Natural Resistance of White Spruce and the Behavior of the Eastern Spruce Budworm

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Abstract

Natural Resistance of White Spruce and the Behavior of the Eastern Spruce Budworm

Brian J. Mader

A white spruce, *Picea glauca* (Moench) Voss. (Pinaceae), plantation in southern Québec contained two distinct types of trees: resistant and susceptible to attack by spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). The primary purpose of this study was to evaluate if and how the pattern of feeding and oviposition preference are modified in response to past nutritional experience. Both lab-reared insects and field-reared insects, tested on artificial diet and foliage, respectively, failed to show differences in the pattern of feeding between groups with different nutritional experience. These findings suggest that the spruce budworm, like other specialists feeders, may not have evolved mechanisms of physiological or behavioral modification in response to previous nutritional stress. That being said, adult budworm, when reared on foliage from resistant trees, preferentially laid more eggs on foliage from susceptible trees but showed no significant preference when reared on foliage from susceptible trees. These findings illustrate that the presence of deterrent compounds on the surface of needles, coupled with larval experience, may be influencing adult oviposition preference. Both these findings have important implications for forest management strategies; plantations using mixed tree phenotypes may slow budworm adaptation to resistant trees.

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Contributions of the Authors

As the author of this work and first author of the manuscripts, I was responsible for the conception, design, setup, data gathering and data analysis of the experiments, and writing. My responsibilities also included field collection of insects and foliage used in all experiments.

Chapters 3 and 4 were coauthored by Dr. Emma Despland (Concordia University), and Dr. Éric Bauce (Université Laval), and M. Simon P. Daoust. Both Dr. Despland and Dr. Bauce served as mentors and supervisors from conception to submission of this thesis. They helped in designing and planning experiments. Dr. Despland further helped in statistical analysis and correcting early thesis versions. M. Daoust aided in data analysis and manuscript writing, providing valuable feedback.

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Chapter 1

General Introduction

Coevolution between plants and one or more of their natural enemies is a reciprocal evolutionary interaction that is said to occur in cycles (Berenbaum and Zangerl, 1988; Ehrlich and Raven, 1964; Futuyma, 2000; Gould, 1988; Janzen, 1980; Rausher 1992; Whittaker, 1971). In the first phase of this cycle, natural selection in response to enemies results in the evolution of plant resistance, reducing enemy attack. The second phase involves the evolution of counter-resistance – characters that avoid or tolerate the newly evolved plant resistance – by those enemies (Rausher, 2001). Consequently, while herbivores have evolved mechanisms for circumventing plant defenses, plants have evolved defense responses in turn.

Adaptation is a fundamental biological process whereby the dominant phenotype within a population is slowly modified in response to a changing environment, allowing organisms to survive and multiply (Mousseau and Dingle, 1991). Accumulation of adaptive changes over time causes species to evolve (Kimura, 1979). Currently, the most widely accepted theory of adaptation is the Darwinian theory of evolution by natural selection. This theory is founded on the principle that evolution is the result of the interplay between phenotypic variation in a trait related to fitness and environmental selective pressures (Kimura, 1979). According to this theory, individuals whose genes give rise to characters that are best adapted to the environment will be the fittest to survive, reproduce and leave survivors that reproduce in turn (Kimura. 1979).

If the process of adaptation to a new plant is to take place, an insect can overcome plant deterrence with selected behavioral or physiological changes (Bernays and

Chapman, 1994). Therefore, behavior, especially variation in behavioral phenotype within a population, is central to understanding the evolutionary relationship between insects and their plant hosts (Bernays and Chapman, 1994). For this reason, it is conceivable that mechanisms of adaptation will act on behavioral phenotypes and, more specifically, on plastic behaviors. There are several factors that can influence plastic behaviors, leading to behavioral modification, such as parental effects and the effect of experience (Bernays and Chapman, 1994; Mousseau and Dingle, 1991).

The mechanisms of behavioral modification have been of great interest to biologists for several centuries (Bernays and Chapman, 1994). Understanding these mechanisms is crucial to unraveling the coevolutionary history of plants and their insect hosts. Furthermore, for economically important species, understanding how behavioral modification can impact population growth and eventual outbreaks would greatly help management programs.

Canada is the world's largest exporter of forest products, generating 1.9% of its gross domestic product in 2009 (NRC, 2009). This important economic commodity is, however, at the mercy of natural disturbances such as epidemics of insect defoliators. Insect damage to Canadian forests is quite substantial, which is why considerable effort has been invested in research aimed at predicting outbreaks and controlling insect pests (Hanover, 1975).

The eastern spruce budworm (*Choristoneura fumiferana*) (Clem.) is a major defoliator of coniferous forests (Miller and Rusnok, 1993). This outbreaking insect is responsible for over 1.3 million hectares of damage to Canada's forests in 2007 (NRC, 2009), and 321 thousand hectares of damage in Québec (2008) (Tanguay, 2009), a number which has been slowly climbing in recent years.

Recently, a white spruce (*Picea glauca*) plantation in Drummondville, Québec, was found to contain two types of trees; one showing a natural resistance to defoliation by spruce budworm, and the other not (Daoust *et al.*, 2010). Trees exhibiting a resistant phenotype to budworm attack were therefore labeled as resistant and those that did not were labeled as susceptible (Bauce and Kumbasli, 2007; Clancy *et al.*, 1993, Daoust *et al.*, 2010).

The primary purpose of this study is to evaluate the processes of behavioral modification in response to past nutritional experience. I will be using the *Picea glauca – Choristoneura fimuferana* (white spruce – eastern spruce budworm) system as a model. The primary objectives are as follows: (1) evaluate and describe the mechanisms by which spruce budworm larvae compensate or modify their feeding behavior in response to past nutritional history by evaluating the pattern of feeding on artificial diet, (2) examine the impact of past nutritional history on larval feeding behavior and adult oviposition behavior of field insects on actual foliage, and (3) place these findings in the broader context of forest-pest management strategies.

It has been known for a long time that individual trees within a population of host trees, can differ in their vulnerability to budworm attack (Fleming, 1983). Theoretically, manipulating certain tree characteristics could be used to control and possibly predict spruce budworm population change. The speed at which the insect can overcome different natural defense mechanisms of its host plant would greatly influence plantation strategies.

Chapter 2

Literature Review

2.1 Insect-host plant coevolution

There are approximately 1.5 million species described to date (Freeman, 2008), and many experts agree that this number represents only a small fraction of the total number of species actually present on earth. The process of coevolution between plants and their natural enemies, including viruses, fungi, bacteria, nematodes, insects and mammals, is believed by many biologists to have generated much of this biological diversity (Berenbaum and Zangerl, 1988; Ehrlich and Raven, 1964; Gould, 1988; Gould, 1991; Janzen, 1980; Rausher 1992; Thompson, 1994; Whittaker, 1971). This cyclical relationship involves the development of plant resistance, and subsequent counter-resistance by herbivores (Rausher, 2001). Over time, these adaptations have led to speciation, contributing to the overall biodiversity of the planet. Furthermore, herbivores, such as insects, provide a major link between primary producers and a multitude of animals across several trophic levels (Bernays and Chapman, 1994). The extraordinary diversity of insect species has led to an equal diversity of organisms preying on them, greatly contributing to the current animal diversity.

Insect-plant coevolution may have contributed to the overall biodiversity of the planet (Grimaldi and Engel, 2006), but it poses significant difficulties when trying to design control programs for insect pest species. Programs aimed at controlling insect pest populations may be unsuccessful because of this cyclical relationship.

2.2 Parental effects

Individual phenotype is frequently and sometimes dramatically influenced by the environmental experience of mothers (Mousseau and Fox, 1998) and fathers (Delisle and Hardy, 1997). Parental effects arise when the mother and/or father makes a contribution to the phenotype of her/his progeny beyond that which results from the genes they contribute to the zygote (Delisle and Hardy, 1997). Possible mechanisms include i) from mother: cytoplasmic inheritance, maternal nutrition, transmission of pathogens, antibodies, hormones and messenger RNAs, ii) from father: nutritional investment via copulatory gifts or nutrients transmitted through the spermatophore (Bernardo, 1996; Mather and Jinks, 1971; Mousseau and Fox, 1998). Environmentally based parental effects have been documented for amphibians, reptiles, fish, marine invertebrates and terrestrial isopods (Rossiter, 1994). For insects, Mousseau and Dingle (1991) found expression of parental effects in nearly 60 genera and noted that when sought, they are usually found.

Parental effects can often be simple and maladaptive. Delisle and Hardy's work (1997) shows that poor paternal larval nutrition can influence the reproductive success of adult spruce budworm. Larvae reared on old foliage (containing less nutritional value) produced fewer offspring. Indeed, many authors have demonstrated negative impacts on offspring through parental effects (Bernardo, 1996; Phelan and Frumhoff, 1991; Rossiter, 1991b; Wellington, 1965).

However, it has been repeatedly suggested that parental effects often provide a mechanism for adaptive trans-generational phenotypic plasticity, in which the environment experienced by the mother/father is translated into phenotypic variation in offspring (Mousseau and Fox, 1998). In other words, the parents' experience can be passed on to offspring and increase their fitness if the modified phenotype of the offspring is beneficial. For example, a hypothetical situation involving parents experiencing drought, could produce offspring with altered diapause behavior leading to their increased fitness. The offspring that are able to survive and pass on their genes to further generations could lead to evolutionary change indirectly influenced by parental effects. Many authors have suggested that nutritional conditions of parents can affect the performance of their offspring over one or many generations (Barbosa and Capinera, 1978; Carisey and Bauce, 2002; Fox, 1993; Rossiter, 1991a; Rossiter, 1994).

2.3 Feeding by Phytophagous Insects

Most animals feed in bouts or meals that are separated from each other by more or less extended periods without feeding (Simpson, 1990; Simpson and Raubenheimer, 2000). Feeding patterns of herbivores are influenced by a range of biotic and abiotic factors (Hughes, 1993; Simpson and Raubenheimer, 1993). Every step in the behavioral chain of events leading to and including plant ingestion is potentially influenced by the chemical composition of plant tissues and is essentially governed by a balance of phagostimulatory and deterrent inputs from the peripheral sense organs to the central nervous system (Bernays and Chapman, 1994; Chapman, 2003; Frazier and Chyb, 1995; Mitchell, 1981; Städler, 1992; Wright *et al.*, 2003). Other factors which influence insect feeding are: time since last meal, size of last meal, and physiological processes such as the activity of gut stretch receptors (Simpson and Raubenheimer, 2000).

Plant feeding insect species make up over one quarter of all macroscopic organisms, and the green plants they feed on make up another quarter (Bernays and

Chapman, 1994). This diversity is attributable, at least in part, to the long history of coevolution of plants and their insect herbivores. However, insect adaptation to new or modified plant hosts can be seen on a smaller scale. Insect herbivores are known to possess various short-term modifications to the temporal availability and chemical variability of host plants (Denno and McClure, 1983; Whitham, 1981). Such mechanisms have been shown to be physiological and behavioral in nature. Physiological modifications can lead to morphological changes, such as the strengthening of mandible musculature (Bernays, 1986). Other physiological modifications in insects involve the alteration of food processing and utilization (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 2000; Simpson *et al.*, 2004; Thompson *et al.*, 2001). For example, Warbrick-Smith *et al.*, (2006) demonstrated that *Plutella xylostella* caterpillars, when reared for multiple generations on a high carbohydrate diet, were able to modify the amount of excess fat stored in their body. Behavioral modifications include dietary self-selection, whereby insects distinguish different nutrient composition of available food items and selectively eat those containing the needed balance of nutrients (Schiff *et al.*, 1989; Simpson *et al.*, 1988; Slansky and Scriber, 1985; Thompson *et al.*, 2001). Insects have also been shown to actively increase their food intake when on a poor food source termed compensatory feeding (Abisgold and Simpson, 1987; Raubenheimer and Simpson, 1993; Simpson *et al.*, 1988, Simpson *et al.*, 1989). A third behavioral modification is known as induction of preference, which involves differential food intake by the insect based on past experience (Bernays and Chapman, 1994; Simpson *et al.*, 1988).

There has been much research in the field on host plant resistance to insects and maintenance of that resistance. The possibility of insect modifications can render genetically modified insect-resistant crops (for example; crops containing endotoxins from *Bacillus thuringiens*) useless in a few insect generations (Gibbons, 1991; Harris, 1991). Researchers have recommended a number of strategies to delay the eventual resistance of insects to these crops. Potentially valuable planting strategies include growing stands of normal crop alongside resistant crop to maintain a 'refugium' of susceptible insect populations (Gould, 1989; Mallet and Porter, 1992).

Parental effects have been considered in this context. Fox *et al.*, (1997) showed that the use of the novel plant host *Chloroleucon ebano* by the seed beetle, *Stator limbatus*, is facilitated by non-genetic effects of parental host plant on progeny survival; parents reared on *Cercidium floridum* (normal host plant) produce progeny with substantially higher survivorship on the new host *C. ebano* than parents reared on *Acacia greggii* (a second normal host plant). Therefore, host-plant-mediated parental effects are likely to be common and thus must be included in studies in order to truly understand herbivorous insect evolution and population dynamics (Fox *et al.*, 1997, Rossiter, 1991b).

2.4 White Spruce, *Picea glauca* (Moench) Voss. (Pinaceae)

White spruce is one of Canada's widest ranging and most commercially important tree species, being the main source of pulpwood and construction grade lumber (OMNR, 1995). Trees can grow up to forty meters tall and attain a trunk diameter of up to one meter. Being part of the Coniferophyta, they possess characteristically long needle shaped leaves with a thick epicuticular wax layer (Campbell and Reece, 2002). Although balsam fir (*Abies balsamea*) is typically the most defoliated host of spruce budworm, white spruce is considered the most suitable food for the insect, since it has been shown to have optimal performance on this host (Albert, 1982; Guertin and Albert, 1992).

The surface of the needle represents a highly complex environment (Muller and Riederer, 2005). The outside layer of the needle consists of the cuticular layer and the cuticle proper, which is covered by epicuticular waxes. These waxes are a complex mixture of long-chain aliphatic and cyclic compounds (Muller and Riederer, 2005). The major wax components are widely distributed across plant species, and therefore are of little relevance for host plant discrimination by insects (Chapman, 1976). However, the feeding behavior of insects is influenced by the low concentration of secondary chemicals contained within, or presented on the waxes (Woodhead and Chapman, 1986). This type of behavior can be highly adaptive because it enables the insect to accept or reject the food item without ingesting potentially toxic substances. In response to insect feeding, plants have evolved several adaptations to protect themselves. These adaptations include: morphological features, presence of chemical repellents, absence of chemical attractants and nutritional status regulators such as developmental inhibitors (Albert, 1991). Plants produce an exuberant amount of defensive compounds which are grouped in 2 different categories: toxic compounds and digestibility reducing compounds (Chown and Nicolson, 2004). One such group of digestibility reducers are the monoterpenes present in the epicuticular wax layer (Chown and Nicolson, 2004). The production of these chemicals has been shown to be stimulated by plant tissue damage and to have a deterrent effect on insect feeding and oviposition (Litvak and Monson, 1998).

2.5 Eastern Spruce Budworm (*Choristoneura fumiferana*)

The eastern spruce budworm is an insect of the lepidopteran order. Being holometabolous, it undergoes complete metamorphosis to become an adult tortricid moth. Larvae are oligophagous; preferred hosts are the balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), red spruce (*P. rubens*) and black spruce (*P. mariana*) (Albert, 1982). The main peripheral taste organs are located on the two maxillae and on the epipharynx of the mouthparts (Albert, 1980). Outbreaks can lead to vast defoliation of coniferous forests and therefore is of major economical interest to the pulp and paper industry (Miller and Rusnok, 1993).

The previous spruce budworm outbreak (1968-1990) resulted in a loss of approximately 180 million m^3 of economically important coniferous trees in eastern North America due to tree mortality and almost an equal amount lost in tree growth (Boulet, 2001; Miller and Rusnok, 1993). A new epidemic has slowly been building in the last few years in different regions across Québec (Outaouais, Lac-Saint-Jean, Mauricie and Bois-Francs) (Bauce *et al.*, 2006a). For these reasons, much research has been conducted on the spruce budworm in an attempt to ascertain the various life history traits as well as environmental conditions that enable populations to reach epidemic proportions (Volney, 1985).

There are several important behaviors associated with the success and survival of the spruce budworm. One such behavior is dispersal (Wellington, 1980). Some sort of dispersal occurs during all active stages of the insect's life. The moth however, is the most important, having been documented to reach distances of 600km from their point of origin and a high proportion of these being egg laying females (Dobesberger *et al.*, 1983).

Not to be overlooked is the dispersal of first and second instar larvae both before and after hibernation, respectively. Dispersal, or ballooning, involves the budworm parachuting with silk threads released by the spinneret and is limited to a few kilometers, depending on wind conditions (Batzer, 1968). This can lead to the spread of outbreaks (Blais, 1952), but could also result in a considerable reduction of population if budworm balloon to inferior or inadequate food sources (Miller, 1958). This in turn can play an important role in budworm population dynamics.

Another important behavior is that of oviposition by adult females. Since larvae have limited mobility, and dispersal conditions may not always be beneficial, larvae depend chiefly on the recognition of an adequate food source by their mothers. Several studies show that egg laying females use a wide range of visual, olfactory, chemotactic, and mechanical cues in this decision-making process (e.g., Rivet and Albert, 1990; Städler, 2002; Wallace *et al.*, 2004).

The final behavior addressed, and the main focus of this study, is that of larval feeding behavior. Spruce budworm larvae are responsible for the defoliation and mortality of their host trees; the main objective of budworm larvae is to eat the most biomass possible and convert this biomass to somatic and gonadal growth for their progression to adulthood. Larvae consume entire current year needles which results in the loss of area used for photosynthesis and visible defoliation of host trees. Adult moths do not consume needles but drink water.

2.6 Host Plant Selection by Spruce Budworm

Although much of the previous work on insect feeding patterns have used grasshoppers as model organisms, this information is still incredibly usefully in explaining caterpillar feeding patterns (Simpson and Raubenheimer, 2000). Many factors affect host-plant selection by budworm; plant acceptance and sustained feeding is ultimately determined by chemical features of the plant and is highly specific (Chapman, 2003; Schoonhoven and van Loon, 2002). Plants can rely on a wide range of chemical defenses against herbivores. Monoterpenes are secondary plant compounds, found on the surface of foliage, that act as feeding and oviposition deterrents to a variety of herbivores, including various species of bark beetles, lepidopteran defoliators, mammals (Litvak and Monson, 1998) and specifically, against the spruce budworm (Bauce *et al.* 1994; Clancy *et al.*, 1993: Chen *et al.*, 2002). Tannins, which are found within the needle, are known to act as feeding deterrents, digestibility reducers, and toxins in numerous insect species (Cardinal-Aucoin, 2007), and more specifically, they have recently been shown to reduce the growth and survival of the spruce budworm (Kumbasli, 2005).

Other than chemical defenses, tree populations often have large variations in time to budburst (Nienstaedt and King, 1969). Trees that consistently budburst earlier or later than similar-aged conspecifics may escape attack from herbivores (Quiring, 1994). Optimal synchrony for the spruce budworm occurs when emergence of the insect precedes budbreak by approximately two weeks. However, it has been shown that performance was high even when emerging 3-4 weeks prior to budbreak. The insects will feed on older foliage until the new buds start growing (Lawrence *et al.*, 1997). These natural plant defenses can exert a strong selective pressure on insect populations, either by affecting the individual's fitness directly (by killing them) or indirectly through parental effects.

2.7 Study Site

Studies conducted in the summers of 2002 - 2004, within a fast growing white spruce plantation (Drummondville, Québec, Canada, 45° 53' 0'' N 72° 29' 0'' W) located in a zone severely infested by spruce budworm (>50 larvae/ 45 cm long branches), revealed the presence of two distinct types of white spruce trees. The two types of trees were deemed susceptible and resistant to spruce budworm attack based on empirical observations of differential levels of defoliation and as per Clancy *et al.*, (1993) (Bauce and Kumbasli, 2007). The two types of trees have chemical differences in secondary metabolites; resistant trees have been shown to have 20-26% more monoterpenes on the surface of the needle, as well as 110% more condensed tannins within the needle (Daoust *et al.*, 2010). Preliminary observations have also demonstrated that resistant trees bud break 3 weeks after susceptible trees (Bauce, personal communication).

Clancy (1993, 2001) has done much research on a resistant-susceptible tree system with the western spruce budworm on balsam-fir. The mechanism underlying host resistance for her case was a quantitative difference in monoterpenes. Her studies, however, involved performance, not behavior (Clancy *et al.*, 1993).

2.8 Previous Research on this System

There are known feeding behavior differences (Daoust *et al.*, 2010) as well as differences with regard to multiple generational survivorship and performance (Bauce *et* *al.*, 2006a) between tree types. Significantly fewer insects transitioned from probing to feeding on resistant needles leading to a reduced number of feeding bouts as well as a significantly shorter first meal. Indeed, 79.3 % of spruce budworm had at least one feeding bout following a probing event on susceptible needles, whereas only 34.1% on resistant needles. Furthermore, when the waxes were removed, the number of insects that fed following a probing event decreased to 31.4 % on susceptible de-waxed needles, whereas increased to 50 % on resistant de-waxed needles. This implies that the monoterpenes present at 20-26% higher levels for resistant needles could possibly serve as a phagodeterrent which supports findings from previous studies (Chen *et al.*, 2002; Clancy *et al.*, 1993; Litvak and Monson, 1998). It is possible that a heritable or parentally-induced difference in behavioral response to monoterpenes underlies the increased success on resistant foliage of caterpillars whose parents were raised on that same foliage.

Bauce *et al.*, (2006b) has demonstrated that the spruce budworm is capable of successfully adapting to various qualities of artificial diet. The offspring of parents reared on a high stress artificial diet (simulating resistant trees), were shown to perform better on both a high stress diet and low stress diet than did the offspring from parents reared on low stress diet (personal communication). Similarly in the field, the resistant trees impose a strong selection pressure with a mortality rate of 64%-98% during the feeding season as opposed to 30%-51% on susceptible trees. The following generation of insects whose parents had survived had much higher over winter survival as first instar larvae on resistant trees (90% as opposed to only 48% of insects from susceptible trees) (Daoust *et al.*, 2010).

Chapter 3

Past nutritional experience does not appear to influence the pattern of feeding of laboratory reared spruce budworm (*Choristoneura fumiferana*) (Clem.) larvae.

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3.1 Abstract

The nutritional quality of available food sources can have drastic effects on many aspects of an insect's life history and insects have evolved mechanisms of adjusting to the nutritional variability of food sources. The purpose of this study was to (1) evaluate and describe the mechanisms, if any, by which spruce budworm larvae (*Choristoneura fumiferana*) (Clem.) compensate or modify their feeding behavior in response to their past experience (both experienced by the individual insect and the parental generation) and (2) place these findings in the broader context of forest-pest management strategies. The microstructure of the pattern of feeding was analyzed for budworm with different past experience: non-stressed and stressed. Although slight trends were observed, budworm from different past experience appeared to show no differences in behavior, suggesting no physiological or behavioral modification. These results suggest that lab-reared spruce budworm larvae do not compensate or modify feeding behavior in response to previous nutritional experience, which has interesting implications for forest management strategies.

3.2 Introduction

Nutritional quality of food sources can have a drastic effect on many aspects of an insect's life history. Food quality can influence larval survivorship and growth rate (Noseworthy and Despland, 2006; Slansky, 1990); subsequently influencing the timing of molts, restricting access to phenotypically appropriate foliage and available mates. Food quality can also directly impact the individual's fitness by influencing mating success, clutch size, nutrient investment in the eggs, oviposition choice sites and even offspring survival (Awmack and Leather, 2002; Carisey and Bauce, 2002; Slansky and Rodriguez, 1987).

However, insects have evolved mechanism of adjusting to inadequate food sources. Such mechanisms have been shown to be physiological and behavioral in nature. Physiological modification can lead to morphological changes, such as the strengthening of mandible musculature, which has been documented in the grass-feeding caterpillar, *Pseudaletia unipuncta* (Bernays, 1986). Individuals reared on harder grasses had head masses twice as great as those reared on softer artificial diet, allowing them to ingest the harder food source. Other physiological modifications in insects involve the alteration of food processing and utilization (Despland and Noseworthy, 2006; Lee *et al.*, 2002; Shi *et al.*, 2008; Simpson and Raubenheimer, 2000; Thompson *et al.*, 2001). Slansky and Wheeler (1989) demonstrated that the generalist caterpillar, *Anticarsia gemmatalis,* can increase the efficiency at which their consumed food is digested and assimilated, allowing them to reach a normal growth rate in response to low nutrient levels in food. Insects can also deal with unbalanced nutrient intake by excreting nitrogen through fecal uric acid (Simpson and Raubenheimer, 2001) or increasing cellular respiration to utilize excess carbohydrates (Zanotto *et al.*, 1997).

As previously mentioned, insects also have behavioral mechanisms enabling them to adjust to food sources of various nutritional quality. One such mechanism is termed dietary self-selection, whereby insects distinguish the different nutrient composition of available food items and selectively eat those containing the needed balance of nutrients (Schiff *et al.*, 1989; Simpson *et al.*, 1988; Slansky and Scriber, 1985; Thompson *et al.*, 2001). Insects have also been shown to actively increase their food intake when on a poor food source. This behavioral mechanism is termed compensatory feeding (Abisgold and Simpson, 1987; Raubenheimer and Simpson, 1993; Simpson *et al.*, 1988, Simpson *et al.*, 1989). Compensatory feeding has been documented in several insect species: locusts (Abisgold and Simpson, 1987; Raubenheimer and Simpson, 1993; Simpson *et al.*, 1988), blowflies (Simpson *et al.*, 1989), and caterpillars (Lavoie and Oberhauser, 2004; Simpson *et al.*, 1988), and has been suggested in the spruce budworm (*Choristoneura fumiferana*) (Clem.) (Albert and Bauce, 1994; Toufexis *et al.*, 1996). The third behavioral mechanism for adjusting to low-quality food is post-ingestive in nature and is termed induction of preference, which involves differential food intakes by the insect, based on its own past experience (Bernays and Chapman, 1994; Simpson *et al.*, 1988). The insect can, in essence, modify the type and quantity of plant material ingested based on what it has preferred in the past or what its current needs are (Bernays and Chapman, 1994). Understanding these mechanisms is crucial to unraveling the coevolutionary history of plants and insects. Furthermore, for economically important pest species, understanding how physiological and behavioral modifications can influence population growth and eventual outbreaks would prove very useful in developing durable insect-pest management strategies.

The purpose of this study was to (1) evaluate and describe the mechanisms by which spruce budworm larvae compensate and adjust feeding behavior in response to their nutritional histories (both experienced by the insect and the parental generation) and (2) place these findings in the broader context of forest-pest management strategies. In order to answer these questions, we analyzed the microstructure of the pattern of feeding of spruce budworm larvae from two different nutritional past experiences: non-stressed and stressed. We hypothesize that when given a good food item (1) budworm from stressed nutritional past experience might have shorter pauses between meals, indicating more efficient food processing and (2) budworm with a stressed nutritional past experience might allocate more time to feeding, in essence taking advantage of an adequate food source and compensating for malnutrition over its developmental history.

3.3 Materials and Methods

3.3.1 Study Site

Field studies conducted in the summers of 2002 - 2004, within a fast growing white spruce, *Picea glauca* (Moench) Voss. (Pinaceae), plantation (Drummondville, Québec, Canada, 45° 53' 0'' N 72° 29' 0'' W) located in a zone of severe infestation by spruce budworm (>50 larvae/ 45 cm long branches), revealed the presence of two distinct types of trees. These trees were labeled as susceptible (S) or resistant (R), based on evaluation of defoliation history (Bauce and Kumbasli, 2007; Clancy *et al.*, 1993, Daoust *et al.*, 2010).

3.3.2 Insects

Spruce budworm larvae were obtained as pupae from (S) and (R) trees from the study site in the summer of 2006. Adults emerged in the laboratory, were sexed and mated following protocols used by Carisey and Bauce, 2002. Couples were made within tree type. Offspring (eggs) were collected and emerging larvae were stored for hibernation following Carisey and Bauce, 2002. Upon emergence from hibernation, in spring 2007, second instar larvae were embedded within strips of cheesecloth and Parafilm®. The cheesecloth was cut into small 2 cm^2 squares and placed in 23 ml plastic milk cups containing artificial diet.

Offspring of parents from (S) trees were placed on normal artificial diet designed by Grisdale and Wilson (1988) (sugar 9.2%, nitrogen 9.4%, cellulose 1.3%) whereas, insects of parents from (R) trees were placed on a stress diet (sugar 1%, nitrogen 6.8%, cellulose 8.9%) (Bidon, 1993). Bauce *et al.*, 2002, demonstrated that the stress diet leads to variations in general performance, such as higher larval mortality, longer development time as well as lower pupal weights (Bauce *et al.*, 2002). These effects are mirrored in the effects seen on budworm reared on foliage from (R) trees (Bauce *et al.*, 2006a). The cups containing the insects and appropriate artificial diet were then sealed with cardboard lids and placed in an incubator under a L16:D8 photoperiod at an ambient temperature of 22 °C and 60% relative humidity. When the larvae reached the fourth or fifth instar (approximately two weeks), they were transferred to Petri dishes with the corresponding diet (described above). Freshly moulted (within the last 24 h), surviving sixth-instar larvae were used for behavioral experiments. Insects were measured for size comparison, and subsequently coded for recording so that all experimentation and video analysis was done under blind conditions.

Before the insects were placed in direct contact with the diet, it was irradiated with a Spectroline 120 volt shortwave Ultraviolet 254 mm light, model XX-15G (Spectronics Corporation, Westbury; NY, USA) for thirty minutes, to sterilize the food.

3.3.3 Behavior

Insects were starved for a period of 4 h, at room temperature, in 3.5 cm Petri dishes prior to experimentation. The Petri dishes were then placed on a 27 cm x 22 cm Styrofoam board. Styrofoam strips were placed between the Petri dishes, isolating insects from each other. Insects were then placed in a 100cm x 150cm x 100cm wooden framed box, covered in black felt. This isolation chamber was continuously lit for the experiment using a fluorescent lamp attached to the inside, upper frame. The larvae were viewed using a Canon, GL2 Video Camcorder, 3CCD Camera System, 20X/100X professional fluorite lens, 1.7 megapixels and recorded onto a computer using Virtual Dub software (1.5.10, 1998-2003, Avery Lee) set at 1 frame/s. The behavioral experiments took place from 14:00 to 09:00 of the following morning.

Normal artificial diet was cut into 1cm x 0.5cm x 1cm blocs and placed in the Petri dish containing the starved insects. All behavioral indices that we monitored were as follows: first contact with food item, initiation of probing event, cessation of probing event, initiation of feeding bout and cessation of feeding bout. A probing event consisted of any contact between the palps (palpating) and/or the mandibles (biting) of the insect with the diet that did not involve ingestion of the material. For the purpose of this experiment we did not distinguish between probing with palps or with mandibles. A feeding bout occurs when food is ingested. Individual feeding bouts are separated by either short probing events or short resting and exploration periods. The behavioral indices were monitored for a duration of 10h. Previous studies revealed that individual meals were separated by pauses that exceeded 10 min (Bernays and Singer, 1998; Daoust *et al.*, 2010). Fifteen replicates were used for each treatment. All insects were tested on normal artificial diet.

Treatments are defined as (1) insects with non-stressed past experience; reared on normal artificial diet and parental generation reared in the field on (S) trees and (2) insects with stressed past experience; reared on stress artificial diet and parental generation reared in the field on (R) trees. Please note that the definition of treatment is focused on the individual insect's own experience and not parental effect; individuals whose parents experienced a stressed diet also experienced a stressed diet, therefore we include parental effects within individual experience.

3.3.4 Statistical analysis

All data were analyzed using SPSS (SPSS Statistics, release 17.0.1, Dec 1, 2008). The data for the number of probing events (PE), mean median-duration of individual probing events, number of feeding bouts (FB), mean median-duration of individual feeding bouts, total duration of feeding bouts, the number of meals and insect length satisfied the conditions for normality (Sokal and Rohlf, 1995). However, the time to first contact, total duration of probing events, number of rest and exploration periods (RE), total duration of rest and exploration periods, and the mean median-duration of individual pauses between meals did not satisfy the conditions for normality (Sokal and Rohlf, 1995) and were rank transformed. t-tests were used to analyze all 11 parameters.

In order to avoid pseudo replication within individuals favoring spruce budworm that probed or fed significantly more often than others, medians were calculated for the duration of individual probing events, feeding bouts, and pauses between meals (because multiple readings were taken for each individual for these parameters). Medians were used because of the strong positive skew in the data set (Sokal and Rohlf, 1995).

Upon obtaining non-significant results for every behavioral indice monitored, equivalence testing was performed for total number and duration of feeding bouts as well as pauses between meals (Brosi and Biber, 2009; Jones *et al.*, 1996). Traditional hypothesis tests failed to demonstrate a significant difference; this does not mean that there is no difference. Equivalence intervals were chosen and compared to calculated confidence intervals. If CI overlap zero, the means are said not to differ significantly (Jones *et al.*, 1996). This test was performed to further support t-test findings.

3.4 Results

3.4.1 Probing

The time taken for spruce budworm larvae to first come into contact with the needles did not differ significantly between treatments (t-test, df = 28, t = 0.955, p = 0.348) (Figure 1) (CI = 1613.06 ±1886.44 s).

Both the mean number (t-test, df = 28, t = 0.540, p = 0.594) (CI = 9.17 ±27.39 probing events) and mean median-duration of individual probing events (t-test, df = 28, t = -0.033, p = 0.975) (CI = -1.15 ±3.51 s) were not significantly different (Figures 2 and 3). Although insects with stressed past experience tended to spend less time probing when compared to insects with non-stressed past experience, these differences were not significant (t-test, df = 28, t = 0.785, p = 0.439) (Figure 4) (CI = 1059.40 ±1385.73 s).

3.4.2 Feeding

There were no differences in the number of insects that successfully transitioned from probing to feeding between both treatments. Only 3 insects failed to transition following palpation: one from non-stressed past experience and two from stressed past experience.

There were no significant differences in the number (t-test, df = 28, t = -0.041, p = 0.968) (Figure 5) (CI = 6.71 ±27.02 rest and exploration periods), the duration of individual pauses between meals (t-test, df = 24, t = -0.841, p = 0.409) (Figure 6) (CI = -707.71 ±1205.74 s), or the total duration of rest and exploration periods (t-test, df = 28, t = 0.245, p = 0.808) (Figure 7) (CI = 231.38 ±2853.60 s). Insects from stressed past experience tended to have longer pauses between meals.

Although the insects from stressed past experience tended to feed less often (Figure 8) compared to those from non-stressed past experience, the observed trends were not significant (t-test, df = 28, t = 0.835, p = 0.411) (CI = 5.20 ±8.81 feeding bouts). There was no significant difference between treatments for the duration of individual feeding bouts (t-test, df = 24, t = -1.16, p = 0.257) (Figure 9) (CI = -27.35 ±31.56 s) or the total duration of feeding (t-test, df = 28, t = 0.565, p = 0.576) (Figure 10) (CI = 737.22 ±1811.01 s). There was also no significant difference in the total number of meals (t-test, df = 28, t = 0.103, p = 0.918) (Figure 11) (CI = 0.33 ±4.15 meals). Insects from different treatments did not vary in size (t-test, df = 28, t = 0.327, p = 0.746). Bauce *et al.*

(2002) had showed differences in pupal mass when reared on stress artificial diet, however pupae were not measured in this study.

Equivalence testing revealed no significant difference between treatments for total number of feeding bouts (CI = 5.20 ±8.81 feeding bouts), total duration of feeding (CI = 737.22 ±1811.01 s), or pauses between meals (CI = -707.71 ±1205.74 s).

3.5 Discussion

Several studies have looked at the impact of nutritional experience on the weight, development, reproductive fitness, and offspring survival of insects (Colasurdo *et al.*, 2009; Despland and Noseworthy, 2006; Fortin *et al.*, 1997; Mousseau and Dingle, 1991; Slansky and Rodriguez, 1987), including spruce budworm (Bauce *et al.*, 2006b; Carisey and Bauce, 2002; Daoust *et al.*, 2010). Carisey and Bauce (2002) showed that parental nutrition clearly affects hatching success and first-instar survival. Progeny fitness was significantly reduced when the parental generation was reared on either low nitrogen or low carbohydrate foods. Similar results were observed with insects on actual foliage from the current study site (Daoust *et al.*, 2010).

Previous works hinted at the possibility that spruce budworm larvae could potentially compensate when confronted with low-quality food by increasing their rate of consumption (Albert and Bauce, 1994; Toufexis *et al.*, 1996). Therefore, we had hypothesized that when given a food item of good quality, budworm with a stressed nutritional history might allocate more time to feeding, compensating for the history of malnutrition. The same budworm might also have shorter pauses between meals, indicating more efficient food processing, seeing as other caterpillar species can increase digestive efficiency when faced with inadequate diets (Slansky and Wheeler, 1989). However, contrary to our predictions we demonstrate that nutritional experience has no significant effect on the pattern of feeding of spruce budworm.

Therefore, this preliminary research demonstrates that budworm do not appear to have any significant physiological or behavioral adjustments in response to different nutritional past experiences in this instance. There was no evidence of insects having different pauses between meals, or different sizes; suggesting that budworm in this study from either stressed past experience or non-stressed past experience did not differ in their food processing efficiency. Furthermore the duration and number of feeding bouts did not differ, suggesting no compensatory strategies. These findings are consistent with the theory that nutritional regulation abilities are less important in specialist herbivores compared to generalists (Despland and Noseworthy, 2006; Lee *et al.*, 2006). In the wild, spruce budworm larvae are generally restricted to the host tree on which they were laid by their mother. Therefore are not exposed to food sources of varying quality and may not have evolved behavioral mechanisms for dealing with poor quality food.

Although there were no significant differences, we did observe several interesting trends that we wish to discuss. Budworm coming from stressed past experience tended to have a shorter time to first contact and a shorter time spent probing. This could possibly be a modification of behavior based on previous nutritional experience. However, individuals from stressed past experience tended to take fewer feeding bouts and spend less time feeding in total. They also tended to have longer pauses between meals. Because budworm have been malnourished for their development, it is conceivable that they do not have the energetic means to breakdown food as fast as budworm coming from normal past experience. This would counter the idea of any sort of beneficial modification.

The study site, origin of the parental generation, contains two types of white spruce: the first susceptible and the second resistant to budworm attack. Chemical attributes of these trees may have the possibility to be altered through selective breeding or cutting and used in plantation strategies (Slansky, 1990). We know from previous research on performance that the resistant trees place a strong selective pressure on developing insects and their offspring (Bauce *et al.*, 2006a; Carisey and Bauce, 2002; Daoust *et al.*, 2010). During the feeding season, there was a recorded mortality rate of 64%-98% on resistant trees as opposed to 30%-51% on susceptible trees. The offspring of parents that survived had much lower over winter mortality as first instar larvae on resistant trees (10% as opposed to only 48% on susceptible) as well as during the growing season; mortality rate of 55% on resistant trees compared to offspring of parents from susceptible trees (75%) (Bauce *et al.*, 2006a; Daoust *et al*, 2010). The present study suggests that budworm do not possess the capabilities of adjusting in response to past nutritional stress, both individual stress and parental stress. Combining these two findings, plantation strategies utilizing resistant trees may be worthwhile, considering that they have been shown to (1) reduce spruce budworm populations and (2) given the fact that the artificial stress in this study has been shown to simulate that of the resistant trees (Bauce *et al.*, 2002, Bauce *et al.*, 2006a), spruce budworm may not show significant physiological and/or behavioral modifications in the following generation. It is important to note here that regardless of parental history, even though the offspring from parents of resistant trees seem to be more fit (with lower motality rates), these individuals do not seem to compensate, conceivably not causing more damage.

As an aside, personal observations demonstrated a completely different pattern of feeding of budworm on artificial diet when compared to actual foliage. On artificial diet, insects appear to take shorter feeding bouts, more often, with shorter pauses, possibly leading to overall longer meals. Whereas insects of foliage are faster to start feeding, have longer feeding bouts with longer pauses (for digestion) but overall shorter meals. This would demonstrate that studies using artificial diet should be carefully compared to those involving actual foliage, however, further studies should be done to elucidate thiese observations.

In conclusion, this exploratory study suggests that the spruce budworm, like other specialists feeders, may not have evolved mechanisms of physiological or behavioral modifications based on previous nutritional stress. This study also suggests that utilizing resistant trees in plantation strategies may be beneficial given this lack of modification and the resulting decrease in population size from selective pressures.

Figures

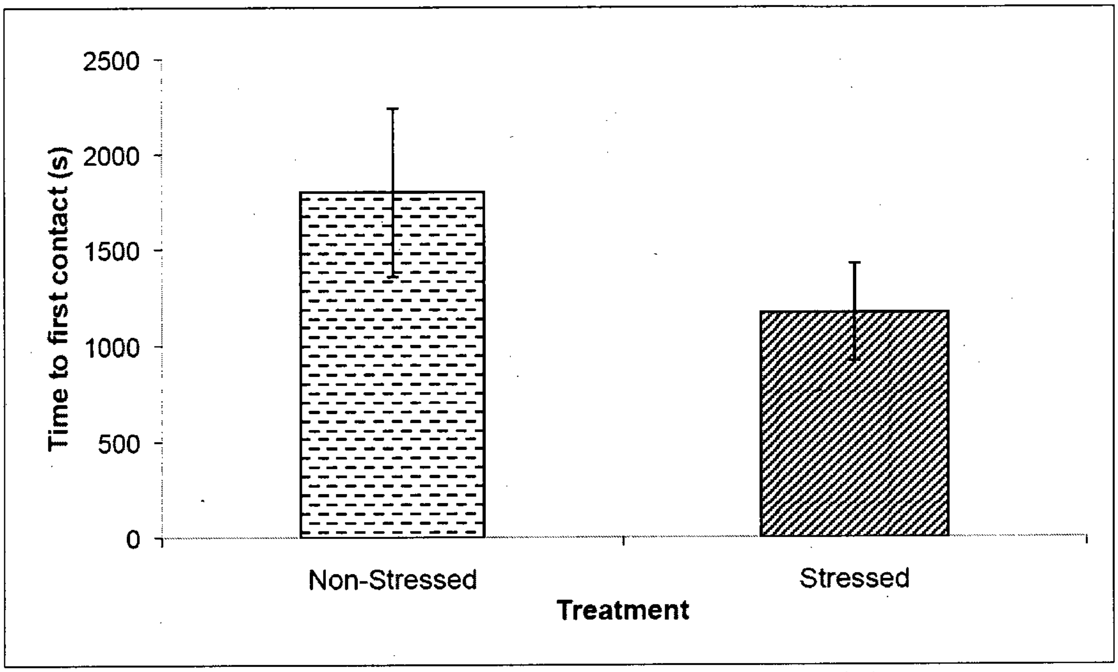

Figure 1. Mean time to first contact of the needle (s) ± SE by sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.955, p = 0.348) (n = 14, 16).

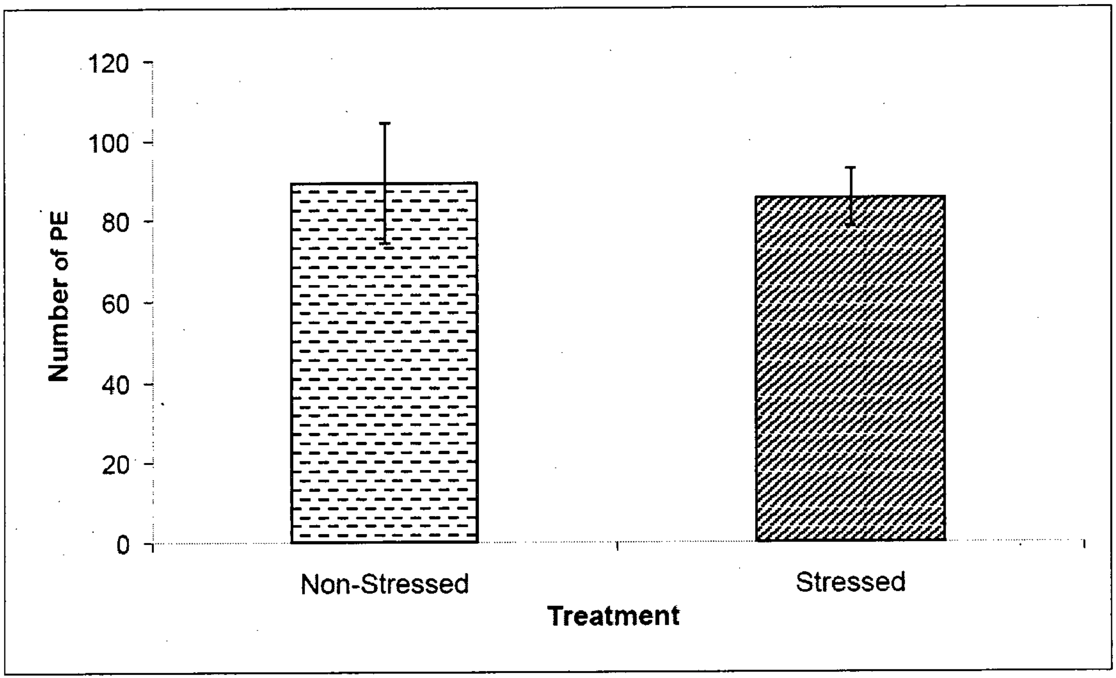

Figure 2. Mean total number of probing events ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.540, p = 0.594) (n = 14, 16).

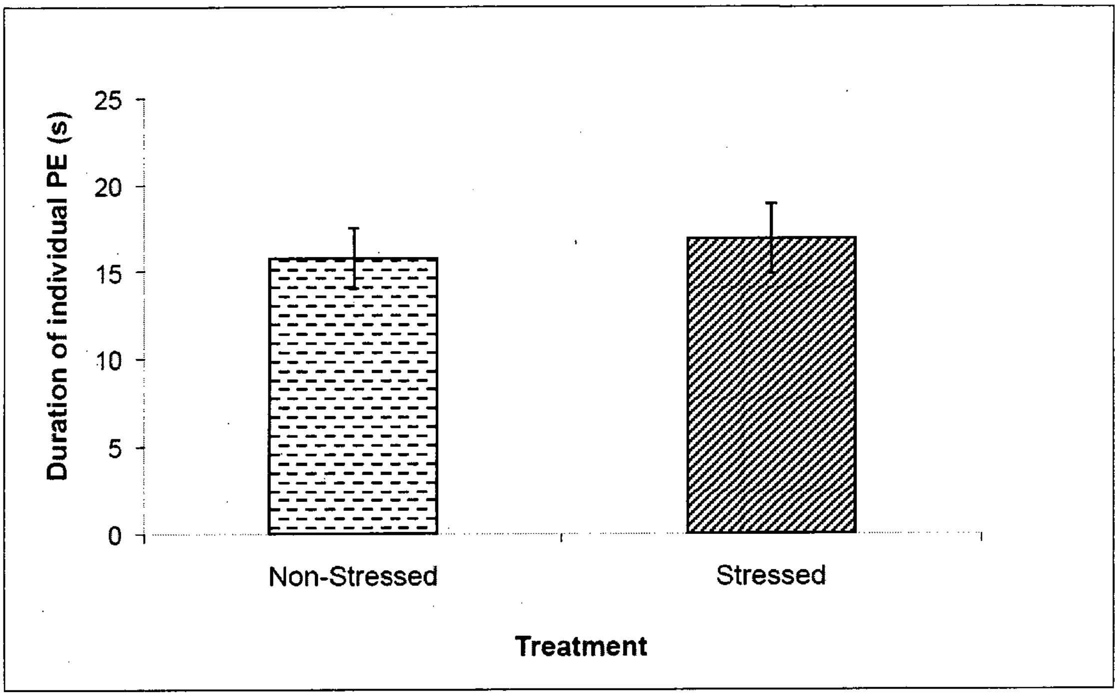

Figure 3. Mean median-duration of individual probing events (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = -0.033, p = 0.975) (n = 14, 16).

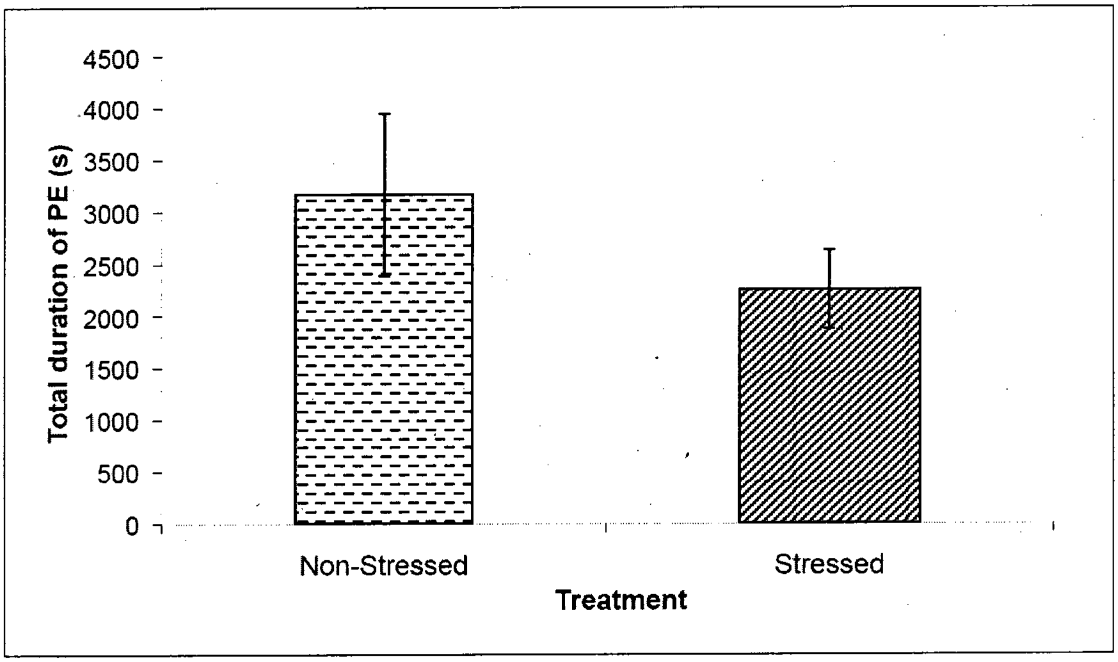

Figure 4. Mean total duration of probing (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.785, p = 0.439) (n = 14, 16).

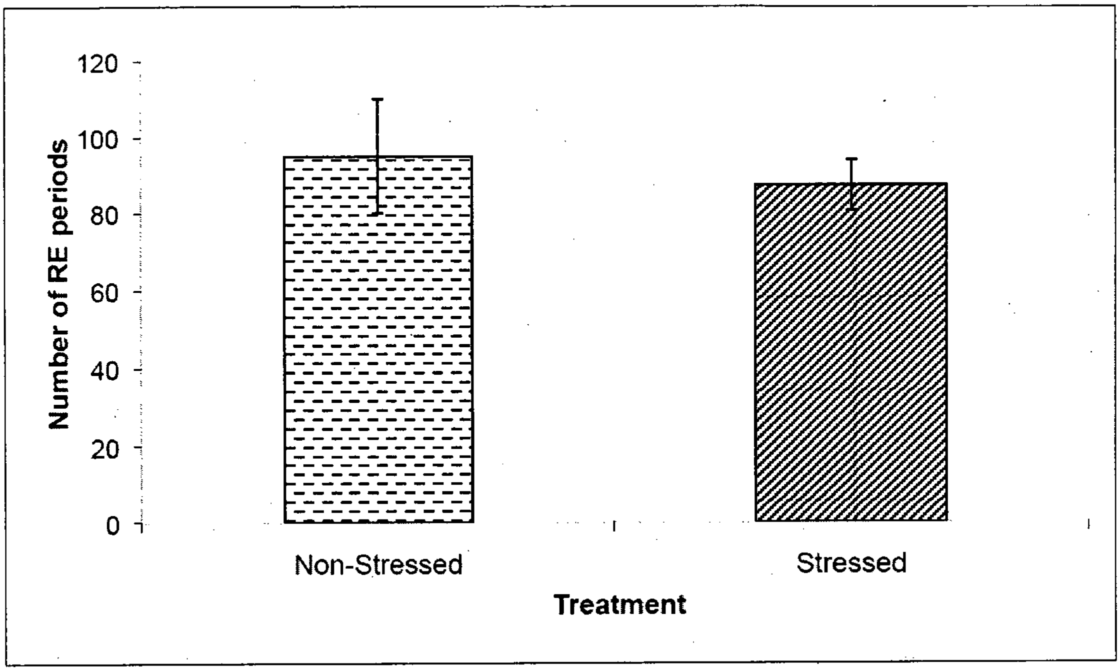

Figure 5. Mean number of rest and exploration periods ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = -0.041, p = 0.968) (n = 14, 16).

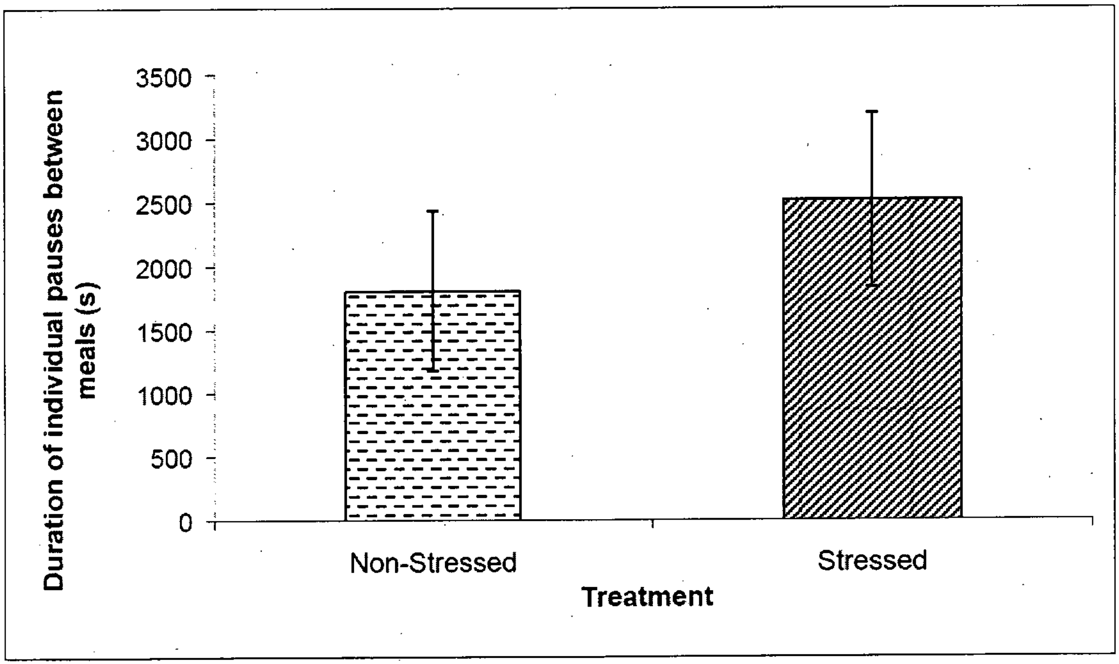

Figure 6. Mean median-duration of individual pauses between meals (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 24, t = -0.841, p = 0.409) (n = 13, 13).

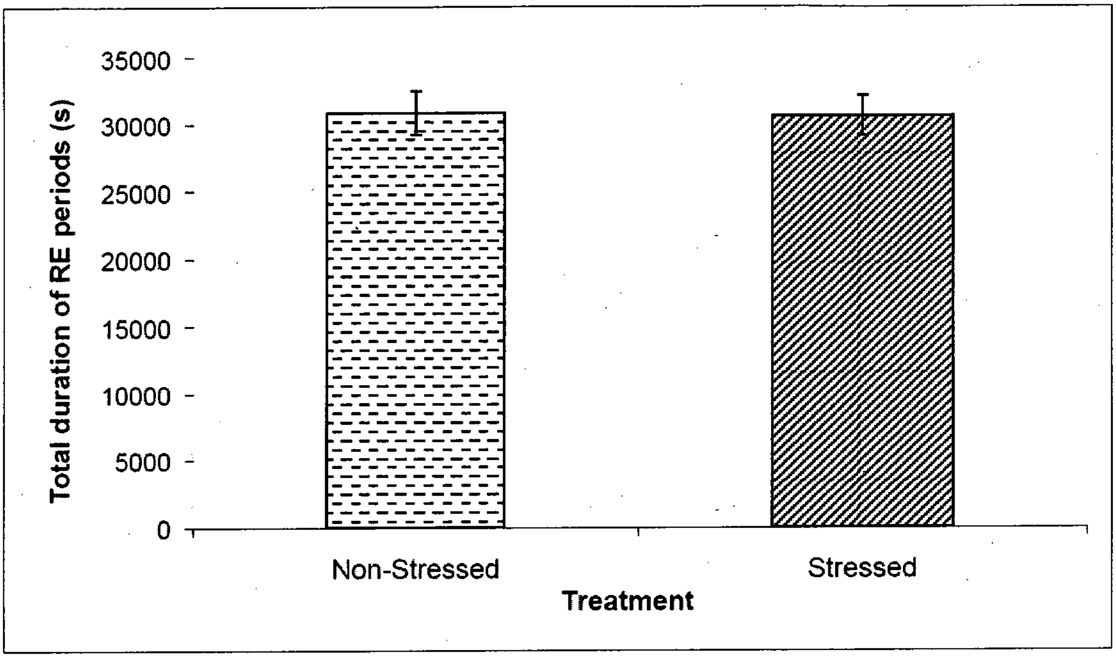

Figure 7. Mean total duration of rest and exploration periods (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.245, p = 0.808) (n = 14, 16).

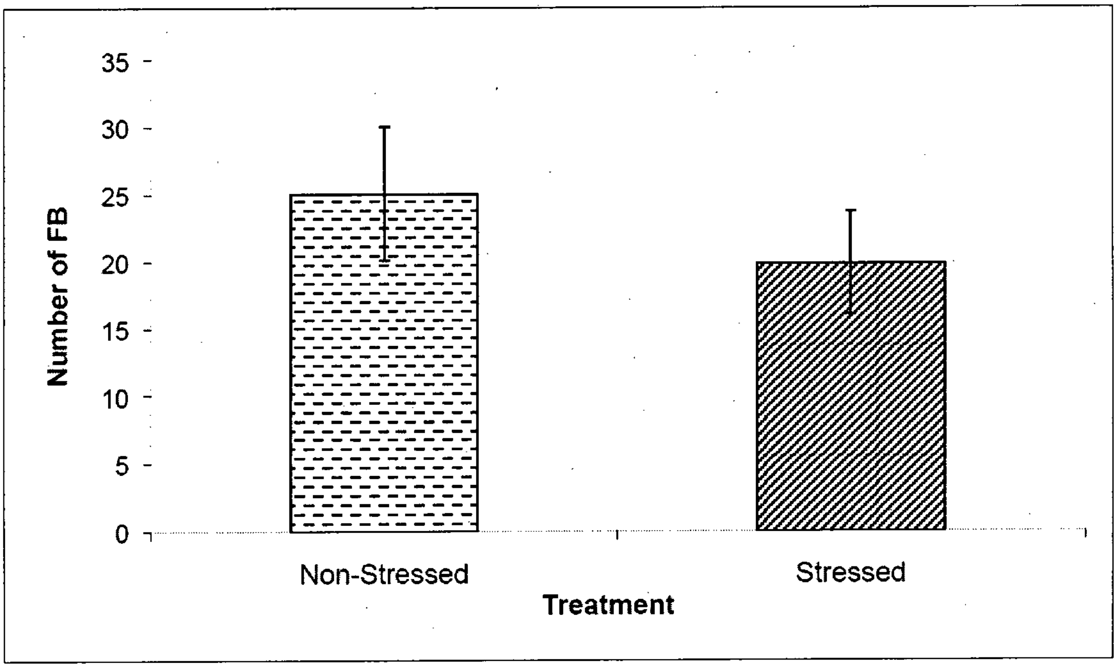

Figure 8. Mean total number of feeding bouts ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.835, p = 0.411) (n = 14, 16).

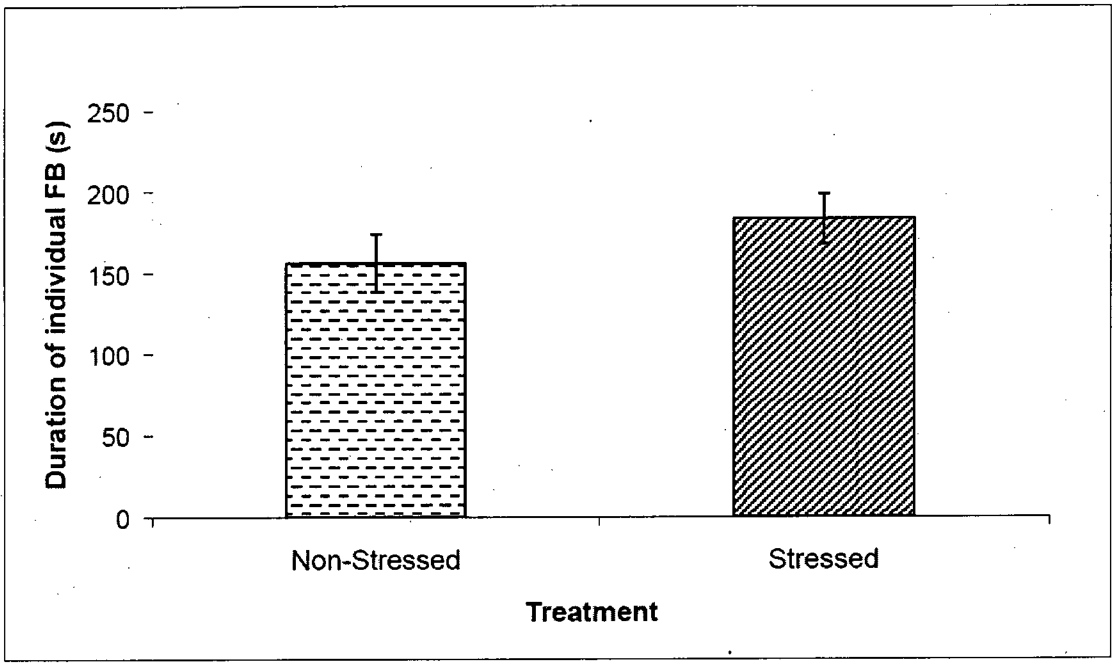

Figure 9. Mean median-duration of individual feeding bouts (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 24, t = -1.161, p = 0.257) (n = 13, 13).

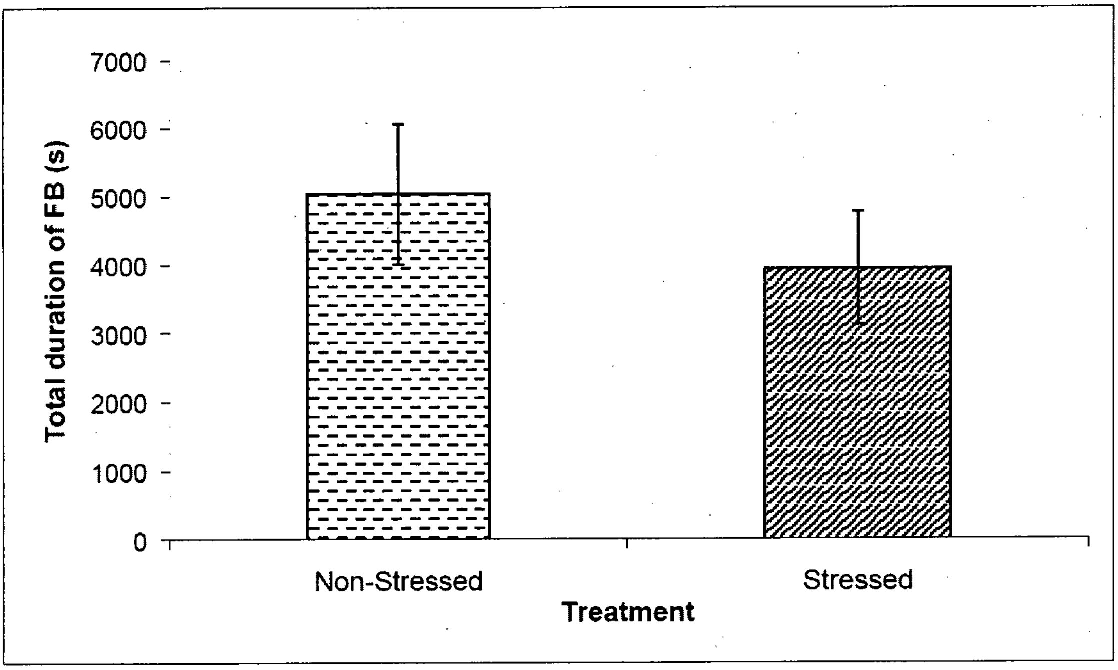

Figure 10. Mean total duration of feeding (s) ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.565, p = 0.576) (n = 14, 16).

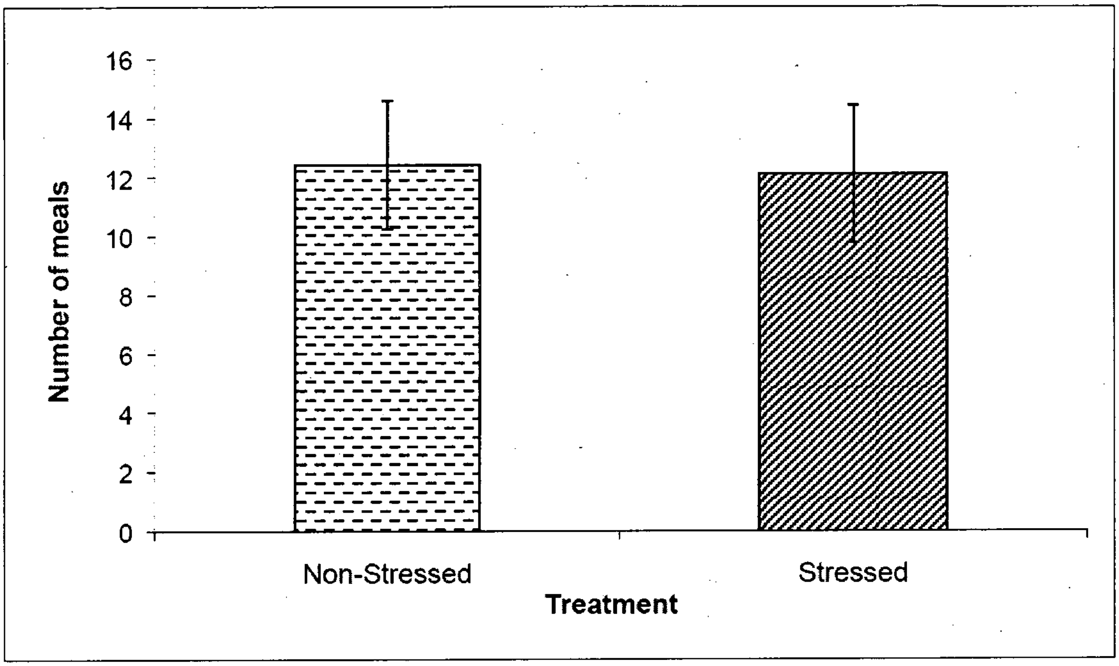

Figure 11. Mean total number of meals ± SE of sixth-instar spruce budworm when reared on artificial diet (t-test, df = 28, t = 0.103, p = 0.918) (n = 14, 16).

Connecting Statement

Chapter 3 examined the pattern of feeding of spruce budworm larvae on artificial diet in order to explore possible mechanisms of physiological and behavioral modification. It was suggested through empirical observations that studies using artificial diet should be carefully compared to studies using actual foliage. In Chapter 4, spruce budworm were both reared and tested on actual foliage. Feeding behavior and oviposition preference were examined, allowing for a better comparison to the field by further exploration of physiological and behavioral modification.

Chapter 4

Does past nutritional experience influence the behavior of spruce budworm (*Choristoneura fumiferana*) (Clem.) from natural populations in response to resistant host trees?

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4.1 Abstract

Here we are interested in the control of insect pest populations by utilizing a host tree's natural resistances. Our study system is composed of two types of white spruce, *Picea glauca* (Moench) Voss. (Pinaceae), one showing a natural resistance to defoliation by spruce budworm (*Choristoneura fumiferana*) (Clem.), the other not. The purpose of this study is to elucidate how feeding and oviposition behaviors of budworm from a natural population may be modified in response to past nutritional experience and parental history with the hopes of applying this knowledge to improve current management strategies. It was demonstrated that those budworm that survived being reared on resistant foliage do not show significant modifications in feeding behavior compared to budworm reared on susceptible foliage. Budworm do however, show a modification in adult female oviposition behavior; adults reared on resistant foliage preferentially lay on susceptible foliage but those reared on susceptible foliage show no preference. These findings illustrates that the presence of deterrents on the surface waxes coupled with larval nutritional experience may be influencing adult oviposition preference. This has important implications for forest management strategies in this system. Plantation strategies favoring mixed tree phenotypes may slow budworm adaptation to resistant trees, where susceptible trees act as buffers or decoys for egg laying females.

4.2 Introduction

Canada's 402.1 million hectares of forest and other wooded marginal habitats represent 10% of the worlds total forest cover. Canada is also the world's largest exporter of forest products, generating 1.9% of its gross domestic product in 2009 (NRC, 2009). This important economic commodity is, however, at the mercy of natural disturbances such as epidemics of insect defoliators. Insect damage to Canadian forests is quite substantial, which is why considerable effort has been invested in research aimed at predicting outbreaks and controlling insect pests (Hanover, 1975). Past insect pest management strategies have relied on the use of insecticides, silvicultural management techniques as well as natural parasites, predators and targeted viruses (Hanover, 1975). That being said, the most efficient and cost effective method to control insect populations is potentially by taking advantage of the host tree's natural resistances (Beck, 1965; Hanover, 1975; Maxwell *et al.*, 1972).

The eastern spruce budworm (*Choristoneura fumiferana*) (Clem.) is a major defoliator of coniferous forests (Miller and Rusnok, 1993). This outbreaking insect is responsible for over 1.3 million hectares of damage to Canada's forests in 2007 (NRC, 2009), and 321 thousand hectares of damage in Québec (2008) (Tanguay, 2009), a number which has been slowly climbing in recent years. There are several important behaviors associated with the success and survival of the spruce budworm, but none more important than feeding behavior. Many factors have been shown to influence the pattern of feeding of spruce budworm larvae, notably the presence of stimulants or deterrents on the surface of needles as well as secondary metabolites within the tissues of the needles (Daoust *et al.*, 2010).

Another important behavior associated with budworm survival is the oviposition decisions made by adult females. Since budworm larvae have limited mobility, and dispersal may not always be advantageous, larvae depend chiefly on the recognition of adequate food sources by their mothers. White spruce (*Picea glauca*) Moench (Voss.) is considered the most suitable host for the organism (Albert, 1982; Guertin and Albert, 1992). Previous work has shown that egg laying females can discriminate between oviposition substrates. In fact, previous experiments show that females preferred to lay eggs on white spruce when given the choice over other acceptable hosts such as balsam fir (*Abies balsamea* (L.) Mill.) (Jayens and Speers, 1949). Furthermore, adult females prefer to lay on substrates treated with host plant extracts as opposed to neutral substrates (Wallace *et al.*, 2004). It has been suggested that adult females use chemosensilla found on the proboscis, tarsi, antennae, as well as the ovipositor (Ramaswamy, 1994; Rivet and Albert, 1990).

Previous research has looked at the effect of individual experience on both larval and adult behavior. Feeding caterpillars often demonstrate a reduced sensory input after experience (Blaney *et al.*, 1986; Chapman *et al.*, 2003; Schoonhoven *et al.*, 1998). Decreased sensory input to deterrents following exposure to those deterrents has been documented and in several cases, this change is seen by a reduction in taste cell responsiveness (Glendinning *et al.*, 1999; Glendinning *et al.*, 2001). Also, when individuals are satiated with carbohydrates or proteins, they experience reduced sensory input to sugars or amino acids, respectively (Simpson and Simpson 1992). Hopkins host selection principle suggests that larval experience is carried across metamorphosis to influence adult host preferences whereby adults will preferentially select the same host experienced as larvae (Barron, 2001). This principle is seen in a variety of insects including Diptera (McCall and Eaton, 2001), Lepidoptera (Anderson *et al.*, 1995), Hymenoptera (Caubet *et al.*, 1992; Smith and Cornell, 1979) and Coleoptera (Solarz and Newman, 2001). There are, however, other studies that do not support this principle (Dethier, 1954; Wiklund, 1974; Wiklund, 1975). It has been repeatedly suggested that parental effects often provide a mechanism for adaptive trans-generational phenotypic plasticity, in which the environment experienced by the mother/father is translated into phenotypic variation in offspring (Mousseau and Fox, 1998), however there are no studies, to my knowledge, that document parental effects on oviposition behavior.

Here we investigate natural resistance to defoliation by spruce budworm within the same white spruce plantation in Drummondville, Québec (Bauce and Kumbasli, 2007; Clancy *et al.*, 1993, Daoust *et al.*, 2010). Insects are field reared and all experiments used fresh, phenotypically appropriate foliage, which has yet to be tested in a behavioral setting. Previous research has demonstrated chemical differences in secondary metabolites between tree types (Bauce and Kumbasli, 2007; Daoust *et al*, 2010). Foliage from resistant trees contain significantly more condensed tannins and monoterpenes, both of which have been shown to have adverse effects on spruce budworm performance and feeding behavior (Bauce *et al.*, 2006a; Cardinal-Aucoin *et al.*, 2009; Chown and Nicolson, 2004; Daoust *et al.*, 2010; Litvak and Monson, 1998; Kumbasli, 2005). Insects reared on resistant foliage experienced significantly higher larval mortality during the feeding season; however, offspring of surviving parents from resistant trees have lower overwinter mortality (Bauce *et al.*, 2006a; Daoust *et al*, 2010). When insects are reared on artificial diet and introduced to both types of foliage, significantly fewer insects transitioned from probing to feeding on resistant foliage (Daoust *et al*, 2010). These findings give insight into how the chemical constituents of resistant trees may be contributing to the alteration of feeding behavior.

The present study aims at furthering our understanding of the pressures placed by resistant trees on budworm and how these insects respond to such pressures. We will be examining the impact of parental rearing diet (resistant or susceptible foliage) and larval rearing diet (resistant or susceptible foliage) on larval feeding behavior and adult oviposition behavior of F1 generation spruce budworm. We propose to elucidate how these behaviors may be modified in response to past experience and parental history and how these changes in behavior may lead us to improve our management strategies.

We hypothesize that feeding behavior will be different between insects of different past experience. Certain differences in the pattern of feeding can suggest either behavioral or physiological modifications in response to a resistant or less suitable host. Differences in number and duration of feeding bouts would indicate some sort of behavioral modification such as compensatory feeding (Colasurdo *et al.*, 2007; Simpson, 1995). Differences in duration of pauses between meals would suggest physiological modifications; food processing differs in efficiency (Colasurdo *et al.*, 2007; Simpson, 1995). For oviposition, there are three possible outcomes: (1) spruce budworm preferentially lay on the foliage type they experienced as larvae (Hopkins host selection principle). If the insects preferentially laid eggs on their rearing tree type, this would strengthen the hypothesis that there exists two distinct populations in the field, one living solely on resistant trees and another living on susceptible trees. (2) Spruce budworm reared on resistant foliage preferentially lay on susceptible foliage. This would involve a modification of behavior based on what insects experienced as larvae; the adults recognize a less suitable host and chose the opposite for their offspring. (3) Adults show no preference and lay eggs equally on each substrate. This would indicate a lack of ability in female moths to discriminate between tree types. The last two scenarios would further suggest that insects are all members of a single population, and mixing is continuously occurring.

4.3 Materials and Methods

4.3.1 Study Site

Please refer to section 3.3.1

4.3.2 Insects

Spruce budworm were obtained as pupae from susceptible (S) and resistant (R) trees from the study site in the summer of 2008. Adults emerged in the laboratory and were sexed and mated following protocols used by Carisey and Bauce, 2002. Couples were formed within tree type. Offspring (eggs) were collected and emerging larvae were stored for hibernation following Carisey and Bauce, 2002. Upon emergence from hibernation, in spring 2009, second instar larvae were placed individually on trees in the field. Offspring of parents from (R) type trees were placed again on (R) type trees, and offspring of parents from (S) type trees were placed on (S) trees. Twenty insects were placed onto branches in the top 2/3 of 14 individual trees (7 per type) and covered in mesh bags and sealed with ribbon to prevent dispersal of and predation on budworm. After two weeks, branches were cut and brought to the lab.

Insects were collected as fourth or fifth instars from branches and placed in 18cm x 12cm x 8cm Tupperware® containers with a mesh covered hole in the lid and fresh foliage. Foliage petiole were placed in small, 1.5mL Eppendorf® tubes filled with water. The Tupperware® with insects and foliage were stored in an incubator under a L16:D8 photoperiod at an ambient temperature of 22 °C and 60% relative humidity. Freshly moulted (within the last 24 h) sixth-instar larvae were used for feeding experiments and mated females were used for oviposition experiments. All insects were coded for recording so that experimentation and video analysis was done under blind conditions.

4.3.3 Foliage

Fresh, phenologically appropriate, current-yr, white spruce (*Picea glauca*) foliage was collected every four days and fed to growing larvae. Insects were fed needles from the tree they were collected from (7 trees per type), however, the trees on both extremes (most defoliated and least defoliated) were chosen for experimentation in order to maximize the possibility of observing an effect on behavior. Trees were sampled in June 2009, which coincided with the presence of sixth-instar larvae on both the (S) and (R) trees. Host current-year cuttings were stored at 4°C and placed in water for the duration of experiments.

4.3.4 Feeding Behavior

4.3.4.a. Experimental set-up

Please refer to section 3.3.3. for set up and recording. Some minor modifications to the aforementioned protocol includes the use of actual foliage in the place of artificial diet. Three needles, all from the same tree, were given to each insect for recording. The first 0.5 cm of each needle was placed into plastic pipetter tips filled with water and sealed with Parafilm®; this allowed the needles to remain moist for the duration of the experiment. Behavior was monitored for a duration of 8 hrs, or until needles were completely eaten.

A second modification to the aforementioned protocol was a change in treatment number. Ten replicates were used for each treatment. Treatments are defined by both rearing tree and test tree; 1) reared on (S), test on (S) 2) reared on (S), test on (R) 3) reared on (R), test on (R) 4) reared on (R), test on (S) (Figure 1).

4.3.4.b. Statistical analysis

All data were analyzed using SPSS (SPSS Statistics, release 17.0.1, Dec 1, 2008). The data for the time to first contact, number of probing events (PE), total time spent probing, number of rest and exploration periods (RE), total duration of rest and exploration, mean median-duration of individual pauses between meals, number of feeding bouts (FB), total time spent feeding and the total time (from first contact until end of the last feeding bout) did not satisfy the conditions for normality and were therefore ranked transformed (Sokal and Rohlf, 1995). The total number of meals did satisfy the conditions for normality. 2-WAY Analyses of variance (2-WAY ANOVA) were used to analyze all data, with the reared diet and the test diet as the two independent variables.

Multiple Kolmogorov-Smirnov tests were performed for each of the behavioral indices to test if the sample distributions of each treatment were significantly different from one another (Crawley, 2009). Similarly, Fisher's *F* tests, were performed to compare variances for each treatment within each of the behavioral indices (Crawley, 2009)

4.3.5 Oviposition Behavior

4.3.5.a. Experimental set-up

Rearing, mating and observation protocol followed that of Wallace *et al.*, 2004. Egg laying females were given a choice between the two tree types and oviposition preference was evaluated. Mating pairs were removed from mating cages. The following day, females were placed in 473-ml containers (Solo Cup Company, Urbana, Illinois) with both foliage types and a free water source. Females were visually observed for the duration of the first oviposition event and the following behaviors were recorded: needle type first contacted and number of switches between needle types. Females were then left in their containers for a period of three days and the following variables were evaluated: number of egg masses laid, number of eggs laid and egg fertility (presence of a developing larvae). There are two treatments in this experiment; insects reared on foliage from (S) vs. (R) trees, both given a two choice test between both foliage types (Figure 1).

4.3.5.b. Statistical analysis

All data were analyzed using SPSS (SPSS Statistics, release 17.0.1, Dec 1, 2008). Only data from successfully mated females (determined by egg fertility) was used for analysis. Fisher's exact test for R x K (FET) was used to examine the difference between treatments on the type of needle first contacted. The data for number of switches between substrates did not satisfy the conditions for normality and were rank transformed (Sokal and Rohlf, 1995). T-tests were used to analyze the effect of rearing diet on the number of switches between needle types as well as the effect of first contact on the number of switches.

The effect of rearing diet on preference was also evaluated by counting both the number of masses laid (which represents host acceptance) and number of eggs laid (which represents offspring number) on each tree type. Number of masses was counted on each type of foliage for both treatments and comparisons were done within treatments. Similarly, number of eggs was counted on each type of foliage for both treatments and comparisons were done within each treatment. All data satisfied the conditions for normality, save egg number data for females reared on (S) type foliage. This data set was also rank transformed (Sokal and Rolf, 1995). Paired t-tests were used to analyze these four parameters.

4.4 Results

4.4.1 Feeding Behavior

Kolmogorov-Smirnov tests revealed no significant differences between distributions of treatments within any of the behavioral indices. Similarly, Fisher's *F* tests revealed no significant differences in variances. Because the distributions and variances were not significantly different, means are reported in all figures as opposed to box plots.

2-WAY ANOVAs revealed no significant differences in the mean time to first contact (Figure 2) (Table I), the number of probing events (Figure 3) (Table I) as well as the total duration of probing (Figure 4) (Table I) between all treatments.

The number of rest and exploration periods, analyzed with a 2-WAY ANOVA, was not significantly different (Figure 5) (Table II). Both the mean median-duration of pauses between meals (Figure 6) (Table II) and the total duration of rest and exploration periods (Figure 7) (Table II) were not significantly different.

A 2-WAY ANOVA revealed no significant differences between treatments for the mean number of feeding bouts (Figure 8) (Table III). There was also no significant difference in the mean total duration of feeding bouts (Figure 9) (Table III). There was no significant difference in the mean number of meals between treatments (Figure 10) (Table III). Insects reared and tested on (R) foliage tended to have a shorter overall duration to completely finish all three needles, which can possibly be attributed to shorter pauses between meals. Regardless, there was no statistical significance (Figure 11) (Table III).

4.4.2 Oviposition Behavior

There was no significant difference between treatments on the type of needle first contacted (FET, $p = 1.000$). There was no significant effect of the needle first contacted on the number of switches between substrates (t-test, $df = 31$, $t = -0.681$, $p = 0.501$). There was also no significant effect of rearing tree type on the number of switches between substrates (t-test, $df = 31$, $t = -0.335$, $p = 0.740$).

The number of egg masses were counted. Each mass laid represents an oviposition event, indicating a choice to lay by adult females. A paired t-test revealed no significant difference in the number of masses laid (Figure 12) on each substrate for insects reared on (S) foliage ($df = 26$, $t < 0.001$, $p = 1.000$). Budworm reared on (R) foliage laid significantly more masses on (S) foliage ($df = 30$, $t = -3.219$, $p = 0.003$). A similar result was found with the total number of eggs laid on each substrate (Figure 13). Insects reared on (S) foliage showed no significant preference ($df = 26$, $t = -0.421$, $p =$

0.677), whereas insects reared on (R) preferentially laid more eggs on (S) foliage (df = 30, t = 2.107, p = 0.044).

The number of eggs (t-test, df = 56, t = 1.746, p = 0.086) as well as the number of fertilized eggs (t-test, df = 56, t = 1.948, p = 0.056) laid in total did not significantly differ between rearing tree type even though adults reared on resistant foliage tended to lay more eggs in total and have more fertilized eggs. Regardless, this indicates no adverse effects of rearing tree type on egg number as well as reproductive success.

4.5 Discussion

This study encompasses both feeding and oviposition behavior of the spruce budworm in response to naturally resistant white spruce trees found in the field. Insects were collected from, reared on, and tested on fresh foliage sampled from the field. Previous studies involving feeding behavior used lab reared insects with no experience to actual foliage (Daoust *et al.*, 2010) and oviposition experiments are lacking in the current system. Our experimental protocols were set up in such a way as to allow direct comparison to individuals in the field, potentially allowing us to use our results in order to improve current management strategies.

Spruce budworm larvae have been shown to demonstrate different feeding patterns when tested on resistant and susceptible foliage (Daoust *et al.*, 2010). When reared on diet, and tested on foliage, significantly fewer insects transitioned from probing to feeding on resistant needles leading to a reduced number of feeding bouts as well as a significantly shorter first meal. Furthermore, Carisey and Bauce (2002) showed, in performance studies, that offspring from parents reared on poor diet are better able to tolerate poor quality foliage. We had therefore predicted that insects, in our study, would display different patterns of feeding. This study, however, revealed no significant differences between treatments for all feeding behavior indices monitored. These results should be interpreted with care; due to the nature of the experimental design, it is quite possible that significant differences were not observed due the low number of replicates. However, based on the current sample, these results suggest that field insects, experienced with actual foliage, do not seem to show differences in their pattern of feeding when tested on foliage from both tree types. This can further suggest that experienced budworm do not seem to utilize behavioral or physiological modifications in response to resistant foliage. That being said, the high variability in spruce budworm behavior would indicate that (1) the selective force placed by the naturally resistant trees on the population is low, (2) there exist many feeding phenotypes in the budworm population or (3) there exists a bias with this study. Budworm were reared from hatching on either resistant or susceptible foliage, and since mortality is higher on resistant foliage, only the surviving sixth-instar budworm were tested. If behavioral modifications were to exist, they may have already been selected for at earlier instars, where surviving spruce budworm on resistant foliage are those exhibiting the feeding phenotype that allows for acceptance of the resistant foliage, which would explain why a significant difference in behavior was not observed. It is also possible that a difference was not observed because of a decrease in sensory input following experience with the needles (previous studies showing differences used inexperienced insects) (Blaney *et al.*, 1986; Chapman *et al.*, 2003; Daoust *et al.*, 2010; Schoonhoven *et al.*, 1998). Regardless, resistant tree phenotypes, may not be selecting for specific feeding behavior traits at the sixth-instar in this budworm population.

Oviposition behavior was also analyzed in order to determine if egg laying females preferentially select one tree type over another and how this behavior might be influencing budworm populations. Adult females have been shown to discriminate between suitable substrates (Jayens and Speers, 1949; Wallace *et al.*, 2004). This ability could conceivably be used in the current field system, allowing females to choose between tree types. Adult moths could therefore be influencing budworm populations. Preliminary observations suggested that tree resistance was not influencing female oviposition behavior, (365-415 eggs per 45cm branch on resistant trees, 323- 375 eggs per 45cm branch on susceptible trees (Bauce, personal communication)). This study, however, did not take into consideration the issue of nutritional past experience on female oviposition behavior. We had predicted three possible outcomes for the current study: (1) spruce budworm preferentially lay on the foliage type they experienced as larvae (Hopkins host selection principle), (2) modification of behavior based on what insects experienced as larvae; the adults recognize a less suitable host and chose the opposite for their offspring, or (3) adults show no preference. Contrary to preliminary observations, and in support of scenario (2) for resistant reared and (3) for susceptible reared, budworm reared on resistant foliage preferentially selected and laid significantly more eggs on susceptible foliage, whereas insects reared on susceptible foliage seemed to show no preference. These results suggest that budworm are all members of a single population in the wild. Furthermore, this experiment illustrates both the importance of larval nutrition on adult egg laying preference, as well as the role that adult females may play in influencing populations of this insect in the wild.

This work shows that larval nutritional experience seems to have an impact on adult host selection, however, not in a Hopkinian manner. This phenomenon has yet to be documented in spruce budworm. Not only is adult preference affected by larval nutritional experience, but it seems to be occurring in an adaptive manner. Budworm have been shown to perform poorly on resistant foliage (Bauce *et al.*, 2006a), and adult females seem to be able to recognize this less suitable host, based on individual and/or trans-generational experience. Resistant foliage was not shown to differ in nutrient content, however certain secondary plant compounds did differ, specifically monoterpenes and tannins, which adversely affect budworm development (Bauce *et al.*, 2006a; Daoust *et al.*, 2010; Kumbasli, 2005). It is possible that memories of these secondary compounds, encountered as larvae or by parents, are retained through metamorphosis and adults are better able to detect and discriminate against these compounds. In fact, monoterpenes on the wax layer have been shown to be oviposition deterrents (Fischer *et al.*, 1994; Litvak and Monson, 1998; Muller and Riederer, 2005; Städler, 1986). However, because there was no observable difference in oviposition of adults reared on susceptible foliage, simply the presence of more monoterpenes on the wax layer on the needle may not be enough to deter eager females. Deterrence coupled with memory or nutritional experience, on the other hand, seems to elicit a response.

Insect-host tree association has important implications for forest management (Lawrence *et al.*, 1997). Breeding for desired tree characteristics of white spruce has been suggested as a method of increasing resistance to spruce budworm (Blum, 1988). That being said, Bernays and Chapman (1994) have suggested that larvae over several generations, assuming genetic variability within the population, may adapt to the selected resistant characteristics. In designing a durable management strategy, one must try to avoid fast adaptation to resistant trees. Based on the results form this study, plantation strategies utilizing resistant type trees may be worth while. There exists a high mortality rate for budworm on resistant foliage (Bauce *et al.*, 2006a), indicating a strong selective pressure placed on insect populations by resistant trees. By designing a plantation solely comprised of resistant type trees, budworm populations would quickly adapt rendering the current control strategies useless. However, plantation strategies using mixed tree phenotypes favoring resistant type trees may be able to slow budworm adaptation and protect plantations. High mortality on resistant trees culls budworm populations, reducing the amount of defoliation. The presence of susceptible trees serving as buffers would potentially alleviate the strong selective pressure placed by resistant trees on budworm populations. This is helped by the finding that females reared on resistant trees preferentially lay on susceptible trees, reducing the selective pressures on the following generation. The budworm population would continuously be mixing, not allowing adaptation to resistant trees to occur too quickly, making the current plantation strategy durable.

In sum, this study has demonstrated that the surviving sixth-instar budworm do not seem to show behavioral modifications in response to resistant foliage when experienced with actual foliage. Budworm do however show a behavioral modification in adult female oviposition behavior, where adults reared on resistant foliage preferentially lay on susceptible foliage but show no preference when reared on susceptible foliage. These findings illustrate that the presence of deterrents on the surface of needles, coupled with larval experience may be influencing adult oviposition preference which has important implications for forest management strategies. Plantation strategies favoring mixed tree phenotypes may slow budworm adaptation to resistant trees, where susceptible trees act as buffers or decoys for egg laying females.

Figures

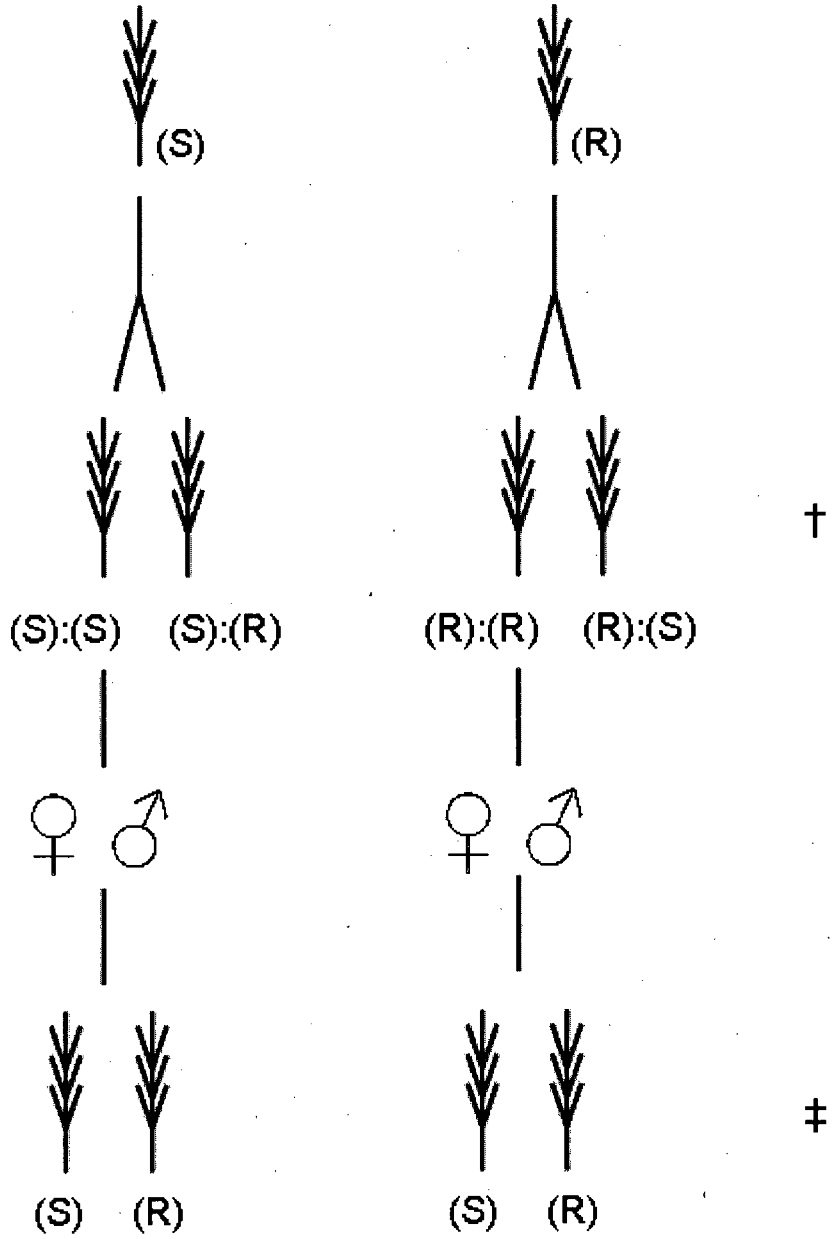

Figure 1. Rearing and experimental protocol on fresh foliage. † Feeding behavior test on sixth-instar larvae (4 treatments, n = 10, 10, 10, 9). ‡ Oviposition behavior choice tests on adults (2 treatments, n = 27, 31).

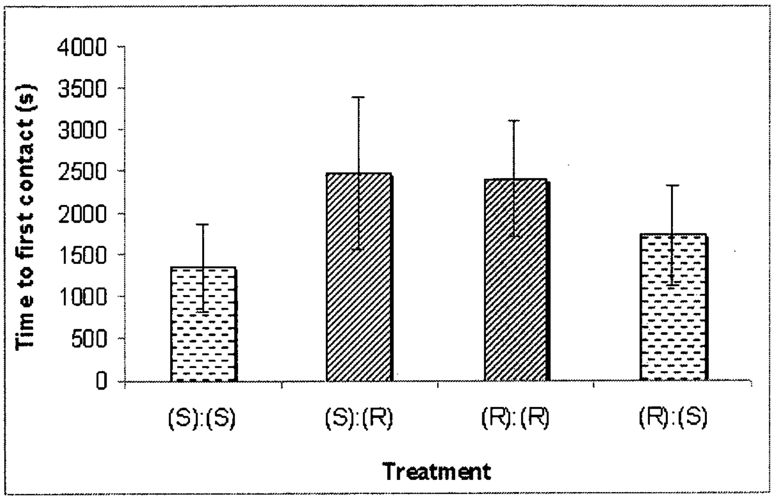

Figure 2. Mean time to first contact of the needle (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table I).

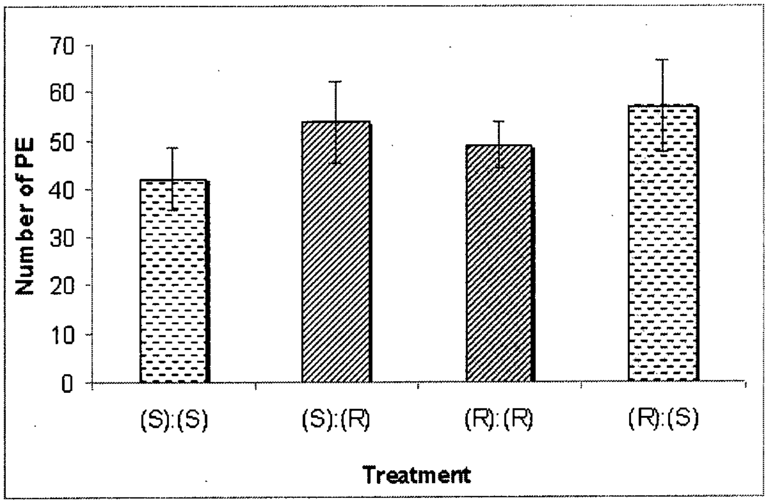

Figure 3. Mean total number of probing events ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table I).

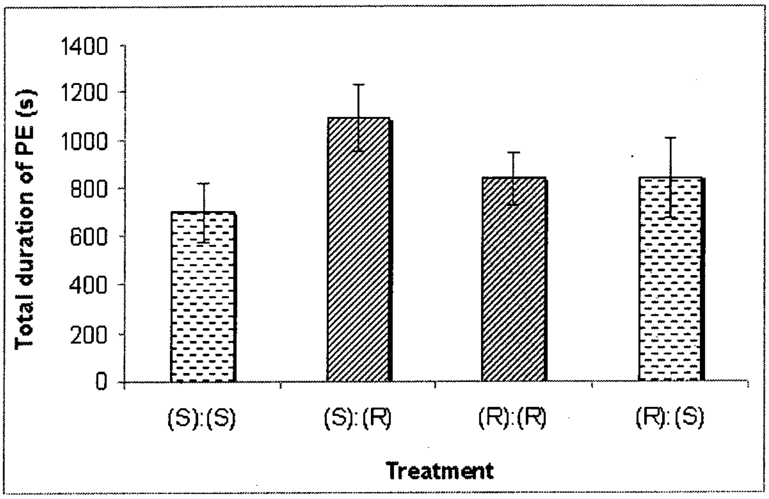

Figure 4. Mean total duration of probing in (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table I).

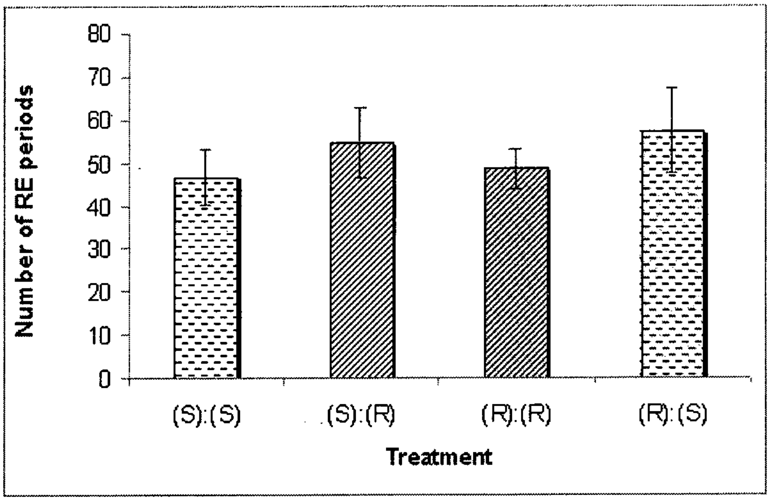

Figure 5. Mean total number of rest and exploration periods ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table II).

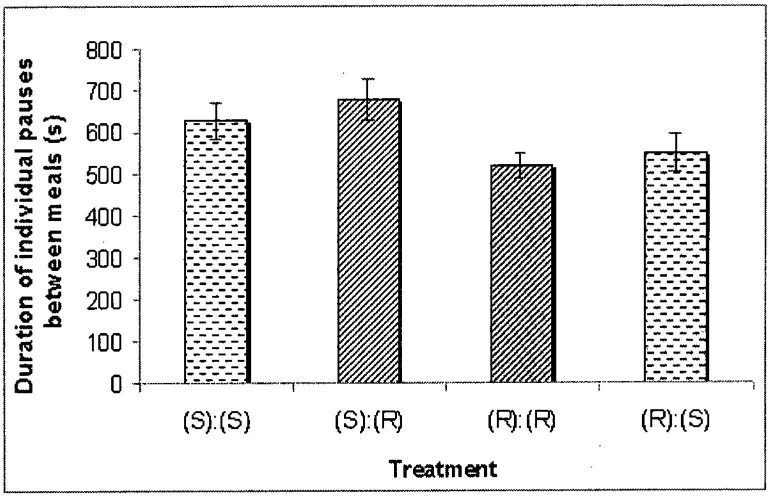

Figure 6. Mean median-duration of pauses between meals (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table II).

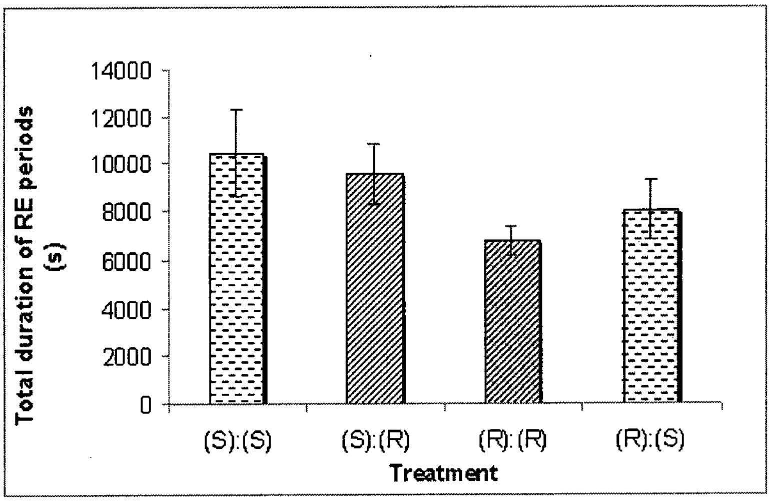

Figure 7. Mean total duration of rest and exploration periods (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table II).

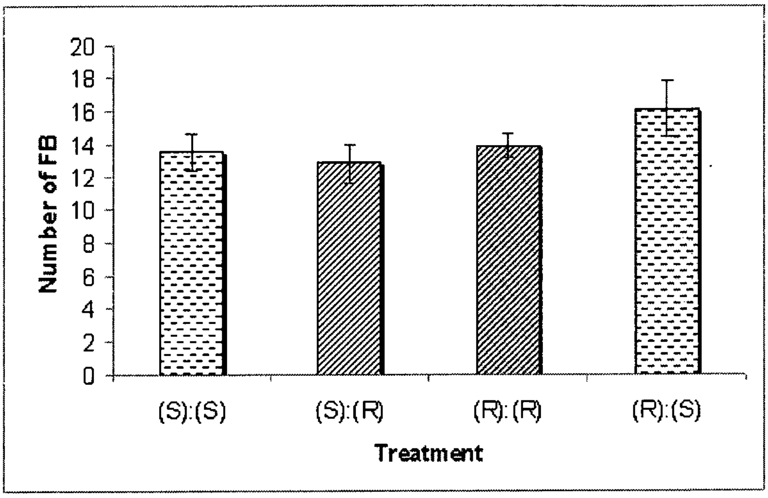

Figure 8. Mean total number of feeding bouts ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table III).

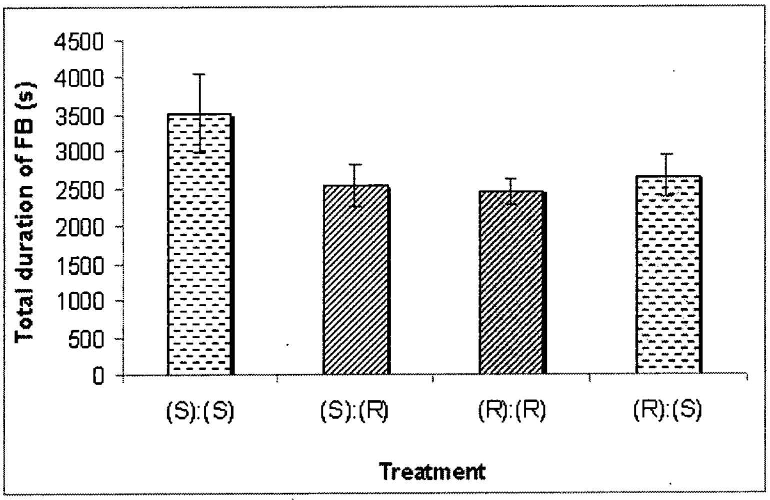

Figure 9. Mean total duration of feeding (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table III).

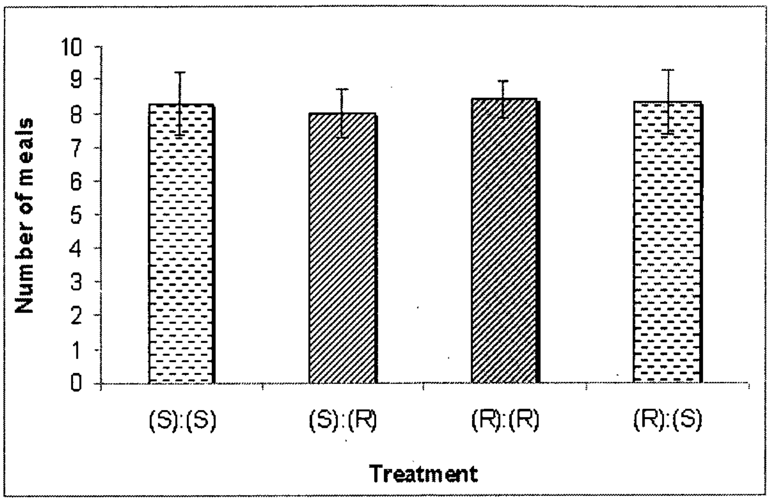

Figure 10. Mean total number of meals ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see alsoTable III).

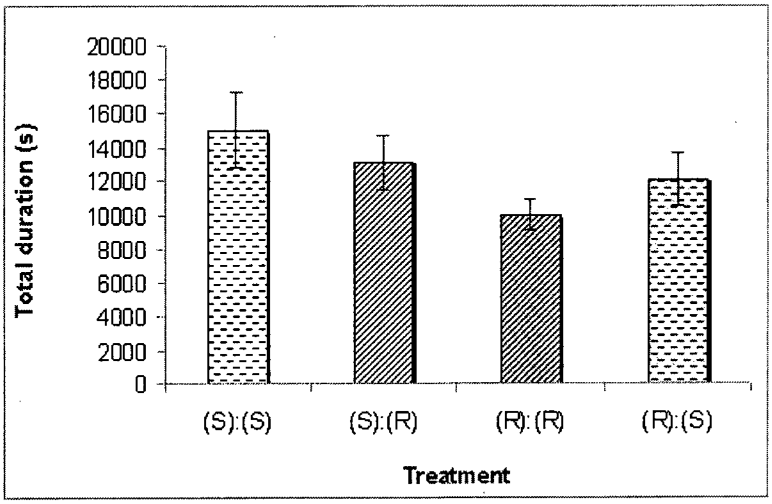

Figure 11. Mean total duration from first contact until end of last feeding bout (s) ± SE of sixth-instar spruce budworm when reared and tested on fresh foliage (see also Table III).

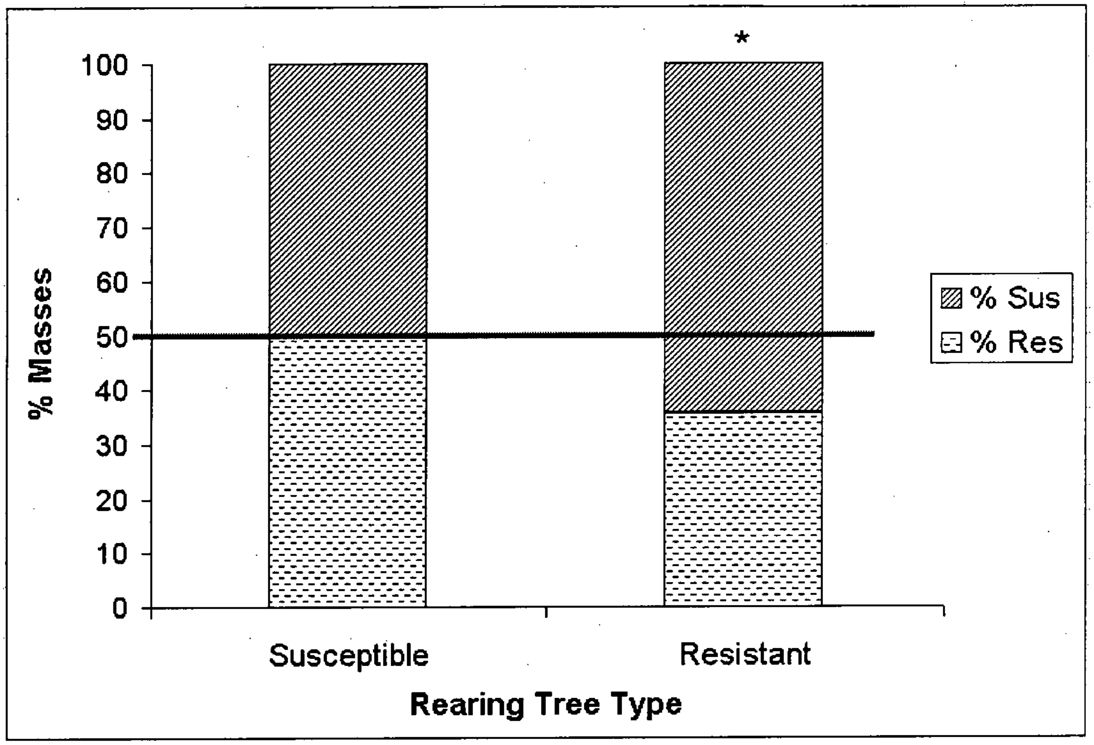

Figure 12. Percent masses laid by adult females on each tree type from different rearing tree types. Reared on (S) foliage (df = 26, t < 0.001, p = 1.000), reared on (R) foliage (df = 30, t = -3.219, p = 0.003*).

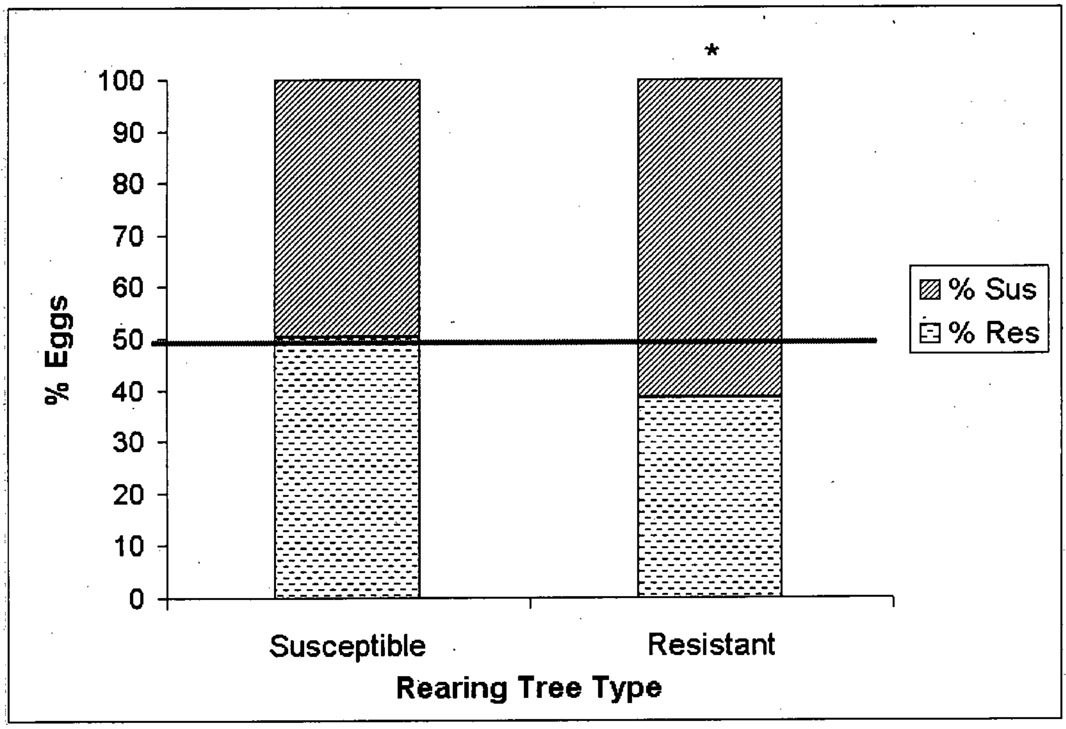

Figure 13. Percent eggs laid by adult females on each tree type from different rearing tree types. Reared on (S) foliage (df = 26, t = -0.421, p = 0.677), reared on (R) foliage (df = 30, t = 2.107, p = 0.044*)

Tables

Table I. Statistical results for probing behavior of sixth-instar spruce budworm when reared and tested on fresh foliage (2-WAY ANOVAs).

Behavior	Sources of Variation	Sum of Squares	Df	F	P
Time to first contact	Rearing diet	37.845	1	0.296	0.59
	Test diet	341.889	1	2.678	0.111
	Interaction	80.267	1	0.629	0.433
	Error	4467.522	35		
Number of PE	Rearing diet	55.784	1	0.41	0.526
	Test diet	26.668	1	0.196	0.661
	Interaction	84.354	1	0.619	0.437
	Error	4766.539	35		
Total duration of PE	Rearing diet	25.342	1	0.199	0.659
	Test diet	259.451	1	2.034	0.163
	Interaction	178.742	1	1.401	0.244
	Error	4464.681	35		

Table II. Statistical results for rest and exploration behavior of sixth-instar spruce budworm when reared and tested on fresh foliage (2-WAY ANOVAs).

Behavior	Sources of Variation	Sum of Squares	Df	F	P
Number of RE periods	Rearing diet	11.892	1	0.085	0.772
	Test diet	8.679	1	0.062	0.804
	Interaction	39.135	1	0.281	0.599
	Error	4870.839	35		
Pauses between meals	Rearing diet	287.82	1	2.461	0.126
	Test diet	82.455	1	0.705	0.407
	Interaction	458.026	1	3.917	0.056
	Error	4093.006	35		
Total duration of RE Periods	Rearing diet	422.401	1	3.315	0.077
	Test diet	28.488	1	0.224	0.639
	Interaction	19.374	1	0.154	0.699
	Error	4459.756	35		

Table III. Statistical results for feeding behavior of sixth-instar spruce budworm when reared and tested on fresh foliage (2-WAY ANOVAs).

Behavior	**Sources of Variation**	**Sum of Squares**	**Df**	**F**	**P**
Number of FB	Rearing diet	171.405	1	1.318	0.259
	Test diet	129.427	1	0.995	0.325
	Interaction	0.227	1	0.002	0.967
	Error	45551.247	35		
Total duration of FB	Rearing diet	31.524	1	0.235	0.631
	Test diet	163.557	1	1.221	0.277
	Interaction	51.47	1	0.384	0.539
	Error	3686.47	35		
Number of meals	Rearing diet	0.457	1	0.075	0.786
	Test diet	0.132	1	0.022	0.884
	Interaction	0.327	1	0.053	0.819
	Error	214.5	35		
Total time	Rearing diet	294.324	1	2.304	0.138
	Test diet	126.097	1	0.987	0.327
	Interaction	35.124	1	0.275	0.603
	Error	4471.8	35		

Chapter 5

General Discussion

The primary purpose of this study was to evaluate the processes of behavioral modification in response to past nutritional experience. The system used as a model for this project involved naturally resistant white spruce trees and the eastern spruce budworm. This work not only evaluated the effect of past experience on behavior but also has important implications for current forest management practices.

This study failed to show a significant difference in the pattern of feeding of larvae with different nutritional experience. Past experience included both individual experience and parental experience. The pattern of feeding was measured in order to determine if insects possessed any physiological or behavioral modifications when faced with a high stress nutrition. This stress comes in the form of resistant trees in this system. The lack of significant difference in pattern of feeding is not supported by previous research with the current system (Daoust *et al.*, 2010) and may be the result of a low replication number. However, with the current sample, this study suggests that the spruce budworm, like other specialists feeders, may not have evolved mechanisms of physiological or behavioral modifications based on previous nutritional stress (Despland and Noseworthy, 2006; Lee *et al.*, 2006). Because budworm are specialist herbivores and are generally restricted to the host tree on which they were laid by their mother, they are not exposed to food sources of varying quality and may not have evolved behavioral mechanisms in dealing with poor quality food.

There was, however a significant difference in oviposition behavior of insects with different nutritional experience. This has yet to be documented in spruce budworm and provides insight into how adult females may be influencing budworm populations. Larvae reared on resistant foliage preferentially lay on susceptible foliage but showed no preference when reared on susceptible foliage. These findings illustrate that the presence of deterrents on the surface of needles, coupled with larval experience and/or parental experience may be influencing adult oviposition preference and in an adaptive manner since resistant trees present a stress on budworm populations.

These findings may prove useful in designing a durable management strategy. Because there exists a high mortality rate for budworm on resistant foliage (Bauce *et al.*, 2006a), plantation strategies using mixed tree phenotypes favoring resistant type trees may be able to slow budworm adaptation and protect plantations by alleviating part of the strong selective pressure placed by resistant trees. High mortality on resistant trees cull budworm populations, lack of physiological or behavioral modification by growing larvae results in a lower the amount of defoliation, and the presence of susceptible trees serving as buffers would continuously mix the budworm population, not allowing adaptation to resistant trees to occur too quickly.

Before a plantation strategy can actually be implemented, further research should be done to confirm these findings. The current study used field reared insects and tested the F1 generation. Past experience included both individual experience and parental experience. Studies evaluating behavior over many (>2) generations and comparing how behavior can change based on parental experience would be greatly informative when comparing to field populations. The effect of parental experience on feeding behavior in insects is also lacking in the current literature. Another spruce budworm behavior that may prove useful in the current system is that of larval dispersal behavior. First and second instar larval dispersal can lead to the spread of outbreaks (Blais, 1952), but could also result in a considerable reduction of population if the budworm disperse to inferior or inadequate food sources (Miller, 1958). This in turn can play an important role in budworm population dynamics. Analyzing different behaviors and evaluating effects of individual experience and parental effects over multiple generations is key to unraveling the coevolutionary history of herbivore-plant interactions and designing durable plantation strategies.

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