# Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest

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ABSTRACT

Neighbouring heterospecific plants are often observed to reduce the probability of herbivore attack on a given focal plant. While this pattern of associational resistance is frequently reported, experimental evidence for underlying mechanisms is rare particularly for potential plant species diversity effects on focal host plants and their physical environment. Here, we used an established forest diversity experiment to determine whether tree diversity effects on an important insect pest are driven by concomitant changes in host tree growth or the light environment. We examined the effects of tree species richness, canopy cover and tree growth on the probability of occurrence, the abundance, and volume of galls caused by the pineapple gall adelgid (*Adelges abietis* L.) on Norway spruce. Although tree diversity had no effect on gall abundance, we observed that both the probability of gall presence and gall volume (an indicator of maternal fecundity) decreased with tree species richness and canopy cover around host spruce trees. Structural equation models revealed that effects of tree species richness on gall presence and volume were mediated by concurrent increases in canopy cover rather than changes in tree growth or host tree density. As canopy cover did not influence tree or shoot growth, patterns of associational resistance appear to be driven by improved host tree quality or more favourable microclimatic conditions in monocultures compared to mixed-stands. Our study therefore demonstrates that changes in forest structure may be critical to understanding the responses of herbivores to plant diversity and may underpin associational effects in forest ecosystems.

INTRODUCTION

Producer diversity has profound ecological consequences on ecosystem function and on the structure of associated communities of consumers (Tilman et al. 1996, Hector et al. 1999, Cardinale et al. 2011). For instance, plant species diversity is frequently observed to decrease the vulnerability of a focal plant to herbivore attack (Jactel and Brockerhoff 2007, Barbosa et al. 2009). This pattern, known as associational resistance, has been well documented and is traditionally explained by two main hypotheses. The enemies hypothesis attributes associational resistance to higher predator recruitment with increasing diversity and thus a stronger suppression of herbivores (Root 1973, Grez and Gonzalez 1995, Hamback and Englund 2005, Muiruri et al. 2016). Secondly, the resource concentration hypothesis states that as herbivores frequently forage in a density-dependent manner, increasing the number of plant species at a constant plant density reduces the probability of finding a preferred host plant species, which in turn lowers herbivore abundance and damage (Root 1973, Letourneau 1987, Tonhasca 1993, Riihimäki et al. 2005). While both hypotheses are well-supported in the literature, associational effects on insect herbivores remain notoriously difficult to predict (Barbosa et al. 2009, Himanen et al. 2010, Axelsson and Stenberg 2012, Muiruri et al. 2015) partly because experimental studies of associational effects often fail to account for factors that co-vary with plant diversity (Huston 1997, Nadrowski et al. 2010). This makes it difficult to establish causal links between producer diversity and insect herbivory and limits mechanistic understanding of associational effects.

While studies reporting diversity effects on herbivores are on the rise in forest ecosystems (Jactel and Brockerhoff 2007, Moreira et al. 2014, Abdala-Roberts et al. 2015, Haase et al. 2015), the underlying biotic and/or abiotic factors driving associational resistance are rarely explored even though the size, complexity and longevity of forests can make for a more

spatially heterogeneous environment. For instance, as tree diversity increases, interactions between plant species of differing growth rates inevitably yields structurally complex forest stands with spatially variable microclimates (Chen et al. 1999). In addition, competitive interactions between species can affect relative growth rates of individual host trees (Piotto 2008, Kaitaniemi and Lintunen 2010), which in turn may influence insect herbivory at the stand-level (Haase et al. 2015). These competitive interactions may also yield changes in the light environment around a focal host tree as the extent of shading they experience by neighbours increases (Lang et al. 2011). Thus, as tree diversity increases, concurrent changes in host tree growth and proximate light conditions may play an important role in insect herbivore distributions and underpin observed patterns of associational resistance.

With growing interest in the mechanistic basis of diversity-ecosystem functioning relationships (Duncan et al. 2015, Moreira et al. 2016), the role of structural and environmental heterogeneity in forests has recently come under scrutiny. For example, Castagneyrol et al. (2013) showed that faster growing and taller trees might be more susceptible to insect herbivory, in accordance with the plant vigour hypothesis (Cornelissen et al. 2008). At the same time, Castagneyrol et al. (2013) demonstrated that associational resistance to leaf-mining insects might be driven by host trees being obscured by taller neighbouring heterospecifics as diversity increases. However, as tree diversity increases, competitive interactions between species may lead to changes in tree crown area, which in turn may affect light availability in the understorey (Lang et al. 2011). Such changes in the light environment are particularly important as they can directly or indirectly influence host plant susceptibility to insect herbivores. For example, Roberts and Paul (2006) demonstrated that shading within and between tree canopies often has a positive effect on insect herbivory due to light modulating foliar quality and increasing anti-herbivore defences. In addition, as shown by Stoepler and Rehill (2012), the effects of light on

insect herbivores may also act independently of the host plant in question. Insects may avoid host plants in high-light environments where natural enemies may be more active and effective at locating prey (Perfecto et al. 2004, Stoepler and Lill 2013) or, they may be more prone to desiccation in the warmer and drier microclimate (von Arx et al. 2012). Thus, changes in the light environment may have overarching consequences for both host trees and their insect pests and may therefore explain patterns of herbivory better than tree species richness per se. Nonetheless, the role of natural variation in the light environment in driving associational effects remains untested.

In this paper, we explore the mechanisms by which tree species diversity affects a galling adelgid (*Adelges abietis*). This adelgid forms pineapple-shaped galls on Norway spruce (*Picea abies*) and can have detrimental impact on tree health and vigour (Havill and Foottit 2007). With no known natural enemies, adelgid responses to diversity are likely to reflect interactions between the host plant and the environment rather than changes in predation risk (Björkman 1998). Previous studies have found that pineapple galls are larger in size on faster growing spruce trees (Flaherty and Quiring 2008). In addition, studies on pineapple gall adelgids have also shown that stem mothers may prefer to initiate galls and oviposit on unshaded branches (Fidgen et al. 1994) indicating that the light environment may be important in the selection and performance of pineapple galls. Therefore, we hypothesised that effects of tree species richness on pineapple gall adelgids are mediated either by changes in tree size or in shading by neighbouring tree species.

Our study was conducted in the Satakunta forest diversity experiment in south-west Finland, where we explored effects of tree species diversity on the (i) presence, (ii) abundance and (iii) volume of pineapple galls on spruce trees growing in 1, 2, 3 and 5-species mixtures. These

5

three variables were chosen to help identify the proximate mechanisms that might influence (i) the selection of trees by stem-mothers, (ii) the accumulation of pineapple gall adelgids on a given tree and, (iii) the performance of mothers and offspring in ensuring gall development. We also measured tree height and diameter as well as canopy cover around each focal spruce tree in pure and mixed-species stands and used structural equation modelling to determine whether gall distributions along richness gradients are driven directly or indirectly by changes in spruce tree size or shading by neighbouring trees. Therefore, by examining herbivore responses to variation in host plant growth and light conditions across the diversity gradient, this study advances our understanding of mechanistic links between plant diversity and insect herbivores.

METHODS

*Study species*

The pineapple gall adelgid, *Adelges abietis* (Hemiptera: Adelgidae), is an aphid-like insect that is monophagous on Norway spruce where it forms pineapple-shaped galls (Havill and Foottit 2007). The entire life cycle is completed on spruce and most individuals stay on the tree on which they were born leading to a highly clustered distribution (Havill and Foottit 2007). The parthenogenetic females, known as fundatrices, overwinter with their stylets inserted in a bud and if attack is successful, the bud develops into a gall (Plumb 1953). Once stem-mothers mature, they oviposit in spring, laying their eggs beneath a wax cover on the swelling bud. The newly-hatched gallicolae crawl into the gall chambers and their feeding enhances further development to form pineapple-shaped galls (Fig. 1a). The resulting multi-chambered pineapple gall grows and eventually dehisces in autumn when the next generation of adelgids emerge, leaving the empty gall behind (Fig. 1b, Havill and Foottit 2007).

We recorded gall presence, gall abundance and gall volume on a given tree. Gall presence reflects not only the probability of stem-mothers selecting a given tree but also the probability of stem-mothers performing well enough to successfully induce gall formation. The second variable, gall abundance, was also used as an indicator of stem-mother abundance on a given tree as all galls are initiated by one or more stem-mothers (Plumb 1953). As most adelgids remain on the same host tree on which they were born, abundance is therefore a measure of past success of gall induction on a given tree. Finally, the third variable, gall volume is considered to be a good indicator of gall performance as larger pineapple galls result from stronger galling stimulus from stem mothers (Flaherty et al. 2010). Previous studies on this adelgid species have also shown that bigger galls host a larger number and size of gallicolae that also have a higher fecundity than gallicolae from smaller galls (McKinnon et al. 1999). Therefore, gall volume indicates the performance of both stem-mothers and their daughters as well as the success of these gallicolae in producing the third generation.


*Experiment*

The study was carried out at the Satakunta forest diversity experiment (www.sataforestdiversity.org) in south-western Finland. Established in 1999, the experiment is located in three separate areas (area 1, 61°42'N, 21°58'E; area 2, 61°39'N, 22°09'E; area 3, 61°40'N, 21°42'E) planted with five tree species: Scots pine (*Pinus sylvestris*); Norway spruce (*Picea abies*); non-native Siberian larch (*Larix sibirica*); silver birch (*Betula pendula*); and black alder (*Alnus glutinosa*). Each area consists of 38 plots (20m x 20m) which are randomly allocated one of 19 treatments representing a richness gradient from monocultures to 2-species, 3-species and a 5-species mixture. Each plot, in turn, consists of 13 rows, with 13 trees planted at 1.5m intervals (total 169 trees) and tree species randomly allocated a position. To ensure establishment of trees in the experiment, all dead seedlings were re-planted in 2000 and, again,

in 2001 in plots where mortality exceeded 10%. No chemical inputs have been used in the experiment but plots have been cleared of naturally regenerating vegetation in 2010 to maintain plot treatment and species densities. During this study, plot thinning also took place in June 2013, selecting trees for removal such that species densities remained constant. However, as gall mothers overwinter on their host tree, effects of thinning on spruce trees and gall abundance were not expected to emerge during the experimental period.

*Gall measurements*

In the present study, two out of the three experimental areas were used (area 1 and area 3). We used all spruce-containing treatments available in the experiment: the spruce monoculture, four 2-species combinations (spruce + alder, spruce + birch, spruce + larch, spruce + pine), three 3-species mixtures (spruce + larch + alder, spruce + pine + birch, spruce + pine + larch) and the five species mixture (spruce + pine + larch + birch + alder). Each treatment was replicated at two plots within each study area (4 replicates in total).

In June 2013, 10 spruce trees were randomly selected from each plot and the presence and abundance of pineapple galls was recorded on eight randomly chosen branches from the mid- and upper-canopy of each tree. Twenty lateral shoots were examined per branch, in keeping with the sampling strategy used for the same adelgid species by Fidgen *et al.* (1994). Trees and shoots found to have pineapple galls were marked for subsequent sampling later in the summer season.

In August 2013, when galls had reached their final size, we assessed gall volume on all experimental spruce trees. Galls occurring on branches with more than 5 galls were excluded from this analysis as both the survival and size of emerging gallicolae has been shown to be

negatively affected by gall densities (Sopow and Quiring 2001). The length and two diameters (measured at right angles to each other) of each gall was measured with callipers and gall volume was calculated using the following equation (McKinnon et al. 1999):

$$4.1888 \times \frac{\text{length}}{2} \times \left(\frac{\text{average diameter}}{2}\right)^2$$

With these data we observed no significant relationship between gall numbers and gall volume ($\chi^2=0.68$, df=1, p=0.409), thus our measures of gall volume were independent of gall abundance. To explore the relationship between gall volume and the number of gallicolae, 50 galls of different size were collected from spruce trees in different plots and treatments. The volume of each gall was recorded as above and galls were dissected to count the number of feeding cavities in each gall. A strong positive relationship was observed ($R^2=0.518$, Supporting Information Appendix 1) confirming that gall volume is a good indicator of stem-mother fecundity.

*Canopy cover and tree size*

To determine changes in canopy cover with tree species richness, we measured the canopy cover around all experimental spruce trees in June 2013. We used the GRS densitometer™ (Geographic Resource Solutions, Arcata, CA, USA) to record the percentage of views obstructed by canopy at 10 evenly-spaced positions around each of the spruce trees. Such visual estimates are commonly used to assess light availability with evidence that canopy cover assessments are well correlated with light intensity (Pannek et al. 2013). Measurements were taken around the edge of the focal tree crowns. As spruce tree crowns are conical in shape, increases in percentage canopy cover reflect increased shading by neighbouring trees and not self-shading.

In August 2013, both the height and diameter at breast height (1.3m, DBH) were measured for all experimental spruce trees as a measure of cumulative growth since the start of the experiment. In addition, on trees harbouring galls, the lengths and diameters of the shoot fostering the gall ("mother shoot") and another shoot on the same branch ("neighbouring shoot") were measured. These measurements were used as indicators of the growth potential of the galled shoot which is assumed to be positively related to adelgid performance (Björkman 1998).

*Statistical analysis*

To aggregate branch-level measurements of galls to the tree-level, we calculated the mean gall volume as well as the total number of galls observed on all sampled branches per tree. Thus, gall presence equates to the presence of at least one gall on one of the eight sampled branches and gall abundance to the total number of galls found on these branches. As only 113 trees of the 353 sampled were infested with galls, we firstly constructed zero-altered and zero-inflated Poisson models to account for the excess zeroes in the data (Zeileis et al. 2008). Results were comparable between zero-altered models, where ungalled trees are excluded from the count part, and zero-inflated models where all trees are included (Supporting Information Appendix 2). However, results differed between the binary and count parts of the models and we therefore performed all analysis on gall presence/absence and gall abundance separately to identify distinct mechanisms driving the presence and density of galls. Additionally, to better separate stem mother preferences for a given host tree (indicated by gall presence) from mechanisms governing the accumulation of pineapple galls on the same host tree, we also omitted ungalled trees in all subsequent analyses of gall abundance.

For each of the three response variables (gall presence/absence, gall abundance, and gall volume), we performed analyses to determine their responses to tree species diversity, changes in canopy cover, and the size of the focal tree. Effects of tree species composition on galls were also tested but as these effects were negligible, we focus on tree species richness only. Initial models were fitted to test for differences in gall presence, abundance and volume between the two study areas (area 1 and 3) and between thinned and unthinned plots. While gall densities were higher in area 3, effects of richness, canopy cover or tree size on galls were independent of study area or thinning (Supporting Information Appendix 3 Table A2). Therefore, we present results from models fit to data pooled from both study areas and both thinned and unthinned plots.

All of the analyses were conducted in R software (R Core Team 2015) using the lme4 package (Bates et al. 2012). All models featured "plot" as a random factor and area as a fixed factor as well as either (1) tree species richness, (2) canopy cover, (3) tree height or (4) tree DBH as additional continuous explanatory variables. Additional models were also constructed to explore whether effects of canopy cover were dependent on tree size by including either tree height or DBH in interaction with canopy cover (canopy cover x height/DBH). Generalized linear mixed models (GLMMs) were used for predicting gall presence/absence and gall abundance. Gall presence/absence was modelled with a binomial error structure and gall abundance with a Poisson error structure in GLMMs. Linear mixed models (LMMs) were used for gall volume as data were successfully log transformed to meet assumptions of homogeneity of variance.

To determine the effect of stand species richness on tree size, we used LMMs to assess changes in tree height or DBH with increasing species richness. Although spruce tree heights and DBH

were positively correlated ($\chi^2$=1081.7, df=1, p<0.001), previous studies have observed stronger effects of tree density and diversity on tree diameter rather than height (Lanner 1985, Piotto 2008). Thus, both variables were tested here separately to isolate any differences in their responses to tree species richness and their effects on pineapple galls. For canopy cover, as it was estimated as a percentage of obstructed canopy (in increments of 10), effects of tree species richness were tested in GLMMs where canopy cover was modelled as a binomial response variable (percentage canopy / percentage sky) bounded between 0 and 100. Relationships between canopy cover and tree size were also explored by repeating these binomial GLMMs with either tree height or tree DBH in place of tree species richness. All models for canopy cover and tree size were finally repeated with gall presence included as an additional explanatory variable. This was done to determine whether infested spruce trees exhibited different properties to ungalled trees across gradients of species richness and canopy cover and, therefore, ascertain whether stem-mother preferences are context-dependent. For these and earlier models, chi-squared and *p* values are reported from an ANOVA of (G)LMMs using the car package in R (Fox and Weisberg 2011).

*Structural equation modelling*

Piecewise Structural Equation Models (piecewise SEM) were used to test the hypothesis that tree species richness effects on pineapple galls are mediated by concurrent changes in spruce tree size or canopy cover around spruce trees. In contrast to traditional SEMs, piecewise SEMs permit the inclusion of hierarchical and non-normally distributed data by piecing multiple (G)LMMs into one causal framework (Lefcheck 2015). However, as piecewise SEMs do not permit inclusion of covariance structures, models were fit to separately test whether tree species richness effects on pineapple gall presence are mediated by changes in either canopy cover, tree height or tree DBH.

The "piecewiseSEM" package in R (available at https://github.com/jslefche/piecewiseSEM) was used to generate the causal network with all component models fit with (G)LMMs as described earlier. Overall fit of the models was assessed using Shipley's test of direct separation which evaluates the probability that none of the paths missing from the hypothesised network contain useful information (Shipley 2009). Models were rejected if a chi-squared test of Fisher's C statistic fell below the significance level ($p < 0.05$) indicating that models are inconsistent with the data. Accepted models were then compared using the second-order Akaike's Information Criterion (AICc) which increases as the relative likelihood of the model decreases (Burnham and Anderson 2004). Models with $\Delta AICc \geq 10$ are considered to be unsupported by the data and can therefore be omitted. For all three pineapple gall variables, attempts to incorporate both canopy cover and tree size variables in the SEM led to a large increase in AICc ($\Delta AICc \geq 10$) and therefore, we only present models including either canopy cover or tree size variables individually.

RESULTS

*Effects of tree species richness, canopy cover and spruce tree size on galling adelgids*

The likelihood of galls being present on spruce trees decreased significantly with the number of tree species per plot (Fig. 2a, Table 1). In contrast, the abundance of pineapple galls on infested spruce trees did not vary with plot species richness (Fig. 2b, Table 1). Gall volume decreased with tree species richness but this effect was only marginally significant (Fig. 2c, Table 1). A similar pattern was observed for canopy cover with the probability of gall presence halving as canopy cover increased from 0 (focal tree completely unshaded) to 100 (focal tree completely shaded) (Fig. 2a, Table 1). As with tree species richness effects on galls, canopy cover had no effect on the number of galls on spruce (Fig. 2b, Table 1) but gall volume

13

decreased as canopy cover increased (Fig. 2c, Table 1). In contrast, effects of tree size were largely positive. Both gall presence and abundance increased with spruce tree height but only gall presence was significantly influenced by tree DBH (Fig. 2a and b, Table 1). Gall volume was not significantly affected by either tree height or DBH (Fig. 2c, Table 1).

In tests of interactive effects of canopy cover and tree size on pineapple galls, we found that effects of canopy cover were dependent on spruce tree diameter but not tree height (Supporting Information Appendix 4). Effects of DBH on gall presence were stronger as canopy cover increased (canopy cover x DBH: $\chi^2$=3.93, df=1, p=0.048), thus galls were least likely to be present on small trees growing under high canopy cover (Supporting Information Appendix 4 Fig. A2a). In addition, for gall abundance, we found that positive effects of tree size were reversed when canopy cover was high (canopy cover x DBH: $\chi^2$=5.95, df=1, p=0.015). Galls were therefore most abundant on large unshaded or, small shaded spruce trees (Supporting Information Appendix 4 Fig. A2b).

Within trees infested by galls, the size of shoots had no effect on the number of galls per tree (p>0.530), but it did have a positive effect on gall volume. Both the average shoot length and diameter were positively related to mean gall volume on individual trees (shoot length: $\chi^2$=5.34, df=1, p=0.021; shoot diameter: $\chi^2$=3.85, df=1, p=0.049). Thus, although tree height had no direct effect on gall volume, differences in shoot size may have impacted gall development. However, as shoot size was unrelated to tree species richness (Supporting Information Appendix 5 Table A5), this cannot explain species richness effects on pineapple galls.

14

Relationships between tree species richness, canopy cover and spruce tree size

Preliminary analysis revealed that canopy cover around spruce trees was significantly lower in area 3 compared to area 1 (Supporting Information Appendix 3 Table A2). Tree species richness had a strong positive effect on canopy cover with focal spruce trees being more shaded in mixed stands than in spruce monocultures ($\chi^2$=11.7, df=1, p<0.001, Fig. 3a). This effect was independent of study area (richness x area: $\chi^2$=0.0, df=1, p=0.947) or thinning (richness x thinning: $\chi^2$=0.01, df=1, p=0.914). Effects of tree species richness on canopy cover were also consistent between galled and ungalled trees (Fig 3a, richness x gall presence/absence: $\chi^2$=0.64, df=1, p=0.425) but the vast majority of gall-infested trees had less than 50% canopy cover by neighbouring trees (Fig. 3a).

At the time of the study, spruce trees averaged 548.7 ± 2.3 cm in height and 59.1 ± 0.3mm in DBH and did not differ in size between study areas or in thinned verses unthinned plots (Supporting Information Appendix 3 Table A2). We observed that tree size generally decreased with tree species richness (Fig. 3b, c), however, this pattern was not significant (height: $\chi^2$=1.40, df=1, p=0.236 and DBH: $\chi^2$=3.09, df=1, p=0.079). Examining galled and ungalled trees separately, we observed that effects of tree species richness on spruce size differed between infested and uninfested trees (Fig. 3b, c). Negative effects of tree species richness on tree size were observed for ungalled trees while trees with galls were equivalent in size regardless of plot species richness. Although this was only significant for tree DBH (Fig. 3c, richness x gall presence: $\chi^2$=4.1, df=1, p=0.046) and not tree height (Fig. 3b, $\chi^2$=2.6, df=1, p=0.108), the consistent pattern suggests that adelgids counteract the negative effects of species richness by selecting the largest trees in more diverse stands. These preferences are reflected in our previous analysis of interactions between canopy cover and tree size (Supporting Information Appendix 4) and in relationships between canopy cover and tree size as we observe

that galled trees are consistently larger than ungalled trees where canopy cover is high (Fig. 3b and c, canopy cover x galls presence/absence: p<0.001).

*Structural Equation Modelling*

For gall presence, only one SEM model was selected demonstrating that effects of tree species richness on the probability of galls being present is almost entirely mediated by changes in canopy cover (Fig. 4a). Comparable models with canopy cover replaced by either tree height or tree DBH were a poor fit to the data (p<0.05, Fig 4a) and more complex models incorporating tree size and canopy cover simultaneously did not improve model fit. In contrast, SEMs for gall abundance did not reveal any direct or indirect effects of tree species richness on gall abundance (Fig. 4b). Although the top model with canopy cover and tree species richness was also selected (p>0.05, $\Delta$AICc$\geq$0.140), it was no different to similar putative models with height or DBH ($\Delta$AICc<10). Even though strong effects of tree species richness on canopy cover were detected, this was uncoupled from any effect of tree size on gall abundance. Finally, SEMs for gall volume revealed a similar pattern to that seen for gall presence in that the top selected model (p>0.05, $\Delta$AICc$\geq$3.63) included tree species richness and canopy cover only (Fig. 4c). As $\Delta$AICc between this top model and other candidate models was low ($\Delta$AICc<10), we could not omit these models entirely (Fig. 4c). However, since estimates for tree species richness effects on either tree height or DBH were small (<0.1), we conclude that tree size is not a strong determinant of gall volume across the diversity gradient.

DISCUSSION

While plant associational effects on insect herbivores have been studied extensively in the literature, experimental data on mechanisms driving patterns of associational resistance (or susceptibility) are lacking (Barbosa et al. 2009). In addition, although plant responses to

herbivory are well known to depend on both plant growth and environmental conditions (Roberts and Paul 2006, Cornelissen et al. 2008), these factors are scarcely ever included in models of biodiversity and ecosystem functioning. Of the few studies that address this discrepancy, Castagneyrol et al. (2013) is the only known study to demonstrate that associational resistance in forest ecosystems can be mediated by simultaneous changes in stand structure with diversity. Here we not only show that changes in stand structure drive associational resistance, but we go one step further with structural equation models to directly test for causal links between tree species richness, changes in canopy cover or tree size and the presence of an important insect pest. We observed that, despite weak positive effects of tree growth on pineapple gall adelgids, changes in canopy cover with tree species richness underpin associational resistance of focal spruce trees to pineapple gall adelgids. Thus, relationships between producer diversity and insect herbivores may be the result of predictable changes in environmental conditions.

*Canopy cover as a mechanism driving associational resistance*

According to the resource concentration hypothesis, the density of pineapple gall adelgids would be expected to decrease with tree species richness as a result of reduced host plant density which, in turn, decreases the likelihood of detection of preferred hosts (Root 1973). While we did observe associational resistance in this experiment, this appeared to be independent of resource concentration as tree diversity had no direct effect on any of the three gall responses. Instead, the effects of tree species richness were mediated by the concurrent increases in canopy cover. Strong relationships between tree species richness and canopy cover were, most likely, due to the fact that spruce was one of the shortest tree species planted in the Satakunta experiment (Muiruri et al. 2015). As a result, spruce trees are frequently shaded by the faster growing tree species in mixed stands (silver birch, larch and Scots pine) and are

therefore less likely to host galls. To the best of our knowledge, this study is the first demonstration of canopy cover acting as a mediator of associational resistance to insect herbivores.

Given that spruce tree growth was unaffected by changes in canopy cover, we suggest that observed tree species richness effects on pineapple galls were driven by some property relating to canopy cover by neighbouring trees. Although we did not directly measure light, a recent study by Pannek et al. (2013) showed that visual estimates of canopy cover correlate well with measures of light intensity in over 100 deciduous forests. Thus, patterns of associational resistance may reflect stem mother preferences for high-light environments leading to selection of trees with canopy cover below 50% (Fig. 3) and initiation of galls in the mid- and upper-canopy branches which are not shadowed by other branches (Fidgen et al. 1994). Similar light-dependent responses of herbivores are documented with light commonly found to suppress herbivory by leaf-chewing insects (Roberts and Paul 2006). However, several studies also find the opposite that light may promote herbivory as a result of concurrent increases in foliar quality or reduced anti-herbivore defences (Roberts and Paul 2006 and references therein). In our study system, for example, stem mothers may choose to oviposit on upper shoots where nitrogen content may be double that in the lower canopy (Tarvainen et al. 2013). In addition, it could be that unshaded spruce trees in monocultures are nutritionally superior compared to spruce trees in mixed stands. However, recent studies exploring changes in host plant quality with diversity have been unable to link observed changes in host chemistry with species richness effects on primary consumers (Mraja et al. 2011, Moreira et al. 2014, Wäschke et al. 2015). Thus, the role of canopy cover in driving patterns of associational resistance may not necessarily be mediated by changes in host plant foliar quality.

In the literature on insect responses to light, there are clear examples of direct herbivore responses to the environment outweighing higher host quality (Sipura and Tahvanainen 2000, Stoepler and Rehill 2012). For instance, work by Sipura and Tahvanainen (2000) demonstrated that, despite better quality foliage in shaded environments, leaf beetles performed better in open habitats where higher and more variable daily temperatures accelerate larval growth. A similar mechanism may occur here as reduced canopy cover in monocultures may result in higher temperatures (Morecroft et al. 1998, Chen et al. 1999) but also increase the variability of the understorey microclimate (Rambo and North 2009, von Arx et al. 2012). At the same time, higher temperatures in unshaded monocultures may increase evaporative demand (Matejka et al. 2004), ensuring the delivery of water and nutrients to stem mothers and developing galls. Even if adelgid offspring are insensitive to the external environment within the gall, they may benefit from the higher transpiration rates in open, species-poor habitats where spruce trees are better nutritive sinks as compared to spruce trees in mixed, shaded stands. More generally, it seems that associational resistance to pineapple gall adelgids may be mediated by changes in canopy cover and microclimatic differences along the species-richness gradient.

*Effects of host tree growth on pineapple galls*

Previous work on pineapple gall adelgids has found that adelgids often prefer and perform better on faster growing modules or trees (McKinnon et al. 1999, Flaherty et al. 2010). Our results support the above conclusions and show that pineapple galls benefit from increased tree growth in accordance with the plant vigour hypothesis (Cornelissen et al. 2008). However, we found that associational resistance of spruce to adelgids was not due to changing spruce vigour as spruce growth was not affected by tree species richness. The only indication that tree species richness and spruce growth may interact was that, even though spruce tree size generally decreased with tree species richness, trees harbouring galls were consistent in size and

19

significantly higher in DBH compared to uninfested trees in mixed plots (Fig. 3c). In contrast to previous studies (Cunningham et al. 2006), we found that spruce tree growth did not generally correlate with canopy cover but galled trees were larger in shaded plots (Fig 3). As galls were also more likely to be found on trees with large DBH, even those growing under high canopy cover (Supporting Information Appendix 4), it appears that stem-mother preferences for larger trees may act to counter the negative effects of tree species richness. However, our findings also suggest that even if stem-mothers settle on small spruce trees in shaded, mixed stands, adelgids may still persist and accumulate (Supporting Information Appendix 4) possibly to avoid the risks associated with migration (Hopper 1999, Havill and Foottit 2007). Thus, although increased canopy cover in high species mixtures reduces the probability of infestation and resulting gall size, it does not guarantee immunity from or prevent the proliferation of adelgids on a given host tree.

*Conclusions*

Results from this study show that although tree growth may be an important determinant of adelgid success, changes in the light environment around spruce predict the presence and fecundity of pineapple gall stem-mothers better than tree species richness per se. Furthermore, tree species richness may have no direct effects on pineapple galls but the associated increase in the density of taller heterospecific neighbours may instead drive changes in pineapple gall presence and volume by modifying canopy cover. From an applied perspective, our findings indicate that mixed planting of spruce trees with faster-growing heterospecifics may offer an alternative and important strategy in adelgid pest management. This is important as few management options exist for gall-forming adelgids because insecticide sprays are ineffective on adelgid offspring sheltered within galls and adelgids also have few natural enemies that could offer sufficient biological control (Havill and Foottit 2007). From a theoretical

viewpoint, our results suggest that canopy cover may be critical to understanding the mechanisms of associational effects in forest ecosystems. Our findings also indicate that future studies may benefit from considering herbivore presence separately from abundance to improve our understanding of plant-insect interactions with changing producer diversity. More generally, this study highlights the importance of examining both direct and indirect effects of plant diversity on consumers in order to develop a mechanistic understanding of diversity-functioning relationships.

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TABLE

Table 1. Factors affecting pineapple gall presence, abundance and volume on spruce. Separate models were run for each of the three gall response variables with either tree species richness, canopy cover, tree height or tree diameter at breast height (DBH) as explanatory variables. Area was included in models (not in interaction) with gall presence and abundance but is omitted here for clarity. Significant effects are shown in bold text.

| Response variable | | $\chi^2$ | df | p |
|---|---|---|---|---|
| Gall Presence | **Richness** | **9.1** | **1** | **0.003** |
| | **Canopy Cover** | **21.7** | **1** | **<0.001** |
| | **Tree Height** | **4.5** | **1** | **0.034** |
| | **Tree DBH** | **8.5** | **1** | **0.004** |
| | | | | |
| Gall Abundance | Richness | 0.40 | 1 | 0.527 |
| | Canopy Cover | 0.23 | 1 | 0.628 |
| | **Tree Height** | **5.17** | **1** | **0.023** |
| | Tree DBH | 0.67 | 1 | 0.413 |
| | | | | |
| Gall Volume | Richness | 3.8 | 1 | 0.052 |
| | **Canopy Cover** | **8.7** | **1** | **0.003** |
| | Tree Height | 1.3 | 1 | 0.263 |
| | Tree DBH | 2.0 | 1 | 0.156 |

FIGURE LEGENDS

Figure 1. The pineapple gall (*Adelges abietis*) in spring (a) and, after dehiscence, the empty gall left-behind the following year (b).


Figure 2. Gall responses to tree species richness, canopy cover, spruce tree height and DBH. Changes in the probability of galls occurring on spruce, the abundance of galls on galled trees and, their volume are shown in panels a, b and c respectively. Smoothed means are shown in red for each plot.


Figure 3. Relationships between tree species richness, canopy cover, tree height and tree DBH. Smooth density estimates are drawn for each variable - (a) canopy cover, (b) tree height) and (c) tree DBH - in the first column. Their responses to tree species richness are shown in the second column, and relationships between canopy cover and tree height/DBH are plotted in the third column. In all cases, data are shown from spruce trees where galls were either absent or present. Overall effects (black, dashed line) are also shown in the second and third columns to illustrate the mean relationships across both galled and ungalled trees.


Figure 4. Structural equation models for effects of tree species richness (RICH), canopy cover (CAN COV) and tree size (HEIG=Height, DBH.=DBH) on either (a) gall presence (GAL PRE) or (b) gall abundance (GAL ABU) and (c) volume (GAL VOL). Blue arrows indicate positive relationships and red arrows indicate negative relationships. Standardised path coefficients are indicated near the arrows and the thickness of arrows corresponds to the magnitude of these coefficients. Overall fit was evaluated using Shipley's test of d-separation: Fisher's C statistic (if p>0.05, then no paths are missing and the model is a good fit) and the second-order Akaike's

Information Criterion (AICc). Models for gall presence were a poor fit with tree height or DBH

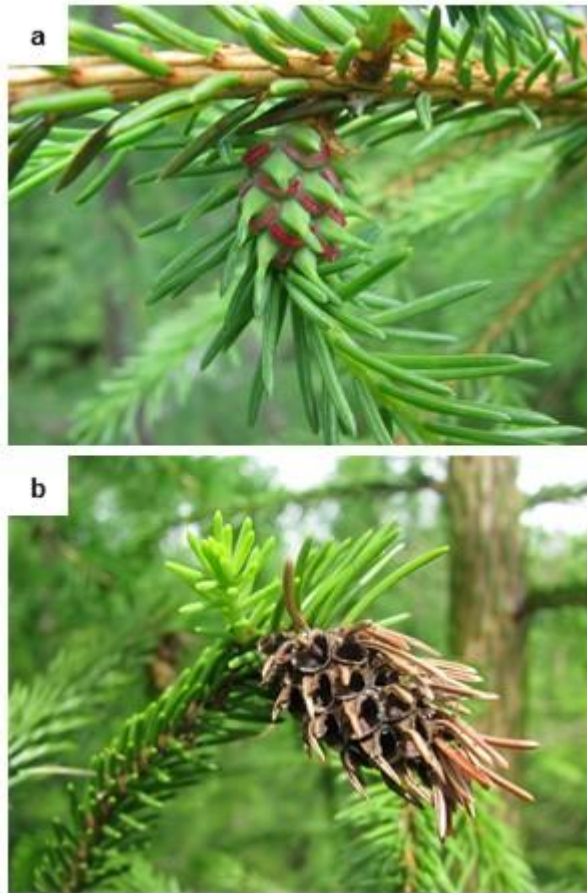(p>0.05) therefore these SEMs are illustrated in grey.

FIGURES

Figure 1

Figure 2

Figure 3

Figure 4



**a) Gall Presence**

CAN COV

RICH — 0.37 → CAN COV
CAN COV — -0.35 → GAL PRE
RICH — -0.09 → GAL PRE

C=1.88, p=0.391
AICc=29.064

HEIG

RICH → GAL PRE

C=6.71, p= 0.035*
AICc=27.353

DBH

RICH → GAL PRE

C=6.39, p=0.041*
AICc=27.033

**b) Gall Abundance**

CAN COV

RICH — 0.44 → CAN COV
CAN COV — -0.01 → GAL ABU
RICH — 0.01 → GAL ABU

C=0.13, p=0.938
AICc=22.308

HEIG

RICH — 0.05 → HEIG
HEIG — 0.12 → GAL ABU

C=0.28, p=0.867
AICc=22.458

DBH

RICH — 0.04 → DBH
DBH — 0.08 → GAL ABU

C=0.27, p=0.875
AICc=22.448

**c) Gall Volume**

CAN COV

RICH — 0.45 → CAN COV
CAN COV — -0.23 → GAL VOL
RICH — -0.09 → GAL VOL

C=1.88, p=0.391
AICc=29.064

HEIG

RICH — 0.06 → HEIG
HEIG — 0.09 → GAL VOL
RICH — -0.20 → GAL VOL

C=5.51, p=0.064
AICc=32.694
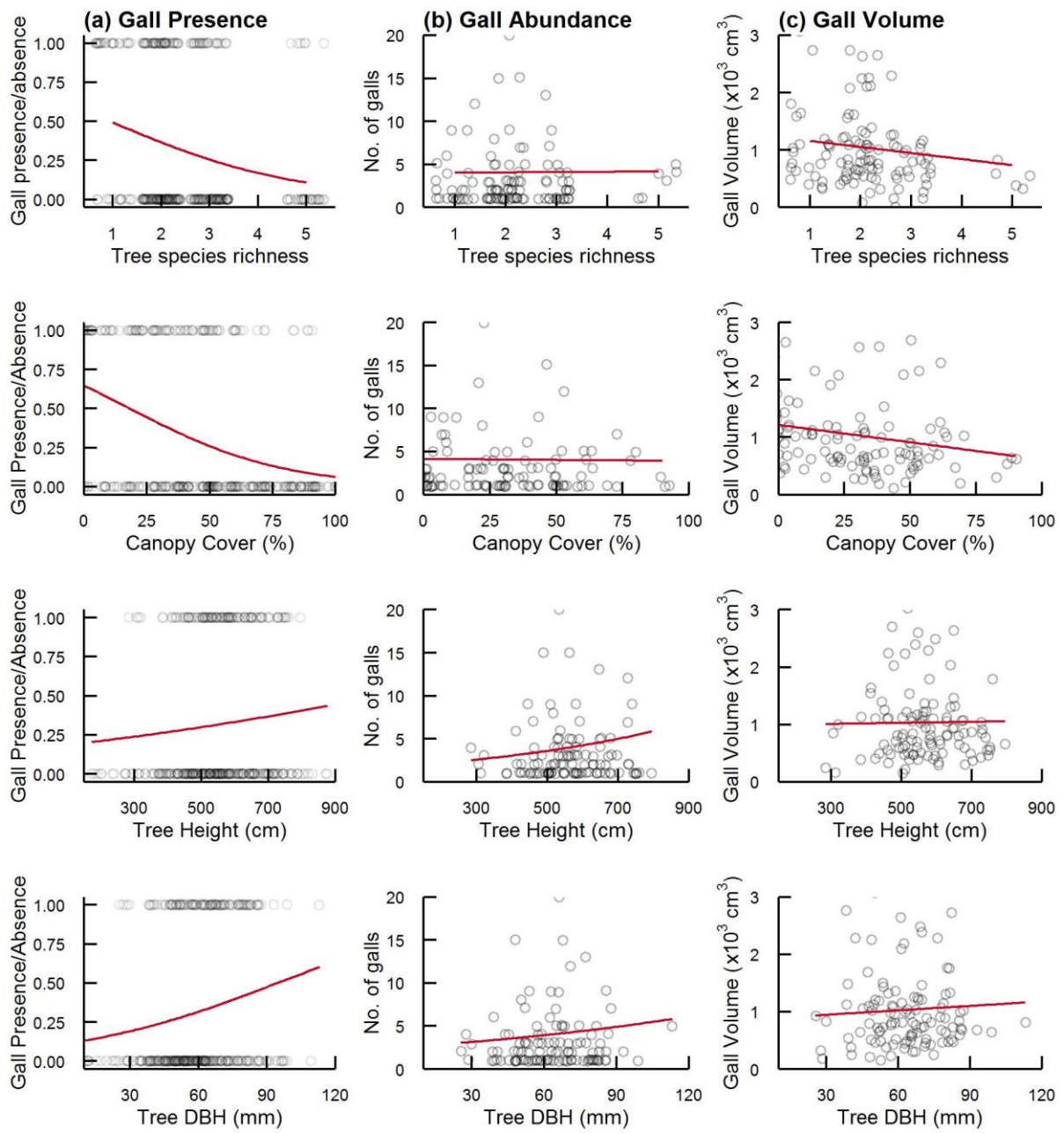
DBH

RICH — 0.05 → DBH
DBH — 0.13 → GAL VOL
RICH — -0.20 → GAL VOL

C=5.64, p=0.061
AICc=32.824
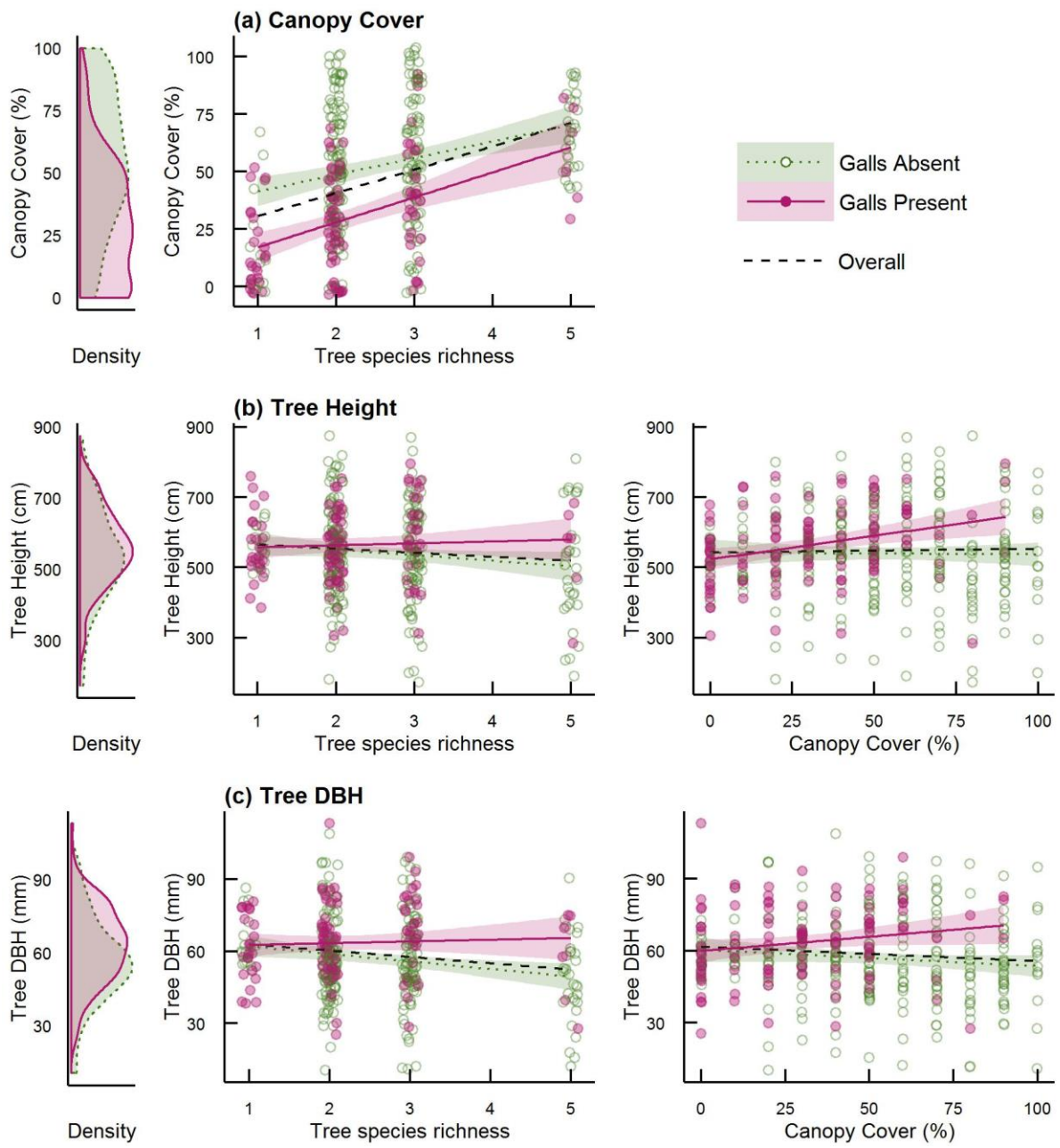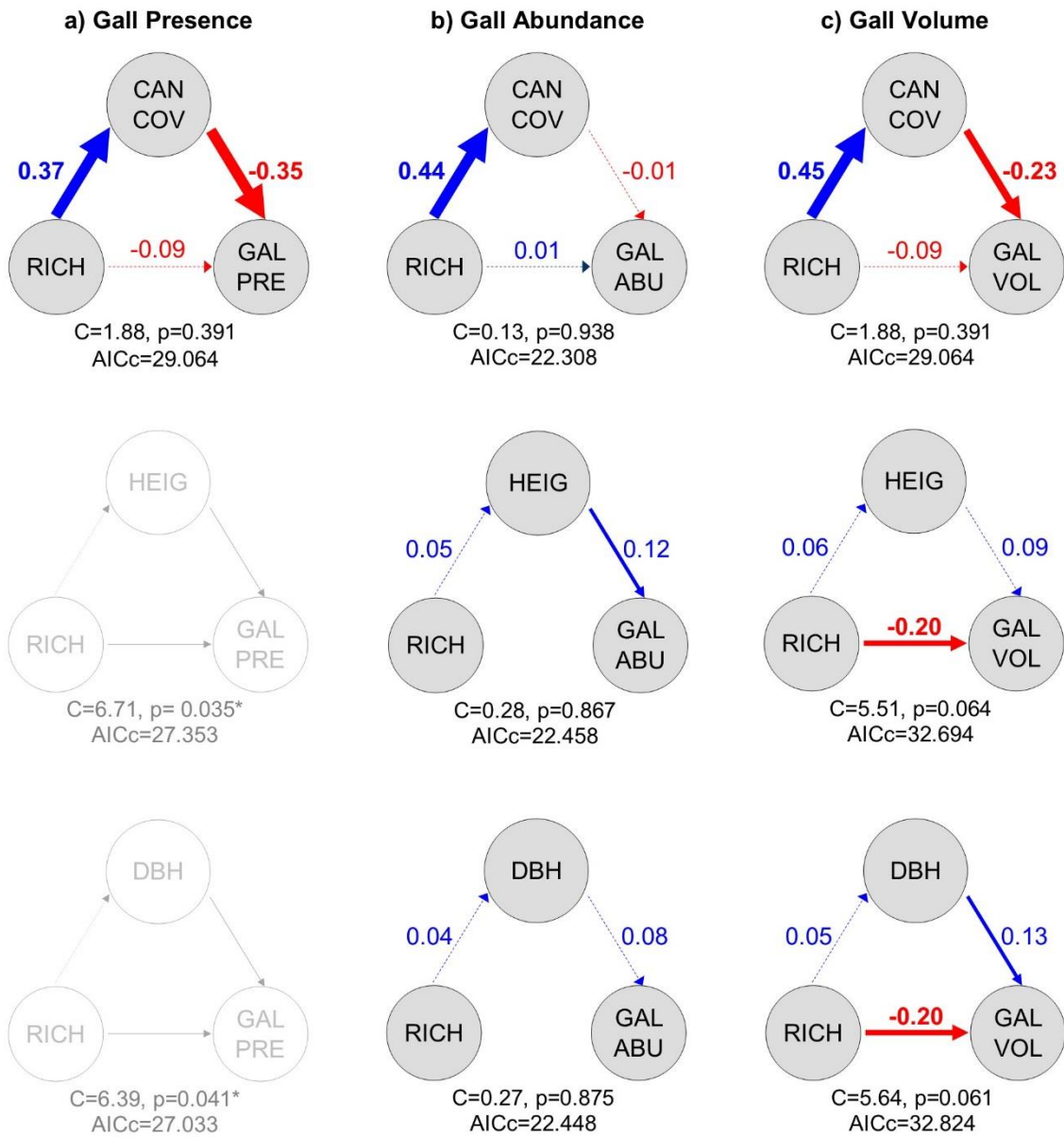
# Going undercover: increasing canopy cover around a host tree drives associational resistance to an insect pest

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## Appendix 1 – Volumetric Relationship

The size of a gall positively correlated with the number of cavities within a gall (Fig. 5.3, $F_{(1,53)}=58.7$, $p<0.0001$) therefore, gall volume can be used as an indicator of fecundity and, therefore, high-performing mothers.
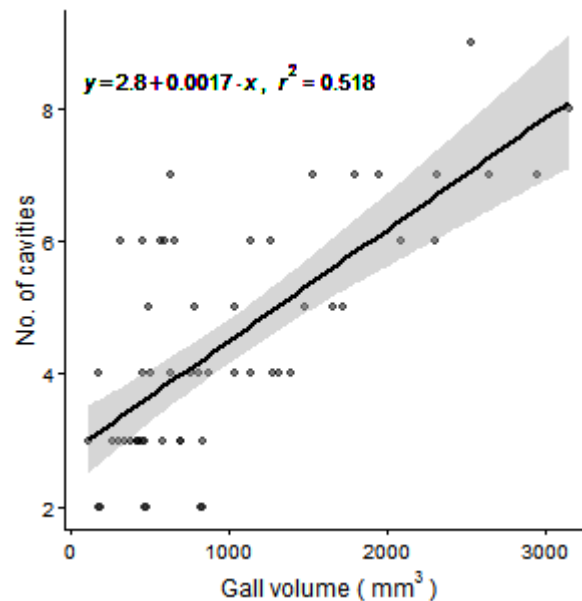


Figure A1. Relationship between gall volume and the number of cavities found in dissected galls. A smoothed mean line (±95% CI) is shown as well as the fitted equation and $r^2$ from a linear model.

**Appendix 2 – Initial analysis of all gall count data**

Using all gall count data, we initially constructed Poisson GLMMs to assess the effects of tree species richness, canopy cover, tree height and DBH. However, all models were a poor fit to the data to an excess of zero values: of the 353 experimental trees, only 113 hosted galls on the sampled branches. We therefore constructed zero-altered Poisson (ZAP) and zero-inflated Poisson (ZIP) regression models using the *pscl* package in R (Zeileis et al. 2008) to account for the large number of zeros in these data. Both ZIP and ZAP models are two part models where the chance of getting a non-zero result is modelled with a binomial distribution, and then, count data are modelled separately. The main difference between them is in a ZIP model zeroes are included in the count model and in the ZAP they are not. Therefore, we are able to test effects of each predictor variable and also investigate whether the increased information in the count data (with ungalled trees included) altered gall responses.

Table A1. Results from zero-altered (ZAP) and zero-inflated poisson (ZIP) models examining effects of tree species richness, canopy cover, tree height and DBH on gall densities. Both model types report results from the binomial and count parts of the model separately.

| | **Binomial** | | | | | **Count** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ZAP** | Estimate | SE | z | p | | Estimate | SE | z | p | |
| Richness | -0.612 | 0.148 | -4.14 | <0.001 | *** | -0.064 | 0.056 | -1.14 | 0.254 | |
| Canopy Cover | -0.850 | 0.146 | -5.81 | <0.001 | *** | 0.043 | 0.064 | 0.67 | 0.503 | |
| Height | 0.299 | 0.130 | 2.30 | 0.022 | * | 0.255 | 0.063 | 4.07 | <0.001 | *** |
| DBH | 0.483 | 0.133 | 3.64 | <0.001 | *** | 0.125 | 0.054 | 2.34 | 0.020 | * |
| **ZIP** | | | | | | | | | | |
| Richness | 0.594 | 0.151 | 3.93 | <0.001 | *** | -0.074 | 0.055 | -1.33 | 0.184 | |
| Canopy Cover | 0.891 | 0.156 | 5.70 | <0.001 | *** | 0.037 | 0.063 | 0.58 | 0.561 | |
| Height | -0.233 | 0.141 | -1.66 | 0.098 | . | 0.244 | 0.063 | 3.87 | <0.001 | *** |
| DBH | -0.489 | 0.145 | -3.38 | <0.001 | *** | 0.112 | 0.054 | 2.08 | 0.038 | * |

NB: The binary part of the models exhibits opposite signs as ZAP models predict the probability of a non-zero response and ZIP models predict the probability of excess zeros.

Comparing ZIP and ZAP models, we found that the inclusion of zeros in the count part of ZIP models yielded similar results for all four predictor variables. In addition, results from ZIP/ZAP models are similar to those reported in the main text with gall abundance influenced by tree size, especially tree height, and gall presence affected by all four predictor variables. However, as these models did not allow for the inclusion of random factors, in the main text, we prefer to report results from separate analyses of gall presence and abundance in (generalized) mixed-effects models where "plot" is specified as a random factor.

## Appendix 3 – Effects of study area and plot thinning

To determine whether observed effects might be confounded by area or thinning, we examined how the three gall response variables, canopy cover and spruce growth varied between the two study areas (area 1 and 3) and between thinned and unthinned plots.

Table A2. Effects of study area and plot thinning on the three gall responses, canopy cover and spruce growth.

|  | Study Area | | | | Thinning | | | |
|---|---|---|---|---|---|---|---|---|
|  | $\chi^2$ | df | p | | $\chi^2$ | df | p | |
| Canopy Cover | 4.36 | 1 | 0.037 | * | 6.61 | 1 | 0.010 | * |
| Tree Height | 0.69 | 1 | 0.405 | | 0.06 | 1 | 0.806 | |
| Tree DBH | 0.05 | 1 | 0.821 | | 0.02 | 1 | 0.888 | |
| Gall Presence | 16.10 | 1 | <0.001 | *** | 1.42 | 1 | 0.234 | |
| Gall Abundance | 17.29 | 1 | <0.001 | *** | 0.00 | 1 | 0.958 | |
| Gall Volume | 0.00 | 1 | 0.973 | | 0.00 | 1 | 0.995 | |

Table A3. Results from models testing whether effects of tree species richness, canopy cover and tree growth on pineapple galls differ between the two study areas. Gall presence/absence was modelled with a binomial error structure, gall abundance with a Poisson error structure in GLMMs and gall volume was log transformed to meet assumptions of homogeneity of variance. (df=1 in all cases)

| *Area\** | Richness | | Canopy cover | | Tree Height | | Tree DBH | |
|---|---|---|---|---|---|---|---|---|
|  | $\chi^2$ | p | $\chi^2$ | p | $\chi^2$ | p | $\chi^2$ | p |
| Gall Presence | 0.91 | 0.340 | 0.02 | 0.889 | 1.89 | 0.169 | 2.35 | 0.125 |
| Gall Abundance | 0.01 | 0.913 | 0.24 | 0.623 | 0.81 | 0.367 | 0.02 | 0.877 |
| Gall Volume | 0.96 | 0.328 | 2.23 | 0.135 | 1.95 | 0.162 | 1.3 | 0.258 |

Canopy cover was reduced by plot thinning but thinning had no effect on any other variable (Table A2). As galls were more likely to occur and were more abundant in area 3 as compared to area 1, we performed further analysis to test whether effects of study area might interact with any of the other variables. However, as none of the interactions with area were found to be significant (Table A3), all subsequent analyses were performed with data pooled across thinned and unthinned plots and, across both study areas.

**Appendix 4 – Interactive effects of canopy cover and tree size on pineapple galls**

We ran models to determine whether effects of canopy cover were dependent on changes in tree height or DBH finding that negative effects of canopy cover on gall presence and abundance are dependent on spruce tree size.

Table A4. Results from models testing the interactive effects of canopy cover and either tree height or diameter at breast height (DBH) on each gall response.

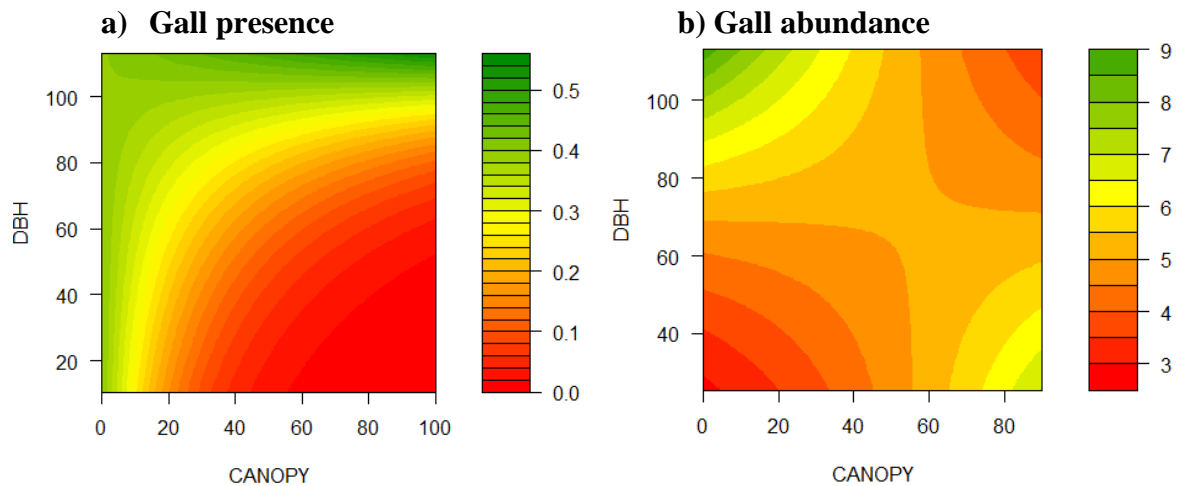| *Canopy Cover\** | Height | | | DBH | | | |
|---|---|---|---|---|---|---|---|
| | $\chi^2$ | df | p | $\chi^2$ | df | p | |
| Gall Presence | 1.66 | 1 | 0.198 | 3.93 | 1 | 0.048 | * |
| Gall Abundance | 0.16 | 1 | 0.690 | 5.95 | 1 | 0.015 | * |
| Gall Volume | 1.01 | 1 | 0.315 | 2.11 | 1 | 0.147 | |



Figure A2. Interactive effects of canopy cover and tree size on a) gall presence and b) gall abundance. Colour scale represents the increased predicted proportion of galled trees (a) or increased number of galls per tree (b) along crossed gradients of canopy cover (CANOPY, %) and diameter at breast height (DBH, mm).

**Appendix 5 – The size of galled "mother" shoots and ungalled "neighbour" shoots**
Spruce shoots infested with galls were consistently larger than ungalled shoots. Mother shoots averaged 181.8mm (±13.7) in length and 4.8mm (±0.3) in diameter while neighbouring ungalled shoots were 127.2mm (±9.2) long and 3.3mm (±0.2) in diameter. The size of galled shoots was positively related to that of neighbouring ungalled shoots (shoot length: $\chi^2$=40.8, df=1, p<0.001; diameter: $\chi^2$=46.1, df=1, p<0.001). In addition, as the height of trees increased, both galled and ungalled shoots decreased in size but tree DBH had no effect on shoot size (Table A5). Similarly, neither tree species nor canopy cover had any effect on shoot size (Table A5).

Table A5. Results from models examining the factors influencing the length and diameter of galled "mother" and ungalled "neighbour" shoots.

|  | Mother Shoot | | | | Neighbouring shoot | | | |
|---|---|---|---|---|---|---|---|---|
| *Shoot length* | $\chi^2$ | df | p | | $\chi^2$ | df | p | |
| Tree species richness | 2.00 | 1 | 0.158 | | 0.24 | 1 | 0.624 | |
| Tree Height | 3.30 | 1 | 0.069 | . | 7.13 | 1 | 0.008 | ** |
| Tree DBH | 1.29 | 1 | 0.257 | | 3.50 | 1 | 0.061 | . |
| Canopy Cover | 0.00 | 1 | 0.951 | | 0.10 | 1 | 0.755 | |
| *Shoot Diameter* | | | | | | | | |
| Tree species richness | 1.48 | 1 | 0.224 | | 0 | 1 | 0.996 | |
| Tree Height | 5.56 | 1 | 0.018 | * | 5.66 | 1 | 0.017 | * |
| Tree DBH | 2.80 | 1 | 0.094 | . | 2.58 | 1 | 0.108 | |
| Canopy Cover | 1.19 | 1 | 0.276 | | 1.19 | 1 | 0.276 | |