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Associational resistance to a pest insect fades with time

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Abstract

Tree diversity is one of the drivers of forest resistance to herbivores. Most of the current understanding of the diversity resistance relationship comes primarily from short-term studies. Knowing whether tree diversity effects on herbivores are maintained over time is important for perennial ecosystems like forests. We addressed the temporal dynamics of the diversity resistance relationship by conducting a 6-year survey of pine attacks by the pine processionary moth *Thaumetopoea pityocampa* (PPM) in a tree diversity experiment where we could disentangle tree composition from host density effects. During the first years after planting the trees, PPM attacks on maritime pine *Pinus pinaster* were reduced in the presence of birch *Betula pendula*, a fast-growing non-host tree (i.e. associational resistance). This effect was maintained but faded with time as the pines eventually grew taller than neighbouring birches. The number of repeated attacks on individual pine trees also decreased in mixed pine–birch stands. Pine density had a positive effect on stand colonisation by PPM and a negative effect on the proportion of trees that were attacked. Pines were less likely to be repeatedly attacked as pine density increased, with attacks being spread over a larger number of host trees. Collectively, these results unravel the independent contribution of tree species composition and host density to tree resistance to herbivores. Both processes had directional changes over time. These results indicate that the resistance of planted forests to herbivores can be improved by carefully choosing the composition of mixed forests and the timing of species planting.

Keywords Associational effects · Forest · ORPHEE experiment · Plant–insect interactions · *Thaumetopoea pityocampa*

Key Message

- Our knowledge of mixed forest resistance to herbivores is largely based on short-term studies.
- We monitored pines for attacks by a pest defoliator for six consecutive years in a large-scale tree diversity experiment.
- Birch caused associational resistance to the pine processionary moth, but this resistance faded over time.
- The resistance of planted forests to herbivores can be improved by carefully choosing both forest composition and the timing of species planting.

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Introduction

Tree diversity has well documented, albeit variable, effects on insect herbivores. Several observational and experimental studies demonstrated that the presence of heterospecific neighbours reduces a tree's risk of being attacked by herbivores, which is known as associational resistance (Barbosa et al. 2009; Moreira et al. 2016). Associational resistance is a common phenomenon in naturally grown and planted forests (Barbosa et al. 2009; Castagneyrol et al. 2014b; Guyot et al. 2016) although the opposite, associational susceptibility, has also been reported (Schuldt et al. 2010; Wein et al. 2016; Castagneyrol et al. 2018). Despite decades of research on associational effects in forest ecosystems, predicting their

direction and strength remains elusive. Three sources of variation can explain discrepancies between studies. First, associational effects may depend more on the composition of tree species assemblages controlling for forest vertical and horizontal heterogeneity than on tree species richness per se (van Schrojenstein Lantman et al. 2018). Second, different herbivores may have contrasting responses to tree diversity, with associational resistance being more common, albeit not always, in specialist herbivore species (Castagneyrol et al. 2014b). Third, tree diversity effects on herbivores may vary with herbivore population density and differ between endemic and outbreak periods (Fernandez-Conradi et al. 2017; Bognounou et al. 2017; Merwin et al. 2017). Forest structure, herbivore population density and community composition also change over time, which may therefore alter the direction and strength of associational effects. Yet, these aspects remain almost unexplored, as most studies were short term (but see Bognounou et al. 2017). Understanding the dynamics of associational effects is therefore of crucial importance for perennial systems such as forests, especially at early stages of their development.

Resource density and frequency are major drivers of associational effects (Verschut et al. 2016). The resource concentration hypothesis predicts that herbivores—in particular specialist herbivore species—are more likely to be attracted to, and to aggregate in, patches with high resource concentration. Yet, for such specialist herbivore species, resource density (i.e. number of host individuals) and frequency (i.e. relative abundance of hosts) often correlate negatively with plant diversity. The resource concentration hypothesis therefore predicts lower herbivore aggregation on host plants surrounded by a greater diversity of non-host plants (Root 1973; Hambäck and Englund 2005; Kim and Underwood 2014; Damien et al. 2016). This hypothesis has been well explored by the literature, but the opposite pattern whereby herbivores aggregate on the fewer number of available plants was also reported (the resource dilution hypothesis, Otway et al. 2005; Damien et al. 2016). The resource concentration and resource dilution hypotheses conflict in what should be the consequences of host plant density and frequency on per capita herbivore load. On the one hand, specialist herbivores may be less attracted by patches where their host plants are diluted among non-host plants. This would result in a lower per capita herbivore load (resource concentration hypothesis). On the other hand, albeit less abundant, herbivores may concentrate on the fewer available host plants, thus increasing per capita herbivore load (resource dilution hypothesis). For the same reasons, in perennial systems, the probability that the same host plants are repeatedly attacked every year by herbivores may be higher in patches where host availability is reduced. Disentangling how the absolute and relative abundance of host and non-host plants contribute to herbivore aggregation among and within patches

therefore emerges as a major challenge in theory for associational effects.

Associational effects depend on the identity of a focal plant's neighbours. Herbivores rely on a mixture of host and non-host cues to identify and orientate towards host plants while avoiding non-host plants. Non-host plants surrounding a focal plant can reduce its physical and chemical apparency to herbivores (i.e. the probability of being found by herbivores, Feeny 1976; Strauss et al. 2015) by emitting repellent cues or masking host's attractive cues (Zhang and Schlyter 2004; Jactel et al. 2011; Castagneyrol et al. 2013). For instance, a focal plant being visually hidden by taller neighbours has proved to be less damaged by herbivores than physically more apparent focal plants (Miller et al. 2007; Dulaurent et al. 2012; Castagneyrol et al. 2013; Damien et al. 2016). Another possibility is that a small focal plant would emit fewer attractive volatile cues than nonattractive or even repellent volatile cues emitted by taller heterospecific neighbours, thus reducing its 'chemical apparency' through higher volatile diversity (*the semiochemical diversity hypothesis*, Zhang and Schlyter 2003; Schiebe et al. 2011). However, isolating the effect of heterospecific neighbours on the physical versus chemical apparency of a focal plant is a difficult task as both are confounded.

Different species in a mixture may have different growth rates such that the physical and chemical apparency of a given plant may change with time as a function of its growth rate and that of its neighbours. For instance, Damien et al. (2016) reported that the initial protection against a specialist herbivore, which was provided to pines by taller neighbouring birches, tended to decrease with time as pine eventually grew taller than birches. Temporal changes in the relative size of plants in a community may further alter competitive and facilitative interactions among plant species, thus altering patterns of resource allocation to growth versus defences and ultimately plant–herbivore interactions (Hakes and Cronin 2012; Kostenko and Bezemer 2013). Although not well documented, both mechanisms are likely to contribute to shifts in the strength and direction of associational effects with time. Yet predicting these effects is not trivial, as tree shape and growth rate of plant species in mixtures also depend on diversity-mediated processes (Jucker et al. 2015).

Recent studies have suggested that the strength and direction of associational effects could be partly determined by the density of herbivore population (Fernandez-Conradi et al. 2017; Merwin et al. 2017). Yet, several herbivore species have eruptive or cyclic population dynamics (Haynes et al. 2014; Li et al. 2015), so that strength and, potentially, even the direction of associational effects may change with herbivore population density. For instance, in a recent study, Bognounou et al. (2017) showed that while damage caused by the spruce budworm to its preferred host species (balsam fir) increased with host concentration and decreased with the

abundance of a less preferred host species (black spruce) at low pest densities, damage was independent of the relative densities of preferred and less preferred host species during an outbreak period. While overall herbivore pressure can hardly be controlled in large-scale studies, controlling statistically for temporal changes in herbivore density may contribute to a better understanding of processes generating associational effects.

We addressed the temporal dynamics of associational effects by using the pine processionary moth *Thaumetopoea pityocampa* (PPM) and its principal host, the maritime pine *Pinus pinaster*, as model species. PPM is an oligophagous insect, feeding mainly on pine species (*Pinus* sp.) and is a major pine defoliator in southern Europe and northern Africa (Battisti et al. 2015). PPM has cyclic population dynamics with a 7-year period (Li et al. 2015). Gravid females flying outside pine stands rely on attractive physical and volatile cues released by pines to select and orientate their flight towards host trees, and are deterred by physical and chemical cues emitted by non-host trees, in particular by broadleaved species (Battisti et al. 2015; Jactel et al. 2015). In a previous study using the same tree diversity experiment associating maritime pine with four different broadleaved species, we showed that pines were less prone to PPM infestation when associated with birch (Castagneyrol et al. 2014c). A likely mechanism was the reduction in pine apparency by the neighbouring fast-growing birch trees (Damien et al. 2016). The objective of this study was to assess the temporal dynamics of tree diversity effects on pine infestation by the PPM during the first years after forest establishment, while controlling statistically for fluctuations in PPM population density. We first hypothesised that associational resistance conveyed by birch decreases over time, as pines grew taller than neighbouring birches. We also asked whether the same individual pines were more often attacked over time in plots where pines were more diluted by broadleaved species. We addressed these questions by quantifying PPM attacks on pines in a long-term tree diversity experiment in South West France in which we could disentangle the effects of tree species composition from pine density (Damien et al. 2016). By doing so, we hoped to provide new insights into our understanding of the mechanisms driving plant–herbivore interactions.

Materials and methods

Experimental design

The ORPHEE experiment (https://sites.google.com/view/orpheeexperiment/home) is located 40 km south of Bordeaux (SW France). It was established in 2008 on a 12 ha clear cut of maritime pine stands. In total, 25,600 trees belonging to five native species were planted (European birch *Betula pendula*; Pedunculate oak *Quercus robur*;

Pyrenean oak *Q. pyrenaica*; Holm oak *Q. ilex*; and Maritime pine *Pinus pinaster*). Eight blocks were established with 32 plots in each block corresponding to the 31 possible combinations of 1–5 species, with an additional replicate of the combination of the five species. In particular, 17 species combinations contained pines, either alone or in association with one, two, three or all of the four other broadleaved species. Plots were separated by a distance of 3 m and were randomly distributed within blocks. Each plot consisted of 10 rows of 10 trees planted 2 m apart, resulting in 100 trees per plot. The plot area was 400 m². Tree species mixtures were established according to a substitutive design, keeping the overall number of trees equal across plots. Within plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbour from each of the other species within a 3 m radius (Castagneyrol et al. 2013).

PPM infestation

During winter, PPM larvae feed at night on pine needles and spend daytime in white silky nests that are visible from the ground (Battisti et al. 2015). The number of winter nests is a common proxy for the level of PPM infestation (Hódar et al. 2002; Régolini et al. 2014). We counted the number of winter nests per tree on every pine of the ORPHEE experiment, every winter from 2013 to 2018, which corresponds to oviposition events that occurred in previous summers (i.e. from 2012 to 2017). We considered dead and broken trees for which there was no assessable crown as missing values. PPM nest count was carried out from the ground by a team of two people carefully looking at the crown of individual pines from two opposite sides. Two people conducted the field observation every year (BC and HJ) but received help from a varying number of people.

Pine density and apparency

We measured tree height from the ground to the highest living bud on every plot from 2008. From 2008 to 2015, we measured the height of the 36 innermost trees per plot. In the period 2015–2018, we repeatedly measured the height of every living oak among these 36 innermost trees per plot, but restricted height measurements of pine and birch to seven individuals of each species, which were haphazardly chosen among these 36 innermost trees.

Previous studies on the same experiment have shown that birch and pines grew much faster than oaks (Damien et al. 2016; Castagneyrol et al. 2018), which were already below the lowest living branches of most pines by 2014 (B. Castagneyrol, personal observations) and partially confounded with the dense and bushy understorey vegetation. We therefore considered the mixtures of pines and oaks,

with no birch, as low-density pine monocultures, forming a gradient of pine density, from 100% in pine monocultures to 25% in plots with pine mixed with three oak species. We used this gradient to test independently the effects of pine density and the pure associational effects provided by the presence of birch, while controlling for pine density (see Fig. 1 in Damien et al. 2016). Overall pine mortality was very low (4.5% dead pines among 1858 surveyed in 2017), so that the number of pines per plot remained virtually the same as when we planted the experiment.

We defined pine apparency (A) as follows: $A = \frac{1}{S} \times \sum_{i}^{S-1} (H_\mathrm{p} - H_i)$, where H_p and H_i were species-specific mean height of pine and associated species i, respectively, and S the number of species planted in the plot (i.e. 1 to 5). We characterised mean pine apparency by averaging tree height at the species level, within plots, as the sample size varied between years and between species. Although they were initially smaller than pines, we retained oaks in the calculation of pine apparency as some individuals were > 2 m in height from 2016 on. Pine apparency therefore quantifies how much taller (positive values) or smaller (negative values) a pine was on average than its neighbours. It should be noted that the design of the ORPHEE experiment does not allow the effect of pine neighbours on pine visual apparency to be disentangled from chemical apparency, as taller neighbours may alter PPM host searching behaviour through both the physical disruption of pine physical cues and the emission of non-host volatiles diluting pine volatiles or acting as repellents.

Resource availability to PPM

In 2013 and 2014, we additionally measured pine diameter at breast height (dbh) on the same seven trees that were measured for height. From 2015 on, we measured the dbh of every living pine within the 36 innermost trees per plot. In order to quantify the amount of resource available to PPM, we used allometric equations to estimate needle biomass at the plot level. The allometric equation was obtained from Shaiek et al. (2011): Needle biomass $= 1.916 \times \mathrm{dbh}^{2.07} \times Age^{-0.67}$, where dbh and Age are mean pine diameter at breast height (cm) and pine age (years), respectively. Then, we multiplied the value obtained by the number of living pines per plot to estimate plot-specific needle biomass, which served as a proxy for resource concentration (i.e. resource available to PPM at the plot level).

Statistical analyses

All analyses were conducted using the R language programming with the *Rstudio* interface. We used the following packages for data analysis: *lmerTest*, *MuMIn* and *multcomp* (Kuznetsova et al. 2015; Bartoń 2019; Hothorn et al. 2016).

Temporal changes in the effects of pine density and the presence of birch on PPM infestations

We analysed two response variables: (1) PPM density, which was the total number of nests per plot, and (2) PPM attack rate, which was the proportion of trees with at least one PPM nest. PPM density informed on plot colonisation by PPM, while PPM attack rate represented how many host trees were damaged once the plot had been colonised (Damien et al. 2016).

We tested the effect of pine age (*Age*, continuous variable), presence of birch (*Birch*, present/absent), pine density (i.e. the number of pines per plot, continuous variable) and all two- and three-way interactions on PPM density and attack rate as fixed effects in generalised linear mixed-effects models (GLMMs). We used *Block*, *Plot* (nested within block) and *Year* (as a factor, crossed with *Block* and *Plot* factors) as random factors. The random *Block* factor accounted for heterogeneity among blocks and non-independence of different plots of the same pine density within blocks (20–50 pines per plot). The individual-level random *Plot* factor accounted for repeated measurements at the plot level. The random effect of *Year* accounted for differences in overall PPM population density between years. All continuous predictors were standardised across years by subtracting the mean and dividing by the standard error to allow direct comparison among model coefficient parameter estimates. We used Poisson and binomial GLMM with *log* and *logit* links to analyse PPM density and attack rate, respectively.

For each response variable, we applied model simplification by sequentially removing non-significant terms, starting with the least significant highest order interaction. We did not simplify the random part of the model, as it was imposed by the experimental design. We tested the significance of predictors with F-tests and Kenward–Roger's approximation for degrees of freedom. We calculated marginal (R_m^2) and conditional (R_c^2) R^2 to estimate the variance explained by fixed effects and fixed plus random effects, respectively.

Number of repeated attacks

In order to test the effect of birch and pine density on the repeated attacks of individual pine trees, we calculated how many times each individual pine was attacked (i.e. presence of at least one nest) during six observation years. Trees that died during the survey were excluded from this analysis. We then analysed the number of repeated attacks (ranging from zero to six) at the level of individual trees using GLMM with Poisson error family and log-link function. Fixed effects were the presence of birch, pine density and their

interaction. Random factors were *Block* and *Plot*, nested within *Block*. Significance of fixed effects was tested using log-likelihood Chi-square tests. We analysed the number of repeated attacks instead of the probability of repeated attack (i.e. number of repeated attacks divided by the number of years) owing to better model residual distribution.

Temporal changes in pine apparency and resource availability

Changes in tree diversity effects on PPM density or attack rate may have been caused by differential dynamics of pine apparency and resource concentration in the presence or absence of birch. Yet, because pine apparency and resource availability were calculated from tree dimensions, they both varied with time. Including these variables together with pine age in statistical models would have caused serious collinearity issues. By contrast, pine density and the presence or absence of birch were imposed by the original design and did not vary with time (see above). We therefore used pine density and the presence/absence of birch to model PPM attacks (see above) and analysed the dynamics of pine apparency and resource availability in mixed plots in separate models, but using the same modelling approach as for PPM attacks.

Results

PPM population density

During the 6-year survey, we counted a total of 5591 PPM nests on 4585 pine trees (the same tree could have been attacked more than once). Pines attacked by the PPM had on average 1.22 PPM nests. Over the time of the survey, only 21 trees had four nests or more in a given year. PPM population density peaked in 2015 (which corresponds to the middle of the survey period) and in 2018 (Fig. 1). There was therefore no risk that fluctuation in PPM population density was confounded with tree growth dynamics.

Dynamics of pine density and birch effects on PPM

PPM density

Overall, PPM nest density (i.e. the number of PPM nests per plot) was reduced in plots associating pine with birch and tended to increase with increasing pine density (Table 1, Fig. 2). However, both the effect of pine density and the presence of birch on PPM density varied over time, as indicated by the significant *Age* × *Birch* and *Age* × *Density* interactions (where '*Birch*' is presence/absence of birch, Table 1). The coefficient parameter estimate (± SE) of the
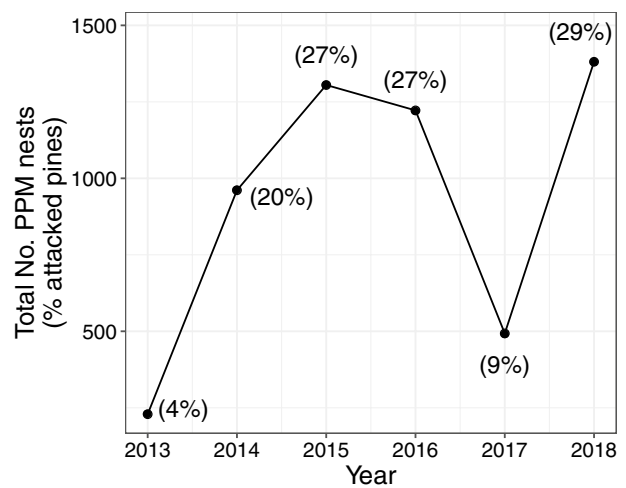

Fig. 1 PPM population dynamics in the ORPHEE experiment consisting of 25,600 trees (including 5120 pines) planted in 256, 400 m² plots over 12 ha in 2008. Dots represent the total number of PPM nests counted on pines. Within parentheses, numbers refer to the percentage of pines with at least one PPM nest

Age × *Birch* interaction was positive (0.16 ± 0.03), indicating that the negative effect of birch on PPM density (-0.57 ± 0.08) decreased in magnitude over time. This result thus indicates that associational resistance conveyed by birch faded with time (Fig. S1). Likewise, the coefficient parameter estimate of the *Age* × *Density* interaction was negative (-0.10 ± 0.02), indicating that the positive effect of pine density on PPM density (0.08 ± 0.04) weakened with time and eventually nullified (Fig. 2). There was no significant interaction between pine density and the presence of birch (Table 1).

PPM attack rate

Overall, PPM attack rate (i.e. the proportion of pines with at least one PPM nest) was reduced in plots associating pine with birch and decreased with pine density (Table 1, Fig. 2). However, both the effect of pine density and presence of birch on PPM attack rate varied with time, as indicated by the significant *Age* × *Birch* and *Age* × *Density* interactions (Table 1). The coefficient parameter estimate of the *Age* × *Birch* interaction was positive (0.15 ± 0.04), indicating that the negative effect of birch on PPM attack rate (-0.63 ± 0.10) decreased in magnitude with time, which corresponds to a fading protective effect of birch over time (Fig. S1). By contrast, the coefficient parameter estimate of the *Age* × *Density* interaction was negative (-0.12 ± 0.02), indicating that the negative effect of pine density on PPM attack rate (-0.29 ± 0.05) strengthened and became even more negative with time (Fig. 2).

Table 1 Summary of (generalised) linear mixed effect models testing the effects of pine age, pine density and presence/absence of birch on PPM density and attack rate and on pine apparency and resource concentration

Explanatory variable	PPM density (no. nests per plot)		PPM attack rate (% attacked trees)		Pine apparency		Resource concentration (needle biomass)	
	χ^2	P value	χ^2	P value	χ^2	P value	χ^2	P value
Age	**2.03**	0.154	1.71	0.191	5604.07	**<0.001**	3082.70	**<0 0.01**
Birch	**39.90**	**<0.001**	28.61	**<0.001**	293.70	**<0.001**	209.88	**<0.001**
Tree density[a]	**3.92**	**0.048**	50.56	**<0.001**	233.41	**<0.001**	82.36	**<0.001**
Age × birch	**23.39**	**<0.001**	12.72	**0.001**	74.33	**<0.001**	8.22	**0.004**
Age × tree density	**40.61**	**<0.001**	45.30	**<0.001**	207.50	**<0.001**	6.23	**0.044**
Tree density *× birch*	**<0.01**	0.979	3.98	**0.046**	24.09	**<0.001**	1.16	0.560
Age × tree density *× birch*	0.42	0.514	0.01	0.900	20.65	**<0.001**	0.86	0.649
$R_\mathrm{m}^2 (R_\mathrm{c}^2)$[b]	0.30 (0.86)		0.07 (0.23)		0.81 (0.98)		0.80 (0.96)	

[a]Density corresponds to realised pine density a given year for PPM density and attack rate (i.e. accounting for pine mortality), while it refers to initial planting density (three-level factor) for pine apparency and resource concentration

[b]Marginal (R_m^2) and conditional (R_c^2) R^2 are given for the simplified models. Characters in bold font refer to significant effects
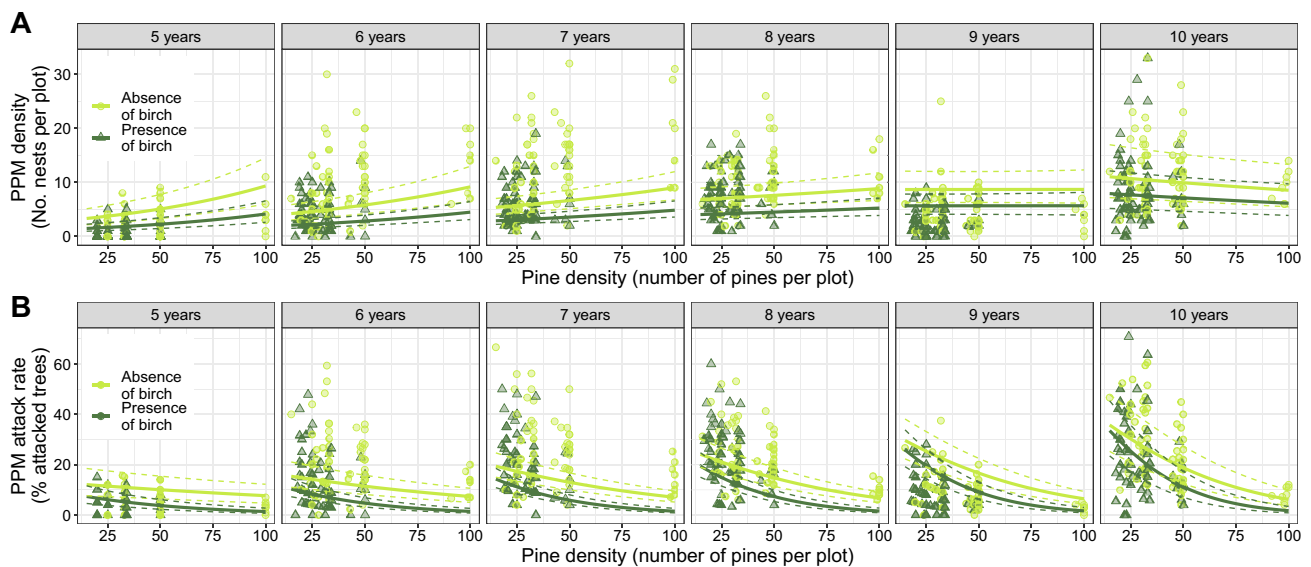

Fig. 2 Temporal changes in the effect of the presence of birch and pine density on PPM density (**a**) and PPM attack rate (**b**). Dots represent PPM density and attack rate at the plot level in the presence (dark green, filled triangle) or absence (light green, square) of birch. Solid and dashed lines represent predictions of the simplified model. Ages above panels correspond to time after planting

Number of repeated attacks

A total of 2118 pines (44%) were attacked at least once during 6-year survey. Conversely, 56% of pines were never attacked. Only 46 individual pines (1%) were attacked every year (i.e. six attack records in the present survey). The number of repeated attacks was lower in the presence of birch ($\chi^2 = 29.98$, $P < 0.001$, Fig. 3) and decreased with pine density ($\chi^2 = 57.89$, $P < 0.001$, Fig. 3). This means that individual pines were more likely to be repeatedly attacked every year in low-density plots and in the absence of birch. There was a significant interaction between the

presence of birch and pine density ($\chi^2 = 3.88$, $P = 0.049$) with a negative model coefficient parameter estimate (-0.28 ± 0.14), indicating that the effect of birch increased in magnitude with increasing pine density (Fig. 3).

Temporal dynamics of pine apparency and resource availability

At the end of the 2016 growing season, birches were on average ($\pm$ SE) 6.48 ± 0.04 m ($n = 1139$), pines were on average ($\pm$ SE) 7.80 ± 0.03 m ($n = 1139$), and oaks (regardless of the species) were on average 1.54 ± 1.51 m tall ($n = 3348$).
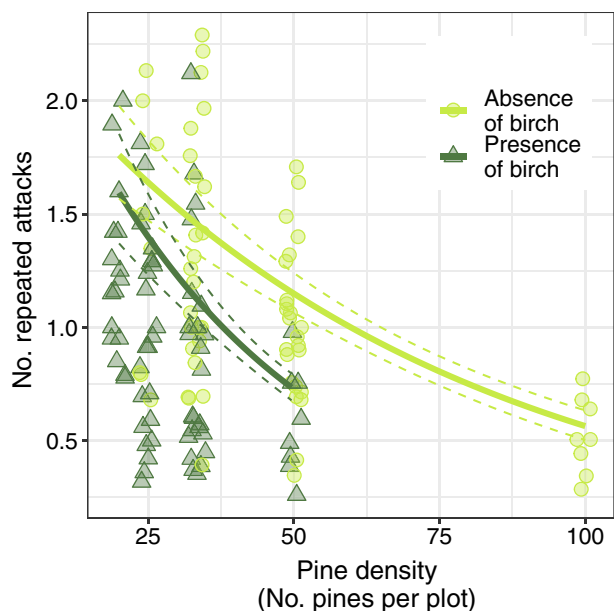
Fig. 3 Effects of pine density and the presence of birch on the probability of repeated attacks. Dots represent individual plots in the presence (dark green, filled triangle) or absence (light green, square) of birch. Solid and dashed lines represent the predictions of the simplified model and their standard error, respectively

The consequence of species-specific differences in growth dynamics is that in the presence of birch, pine apparency—that is the extent to which pines were apparent and easily perceived by herbivores—changed drastically over time (Fig. 4a), but in a different manner depending on initial pine density and on the presence of birch as indicated by the significant $Age \times Density \times Birch$ interaction (Table 1). In particular, pine apparency was on average lower in the presence of birch, and the effect of birch was more pronounced in denser plots (Fig. 4a). Comparisons between slopes of the regression of pine apparency against time (Table S1) indicated that pine apparency increased faster in the absence of birch than in the presence of birch (Fig. 4a) and faster in plots with lower density.

The amount of resource available to PPM (estimated pine needle biomass at the plot level) increased with time, but in a different manner depending on initial pine density (Table 1) and on the presence of birch (Table 1). Resource increased significantly faster in plots with initial density of 50 pines per plots than in plots with initial density of 25 pines per plot (Table S1) and in plots with initial density of 33 pines per plots than in plots with initial density of 25 pines per plot (Table S1). However, resource dynamics were not significantly different in plots with initial density of 50 versus

Fig. 4 Pine apparency (**a**) and resource concentration (**b**) in plots with initial density of 25, 33 or 50 pines per plot in the presence or absence of birch for four consecutive years. Pine apparency (cm) is the mean difference between mean pine height and the mean height of each associated species in mixture. Resource concentration (kg) is the estimated needle biomass at the plot scale. Dots and error bars represent mean ± SD
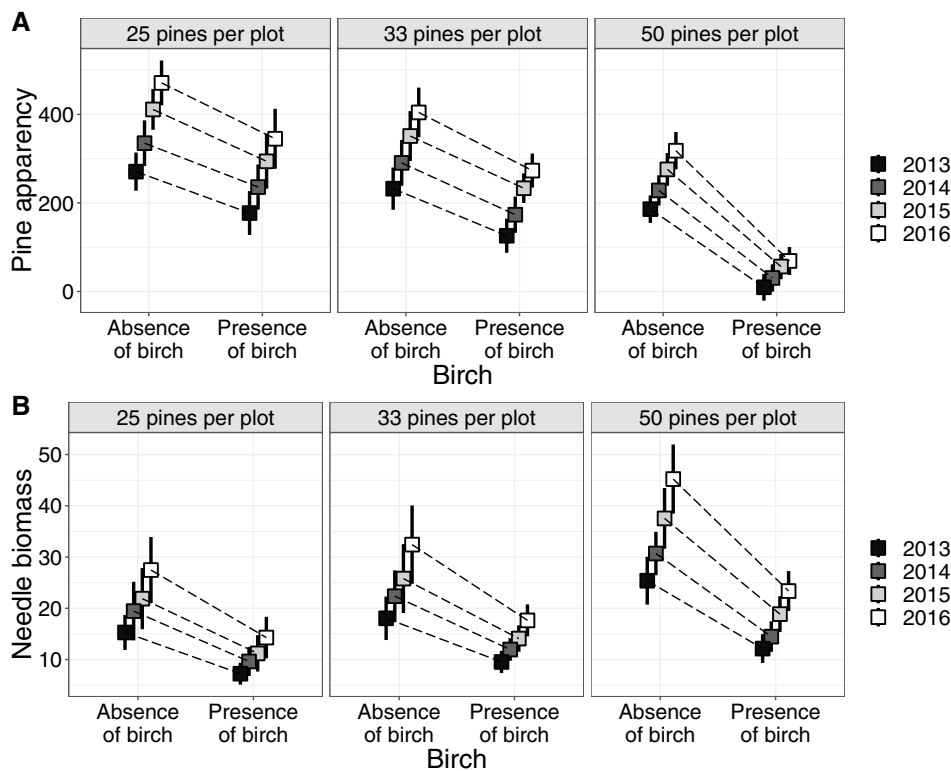
33 pines per plot (Table S1). Since the $Age \times Density \times Birch$ interaction was not significant ($\chi^2 = 0.16$, $P = 0.922$), the difference in the slope of resource amount over time between plots with versus without birch was the same, regardless of pine initial density (Table S1). Likewise, differences in slopes among the three initial pine density levels were the same, regardless of the presence or absence of birch.

Discussion

In this study, the experimental design and modelling approach allowed the distinction between two mechanisms driving tree diversity effects on herbivores, namely resource density and host apparency, while accounting for fluctuation in herbivore population density. More importantly, we were able to analyse the temporal dynamics of such effects at the time of intensive growth of young forest. In particular, we found that associational resistance against PPM conveyed by birch (a non-host species for PPM) faded with time due to contrasting growth rates between PPM host and non-host tree species. We also found that host density had opposite effects on the recruitment of PPM and on their distribution among individual host trees. In particular, we found that pine stand colonisation by PPM increased with pine density, but that this effect decreased and eventually nullified with time, suggesting that factors other than host density drove variability in pine stand colonisation as stands aged. On the other hand, the PPM attack probability of individual pine trees decreased as pine density increased, and this effect strengthened over time. Our study therefore highlights the mechanisms driving insect herbivory on trees in mixed forests during the first decade after tree plantation.

Birch protected pines against PPM attacks, but this effect faded with time

Pine attacks by PPM were reduced in the presence of birch. Both PPM density (i.e. the total number of PPM nests at the plot level) and PPM attack rate (i.e. the proportion of pines attacked by the PPM) were reduced in the presence of birch. This finding is consistent with previous studies conducted on the ORPHEE experiment (Castagneyrol et al. 2014c; Damien et al. 2016) and with observational or experimental studies in the same area (Jactel et al. 2011; Dulaurent et al. 2012; Castagneyrol et al. 2014a). Associational resistance conveyed by birch can be explained by fast-growing non-host trees disrupting host recognition by PPM (Jactel et al. 2011, 2015; Damien et al. 2016). This phenomenon has been found to be dependent on the relative size of pines versus broadleaved species, whereby stronger protection was provided by broadleaved trees taller than pines (Dulaurent et al. 2012; Damien et al. 2016). Consistently, we reported that, for a given pine density, the presence of birch in experimental plots reduced pine apparency.

Associational resistance conveyed by birch faded with time. This finding mirrors the regular increase in pine apparency in every plot, including two-species mixtures associating pines with birch (i.e. densest mixed plots). Such a general increase in pine apparency can explain why associational resistance provided by birch faded over time: pines having eventually grown higher than birches, they were less physically hidden and may have been more easily found by gravid PPM females foraging for oviposition sites. Although the strength of associational resistance decreased with time, plots associating pines with birch remained less attacked than plots of comparable pine density in which birch was absent. Although birches did not contribute to reducing pine physical apparency after pines grew taller, they may have continued contributing to the reduction in pine chemical apparency. Specifically, the maintenance of associational resistance may be explained by birch still releasing volatile organic compounds interfering with the mating behaviour and host searching behaviour of PPM adults (Jactel et al. 2011, 2015). Further studies should aim at quantifying the relative importance of the physical and chemical disruptive cues emitted by broadleaved species.

The presence of birch reduced the probability that the same pines were repeatedly attacked by the PPM. Non-hosts act upon host colonisation by herbivores through a two-step process involving first patch selection and then resource selection within patches (Finch and Collier 2000; Hambäck et al. 2014; Verschut et al. 2016). It is therefore conceivable that birch altered pine colonisation both among and within patches, thus increasing year-to-year variability in the individual pine attack rate. The occurrence of repeated attacks on the same tree has detrimental effects on pine growth (Jacquet et al. 2013). Our finding therefore suggests that although the effect of birch on PPM infestation at the stand level weakened as pines grew, birch may have long-lasting beneficial effects on pines by reducing the number of repeated attacks. The presence of this fast-growing non-host species may allow individual trees to recover from previous PPM attack, thus improving the resilience of mixed pine plantations to PPM attacks. To the best of our knowledge, the risk of repeated attacks by herbivores has thus far never been studied and its consequences in terms of damage and tree growth clearly deserve further attention.

Pine density had opposite effects on PPM density and PPM attack rate

PPM density increased with pine density. Yet, needle biomass also obviously increased with pine density, regardless of the presence or absence of birch in experimental plots. This result is therefore fully in line with the resource concentration hypothesis that predicts an increase in (specialist)

herbivore density with an increase in herbivores' resource concentration (Root 1973; Hambäck and Englund 2005). It can be explained by plots with higher pine density emitting a greater amount of attracting cues, thereby recruiting more herbivores. Alternatively, we cannot rule out the hypothesis that the higher PPM density in plots with higher pine density was simply the result of a passive interception of flying PPM females, whereby more pines would have intercepted more moths.

By contrast, PPM attack rate decreased with increasing pine density. This result indicates that although denser pine stands attracted or arrested more PPM females, the greater number of individuals was spread among an even greater number of pine trees, thus causing the dilution of herbivores among more abundant food items. Similarly, in less dense pine stands, PPM attacks may have concentrated on the fewer pine individuals (i.e. resource dilution hypothesis, Otway et al. 2005; Bañuelos and Kollmann 2011). This result is compatible with the observation that the number of repeated attacks decreased with pine density.

The effect of pine density on PPM density weakened with time, while its effect on PPM attack rate strengthened with time. Although pine density remained roughly constant over time, PPM resource (i.e. needle biomass) consistently increased with time, regardless of pine density or the presence/absence of birch. However, the increase in needle biomass was markedly stronger in denser plots (i.e. two-species mixtures), which may have strengthened the resource dilution effect of PPM attack rate. A possible mechanism explaining these trends is that tree canopies closed faster in pure pine stands (with high pine density) in such a way that the whole plot may be perceived by flying PPM females as a single patch of resource. By contrast, in low-pine-density plots, individual pine trees that are still spatially isolated but with a large crown size may be detected as several host patches by PPM moths responding to a silhouette effect. More individual trees would thus be attacked in low-pine-density plots over time, resulting in a higher percentage of attacked trees (attack rate), whereas the number of PPM nests would be more equally distributed across plots (within a block), resulting in more uniform PPM abundance variation along the pine density gradient. This suggests that spatial among versus within patch host selection is another important dimension that has to be better taken into account in further studies in order to understand associational resistance mechanisms (Bommarco and Banks 2003; Hambäck et al. 2014). In particular, they should address at which spatial scale host choice is made by gravid OPM females and whether the same cues act equally on males and mated versus unmated females.

Conclusion and implication for the management of mixed forests

With this study, we demonstrated for the first time that tree diversity effects on insect herbivores show directional changes as the forest grows. Associational resistance faded, while effects of host density strengthened with time. Not only do our findings question the ability of short-term studies conducted in young tree diversity experiments to draw general conclusions about associational effects (including ours, Castagneyrol et al. 2013; Setiawan et al. 2014; Wein et al. 2016), but they also have potential implications for the long-term management of planted forests.

In the context of planted forests, our results demonstrate that the relative growth rate of associated tree species is a key driver of the observed temporal dynamics of associational effects. In particular, initial associational resistance triggered by fast-growing species can be offset following height dominance shifts in forest structure. A management option could thus consist in planting fast-growing non-host trees before planting the target species in order to prolong associational resistance. However, such a strategy may come with increased competition for light and nutrients. Further quantification of herbivory consequences on the yield of the target species in mixtures versus monocultures is therefore needed to evaluate critically the costs and benefits of such tree mixing strategies. Finally, whether the same processes are at play in less intensively managed forests is still an open question that should be considered by future studies addressing the biodiversity resistance relationship.

Authors' contributions

BC and HJ designed the study. BC, HJ, MT and CP collected the data. BC analysed the data, with advices from MT, MVK and CP. BC wrote the first draft. MVK, HJ and all authors contributed critically to the final version.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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