

# Temporal variation in plant neighborhood effects on the defoliation of primary and secondary hosts by an insect pest

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**Abstract.** Plant neighborhood effects on herbivore damage have been observed in many systems although few studies have assessed the different component effects of the neighborhood (conspecific neighbors vs. heterospecific neighbors) on defoliation. No earlier studies have monitored how temporal scale influences neighborhood effects. We tested hypotheses on resource concentration/dilution and associational effects in the eastern spruce budworm (*Choristoneura fumiferana*)–forest system over a 10-yr period across different stands including stands dominated by highly vulnerable hosts (balsam fir), stands dominated by a species with low vulnerability (black spruce), and mixed composition stands (fir and spruce). We observed persistent resource concentration effects on the primary host (balsam fir) during the increasing phase of the outbreak in balsam fir-dominated and mixed stands, while both resource dilution and associational susceptibility effects were observed on the secondary host (black spruce) and the strength of associational susceptibility increased with an increase in resource dilution. We did not observe associational effects on the primary host in mixed and secondary host-dominated stands but in stands dominated by the primary host, we observed associational resistance when the resource was highly depleted. Therefore, the complexity of neighborhood effects suggests that future studies should consider the separate effects of conspecific and heterospecific neighbors, as well as changes through time in order to predict herbivore damage in different systems, and provide better preventive and reactive strategies to manage herbivore outbreaks.

**Key words:** associational resistance; associational susceptibility; insect herbivory; outbreak herbivore; plant neighborhood; plant–herbivore interactions; primary host; resource concentration; resource dilution; secondary host; stand composition; temporal scale.

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## INTRODUCTION

Interactions between plants and defoliating insects are key processes that shape ecosystem functions and life history traits (Crawley 1983). The strength of the interactions between host plants and their defoliators can vary with host traits, the density of conspecific or heterospecific

neighbors (Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015), diet breadth of herbivores (Barbosa et al. 2009, Plath et al. 2012), and patch composition (Hambäck et al. 2014). Host density can either increase (resource concentration effects; Root 1973) or decrease attack by herbivores (dilution effects; Otway et al. 2005) through herbivore load and

feeding behavior (Root 1973). The resource concentration hypothesis holds that high herbivore abundance in pure and low-diversity mixture stands is due to the high probability of locating hosts, longer tenure time, higher feeding rates, and therefore greater reproductive success of specialist herbivores with narrow host ranges (Root 1973, Long et al. 2003). The resource dilution hypothesis, on the other hand, suggests that host plants in high-diversity mixtures experience greater herbivore loads than in pure stands (Yamamura 2002, Otway et al. 2005). However, these authors also question whether resource concentration or dilution effects change through time, suggesting that resource concentration effects may increase over multiple years of herbivory.

Other researchers have investigated the effects of compositional diversity of host plants and neighbors positing that the frequency of the neighboring heterospecific plants can either increase (associational susceptibility; Letourneau 1995) or decrease herbivore attack (associational resistance; Tahvanainen and Root 1972) on the focal host plant (Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015). These positive and negative associational effects result from indirect interactions among host species in the presence of herbivores (Barbosa et al. 2009). In contrast to resource dilution, associational resistance (Tahvanainen and Root 1972) refers to a focal host's increased resistance to defoliation by a specific defoliator that exceeds the plant's specific morphological and chemical defense traits. This is usually conferred by higher compositional heterogeneity in the surrounding plants and explained by a gain in physical or chemical resistance from the neighboring plant species and/or greater diversity and abundance of natural enemies finding habitat in the diverse neighboring species (Mello and Silva-Filho 2002). Associational susceptibility, on the other hand, involves an increase in the susceptibility of a focal host to insect damage in the presence of another plant species. It has been suggested that this could occur during outbreaks when pests reach high densities and there is spillover from a highly preferred host onto a less preferred one (Atsatt and O'Dowd 1976). However, the temporal dynamics of when this spillover occurs (at the beginning or only late in an outbreak) have not been elucidated.

Herbivore diet breadth can determine whether neighborhood effects will result in associational resistance or associational susceptibility (Andow 1991, Jactel and Brockerhoff 2007), as well as in resource concentration or resource dilution (Otway et al. 2005). Specialist herbivores appear to discriminate host plant quality more effectively than generalist herbivores and specialists have greater efficiency than generalists in finding hosts (Barbosa et al. 2009). Further, specialist herbivores are likely to exert a higher herbivory pressure on their host plants than generalists (Jactel and Brockerhoff 2007). Specialists may also be more sensitive to temporal changes in the amount of host plants as they are not able to switch to alternative hosts and are therefore more likely to respond to resource concentration (Tahvanainen and Root 1972, Root 1973) and resource dilution (Andow 1991, Yamamura 2002, Otway et al. 2005) than generalists. In contrast, generalists are more likely to be influenced by associational effects because they can occur in many stand types including in mixed species stands where there is a greater natural enemy complex to control them (Jactel and Brockerhoff 2007).

Understanding the mechanisms that govern the direction of resource concentration/dilution and associational effects requires determining how tree composition (i.e., patch diversification) affects host neighborhood effect. Previous studies of forest systems reported contrasting results, showing that mixed stands can suffer lower, higher, or similar herbivore damage compared to single-species stands (Jactel and Brockerhoff 2007, Plath et al. 2012). These equivocal results may be observed because finding host plants may be a multi-scale process for specialist herbivores (both monophagous and oligophagous; Hambäck et al. 2014), involving both among-patch and within-patch host selection, and variations through time. Composition at stand level and neighborhood may set the initial conditions for insect perception and movement. At the stand scale, patches with higher concentrations of primary hosts may attract a greater number of egg-laying females, whereas within-patch host distribution may influence the success of larval movement.

While the effects of neighboring plants on insect herbivory have been studied (Barbosa et al. 2009, Plath et al. 2012), the majority of the work on

neighborhood effects (i.e., resource concentration, resource dilution, associational resistance, associational susceptibility) comes from insect pests of agricultural crops in annual plant communities (Letourneau 1995, Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015) while there is much less information from natural forest communities (Jactel and Brockerhoff 2007). Only a few studies attempted to assess how different components of the neighborhood (conspecific neighbors vs heterospecific neighbors) affect focal host plant damage (Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015). None of the published data have monitored how neighborhood effects vary with stand composition and temporal scale to see whether the effects of the different components of local neighborhood persist or change. For example, neighborhood effects within a stand dominated by a primary host may be strongest at the beginning of an outbreak, gradually weakening as the outbreak develops due to increasing population densities of herbivores that overcome the protective effects offered by the less preferred host, or these relationships may persist consistently throughout the outbreak. On the other hand, neighborhood effects within a stand dominated by a primary host may only develop on the secondary host as the outbreak progresses and the primary host food resource is depleted and spillover occurs. Changes in damage to primary and secondary host trees as herbivore populations increase can be interpreted as the “strength of neighborhood effects.” If this effect is small, then it should be observed at the beginning of an outbreak and dissipate quickly as the outbreak progresses. However, if the effects are strong, then they should persist or augment as the outbreak increases.

Evaluating temporal scale and stand composition implications of resource concentration/dilution and associational effects for host plants is important in managing herbivore outbreaks. This knowledge is relevant to determine both the vulnerability of different forests (i.e., based on their composition) to herbivore damage and the choice of control measures against herbivore outbreaks either by planning preventive strategies through manipulation of forest composition (e.g., cleaning) and dominance (e.g., thinning) that may provide long-lasting and low cost control with low environmental impact (Muzika and Liebhold 2000) or

through reactive strategies (e.g., spraying insecticide or harvesting host plants). The evaluation of resource concentration/dilution and associational effects for host plant may provide forest managers with essential knowledge to plan forest operations and more importantly to manage for more resistant mixtures and dominances of species to herbivore damage in natural forests and plantations.

In this study, we tested resource concentration/dilution effects of conspecific neighbors and the associational effects of heterospecific neighbors on eastern spruce budworm, *Choristoneura fumiferana* Clem (Lepidoptera: Tortricidae), in three stand types (black spruce-dominated stands, mixed spruce–fir stands, and balsam fir-dominated stands) over a 10-yr period from the beginning of the outbreak. It is well recognized that balsam fir, the primary host (*Abies balsamea* [L.] Mill.), is the most vulnerable host species, while black spruce, the secondary host (*Picea mariana* [Mill.] Britton, Sterns & Poggenb.), is the least vulnerable (Hennigar et al. 2008).

We predict that (1) the effect of resource concentration/dilution on defoliation will decrease over the outbreak as insect populations increase and all hosts are located, (2) associational susceptibility will increase throughout the development of the outbreak as defoliators spill over onto secondary hosts, (3) associational effects will be strongest in mixed spruce–fir stands, while resource concentration and dilution effects will be strongest in balsam fir- and black spruce-dominated stands, respectively.

## METHODS

### Study system

The study was carried out in the boreal forest of Quebec’s North Shore region, 50 km north of Baie-Comeau (Fig. 1). The forests of this region are dominated by conifer species, mainly black spruce (*P. mariana*) and balsam fir (*A. balsamea*; De Grandpre et al. 2000). Every 30 to 40 yr, populations of the eastern spruce budworm (*C. fumiferana* Clem.) increase to outbreak levels in eastern North American boreal forests (Boulanger and Arseneault 2004, Boulanger et al. 2012). During epidemic phases, defoliation may last locally for 5–8 yr (Morris and Mott 1963, MacLean 1980, Boulanger and Arseneault 2004), during which time extensive tree mortality is observed. Eastern

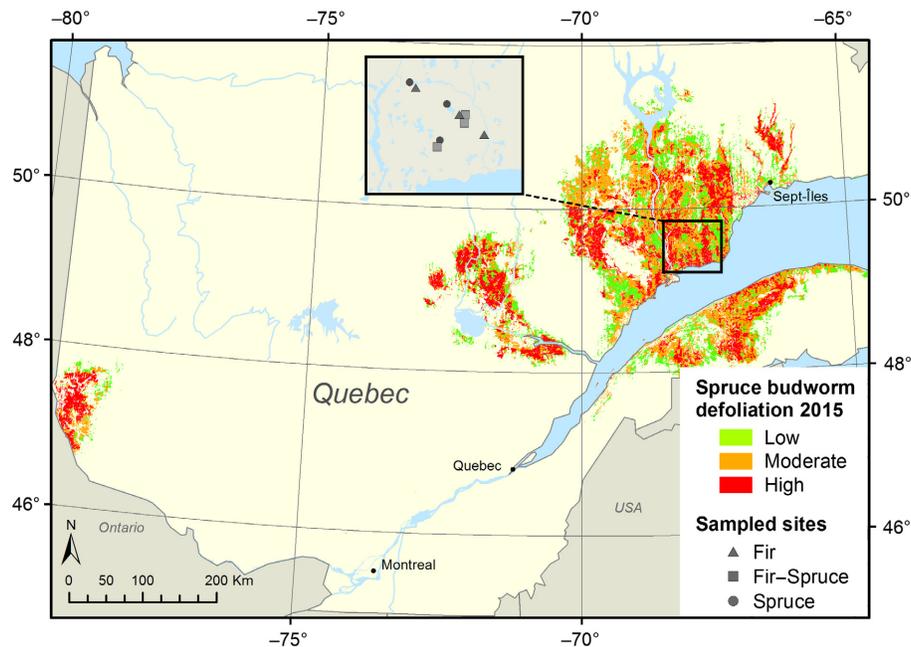


Fig. 1. Map of study location and sites with the three black spruce relative abundance classes: fir, mixed (fir–spruce), and spruce. Green: low defoliation; gray: moderate defoliation; red: severe defoliation. Triangles: balsam fir-dominated stands; squares: balsam fir and black spruce mixed stands; circles: black spruce-dominated stands.

spruce budworm is a specialist (oligophagous) herbivore, and it is known to feed on different genera, such as *Abies*, *Picea*, *Tsuga*, and *Larix*.

In 2006, the first signs of a spruce budworm outbreak were observed in the North Shore, with 2319 ha of forest defoliated (Ministère des Ressources naturelles et de la Faune du Québec 2006). In subsequent years, the area defoliated by the insect increased almost exponentially to reach 3.7 million ha in 2015 in the North Shore and 6.3 million ha in the Province of Quebec (Ministère des Forêts de la Faune et des Parcs du Québec 2015).

### Sampling

In the present study, we sampled stands that differed in their composition of balsam fir and black spruce ranging from pure black spruce, mixed spruce-fir, and pure balsam fir (Table 1). Three classes of relative abundance were considered: fir = stands with more than 74% of balsam fir; mixed = 26–74% black spruce or balsam fir; and spruce: more than 74% black spruce. Ten permanent sample plots (six plots of 40 × 100 m

and four plots of 60 × 100 m) were established between 2006 and 2009, in the epicenter of the outbreak to study long-term forest response to the spruce budworm outbreak. Three permanent plots were established in each stand type except in spruce-dominated stands where there were four permanent plots. Beginning in 2010 in all plots, two parallel transects were established on each side of the 100-m axes of each plot. Every

Table 1. Relative abundance (mean ± SE) of tree species composition in the different stand types (expressed as percentage).

Species	Balsam fir stands ( <i>n</i> = 3 plots)	Mixed fir-spruce stands ( <i>n</i> = 3 plots)	Black spruce stands ( <i>n</i> = 4 plots)
Balsam fir	69.52 ± 7.07	56.94 ± 5.73	5.01 ± 2.14
Black spruce	16.99 ± 11.64	41.74 ± 5.73	93.33 ± 1.50
White spruce	3.52 ± 1.57	0.11 ± 0.07	0.10 ± 0.07
White birch	6.96 ± 1.68	1.21 ± 0.61	0.23 ± 0.10
Aspen	4.50 ± 3.93	...	0.30 ± 0.30
Larch	...	...	0.96 ± 0.65

Note: Only living individuals with dbh ≥ 9.1 cm were taken into account in this characterization.

Table 2. Number of trees sampled per species within the three stand types.

Species	Fir stands			Mixed stands			Spruce stands			
	Fir A	Fir B	Fir C	Mixed A	Mixed B	Mixed C	Spruce A	Spruce B	Spruce C	Spruce D
Balsam fir	19	28	21	20	25	33	3	10	3	4
Black spruce	18	4	7	21	13	21	19	19	22	38

10 m along each transect, a tree of each species was sampled within a 5 m radius (if present; Table 2). The same trees were followed for the entire study. Dominance (i.e., mean basal area at breast height for species  $x$  times the density of species  $x$ ) and relative dominance (i.e., dominance of species  $x$  divided by the sum of dominance for all species) within a 5 m radius circle around each chosen tree were calculated. We used dominance instead of density (Kim and Underwood 2015) because tree dominance takes into account both the density and the size of plants. In forests where tree size differs, this measurement is more accurate than plant density alone, because the latter gives the same weight to each individual plant, while dominance measured by basal area weights individuals by their size. We considered dominance and relative dominance of focal plant neighbors within a 5 m radius ( $\approx 80 \text{ m}^2$ ) because larval dispersal from tree to tree is most effective in the vicinity of the focal plant. The fate of all trees in the permanent plots was followed yearly, and there was little basal area loss due to mortality and growth increase. In fact, tree mortality usually starts after 4–5 yr of severe defoliation (MacLean 1980) and this would correspond to 2015, the last year of sampling or later. Thus, we can consider that neighborhood changes in tree composition were minimal over the 10 yr of this project.

From the beginning to the end of the study in 2015, a 45-cm branch from each tree was sampled in August of each year (after all larvae had metamorphosed into moths, which typically occurs in mid-July in this part of the spruce budworm's range) to quantify annual defoliation. The branch was cut with a pole pruner from the mid-crown of each tree. In the laboratory, a maximum of 75 terminal shoots per branch were randomly chosen for defoliation assessment using Fettes' method (Fettes 1950). This method consists of visually estimating the percentage of needles removed from each year's shoot due to larval feeding. Shoots

were assigned a defoliation percentage (between 0% and 100%, in increments of 10%) for the amount of foliage that was consumed. The percentage of defoliation per branch was then calculated as the average of the shoots' percentage of defoliation. For the 2010 samples, defoliation was quantified as far back as 2006, which was the year when aerial surveys first reported defoliation in the region. This is a common technique for defoliators like spruce budworm that feed primarily on current-year foliage (MacLean and Lidstone 1982). Since balsam fir and black spruce produce new needles annually on the apices of leader shoots, we were able to estimate the percentage of needles removed from internodes before 2010. Both species keep their needles for a period that surpasses four years (MacLean and Lidstone 1982). Thus, annual defoliation refers to the percentage of needles removed from an annual needle cohort for each year, from 2006 to 2015.

#### Data analyses

In order to assess the effect of the co-occurrence of spruce budworm's primary and secondary hosts on defoliation over time, we modeled annual defoliation of both species as a function of species, year, stand composition using a linear mixed-effects model for repeated measures and nested data. The fixed factors were species, year of defoliation, stand composition, and their interaction. The random factor was the nested effect Stands/Plot/Tree. Serial correlation structure CorAr1 was built into the model error term to control for temporal autocorrelation.

Prior to modeling balsam fir and black spruce annual defoliation as function of dominance of conspecific neighboring plants, relative dominance of heterospecific neighboring plants, and stand composition, we assessed collinearity between balsam fir and black spruce dominance using scatterplot (Fig. 2) and variance inflation factors (VIF) of the predictors. All VIF values were below 2.5 (balsam fir:  $\text{GVIF}^2(1/(2 \times \text{df})) = 1.37$ ;

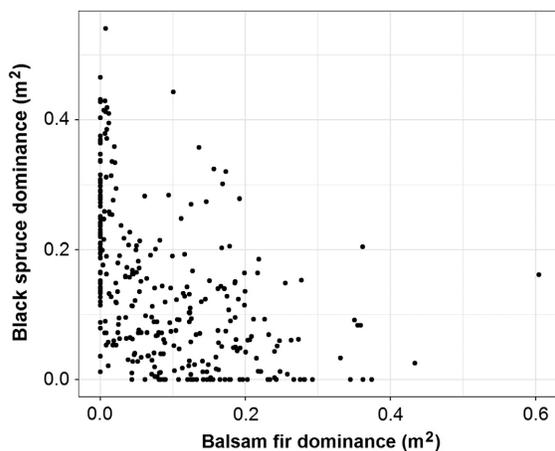


Fig. 2. Balsam fir against black spruce dominance within a 5 m radius circle (78.5 m<sup>2</sup>).

black spruce:  $\text{GVIF}^{\wedge}(1/(2 \times \text{df})) = 1.28$ ; stand:  $\text{GVIF}^{\wedge}(1/(2 \times \text{df})) = 1.22$ ), indicating that there was no collinearity between balsam fir, black spruce dominance, and stand composition (Zuur et al. 2010). We evaluated balsam fir and black spruce defoliation in relation to both dominance (resource concentration or dilution effect) and relative dominance (associational effects) of neighboring plants within a neighborhood of 5 m radius over 10 yr in stands that differed in relative abundance of black spruce. The effects of balsam fir dominance and black spruce relative dominance on balsam fir defoliation over 10 yr, and also the effect of black spruce dominance and balsam fir relative dominance on black spruce defoliation over 10 yr, were analyzed using linear mixed-effects models for repeated measures and nested data. The fixed factors were year of defoliation, stand composition, conspecific plant dominance, and heterospecific plant relative dominance. The random factor was the nested effect Stands/Plot/Tree, and the serial correlation structure to control the temporal autocorrelation was  $\text{CorAr1}$ . Balsam fir and black spruce annual defoliation were used separately as response variables. When the four-way interaction was significant, we separately analyzed the data for each stand type (in order to better interpret the relationship) using the same modeling techniques, that is, the nested effect Plot/Tree as a random factor. However, when the four-way interaction was not significant, we removed the term from the model

before re-running the final analysis (Engqvist 2005). Significant effects of balsam fir and black spruce dominance indicate resource concentration or resource dilution effects, while significant effects of balsam fir and black spruce relative dominance indicate associational resistance or associational susceptibility. All calculations, analyses, and graphs were performed in the R statistical and programming environment (R Core Team 2016) using dplyr (Wickham and Romain 2015), plyr (Wickham 2011), nlme (Pinheiro et al. 2016), and ggplot2 (Wickham 2009) packages.

## RESULTS

### Defoliation trends

Defoliation above background levels of approximately 20% started earlier, progressed significantly faster, and peaked at higher levels in balsam fir than in black spruce (Fig. 3, Table 3). The increase in defoliation in each species was more rapid in stands dominated by fir and slowest in spruce-dominated stands ( $F_{18,1828} = 3.97$ ,  $P < 0.001$ ; Fig. 3A). The increase in balsam fir defoliation in stands dominated by fir was similar to the defoliation increase in mixed stands. The peak of balsam fir (80% in 2014) and black spruce (47% in 2013 and 2014) defoliation was lowest in black spruce stands, but followed the same trend with a lag of one year behind the other stand types (Fig. 3). In the two other stand types, peak defoliation in balsam fir (90% in 2013) occurred a year earlier and was approximately 10% higher than in black spruce-dominated stands. Black spruce defoliation peaked in 2013 in all stand types, but in black spruce-dominated stands (47%), it was lower than in the other stand types (55% in mixed stands and 75% in balsam fir-dominated stands).

### Resource concentration and associational effect on balsam fir defoliation

The four-way interaction effect of year of defoliation, stand composition, conspecific plant dominance, and heterospecific plant relative dominance on the annual defoliation of balsam fir was significant ( $F_{18,724} = 1.75$ ,  $P = 0.028$ ); thus, we separately analyzed the data for each stand type. The separate analyses revealed that the effect of balsam fir dominance was moderated by black spruce relative dominance over time only in balsam

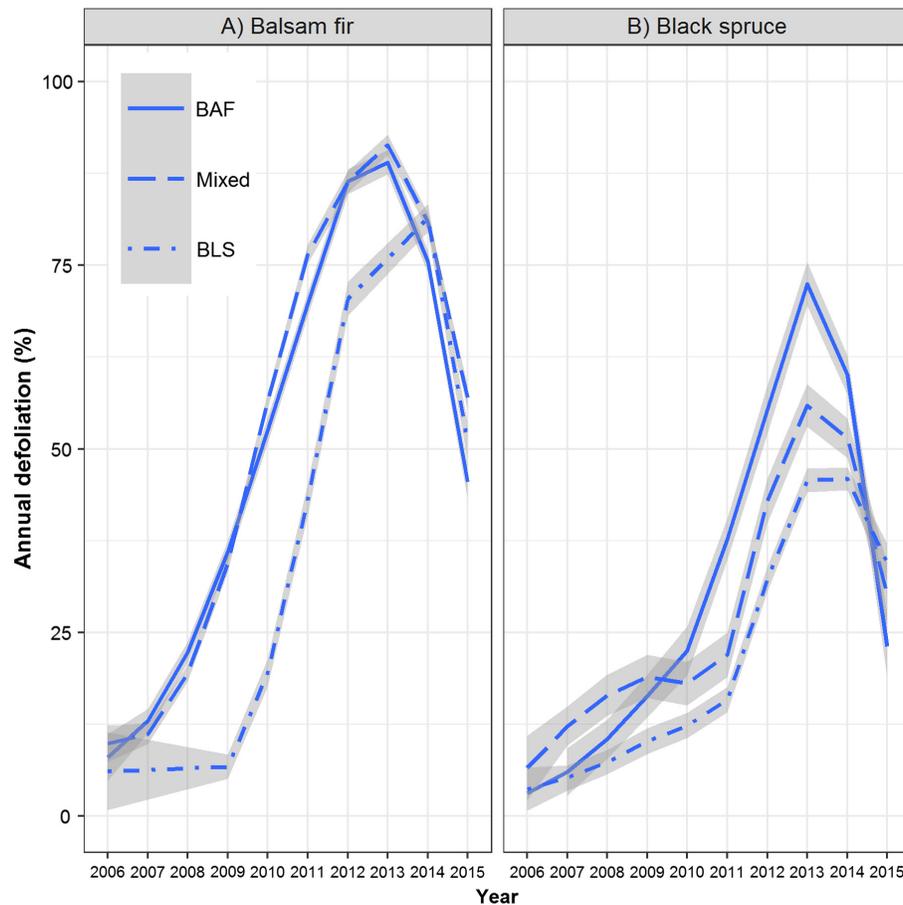


Fig. 3. Defoliation (mean  $\pm$  SE) trends of balsam fir (A) and black spruce (B) within different stand compositions (BAF: balsam fir stands; BLS: black spruce stands; mixed: balsam fir and black spruce mixed stands).

fir-dominated stands (Table 4A, Fig. 4). Balsam fir defoliation increased significantly as its dominance increased (i.e., resource concentration) during the increasing phase of the outbreak (2007–2010) and

Table 3. Summary of linear mixed-effects model for repeated measures and nested data to compare balsam fir and black spruce defoliation progression from 2006 to 2015.

Parameters	df	F	P
Species	1	172.47	<0.001
Stands	2	2.48	<0.001
Year	9	299.39	<0.001
Species: Stands	2	4.08	0.018
Species: Year	9	40.78	<0.001
Stands: Year	18	4.76	<0.001
Species: Stands: Year	18	3.97	<0.001

no relationship between dominance of conspecifics and balsam fir defoliation could be observed after 2010 (Fig. 4A; Appendix S1: Table S1A). The effect of black spruce relative dominance on balsam fir defoliation over time was also observed during the outbreak (Fig. 4B). During two years of the increasing (2010 and 2011) and one year of the decreasing (2014) phases of the outbreak, the annual defoliation decreased with the relative dominance of black spruce (i.e., associational resistance), while in the last year of observation, the annual defoliation increased with the relative dominance of black spruce (i.e., associational susceptibility; Fig. 4A; Appendix S1: Table S1B). Resource concentration and associational resistance effects co-occurred only in 2010.

In mixed stands, the resource concentration effect appeared in the early phase of the outbreak,

Table 4. Summary of linear mixed-effects models for repeated measures and nested data to test the effect of balsam fir dominance and black spruce relative dominance over time on balsam fir annual defoliation within (A) balsam fir-dominated stands, (B) mixed stands, and (C) black spruce-dominated stands.

Parameters	df	F	P
<b>A. Balsam fir-dominated stands</b>			
Balsam_Dom	1	0	0.905
Spruce_RelDom	1	1	0.404
Year	9	1343	<0.001
Balsam_Dom : Spruce_RelDom	1	1	0.337
Balsam_Dom : Year	9	1	0.503
Spruce_RelDom : Year	9	3	0.004
Balsam_Dom : Spruce_RelDom : Year	9	3	<0.001
<b>B. Mixed stands</b>			
Balsam_Dom	1	0.73	0.396
Spruce_RelDom	1	0.36	0.549
Year	9	269.14	<0.001
Balsam_Dom : Spruce_RelDom	1	<0.01	0.996
Balsam_Dom : Year	9	2.47	0.010
Spruce_RelDom : Year	9	1.04	0.406
Balsam_Dom : Spruce_RelDom : Year	9	1.56	0.126
<b>C. Black spruce-dominated stands</b>			
Balsam_Dom	1	0.59	0.455
Spruce_RelDom	1	0.16	0.699
Year	9	57.46	<0.001
Balsam_Dom : Spruce_RelDom	1	0.04	0.849
Balsam_Dom : Year	9	0.26	0.984
Spruce_RelDom : Year	9	0.52	0.857
Balsam_Dom : Spruce_RelDom : Year	9	0.51	0.863

but disappeared in 2011, 2012 (i.e., before defoliation peak) and 2014 (after defoliation peak) (Table 4B, Fig. 5; Appendix S1: Table S2). Neither balsam fir dominance nor black spruce relative dominance had a significant effect on balsam fir annual defoliation in stands dominated by black spruce (Table 5C).

#### *Resource concentration and associational effect on black spruce defoliation*

The four-way interaction effect of year of defoliation, stand composition, conspecific plant dominance, and heterospecific plant relative dominance on the annual defoliation of black spruce was not significant ( $F_{18,872} = 0.69$ ,  $P = 0.818$ ); thus, we dropped the four-way interaction term from the model. The effect of black spruce dominance on the annual defoliation of black spruce was significantly moderated by balsam fir relative dominance

over time (Table 5). The annual defoliation of black spruce decreased significantly with black spruce dominance (i.e., resource dilution) over time except in 2008 and 2010, while the annual defoliation increased with the relative dominance of balsam fir over time (i.e., associational susceptibility) except in the beginning of the outbreak (2006) and in the last two years of our observation (2014 and 2015) (Fig. 6A, Table 5; Appendix S1: Table S3A).

However, the effect of balsam fir relative dominance on black spruce annual defoliation over time depended significantly on stand composition (Fig. 7, Table 5). A constant positive relationship (i.e., associational susceptibility) between defoliation and relative dominance of balsam fir was observed in mixed stands (Fig. 7; Appendix S1: Table S4B). In fir-dominated stands, there was no evidence of associational susceptibility in the early years of the outbreak but as the outbreak progressed, a strong effect was observed (Fig. 7; Appendix S1: Table S4A). At the peak of the outbreak, the relationship inverted and a reduction in black spruce defoliation was observed as the relative dominance of balsam fir in the surrounding target trees increased (i.e., associational resistance). In black spruce-dominated stands, increasing relative dominance of balsam fir significantly decreased black spruce defoliation up to 2012. In the following years, the relationship switched toward a significant increase in black spruce defoliation with increasing relative dominance of balsam fir in the immediate neighborhood of the focal tree (Fig. 7; Appendix S1: Table S4C).

## DISCUSSION

The novelty of our work is not that it reiterates the host preferences of spruce budworm, but rather lies in teasing apart resource concentration/dilution effects from associational effects, providing an overall understanding of these two neighborhood components over the course of an insect outbreak. Although these two neighborhood components are often confounded (Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015), we examined the influence of conspecific dominance and heterospecific relative dominance on spruce budworm damage in order to tease apart their effects over time and across stand compositions. We observed a complex combination of resource concentration and dilution

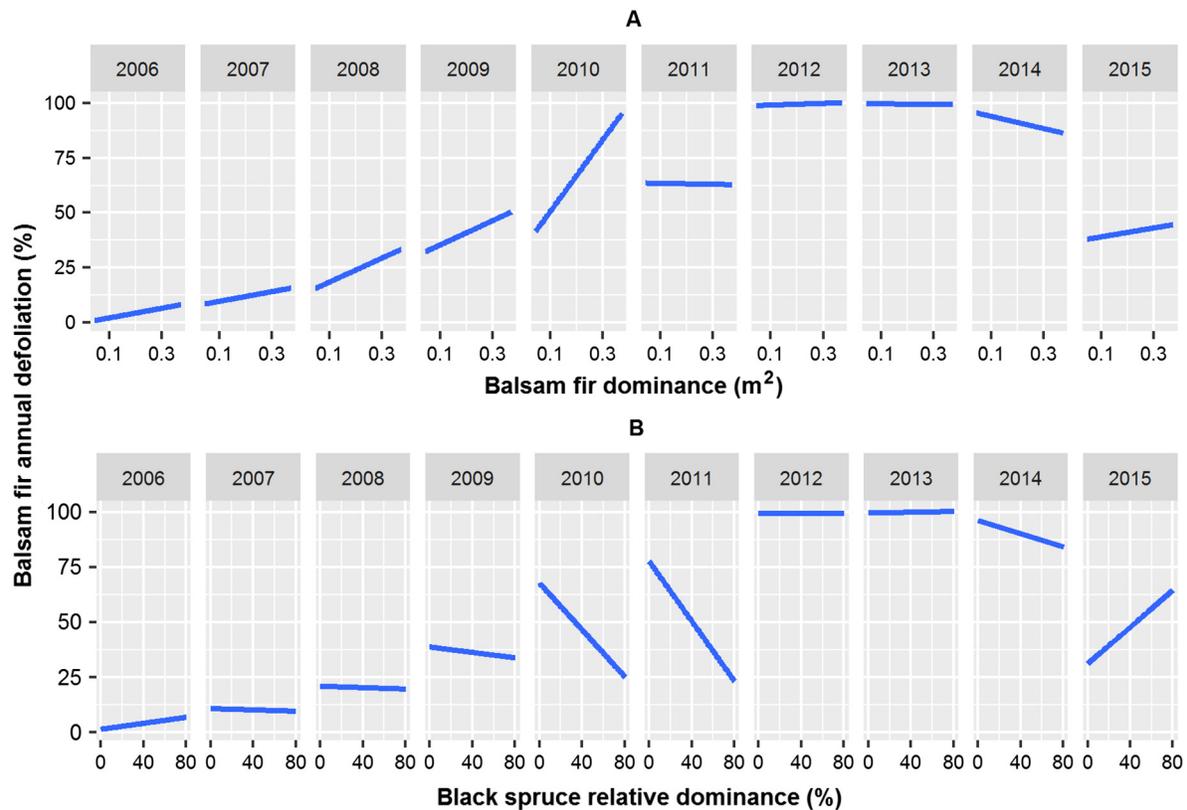


Fig. 4. Fitted linear relationships from mixed-effect modeling of nested data model for balsam fir defoliation and both balsam fir dominance (A) and black spruce relative dominance (B) data within balsam fir-dominated stands.

effects of conspecific neighbors, occurring concurrently with associational effects of heterospecific neighbors (Figs. 4 and 6). The relative strength of relationships changed as the resource is consumed (Kneeshaw et al. 2015).

#### Resource concentration effects

There was a positive relationship between balsam fir defoliation and its conspecific neighbor dominance in balsam fir-dominated and mixed stands as predicted by the resource concentration hypothesis (Long et al. 2003). A negative relationship between black spruce defoliation and its conspecific neighbor dominance was also observed, which supports the resource dilution hypothesis (Yamamura 2002, Otway et al. 2005). In contrast with our hypothesis that the effect of resource concentration would decrease as the outbreak progresses, we instead observed that resource concentration effects on balsam fir

increased slowly as the defoliator population increased. This relationship was persistent over the first five years of the outbreak and then became stronger (e.g., in balsam fir-dominated stand, the slope of the regression line was 0.002 in 2006 and then increased to 0.016 in 2010) until food resources (i.e., foliage) were highly depleted. It appears that as spruce budworm populations reach high levels, dispersing moths located more isolated trees and thus weaken (in mixed stands) or cancel out (in balsam fir-dominated stands) the relationship (Städler 1974).

Resource dilution was supported by decreased black spruce defoliation in the presence of conspecific neighbors within all stands. The pattern of this relationship overtime was consistent with our hypothesis that the effect of resource dilution would decrease over the outbreak (the slope of the regression line was 0.002 in 2007, increased to 0.016 in 2009, and then decreased to 0.004 in

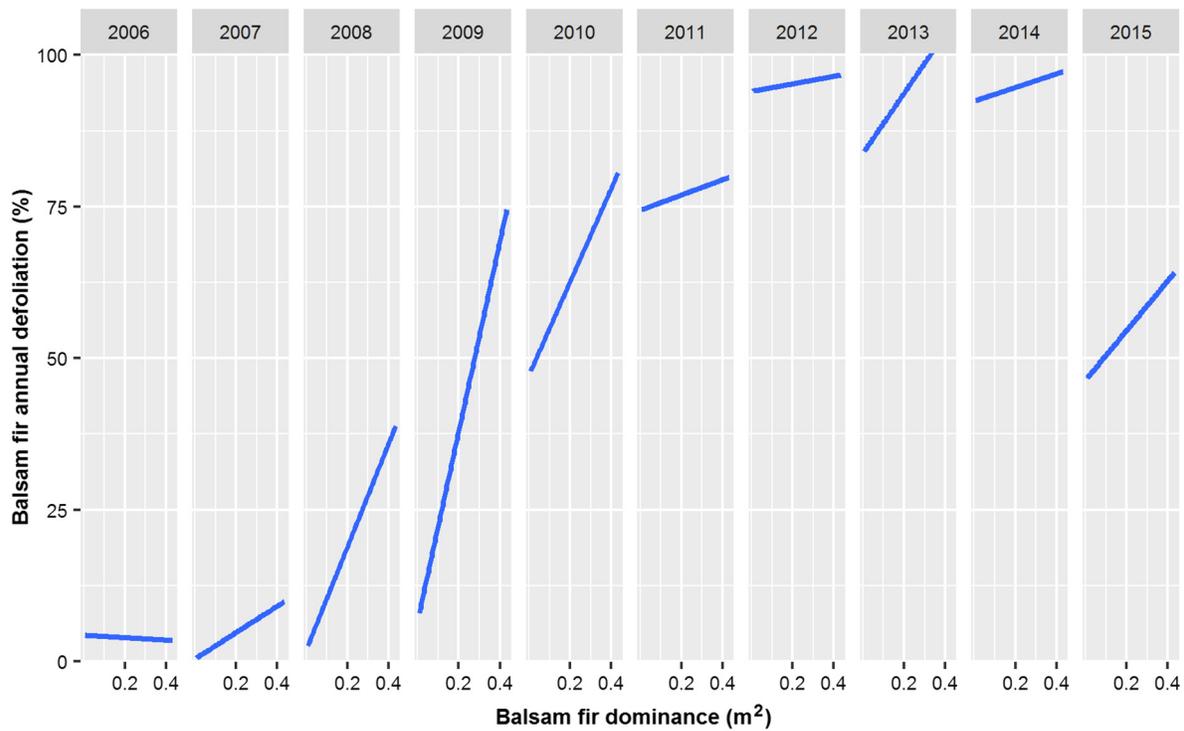


Fig. 5. Fitted linear relationships from mixed-effect modeling of nested data model for the balsam fir defoliation and balsam fir dominance data within mixed stands.

Table 5. Summary of linear mixed-effects models for repeated measures and nested data to test the effect of black spruce dominance and balsam fir relative dominance over time and spatial scale on black spruce annual defoliation.

Parameters	df	F	P
Spruce_Dom	1	6.92	0.009
Balsam_RelDom	1	17.15	<0.001
Stands	2	1.18	0.361
Year	9	143.96	<0.001
Spruce_Dom : Balsam_RelDom	1	0.05	0.815
Spruce_Dom : Stands	2	5.07	0.006
Balsam_RelDom : Stands	2	1.96	0.141
Spruce_Dom : Year	9	2.70	0.004
Balsam_RelDom : Year	9	5.59	<0.001
Stands : Year	18	3.47	<0.001
Spruce_Dom : Balsam_RelDom : Stands	2	1.36	0.256
Spruce_Dom : Balsam_RelDom : Year	9	1.92	0.046
Spruce_Dom : Stands : Year	18	0.48	0.965
Balsam_RelDom : Stands : Year	18	1.79	0.022

2015). The weakening and disappearing of this relationship may be due to the redistribution of late-instar larvae from tree to tree (Morris and Mott 1963, Nealis and Régnière 2004) and mass dispersal of female moths that prefer to oviposit on shoots with the greatest needle density (Städler 1974).

#### Associational effects

Consistent with our hypotheses, we observed associational susceptibility (i.e., the relative dominance of heterospecific neighbors [balsam fir]) in black spruce defoliation (Atsatt and O'Dowd 1976). Spillover from a primary host to the secondary host may drive this relationship as spruce budworm populations and defoliation of balsam fir increase (Blais 1957, Morris and Mott 1963, Nealis and Régnière 2004). Associational susceptibility (measured as the relative dominance of black spruce) increased balsam fir defoliation in

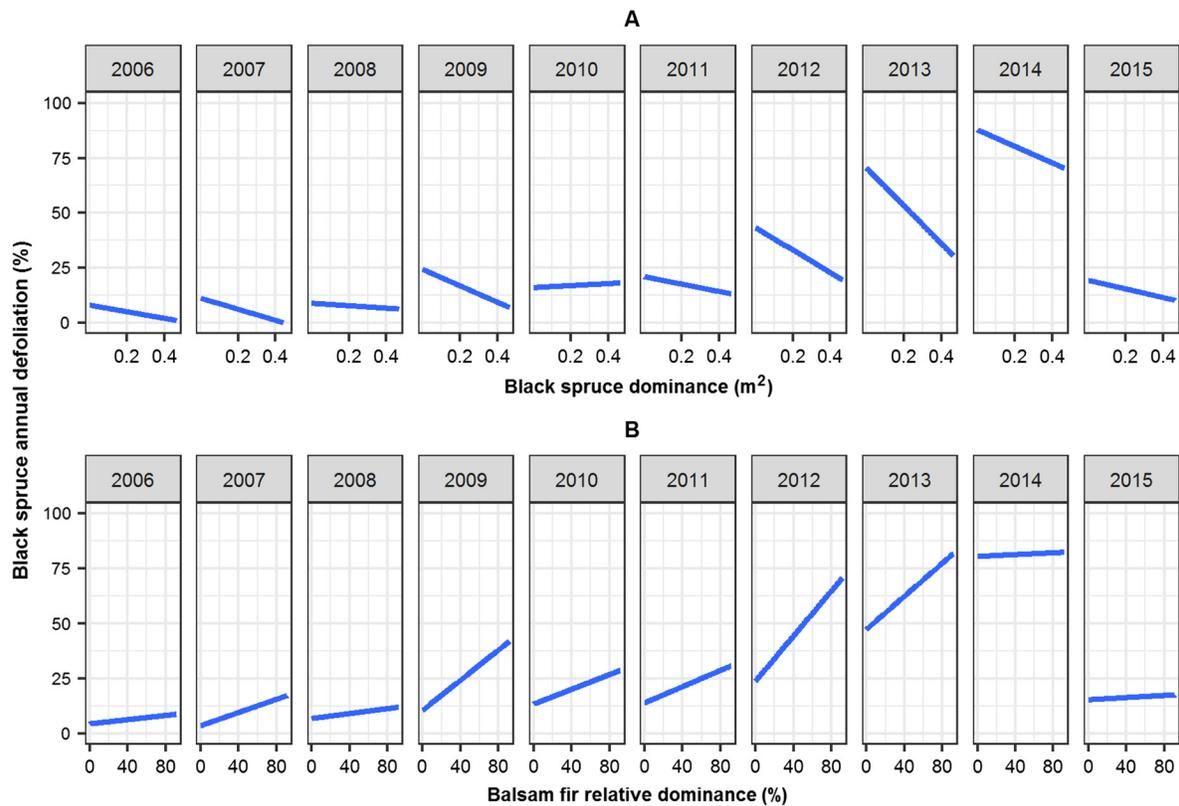


Fig. 6. Fitted linear relationships from mixed-effect modeling of nested data model for black spruce defoliation and both black spruce dominance (A) and balsam fir relative dominance (B) data.

balsam fir-dominated stands at the end of our observation period. In this case, associational susceptibility may be due to the redistribution of larvae on the secondary host because its bud flush is not well synchronized with larval emergence.

On the other hand, there was a negative relationship between heterospecific neighbors (black spruce relative dominance) and balsam fir defoliation in balsam fir-dominated stands, a relationship that appeared in the increasing and decreasing phases of the outbreak. This result is surprising as it suggests that balsam fir may benefit from associational resistance with a secondary host species, black spruce (Tahvanainen and Root 1972), at two different phases in the outbreak. Further, the associational resistance and resource concentration effects co-occurred in one year (2010). A potential explanation is that black spruce acts as a sink (Barbosa et al. 2009), which is strengthened by resource depletion of balsam fir that may enhance larval movement from balsam fir to black spruce

(Nealis and Régnière 2004). In fact, both balsam fir and black spruce are good hosts for female moths to lay their eggs (Nealis and Régnière 2004). As budburst of black spruce is not well synchronized with spruce budworm larval emergence, there is higher mortality of larvae feeding on black spruce trees than on balsam fir.

However, this relationship of associational resistance eventually reverses at very high defoliation levels (high resource depletion) when spruce budworm started moving back from black spruce onto balsam fir (i.e., associational susceptibility). Although many studies have considered associational effects as a static or consistent process (Agrawal et al. 2006, Barbosa et al. 2009, Plath et al. 2012), our results are consistent with studies reporting that neighbors that are hosts initially act as sinks, but as outbreaks continue, may then lead to associational susceptibility of focal hosts via a spillover effect of herbivores from sinks to focal host (Wada et al. 2000, White and Whitham

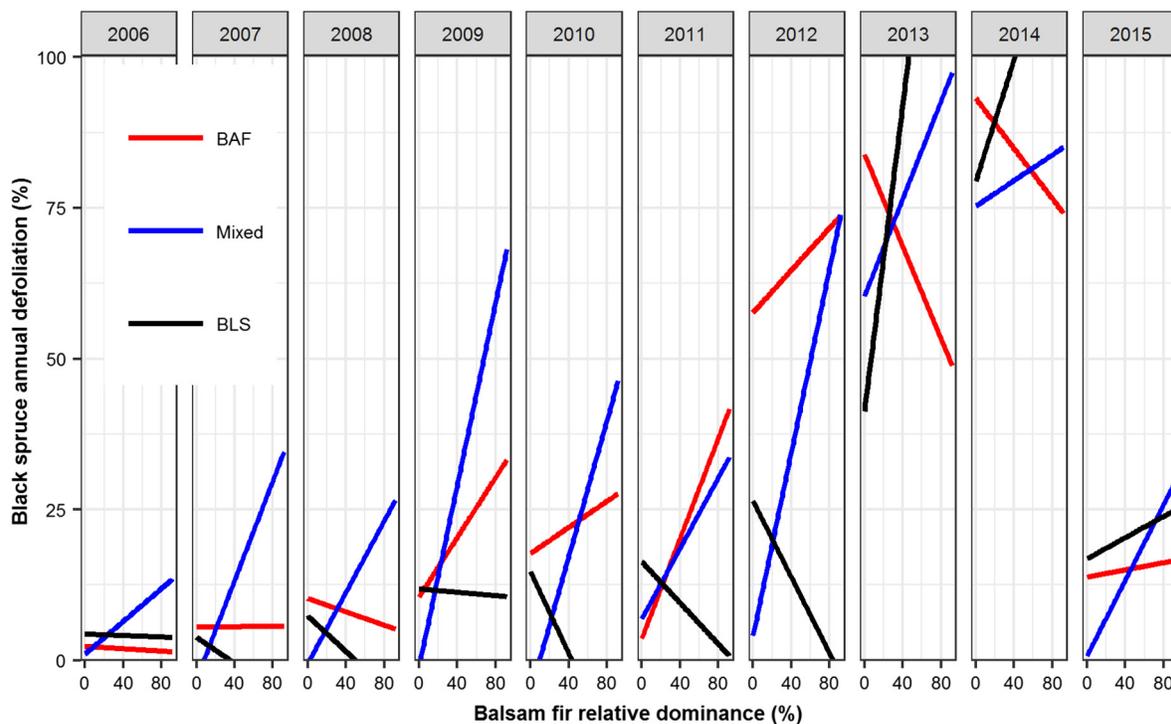


Fig. 7. Fitted linear relationships from mixed-effect modeling of nested data model for the black spruce defoliation and balsam fir relative dominance data within different stand composition (BAF, balsam fir stand composition; BLS, black spruce stand composition; mixed, balsam fir and black spruce mixed stand composition).

2000). This temporal shift may, at least in part, lead to equivocal interpretations on the presence/absence and strength of associational effects (i.e., Koricheva et al. 2006 vs. Jactel et al. 2005).

#### Stand composition effects

Contrary to our prediction that associational effects would be strongest in mixed spruce-fir stands, the effect of heterospecific neighbors varied with no observed effect on balsam fir defoliation in mixed and black spruce-dominated stands, but with a significant effect on black spruce defoliation across stand types. Our findings confirm the theory that systems in which herbivore movement processes occur both among- and within-patch levels should show different patterns of associational effects (Hambäck et al. 2014). The complexity of understanding how species mixtures affect defoliation was exemplified as neighborhood effects changed depending on the overall stand composition. Neighborhood effects of conspecifics were most important when the surrounding matrix included a mix or dominance

of the primary host, thus supporting resource concentration (Long et al. 2003) but also emphasizing the importance of the identity/species of the target tree (Plath et al. 2012). Balsam fir as the primary host and most vulnerable depended more on immediate neighbors and thus associational effects. On the other hand, black spruce as the least preferred host was under a greater effect of resource dilution effects in the context of its immediate neighbors.

Understanding the repercussions of resource concentration/dilution and associational effects on spruce budworm outbreak dynamics over time has major implications in planning preventive and reactive strategies in forestry to reduce the damage of spruce budworm or other outbreaking herbivore damages. For example, in forests dominated by primary hosts with sparse distribution of secondary hosts, our results suggest that secondary hosts offered protective effects to primary host when the resource is highly depleted, but during the decreasing phase of the outbreak, the primary host suffers

associational susceptibility that may prolong the outbreak. Stands to be targeted by aerial spraying should thus change throughout the course of an outbreak. Understanding which mixtures of species at different spatial scales results in more damage by the herbivore can provide forest managers with essential knowledge to plan proactive silvicultural strategies to limit damage.

#### *Study limitations and perspectives*

As an observational study, we acknowledge that experimental manipulation would be useful to validate our findings. Although, in the last ten years, there are few experimental studies that assessed neighborhood effects on herbivore damage, none of this previous work has studied neighborhood effects over time. Our observations show that neighborhood change over time was in accordance with stand composition when trees are attacked by a persistent multi-year defoliator.

The system we studied was a simple two-species system, and thus, interpretations for systems with a greater number of species need to be performed due to herbivore behavior that would probably change with the number of host and non-host species. Our system is also unique in that outbreaks occur over multiple years with multiple generations of herbivores, and thus, results cannot be directly applied to annual crops due to the multiple generations of herbivore populations and also the persistence of the host plant resource in forest systems. Nonetheless, our study integrates what a growing body of literature is, suggesting that herbivore–host systems are controlled by multiple relationships (Hambäck et al. 2014, Underwood et al. 2014, Kim and Underwood 2015). For instance, we observed simultaneous occurrence of resource concentration/dilution and associational effects in spruce budworm–forest system. These findings suggest that it is not only experimental design that matters, as reported by Hambäck et al. (2014), but that in observational studies, sampling design also matters. Our study shows that when considering a multi-year defoliator, a single-year study could provide misleading results. If the neighborhood effect takes time to develop, then early results will not detect any effect and conclude that the relationship is neutral. Since the temporal scale and stand implications of neighborhood effects on host plant are important for the ecology of both

herbivores and their host plants, future studies in other systems (i.e., agriculture) should take into account these variations.

## CONCLUSIONS

In conclusion, the long-term effects of neighborhood on insect outbreak impacts in the spruce budworm–forest system are dynamic and combine resource concentration and/or resource dilution effects with associational resistance and/or associational susceptibility. The complexity of neighborhood effects observed in this study suggests that future studies, in order to understand the long-term implications of neighborhood effects on forest systems, should separate the effects of conspecific and heterospecific neighbors, and use long-term experiments with a large range of conspecific neighbor dominance and heterospecific neighbor relative dominance within plant communities with different species composition.

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