attack and develop successfully on healthy trees and are therefore considered primary pests (Jactel et al., 1996; Régolini et al., 2014). PPM is the main defoliator of pine species around the Mediterranean Basin (Roques et al., 2015).

In both species, adults emerge in early summer. After mating, females choose a host pine on which to lay their eggs. PPM females deposit their eggs on the needles of the selected host. Larvae develop throughout autumn and winter and feed on needles causing low to extreme defoliation, leading to more or less severe growth loss the next two years (Jacquet et al., 2013; Sangüesa-Barreda et al., 2014). In winter when temperature is low, larvae weave dense silk nests (white and easily detectable from the ground, see Fig. 1a) to protect themselves from the frost. At the end of winter, caterpillars move down from the tree crown to the ground in procession and bury themselves in the soil where they pupate until next summer.

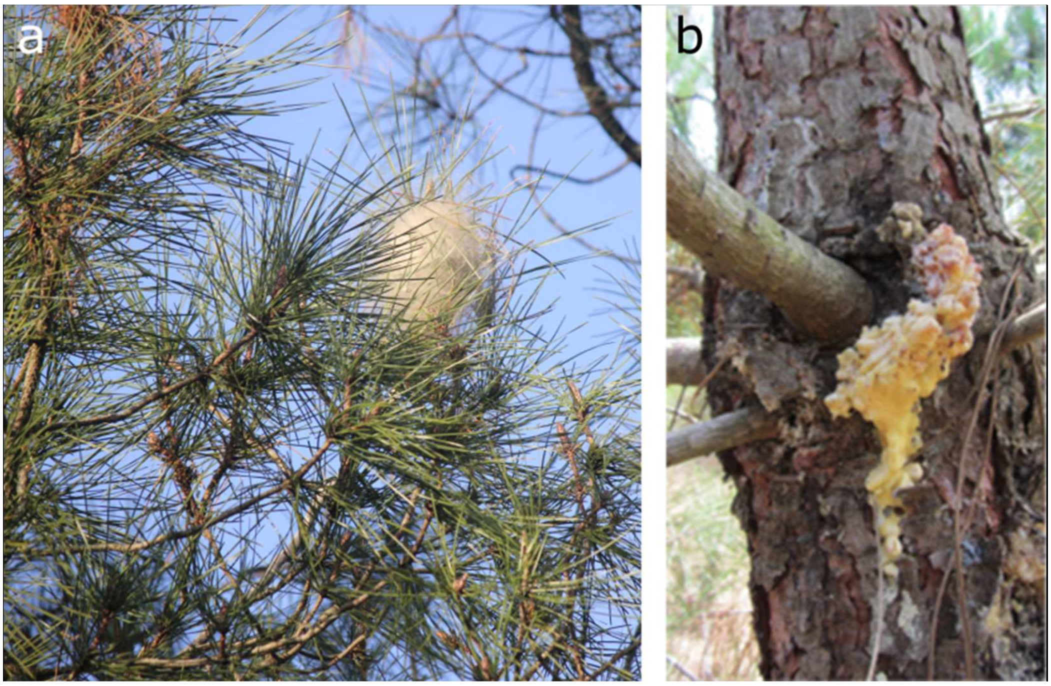

Figure 1. *Pine processionary moth (Thaumetopoea pityocampa) nest in the crown of a maritime pine during winter (a) and the entry point of a pine stem borer (Dioryctria sylvestrella) gallery with a typical resin-and-frass exudate (b).*

PSB females are attracted by volatile terpenic compounds of the resin and preferentially choose vigorous host pines with more bark cracks and a stronger resin smell (Jactel et al., 1996, Kleinheintz et al. 1999). Eggs are laid individually in bark cracks of the trunk during the summer. Young larvae tunnel through the bark into the phloem where they feed until autumn. They overwinter in their gallery and resume boring activity in early spring until the pupal stage in late spring. The horizontal, subcortical larval galleries break resin ducts, inducing resin flow mixed with frass, through the entry hole (see Fig. 1b). Yellow to pinkish coagulated resin on a tree trunk indicate a recent PSB attack. Larval galleries interrupt the flow of sap, resulting in deformation of the trunk and an increased risk of breakage in the event of windstorm (Baronio & Butturini, 1988).

Experimental design (ORPHEE)

The ORPHEE tree diversity experiment (https://sites.google.com/view/orpheeexperiment/home) is located in Southwest of France (44°44’24.9”N; 00°47’48.1”W) and consists of a 25600 trees plantation covering 12 hectares. It was planted in 2008 on a sandy soil. At ORPHEE location, mean annual temperature is 13.6 °C and mean annual cumulated rainfall is 893 mm (1979-2013 average climate from the CHELSA database; https://chelsa-climate.org/; Karger et al., 2017).

The experimental set up consists of 8 replicated blocks, of which half have been irrigated since 2015. Each of the four irrigated blocks is sprinkled at night with 42m^3 of water (block area of 1.28 ha), daily from May to October, which significantly reduces tree water stress as compared to non-irrigated blocks (Castagneyrol et al. 2017; Maxwell et al., 2020). In view of the ombrothermic data (Maxwell et al., 2020 Fig. S1) and the absence of severe drought-induced damage to pines and birches (in 2016, the mean ± sd predawn leaf water potential of maritime pines in non-irrigated blocks was -1.54 ± 0.43 MPa), drought conditions in non-irrigated blocks were considered moderate.

The ORPHEE experiment involves 5 tree species: maritime pine (*Pinus pinaster*), silver birch (*Betula pendula*), pedunculate oak (*Quercus robur*), Pyrenean oak (*Quercus pyrenaica*) and green oak (*Quercus ilex*). Each block of the experiment consists of 32 randomly distributed plots corresponding to all the possible combinations of one to five tree species: each species monoculture and each 2- to 5-species mixture, the 5-species mixture being duplicated. Each plot contains 100 trees planted in 10 rows of 10 trees spaced 2m apart. Mixed plots were planted following a substitutive design with a regular alternate pattern.

At the time of sampling (8 years after ORPHEE plantation), oaks were still very small (less than 1m tall) compared to pines and birches (about 5.5m tall) (Damien et al., 2016). They were considered to belong to the understorey vegetation of the plots, with very limited impact on tree (pine and birch) growth. Therefore, mixtures of pines and oaks were considered pine monocultures, and mixtures of pines, birches and oaks were considered pine-birch mixtures, both with different pine densities depending on the number of oak species in the plot (see Supporting Information Table S1). In each block, we selected all the plots with maritime pine, except the true pine monoculture and the 5-species mixtures, to have both pine monocultures and pine-birch mixtures at the three levels of pine density considered, i.e. 50, 33 and 25 pines per plot (Damien et al. 2016). In total, we surveyed 14 plots per block, representing 112 plots in total, half of which were irrigated (4 irrigated blocks and 4 non-irrigated blocks). We removed 8 plots where data from more than 30% of the trees were missing for either pest species, resulting in a total of 104 plots for the analyses of both pest species.

We measured the circumference at breast height (CBH) of each pine in the central part (i.e. 36 inner trees) of all plots, and computed the basal area (BA) in cm² of each pine as follows:

$$BA = \frac{CBH^2}{4\pi}$$

We computed a proxy of the total pine basal area of the plot as the sum of the basal area of all pines in the inner part of the plot, where CBH was measured. Because the plots of the ORPHEE experiment were planted in a regular pattern, the density of pines in the inner portion of the plot was the same as that of the entire plot. We did the same for *B. pendula* in plots were it was present (see Supporting Information Table S2 for details per plot type). Since all trees are of the same age in the ORPHEE experiment, we believe it was reasonable to consider total basal area per plot to be a good proxy of the concentration of pine resources in a plot.

We assessed the total number of PPM and PSB attacks (*i.e.* nests and resin-and-frass exudates, respectively) in each plot, considering all the pines present for PPM and pines of the inner part of plots for PSB (as for BA). We counted the total number of PPM nests in pine crowns in January 2017, when the nests corresponding to summer 2016 egg laying were detectable (*i.e.* white silk tents). In late summer 2016, we counted the total number of fresh (from the current year) PSB attacks on tree trunks.

Statistical analysis

We used structural equation models (SEM; Grace, 2006) to investigate the direct (association with birch) and indirect (i.e. mediated by the concentration of host resources, here basal area) effects of tree diversity, climate (drought) and their interaction on the number of PPM and PSB attacks in pine plots, while accounting for pine density (i.e. number of host trees).

We built one SEM per pest species at the plot level, each composed of two linear mixed-effect models (M1 and M2) in which we added the block and the irrigation treatment as nested random intercept effects, to account for the non-independence of the plots due to the experimental design (Zuur et al., 2009). Because the application of traditional SEM is restricted to normally distributed data, we used the piecewise SEM approach (Shipley, 2009; Lefcheck et al., 2016), which generalises the method to a larger range of distribution families and to mixed effect models. The SEMs were written as follow in R syntax:

```
M = psem
(M1 = glmer (Total number of attacks per plot ~ (Birch presence + Pine density) * Irrigation + Total
pine basal area + (1|Block:Irrigation), data, family="poisson"),
M2 = lmer (Total pine basal area ~ (Birch presence + Pine density) * Irrigation + (1|Block:Irrigation),
data))
```

We identified the significant relationships and computed an unstandardized coefficient for each path. Unstandardized coefficients are expressed in the same unit as the response variable and are therefore useful to interpret the magnitude of the effects. In our case, they also allowed comparison of the PPM and PSB models. We also computed the marginal R^2 (R^2m, *i.e.* the variance explained by fixed effects) and the conditional R^2 (R^2c, *i.e.* the variance explained by both fixed and random effects) as indicators of the goodness-of-fit of each sub-model (Nakagawa and Schielzeth, 2013).

All analyses were run using R (R Core Development Team, 2013) and the lmer4 (Bates et al., 2015) and piecewiseSEM (v2.1.0, Lefcheck et al., 2016) packages.

Results

The total number of attacks per plot varied from 0 to 12 for PPM and from 0 to 7 for PSB. On average, there were more PPM than PSB attacks per plot: 3.50 ± 2.73 nests *vs* 1.54 ± 1.59 resin-and-frass exudates per plot, respectively (see Supporting Information Table S2 for detail per plot type).

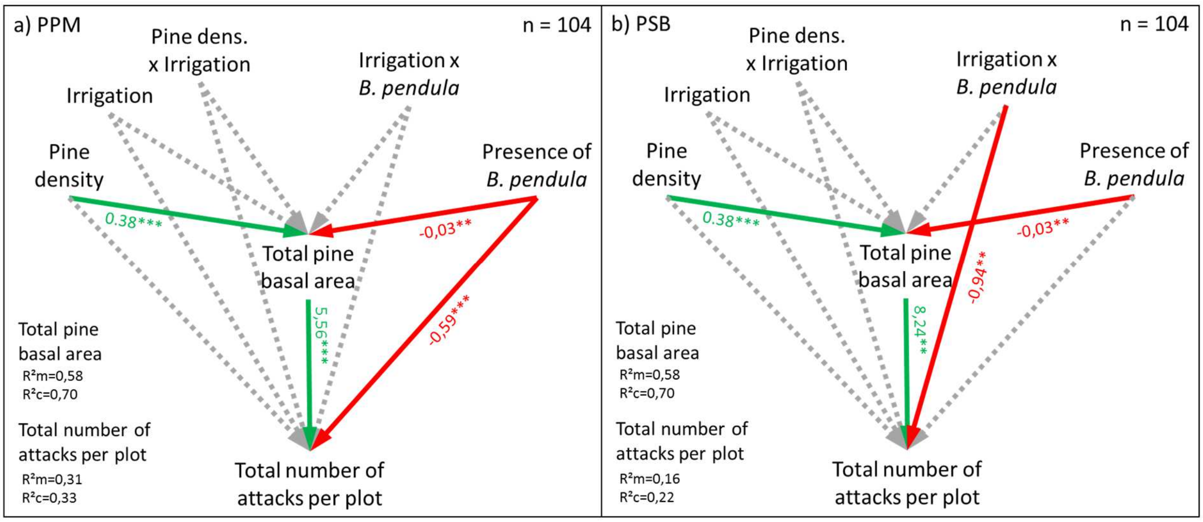

Figure 2*. Structural equation models (SEM) for a) the pine processionary moth (PPM) and b) the pine stem borer (PSB) at the plot level. Grey dashed arrows represent non-significant paths. Red and green arrows represent significant paths, respectively associated with a negative or a positive coefficient. The path coefficients correspond to unstandardized coefficients interpretable in the unit of the variables. Stars indicate the significance level as follow: "***" when p-value is ≤ 0.001, "**" when p-value is between 0.001 and 0.01, and "*" when p-value is between 0.01 and 0.05. Details are provided in Table S3.*

Logically, total pine basal area per plot increased with increasing pine density (Fig. 2). Total pine basal area per plot was not affected by the irrigation treatment (Supporting Information Fig. S1a and Table S3), but it significantly decreased with the presence of birch trees in the plot after pine density was accounted for (i.e. not due to dilution; Fig. 2; Supporting Information Fig. S1b and Table S3). The effect of pine density on the total number of both PPM and PSB attacks was entirely mediated by the total pine basal area.

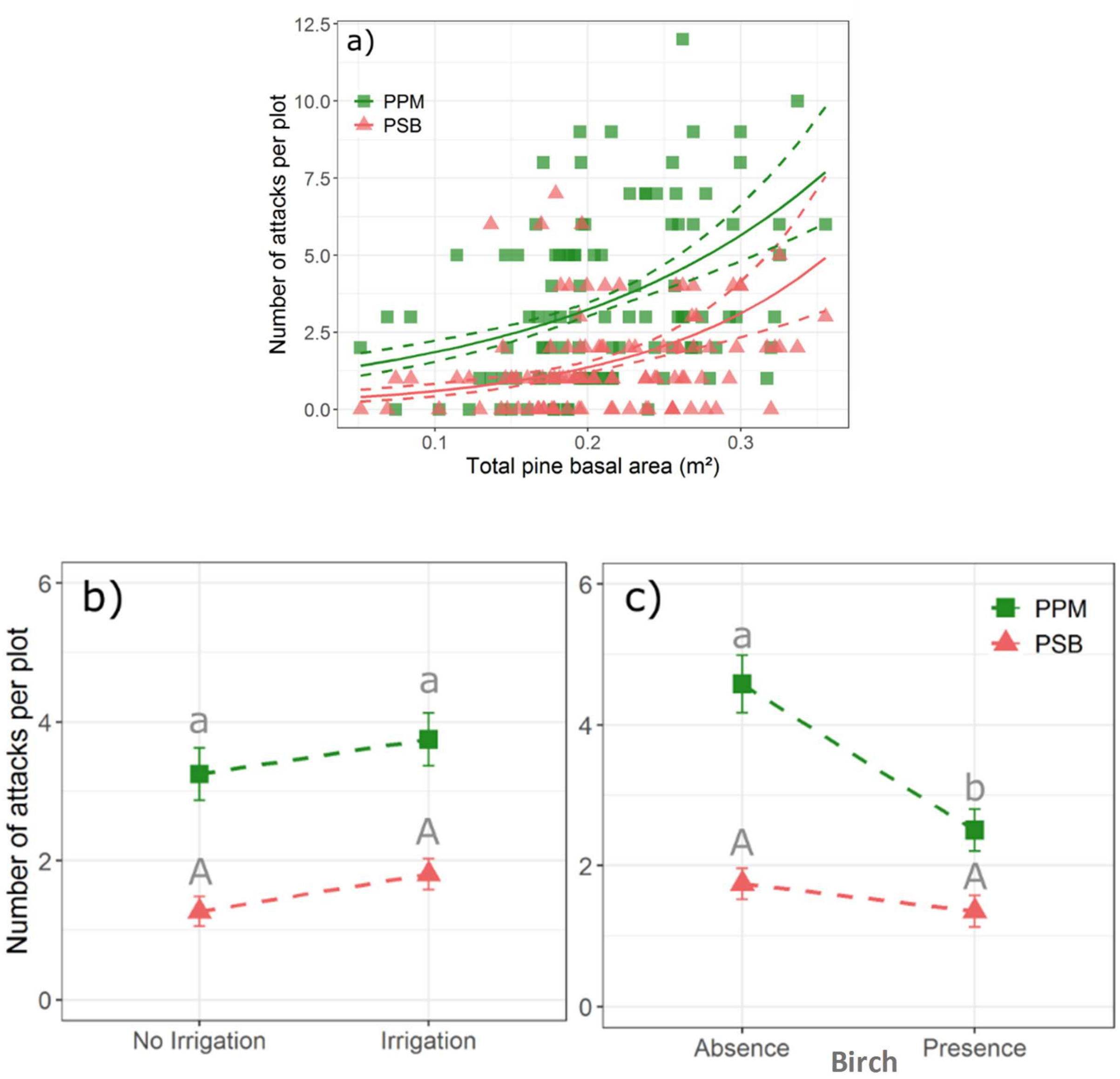

***Figure 3**. Effects of total pine basal area per plot (a), irrigation (b) and birch presence (c) on pine processionary moth (PPM) and pine stem borer (PSB) total number of attacks per plot. On panel a, solid lines represent model predictions and dashed lines the standard deviation around these predictions. On panels b and c, dots and error bars represent raw means and corresponding standard errors, respectively, and letters indicate whether subgroup means differ significantly (different letters) or not (same letter).*

Both the total number of PPM and PSB attacks per plot significantly increased with total pine basal area per plot (Figs. 2 and 3a; Supporting Information Table S3).

The total number of PPM attacks per plot did not significantly vary with irrigation (Figs. 2a and 3b; Supporting Information Table S3). The presence of birch had both direct and indirect (through reduced total pine basal area) negative effects on the total number of PPM attacks per plot (Fig. 2a). On average, there were half as many PPM attacks when birch trees were present in the plot (Fig. 3c). This associational resistance to PPM was observed in both irrigated and non-irrigated conditions (Fig. 4a).

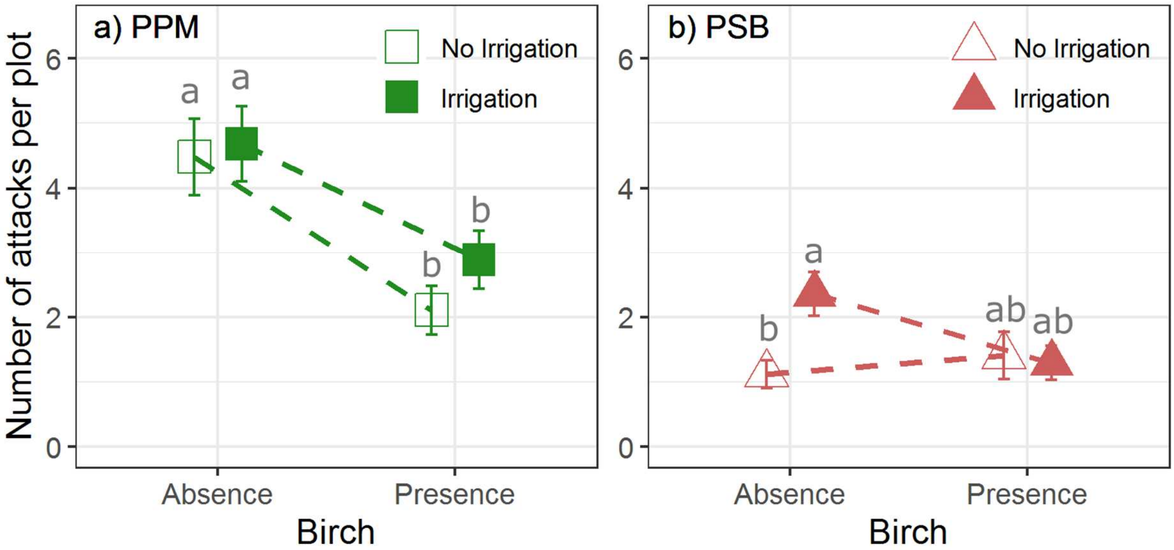

***Figure 4**. Combined effects of irrigation and birch presence on (a) pine processionary moth (PPM) and (b) pine stem borer (PSB) total number of attacks per plot. Dots and error bars represent raw means and corresponding standard errors, respectively, and letters indicate whether subgroup means differ significantly (different letters) or not (same letter).*

There was an interactive effect of irrigation and the presence of birch on the total number of PSB attacks, whereby the presence of birch reduced the total number of PSB attacks per plot in irrigated conditions only (Figs. 2b and 4b; Supporting Information Table S3). On average, there were half as many PSB attacks when birch trees were present in irrigated plots (Fig. 4b). The presence of birch also had an indirect, negative effect on the total number of PSB attacks, through the reduction of total pine basal area (Fig. 2b).

Overall, irrigation, total pine basal area, pine density and the presence of birch explained 31% and 16% of the variance of the total number of PPM and PSB attacks, respectively.

Discussion

With this study, we showed that pine-birch mixtures are more resistant to both PPM and PSB attacks than pine monocultures, a case of associational resistance to several pests (i.e. when attacks decrease with increasing tree species diversity). Furthermore, we found that associational resistance was due not only to direct effects of birch trees, possibly related to repellent or disrupting non-host volatiles (NHVs), but also to indirect, growth-mediated effects whereby the presence of birch trees reduced host pine resources for the pests. The influence of drought was limited to an interference with the direct effect of birch on the number of PSB attacks.

Primary pests responded to the concentration of host resource

In line with resource concentration hypothesis (Root 1973), the total number of attacks per plot of the two pests increased with total pine basal area. The effect of pine density per plot on the total number of attacks was mediated by the effect on total pine basal area, suggesting that the resource concentration effect is due more to the total amount of resources than the total number of individual trees (Hambäck and Englund, 2005). Total pine basal area is an indicator of the food resources available to both pests, being directly correlated to the volume of tree trunks where PSB larvae can develop, but also allometrically and positively related to the biomass of needles (Shaiek et al., 2011) that PPM larvae feed on. Pine basal area is also a proxy for the visual and olfactory apparency of host pines to the pests. Because all trees were of the same age in our experiment, it is reasonable to consider that pine basal area was also positively correlated with pine growth rate (i.e. vigour), which can be considered a predictor of bark cracking and thus terpene emission, an olfactory cue used by PSB to choose their host tree (Jactel et al. 1996). Similarly, the attraction of female PPM was probably enhanced by the volume of crowns, through increased visual apparency and higher release of host odours (Jactel et al. 2015).

Resource-mediated associational resistance in pine-birch forests

We found that the presence of birch resulted in lower PPM and PSB attacks on pines, i.e. a case of associational resistance, which is consistent with predictions for oligophagous insect herbivores in mixtures of broadleaved and conifer trees (e.g. Castagneyrol et al. 2014; Jactel et al. 2021). For both pests, this was partly explained by an indirect effect of birch on attacks whereby the presence of birch trees in the plot induced a reduction of pine basal area, likely making the pine resources less abundant and attractive to the two primary pests. By focusing on the plot scale (total number of attacks per plot and total basal area per plot) we could clearly demonstrate that a reduction of feeding substrate in mixed species forests can lead to reduced infestation by primary pests, providing support to the resource concentration hypothesis. This associational resistance effect was not due to increased dilution of host tree resources within mixed stands (Hamback et al. 2014) because there was no direct effect of the number of individual trees (pine density) on total number of attacks. It was due to the fact that the non-host trees reduced the growth of neighbouring host trees, probably mainly through competition for light as both pine and birch are light demanding species and not through competition for water since the presence of birch resulted in lower pine basal area in both irrigated and non-irrigated blocks.

We found no significant effect of irrigation on total pine basal area. More precisely, there was a tendency for pines to grow bigger in irrigated plots; however, this was not significant at the time of sampling. It is likely that the benefit of irrigation in terms of water stress reduction (Maxwell et al., 2020 Fig. S1) was not sufficient after only two growing seasons to be significant in terms of tree growth, and perhaps tree nutritional quality.

Growth-mediated effects of birch on pest attacks occurred independently of drought conditions and there was no direct effect of irrigation on the level of pest infestation. The responses of forest insects to drought seem to differ between feeding guilds and drought intensity, which can be explained by the main physiological mechanisms of tree resistance (Jactel et al., 2012; Gely et al., 2020). Under moderate water stress, leaf chewers (e.g. PPM) would increase their damage because of higher quality of leaf tissues, in particular higher nitrogen content (Gely et al., 2020). On the contrary, the damage of bark-and phloem feeders (e.g. PSB) would be negatively affected by moderate drought because reduced vegetative growth would induce reallocation of carbohydrates to secondary metabolism, leading to a decrease in the quality of wood tissues and a strengthening of induced defence reactions involving terpenes and phenols (Jactel et al. 2012). Since we did not analyse the chemical composition of pine needle and phloem, we could not determine whether the effect of heterospecific neighbours on pine growth was accompanied by a reduction in host suitability, i.e. through increased allocation of resources to chemical defences against herbivores, following the growth-defence trade-off (Herms and Mattson, 1992). However, in our study, the change in nutrient quality of trees induced by their heterospecific neighbours (e.g., nitrogen or carbohydrate concentration) that might have affected PPM or PSB attacks was probably not strong enough or was largely offset by the unlimited availability of needles and stems.

Direct associational effects of birch

We also found that the presence of birch directly reduced the number of both primary pests' attacks, which confirms previous results (Jactel et al., 2011, 2019; Castagneyrol et al., 2019). This "protective" effect was possibly due to the emission of non-host volatile compounds by birch trees, and in particular methyl salicylate (MeSa) that was shown to repel PPM females (Jactel et al. 2011) and has been since found to be repellent against the pine weevil *Hylobius abietis* (Azeem et al. 2015) and the shot hole borer *Euwallacea fornicatus* (Byers et al. 2021).

However, this associational resistance effect likely related to NHVs released by birch trees did not operate on PSB attacks in drought conditions (Fig.4). Birch basal area was marginally and non-significantly higher in irrigated plots than in non-irrigated plots (see Supporting Information Table S4 and Fig. S2), maybe leading to slightly higher release of NHVs in irrigated blocks.

Conclusion and Perspectives

In this study, using structural equation modelling, we show that associational resistance of pine-birch forests to two pine primary pests was explained by both direct and indirect, growth-mediated effects of birch. Our findings also confirmed that associational effects could be modulated by drought conditions. In fact, the predicted increase in drought frequency and intensity could reduce the resistance of pine-birch mixed forests to PSB attacks, but not to PPM attacks. Future studies should investigate the effect of drought on tree growth and tree diversity-herbivory relationship on a longer term basis, to verify whether the growth-mediated associational effects of birch can be really modulated by drought.

We detected a negative effect of heterospecific neighbours on host tree growth, and a tendency for host tree growth to decrease in drought conditions, but we could not determine whether this was accompanied by an increase in chemical defences according to the growth-differentiation balance hypothesis. The next step is to analyse the combined effects of heterospecific neighbours and drought on tree traits involved in associational resistance to primary pests, focusing on host and non-host VOCs release and resource nutritional quality.

Finally, birch improved pine resistance to primary pests, but this was partly due to pine growth limitation that may not be desirable from a forester point of view. Future studies, should assess the benefit of birch-induced pest resistance against the negative effect of the presence of birch on pine biomass production to determine the true benefit of diversification.

We hope this work will encourage researchers to further disentangle the mechanisms behind associational resistance, which should help build toward a more general predictive framework of tree diversity-herbivory relationships.

Credit authorship contribution statement

Charlotte Poeydebat: Methodology, Formal analysis, Visualization, Writing – Original draft. **Bastien Castagneyrol**: Methodology, Writing – Review and editing. **Inge Van Halder**: Investigation, Writing – Review and editing. **Hervé Jactel**: Supervision, Investigation, Methodology, Writing – Original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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