

**DETERMINING THE MECHANISM OF IMPACT OF HARDWOOD CONTENT
ON SPRUCE BUDWORM DEFOLIATION OF BALSAM FIR**

by

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A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

Doctor of Philosophy

in the Graduate Academic Unit of Forestry and Environmental Management

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This dissertation is accepted by the Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

June, 2020

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ABSTRACT

I investigated effects of hardwood content on eastern spruce budworm (*Choristoneura fumiferana* Clem.) defoliation in mixedwood forests during the first 5 years of an outbreak. I sampled 27 balsam fir (*Abies balsamea* (L.) Mill.) - hardwood plots representing three percent hardwood basal area classes (0%–25% (termed softwood), 40%–65% (mixedwood), and 75%–95% (hardwood)) near Amqui, Québec, Canada. Balsam fir defoliation was significantly lower ($p < 0.001$) as hardwood content increased, but the relationship varied with overall defoliation severity each year. We formulated three predictive models to estimate defoliation in fir-hardwood plots, among which a Random Forests procedure incorporating 11 predictor variables generated the best prediction ($r = 0.92$). Accurate estimation of defoliation in fir-hardwood stands requires data about average defoliation level in that year in addition to percent hardwood content. The observed reduction of defoliation associated with hardwood content could be explained by two hypotheses: habitat fragmentation and/or natural enemy hypotheses. The first one posits that higher early-instar larval dispersal loss occurs in fir-hardwood stands with higher hardwood content while the second suggests that higher parasitism occurs in mixed stands. First- and second-instar larval dispersal loss, stage-specific budworm density, and parasitism rates were assessed in the three stand types through field sampling. Second-instar dispersal losses were significantly influenced by stand type; higher dispersal losses occurred in plots with higher hardwood content. These results suggested that the habitat fragmentation hypothesis is a plausible explanation for the lower spruce budworm density and balsam fir defoliation associated with increasing hardwood content. There was no significant relationship between parasitism and stand

type, not supporting the natural enemy hypothesis. Diversity and structure of the primary parasitoid assemblage of spruce budworm was assessed in the three stand types. Species diversity (α -diversity) did not show significant differences between the three stand types. The community structures of parasitoid assemblages differed significantly among the three stand types (β -diversity). Elevation ($p = 0.007$), population density of spruce budworm ($p = 0.065$), and percent hardwood content ($p = 0.132$) were the top three variables correlated with the parasitoid community structure among seven selected explanatory variables.

DEDICATION

To my wife, Mai, my two lovely daughters Joanna and Leah

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. David MacLean, and advisory committee, Dr. Rob Johns and Dr. Eldon Eveleigh, for their guidance, support, and mentorship. Thanks to Dr. Chris Hennigar, Dr. Steve Heard, and Dr. Dan Quiring who provided helpful advice, encouragement, and feedback. I am grateful to my wife for her support and patience. Thanks also go to my lab-mates for comradery.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Spruce budworm (*Choristoneura fumiferana* Clem.) is a native defoliator in boreal and New England-Acadian forests in eastern North America (Miller, 1975; Belle-Isle and Kneeshaw, 2007; Royama et al., 2017). In eastern Canada, budworm outbreaks are cyclical and population levels peaked every 30-40 years during the 20th century (Royama, 1984; Royama et al., 2005). In order of preference, balsam fir (*Abies balsamea* (L.) Mill.), white spruce (*Picea glauca* (Moench) Voss), red spruce (*P. rubens* Sarg.), and black spruce (*P. mariana* (Mill.) BSP) are the major host tree species of spruce budworm (Greenbank, 1963). Outbreaks last 5-15 years and severe defoliation causes tree growth reduction, loss of seed production, impeded regeneration and tree mortality (Hennigar et al., 2008; MacLean, 2016; Nie et al., 2019). Budworm outbreaks typically have a very large spatial extent (Bouchard and Auger, 2014): outbreaks that began in ca. 1910, 1940 and 1970 covered areas of 10, 25 and 55 million ha, respectively, in eastern Canada (Blais, 1983). As a major ecological disturbance in fir-spruce forests, budworm outbreaks cause large volume losses of timber and pulp production and significant economic impacts (Natural Resources Canada, 1995; Chang et al., 2012; Liu et al., 2019).

Understanding spruce budworm defoliation requires knowledge of budworm phenology. Spruce budworm has a 1-year life cycle comprising nine developmental stages. The time of each developmental stage depends on temperature and geography (Miller, 1975). In eastern North America, budworm adults deposit eggs in July and August. Eggs hatch in about 10 days to 2 weeks. The emerging first-instar larvae (L1) immediately disperse by walking or ballooning (on silken threads) in search of suitable sites such as old flower scars, bark scales, or lichen on host or non-host trees (Johns and

Eveleigh, 2013; Royama et al., 2017). L1 rarely feed but spin cocoon-like shelters within which they molt to L2 and enter diapause through the winter. L2 emerge in the spring and they may disperse while searching for viable foliage. L1 and L2 are the two major larval dispersal periods; mortality occurs during these airborne redistributions when the larvae are blown to the ground or non-host environment. The subsequent L3 to L6 feed voraciously on new foliage and defoliate host trees. Pupation occurs in July and lasts for 8 to 12 days. Adult moths live about 2 weeks and can travel long distances (depending on weather conditions) to lay eggs (Miller, 1975; Royama et al., 2017).

Population density of spruce budworm is commonly assessed by sampling: 1) moths using light or pheromone traps; 2) pupal cases; 3) egg masses; 4) over-wintering L2; and 5) large larvae and pupae (Sanders, 1980; Dorais and Kettela, 1982). Except moth sampling, budworm specimens are collected by cutting a branch tip using extended pole pruners commonly from the mid-crown of dominant and co-dominant host trees. The length of the branch tip sampled could be 45 cm, 75 cm, or a whole branch and the density is normally expressed per m² of branch surface area or per kg of foliage (Sanders, 1980; Régnière et al., 1989). Defoliation of spruce budworm can be assessed by: 1) aerial survey (sketch-mapping foliage color change from aircraft); 2) ground assessment with binoculars; or 3) branch sampling with pole pruners (Sanders, 1980). The latest remote-sensing technology such as hemispherical imagery, multispectral imagery, and hyperspectral imagery is experimentally being tested for assessment and detection of budworm defoliation but is not yet practically adopted by industry and government agencies (Huang, 2015; Donovan et al., 2018; Rahimzadeh-Bajgirani et al., 2018). Branch sampling with pole pruners and rating defoliation on individual shoots is considered to be

the most accurate technique and is widely used for assessing spruce budworm defoliation (MacLean and MacKinnon, 1998; Donovan et al., 2018).

A meta-analysis of 119 studies worldwide showed that insect herbivory was less severe with increasing forest diversity (Jactel and Brockerhoff, 2007). For spruce budworm, mature and over-mature forest stands with a large proportion of balsam fir, especially in contiguous softwood landscapes, have the highest susceptibility (defoliation) and vulnerability (growth reduction and mortality) to spruce budworm outbreaks (MacLean, 1984; Nealis and Régnière, 2004). Several studies have found that outbreak impacts were less severe in fir-hardwood mixed stands than in pure softwood stands. For example, in the peak and declining phase of an outbreak in the 1990s, balsam fir defoliation was 12–32% in stands with >40% hardwood versus 58–71% in stands with <40% hardwood (Su et al., 1996). A tree-ring study suggested that budworm-caused growth reductions on balsam fir were 40% versus 20% in stands with < or >50% hardwood contents, respectively (Campbell et al., 2008). During severe defoliation, fir regeneration seedlings could sustain higher damage, as much as 85%, in softwood stands (<25% hardwood) than in hardwood stands (>75% hardwood) (Nie et al., 2018). Budworm-caused mortality on balsam fir was 14-30% less in fir-hardwood mixed stands (~ 30% hardwood content) than in fir dominant stands (Turner, 1952; MacLean, 1980; Bergeron et al., 1995).

Defoliation severity is determined by budworm population density, so one possible explanation for differences in defoliation level is that smaller densities of herbivorous insects are directly related to less abundant host trees (Mazzi and Dorn, 2012). This simple relationship would be affected by other ecological mechanisms that

cause mortality and reduced budworm density. Examining population densities at per-unit-host scale (e.g., per unit basal area or biomass of host trees) would avoid this confounding effect. Previous research has suggested two hypotheses to explain the reduced insect herbivory associated with tree diversity. The “habitat fragmentation” hypothesis (also known as resource concentration hypothesis (Mazzi and Dorn, 2012)) suggests that diverse vegetation could negatively affect the ability of the insect to locate and utilize its host plant. Diverse plant communities reduce host tree availability and increase the level of habitat fragmentation for herbivore insects, thereby mitigating impacts on host trees through hindering foraging, dispersal, and mating success (Cappuccino et al., 1998; Yamamura, 2002). Kemp and Simmons (1979) found higher mortality of spruce budworm L1 and L2 associated with the abundance of non-host tree species. The insects would have to spend more time and energy to search for suitable sheltering or feeding sites in heterogeneous environments due to the presence of physical barriers (non-host trees). The chance of being preyed upon also would increase due to longer exposure to predators (Schowalter, 1996). The “natural enemy” hypothesis argues that more diverse plant communities, non-host trees, or more diverse understories associated with mixed stands provide additional hosts or other resources, such as nectar (Leius, 1968; Simmons et al., 1975) to the natural enemies of the herbivore insects, and thus, enhance parasitism activities by providing alternative and alternate prey, more predation opportunities, or better sheltering conditions (Siemann et al., 1998; Quayle et al., 2003; Cardinale et al., 2006). Some spruce budworm parasitoids, such as *Meteorus trachynotus* Vier. (Maltais et al., 1989) and *Itoplectis conquisitor* Say (Cappuccino and Martin, 1997), have shown dependent relationships with other host insects that feed on

hardwood trees. The tachinid fly *Actia interrupta* Curran has higher attack rates on budworm in more mixed stands or landscapes (Cappuccino et al., 1998; Quayle et al., 2003). Natural enemies of spruce budworm are more abundant or more effective in mixedwood forests than in fir-spruce forests (Bergeron et al., 1995; Cappuccino et al., 1998). Eveleigh et al. (2007) analyzed the spruce budworm food web structure and found strong interactions between softwood and hardwood sub-webs; softwood-hardwood content influenced parasitoid diversity and movement.

The spruce budworm food web is complex, with more than 50 parasitoid species that can cause budworm mortality (Eveleigh et al., 2007; Royama et al., 2017). Biodiversity and community composition influence the structure and function of ecosystems (Hooper et al., 2005) and spatial variation in biodiversity and community structure has been a major theme in studies of agroecosystems, grasslands, or microbial communities (Tilman et al., 2001; Cardinale et al., 2006; Andrade et al., 2015; Shen et al., 2016). Yet, potential correlations between diversity and community structure of higher trophic level communities, e.g., parasitoid, and vegetational characteristics, have not been thoroughly studied (Fraser et al., 2007). Budworm and its complex natural enemy community offers an excellent system for exploring influences of forest tree composition on parasitoid communities.

In the 20th century, pest management strategies for fighting spruce budworm outbreaks were mainly reactive, including insecticide spray, salvage harvesting of dead and dying trees, or re-planning of management schedules to minimize impacts on timber supply (MacLean et al., 2019). To fight spruce budworm, a total of 6280 tons of dichlorodiphenyltrichloroethane (DDT) was sprayed in New Brunswick, Canada between

1952 and 1968 (Kurek et al., 2019). The intense insecticide application caused long-lasting environmental impacts to the lakes and aquatic communities (Carson, 1962; Kurek et al., 2019). In the 21st century, proactive approaches such as an Early Intervention Strategy (EIS) in New Brunswick, Canada are being tested to battle the spruce budworm outbreaks and have received positive results to date (Johns et al., 2019; MacLean et al., 2019). The EIS includes intensive monitoring of budworm populations, targeted insecticide treatment, and interactive public involvement. The proactive approach is better aligned with modern, internationally accepted Integrated Pest Management (IPM) concepts than the reactive approach (Peshin et al., 2009). Some key IPM concepts such as “pesticides should only be applied as a last resort when there are no adequate non-chemical alternatives and use of pesticides is economically justified” were conceptualized into these proactive strategies (Vapnek et al., 2007). The concept that using natural enemies, e.g., parasitoids to suppress herbivory insects as a biological control is inherently an integral component in the IPM. Altering tree species composition, and thus, influencing the interactions between spruce budworm and its parasitoids could be an IPM strategy in combating spruce budworm outbreaks.

Determining how the hardwood component influences spruce budworm defoliation in mixedwood forests during the rise of budworm outbreaks is essential for understanding the interaction between spruce budworm and its hosting environment. The initiation stage is key in determining whether the overall rise or spread of an outbreak can be suppressed. This knowledge is especially important for developing proactive pest management strategies, such as EIS, for preventing outbreaks. From ecological and entomological standpoints, it is also important to understand the mechanisms behind the

phenomenon that less severe fir defoliation occurred in mixedwood forests with more hardwood content, and thus, to better understand how forest composition influences insect herbivory and outbreak intensity.

1.2 Overall goal and specific objectives

This study was part of the project “Early Intervention Strategy to Suppress Spruce Budworm Outbreak” (EIS) funded by the Atlantic Innovation Fund. The EIS focuses on suppressing rising spruce budworm populations before major defoliation occurs and this study aided in the construction of the EIS treatment priority algorithm (MacLean et al., 2019). The overall goal of this study was to examine the relationship between balsam fir defoliation and hardwood content during the initiation and building phases of a spruce budworm outbreak and to explore the biological mechanisms behind this benefit, i.e., reduction of defoliation associated with hardwoods. Specific objectives were to:

1. Determine the relationship between balsam fir defoliation and hardwood content during the initiation and building phases (first 5 years) of a spruce budworm outbreak; compare accuracy of predictions of spruce budworm defoliation in fir-hardwood stands based on three alternative model formulations: a simplified linear model, a generalized linear model with mixed effects, and a machine learning (Random Forests) formulation.
2. Examine the density of spruce budworm during four different life stages using field data collected over 3 years in 27 plots representing a balsam fir-hardwood composition gradient; measure L1 and L2 larval dispersal using ground traps in these plots and determine parasitism rates by rearing sampled spruce budworm

larvae, pupae, and eggs.

3. Investigate the spruce budworm parasitoid community in the 27 forest plots with varied balsam fir-hardwood tree composition; examine diversity (α -diversity) and dissimilarity of the spruce budworm parasitoid community assemblage (β -diversity) in forest stands with varied balsam fir-hardwood tree composition.

1.3 Study area

The study area was situated on the north side of Lake Matapédia in the Gaspé area, Québec (48°32'N–48°35'N, 67°25'W–67°34'W); within the Témiscouata-Restigouche section of the Great Lakes-St. Lawrence forest region (Rowe, 1972). Government-operated regional spruce budworm aerial defoliation survey of the area started in 2010 and defoliation in the study area was first detected in 2012 (Ministère des Forêts de la Faune et des Parcs, 2012). Defoliation estimates were classified by aerial observers using a three-level scale: light, moderate, and severe. Most of the study area was classified as light or light-moderate defoliation in 2012 and 2013, and as severe or moderate-severe defoliation in 2014 and 2015. Defoliation from the aerial survey declined to light or light-moderate in 2016 (Ministère des Forêts de la Faune et des Parcs, 2017).

Twenty-seven balsam fir-hardwood sample plots were established in 2014 within an ~100 km² area. Nine 0.05 ha sample plots were established in each of the three balsam fir-hardwood stand types classified by percent basal area of hardwood content: 0-25% (termed softwood), 40-65% (mixedwood), and 75-95% (hardwood). Sample plots were

located at least 50 m apart and at least 50 m away from forest edges. The most common hardwood tree species in the plots were sugar maple (*Acer saccharum* Marshall), yellow birch (*Betula alleghaniensis* Britton), white birch (*Betula papyrifera* Marshall), and red maple (*Acer rubrum* L.). Stand density ranged from 520 to 2975 stems/ha, with an average of 1346 stems/ha. Percent basal area of balsam fir averaged 70%, 39%, and 11% in softwood, mixedwood, and hardwood plots, respectively. Percent basal area of hardwoods averaged 15%, 55%, and 88% in the three stand types, respectively. White spruce (*Picea glauca* [Moench] Voss), another host species of spruce budworm, comprised on average 3.3% of basal area in each plot and existed only in about one half of the plots. All plots except three were on flat ground with <10 degrees of slope. Elevation ranged from 165 to 357 m, with an average of 261 m. A detailed description of sample plots can be found in Chapter 2 of this thesis.

1.4 Thesis structure

This thesis is presented in an “article” format. The body of the thesis consists of three chapters (Chapters 2, 3, and 4), which are described below. The last chapter (Chapter 5) presents a general discussion of the conclusions from these chapters.

Chapter 2 quantified the relationship between balsam fir defoliation and hardwood content during the initiation and building phases (first 5 years) of a spruce budworm outbreak in the 27 plots along a balsam fir-hardwood composition gradient. It also compared the accuracy of predictions of spruce budworm defoliation in fir-hardwood stands based on three alternative model formulations: a simplified linear model, a

generalized linear model with mixed-effects, and a machine learning (Random Forests) formulation. This chapter has been published in the journal *Forests* (Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., 2018. *Effects of hardwood content on balsam fir defoliation during the building phase of a spruce budworm outbreak. Forests* 9, 530).

Chapter 3 tested the habitat fragmentation and natural enemy hypotheses by sampling spruce budworm early-instar larval dispersal and parasitism between 2014 and 2017 from the 27 plots. This chapter has been published in the journal *Forest Ecology and Management* (Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., Edwards, S., 2020. *Hardwood-softwood composition influences early-instar larval dispersal mortality during a spruce budworm outbreak. Forest Ecology and Management* 463, 118035).

Chapter 4 investigated the spruce budworm parasitoid community in the 27 plots and compared diversity (α -diversity) and dissimilarity of the spruce budworm parasitoid community assemblage (β -diversity) in the three forest stand types. This chapter is planned for submission for publication as: Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., 2020. *Comparison of community structure of the spruce budworm (Lepidoptera: Tortricidae) parasitoid assemblage in softwood, mixedwood, and hardwood forest stands during the rise of an outbreak.*

I was the primary author for the manuscripts in the body of this thesis (Chapters 2, 3, and 4). Dr. David A. MacLean, Dr. Rob C. Johns, Dr. Eldon S. Eveleigh, and Dr. Sara Edwards are included as co-authors on these manuscripts.

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**CHAPTER 2: EFFECTS OF HARDWOOD CONTENT ON BALSAM
FIR DEFOLIATION DURING THE BUILDING PHASE OF A
SPRUCE BUDWORM OUTBREAK**

Paper published as:

Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., 2018. Effects of hardwood content on balsam fir defoliation during the building phase of a spruce budworm outbreak. *Forests* 9, 530.

2.1 Abstract

Defoliation by spruce budworm (*Choristoneura fumiferana* Clem.) on balsam fir (*Abies balsamea* (L.) Mill.) is more severe in fir than in mixed fir-hardwood stands. Previous studies assumed that defoliation in fir-hardwood stands was reduced in proportion to percent hardwood regardless of outbreak severity. We tested the influence of stand composition on defoliation during the first 5 years of a spruce budworm outbreak near Amqui, Québec, by sampling 27 fir-hardwood plots selected to represent three percent hardwood basal area classes (0%–25%, 40%–65%, and 75%–95%). Balsam fir defoliation was significantly lower ($p < 0.001$) as hardwood content increased, but the relationship varied with overall defoliation severity each year. Annual plot defoliation in fir-hardwood plots, estimated using: (1) defoliation in pure fir plots and the assumption that defoliation in fir-hardwood plots was reduced in proportion to percent hardwood; (2) a generalized linear mixed-effects model with defoliation in pure fir plots, percent hardwood, and interaction as fixed-effects; and (3) Random Forests prediction incorporating 11 predictor variables, resulted in $r = 0.77, 0.87, \text{ and } 0.92$ versus measured defoliation, respectively. Average defoliation severity in softwood plots and percent hardwood content were the most important variables in Random Forests analysis. Data on average defoliation level in softwood stands, as an indicator of overall outbreak severity, improves prediction of balsam fir defoliation in mixed stands.

2.2 Introduction

Effective forest pest management in heterogeneous landscapes and in mixed-species forest stands requires knowledge about how tree diversity affects insect herbivory. A meta-analysis of a worldwide data set of 119 studies by Jactel and Brockerhoff (2007) showed a significant reduction in herbivory with increasing forest diversity for oligophagous insects (i.e., species that exploit one or a few closely related genera of hosts). This seems also to be the case for spruce budworm (*Choristoneura fumiferana* Clem.), which is the major defoliator of balsam fir (*Abies balsamea* (L.) Mill.) and spruce (*Picea* spp.) in boreal and New England-Acadian forests in eastern North America (Morris et al., 1958; Belle-Isle and Kneeshaw, 2007; Morin et al., 2007). Budworm outbreaks are cyclical and have occurred at 30-40-year intervals in eastern Canada during the past century (Royama, 1984; Royama et al., 2005). Outbreaks usually last 5-15 years and severe defoliation causes growth loss and tree mortality over large areas (Piene and MacLean, 1999; Hennigar et al., 2008), peaking at over 52 million ha of defoliation of forests in eastern Canada (Natural Resources Canada, 1995). Spruce budworm defoliation can be assessed by conducting aerial survey, ground assessment with binoculars, and branch sampling with pole pruners, of which branch sampling with pole pruners and rating defoliation on individual shoots is considered to be the most accurate technique (Sanders, 1980; MacLean and MacKinnon, 1998; Donovan et al., 2018).

Several studies have reported lower spruce budworm-caused defoliation of balsam fir, and lower resulting growth reduction and mortality, in stands or forest landscapes associated with higher percentage of hardwood tree species (MacLean, 1980; Bergeron et al., 1995; Su et al., 1996; Campbell et al., 2008). Mature stands with a large

proportion of balsam fir, especially in contiguous softwood landscapes, have the highest susceptibility and vulnerability to spruce budworm outbreaks (MacLean, 1984; Nealis and Régnière, 2004). Balsam fir defoliation assessed using the branch sampling method was 12%–32% in fir-hardwood stands with >40% hardwood content versus 58%–71% in stands with <40% hardwood content (Su et al., 1996). Tree-ring analysis showed that budworm-caused growth reductions averaged 40% in stands with <50% hardwood content versus 20% in stands with >50% hardwood content (Campbell et al., 2008). Mortality of balsam fir resulting from budworm-caused defoliation was 14%–30% less in fir-hardwood mixed stands (~30% hardwood content) than in fir-dominated stands (MacLean, 1980; Bergeron et al., 1995).

Two hypotheses have been proposed to explain less severe insect herbivory associated with higher tree diversity. The “natural enemy” hypothesis (Riihimäki et al., 2005) argues that more diverse plant communities support more abundant natural enemies of herbivore insects by providing alternative prey, more predation opportunities, or better sheltering conditions (Siemann et al., 1998; Quayle et al., 2003; Cardinale et al., 2006). Alternatively, the “habitat fragmentation” hypothesis argues that reduced host tree availability increases the degree of habitat fragmentation for the insects and creates barriers for foraging, dispersal, and mating success (Kemp and Simmons, 1979; Cappuccino et al., 1998; Yamamura, 2002).

Past studies of how hardwood content influences spruce budworm defoliation have all focused on severely defoliated stands at the peak and declining phases of outbreaks. Su et al. (1996) reported that defoliation of balsam fir decreased as hardwood content increased in the declining phase (last 5 years) of the last outbreak, but also noted

that the relationship between defoliation and hardwood content may well vary during different stages of outbreak. Information is lacking on the building phase of an outbreak, as budworm populations increase from low to peak density.

Su et al. (1996) also proposed a direct linear relationship for use in predicting defoliation in fir-hardwood stands, using percent hardwood and defoliation in a pure fir stand. The relationship can be expressed succinctly as $y = D_0 \cdot (1 - x)$ (which we term the simplified linear model), where fir defoliation in a mixedwood stand (y) is a function of percent hardwood (x) and fir defoliation level in a pure fir stand (D_0). The relationship was quantified using defoliation data collected in the declining years (1989–1993) of the last outbreak and were subsequently used in Needham et al. (1999) and Sainte-Marie et al. (2014). In this study, we examine whether this relationship holds true in the building phase of an outbreak.

Insect herbivory is influenced by other variables in addition to tree diversity. For example, Douglas-fir tussock moth (*Orgyia pseudotsugata* McDunnough) defoliation varied with slope, stand density, and site index (Stoszek et al., 1981). Scots pine (*Pinus sylvestris* L.) defoliation during common pine sawfly (*Diprion pini* L.) outbreaks was correlated with forest site class (De Somviele et al., 2004). Studies conducted in the declining phase of the last spruce budworm outbreak suggested that hardwood content was significant in predicting defoliation (MacKinnon and MacLean, 2003; Colford-Gilks et al., 2012), but other factors can include outbreak stage and severity, soil drainage, and site conditions (MacLean and MacKinnon, 1997; MacKinnon and MacLean, 2003). In the above mentioned simplified linear model, percent hardwood was used as a variable and defoliation in pure fir stand was used as a constant for predicting defoliation in

mixedwood stand in a given year. In this study, we also test whether adding other biotic and abiotic variables improve the accuracy of defoliation prediction.

The objectives of this study were to: (1) determine the relationship between balsam fir defoliation and hardwood content during the initiation and building phases (first 5 years) of a spruce budworm outbreak, and (2) compare accuracy of predictions of spruce budworm defoliation in fir-hardwood stands based on three alternative model formulations: the simplified linear model, a generalized linear model with mixed-effects, and a machine learning (Random Forests) formulation. We evaluated two predictions: (1) fir-hardwood stands with higher hardwood content will have less severe annual defoliation of balsam fir; and (2) the simplified linear model can be used to estimate budworm defoliation in fir-hardwood mixed stands if other predictor variables are not available, but incorporating more variables will improve the accuracy of predictions.

2.3 Materials and methods

2.3.1 Study area and stand sampling

Study sites were located on the north side of Lake Matapédia in the Gaspé area, Québec (48°32'N–48°35'N, 67°25'W–67°34'W) (Figure 2.1). The area is within forest section L6, Témiscouata-Restigouche, in the Great Lakes-St. Lawrence forest region (Rowe, 1972). Aerial survey of spruce budworm defoliation conducted by the Québec provincial government since 2010 indicated that defoliation was first detected in the study area in 2012 (Ministère des Forêts de la Faune et des Parcs, 2012). Defoliation was classified by aerial observers using a three-level scale: light, moderate, and severe. Most of the study area was classified as light or light-moderate defoliation in 2012 and 2013,

and as severe or moderate-severe defoliation in 2014 and 2015. Defoliation estimates from the aerial survey declined to light or light-moderate in 2016 (Ministère des Forêts de la Faune et des Parcs, 2016).

Given that previous studies suggested that less severe defoliation would be observed in fir-hardwood stands with >40%–50% hardwood during a spruce budworm outbreak (Su et al., 1996; Needham et al., 1999; Campbell et al., 2008), mature (age >40 years) fir-hardwood stands were selected within three percent basal area of hardwood content classes: 0%–25% (termed softwood), 40%–65% (mixedwood), and 75%–95% (hardwood) (Figure 2.1). Nine 12.6 m radius circular (0.05 ha) sample plots were established, at least 50 m apart and at least 50 m away from stand edges within each of the three classes. Within each plot, all trees with diameter at breast height (DBH, 1.3 m above ground) >4 cm were numbered, and DBH and total height were measured. Elevation, slope, tree density, dominant ground vegetation, and GPS coordinates of plot center were recorded. All sample plots were established in spring 2014 except plots 13a, 14a, and 15a, which were established in 2015 to replace the corresponding original plots that were harvested during winter 2014, in an area with similar stand and defoliation conditions. Therefore, defoliation from 2012 to 2014 was collected in plot 13, 14, and 15 whereas defoliation for 2015 and 2016 was collected in plot 13a, 14a, and 15a.

Balsam fir was the dominant softwood species in all 27 plots, ranging from 64%–95% of the basal area in softwood plots, 20%–57% in mixedwood, and 5%–24% in hardwood (Table 2.1). White spruce (*Picea glauca* (Moench) Voss) occurred in about one-half of the softwood and mixedwood plots, and in one hardwood plot, but comprised <10% of the basal area in most plots. Percent basal area of hardwoods ranged from 1% to

95%, and averaged 12%, 56%, and 88% in the softwood, mixedwood, and hardwood classes, respectively (Table 2.1). Sugar maple (*Acer saccharum* Marshall) is a late successional species that was commonly found in both hardwood and mixedwood plots but was absent in softwood plots. The most abundant hardwood species in the mixedwood plots were generally yellow birch (*Betula alleghaniensis* Britton), white birch (*Betula papyrifera* Marshall), and red maple (*Acer rubrum* L.). Stand density ranged from 520 to 2975 stems/ha, with an average of 1346 stems/ha. Density of softwood and mixedwood plots was approximately double that of hardwood plots. Mean DBH and height of softwood, mixedwood, and hardwood plots was 15.7, 16.3, and 19.3 cm, and 14.0, 13.6, and 15.4 m respectively. Average diameter of balsam fir always exceeded the plot mean diameter in the softwood plots, but this relationship was variable in the mixedwood and hardwood plots. All plots except three were on flat ground with <10 degrees of slope. Elevation ranged from 165 to 357 m, with an average of 261m.

2.3.2 Defoliation measurements

Current year's defoliation was estimated on four trees per plot, one branch per tree, and 25 current-year shoots per branch after annual defoliation ceased (in August) each year from 2014 to 2016. Mean plot defoliation was computed as the averages of four trees. In each sample plot, four co-dominant balsam fir trees were randomly selected, and one mid-crown branch (MacLean and Lidstone, 1982) was collected using pole pruners from each tree. Cardinal direction was not considered as it does not significantly influence spruce budworm density (Morris, 1955) and therefore not defoliation. Twenty-five current-year shoots per branch were randomly selected beginning at the branch tip

and back along the branch length and assessed for annual defoliation using the shoot-count (Fettes) method (Sanders, 1980). Total number of needles of an individual shoot was visually compared to the regular pattern of needle positions (phyllotaxis) and the shoot was assigned to one of the seven defoliation classes (0%, 1%–20%, 21%–40%, 41%–60%, 61%–80%, 81%–99%, and 100%) in the field to prevent physical needle removal during transport. The mid-point of each defoliation class was used to calculate mean defoliation per branch and per plot. Required sample sizes (25 shoots per branch) were determined based on MacLean and MacKinnon (1998) to achieve 90% accuracy under most defoliation levels.

In 2014, branch samples were also collected in the spring prior to current year defoliation, and used to measure the annual defoliation that occurred in 2012 and 2013. Needle losses on 2012 and 2013 foliage were deemed to be the defoliation that occurred in the corresponding years, since aerial surveys indicated that defoliation was light to light-moderate in both years and back-feeding (late instar larvae feeding on older, non-current-year age classes of foliage) only occurs when very high insect populations consume all current year foliage (Blais, 1953).

2.3.3 The simplified linear model

As described in the introduction, the simplified linear model was a direct linear relationship proposed by Su et al. (1996):

$$y = D_0 \cdot (1 - x), \quad (2.1)$$

where percent hardwood (x) and fir defoliation in a pure fir stand (D_0) were used to predict fir defoliation in fir-hardwood mixed stands (y). In Su et al. (1996), the intercept

from simple linear regression for defoliation as a function of percent hardwood content each year was used as D_0 . We used the average plot defoliation in our softwood plots (i.e., with 0%–25% hardwoods) calculated each year, D_{sw} , as a substitute for D_0 . In our data, preliminary analysis suggested that average plot defoliation in softwood plots D_{sw} was highly correlated ($r = 0.995$) with the intercepts from simple linear regressions D_0 . We chose to use the empirical average plot defoliation in softwood plots instead of the theoretical extrapolated “defoliation-with-zero-hardwood” from the statistical models, because forestry agencies and industries can easily obtain D_{sw} without sampling mixedwood and hardwood stands.

2.3.4 Analyzing the relationship between annual balsam fir defoliation and hardwood content using generalized linear mixed-effects model

Average balsam fir defoliation at the plot level has two biological limits. At the lower end of the scale, needle loss can occur due to factors other than spruce budworm feeding. This and the lower threshold of measurement (i.e., use of 0% and 1%–20% defoliation classes, the latter with a mid-point of 10% defoliation) prevent defoliation from averaging 0% even in the absence of budworm. At the upper end, competition for food occurs among larvae, and defoliation reaches a near-100% plateau when budworm populations are extreme. Yet, average plot defoliation rarely reaches 100% because usually some needles escape complete defoliation. Hence, while a linear relationship between defoliation and hardwood content could be expected at mid-range defoliation severity levels, non-linear relationships would be expected at very light and at extreme defoliation levels. For these reasons, we analyzed defoliation through a logit link function

(McCullagh and Nelder, 1989) using a generalized linear mixed-effects model (GLMM):

$$\text{logit}(\text{defoliation}) = \beta_0 + b_0 + \beta_1 x + \beta_2 D_{sw} + \beta_3 x \times D_{sw} , \quad (2.2)$$

where $\text{logit}(\text{defoliation})$ is the logit link function ($\ln(p/(1 - p))$) for defoliation in a fir-hardwood mixture; x = percent hardwood content; D_{sw} = average plot defoliation in softwood plots; β_i 's are the fixed effects parameter estimates; and b_0 is the random effects. Year as a categorical variable was not included in the model since the annual differences were already incorporated in D_{sw} . Plot was included as a random effect and a serial correlation structure CorAr1 was included in the model error term to control for temporal autocorrelation. Averaged annual defoliation for each stand type was calculated and differences among the three stand types were tested using Kruskal-Wallis analysis by ranks.

2.3.5 The Random Forests model

Random Forests (Breiman, 2001), an ensemble regression tree statistical procedure, was also used to predict spruce budworm defoliation. Random Forests has been used extensively in ecological and forestry studies (e.g., Candau and Fleming, 2011; Penner et al., 2013; Lopatin et al., 2016) and has shown advantages in dealing with small sample size (Chen and Ishwaran, 2012) and complex interactions between factors (Prasad et al., 2006; Cutler et al., 2007). Random Forests was developed from classification and regression trees (De'ath and Fabricius, 2000). We used the Random Forests routine (Liaw and Wiener, 2002) in R (R Core Team, 2018) and the default of 500 trees (parameter “ntree”) was applied as error rate stabilized at 100-150 trees. A random one-third of predictor variables were used to perform data partitioning at each node (parameter

“mtry”). Two-thirds of the overall dataset was randomly selected and used to build the Random Forests model, and the other one-third retained for testing the model. The importance of each predictor variable was measured as the change in prediction accuracy (increase in mean square error, function “importance”), computed by permuting (value randomly shuffled) the variable with out-of-bag data in the Random Forests validation approach (Liaw and Wiener, 2002). A larger percent increase in mean square error indicates higher importance of a variable in prediction.

Initially, a total of 14 variables were assessed for multicollinearity using a correlation matrix: hardwood percent basal area (HW%), balsam fir percent basal area (bF%), D_{sw} , mean DBH, mean height, basal area (BA), mean DBH of bF, mean height of bF, standard deviation of bF height, mean bF crown base height, standard deviation of bF crown base height, tree density, elevation, and slope. Random Forests does not hold formal distributional assumptions of data and is relatively insensitive to multicollinearity (Breiman, 2001), but removing multicollinearity and redundancy to improve predictive power is recommended (Murphy et al., 2010; Dormann et al., 2013). Correlation analysis indicated that HW% and bF%, mean DBH of bF and mean height of bF, and mean DBH and density were highly correlated with coefficients (r) of -0.97, 0.87 and -0.84, respectively. HW%, mean DBH of bF, and mean DBH were selected over their counterpart variables because HW% should be included as a predictor in attempts to estimate defoliation reduction caused by hardwood; and mean DBH is readily available in most forest inventories and can be quickly assessed with an efficient sampling design. Therefore, 11 variables were retained and tested using Random Forests: HW%, D_{sw} , mean DBH, mean height, mean DBH of bF, BA, standard deviation of bF height, mean

bF crown base height, standard deviation of bF crown base height, elevation, and slope.

2.3.6 Statistical analyses

Balsam fir defoliation was estimated using the three alternative models: (1) the simplified linear model, (2) the GLMM model, and (3) the Random Forests model. The annual plot defoliation estimated using each model was plotted against actual defoliation measured in the field. Pearson correlation analysis was used to compare the accuracy of the estimates of the three models to the measured defoliation.

All statistical analyses were performed using R software (R Core Team, 2018), with $p = 0.05$ used to indicate significance. Kruskal-Wallis test and Pearson correlation analysis were performed using the R “stats” package (R Core Team, 2018), GLMM model using “nlme” (Pinheiro et al., 2018), and Random Forests using the “randomForest” (Liaw and Wiener, 2002) packages.

2.4 Results

2.4.1 Relationship between defoliation and hardwood content

Both percent hardwood content and D_{sw} had significant effects on balsam fir defoliation (Table 2.2), with a significant interaction between percent hardwood and D_{sw} , indicating that the relationship between defoliation and percent hardwood varied significantly with overall defoliation severity each year. Examining the fitted relationships from the GLMM, balsam fir defoliation was negatively related to percent hardwood content each year from 2012 to 2016 (Figure 2.2). The fitted lines indicated that the relationship between defoliation and hardwood amount was weak in 2012 and

became stronger in 2013 and 2014, the second and third years of defoliation, then declined in 2015 and 2016. Defoliation was highest in softwood plots in 2014 and 2015 (means of 79% and 87%, respectively), and in those years mean defoliation in hardwood plots was 12% and 55%, respectively (Figure 2.3). In 2012, the first year of defoliation, mean defoliation of balsam fir in softwood, mixedwood, and hardwood plots was 27%, 14%, and 12%, respectively. Average plot defoliation was significantly different among stand types (softwood > mixedwood > hardwood) in 2013 and 2014, when defoliation rapidly increased in softwood and mixedwood plots (Figure 2.3). Defoliation in softwood was significantly higher than in hardwood plots in all 5 years, but was significantly higher than in mixedwood plots only in 2013 and 2014. Defoliation peaked in 2015 in all three stand types, with means of 87%, 70%, and 55% defoliation in softwood, mixedwood, and hardwood plots, respectively (Figure 2.3). Defoliation declined in 2016, to 47% and 42% defoliation in softwood and mixedwood and 15% defoliation in hardwood plots, comparable to years prior to 2015. Over the 5 years, defoliation in softwood plots averaged 14% higher than in mixedwood plots, and defoliation in mixedwood plots averaged 20% higher than in hardwood plots. Average fir defoliation in hardwood plots remained below 20% in all years except 2015, the year with the highest defoliation in all stand types (Figure 2.3).

2.4.2 Defoliation estimated using three model formulations

The Random Forests model demonstrated the best performance among all three models, yielding a correlation (r) of 0.92 with defoliation measured in the field (Figure 2.4C). The GLMM model produced an intermediate correlation ($r = 0.87$) among the

three models (Figure 2.4B), while defoliation estimated using the simplified linear model showed the lowest correlation ($r = 0.77$; Figure 2.4A). The GLMM described the effects of hardwood content and D_{sw} on balsam fir defoliation well ($R^2 = 0.85$ considering fixed effects only and 0.94 considering both fixed and random effects (Nakagawa and Schielzeth, 2013)).

The simplified linear model that estimated defoliation using Equation (2.1) largely underestimated defoliation in mixedwood and hardwood plots under moderate and severe defoliation scenarios (Figure 2.4A). The GLMM performed well but still underestimated defoliation in some hardwood and mixedwood plots under mid-range defoliation level (Figure 2.4B). Among the three models, the Random Forests model provided the most accurate defoliation estimates, but a slight underestimation in softwood plots when actual defoliation was $>80\%$ (Figure 2.4C). Interestingly, both the Random Forests and GLMM models slightly overestimated defoliation in some hardwood plots under light defoliation levels ($<20\%$) (Figure 2.4B, C).

Amongst the 11 variables used to predict annual defoliation with Random Forests, average annual defoliation in softwood plots (D_{sw}) and percent hardwood content were the most important predictor variables, at increases in mean square error of 43% and 18%, respectively (Figure 2.5). D_{sw} was important as an indicator of the overall spruce budworm outbreak severity in a given year, while inclusion of percent hardwood content confirmed its significance in predicting budworm defoliation. Mean DBH, mean height, elevation, and standard deviation of bF height ranked as the third to the sixth most important predictors, at 8-9% increases in mean square error (Figure 2.5). Other predictor variables included in the model (BA, slope, mean DBH of bF, standard deviation of bF

crown base height, and mean bF crown base height) each had <5% increase in mean square error (Figure 2.5). We tried running the Random Forests model eliminating some of the variables with little contribution to accuracy (e.g., using only the top several variables in Figure 2.5), and the resulting correlation between measured defoliation and the model estimate dropped by 2-3%. Variables in the Random Forests model and the GLMM essentially converged, along with the addition of DBH or Height and Elevation in the Random Forests model.

2.5 Discussion

2.5.1 Relationships between defoliation and hardwood content during building phase of a spruce budworm outbreak

Our results on the relationship between spruce budworm defoliation of balsam fir and hardwood content during the building phase of an outbreak generally conformed to observations in the literature that tree diversity reduces herbivory by oligophagous insects (Jactel and Brockerhoff, 2007). Defoliation of fir was lower in plots with higher hardwood content in each of the 5 years of our study. Density of softwood and mixedwood plots was approximately twice that of hardwood plots. Under dense host conditions, natural enemies of budworm may have more predation opportunities since budworm as the prey may be more concentrated. Average balsam fir diameter always exceeded the plot mean diameter in the softwood plots, but this relationship was variable in the mixedwood and hardwood plots. Female budworm moths may have less chance of landing and laying eggs on balsam fir trees in mixedwood and hardwood plots as balsam fir was less dominant in these plots.

Relationships between defoliation and hardwood content varied significantly with average defoliation in softwood plots each year in the GLMM. The relationship was weak in 2012 and became stronger in 2013 and 2014 before declining again in 2015 and 2016. Similarly, a varying relationship between defoliation and hardwood content was found in the declining phase (1989-1993) of the last outbreak (Su et al., 1996). Relationships were stronger in the first 3 years of the sampling period (1989-1991) and declined gradually in the last 2 years (1992 and 1993). In the building phase of a spruce budworm outbreak, populations increase and eventually reach epidemic level. Likewise, populations gradually decrease to endemic levels in the declining phase. Royama et al. (2017) suggested that the budworm outbreak cycle was mainly determined by postdiapause mortality caused by parasitism. The varying relationship between defoliation and hardwood content among years may reflect varying parasitism rates associated with hardwoods. For example, in 2012 when budworm population density was low, the assemblage of hyperparasitoids was smaller than in years with high budworm density (Eveleigh et al., 2007); parasitism rates may be similar in softwood and hardwood plots. When budworm population density rapidly increased in 2013 and 2014, “switching behavior” (Křivan and Schmitz, 2003; Eveleigh et al., 2007) of mobile generalist parasitoids may have been stronger in a heterogeneous environment than in homogeneous forest plots, resulting in higher parasitism rates with more hardwoods than in pure fir plots. Once the budworm population reached its peak in 2015, neither the “birdfeeder effect” (Eveleigh et al., 2007) nor “switching behavior” could stabilize the food web or regulate the severity of the outbreak under the high prey density; thus, parasitism rates may have been similar again and the defoliation reached its highest level in all three stand

types.

Judged based on dispersion of data points around the graphed defoliation versus hardwood content relationships (our Figure 2.2 versus Figure 2 in Su et al. (1996)), our results showed somewhat weaker relationships in the spruce budworm building phase compared with the declining phase. Budworm annual defoliation is significantly affected by budworm outbreak status (MacLean and MacKinnon, 1997). It is unclear why a stronger relationship and larger impact between budworm defoliation and hardwood content occurred in the declining phase than it is in the building phase, but there are two possible explanations. First, in the declining phase of a budworm outbreak, sudden declines in the spruce budworm population in a given year can occur (e.g., third to fourth instar larval population decreased about 20 times in 1988 for Plot 1 and Plot 2 in Royama et al. (2017)). Given that the density of natural enemies (parasitoids) in year X is determined by their density in the previous year (X-1) when the budworm population was higher, parasitism and its impact on budworm populations in year X would be disproportionately large (Royama et al., 2017), resulting in less defoliation and, thus, a strong relationship between defoliation and hardwood content. The second possible explanation is based on the “habitat fragmentation” hypothesis, because tree mortality occurs during the declining phase of a budworm outbreak, resulting in an increasing degree of habitat fragmentation for budworm larvae. After the peak of an outbreak, balsam fir trees and branches as the main food source for budworm would be sparser and patchier than earlier in the outbreak. The worsened host condition in the declining phase could have greater impact on budworm population, and thus defoliation, resulting in a stronger relationship between budworm herbivory and tree diversity. These hypotheses

could be tested by further studies on spruce budworm population dynamics.

2.5.2 Which model provides the “best” defoliation estimates?

The Random Forests model yielded the most accurate defoliation estimates among the three tested models. It incorporated 11 predictor variables, with percent hardwood the second most important predictor, following only average annual defoliation in softwood plots (D_{sw}), reflecting average regional severity of defoliation in a particular year. Hardwood content was a significant factor in predicting budworm defoliation, similar to findings of MacKinnon and MacLean (2003) and Colford-Gilks et al. (2012). Mean DBH and mean height ranked as the third and fourth important variables, suggesting that average tree size had some importance in predicting budworm defoliation. It has repeatedly been observed that mature and over-mature stands have the highest susceptibility and vulnerability to budworm outbreaks (e.g., MacLean, 1984). Standard deviation of bF height was the only variable with >5% increase in mean square error among variables related with canopy structure, indicating that canopy structure had little importance in predicting defoliation in these plots, which were mature with a single canopy layer. We conclude that incorporating more independent variables improved the accuracy of defoliation predictions, but at the cost of constructing a larger and more complex over-fitted model. Such models could be difficult to construct using traditional parametric approaches due to complex interactions among variables and violation of distributional assumptions. Nevertheless, hardwood content and D_{sw} as the two most important independent variables were not correlated and we suggest that they should be included in any budworm defoliation modeling attempts.

Defoliation estimates from the GLMM were less accurate than the Random Forests model but better than estimates from the simplified linear model. Like the Random Forests model, the GLMM model was constructed using sampled defoliation data from all three stand types, but fewer predictor variables were included. The GLMM modified the form of the relationship and included the different relationships between defoliation and percent hardwood content under varying defoliation severities. In contrast, the simplified linear model assumed a constant relationship, but did have the benefit of being able to estimate defoliation in fir-hardwood stands without conducting comprehensive sampling, model construction, or parameter estimation. Using this model, foresters and resource managers can quickly estimate defoliation in mixedwood and hardwood stands using defoliation data from softwood stands. Percent hardwood content is readily available in all forest inventories and D_{sw} as an indicator of average annual defoliation severity can be estimated relative easily from either regional defoliation surveys carried out by government agencies in softwood stands, or regional spruce budworm population sampling such as second instar larval or moth sampling. With these data and Equation (2.1), defoliation in fir-hardwood stands could be quickly estimated. However, either traditional parametric (GLMM) model or non-parametric Random Forests model provided more accurate defoliation estimates than the simplified linear model. In similar analyses, we suggest that data on the average defoliation level, or another indicator of annual outbreak severity, should be included in addition to percent hardwood content.

2.6 Conclusions

Balsam fir defoliation caused by spruce budworm during the initiation and building phase of the outbreak (2012-2016) was significantly lower in plots with higher percent hardwood content. The relationship between defoliation and percent hardwood varied significantly with overall defoliation severity each year. Average defoliation in softwood was significantly higher than in hardwood plots in all years during the studied period, but was significantly higher than in mixedwood plots only in 2013 and 2014 when overall defoliation rapidly increased. Average defoliation severity in softwood plots and percent hardwood content were the two most important variables for predicting balsam fir defoliation caused by spruce budworm. Excluding average defoliation severity in softwood plots as a predictor variable decreased the accuracy of prediction of fir defoliation. A simplified linear model could be used to quickly estimate spruce budworm defoliation in mixedwood stands, but with lower accuracy than parametric or non-parametric modeling approaches that include percent hardwood content and an indicator of average defoliation level or overall outbreak severity. The varying relationship between defoliation and forest stand composition indicated the complexity of the ecological processes that govern the spruce budworm population dynamics (Régnière and Nealis, 2008). The “natural enemy” and/or “habitat fragmentation” hypotheses could vary not only in time and with prey density, but also with changing forest condition.

2.7 Acknowledgments

This research was funded by the Atlantic Innovation Fund project “Early Intervention Strategy to Suppress a Spruce Budworm Outbreak” grant to D.A.M. The

authors provided equal contribution towards decisions regarding methodology and study design. B.Z. wrote the manuscript draft; D.A.M., R.C.J., and E.S.E. contributed to manuscript revision and development. We thank Wayne E. MacKinnon of the Canadian Forest Service for providing inventory data for stand selection, Dr. Chris R. Hennigar for input on statistics, and Annik Proulx of Québec Ministère des Forêts, de la Faune et des Parcs for excluding our sample plots from harvesting and insecticide treatment. Allison Dykstra, Evan Dracup, Matt Hill, Shawn Donovan, Jessica Cormier, Craig Wall, and Maggie Brewer assisted with field data collection.

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Table 2.1. Description of the 27 plots sampled near Amqui, Québec.

Plot no. ^b	Density (stems/ha)	DBH ^c (cm)	Ht ^c (m)	DBH bF ^c (cm)	BA ^c (m ² /ha)	Species composition % basal area ^d					Total HW ^d (%BA)	
						bF	wS	OS	sM	yB		IH
Softwood ^a												
1	1020	20.0±1.1	16.3±0.6	20.7±1.1	37	78	4	9	–	2	8	9
2	2800	13.6±0.5	12.8±0.4	14.1±0.6	49	78	3	–	–	4	15	19
3	920	21.5±1.4	15.7±0.8	23.4±1.3	39	64	17	16	–	–	3	3
4	2975	13.1±0.5	12.9±0.3	13.1±0.5	47	84	3	12	–	–	1	1
5	1650	16.1±0.9	14.6±0.7	15.8±1.0	40	81	–	5	–	–	14	14
6	2125	15.6±0.5	15.8±0.3	15.8±0.6	44	95	–	2	–	–	3	3
7	2100	15.6±0.8	13.9±0.4	16.0±0.8	50	69	7	8	–	1	16	16
8	1100	16.5±1.7	12.7±0.8	21.5±2.2	38	81	–	–	–	–	19	19
9	1380	16.4±1.1	14.1±0.7	22.5±1.4	39	79	–	–	–	8	13	21
Mixedwood												
10	2540	11.2±0.7	9.7±0.5	7.3±0.7	36	20	10	7	–	34	30	64
11	760	19.2±2.1	12.5±1.0	20.8±2.6	32	36	2	–	29	19	13	62
12	1620	14.1±1.1	10.7±0.6	14.1±1.3	37	45	–	–	1	23	31	55
13	960	20.1±1.5	18.7±1.0	20.9±1.7	39	33	11	7	–	1	49	49
14	980	21.5±1.9	17.5±1.2	19.0±1.8	49	27	1	17	–	7	55	55
15	1280	17.8±1.0	17.5±0.9	18.1±1.5	39	42	5	2	–	2	53	53
13a	1980	11.0±0.7	9.7±0.5	10.0±1.3	26	40	–	–	–	–	59	60
14a	1620	13.7±0.9	11.0±0.6	13.3±1.3	32	44	–	–	–	–	56	56
15a	1780	15.1±0.8	12.0±0.5	14.4±0.9	40	57	4	–	–	5	35	40
16	1260	17.7±1.1	15.0±0.5	19.0±1.2	38	48	–	–	9	16	28	52
17	1200	16.9±1.1	14.5±0.6	18.9±2.3	33	43	–	–	8	22	28	57
18	1200	17.3±1.4	14.0±0.6	16.7±1.9	40	33	4	–	6	17	40	63
Hardwood												
19	800	18.2±1.8	16.7±0.8	26.9±7.3	28	10	–	–	90	–	–	90
20	1080	16.8±1.4	14.5±0.8	21.3±3.7	33	24	–	–	35	9	31	76
21	520	22.8±2.3	16.5±1.0	20.0±2.3	27	12	–	–	51	8	29	88
22	1000	18.5±1.7	12.0±0.7	11.4±2.0	38	12	3	–	34	24	26	84
23	620	22.6±2.7	17.2±1.4	26.8±4.1	35	14	–	–	86	–	–	86
24	640	18.7±2.4	12.6±1.0	25.7±1.2	27	8	–	–	16	28	49	92
25	1120	13.7±1.0	15.2±0.7	22.9±3.9	22	8	–	–	81	2	10	92
26	800	17.2±1.7	16.5±0.8	19.7±4.4	25	5	–	–	79	14	2	95
27	560	24.8±2.3	17.2±0.6	19.6±2.8	33	10	–	–	44	28	18	90

^a Softwood, mixedwood, and hardwood stand types were classified by hardwood basal area percentage: softwood (0%–25%), mixedwood (40%–65%), and hardwood (75%–95%). ^b The ‘a’ suffix denotes three sample plots established 1 year after initial sampling to replace original plots which were harvested. ^c Abbreviations: DBH = mean diameter at breast height; Ht = mean total height; DBHbF = mean DBH of balsam fir; BA = basal area. DBH, Ht, and DBHbF are shown as plot average ± one standard error of the mean. ^d Species abbreviations: bF balsam fir; wS white spruce; OS other softwood = black spruce (*Picea mariana* (Mill.) BSP), eastern white-cedar (*Thuja occidentalis* L.), and eastern larch (*Larix laricina* (Du Roi) K. Koch); sM sugar maple; yB yellow birch; IH intolerant hardwood = red maple, white birch, trembling aspen (*Populus tremuloides* Michx.), and balsam poplar (*Populus balsamifera* L.), HW hardwood = sM + yB + IH.

Table 2.2. Results of generalized linear mixed-effects model to test the effects of percent hardwood content (HW%) and average annual defoliation in softwood plots (D_{sw}) on defoliation for 27 plots from 2012 to 2016 in Québec.

Fixed Effects	Parameter Estimates			Analysis of Deviance	
	Par.	Est.	SE	X^2	p
Intercept	β_0	-2.1225	0.3434		
HW%	β_1	-0.0089	0.0061	46.68	<0.001
D_{sw}	β_2	0.0490	0.0055	175.70	<0.001
HW% \times D_{sw}	β_3	-0.0002	0.0001	6.51	0.011

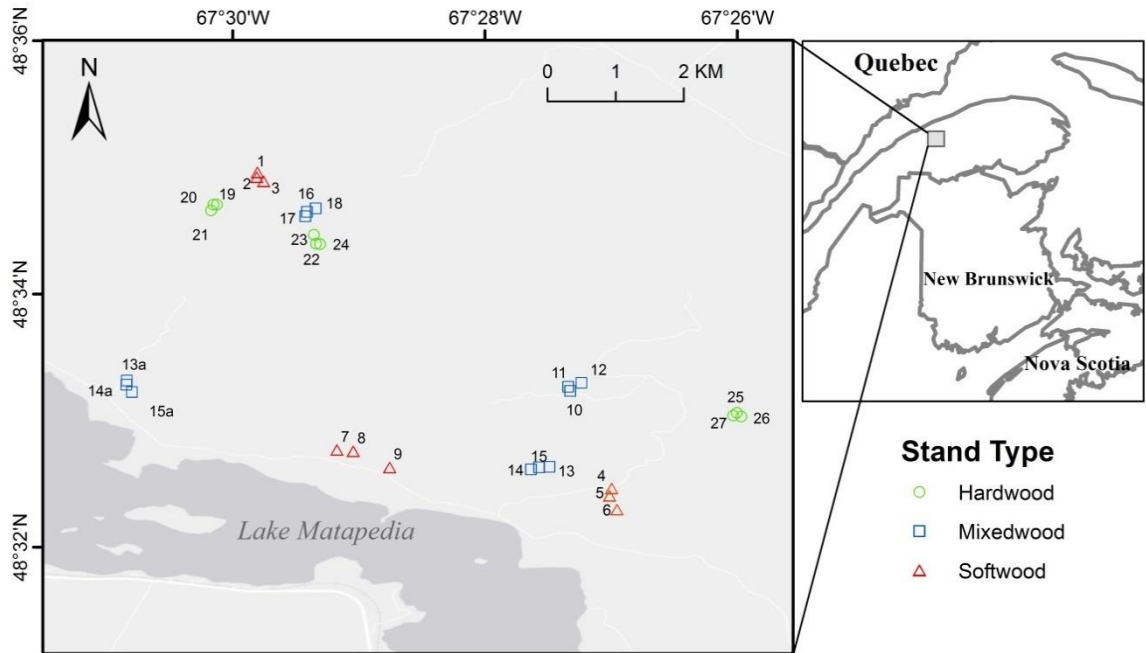


Figure 2.1. Locations of 27 plots sampled near Amqui, Québec, by stand type.

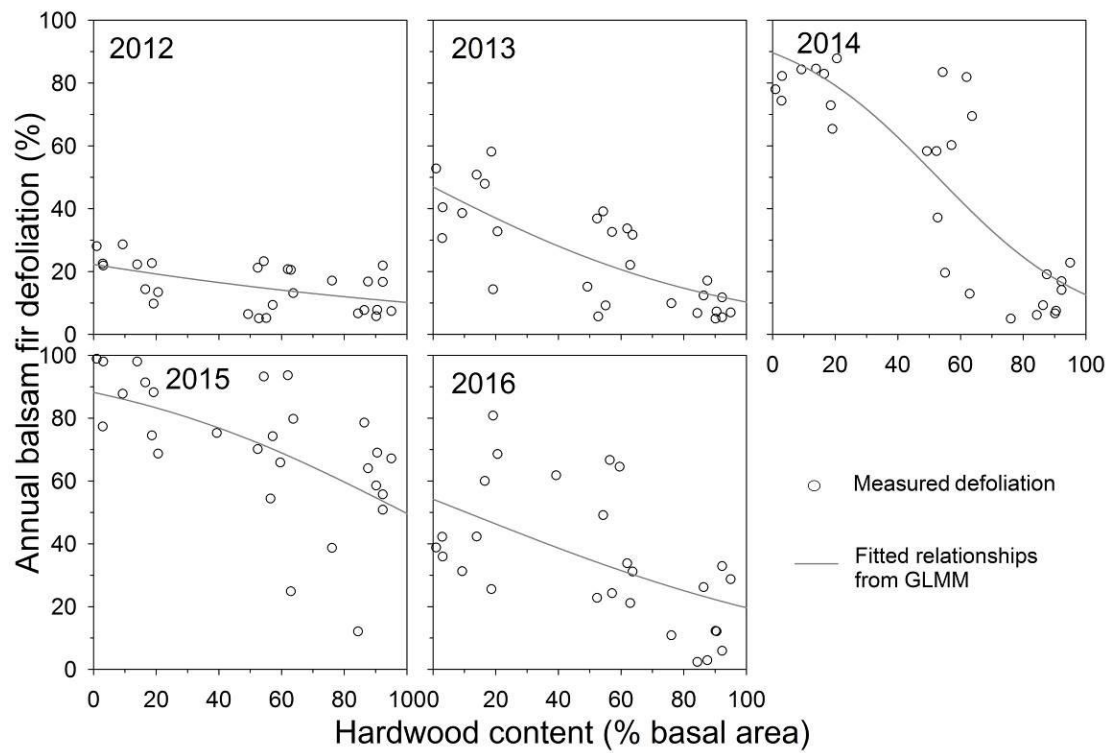


Figure 2.2. Measured balsam fir defoliation and fitted relationships from generalized linear mixed-effects model to test the effects of percent hardwood content on annual plot defoliation from 2012 to 2016, for 27 plots near Amqui, Québec.

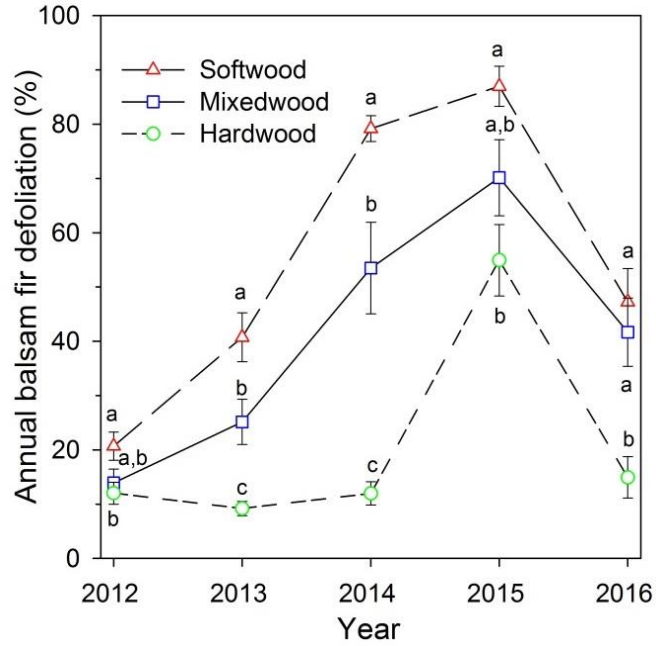


Figure 2.3. Average annual balsam fir defoliation (\pm one standard error) from 2012 to 2016, for nine plots in each of three stand types with varied hardwood contents, near Amqui, Québec. Different letters indicate significant differences among stand types in each year.

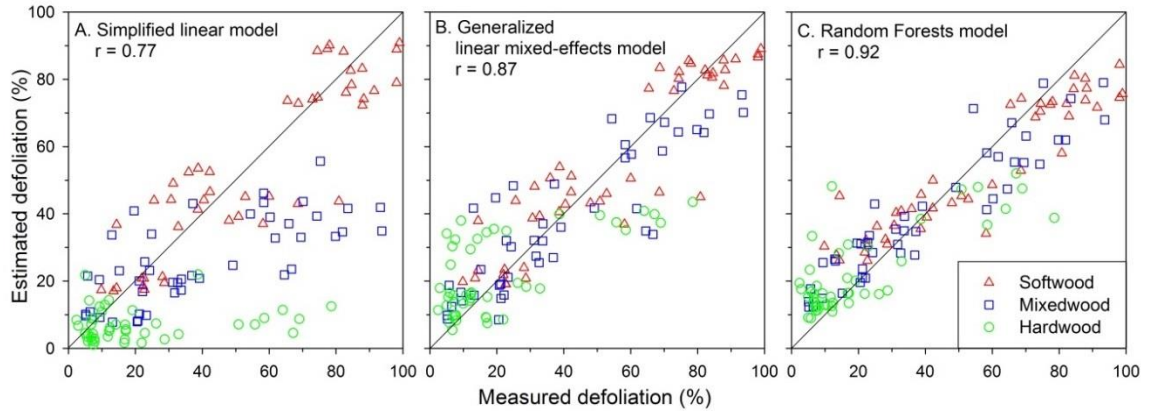


Figure 2.4. Annual plot defoliation, estimated with: (A) the simplified linear model (Equation (2.1)); (B) a generalized linear mixed-effects model; and (C) the Random Forests model, plotted against measured defoliation for 27 plots each year from 2012 to 2016.

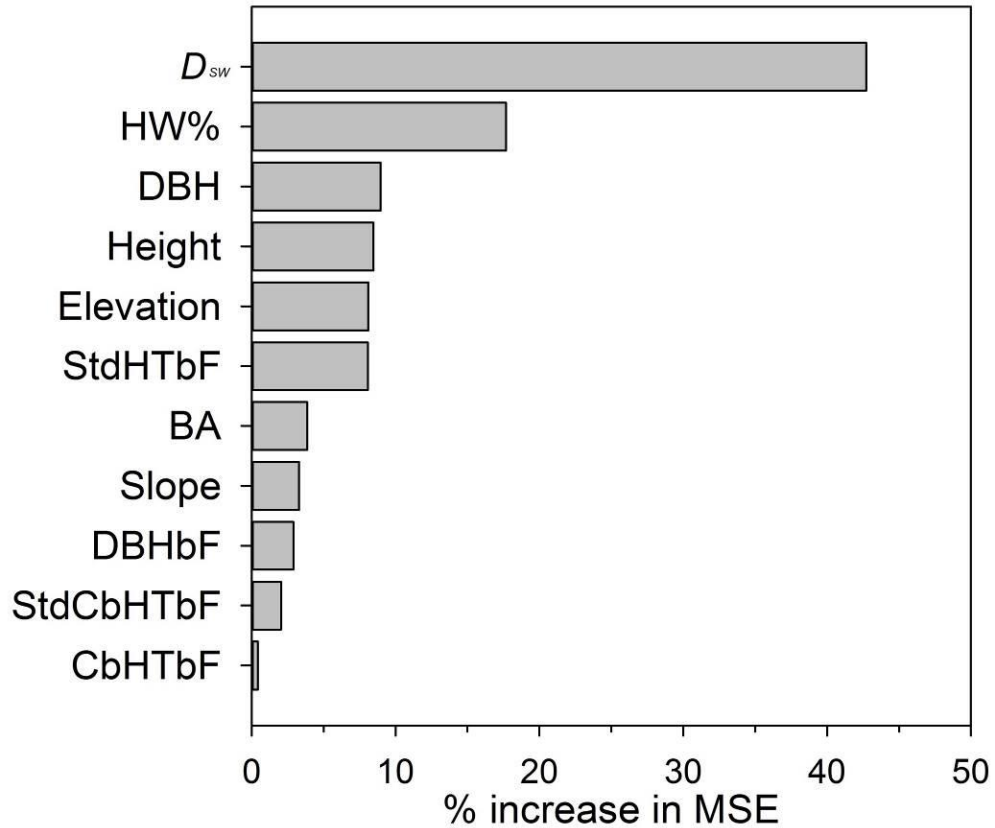


Figure 2.5. Variable importance (percent increase in mean square error of the Random Forests model when the data for that variable were randomly permuted) of the 11 predictors used to predict spruce budworm defoliation in fir-hardwood mixed stands. High values of percent increase in mean square error indicate more important variables in the Random Forests model. The 11 predictor variables were: average annual defoliation in softwood plots (D_{sw}), percent hardwood basal area (HW%), mean DBH (DBH), mean height (Height), elevation, standard deviation of balsam fir (bF) height (StdHTbF), basal area (BA), slope, mean DBH of bF (DBHbF), standard deviation of bF crown base height (StdCbHTbF), and mean bF crown base height (CbHTbF).

**CHAPTER 3: HARDWOOD-SOFTWOOD COMPOSITION
INFLUENCES EARLY-INSTAR LARVAL DISPERSAL
MORTALITY DURING A SPRUCE BUDWORM OUTBREAK**

Paper published as:

Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., Edwards, S., 2020. Hardwood-softwood composition influences early-instar larval dispersal mortality during a spruce budworm outbreak. *Forest Ecology and Management* 463, 118035.

3.1 Abstract

Spruce budworm (*Choristoneura fumiferana* Clem.) defoliation of balsam fir (*Abies balsamea* (L.) Mill.) has been shown to be less severe in stands with higher hardwood (broadleaved trees) content during both building and declining phases of budworm outbreaks. Reduced defoliation associated with forest composition could be explained by habitat fragmentation and/or natural enemy hypotheses, which posit higher early-instar larval dispersal loss or more intense parasitism in hardwood-softwood mixed stands than in pure balsam fir stands, respectively. We carried out field studies in 27 hardwood, mixedwood, and softwood plots with varied balsam fir-hardwood composition to assess effects of forest composition on: 1) first- and second-instar larval dispersal loss, and 2) stage-specific spruce budworm density and parasitism rates. Results indicated that increasing hardwood content increased second-instar (L2) dispersal losses. Stand type significantly affected dispersal loss of L2 larvae, both measured directly for three years and sampled on balsam fir seedlings. Post-hoc pair-wise comparisons indicated that L2 dispersal loss was less in softwood than in mixedwood or hardwood stand types based on measurements on ground traps and less in softwood and mixedwood than in hardwood stands based on sampling budworm larvae on regeneration. This suggested that high early-instar larval dispersal loss is a plausible explanation for the decreased spruce budworm density and balsam fir defoliation associated with increasing hardwood content. Stand type did not significantly affect parasitism rates.

3.2 Introduction

Herbivory by forest insects tends to be lower in forests with diverse plant communities compared with those that are more homogeneous (Jactel and Brockerhoff, 2007; Colford-Gilks et al., 2012; Aoki et al., 2018). Two main hypotheses have been proposed to explain apparent effects of forest composition on insect herbivores. First, the ‘habitat fragmentation’ hypothesis argues that diverse plant communities reduce host tree availability and increase the level of habitat fragmentation for herbivore insects, thereby mitigating effects on host trees through hindered foraging, dispersal, or mating success (Kemp and Simmons, 1979; Cappuccino et al., 1998; Yamamura, 2002). Alternatively, the ‘natural enemy’ hypothesis (Riihimäki et al., 2005) posits that more diverse stand structure supports a greater variety of prey, greater predation opportunities, or better sheltering conditions that confer benefits to natural enemies (Siemann et al., 1998; Quayle et al., 2003; Cardinale et al., 2006), and thus increases prey mortality rates.

In this article, we tested the ‘habitat fragmentation’ and ‘natural enemy’ hypotheses to explain the negative relationship between defoliation caused by spruce budworm, *Choristoneura fumiferana* (Clem.), and increasing hardwood content in fir-hardwood mixed stands (Su et al., 1996; Zhang et al., 2018). During a budworm life cycle, the two processes function in different time periods: mortality from natural enemies occurs during late instars whereas larval dispersal loss caused by habitat fragmentation occurs during early instars. Yet the two processes both affect budworm survivorship within one budworm generation. Spruce budworm outbreaks are a major ecological disturbance throughout the Boreal Shield, Atlantic Maritime, and Mixedwood Plains ecozone forests of North America (Bouchard and Auger, 2014; MacLean, 2016).

Outbreaks inflict substantial economic impacts on the forest sector (Chang et al., 2012; Liu et al., 2019) due to tree growth reduction and extensive tree mortality (MacLean, 2016). Defoliation by spruce budworm larvae on their primary host, balsam fir (*Abies balsamea* (L.) Mill.), tends to be higher in pure balsam fir stands compared with those more intermixed with hardwoods (Su et al., 1996; Zhang et al., 2018). Defoliation severity associated with fir-hardwood content reflects differences in density of spruce budworm larvae (Miller, 1977; Régnière and You, 1991).

For spruce budworm, habitat fragmentation is likely to have the largest impact on early-instar, pre-feeding larvae that undergo periods of dispersal both before and after overwintering. In late summer, following egg hatch, first-instar larvae (L1) disperse to find overwintering sites. In the spring, second-instar larvae (L2) emerge from hibernation and undergo a second bout of dispersal in search of suitable new buds for feeding. In both instances, dispersal occurs via walking or “ballooning” on silken threads and is normally restricted to within or among nearby tree crowns (Miller and Kettela, 1972; Régnière et al., 1989). Subsequent stages of feeding larvae (i.e., third to sixth instar, L3 to L6) rarely disperse unless foliage is heavily depleted (Royama et al., 2017). Losses during these two periods of dispersal are a major source of larval mortality, as dispersing larvae may be carried away by wind to land on the ground or non-host vegetation where death is likely unless they can climb back onto host trees (Régnière and Fletcher, 1983; Régnière and Nealis, 2008). This pre-emergence survivorship (from egg-laying until L2 settle in feeding sites), the loss of larvae during their pre- and post-winter dispersal, is variable due to host-plant feedback (Régnière and Nealis, 2007, 2008). We hypothesize that the risks associated with dispersal during these stages may be higher in stands with greater

hardwood content as dispersers are more likely to land on unsuitable hosts.

Several studies also suggest that increased natural enemy diversity is likely to enhance parasitism in more diverse forests (Quayle et al., 2003; Jäkel and Roth, 2004; Marrec et al., 2018). The spruce budworm food web is very complex, with 66 primary parasitoids and 23 secondary parasitoids identified (Eveleigh et al., 2007). Parasitoids may attack L1 and L2 though they do not actually kill larvae until about L4 (Royama et al., 2017). There is little information available on L1 and L2 mortality caused by diseases, pathogens, or predators. From L3 to L6, post-diapause (after L2 settle in feeding sites until adult eclosion) survival is mainly related to natural enemies, with Hymenopteran and Dipteran parasitoids major sources of mortality (Régnière and Nealis, 2007; Royama et al., 2017). Most parasitoids of spruce budworm cause mortality in the late larval or pupal stages (McGugan and Blais, 1959; Miller, 1963; Régnière and Nealis, 2007; Royama et al., 2017). Most recent studies agree that post-diapause survivorship is the primary driver of the spruce budworm population (Régnière and Nealis, 2007; Royama et al., 2017), while the rate of egg recruitment regulates secondary deviations of the cycle (Royama et al., 2017). We hypothesize that less severe balsam fir defoliation in stands with higher hardwood content may be caused by higher parasitism of L3 to L6 budworm larvae in mixed fir-hardwoods compared with that in more homogenous balsam fir stands.

In this study, we first examined the density of spruce budworm during four different life stages using field data collected over 3 years in 27 plots representing a balsam fir-hardwood composition gradient near Amqui, Québec. Secondly, we measured L1 and L2 larval dispersal using ground traps in these plots and determined parasitism

rates by rearing sampled spruce budworm larvae. For the ‘habitat fragmentation’ hypothesis, we tested the prediction that L1 and/or L2 larval dispersal loss (L1 and L2 that disperse from the upper crown layer and that are generally unable to cause overstory tree defoliation (Régnière and Fletcher, 1983; Johns and Eveleigh, 2013)) will be higher in stands with larger amounts of hardwood content. For the ‘natural enemy’ hypothesis, we tested the prediction that parasitism of spruce budworm will be higher in balsam fir-hardwood mixed plots than in pure fir plots. Spatially, our analyses tested the two hypotheses at the forest stand level.

3.3 Materials and methods

3.3.1 Spruce budworm life history

Spruce budworm is a native defoliator of balsam fir and spruce (*Picea* spp.) species in eastern North America (Morin et al., 2007). In eastern Canada, spruce budworm moths lay eggs in July and August. The L1 larvae disperse to find suitable overwintering sites and molt to L2 for hibernation. L2 emerge in the spring and disperse again to feeding sites. After settling in feeding sites, budworm in the subsequent post-diapause stages (final part of L2 to L6 and pupae) rarely move (Morris, 1963; Royama et al., 2017). Adults emerge in July and can fly short or long distances to lay eggs (Miller, 1975).

3.3.2 Study plots

Field sampling was carried out during the initial years of a spruce budworm outbreak near Amqui, Québec. Annual aerial surveys of spruce budworm defoliation

indicated that defoliation was first detected in the study area in 2012 (Ministère des Forêts de la Faune et des Parcs, 2012). We established 27 0.05-ha circular plots representing a balsam fir-hardwood gradient in spring 2014, with nine plots selected in each of three percent basal area of hardwood content classes: 0-25% (termed softwood), 40-65% (mixedwood), and 75-95% (hardwood) (Table 3.1). Softwood, mixedwood, and hardwood plots averaged 70%, 39%, and 11% balsam fir and 15%, 55%, and 88% hardwoods, respectively (Table 3.1). White spruce (*Picea glauca* [Moench] Voss), another host species of spruce budworm, comprised on average 3.3% of basal area in each plot and existed only in about one half of the plots and thus we did not conduct sampling on white spruce. Plots were located at least 50 m apart, away from stand edges (> 50 m), and collectively were located within a 6.1 km x 4.8 km area on the north side of Lake Matapédia near Amqui (see Zhang et al., 2018 for a map showing plot locations). Within plots, diameter at breast height (DBH) and height of each tree (using an ultrasound hypsometer) were measured. Elevation, slope, tree density, and dominant ground vegetation were recorded. Total basal area, basal area for balsam fir, and basal area for hardwood were calculated for each plot (Table 3.1). Annual balsam fir defoliation, spruce budworm population density in four life stages (L3-L4, L6, pupae, and eggs), L1 and L2 dispersal losses, and parasitism rates per life stage were sampled in each plot. A more detailed description of plot establishment and defoliation sampling is given in Zhang et al. (2018). Previous results from the same set of plots showed that balsam fir defoliation was significantly lower ($p < 0.001$) as percent hardwood content increased but that the relationship varied with overall defoliation severity each year (Zhang et al., 2018).

3.3.3 Spruce budworm sampling

Four balsam fir trees were randomly selected from the dominant or co-dominant crown layer (Smith et al., 1997) in each plot and were sampled at the peak of each of four spruce budworm developmental stages, each year from 2014 to 2016: L3-L4 (sampled between the peaks of the third and fourth instar), L6, pupae (including exuviae, as an estimate of the number of adults; Royama et al., 2017), and eggs (egg masses \times 20 eggs/mass) (Eveleigh and Johns, 2014). One mid-crown branch from each sample tree was collected using pole pruners and shortened to 45 cm from the branch tip (Sanders, 1980; Régnière et al., 1989). Mid-crown branches are widely used for spruce budworm sampling because intra-tree variation is small (Régnière et al., 1989; Eveleigh and Johns, 2014). Cardinal direction does not affect population density (Morris, 1955) and was not controlled in this study. The total spruce budworm density sample analyzed was 1296 branches: 4 branches/plot for each of 4 stages = 16 branches/plot/year \times 3 years \times 27 plots.

Branch tips were bagged and number of spruce budworm L3-L4, L6, pupae, and egg masses per branch tip were counted. A collecting basket was attached to pole pruners during L6 sampling to catch dropping larvae (Sanders, 1980). Branch tips were cut into small pieces and arranged into a 45 cm long rectangle to calculate branch surface area, and spruce budworm density was expressed as number per branch surface area (length \times width) (Royama et al., 2017). Spruce budworm density per life stage was calculated as the average of four branches per plot. Sampling of each stage was conducted within 2 days of the peak of each developmental stage, predicted by: 1) pre-sampling head-capsule width (McGugan, 1954) on up to 50 larvae or pupae, 2) calculating accumulated degree-

days each year using temperature data (Baskerville and Emin, 1969; Dorais and Kettela, 1982) from the Amqui weather station (World Meteorological Organization Identifier 71386), and 3) using the spruce budworm seasonal biology model (Régnière et al., 2012) running under BioSIM-10 software simulation (Régnière et al., 2014). Together, estimation of peak date of each developmental stage was achieved within ± 1 day (Eveleigh and Johns, 2014).

3.3.4 Sampling early-instar dispersal loss and parasitism

Early-instar larval dispersal loss was sampled using nine ground traps (Régnière and Fletcher, 1983) per plot, set out throughout the L1 and L2 dispersal periods. Ground traps were placed evenly on four cardinal direction axes (far-end and mid-point) and at the plot center. Each trap consisted of a 0.25 m² square white plastic board coated with petroleum jelly and fixed horizontally 1 m above ground on a wooden stake, which was also coated with petroleum jelly to prevent larvae climbing up. Trapped larvae were collected and counted 1-2 times every week and the plastic boards were cleaned and recoated if necessary throughout the dispersal periods. Dispersal loss of L1 and L2 larvae was quantified as the number of larvae per m² ground trap that dispersed from one m² basal area of overstory balsam fir, to account for varying host tree populations (i.e., larvae · m² ground trap⁻¹ · m² balsam fir basal area⁻¹).

In addition to ground trap sampling, in summer 2015 we sampled late-instar larvae (L3 to L6) on regenerating balsam fir as another estimate of larval dispersal loss from the overstory tree layer. In the years prior to 2015, we did not observe any larvae on regeneration. Ten large balsam fir seedlings or saplings (height >45 cm and DBH <4 cm)

were sampled in each plot (nine plots in each stand type) and a total of 867 spruce budworm larvae and pupae (3 L3, 82 L4, 267 L5, 508 L6, and 7 pupae) were collected on the balsam fir regeneration (Nie et al., 2018). Ovipositing moths prefer the tallest and most exposed trees and generally do not lay eggs on regeneration (Greenbank, 1963; Batzer, 1968). Therefore we assumed these larvae and pupae developed from L1 or L2 that fell from the overstory tree layer (e.g., Batzer, 1968) because late-instar movements beyond tree crowns are rare except under very high defoliation levels (Royama et al., 2017). Dispersal was quantified as the number of budworm \cdot seedling⁻¹ \cdot m² balsam fir basal area⁻¹, to account for varying overstory host tree populations. These larvae and pupae were completing (or had completed) their late-larval life stages on the regeneration, and thus were used to provide an additional estimate of dispersal losses from the overstory.

Sampling of spruce budworm to assess parasitism was conducted in 2015 and 2016 during the L3-L4, L6, pupae, and egg life stages. Three randomly-selected larvae from each of the L3-L4 and L6 stages were carefully collected using a paintbrush from each of four sample branches per plot. Larvae were reared in creamer cups on an antibiotic-free artificial diet (modified from McMorran (1965), obtained from Canadian Forest Service, Great Lakes Forest Research Center, Sault Ste. Marie, ON, Canada) until parasitoids emerged or moth eclosion occurred. Three pupae or egg masses per branch (in total 12 per stage per plot) were also collected and kept in lidded vials until parasitoids, adults, or L1 larvae emerged. A total of 2592 spruce budworm were reared for parasitism analyses: 12/stage/plot x 4 stages x 2 years x 27 plots. We observed only a few cases where mortality was caused by diseases or unknown sources and discarded those samples

from analyses.

3.3.5 Statistical analyses

All statistical analyses were run in R version 3.6.1 (R Core Team, 2018). Mixed effects models – linear and generalized linear – were conducted using the *lmer* and *glmer* functions (respectively) from the *lme4* package version 1.1-21 (Bates et al., 2015). The *lmerTest* package version 3.1-0 (Kuznetsova et al., 2017) was used to estimate the significance of fixed effects from mixed effect models.

To determine effects of stand type on budworm density we used a linear mixed effect model fit with fixed effects of year, stand type, budworm stage, their interactions (2 and 3-way), and a random plot effect (to account for repeated measures). Density was $\log(x + 1)$ transformed prior to analysis to normalize the data. All 2-way interactions were significant making the effect of stand type difficult to interpret. As such, models were simplified and broken up by budworm life stage (L3-L4, L6, pupae, and eggs). For each individual stage a linear mixed-effect model was fit as described above but with effect of life stage and the 3-way (year x stand type x life stage) interaction removed. For this test and subsequent linear models described below, we inspected residual plots and did not find obvious patterns or heteroscedasticity. R^2 was estimated using the *r.squaredGLMM* procedure from the *MuMIn* package version 1.43.6 (Barton, 2019). Post-hoc testing to further investigate the effect of stand type was carried out using pairwise comparisons of stand type using the *emmeans* function (adjustment = bonferroni) from the *emmeans* package version 1.4.2 (Russell, 2019).

To examine the effect of stand type on dispersal loss of L1 and L2 larvae we used

linear mixed effect models for each larval stage, fit with a fixed effect of year, stand type and their interaction, and a random effect of plot. Dispersal values were transformed using a $\log(x + \frac{1}{2} \text{LDL})$ transformation, where LDL was the lowest detected dispersal level (LDL = 0.07) to normalize the data prior to analysis. R^2 values for each model were estimated using the *r.squaredGLMM* procedure (Barton, 2019). Effect of stand type on dispersal loss quantified based on number of late instar larvae on regeneration was tested with a linear model using the *lm* function (R Core Team, 2018). Pair-wise comparisons of stand types were conducted using the *emmeans* function (Russell, 2019). The dispersal loss was log transformed prior to analysis.

Parasitism data were analyzed using a binomial generalized linear model (link = logit). The dependent variable was a two-column response variable of successes and failures (parasitized and non-parasitized individuals, respectively) per plot. Model parameters included fixed effects of year, stand type, budworm stage targeted, and their interactions (2- and 3-way).

Although the predictor variable stand type by nature is categorical since no plots were sampled with percent hardwood content between each class, i.e., 25-40% and 65-75%, we also tried the analyses of dispersal loss and parasitism using percent hardwood content (%HW) as a continuous predictor variable instead of categorical stand type. This analysis for dispersal yielded similar results in terms of variable significance (for L1: year $p = 0.108$, %HW $p = 0.179$, year x %HW $p = 0.011$; for L2: year $p < 0.001$, %HW $p < 0.001$, year x %HW $p = 0.877$; for Regen: %HW $p < 0.001$) and thus did not affect our interpretations. The parasitism model also produced similar results and did not help elucidate effects of hardwood content on budworm parasitism, and as such we only

present results from the analyses by stand type in the results

3.4 Results

3.4.1 Differences in spruce budworm density among stand types

There were significant effects of year, stand type, and their interactions on density of L3-L4, L6, and pupae (Table 3.2, Figure 3.1). Spruce budworm density per m² of branch surface area was consistently highest in softwood (balsam fir), intermediate in mixedwood, and lowest in hardwood stand types (Figure 3.1). However, pair-wise post hoc comparisons of stand types among years suggested no significant differences in budworm density between softwood and mixedwood stands, but hardwood stands had significantly lower density than both softwood and mixedwood stands in eight out of 18 contrasting pairs (Table 3.3). Especially in 2014, the year before peak density (Figure 3.1), budworm density in hardwood stands was significantly lower than in softwood or mixedwood stands in all contrasting pairs except for L6 in mixedwood-hardwood (Table 3.3). Egg density was significantly affected by year but stand type was not significant, although near the boundary of significance ($p = 0.073$, Table 3.2). The models explained 70%, 37%, 39%, and 17% of the variance in budworm density of L3-L4, L6, pupae, and eggs, respectively, or including random effects, 82%, 47%, 46%, and 42%, respectively.

3.4.2 Effects of hardwood content on early-instar larval dispersal loss

L1 dispersal loss averaged 0.23 ± 0.03 , 0.60 ± 0.09 , and 0.80 ± 0.26 larvae \cdot m² ground trap⁻¹ \cdot m² balsam fir basal area⁻¹ for softwood, mixedwood, and hardwood plots, respectively (Figure 3.2). L1 dispersal loss differed significantly among years and there

was a significant interaction between year and stand type, but effect of stand type was non-significant ($p = 0.057$) (Table 3.4). Because there was a significant year x stand type interaction, we conducted post-hoc pair-wise comparisons of L1 dispersal among stand types by year, but the comparisons did not show significant differences among stands (Table 3.5, Figure 3.2). The model explained 41% (52% including random effects) of the variance in L1 dispersal loss.

Dispersal loss of L2 was much higher, and averaged 0.92 ± 0.21 , 2.25 ± 0.91 , and 4.52 ± 1.20 larvae \cdot m² ground trap⁻¹ \cdot m² balsam fir basal area⁻¹ for softwood, mixedwood, and hardwood plots, respectively (Figure 3.2). L2 dispersal loss differed significantly among years and stand types, but the year x stand type interaction was not significant (Table 3.4). Pair-wise comparisons of dispersal among stand types showed that mixedwood and hardwood stands had significantly higher levels of L2 dispersal loss than softwood stands (Table 3.5, Figure 3.2), but that mixedwood and hardwood stands were not significantly different. The model explained 74% (81% including random effects) of the variance in L2 dispersal loss. Averaging all plots and years, L2 dispersal loss was, on average, 4.5 times as large as for L1 (Figure 3.2).

Stand type also had a significant effect on dispersal loss quantified based on number of late instar larvae counted on balsam fir regeneration ($r^2 = 0.39$, Table 3.4). Softwood and mixedwood stands had significantly fewer larvae counted on understory balsam fir trees than in hardwood stands (Table 5, Figure 3.3). On average, 0.14 ± 0.02 , 0.28 ± 0.07 , and 0.66 ± 0.14 larvae \cdot seedling⁻¹ \cdot m² balsam fir basal area⁻¹ occurred in softwood, mixedwood, and hardwood plots (Figure 3.3).

3.4.3 Effects of stand type on parasitism rates

Median parasitism rates of each of the L3-L4, L6, pupae, and egg life-stages in the three stand types ranged from 0-20% in 2015 and from 0-29% in 2016 (Figure 3.4). Parasitism rates varied significantly among years ($p = 0.012$) and budworm stage attacked ($p < 0.001$), but there was no effect of stand type ($p = 0.40$) (Figure 3.4). Additionally, there were no significant interactions among fixed effects (year, stand type and budworm life stage).

3.5 Discussion

Our results supported the ‘habitat fragmentation’ hypothesis, that diverse plant communities reduce host tree availability and increase the level of habitat fragmentation for herbivore insects, thereby mitigating effects on host trees through hindered foraging, dispersal, or mating success (Kemp and Simmons, 1979; Cappuccino et al., 1998; Yamamura, 2002). Diverse tree species will maintain the overall functioning of an ecosystem when biotic and abiotic conditions are temporally disturbed. This has been termed the ‘insurance hypothesis’: biodiversity insures ecosystems against declines in their functioning because many species provide greater guarantees that some will maintain functioning even if others fail (Yachi and Loreau, 1999). Stand type significantly affected dispersal loss of L2 larvae, both measured directly in three years and sampled on balsam fir seedlings. Post-hoc pair-wise comparisons indicated that L2 dispersal loss, quantified as larvae \cdot m⁻² of overstory balsam fir basal area, was less in softwoods than in mixedwood or hardwood stand types based on measurements on ground traps and less in softwood and mixedwood than in hardwoods based on sampling

budworm larvae on regeneration. This suggested that high early-instar larval dispersal loss is a plausible explanation for the decreased spruce budworm density and balsam fir defoliation associated with increasing hardwood content. L2 dispersal loss was, on average, 4.5 times as large as for L1, perhaps because L1 disperse mostly within crowns whereas L2 more readily disperse between tree crowns (Régnière and Fletcher, 1983; Johns and Eveleigh, 2013). Overall, L2 dispersal loss declined over the three years sampled, corresponding to declining defoliation estimates for the same areas (Zhang et al., 2018).

Our results are consistent with those of Régnière and Fletcher (1983) that dispersal loss due to “wastage” of dispersing larvae on non-host material mostly occurred for L2 in spring, and dispersal loss was influenced by stand structure, host species, and previous defoliation. Prolonged defoliation can cause significant decreasing trends of early-instar survival due to the worsened forest conditions (Régnière and Nealis, 2007). This host-plant feedback mechanism, i.e., changes in host condition adversely affecting budworm survival via an increase in dispersal mortality (Nealis et al., 2003; Nealis, 2015), was observed in spruce budworm (Régnière and Nealis, 2008; Nealis, 2016) as well as two other conifer feeding *Choristoneura* species: jack pine (*Choristoneura pinus pinus* Free) (Nealis and Lomic, 1994) and western spruce budworm (*Choristoneura occidentalis* Free) (Nealis and Régnière, 2009). Repeated defoliation in primarily conifer stands reduced viable foliage, changed stand characteristics, and caused higher dispersal mortality (Régnière and Nealis, 2007, 2008; Nealis and Régnière, 2009), whereas in our study balsam fir foliage was inherently less abundant in fir-hardwood plots with large amount of hardwoods. Although higher dispersal mortality resulted from two different

mechanisms: one through host-plant feedback and the other caused by differences in forest composition, they essentially demonstrated a similar ecological process in that a higher degree of “habitat fragmentation” increased dispersal losses. Contribution of early-instar mortality to budworm population change may warrant more attention (Campbell, 1993; Nealis and Régnière, 2009).

Analysis of L1 dispersal loss based on ground trap sampling showed a significant stand type x year interaction, indicating that the relationship between dispersal loss and hardwood content varied among years, but post-hoc tests within years revealed no significant differences among stand types. In contrast, L2 dispersal loss was significantly influenced by year and by stand type, but interaction terms were not significant. Therefore, although L2 dispersal losses varied among years, the effect of hardwood content on dispersal loss was consistent among years. Higher L2 dispersal losses in plots with more hardwoods, and thus a higher degree of habitat fragmentation, were associated with lower spruce budworm density and defoliation. Even if dispersing larvae do not land on the ground or a nonhost tree, they may be subject to higher risk of parasitism or predation than stationary larvae due to exposure during dispersal (Johns and Eveleigh, 2013). Indirectly, L1 and L2 dispersal loss can be estimated by comparing L3 density to egg density. Measuring dispersal loss directly is problematic, as the proportion of the population that is involved in dispersal, but did not land on the ground, is very difficult to measure by direct methods (Morris and Mott, 1963). Those larvae that land on non-host trees are likely to continue to disperse to find suitable foliage (Johns and Eveleigh, 2013). Therefore tree and branch density per plot could affect the probability of dispersal success in a series of “balloonings”. However, no information is available on L1 or L2

dispersal loss before landing on the ground. To make direct measurement of L1 and L2 dispersal loss possible, the best assumption is that dispersal continues until larvae reach a host species or fall onto the forest floor; once on the ground, they would be deemed “loss” as they would be unable to damage upper tree layers unless they can climb back onto host trees. L1 do not climb back onto host trees once on the ground, but about 15% of L2 could be “recovered” this way (Régnière and Fletcher, 1983). Our results disregard this recovery behavior and assumed that dispersal loss would be proportionally higher with higher larval catch on the ground traps. We placed ground traps evenly in our plots and compared larval dispersal at the plot level for the three stand types. Future study could examine each ground trap in relation to its surrounding tree and branch conditions to investigate effects of vegetational and structural characteristics on dispersal loss at the within-plot scale. Insect dispersal is affected by the spatial scale under which it is examined. Yet the distance of L1 and L2 dispersal is limited (Johns and Eveleigh, 2013) and thus is less affected by spatial scale. Still, further study could consider the effects of surrounding landscape on early-instar dispersal. Although hardwood content in fir-hardwood stands causes habitat fragmentation for budworm, we did not directly measure the degree of habitat fragmentation nor establish a quantitative relationship between fragmentation and percent hardwood content. Further study could investigate this relationship and quantify the relationship between early-instar dispersal loss and degree of habitat fragmentation.

Our results did not support the ‘natural enemy’ hypothesis (Riihimäki et al., 2005) that more diverse stand structure supports a greater variety of prey, greater predation opportunities, or better sheltering conditions that confer benefits to natural enemies. We

did not detect a significant difference between budworm parasitism rates and forest composition. Parasitoids are known to play an important role in the spruce budworm food web (Eveleigh et al., 2007), and many parasitoid species have been reported to be higher in mixedwood forests (Simmons et al., 1975; Houseweart, 1984; Cappuccino et al., 1998; Quayle et al., 2003). However, interaction between the parasitoid community and spruce budworm is highly complex, with nearly 100 specialist and generalist parasitoids involved (Eveleigh et al., 2007), and parasitism by some parasitoids, e.g., *Glypta fumiferanae* (Viereck), has been found to be negatively related to forest diversity (Legault and James, 2018). Thus, differing parasitoid species may be involved in different stand types and the aggregated response of the parasitoid communities may respond differently to differing tree species composition. Our sampling and rearing sample sizes in this study were limited owing to sampling across 27 plots, and it is possible that increased rearing sample sizes over more years might reveal stronger relationships of specific parasitoid species to stand type. Parasitism is affected by the spatial scale under which it is examined (Legault and James, 2018; Marrec et al., 2018). We conducted an empirical field study at the plot level, with all plots restricted to a relatively small area (29 km²) so that all plots would be at the same stage of the spruce budworm outbreak. If sampling had been done in a larger area where landscape heterogeneity could be taken into account, an alternative approach could be adopted to study the relationships between insect parasitoids and forest structure under multiple spatial scales (Roland and Taylor, 1997). Dispersal distance of parasitoids varies greatly from meters to a scale of kilometers, affected by many factors including host density, body size of parasitoids, forest structure, and parasitoid perception of spatial scale.

(Elzinga et al., 2007; Xu et al., 2016; Legault and James, 2018; Marrec et al., 2018). Using this approach, parasitoids sampled at the plot level were related to predictor variables extracted at the landscape level. For spruce budworm, Legault and James (2018) found that the two major parasitoids *Apanteles fumiferanae* (Viereck) and *Glypta fumiferanae* were affected oppositely by forest diversity and responded to forest diversity at different spatial scales, perhaps due to differences in flying capacity and body size (Legault and James, 2018). A patchy landscape with non-host species (such as hardwood stands) could restrain the spread of defoliation possibly due to hindered moth dispersal (Cappuccino et al., 1998; Nealis and Régnière, 2004; Bouchard and Auger, 2014). It is likely that dispersal of parasitoids could be affected at the landscape level if they aggregate to a high host budworm density, especially in epidemic populations. However, at the plot level, effects of dispersal of parasitoids and their response to forest composition is less clear. Within a relatively small area, it is possible that individual parasitoids move frequently from high predation pressure (competition) to low pressure areas. Eventually, equilibrium is reached and parasitism on budworm will be more or less equally applied within the small area regardless of forest stand composition. Although positive or negative relationships between certain parasitoid species and forest composition have been observed (Quayle et al., 2003; Eveleigh et al., 2007; Legault and James, 2018), we did not detect a relationship between the aggregate parasitism and forest composition at the plot level. To thoroughly understand the dispersal behavior and capability of parasitoids, molecular marking or tracking could be the best approaches for the purpose at either landscape or plot level (Lavandero et al., 2004; Kankare et al., 2005).

Predatory birds are unlikely to affect the budworm outbreak cycle (Royama et al., 2017), but could contribute to differences in budworm survivorship between stand types (Drapeau et al., 2000), especially when budworm levels are low. Crawford and Jennings (1989) reported that the bird community showed significant functional response at low and transitional budworm density but provided ineffectual predation at high budworm density. It is not known whether budworm mortality caused by pathogens, entomopathogenic fungi, viruses, or mammalian predators differ with forest composition, but diverse forest composition provides enhanced ecosystem functions and services and might support more diverse communities (Berg, 1997; Forrester and Pretzsch, 2015; Sayer et al., 2017).

Spruce budworm density was significantly influenced by stand type and by significant year x stand type interactions for the L3-L4, L6, and pupae life stages. The trends of higher spruce budworm densities in softwood (balsam fir) than in mixedwood and higher in mixedwood than in hardwood stands were consistent with significant differences in defoliation among stand types (Zhang et al., 2018). Other factors, including year and budworm life stage, showed significant interactions in our analysis. Spruce budworm density was highest in L3-L4 in 2015, when defoliation averaged about 87% in softwood stands, the most severe among the years sampled (Zhang et al., 2018). We observed a high survival from L3-L4 to pupae in 2014, then the survival decreased in 2015 and 2016. Régnière et al. (2019) studied budworm populations from 2012 to 2014 in a nearby geographic region and also found high survival in 2014, increased from previous years. They considered this sudden shift in survival as evidence supporting the “double-equilibrium theory” (Ludwig et al., 1978), one of the two most popular theories

explaining the start, termination, and pattern of spruce budworm outbreaks.

Determining the ecological mechanisms underlying the less severe balsam fir defoliation by spruce budworm associated with hardwoods would help ecologists and entomologists better understand how forest composition and structure affect insect herbivory. It also provides insights for successful mixedwood management and pest control during spruce budworm outbreaks (Johns et al., 2019). Our results suggested that hardwood mixed with balsam fir reduced spruce budworm early-instar larval dispersal success and contributed to the observed reduced defoliation level (Zhang et al., 2018). Parasitism of spruce budworm was not significantly related to stand type. Altering tree species composition or stand structure (Régnière and Fletcher, 1983) to hinder early-instar dispersal success could therefore reduce stand susceptibility to spruce budworm outbreaks.

3.6 Conclusions

Early-instar larval dispersal losses, especially during the spring as L2 searched for suitable feeding sites, increased with increasing forest hardwood content. Higher early-instar larval dispersal loss caused by ‘habitat fragmentation’ is thus a plausible hypothesis to explain the decreased defoliation associated with hardwood content in fir-hardwood mixed stands. Parasitism of spruce budworm, however, was not significantly influenced by stand type in our study, and we were thus unable to lend support to the ‘natural enemy’ hypothesis.

3.7 Acknowledgments

This research was funded by the Spruce Budworm Early Intervention Strategy

project supported by the Healthy Forest Partnership, Atlantic Innovation Fund, Canada, and by Natural Resources Canada, with grant number 203544 to Dr. David A. MacLean. We thank the Atlantic Forestry Centre, Canadian Forest Service and the New Brunswick Department of Energy and Resource Development for assistance with budworm sampling. Drs. Stephen Heard and Dan Quiring provided valuable comments on the results. Allison Dykstra, Evan Dracup, Zhuoyi Nie, Matt Hill, Shawn Donovan, Garrett Brodersen, Jessica Cormier, Craig Wall, Maggie Brewer, and Yanyi Li assisted with field data collection.

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Table 3.1. Average stand density, basal area, percent balsam fir content, and percent hardwood content (\pm one standard error, $n = 9$) of the softwood, mixedwood, and hardwood stand types sampled near Amqui, Québec.

Stand type	Softwood	Mixedwood	Hardwood
Density (stems ha ⁻¹)	1771 \pm 273	1432 \pm 145	793 \pm 76
Basal area (m ² ha ⁻¹)	44.4 \pm 2.0	36.7 \pm 1.6	29.8 \pm 1.8
Balsam fir (% basal area)	70.3 \pm 3.0	38.9 \pm 2.8	11.4 \pm 1.8
Hardwood (% basal area)	14.5 \pm 2.4	55.5 \pm 2.0	88.0 \pm 1.9

Table 3.2. Effects of year and stand type on the density of spruce budworm at various life stages. Significant p values are shown in bold font.

Dependent variable	Fixed effect	Mean square	F	p
L3-L4	Year	14.32	82.50	<0.001
	Stand type ^a	3.28	18.90	<0.001
	Year × Stand type	0.89	2.11	0.002
L6	Year	2.81	11.23	<0.001
	Stand type	1.36	5.44	0.010
	Year × Stand type	1.00	1.01	0.007
Pupae	Year	1.97	3.81	0.023
	Stand type	4.41	8.47	0.001
	Year × Stand type	3.35	6.44	<0.001
Eggs	Year	6.25	3.18	0.050
	Stand type	5.67	2.89	0.073
	Year × Stand type	0.84	0.43	0.786

^a Stand types were defined based on % hardwood content: softwood 0-25%, mixedwood 40-65%, and hardwood 75-95%.

Table 3.3. Results (p values) of post-hoc pair-wise comparisons of budworm density among stand types for three budworm life stages in which a significant effect of stand type was observed. Significant p values are shown in bold font.

Year	Pair-wise stand types	L3- L4	L6	Pupae
2014	Softwood – mixedwood	0.480	0.941	0.999
	Softwood – hardwood	<0.001	0.004	<0.001
	Mixedwood – hardwood	<0.001	0.128	<0.001
2015	Softwood – mixedwood	0.915	1.000	0.915
	Softwood – hardwood	<0.001	1.000	0.999
	Mixedwood – hardwood	0.012	1.000	1.000
2016	Softwood – mixedwood	0.990	0.803	0.999
	Softwood – hardwood	0.461	0.807	0.973
	Mixedwood – hardwood	0.951	0.052	0.681

Table 3.4. Effects of year and stand type on the dispersal loss (larvae · m² ground trap⁻¹ · m² balsam fir basal area⁻¹) of spruce budworm larvae. Dispersal loss was measured during the L1 and L2 life stages using ground traps and also quantified by sampling budworm found on regenerating balsam fir trees (“Regen”). Dispersal loss on regenerating trees was only quantified in one year, therefore, year was not included as a model parameter. Significant p values are shown in bold font.

Dependent variable	Fixed effect	Mean square	F	p
L1	Year	21.47	22.97	<0.001
	Stand type ^a	2.99	3.20	0.057
	Year × Stand type	2.54	2.72	0.034
L2	Year	97.09	131.22	<0.001
	Stand type	6.86	9.27	0.001
	Year × Stand type	0.95	1.28	0.290
Regen	Stand type	4.59	12.81	<0.001

^a Stand types were defined based on % hardwood content: softwood 0-25%, mixedwood 40-65%, and hardwood 75-95%.

Table 3.5. Results (p values) of post-hoc pair-wise comparisons of budworm dispersal loss among stand types. Dispersal loss was quantified for L1 and L2 life stages using ground traps as well as quantified using budworm individuals found on regenerating balsam fir trees (“Regen”). Significant p values are shown in bold font.

Pair-wise stand types	L1 ^a			L2	Regen
	2014	2015	2016		
Softwood – mixedwood	0.930	0.845	0.396	0.023	0.128
Softwood – hardwood	0.996	0.963	0.157	<0.001	<0.001
Mixedwood – hardwood	1.000	0.178	1.000	0.378	0.016

^a Effects of stand type were marginally non-significant in linear mixed effects model. Pair-wise comparisons were conducted within each year because of significant interaction Year × Stand type (Table 3.4).

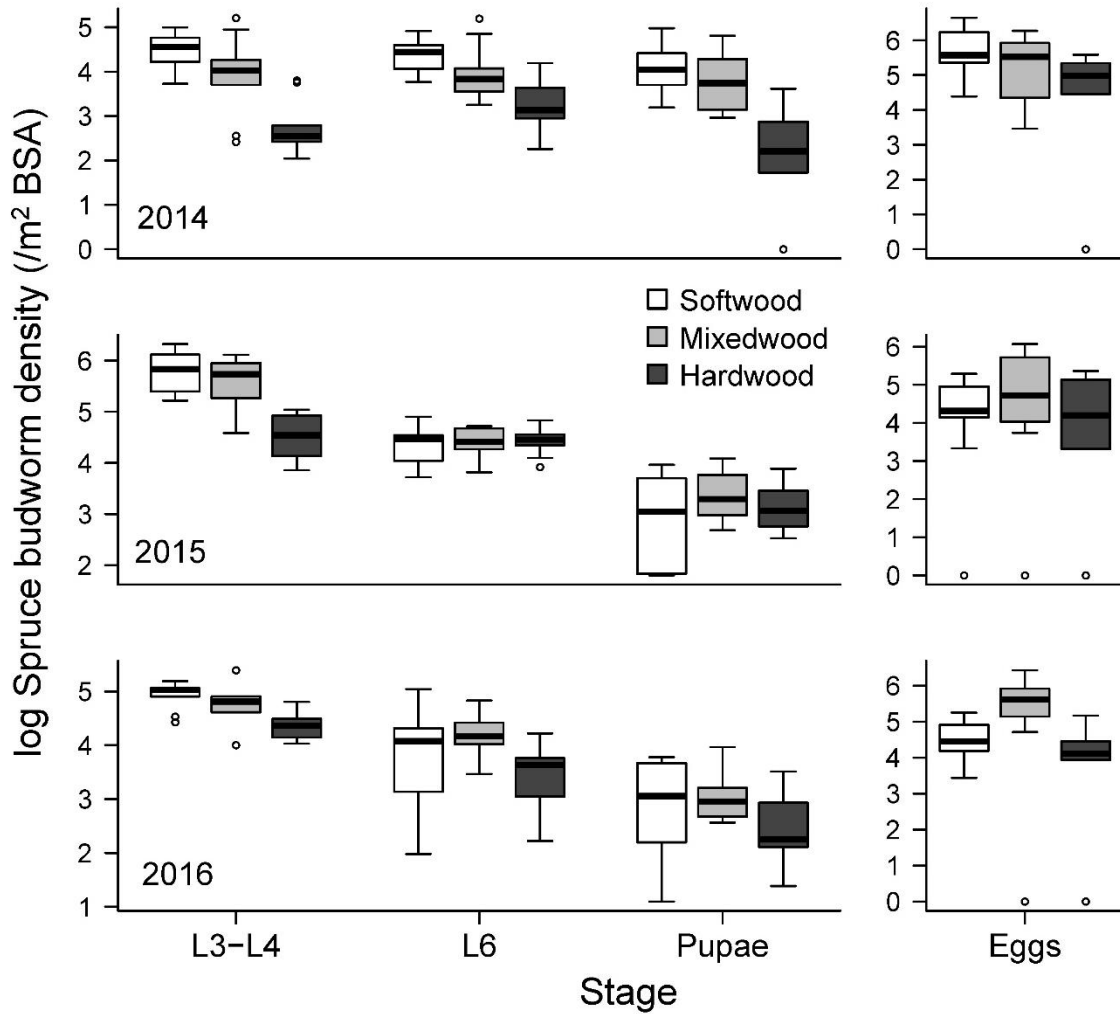


Figure 3.1. Spruce budworm density (log number per m² branch-surface-area) among stand types sampled from 2014-2016, for nine plots in each of three stand types (based on % hardwood content: softwood 0-25%, mixedwood 40-65%, hardwood 75-95%) near Amqui, Québec. Boxes represent 25 and 75% quartiles and the middle lines represent the median. Whiskers show 2.5 and 97.5% quantiles and points depict any outliers.

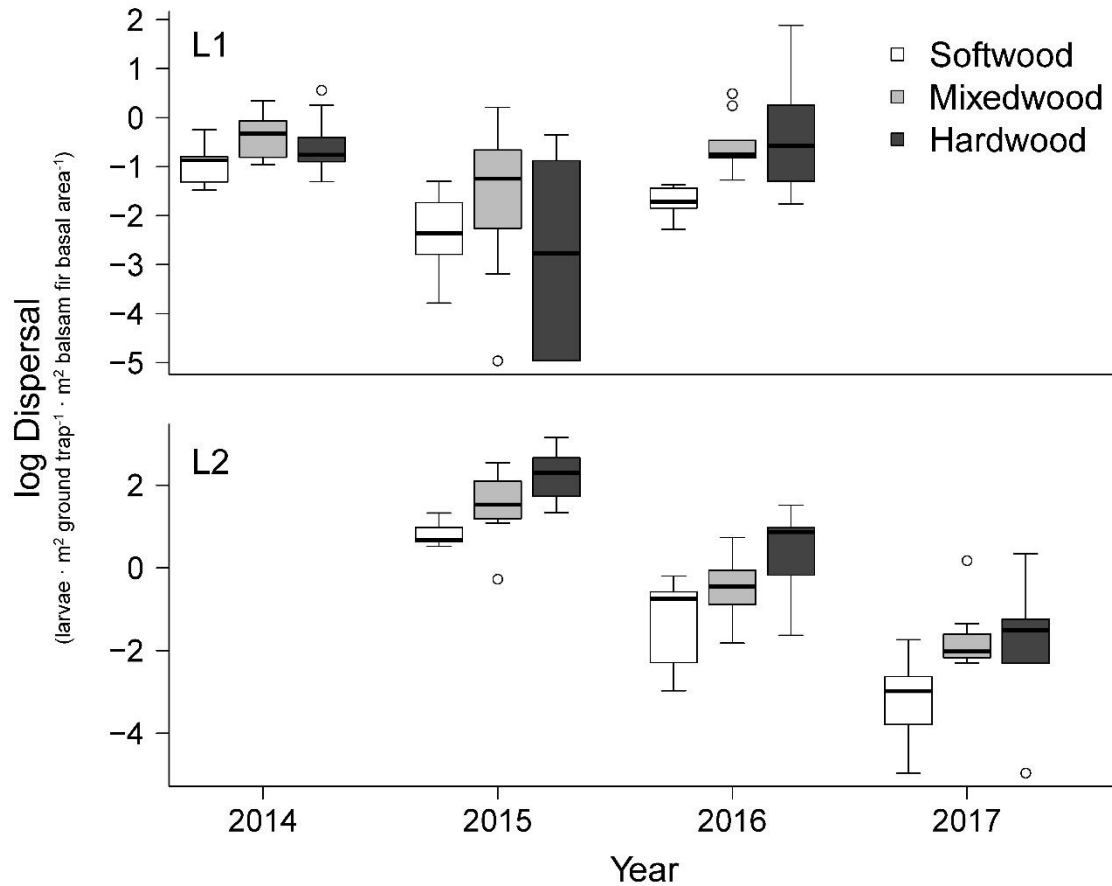


Figure 3.2. Spruce budworm first instar (L1) and second instar (L2) larvae dispersal loss ($\log \text{larvae} \cdot \text{m}^2 \text{ ground trap}^{-1} \cdot \text{m}^2 \text{ balsam fir basal area}^{-1}$) among three stand types in three consecutive years. Boxes represent 25 and 75% quartiles and the middle lines represent the median. Whiskers show 2.5 and 97.5% quantiles and points depict any outliers. Dispersal loss of larvae was presented per m^2 of overstory balsam fir basal area to account for differences in host tree density, and was log transformed because of skewness. We assumed based on Régnière and Fletcher (1983) that most early-instar larvae caught on ground traps would be unable to relocate onto host trees and thus a high proportion would die.

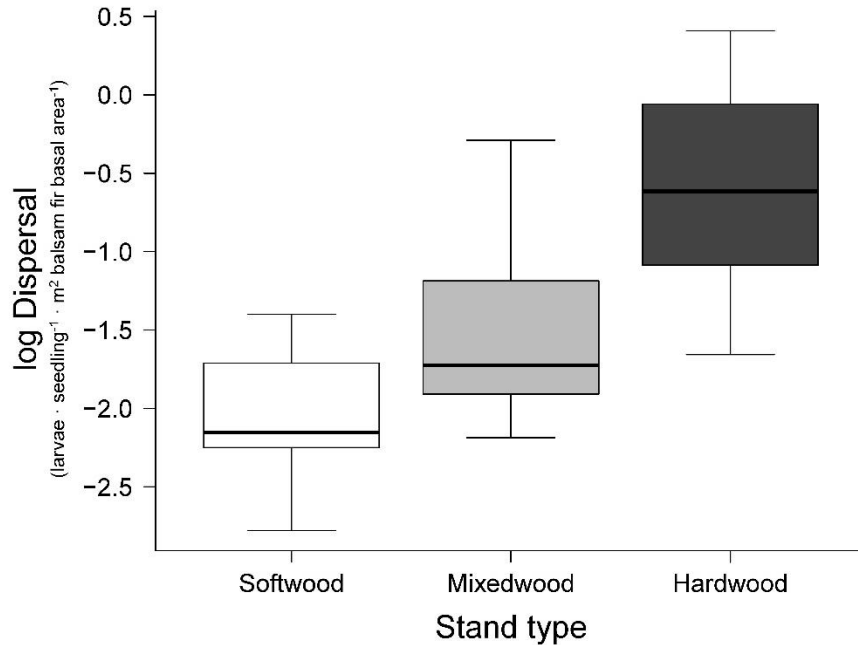


Figure 3.3. Dispersal ($\log \text{ budworm} \cdot \text{seedling}^{-1} \cdot \text{m}^2 \text{ balsam fir basal area}^{-1}$) of spruce budworm larvae from overstory trees to the understory balsam fir regeneration (height >45 cm and DBH <4 cm) in fall 2015, for 27 plots near Amqui, Québec. Boxes represent 25 and 75% quartiles and the middle lines represent the median. Whiskers show 2.5 and 97.5% quantiles. Budworm larvae or pupae (L3 to pupae) on balsam fir regeneration typically originate from L1 or L2 in overstory trees (Batzer, 1968), and here were assumed to be dispersal losses from the overstory population because they will complete their life stages on the regeneration and thus would not cause defoliation of overstory trees.

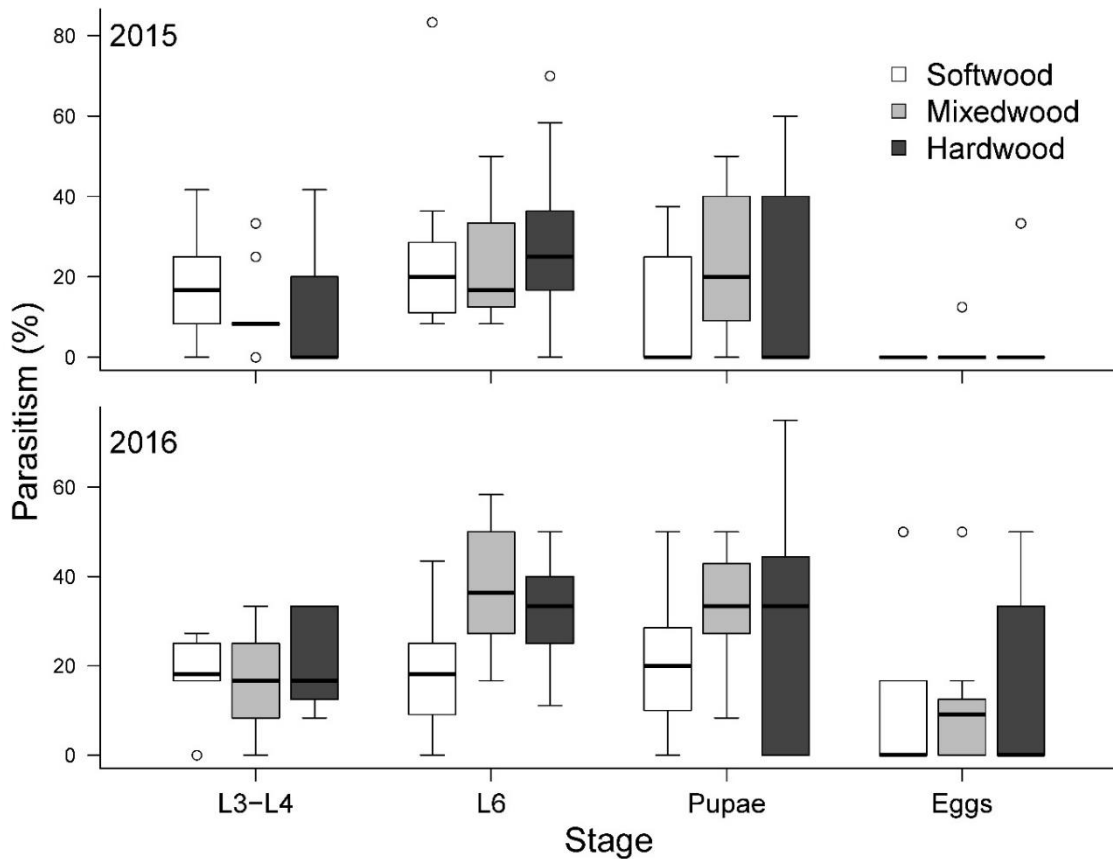


Figure 3.4. Percent parasitism in four spruce budworm life stages in two years, for nine plots in each of the three stand types near Amqui, Québec. Boxes represent 25 and 75% quartiles and the middle lines represent the median. Whiskers show 2.5 and 97.5% quantiles and points depict any outliers. Percent parasitism was determined by collecting and rearing ca. 2590 randomly-selected spruce budworms (3 samples per life stage x 4 sample branches/plot x 4 stages x 2 years x 27 plots). Eggs sampled at the end of 2015 and 2016 sampling seasons belonged to budworm generations in 2016 and 2017, respectively.

**CHAPTER 4: COMPARISON OF COMMUNITY STRUCTURE OF
THE SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE)
PARASITOID ASSEMBLAGE IN SOFTWOOD, MIXEDWOOD,
AND HARDWOOD FOREST STANDS DURING THE RISE OF
AN OUTBREAK**

Paper planned for submission as:

Zhang, B., MacLean, D.A., Eveleigh, E.S., Johns, R.C., 2019. Comparison of community structure of the spruce budworm (Lepidoptera: Tortricidae) parasitoid assemblage in softwood, mixedwood, and hardwood forest stands during the rise of an outbreak.

4.1 Abstract

Diversity and structure of high trophic level communities such as parasitoids of herbivore insects in a forest environment are thought to be influenced by vegetational characteristics. We examined and compared diversity and structure of the primary parasitoid assemblage of spruce budworm (*Choristoneura fumiferana* (Clem.)) (Lepidoptera: Tortricidae) in 27 plots representing three different forest stand types: softwood, mixedwood, and hardwood, i.e., balsam fir (*Abies balsamea* (L.) Mill.) – hardwood (broadleaved trees) mixed stands with varied amounts of hardwood content. Parasitoids were assessed in terms of density of eight morphospecies groups: *Apanteles* spp., *Glypta* spp., Ichneumonids, *Meteorus trachynotus*, pupal Chalcids, pupal Ichneumonids, Tachinids, and *Trichogramma* spp. Among these, *Apanteles* spp., *Glypta* spp., and *M. trachynotus* were the most common taxa. Species diversity (α -diversity) did not show statistical differences between the three stand types. The community structures of parasitoid assemblages differed significantly among the three stand types (β -diversity). Among the seven explanatory variables (elevation, slope, percent hardwood content by basal area, diameter at breast height (DBH), DBH of balsam fir, basal area, and spruce budworm population density), elevation ($p = 0.007$), population density of spruce budworm ($p = 0.065$), and percent hardwood content ($p = 0.132$) were the top three variables correlated with the parasitoid community structure. These results are helpful for understanding the interactions between the parasitoid community and the forest vegetational and environmental characteristics in the rise of a spruce budworm outbreak.

4.2 Introduction

Biodiversity and community composition are central factors influencing the structure and function of ecosystems (Hooper et al., 2005). Spatial variation in biodiversity and community structure has been a major theme in studies of agroecosystems, grasslands, or microbial communities (Tilman et al., 2001; Cardinale et al., 2006; Andrade et al., 2015; Shen et al., 2016); however, few comparable studies have been carried out in forest ecosystems for higher trophic level insect host-parasitoid communities (Sääksjärvi et al., 2006; Gould et al., 2013; Peralta et al., 2018). In particular, we lack information on potential correlations between parasitoid diversity, community structure and forest vegetational characteristics (Fraser et al., 2007). This is a notable gap as insect parasitoids comprise a large portion of terrestrial biodiversity (Heraty, 2017).

Spruce budworm (*Choristoneura fumiferana* (Clem.)) (Lepidoptera: Tortricidae) and the complex natural enemy community it supports offers an excellent system for exploring influences of forest tree composition on parasitoid communities. The population dynamics of this native defoliator in eastern North America have been intensively studied (Royama, 1984; Morin et al., 2007; Royama et al., 2017; Régnière et al., 2019). Its population follows a predictable 30-40-year outbreak cycle (Royama et al., 2005) and the primary driver of the population cycle is believed to be its natural enemies, especially Hymenopteran and Dipteran parasitoids (Régnière and Nealis, 2007; Royama et al., 2017). During spruce budworm outbreaks, repeated annual defoliation occurs on balsam fir (*Abies balsamea* (L.) Mill.) and spruce species (*Picea* spp.), which causes major ecological and economic impacts (Chang et al., 2012; MacLean, 2016; Liu et al.,

2019). Several studies have suggested that homogenous softwood stands sustained more severe damage from spruce budworm infestations than softwood-hardwood mixed stands (Su et al., 1996; Cappuccino et al., 1998; Zhang et al., 2018). It is reasonable to speculate that the parasitoid community functions differently in different forest stand types.

Traditional approaches for studying spruce budworm parasitoids focused on the response of individual species to specific environmental conditions, such as forest composition and tree diversity. Parasitism of some parasitoids was found to be influenced by forest composition, e.g., *Apanteles fumiferanae* Vier. (Legault and James, 2018) and tachinid fly (*Actia interrupta* Curran) demonstrated higher attacking rates on spruce budworm in mixed stands or landscapes (Cappuccino et al., 1998; Quayle et al., 2003), whereas *Glypta fumiferanae* Vier. was negatively related to forest diversity (Legault and James, 2018). Some parasitoids, such as *Meteorus trachynotus* Vier. (Maltais et al., 1989) and *Itopectis conquisitor* Say (Cappuccino and Martin, 1997), have dependent relationships with other hosting caterpillars that feed on hardwood trees. The collective response of a parasitoid community is not clear due to varied response of individual parasitoid species.

The parasitoid-host food web centered on spruce budworm is highly complex (Eveleigh et al., 2007). Royama et al. (2017) identified 50 parasitoid species that were responsible for budworm mortality. In the endemic (non-outbreak) stage, spruce budworm populations were nearly undetectable, while in the epidemic stage, >100 feeding larvae were commonly found on one m² of a mid-crown balsam fir branch surface area (Eveleigh and Johns, 2014; Royama et al., 2017). This drastic change in host population density alters the community structure of parasitoids associated with spruce

budworm possibly through two hypothesized mechanisms. Firstly, the immense increase of prey density may trigger an aggregational response of the parasitoids or predators (Holling, 1959a, b), i.e., parasitoids are attracted across the landscape to the area with high budworm density (also known as a “birdfeeder effect”) (McCann et al., 2005; Eveleigh et al., 2007). Secondly, individual predators and parasitoids may actively switch diet to the most abundant resource (also known as “switching behavior”), to maximize energy intake and to improve fitness (Murdoch et al., 1975; Abrams and Kawecki, 1999; Elliott, 2004). Collectively, the parasitoid community may also demonstrate movement to areas with a higher density of viable hosts.

Spruce budworm population density varies both temporally and spatially (Eveleigh et al., 2007; Royama et al., 2017). As an outbreak progresses, population density rises, reaches its peak, and then declines. Spatially, higher density naturally occurs in softwood stands due to the abundance of host balsam fir and spruce trees. In general, compared with pure softwood stands, mixed softwood-hardwood stands contain fewer host trees and thus lower overall spruce budworm density. Based on the above-mentioned adaptive foraging behaviors (Křivan and Schmitz, 2003), i.e., “bird feeder” and “switching behavior”, during spruce budworm outbreaks the community structure of parasitoids could change drastically in response to fluctuating budworm population density. Different parasitoid community structures could emerge during different outbreak stages and in different areas with low or high host densities. Eveleigh et al. (2007) identified 66 primary and 23 secondary parasitoid species associated with the spruce budworm food web and reported that the parasitoid community varied greatly in different outbreak stages. Marrec et al. (2018) counted 32 primary parasitoids of spruce

budworm and found that forest structure explained 61% of variation in the parasitoid community, but more than half of this variance was explained collectively by non-environmental spatial factors such as geographic distance between sample plots. Marrec et al. (2018) also found that spruce budworm outbreak status explained little of the variation in community structure.

Descriptions of community structure are based on quantitative and qualitative characteristics such as number of species and distribution of individuals (Hanson, 1950; Adey and Loveland, 2007). Biodiversity is described spatially in terms of α - and β -diversities (Whittaker, 1972). The α -diversity refers to the average species diversity in a habitat or specific area, while β -diversity represents the differences in species composition among sites (Anderson et al., 2011). In biodiversity studies, morphospecies have been used as surrogates for taxonomic species to overcome the logistical difficulties associated with identification of invertebrate species (Derraik et al., 2010). The morphospecies technique involves the separation of taxa based on easily observable morphological characters. It has been used in various invertebrate inventories including Hymenoptera and Lepidoptera and has been suggested to be an acceptable surrogate in environmental monitoring, conservation, and diversity estimates (Derraik et al., 2010; Vieira et al., 2012; Alves et al., 2016).

We investigated the spruce budworm parasitoid community in 27 forest plots representing a gradient of balsam fir-hardwood tree composition during a rise of an outbreak. Spatially, we examined the parasitoid community at the forest stand level. Parasitoids were assessed in terms of density of eight morphospecies groups: *Apanteles* spp., *Glypta* spp., Ichneumonids, *Meteorus trachynotus*, pupal Chalcids, pupal

Ichneumonids, Tachinids, and *Trichogramma* spp. The objective was to determine diversity (α -diversity) and dissimilarity of the spruce budworm parasitoid community assemblage (β -diversity) in forest stands with varied balsam fir-hardwood tree composition. We predicted that diversity of the spruce budworm parasitoid community would be higher in mixed balsam fir-hardwood stand types than in softwood or hardwood types, and that the parasitoid community would be different in different forest stand types, based on the assumption that diverse plant environment supports a more diverse caterpillar hosts and parasitoid community.

4.3 Materials and methods

4.3.1 Study area

We established 27 balsam fir-hardwood sample plots in 2014 within ~100 km² area north of Lake Matapedia in the Gaspé region, Québec (48°32'N–48°35'N, 67°25'W–67°34'W). Government-operated aerial defoliation surveys of the study area began in 2010 and defoliation was first detected in 2012 (Zhang et al., 2018). Nine 0.05 ha sample plots were established in each of the three balsam fir-hardwood stand types classified by percent basal area of hardwood content: 0-25% (termed softwood), 40-65% (mixedwood), and 75-95% (hardwood). Sampling plots were located at least 50 m apart and at least 50 m away from forest edges. The most common hardwood tree species in the sample plots were sugar maple (*Acer saccharum* Marshall), yellow birch (*Betula alleghaniensis* Britton), white birch (*Betula papyrifera* Marshall), and red maple (*Acer rubrum* L.). Balsam fir averaged 70%, 39%, and 11% of the basal area in softwood, mixedwood, and hardwood plots respectively. Hardwoods averaged 15%, 55%, and 88%

of the basal area in the three stand types, respectively. White spruce (*Picea glauca* [Moench] Voss), another host species of spruce budworm, comprised on average 3.3% of basal area in each plot and existed only in about one half of the plots. A detailed description of plot establishment is in Zhang et al. (2018).

Various biotic and abiotic attributes of the sample plots were measured in the field (Table 4.1). Elevation and slope were recorded in each circular plot at the plot center. Biotic attributes such as diameter at breast height (DBH), basal area (BA), and tree species composition were measured in 2013 for all trees with DBH > 4 cm. Methods of plot measurements were described in Zhang et al. (2018). Average densities of the third to fourth instar larvae (L3-L4) in 2015 and 2016 were sampled and used to indicate spruce budworm population density in this study as most parasitoids are associated with late-instar larval and pupal stages (Régnière and Nealis, 2007; Royama et al., 2017). We assessed larval density in each plot on four 45-cm balsam fir mid-crown branch tips (from four separate dominant balsam fir trees) during L3-L4 and expressed as number per branch surface area (BSA, length × width) (Sanders, 1980; Royama et al., 2017). Time of collection of the budworm developmental stage was determined by predicted phenology (Dorais and Kettela, 1982; Régnière et al., 2014).

4.3.2 Parasitoid sampling

Spruce budworm from various life stages were sampled from each of the three types of forest plots and reared to examine the parasitoid communities. Spruce budworm specimens were collected in nine time periods through the developmental stages between 2014 and 2016: 2014 (egg); 2015 (L3-L4, L6, pupa, and egg); 2016 (L3-L4, L6, pupa,

and egg). For each sample plot and collection, three budworms were collected from each of the four sample trees (four separate dominant balsam fir trees) and were reared on antibiotic-free artificial diet (modified from McMorran (1965), obtained from Canadian Forest Service, Great Lakes Forest Research Center, Sault Ste. Marie, ON, Canada) until parasitoids emerged or moth eclosion occurred. Rearing spruce budworm larvae on antibiotic-free artificial diet does not affect parasitism (Seehausen et al., 2013).

Emerged parasitoids were morphologically identified into eight morphospecies groups as a surrogate for taxonomic species (Oliver and Beattie, 1996; Derraik et al., 2002). Morphospecies were grouped based on prevalence/dominance, emergence life stage, and possible associations with non-host vegetation. *Apanteles fumiferanae* and *Glypta fumiferanae* dominate parasitism of early larval budworm (Legault and James, 2018) and these two species collectively could control budworm population effectively (McLeod, 2012). As in Marrec et al. (2018), we assumed that under spruce budworm outbreak conditions, *A. fumiferanae* and *G. fumiferanae* comprised most our *Apanteles* spp. and *Glypta* spp. samples, respectively. *Meteorus trachynotus* has a dependent relationship with other hosting caterpillars that feed on hardwood trees (Maltais et al., 1989). Parasitism of *Trichogramma minutum* Riley was related to proportion of non-host tree basal area (Quayle et al., 2003) possibly because of the nectar provided by diverse understory vegetation (Bai and Smith, 1993). We assumed that *T. minutum* comprised a major portion of our *Trichogramma* spp. under outbreak population. Morphospecies that were grouped based on the spruce budworm emergence life stage were: Ichneumonids emerging from larvae (Ichneumonids) and from pupae (pupal Ichneumonids); and Chalcids emerging from pupae (pupal Chalcids). In total, parasitoids were grouped into

eight morphospecies: *Apanteles* spp., *Glypta* spp., Ichneumonids, *Meteorus trachynotus* Viereck, pupal Chalcids, pupal Ichneumonids, Tachinids, *Trichogramma* spp. In total, ca. 2800 spruce budworm specimens were collected and reared from the three stand types, as some branch samples contained fewer than three budworms (target sample of 3 budworms \times 4 trees \times 27 plots \times 9 collections = 2916). A total of 404 parasitoid individuals were found from all reared budworm specimens.

To confirm the accuracy of the morphospecies identification procedure, we randomly selected 60 parasitoid samples and sent them to a DNA barcoding laboratory (Department of Integrative Biology and the Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario). These parasitoid specimens were identified to taxonomic species using a DNA barcoding procedure (Smith et al., 2011; Smith et al., 2013). Comparing the taxonomic species results to the morphospecies that identified using morphological characters, all specimens were correctly identified to their morphospecies group, confirming that our morphospecies identification procedure was accurate. The DNA barcoding results showed that all *Apanteles* spp. were *Apanteles fumiferanae*, all *Glypta* spp. were *Glypta fumiferanae*, two species were identified as pupal Ichneumonids, and three species were identified as Tachinids.

4.3.3 Statistical analyses

Two species diversity indices were calculated for the parasitoid community of spruce budworm in each plot (α -diversity): Shannon-Wiener diversity index (H):

$$H = - \sum_{i=1}^s p_i \ln p_i, \quad (4.1)$$

and Shannon Evenness index (E_H):

$$E_H = H/\ln S, \quad (4.2)$$

where p is the proportion (n/N) of individuals of one particular species found divided by the total number of individuals found (N), and S is the number of species. H is the most common used diversity index and E_H provides a separate evenness measure (Magurran, 2004). As our study focused on the overall parasitoid community structures in different forest stand types, parasitoids collected in the nine time periods through budworm developmental stages were aggregated and treated as a whole assemblage of parasitoid community despite the fact that the composition of parasitoids might differ over time within a season (Eveleigh and Johns, 2014; Marrec et al., 2018). Abundance of each morphospecies in each plot was calculated by summing the total occurrence during the nine sampling periods. The two species diversity indices of the three stand types ($n = 9$) were compared using analysis of variance (ANOVA) in R (R Core Team, 2018). ANOVA distributional assumptions were checked and accepted. We did not statistically test the abundance of individual parasitoid species among the three stand types with the morphospecies data. Although using morphospecies is a practical technique for invertebrate studies when time and financial constraints exist (Derraik et al., 2010), biological implications of individual morphospecies should not be “over-interpreted” (Derraik et al., 2002; Krell, 2004). Our study focused on the overall diversity and community structure.

Dissimilarity of the parasitoid communities of spruce budworm between the three stand types (β -diversity) was examined using non-metric multidimensional scaling (nMDS), an ordination method for community analysis that does not require distributional assumptions about the data (Minchin, 1987). The nMDS applies an iterative

algorithm and ordines the Bray-Curtis dissimilarity matrix (Anderson et al., 2006) that was built from the abundance of each parasitoid species. The nMDS presents the multidimensional data of a dissimilarity matrix in a two-dimensional space and delineates each sample plot by positioning it according to its distance from all other sample plots. The Bray-Curtis dissimilarity and nMDS were performed using the “vegan” package (“metaMDS” function) in R (Oksanen et al., 2018). We used permutational, nonparametric multivariate analysis of variance tests (perMANOVA) to quantify the relationship between dissimilarity measures of the parasitoid communities and stand types as a categorical explanatory variable (“adonis” function in “vegan” package in R (Oksanen et al., 2018)). The perMANOVA allows an ANOVA-like test of variance in β -diversity explained by explanatory variables. It is more powerful than other resemblance-based permutation methods in detecting changes in community structure and less sensitive to heterogeneity and correlation structure (Anderson and Walsh, 2013). We further used the PERMDISP2 procedure (Anderson, 2006; Anderson et al., 2006) to analyze the multivariate homogeneity of group dispersions (“betadisper” function in “vegan” package in R (Oksanen et al., 2018)) to ensure that the perMANOVA results were not influenced by heterogeneous dispersions within each stand type. Abundance of the parasitoids was $\log(x + 1)$ transformed prior to the nMDS, perMANOVA, and PERMDISP2 procedures to weight the contributions of common and rare species in the (non-parametric) multivariate representations (Clarke et al., 2014).

Limited information is available on what biotic and abiotic variables could influence parasitoid community structure (Eveleigh et al., 2007; Marrec et al., 2018). We selected seven variables: elevation, slope, percent hardwood content by basal area, DBH

of all trees, DBH of balsam fir, basal area of all trees, and spruce budworm population density (average L3-L4 density in 2015 and 2016) and fitted them on the nMDS ordination diagram based on the correlations of the variables with the ordination axes using “envfit” function in “vegan” package (Oksanen et al., 2018). This helped to identify variables that drive the parasitoid community structure.

4.4 Results

4.4.1 Comparison of the abundance and species richness of parasitoids

All eight parasitoid morphospecies of spruce budworm were identified in the softwood, mixedwood, and hardwood stand types (Figure 4.1). In total, 404 (15.0 ± 0.9 per plot) parasitoid individuals were found from all reared budworm specimens, and only four specimens could not be identified to morphospecies (Figure 4.1). Of these parasitoid samples, *Apanteles* spp., *Glypta* spp., and *Meteorus trachynotus* were the most common taxa in the parasitoid community (Figure 4.1). In each plot, ca. 5 or 6 morphospecies were found; species richness (number of morphospecies) did not differ significantly between the three stand types ($F = 1.43$; $df = 2, 24$; $p = 0.26$) (Table 4.1). Both the Shannon-Wiener ($F = 0.42$; $df = 2, 24$; $p = 0.66$) and Shannon Evenness ($F = 1.88$; $df = 2, 24$; $p = 0.18$) indices showed tendencies to be higher in mixedwood plots, but neither of them reached statistical significance (Table 4.1).

4.4.2 Comparison of the parasitoid community assemblage

The non-metric multidimensional scaling (nMDS) analyses showed separation of clusters of parasitoid abundance patterns for the softwood, mixedwood, and hardwood

stand types (Figure 4.2A). The community structures of parasitoid assemblages differed significantly among the three stand types ($F = 3.171$; $df = 2, 24$; $p = 0.005$; permutation = 999; perMANOVA). The PERMDISP2 procedure suggested that the multivariate group dispersions (within-group differences) were not different among the three stand types ($F = 1.279$; $df = 2, 24$; $p = 0.30$).

4.4.3 Correlations of the parasitoid community with seven selected variables

Elevation ($p = 0.006$; permutation = 999), spruce budworm population density ($p = 0.068$), and percent hardwood content by basal area ($p = 0.119$) were the most influential variables on the parasitoid community structure (as indicated by the length of arrows in Figure 4.2B), with lower correlations for slope ($p = 0.349$), DBH ($p = 0.595$), DBH of balsam fir ($p = 0.468$), basal area ($p = 0.718$). Examining the two-dimensional ordination plot, elevation and percent hardwood were associated with mixedwood and hardwood plots, whereas spruce budworm population density was associated with softwood plots (also see in Table 4.1).

Glypta spp., *Meteorus trachynotus*, and *Apanteles* spp. were the closest to the center of the two-dimensional ordination plot among all morphospecies (Figure 4.2B), indicating that they were the most abundant morphospecies as we found in Figure 4.1. *Trichogramma* spp., *Meteorus trachynotus*, and Ichneumonids were situated in the lower-right quadrant in Figure 4.2B which associated primarily with hardwood plots.

4.5 Discussion

Our comparison of species abundance and richness among the three stand types did not statistically support our first prediction that diversity of parasitoid community

would be higher in more mixed forest stands. We considered the three following explanations. Firstly, we used morphospecies instead of taxonomic species in this study. The taxonomy of nearly all primary parasitoids of spruce budworm is known and available, but accurate microscopic identification of a specimen can be onerous and requires that specimens be in good shape to see distinguishing characteristics. Even though morphospecies was suggested to be an acceptable surrogate of taxonomic species in environmental monitoring and diversity estimates (Derraik et al., 2010; Vieira et al., 2012), it still conveyed less taxonomic information than those from a traditional taxonomic species identification procedure. Overall, fewer taxonomic units would be identified with the morphospecies technique and the estimate of species richness could be influenced (Goldstein, 1997; Oliver and Beattie, 1997). Secondly, it is also possible that the dissimilarity of budworm parasitoid communities among the three stand types was mainly reflected by the variation of the species composition of assemblages (β -diversity) rather than the abundance and richness (α -diversity). Gould et al. (2013) also found that the parasitoid Ichneumonid community structures differed between two forest habitats but that abundances were similar. Thirdly, parasitism is affected by the spatial scale under which it is examined (Legault and James, 2018; Marrec et al., 2018). Flying distance of parasitoids varies greatly from meters to a scale of kilometers, and is affected by host density, body size of parasitoids, forest structure, and parasitoid perception of spatial scale (Elzinga et al., 2007; Xu et al., 2016; Legault and James, 2018; Marrec et al., 2018). Examining parasitoid diversity under large spatial scales may produce different results. However, parasitoids have critical habitat requirements in addition to food source (Miller and Varty, 1975). The food source (budworm density in different areas) may play

an important role at a large spatial scale, but at the local (stand) scale, habitat may have strong effects on the parasitoid community.

The perMANOVA test of dissimilarity measures supported our second prediction that the assemblage of the parasitoid community was different in the three forest stand types. Even though, in Figure 4.2A, we could identify some outliers from the clusters of parasitoid abundance patterns for the three stand types, the PERMDISP2 procedure confirmed that differences in parasitoid community assemblage resulted from differences between stand types rather than within each stand type. Our result obtained using the ordination procedure on empirical community data was similar to previous studies that the parasitoid community and food-web structure of spruce budworm are influenced by forest composition (Eveleigh et al., 2007; Marrec et al., 2018). Similar studies in other forest ecosystems also found that the parasitoid community of herbivorous caterpillars differed significantly between various forest habitats (Sääksjärvi et al., 2006; Gould et al., 2013; Peralta et al., 2018).

The dissimilarity of spruce budworm parasitoid communities among the three stand types was reflected mainly by the variation of the species composition of assemblages (β -diversity) rather than species abundance and richness (α -diversity). The β -diversity, which defines the amount of variation in community composition, or degree of community differentiation in relation to a complex-gradient of environment or a pattern of environments (Whittaker, 1960), can be further partitioned into separate components. We suggest partitioning β -diversity into turnover and nestness-resultant components (Baselga, 2010; Baselga and Leprieur, 2015) where taxonomic species data are available. It could help to further understand the patterns of species replacement and

species loss associated with certain forest stand types. The scale of sampling and rearing in this study was limited owing to sampling across 27 plots. Because our intention was to compare the parasitoid communities in the three stand types, we collected data in nine plots in each stand type over two years to minimize the within-group variation. A similar study conducted in fewer plots but over a larger study area could determine how spatial forest structure at the landscape scale influenced parasitoid communities (Marrec et al., 2018). It is possible that increased rearing sample sizes over more years or at a later stage of the outbreak might reveal stronger relationships of diversity and structure of the parasitoid community to stand type.

Elevation, population density of spruce budworm, and percent hardwood content were the top three variables correlated with the parasitoid community structure among the seven variables fitted onto the ordination plot. Studies suggested that parasitism and parasitoid communities are significantly influenced by elevation, which is important given projected temperature fluctuation associated with global warming (Péré et al., 2013; Maunsell et al., 2015). Spruce budworm population density varies greatly in different outbreak stages and was correlated with parasitoid community structure, indicating that the parasitoid-host food-web structure could differ substantially among outbreak stages (Eveleigh et al., 2007). Percent hardwood content is the main biotic factor that differentiates the three forest stand types. This correlation between percent hardwood content as an explanatory variable and the nMDS ordination supplemented the distance matrix itself (Clarke, 1993; Legendre and Legendre, 2012) and confirmed that the parasitoid community assemblages were different in the three stand types with differing amount of hardwood content.

We did not statistically compare abundance of individual parasitoid species among the three stand types in this study. Individual parasitoid species of spruce budworm have been studied in the past (Maltais et al., 1989; Quayle et al., 2003; Legault and James, 2018). Examining the trend of abundance of individual morphospecies, we found congruence with previous studies that some species, e.g., *Meteorus trachynotus*, were more abundant in more mixed forest stands (Maltais et al., 1989).

Only primary parasitoids of spruce budworm were collected and examined in our study. Eveleigh et al. (2007) examined the spruce budworm food web under different host densities and found that hyperparasitoid (higher trophic level) mobile generalists responded rapidly to the changing spruce budworm abundance. Even though hyperparasitoids influence the food web stabilization (Kondoh, 2003; McCann et al., 2005; Rooney et al., 2006), only those primary parasitoids that attack spruce budworm influence the host mortality directly. Hence, variation of the assemblage of the primary parasitoids is more crucial in terms of determining outbreak impacts in various forest stand types. Still, we would suggest including hyperparasitoids and examining the cross-trophic level parasitoid community assemblage in different forest stand types where data are available. It could help to further understand the adaptive foraging behavior (Křivan and Schmitz, 2003) of parasitoid communities based on the theory that hyperparasitism has greater response to the fluctuation of prey density (McCann et al., 2005).

Less severe spruce budworm defoliation has been observed in softwood-hardwood mixed forest stands than in pure fir stands (Su et al., 1996; Zhang et al., 2018). The “natural enemy” hypothesis suggests that more diverse plant conditions support higher predation/parasitism by providing alternative and alternate prey, more predation

opportunities, or better sheltering condition to natural enemies of herbivore insects (Riihimäki et al., 2005; Cardinale et al., 2006). However, in our previous study conducted in the same sites, we did not find a positive relationship between parasitism and stand type (Zhang et al., 2020). Given the dissimilarity of the parasitoid community structure among stand types observed in this study, forest composition influences parasitoid community structure, but it is possible that the overall impact of parasitoid communities on spruce budworm mortality could remain more or less the same, particularly when sampling was conducted in a small area as in this study. The correlations between explanatory variables and community structure in the nMDS ordination help us understand biotic and abiotic constraints of the parasitoid-host interaction in the rise of a spruce budworm outbreak and possibly provide useful information for pest management planning, e.g., elevation was significantly correlated with parasitoid community assemblage.

4.6 Acknowledgments

This research was funded by the Atlantic Innovation Fund project “Early Intervention Strategy to Suppress Spruce Budworm Outbreak” grant to D.A.M. Allison Dykstra, Evan Dracup, Zhuoyi Nie, Matt Hill, Shawn Donovan, Garrett Brodersen, Jessica Cormier, Craig Wall, Maggie Brewer, and Yanyi Li assisted with field data collection.

4.7 References

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Table 4.1. Description of forest stand attributes and diversity measures of the parasitoid assemblage of spruce budworm (\pm one standard error, $n = 9$ plots) of the softwood, mixedwood, and hardwood stand types sampled in Gaspé, Québec.

Stand type	Softwood	Mixedwood	Hardwood
Elevation (m)	220.9 \pm 12.7	270.6 \pm 20.9	316.6 \pm 9.6
Slope (degree)	3.4 \pm 0.8	6.6 \pm 1.8	5.6 \pm 2.1
Basal area* (m ² /ha)	44.4 \pm 2.0	36.7 \pm 1.6	29.8 \pm 1.8
DBH* (cm)	16.8 \pm 1.0	15.1 \pm 1.0	19.2 \pm 1.2
DBH of balsam fir (cm)	18.5 \pm 1.3	14.9 \pm 1.5	21.6 \pm 1.6
Hardwood (% basal area)	14.5 \pm 2.4	55.5 \pm 2.0	88.0 \pm 1.9
Population density [†] (/m ² BSA)	240.7 \pm 24.4	210.1 \pm 27.5	88.1 \pm 8.0
Number of morphospecies	5.2 \pm 0.4	5.9 \pm 0.3	5.0 \pm 0.4
Shannon-Wiener index (H)	1.45 \pm 0.07	1.51 \pm 0.06	1.43 \pm 0.07
Shannon Evenness index (E _H)	0.90 \pm 0.02	0.87 \pm 0.02	0.91 \pm 0.02

* Basal area and DBH represent all tree species.

[†] Population density was the average L3-L4 budworm larval density in 2015 and 2016.

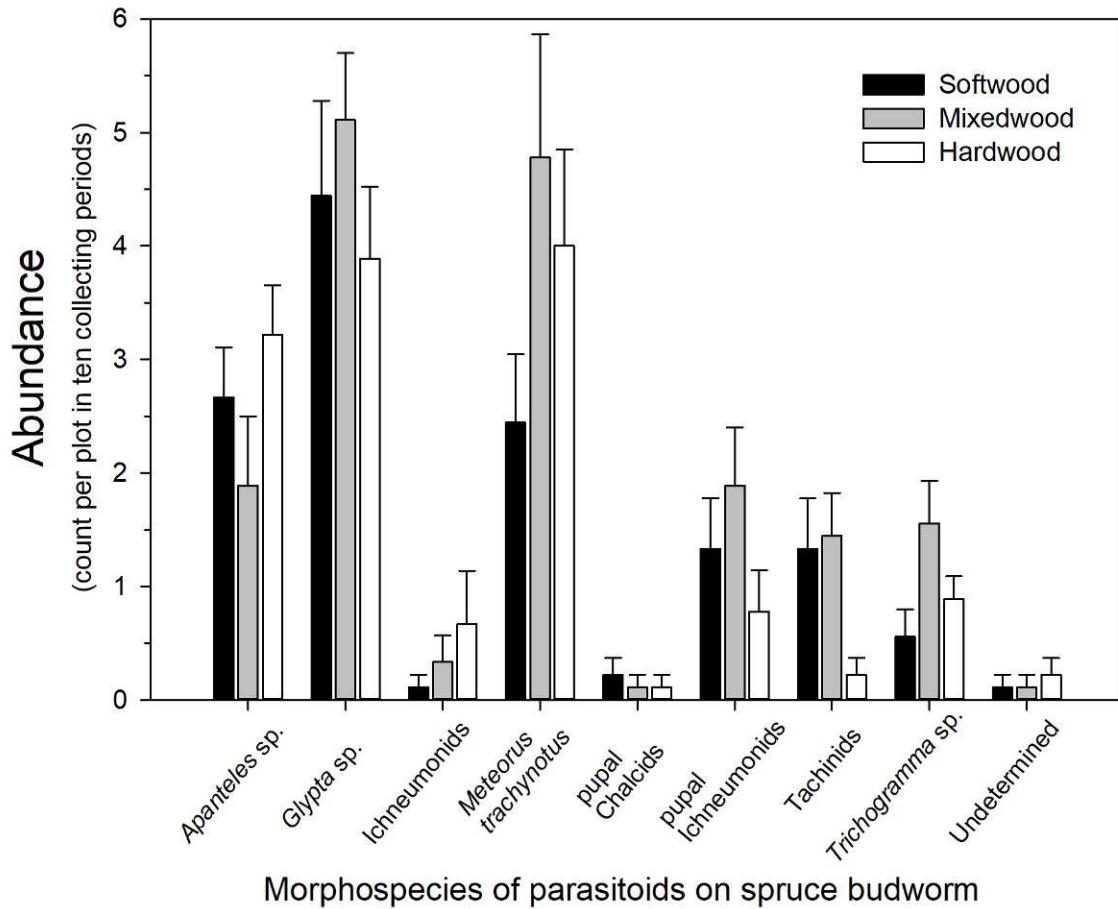


Figure 4.1. Abundance of the eight parasitoid morphospecies on spruce budworm (\pm one standard error, $n = 9$ plots) in softwood, mixedwood, and hardwood stand types sampled in ten collecting periods through budworm developmental stages between 2014 and 2016 in Gaspé, Québec.

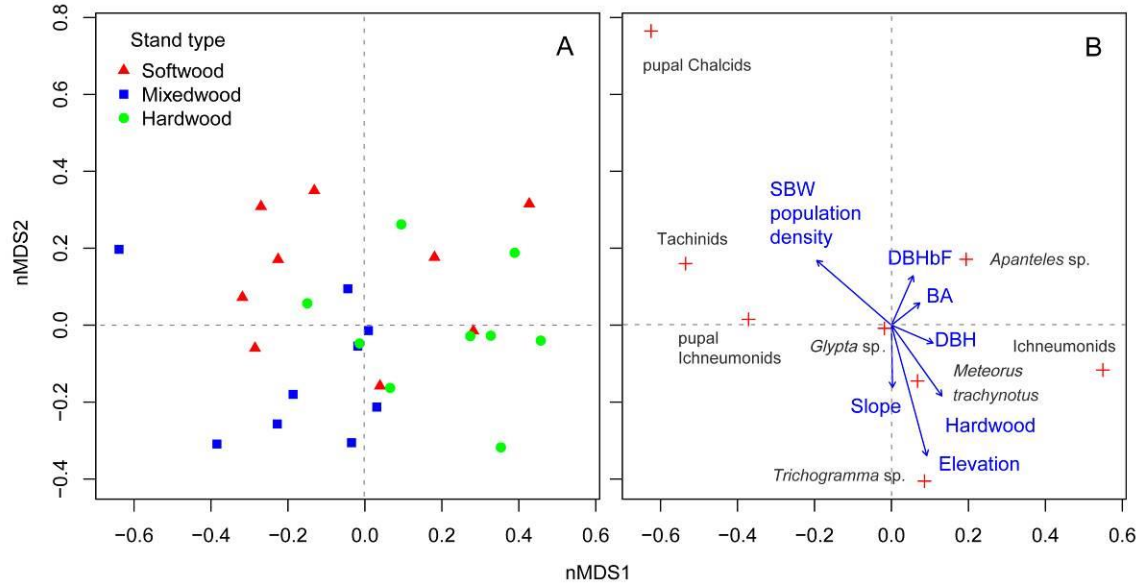


Figure 4.2. (A) The non-metric multidimensional scaling (nMDS) ordination plot comparing spruce budworm parasitoid communities in the three forest stand types. Each point represents the parasitoid community from one of the 27 sample plots. The Bray-Curtis distance metric based on the abundance ($\log(x + 1)$) of each parasitoid species was used to rank distances. Two-dimensional stress = 0.189. (B) The nMDS ordination plot showing the correlations between parasitoid community structure and Elevation, Slope, Percent hardwood content by basal area (Hardwood), DBH, DBH of balsam fir (DBHbF), Basal area (BA), and Spruce budworm population density (SBW population density). The correlations between the seven variables and the two ordination axes are represented by the length and angle of the arrows. The “+” delineates the position of each parasitoid morphospecies on the two-dimensional plot.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

5.1 Introduction

Spruce budworm outbreaks have caused substantial impacts on the ecological processes of forests in eastern North America for a long period (Royama, 1984; Royama et al., 2017). Researchers and forest agencies have strived to shift passive, reactive pest management strategies to proactive alternatives (Johns et al., 2019; MacLean et al., 2019). During the decline of the last outbreak in the 20th century, multiples studies observed less severe outbreak impacts in mixedwood forests with a hardwood component than in pure softwood forests, including less severe defoliation, less growth reduction, and less tree mortality (Bergeron et al., 1995; Su et al., 1996; Campbell et al., 2008). Knowledge of how hardwood content influences spruce budworm outbreak impacts is needed for proactive pest management planning during the rise of spruce budworm outbreaks. Appropriate sampling, monitoring, and treatment strategies can be assigned to forest stands according to their softwood-hardwood composition. It is also important to investigate which ecological processes are involved in the reduced defoliation associated with hardwood content. This knowledge provides insights to ecologists and entomologists on the fascinating interaction between the insect and its hosting environment. Here I offer a general discussion of the results and conclusions of this study and discuss the applications and limitations.

5.2 Summary of results

5.2.1 Effects of hardwood content on balsam fir defoliation during the building phase

I quantified the relationship between balsam fir defoliation and hardwood content

in the initiation and building phase (first 5 years) of a spruce budworm outbreak. Balsam fir defoliation was negatively related to percent hardwood content each year during the 5 sampling years (2012 to 2016). Balsam fir defoliation was significantly related to percent hardwood content and overall defoliation severity (average defoliation in pure softwood stands). There was also a significant interaction between these two variables, indicating that the relationship between defoliation and percent hardwood varied significantly with overall defoliation severity each year. The relationship between defoliation and hardwood amount was weak in the first year and became stronger in the second and third year, then declined in the last 2 years of the sampling period. Balsam fir defoliation in hardwood plots remained below 20% in all years except 2015, the year with the highest defoliation in all stand types.

Three model formulations of the relationship between fir defoliation and hardwood content were compared. The simplified linear model (based on a simple assumption that defoliation in fir-hardwood stands with $x\%$ hardwood was $x\%$ less than in pure fir stands) was the least accurate among the three models, with a correlation (r) of 0.77 between the predicted defoliation and defoliation measured in the field. A generalized mixed effects model that incorporated hardwood content and overall defoliation severity produced a correlation ($r = 0.87$) intermediate among the three models. The Random Forest model (an ensemble regression tree statistical procedure) generated the most accurate prediction among the three models ($r = 0.92$). Overall defoliation severity and percent hardwood content were the most important predictors among the 11 variables incorporated in the Random Forest model.

5.2.2 Influence of hardwood content on mortality associated with early-instar larval dispersal and parasitism

Defoliation and population density are directly influenced by budworm mortality. Spruce budworm densities were higher in sampled softwood (balsam fir) than in mixedwood and higher in mixedwood than in hardwood stands, which was consistent with significant differences in defoliation among stand types (Zhang et al., 2018). Spruce budworm early-instar larvae (L1 and L2) dispersal loss and mortality caused by parasitism (which often occurs in late larval and pupal stage) are the major sources of mortality throughout the budworm life-cycle (McGugan and Blais, 1959; Régnière and Fletcher, 1983; Royama et al., 2017). Hardwood content in fir-hardwood stands significantly ($p < 0.001$) influenced L2 dispersal losses. L2 dispersal loss was less in softwoods than in mixedwood or hardwood stand types based on measurements on ground traps and less in softwoods and mixedwoods than in hardwoods based on sampling budworm larvae on regeneration. These results supported the ‘habitat fragmentation’ hypothesis and suggested that high early-instar larval dispersal loss is a plausible explanation for the decreased spruce budworm density and balsam fir defoliation associated with increasing hardwood content. Budworm parasitism rates were not significantly related to forest composition, and thus results did not support the natural enemy hypothesis that higher parasitism occurs in more diverse forest environment than in homogenous forests. The scale of sampling and rearing in this study were limited owing to sampling across 27 plots, and it is possible that increased rearing sample sizes over more years might reveal stronger relationships of specific parasitoid species to stand type.

5.2.3 Parasitoid communities of spruce budworm in forest stands with varied amount of hardwood content

All eight parasitoid morphospecies of spruce budworm were identified in each stand type, in which *Apanteles* spp., *Glypta* spp., and *Meteorus trachynotus* were the most common taxa in the parasitoid community. I did not find significant differences in species richness (number of morphospecies), Shannon-Wiener ($F = 0.42$; $df = 2, 24$; $p = 0.66$), or Shannon Evenness ($F = 1.88$; $df = 2, 24$; $p = 0.18$) diversity indices between the hardwood, mixedwood, and softwood stand types. Abundance and species richness of the parasitoid communities did not show statistical differences between the three stand types. The community structures of parasitoid assemblages differ significantly among the three stand types ($F = 3.016$; $df = 2, 24$; $p = 0.004$; permutation = 999; perMANOVA). The PERMDISP2 procedure suggested that the multivariate group dispersions (within-group difference) were not different among the three stand types ($F = 1.08$; $df = 2, 24$; $p = 0.37$). Among the seven selected biotic and abiotic variables, elevation ($p = 0.007$; permutation = 999), budworm population density ($p = 0.065$), and percent hardwood content ($p = 0.132$) were most influential on the parasitoid community structure.

5.3 Application of study results

Understanding the relationship between spruce budworm and its hosting environment is important for comprehending the ecological and economic impacts caused by budworm outbreaks. Outbreak impacts were substantially different in forest stands with varied fir-hardwood composition (MacLean, 1980; Bergeron et al., 1995; Su et al., 1996; Campbell et al., 2008). The relationship between fir defoliation and hardwood was

previously studied in the declining phase of the previous outbreak (Su et al., 1996). During the rise of an outbreak, a quantified relationship between fir defoliation and hardwood content in fir-hardwood forest stands is especially essential for proactive pest management strategies such as active population monitoring and targeted insecticide treatment (MacLean et al., 2019). My results (Chapter 2) showed that defoliation of balsam fir was negatively related to hardwood content in fir-hardwood mixed stands during the initiation and building phases of a spruce budworm outbreak. This conformed to the study that was conducted in the declining phase of an outbreak (Su et al., 1996) and the general observations in the literature that tree diversity reduces herbivory by oligophagous insects (Jactel and Brockerhoff, 2007).

Results from this study showed somewhat weaker relationships between fir defoliation and hardwood content in fir-hardwood forest stands in the initiation and building phase compared with in the declining phase (Su et al., 1996); the reason is unclear. I considered two possible explanations for this weaker relationship in the building phase. First, in the declining phase of a budworm outbreak, sudden declines in the spruce budworm population in a given year can occur (e.g., Royama et al., 2017). Given that the density of natural enemies (parasitoids in particular), the major source of natural control of budworm population (Eveleigh et al., 2007), in year X is determined by their density in the previous year ($X - 1$) when the budworm population was higher, parasitism and its impact on budworm populations in year X would be disproportionately large, resulting in less defoliation and, thus, a strong relationship between defoliation and hardwood content. The second possible explanation is that because tree mortality occurs during the declining phase of a budworm outbreak, resulting in an increasing degree of

habitat fragmentation for budworm larvae. After the peak of an outbreak, balsam fir trees and branches as the main food source for budworm would be sparser and patchier than earlier in the outbreak. The worsened host condition in the declining phase could have greater impact on the budworm population, and thus defoliation, resulting in a stronger relationship between budworm herbivory and tree diversity.

There are distinct views on the ecological processes that govern the initiation, termination, and recurrence of budworm outbreaks. The “double-equilibrium theory” (Ludwig et al., 1978) presumed that the eruption of budworm populations was triggered by or coincided with certain conditions (e.g., meteorological, host conditions). The “oscillatory theory” (Royama, 1984) asserted that a periodic predator-prey cycling process dominated the outbreak cycles; the budworm population was regulated mainly by its natural enemies (parasitoids in particular) (Régnière and Nealis, 2007; Royama et al., 2017). Recent study found that outbreak population density must be elevated via immigration (Régnière et al., 2019). My results suggest that early-instar mortality may play a significant role in budworm population change and differences in budworm density and defoliation between stand types.

Practically, my results suggest that: 1) it could be beneficial to incorporate the effects of hardwood content (at the cost of reduced fir volume) in proactive pest management strategies, as it is clear that reduced fir defoliation associated with hardwood content occurs not only in the declining phases but also in the initiation and building phase in an outbreak; and 2) less insecticide treatments may be needed in mixed stands, as the effects of hardwood content were stronger in the declining stage of an outbreak possibly due to the ineffectiveness of the natural enemies in the initiation and building

stage.

The simplified linear model (a simple equation based on the assumption that defoliation in fir-hardwood stands with $x\%$ hardwood was $x\%$ less than in pure fir stands) can be used to predict reduced defoliation associated with hardwood content for convenience at the cost of accuracy. Accurate prediction of budworm defoliation in fir-hardwood stands requires the incorporation of two predictor variables: hardwood content and overall defoliation severity. Predicting spruce budworm defoliation with a Random Forests procedure (incorporating 11 predictor variables) and a generalized mixed effects model (included outbreak severity and percent hardwood content) yielded more accurate results than the simplified linear model. The benefits of using the simplified linear model is that defoliation in fir-hardwood stands can be estimated without conducting comprehensive sampling, model construction, or parameter estimates. However, the accuracy of the prediction may be variable as the relationship between defoliation reduction and percent hardwood is dynamic rather than constant under varied outbreak severity. To predict spruce budworm defoliation in fir-hardwood mixedwood stands, besides percent hardwood content, I suggested including an indicator of average regional outbreak severity, e.g., regional defoliation surveys carried out by government agencies in softwood stands or regional spruce budworm population sampling such as second instar larval or moth sampling.

My results in Chapter 3 can help ecologists and entomologists to better understand how forest composition affects insect herbivory. Mixing hardwood with balsam fir could reduce spruce budworm early-instar dispersal success and thereby reduce the population and resulting defoliation. Results of parasitism can be affected by

the spatial scale at which it is examined. Legault and James (2018) found that the relationship between parasitism of *G. fumiferanae* and *A. fumiferanae* and forest diversity varied at different spatial scales. We sampled parasitism within a relatively small study area to minimize the variance in outbreak severity and spatial structure among the three stand types. The non-significant relationship between parasitism and stand type at the local scale may suggest that the natural enemy hypothesis may not occur at the stand level where most forest management strategies are applied. Or it may be that the parasitoid community had not yet reached maximum capacity at this early stage of the spruce budworm outbreak.

My results in Chapter 4 indicated that dissimilarity of spruce budworm parasitoid communities between forest stand types was reflected mainly by variation of species composition of assemblages (β -diversity) rather than species abundance and richness (α -diversity). In Chapter 3, I hypothesized that higher parasitism would occur in fir-hardwood stands with higher hardwood content but my results did not support it. This suggests that forest composition mainly influences parasitoid community structure, rather than the overall abundance of parasitoids and its impact on budworm mortality, and thus defoliation. Marrec et al. (2018) found that landscape-level forest structure explained 61% of the variation in the parasitoid community. However, more than half of the variance was collectively explained by non-environmental spatial structures. We sampled the parasitoid community at a local (stand) scale and thus minimized the effects of landscape spatial structure and differences in outbreak status. Our results implied that spatial structure such as distance, physical barriers (lake, clearcut patch) may play an important role in budworm parasitoid community.

The correlations between explanatory variables and community structure in the nMDS ordination help us understand biotic and abiotic constraints of the parasitoid-host interaction in the rise of a spruce budworm outbreak.

5.4 Limitations of the study

Spruce budworm outbreaks cover large land area with long-lasting economic and ecological impacts (MacLean, 2016; Liu et al., 2019). In the 20th century, researches on spruce budworm ecology were largely conducted in peak and declining phase of outbreaks. I focused on the initiation and building phase of a spruce budworm outbreak in order to expand our understanding on the entire outbreak period. Due to logistic and time constraints, the thesis examined the first 5 years of the outbreak using empirical data collected in the field. This time frame generally covered the initiation and building phase considering that repeated defoliation typically lasting up to 10 years in an outbreak (MacLean et al., 2019). This research was not designed to study the entire outbreak cycle and therefore generalizations of results beyond the 5 sampling years considered in this study should be assumed with caution.

Chapter 3 of this thesis assessed spruce budworm population density during the sampling period. The population density reflected the annual defoliation severity caused by budworm in each year. Analyzing population dynamics generally requires long-term observations of a targeted population. This thesis was not designed to reveal spruce budworm population dynamics but could be used in future study for such purpose as using a meta-analysis procedure. Again, to fully understand how spruce budworm population density fluctuates in various forest environments, long-term observations of

population and survival are needed.

The morphospecies technique was used in Chapter 4 of the thesis to study parasitoid community of spruce budworm. The taxonomy of nearly all primary parasitoids of spruce budworm is known and available, but accurate microscopic identification of a specimen can be onerous and requires that specimens be in good shape to see distinguishing characteristics. Morphospecies was suggested to be an acceptable surrogate of taxonomic species in environmental monitoring and diversity estimates (Derraik et al., 2010; Vieira et al., 2012), however it still conveyed less taxonomic information than those from a traditional taxonomic species identification procedure. β -diversity of spruce budworm parasitoid community has not been extensively studied. Spruce budworm is an excellent candidate for studying influences of forest tree composition on parasitoids communities as population dynamics of the insect have been studied intensively during the 20th century (Royama, 1984; Morin et al., 2007; Royama et al., 2017). I suggested more in-depth investigations on parasitoid community of spruce budworm with taxonomic species datasets.

In this thesis, I quantified the relationship between budworm defoliation and hardwood content in fir-hardwood forests during the initiation of an outbreak for the first time. It provides important knowledge for tactical planning in proactive pest management, e.g., selecting targeted insecticide spray areas. In the two years (2013 and 2014) immediately prior to the peak defoliation in all stand types, defoliation in softwood plots were 21% and 49% higher than in mixedwood and hardwood plots, respectively. Fir-hardwood composition significantly influenced budworm early-instar dispersal. Altering tree species composition or stand structure (Régnière and Fletcher, 1983) to

hinder early-instar dispersal could reduce stand susceptibility to budworm outbreaks. Fir-hardwood composition significantly influences community structure of budworm parasitoids, rather than the abundance of parasitoids and overall parasitism on budworm.

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This article/chapter was published in Forest Ecology and Management, 463, Zhang, B., MacLean, D.A., Johns, R.C., Eveleigh, E.S., Edwards, S., Hardwood-softwood composition influences early-instar larval dispersal mortality during a spruce budworm outbreak, 118035, Copyright Elsevier (2020). DOI link to the formal publication:

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