Pre-ingestive effects of tannins on the Spruce Budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae)

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Abstract

Pre-ingestive effects of tannins on the Spruce Budworm,
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The spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is considered the most important pest threatening the coniferous forests of eastern North America. Its major hosts are balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), red spruce (*Picea rubens*), and black spruce (*Picea mariana*), all of which contain defensive compounds including tannins. Tannins are known to act as feeding deterrents, digestibility reducers, and toxins in numerous insect species and specifically to reduce growth and survival in the spruce budworm. Using two-choice feeding tests, it was discovered that hydrolysable tannins act as a feeding deterrent and condensed tannins extracted from white spruce act as a phagostimulant for the spruce budworm. To my knowledge, this is the first time condensed tannins have been demonstrated to be phagostimulatory in any insect. It was shown that both types of tannins function via different mechanisms: spruce tannins directly stimulate feeding whereas tannic acid interferes with sucrose detection. A dose-response relationship was demonstrated for both types of tannin. Concentrations of defensive compounds and of tannins are affected by certain forest management techniques. Polar extracts from foliage sampled from 3 different thinning regimes were investigated for their affect on spruce budworm feeding, again using the two-choice feeding tests. Differences between trees appear to be more important than the effects of the thinning treatments but more data are

necessary in order to make any firm conclusions. Tannin concentrations should be considered in forest management aimed at controlling spruce budworm populations.

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To my parents, whose infinite and unconditional love and support have made everything possible. I love you always.

And to Kari:

When we are together, we stay up all night.

When you are gone, I cannot sleep.

Praise God for these two insomnias!

And the difference between them.

-Rumi,

13th century Sufi poet and mystic

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"There are no applied sciences, there are only applications of Science and this is a very different matter. The study of the applications of Science is very easy to anyone who is master of the theory of it." -Louis Pasteur, whose father was a tanner.

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Introduction

Almost half of all insects feed on plants (Triplehorn and Johnson, 2005) and, as the primary herbivores in many ecosystems (Schowalter, 2006), phytophagous insects play an essential role in shaping, altering, and maintaining important ecological processes. Some phytophagous insects, such as the spruce budworm, *Choristoneura fumiferana* (Clemens), feed on economically important plants and we therefore find ourselves in direct competition with these so called pests. One striking aspect of insect-plant interactions is the insect's ability to select a host plant from the myriad choices available and the high degree of food specialization these insects demonstrate (Schoonhoven *et al.*, 1992). Factors such as light, temperature, humidity, and leaf physical characteristics can affect host-plant selection but food plant acceptance and maintenance of feeding is ultimately determined by the chemical features of the plant (Heron, 1965; Schoonhoven and van Loon, 2002; Chapman, 2003).

The plant's chemical composition is considered the most important source of information used by phytophagous insects to determine the suitability of a potential host-plant or food item (Schoonhoven and Van Loon, 2002). Phytophagous insects possess an array of chemosensilla that they use to detect and assess the chemical composition of a potential host-plant prior to ingestion (Chapman, 2003). More specifically, phytophagous caterpillars are endowed with some 118 chemoreceptory cells distributed in sensilla on the maxillae and epipharynx (Schoonhoven and van Loon, 2002). There is evidence for some possible chemoreceptors in the hypopharynx and deeper portions of the buccal cavity but these are mainly involved in swallowing (Schoonhoven and van

Loon, 2002). It is through the chemosensilla that the chemical information is translated into signals that lead to behavioural responses (Simmonds and Blaney, 1990).

The basic structure of the insect taste sensillum is remarkably conserved – in general consisting of a uniporous hair with both chemosensory and mechanosensory cells (Hallem, *et al.,* 2006). Most insects possess receptor cells specialized for primary nutrients such as sugars, amino acids, and water, and another group of cells that is sensitive to a variety of secondary plant substances and is therefore responsible for host plant recognition (Hansen, 1978; Schoonhoven and van Loon, 2002). However, individual gustatory cells may respond to more than one class of compounds (Chapman, 2003). In fact, Chapman (2003) suggests that gustatory sensilla almost always contain both phagostimulatory and phagodeterrent cells. Depending on the insect and the compound, stimulation of a neuron might send positive or negative signals to the brain, resulting in feeding stimulation or inhibition, respectively. It is generally accepted that a strict combination of stimulants and deterrents is important in determining feeding (Jermy, 1961; Dethier, 1980; Schoonhoven and van Loon, 2002; Chapman, 2003). In other words, it is the blend of positive and negative signals received by the insect and interpreted by the central nervous system that ultimately determines the behavioural response.

Schoonhoven and van Loon (2002) point out that our knowledge of coding and of central integration processes in insect chemosensory systems is still very limited. Two main types of sensory coding have been described: a labeled line and an across-fiber pattern (Schoonhoven *et al.*, 1992). In a labeled line, each neuron conveys a specific message that is understood by the central nervous system without additional information

from other neurons. The across-fiber pattern is transmitted by two or more neurons possessing different stimulus spectra and the message from each is synthesized and integrated in the central nervous system and must be read together in order to produce a response. Current evidence suggests that, among others, deterrent cells are coded through labeled lines (van Loon, 1996; Schoonhoven and van Loon, 2002) but it is the across-fiber pattern of firing that determines the considerable subtlety in host-plant selection by phytophagous caterpillars (van Loon, 1996). These coding types are not mutually exclusive and likely operate simultaneously within the same organism (Schoonhoven and van Loon, 2002). Acceptance of a host-plant or food item has been interpreted as the matching of the chemical profile of the plant as encoded by the gustatory receptors with a hypothetical template in the brain of the insect (van Loon, 1996).

A basic assumption of all studies of chemosensory coding is that there exists a simple and direct relationship between stimulation of the sensory neurons and feeding behaviour (Schoonhoven *et al.*, 1992). However, the chemosensory system of an insect may vary in its sensitivity because of a number of factors: age, time of day, feeding history (diet), effect of food deprivation, adaptation rate, and individual variability (Schoonhoven, 1977; Simmonds and Blaney, 1990; van Loon, 1996). In addition to the innate variability of insect chemosensory equipment, the food is chemically complex and the response characteristics of a sensillum to individual compounds alone is not sufficient to enable one to predict the nature of the response to the total plant (Dethier and Kuch, 1971). Some studies have shown that combinations of chemicals can produce unexpected results when compared with responses to individual components

(Schoonhoven *et al.*, 1992; van Loon, 1996; Chapman, 2003). Compounds in mixtures may interact in an inhibitory or synergistic fashion (Dethier, 1980; Chapman, 2003). To further confuse matters, the insect is capable of adapting to noxious compounds in its diet through both pre-ingestive (i.e. gustatory) and post-ingestive mechanisms (e.g. production of detoxication enzymes) (Glendinning *et al.*, 2001; Glendinning, 2002).

It is possible for an insect to become habituated to plant secondary compounds, enabling them to eat food that is initially distasteful (Chapman, 2003). In some insects, rearing of early instars on a compound or plant of interest can produce differences in preferences for that compound or plant as compared to control animals. This process of induction has been demonstrated for several caterpillar species (Chapman, 2003). For example, after being reared on artificial diet containing the deterrents caffeine and salicin, *Manduca sexta* caterpillars readily accept food containing them (Chapman, 2003). There is also some evidence that secondary plant compounds might be involved in associative learning in caterpillars (Chapman, 2003).

Within the phytophagous insects we can define three feeding habits: polyphagous, a generalist feeding on a wide variety of plants; oligophagous, feeding on a limited range of species usually within the same family or genus; and monophagous, a specialist restricting its diet to one or two closely related species. Monophagy and oligophagy most likely developed in conjunction with detoxication mechanisms by turning earlier deterrent agents into phagostimulating key substances for host plant recognition (Feeny, 1975; Hansen, 1978). The idea that a change from polyphagy to oligophagy (or the reverse) involves a general increase (or reduction) in sensitivity to deterrent compounds is supported by behavioural evidence (Chapman, 2003). However, physiological

evidence points to a change within the central nervous system rather than the peripheral receptors as the first step in such a transition (Chapman, 2003). Some insects use host plant-specific compounds as sign or token stimuli. These compounds inform the insect of a potential host-plant and in some cases are required for initiation of feeding (Fraenkel, 1959). This could be achieved by expressing the appropriate receptor on a phagostimulatory cell instead of a deterrent cell (Chapman, 2003).

All phytophagous insects studied so far are capable of detecting some secondary plant compounds, which usually produce a negative effect (Chapman, 2003). Possession of a deterrent cell is likely to be the basal condition in insects that has perhaps become emphasized in phytophagous species (Chapman, 2003). In a number of caterpillars, secondary compounds known to inhibit feeding have been shown to stimulate gustatory receptor cells in the mouthpart sensilla (Chapman, 2003). Feeding deterrent neurons have been reported in a variety of larval lepidopterans including *Bombyx mori*, *Lymantria dispar*, *Operopthera brumata*, *Manduca sexta*, *Pieris brassicae*, *Spodoptera exempta*, and *Spodoptera littoralis* (Schoonhoven *et al.*, 1992). Some authors suggest that the "salt cells" of phytophagous insects should be equated with "deterrent cells" (Bernays and Chapman, 2001), but Dethier (1980) rejects the idea of generalized deterrent receptors. No deterrent cell has yet been identified in the spruce budworm (Dr. P. J. Albert, personal communication). However, certain compounds have been found to act as feeding deterrents for the spruce budworm. Heron (1965) reported a feeding deterrent effect of pungenin, a glucoside. The amino acid l-valine has also been reported to inhibit feeding but is known to stimulate a phagostimulatory cell (Panzuto and Albert, 1998) and Chapman (2003) believes this implies the existence of a deterrent cell. In general, the

fact that a secondary compound is deterrent to an insect does not imply that the insect does not eat plants containing that compound (Chapman, 2003). Usually, insects with more restricted diet breadths (specialists) do not possess deterrent cells that respond to the secondary compounds of their hosts (Chapman, 2003).

In his seminal paper, "The raison d'être of secondary plant substances", Fraenkel (1959) suggested that plant secondary metabolites evolved as protection against insect herbivory and are now responsible for determining host-plant specificity. After hundreds of millions of years of evolution and co-evolution – insect herbivores have been attacking plants at least since the Devonian (~416-359 mya) – plants today produce a bewildering array of secondary compounds which are often unique to specific families, genera, or species, expressing the chemical individuality of their manufacturer (Haslam, 1988). Secondary plant substances influence insect herbivores behaviourally and metabolically (Dethier, 1980) by acting as feeding stimulants or deterrents, affecting nutrient acquisition or producing toxic effects. Secondary plant compounds can affect feeding by stimulating chemosensilla in several ways: directly stimulating neurons (deterrent or stimulant), inhibiting phagostimulant neurons, and distorting the sensory code (Schoonhoven *et al.*, 1992).

Tannins are not the only compounds responsible for feeding aversion to plants but, according to Swain (1979), they appear to be the most important. As Swain (1979) concluded, "the ability to sense the presence of undesirable amounts of tannins in foods has been of significant importance in plant-animal co-evolution."

Tannins

A fundamental property of tannins, which is responsible for their capacity to tan (i.e. convert to leather) animal hides, is their ability to combine with proteins and other polymers such as cellulose or pectin (Ribéreau-Gayon, 1972). The tannins, also referred to as polyphenols, are difficult to define precisely since they form such a large group and include structurally varied compounds. According to Haslam (1999), the simplest, most concise, and widely used definition of vegetable tannins was given by Bate-Smith and Swain in 1962:

> "water-soluble phenolic compounds, have molecular weights between 500 and 3000, and, besides giving the usual phenolic reactions have the special properties such as the ability to precipitate alkaloids, gelatin and other proteins."

Polyphenols are multidentate ligands able to bind simultaneously at more than one point to the protein surface (Haslam, 1988). Differences in tannin and protein structure have been shown to affect the efficiency of tannin-protein binding (Swain, 1979; Haslam, 1989).

The tannins can be further classified into two groups originally proposed by Freudenberg (1920) as the hydrolysable and the condensed tannins (Figure 1) (Nierenstein, 1934). Hydrolysable tannins, so named because they are readily hydrolyzed by acids and enzymes, consist of a carbohydrate core, usually glucose, esterified by gallic acid or one of its derivatives. Condensed tannins are formed by the condensation of hydroxyflavans, e.g. catechins (i.e. flavan-3-ols) or leucoanthocyanins (i.e. flavan-3,4-

diols), and are resistant to hydrolysis. When treated with hydrochloric acid, condensed tannins yield anthocyanidins and are therefore often referred to as proanthocyanidins. One of the chief commercial hydrolysable tannins is tannic acid from plant galls, a mixture of gallic acid, *m*-digallic acid, ellagic acid, pentagalloylglucose, trigallic acid, and several other minor compounds (Ribéreau-Gayon, 1972). The condensed tannins are a mixture of polymers of the flavans mentioned above. The tannins are formed biosynthetically from the products of the shikimic acid pathway (Figure 2) (Swain, 1979).

The tannins are distributed throughout the plant kingdom but are first encountered as widespread and important plant products in the spermatophytes (Nierenstein, 1934; Swain, 1979). The condensed tannins are much more widely distributed in the vascular plants than the hydrolysable tannins which are restricted to the dicotyledons of the angiosperms (Swain, 1979). The gymnosperms contain only condensed tannins (Swain, 1979). Tannins are the fourth most abundant biochemical produced by vascular plants, after cellulose, hemicellulose, and lignin (Kraus *et al.*, 2003) and are found in all parts of the plant, from roots and rhizomes to leaves, shoots, and fruits (Nierenstein, 1934; McKey, 1979). They can be found freely soluble in the plant sap but are more commonly sequestered within vesicles or vacuoles (Nierenstein, 1934). Tannin-containing vacuoles have, for example, been reported in white spruce, *Picea glauca* (Chafe and Durzan, 1973), a common host-plant of the spruce budworm. Because they are widely distributed, complex, and costly (to the plant) to produce, it is believed that tannins play an important role in plant function and evolution.

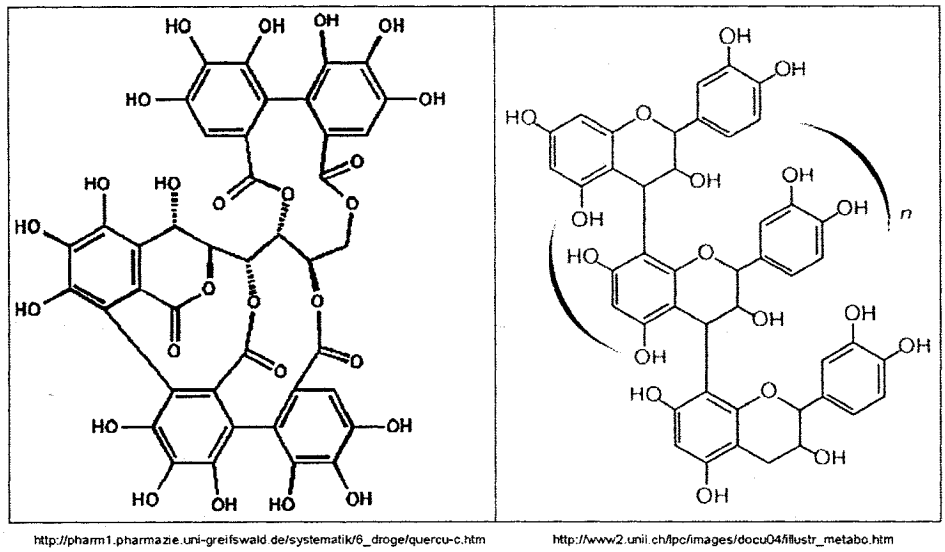

Fig. 1. Left: Example of a hydrolysable tannin, castalagin from oak; Right: Example of a condensed tannin, proanthocyanidin (n=1-30).

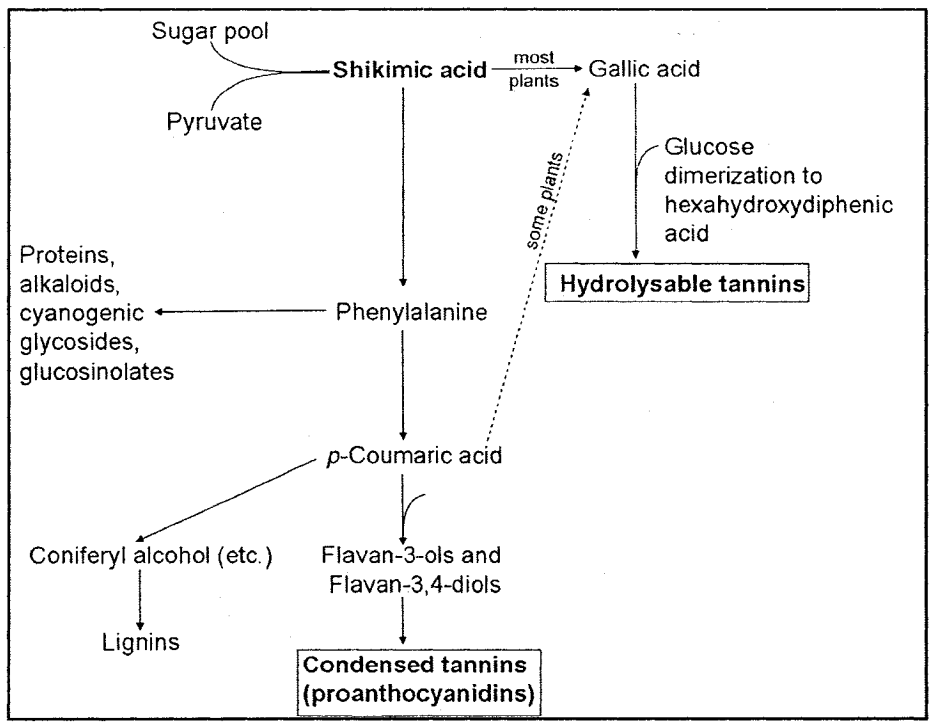

Fig. 2. Biosynthetic origins of the hydrolysable and condensed tannins (after Swain, 1979).

Tannins and insect herbivores

If tannins do indeed serve a function in plant defense the question arises as to their mode of action: how do tannins defend against insect herbivores? Appendix 1 summarizes the tannin-insect herbivore literature. I chose not to include electrophysiological experiments in Appendix 1 for two reasons: first, they offer no new information regarding possible mechanisms of action of tannins and second, they only elucidate possible mechanisms to explain one particular mechanism of tannin action, which serves to further confuse things. Three modes of action have been attributed to tannins: 1) reduction of the digestibility and availability of a limiting nutrient (usually nitrogen and protein) (Feeny, 1970; Nomura and Itioka, 2002), 2) production of overt toxic effects (Steinly and Berenbaum, 1985), and 3) perception as feeding deterrents (Bennett, 1965; Muir *et al.*, 1999). However, some insects appear to have evolved a preference for some hydrolysable tannins rendering these compounds phagostimulatory (Görnitz, 1954; Foss and Rieske, 2003) while others appear to be unaffected by the presence of tannins in their diet (Lawson *et al.*, 1984).

The role of tannins in insect-plant interactions was particularly emphasized in the work of Feeny (1968; 1969; 1970). His studies of the effects of oak (*Quercus robur*) leaf tannins on the winter moth, *Operophthera brumata*, highlighted the negative effects of protein-precipitation by tannins. He showed that tannins chelate nitrogen-bearing molecules, such as enzymes and other proteins, to form indigestible complexes (Feeny 1969). He concluded that insects incapable of catabolizing tannins or preventing chelation suffer gut damage and are unable to assimilate nitrogen from food (Feeny, 1970). He surmised that polyphenols act as quantitative, dosage-dependent barriers, even

to insects that normally feed on leaves containing them. Subsequent studies have supported Feeny's original conclusions that the relevant physiological effects of polyphenols upon herbivores derive from their ability to form complexes with proteinaceous materials (Bate-Smith, 1973; Swain, 1977; Nomura and Itioka, 2002).

Some caterpillars exposed to dietary tannins have been shown to suffer effects similar to those produced by the endotoxin of *Bacillus thuringiensis* and it has therefore been proposed that tannins function as toxins rather than general purpose digestibility reducers (Bernays, 1978; Bernays *et al.* 1980; Steinly and Berenbaum, 1985). Others have suggested that tannins act as deterrents via the contact chemoreceptors, rendering the plant tissue unpalatable rather than indigestible (Bennett, 1965; Schoonhoven and Derksen-Koopers, 1973; Chapman and Bernays, 1977; Muir *et al.*, 1999). These several postulated modes of action of tannins are not mutually exclusive and might be found in various combinations in different insects and for different tannins.

As mentioned above, some authors have reported a possible phagostimulant role for tannins. One of the first detailed examinations of the effects of tannins on insect feeding was performed by Görnitz (1954) who sites the earlier works of Lagerheim (1900) and Grevillius (1905) as preliminary investigations that demonstrated that polyphagous caterpillars are "tannin specialists that prefer plants with high tannin substance" and that tannins can be recognized by the caterpillar by "some sort of receptor system". Görnitz showed that some caterpillars could be induced to feed, in some cases vigorously, on tannin-treated plants outside their host range and that this effect was dose-dependent (Görnitz, 1954). However, he also concludes that this tendency does not hold true for all caterpillars.

At least four mechanisms have been proposed by which insects can counteract the effects of tannins. Alkaline gut pH (Berenbaum, 1980), adsorption onto the peritrophic membrane (Bernays, 1978), gut surfactants (Martin and Martin, 1984; Martin *et al.*, 1985), and digestive enzymes (Bernays and Woodhead, 1982) act to reduce or prevent the deleterious consequences of feeding on a tannin-containing plant. More recently, gut antioxidants have been added to the array of insect anti-tannin defense mechanisms (Barbehenn *et al.*, 2003).

The literature dealing with tannin-insect herbivore interactions is vast, often controversial, and confusing. The results presented in Appendix 1 are necessarily generalized and simplified; specific details have been omitted in order to clarify the main issues and focus attention on the overall situation. The table represents a century of research, including nearly 40 separate publications, covering over 50 species in 20 different families and 5 orders. However, the great variety of insects studied, tannins used, concentrations tested, and experimental designs employed makes it very difficult to extract any meaningful inferences or overarching themes. I can draw two simple conclusions from the extensive research published on the subject of tannins and insect herbivores: 1) that the overwhelming structural diversity of the tannins reflects qualitative and quantitative functional differences with regards to their deterrent, toxic, and digestibility reducing effects, and 2) that the incredible diversity of phytophagous insects and their myriad feeding habits have resulted in an unpredictable array of adaptations and counter-adaptations to specific host-tannins as well as to general polyphenolic compounds. Therefore, the only way to assess with certainty the effect of a given tannin on a particular insect is to test that specific tannin on that specific insect.

The spruce budworm, *Choristoneura fumiferana*

The spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) is widespread throughout eastern North America, including the eastern States from Virginia to Minnesota and all the forested regions of Canada from Newfoundland to Alberta and the southern parts of the Yukon and North West Territories (Prebble, 1975). In most instances the spruce budworm can be described as uncommon and even as a rare species (Morris, 1963). Endemic levels of spruce budworm can be less than 5 larvae per tree (Morris, 1963). However, in an outbreak year the population can swell to as much as 1000 times the minimum level and the budworm can devour all the new needles produced by a forest of balsam fir and ultimately kill 80% of the mature trees in the forest (Morris, 1963). This incredible destructive capacity has made the spruce budworm one of the most important threats to our softwood forests and forestry industry. A better understanding of spruce budworm biology is definitely an asset when designing and implementing strategies to help control their populations.

Miller (1963) gives an account of the life cycle of the spruce budworm (Figure 3). The eastern spruce budworm completes one generation per year. After overwintering in diapause, the second instar larva emerges from its silken hibernaculum between April and May and begins to feed and grow. Second instars will eat staminate cones, current-years' needles or mine into previous-years' needles. Larvae molt through six instars before pupation and finally emerge as adult moths around July. The adult moths are vehicles for dispersal and reproduction but do not feed with their severely reduced mouthparts. Toward the end of July, batches of eggs are laid on the underside of needles and hatch within days to weeks. The emerging first instar larvae do not to feed but immediately

seek out suitable shelter to spin a hibernaculum and prepare for winter diapause. Dispersal occurs at three separate stages during the life of the spruce budworm: first instar, second instar, and adult moth. First and second instar larvae can drop from their host tree on a thread of silk to be dispersed by the wind. Although winged adults can fly, long distance dispersal is usually assisted by the wind as well. The larger fifth and sixth instars are responsible for the most intense feeding and therefore for most of the defoliation. Sixth instars usually confine their feeding to newly developed shoots except in cases of severe infestation.

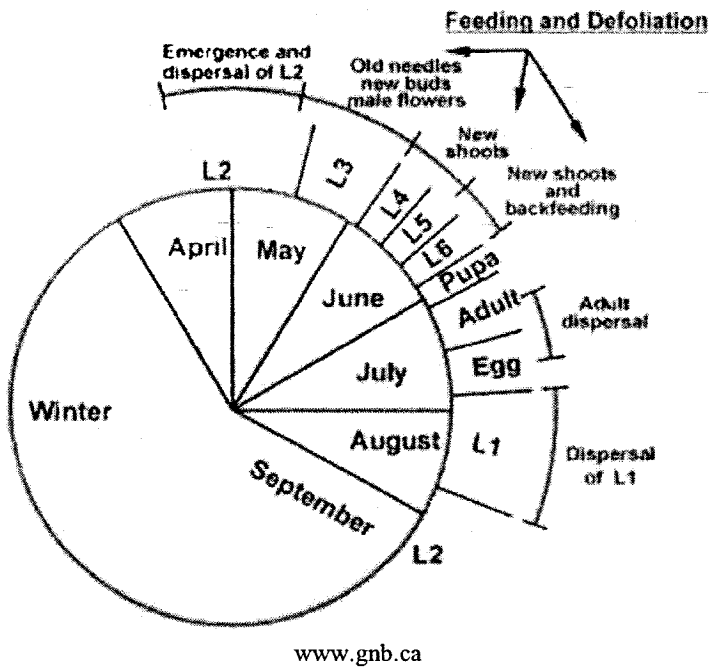

Fig. 3. Life cycle of the spruce budworm, *Choristoneura fumiferana* (Clemens).

The spruce budworm is an oligophagous species which feeds on white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), and black spruce

(*Picea mariana*). Emergence of second instar larvae from their hibernacula corresponds with budbreak of balsam fir and white spruce. The lower number of early instar larvae on red and black spruce most likely reflects differences in phenology (Albert, 1982). Phenology is important because it affects the availability of early spring feeding sites for the second instar larvae and also the levels of nutrients and allelochemicals (Mattson *et al.*, 1991; Nealis and Régnière, 2004).

The polar extracts from its four major host trees are important in determining the feeding behaviour of the spruce budworm; the sugar/glycoside fraction was shown to be most stimulating (Albert and Jerrett, 1981; Albert, 1982). Heron (1965) found that sugars and the amino acid L-proline stimulated feeding by the spruce budworm. Shikimic acid and caffeic acid each stimulated feeding when present in mixture with sucrose. Pungenin, a glucoside, deterred feeding. Sucrose was the most stimulating sugar of those tested and larvae were most stimulated at a concentration of 25 mM which corresponds well with concentrations normally found in the host plant (Albert *et al.*, 1982). Amino acids were less stimulatory than sugars (Albert, 1982) but when mixed produced a positive synergistic effect on larval feeding (Albert and Parisella, 1985b). Organic acids had either no effect or were deterrent (Albert, 1982).

The chemical composition of plants, including concentrations and distributions of primary and secondary compounds, changes between and within individuals. The effects of tree species (Albert, 1982; Mattson *et al.*, 1991), age (Bauce *et al.*, 1994), nutrient levels (Little, 1970; Mattson *et al.*, 1991), defensive compounds (Mattson *et al.*, 1991), inducible defenses (Mattson *et al.*, 1991), stress (Mattson *et al.*, 1991), seasonal changes (Little, 1970), moisture content (Little, 1970), crown level (Carisey and Bauce, 1997),

and anthropogenic impacts (Bauce, 1996; Lamontagne *et al.*, 2002) on the nutritional ecology of the spruce budworm have all been explored at the chemical level for some of its host trees. In terms of tannins, Kirchhoff (1915) found that the quantity of tannins in conifers always increased with the age of the needle (Nierenstein, 1934). Young balsam fir (30 years old) was found to contain a lower nitrogen:tannin ratio and a higher concentration of monoterpenes compared with old balsam fir (70 years old) (Bauce *et al.*, 1994). These differences are directly related to increased mortality, decreased pupal weight, and longer larval development of the spruce budworm (Bauce *et al.*, 1994).

Ecological examination of the budworm-forest system leads to the conclusion that free from the influences of man, it possesses self-regulating, stabilizing mechanisms (Baskerville, 1975). Many entomologists and foresters have suggested that the damage caused by the spruce budworm was largely due to the disruption of natural ecosystems by forestry practices and could therefore be significantly reduced through appropriate forest management and sylvicultural practices (Miller and Rusnock, 1993). One year after a selective cut, the remaining trees experience a decrease in concentrations of defensive compounds such as monoterpenes and tannins (Bauce, 1995). However, several years after the treatment (i.e. 2 or 3 years) the trees react by increasing the production of foliage which gradually compensates for any prior positive effects to the insect and eventually results in increased resistance (Bauce *et al.*, 2001). It has recently been shown that tannins (specifically white spruce condensed tannins), at levels found in thinned spruce stands, negatively affect the growth and survival of the spruce budworm, *C. fumiferana*, and that the decreased levels of tannins observed in white spruce during precommercial thinning likely translate into positive effects on the spruce budworm (Kumbaşli, 2005).

In his research, Kumbaşli addressed the post-ingestive effects of tannins on the spruce budworm and found that the spruce tannins negatively affected the insect's biological performance (Kumbaşli, 2005).

In the present study, I will investigate the pre-ingestive chemoreceptive responses of the spruce budworm to tannins using behavioural techniques and examine whether these plant secondary compounds play a role in host-plant preferences in a selectively-cut, managed forest. In other words, can the spruce budworm 'taste' (i.e. detect) tannins in its host-plant and, if so, how does it respond? And, are tannins responsible for differences in host-plant selection in selectively cut forests? Furthermore, what are the effects, if any, of rearing the spruce budworm larvae on diets containing various concentrations of tannin? I suspect that tannins, both hydrolysable and condensed, can be detected by the insect and will most likely act as feeding deterrents, perhaps with a dose-response relationship. Additionally, I believe that lower tannin concentrations in its host-plant due to forest management techniques will translate into increased feeding by the spruce budworm, besides increased growth and survival as already shown by Kumbaşli (2005).

Materials and Methods

Insect rearing

Insects were received as emerging second instar larvae from the Forest Pest Management Institute, Sault Ste Marie, Ontario. Animals were reared according to the methods of Grisdale (1970) and Wilson and Grisdale (1988). Hotpack (Waterloo, Ont.) incubators provided a controlled environment with an L16:D8 photoperiod and an ambient temperature of 22°C and 60% humidity. Second instar larvae were placed in Winpack Portion Packaging 23 ml creamer cups prepared by filling them approximately 1/3 with diet (McMorran, 1965; Wilson and Grisdale, 1988) and covered with Stanpac waxless cardboard creamer covers. In the 'Two-choice feeding test with spruce tannins and tannic acid' diets containing tannic acid were used as explained below. The creamers were placed upside down so that the diet is at the top, since larvae are positively phototactic (Wellington, 1948). After 2 weeks, when most larvae reached at least the fourth instar, insects were transferred from the creamers to Petri dishes containing diet, where they remained for the rest of their development. Sixth instar larvae were used in all experiments.

Tannin and tannic acid

Tannic acid was purchased from Sigma-Aldrich Chemical Company (A.C.S. reagent, 403040-500g, Batch #: 06723ED). Spruce tannins were supplied by the laboratory of Éric Bauce (Université Laval) and were extracted and purified from white spruce, *Picea glauca*, foliage following the methods described by Hagerman and Butler (1980) and Hagerman (1988). A more detailed description of the extraction and purification procedures is found in Appendix 2. The spruce tannins obtained in this way

are 97-98% pure and any impurities are proteins (Hagerman and Butler, 1980; Hagerman, 1988). Preliminary analysis of the extracted spruce tannins revealed that the mixture comprises over 350 types of tannins and that the condensed tannins represent 95% of the total mixture in leucocyanidin equivalents (Éric Bauce, personal communication).

Two-choice feeding tests with spruce tannins and tannic acid

Animals used for the two-choice feeding tests were reared, as described above, from emergence of the second instar larvae on either normal (tannin-free) diet (McMorran 1965; Wilson and Grisdale, 1988) or diet containing various concentrations of tannic acid: 8% or 15% dry weight. A diet containing 30% dry weight tannic acid was attempted but the tannins failed to mix properly at that concentration. It was not possible to prepare diet containing spruce-tannins due to severely limited supplies. Tannic acid-containing diet was prepared by first weighing out the required amounts of dry components (e.g. sucrose, wheat germ, etc.) and then adding an appropriate amount of tannic acid to arrive at the desired concentration. Therefore, the relative amounts of all other components (e.g. sugars, proteins, etc.) remained constant while only the tannin concentration varied.

All tannin and tannic acid solutions were prepared in 25 mM sucrose unless otherwise indicated as 'alone' in which case they were prepared in distilled water. Concentrations are expressed as percent dry weight to be consistent with the literature. A concentration of 0.75% corresponds to 1.88 mg/ml, that of 8% to 0.020 g/ml, 15% to 0.0375 g/ml, and 30% to 0.075 g/ml. A 30% solution of spruce tannins was not used because the tannins do not completely dissolve at that concentration. The concentration

normally found in the tree is around 8% dry weight (Kumbaşli, 2005). Table 1 summarizes the combinations of diet, control solution, and test solution used during the two-choice feeding tests.

Table 1. Combinations of diet, control solution, and test solution used in two-choice feeding tests. TA: tannic acid; ST: spruce tannins.

Diet	Control solution	Test solution
Normal (0%)	25 mM sucrose	8% TA alone 8% ST alone
	dH_2O	8% TA alone 8% ST alone
	25 mM sucrose	25 mM sucrose 0.75% ST 8% ST 15% ST 0.75% TA 8% TA 15% TA 30% TA
8% TA diet		0.75% ST 8% ST 15% ST 0.75% TA 8% TA 15% TA 30% TA
15% TA diet		0.75% ST 8% ST 15% ST 0.75% TA 8% TA 15% TA 30% TA

Discs were punched, using a regular hole-puncher, from cellulose nitrate filter discs (Sartorius 0.45 µm millipore cellulose nitrate filter paper) to obtain discs with a surface area of 31.74 ± 0.05 mm^2. The discs were pinned with stainless steel 2.5 cm sewing pins approximately 5 mm above the floor of a Styrofoam sheet (Cascade 12S) and arranged in a circle of 21 mm diameter (Figure 4). Each behaviour arena containing 8 discs was covered by a 3.5 cm diameter by 1 cm high Petri dish (Figure 4). The discs were wetted with a 15 µl aliquot of the appropriate solution (Table 1) alternating between control and test discs in an A-B-A-B fashion (Figure 4). Coloured pins were used to distinguish between solutions (i.e. red=test, yellow=control). Colours were alternated between control and test solutions to control for possible colour preference.

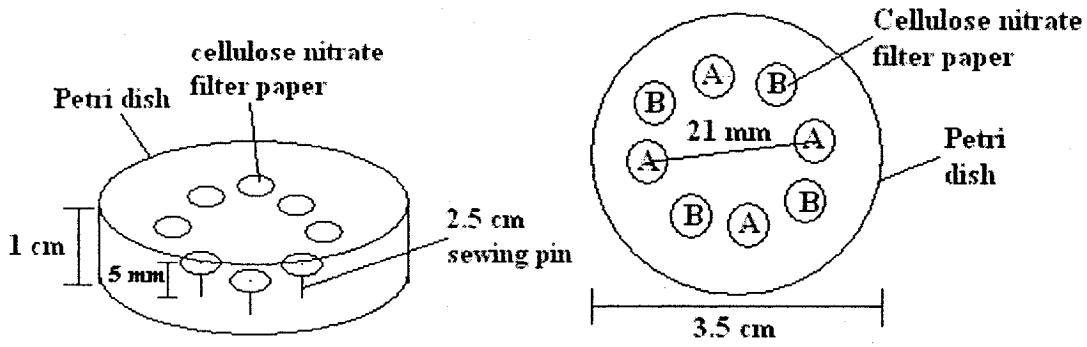

Fig. 4. Experimental set-up of behaviour arenas for two-choice feeding tests. *Left*: side-view, *Right*: view from above.

Larvae were starved individually for 24 h prior to being individually placed into a behaviour arena. Tests ran for 24 h. In most cases, a minimum of n=20 insects were tested for each experiment. Animals that ate more than 375% or less than 25% of either the control or test discs respectively (i.e. 1 disc=100%) were discarded along with

animals that pupated or died during the experiment. Approximately 5% of test animals were discarded for the abovementioned reasons. After completion of the test, the remains of the discs were removed, glued to a black background and photographed, along with a ruler for scale. The area of each disc was calculated using ImageJ 1.36b software (Wayne Rasband, National Institutes of Health, USA). Mean percent consumption of control and test discs was calculated by dividing the amount of control or test discs eaten by the total amount of discs consumed (Figure 5). Feeding rate was calculated by dividing the total amount of discs consumed by the duration of the test (i.e. 24 h) (Figure 5). The values obtained for mean percent consumption were arcsine transformed prior to analysis, as prescribed by Sokal and Rohlf (1995) for percent data. Normality was assessed by the Kolmogorov–Smirnov test. A paired Student's t-test or Wilcoxon's signed rank test was initially performed on all sets of mean percent consumption data for parametric or non-parametric data respectively. Two-way analysis of variance (2-way ANOVA) was performed with diet and concentration as treatments, once for tests with spruce tannins and once for tests with tannic acid. Feeding rates were similarly analyzed by 2-way ANOVA but not arcsine transformed. Linear regression was performed on arcsine transformed mean percent consumption data for test discs for each of the six diet-tannin/tannic acid combinations (3 diets × 2 types of tannin = 6 regressions).

$$MPCT = \frac{CT}{CC + CT} \times 100$$

$$MPCC = \frac{CC}{CC + CT} \times 100$$

$$FR = \frac{CT + CC}{24\ h}$$

Where

MPCT = mean percent consumption of test discs
MPCC = mean percent consumption of control discs
CT = consumption of test discs
CC = consumption of control discs
FR = feeding rate

Fig. 5. Formulae used to calculate mean percent consumption and feeding rate.

During the two-choice feeding tests with larvae reared on normal (i.e. 0%) and 8% tannic acid diet, larvae were sexed by presence or absence of testes which appear as two dark kidney-shaped spots visible in the posterior abdomen of males. Data were analyzed by three-way ANOVA, adding sex as a treatment along with diet and tannin concentration as above. Since it was found that sex had no significant effect on larval preference or feeding rate (Table 2), sex was not recorded for tests with larvae reared on 15% tannic acid diet.

Caterpillar weighing

To determine the effects of different diets on larval weight, n=20 sixth instar caterpillars reared respectively on each of the 3 different diets (i.e. 0% tannic acid diet, 8% tannic acid diet, and 15% tannic acid diet) were dried in an oven at 75°C until the weight of the larvae was constant (approximately 72 h). Larvae were individually weighed. Average weights of the larvae reared on the different diets were compared by ANOVA.

Table 2. Sex has no significant effect on larval preference (MPCT: mean percent consumption test) or feeding rate (FR) (α=0.05). Although p=0.05 for MPCT on tannic acid, a *post hoc* Bonferroni correction indicated no significant difference between male and female consumption. A significant interaction between concentration, diet, and sex was detected for FR tested with tannic acid. However, since no other significant interactions were found with sex there is likely no biological significance to this result.

Compound	Test factor	Treatment	df	F	p
Spruce tannin	MPCT	A: Diet	1	12.19	<0.001
		B: Conc.	2	0.32	0.72
		A×B	2	7.46	<0.001
		C: Sex	**1**	**0.15**	**0.70**
		A×C	1	0.00	0.97
		B×C	2	0.97	0.38
		A×B×C	2	1.02	0.36
	FR	A: Diet	1	8.67	0.004
		B: Conc.	2	10.48	<0.001
		A×B	2	6.54	0.002
		C: Sex	**1**	**1.94**	**0.17**
		A×C	1	1.84	0.18
		B×C	2	0.49	0.61
		A×B×C	2	0.47	0.63
Tannic acid	MPCT	A: Diet	1	2.58	0.11
		B: Conc.	3	14.32	<0.001
		A×B	3	0.36	0.78
		C: Sex	**1**	**3.97**	**0.05**
		A×C	1	0.33	0.57
		B×C	3	0.14	0.93
		A×B×C	3	0.60	0.61
	FR	A: Diet	1	1.91	0.17
		B: Conc.	3	2.15	0.09
		A×B	3	0.09	0.97
		C: Sex	**1**	**0.11**	**0.74**
		A×C	1	1.03	0.31
		B×C	3	0.26	0.85
		A×B×C	3	2.96	0.03

Foliar extracts

Foliage of white spruce (*P. glauca*) and balsam fir (*Abies balsamea*) from three different thinning regimes (Table 3) were obtained from the experimental Montmorency forest of the Université Laval and were supplied by Dr. Éric Bauce (Université Laval). All samples were collected from sites with mesic drainage. Foliage was immediately frozen upon collection and later freeze-dried for long-term storage. All samples were collected in July 2004, when sixth instar spruce budworm were active. For detailed descriptions of sites and thinning regimes refer to Kumbaşli (2005).

Table 3. Treatment regimes for white spruce and balsam fir used for extractions.

Species	Selective Cutting*	Soil Drainage
White spruce	0% thinning (control) 25% thinning 40% thinning	Mesic
Balsam fir	0% thinning (control) 25% thinning 40% thinning	

*2 replicates of each thinning regime were collected.

Extraction and separation of polar and non-polar compounds from foliage of white spruce and balsam fir

The extractions were performed according to the technique proposed by Dr. Éric Bauce (personal communication). Freeze-dried foliage was first manually crushed with a mortar and pestle and extracted with a 12 methanol: 5 chloroform: 3 water (v:v:v) solvent. 5 ml of solvent were added to 0.2 g of foliage powder and mixed thoroughly.

The solution was then centrifuged (IEC CENTRA CL2, Fischer Scientific) at 3500 rpm at ambient temperature for 5 min. The supernatant was recovered in a second tube and the sediment was resuspended in another 5 ml of solvent. The resuspended solution was then mixed and centrifuged. The supernatant was once again recuperated and the sediment resuspended in 5 ml of solvent. This resuspended sediment was mixed and centrifuged one last time. The remaining sediment (white pellet) was discarded. 5.5 ml of distilled water were added to the supernatant in order to separate the polar (on top: soluble amino acids, soluble sugars, phenols, tannins) and non-polar (on bottom: lipids, terpenes) phases. The mixture was centrifuged and the two phases isolated by pipetting off the polar phase. The water was evaporated from the polar phase by roto-evaporation (Büchi Rotovapor Collegiate, Brinkman, an Eppindorf company).

The extracts were redissolved in an appropriate amount of distilled water to return all test solutions to the original foliar concentrations, considering that the foliage is ~80% water (Albert and Parisella, 1988).

Two-choice feeding tests with foliar extracts

The two-choice feeding tests proceeded as described above with substitution of the spruce tannin and tannic acid solutions for solutions of foliar extract (Table 4). Mean percent consumption data were arcsine transformed as described above and analyzed by Student's t-test for parametric data or Wilcoxon's signed rank test for non-parametric data.

Table 4. Foliar extract solutions for two-choice feeding tests. All tests were performed n=2 times for white spruce and balsam fir, respectively, each time with foliage from a different tree.

Test Solution #1	Test Solution #2
0% thinning	25% thinning
0% thinning	40% thinning
25% thinning	40% thinning

All statistical analyses were performed using NCSS 97 (©1996 by Jerry Hinze) and reported as significant at α=0.05.

Results

Two-choice feeding tests with spruce tannins and tannic acid

In the first series of experiments, TA (tannic acid) and ST (spruce tannins) were dissolved alone (i.e. no added sucrose) in distilled water and tested against either water or a 25 mM sucrose solution (Figure 6). When 8% TA alone was tested against water, larvae did not differ significantly in the amount they consumed (t-test; $t=1.376$; $p=0.19$). The same solution tested against 25 mM sucrose yielded a preference for sucrose of roughly 70% (Wilcoxon signed rank test; $Z=4.1069$; $p<0.001$). The 8% ST solution was preferred to water (t-test; $t=-3.374$; $p=0.003$) but produced no significant difference in consumption when tested against the 25 mM sucrose solution (t-test; $t=-0.224$; $p=0.82$). In all cases, the feeding rate was low (Table 5), about half that of control insects reared on normal diet and tested with 25 mM sucrose (Table 6); around 2 mm^2/h compared to 4 mm^2/h.

Table 5. Feeding rates and standard errors (SE) for insects reared on 0% TA diet and tested with solutions of TA or ST alone (i.e. in distilled water).

Diet	Control Solution	Test Solution	Feeding rate (mm^2/h)	±SE
0% TA(normal)	Sucrose	8% TA alone	2.3	0.3
		8% ST alone	2.6	0.4
	Distilled water	8% TA alone	1.6	0.1
		8% ST alone	2.5	0.4

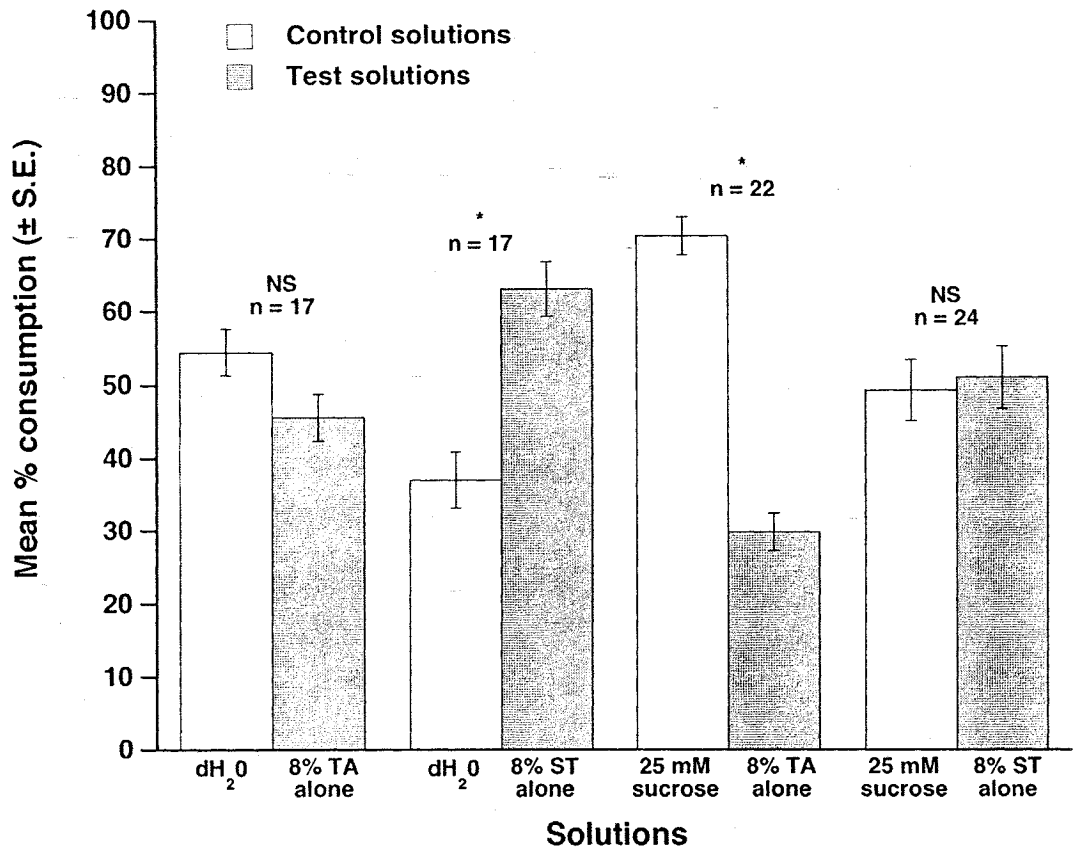

Fig. 6. Mean percent consumption (±SE) for insects reared on 0% TA (normal) diet and tested with solutions of tannic acid (TA) or spruce tannin (ST) alone (i.e. in distilled water). n=sample size; * = $p<0.05$; NS = $p>0.05$.

Table 6. Mean feeding rates and standard errors for insects reared on different diets and tested with TA or ST.

Diet	Control solution	Test solution	Mean feeding rate (mm^2/h)	±SE
0% TA diet	25 mM sucrose	25 mM sucrose	4.3	0.3
		0.75% ST	3.9	0.3
		8% ST	4.4	0.5
		15% ST	4.7	0.5
		0.75% TA	3.7	0.3
		8% TA	3.2	0.3
		15% TA	3.5	0.4
		30% TA	2.5	0.3
8% TA diet		25 mM sucrose	3.9	0.3
		0.75% ST	4.0	0.5
		8% ST	3.7	0.4
		15% ST	2.0	0.3
		0.75% TA	4.1	0.3
		8% TA	3.8	0.3
		15% TA	3.6	0.4
		30% TA	3.1	0.4
15% TA diet		25 mM sucrose	2.9	0.3
		0.75% ST	1.8	0.2
		8% ST	2.7	0.3
		15% ST	2.9	0.4
		0.75% TA	2.1	0.4
		8% TA	1.9	0.2
		15% TA	2.0	0.2
		30% TA	1.4	0.2

In the second series of experiments, various concentrations of TA and ST were prepared in a solution of 25 mM sucrose. In other words, the test solution always consisted of a mixture of a certain concentration of either TA or ST with 25 mM sucrose. A solution of 25 mM sucrose was always used as the control solution. These solutions were tested on insects reared on either 0% TA diet, 8% TA diet or 15% TA diet. A dose-

response trend is apparent in the graphs of MPC for tests with TA (Figure 7) and ST (Figure 8). In every case, a significant effect was detected above the 0.75% concentration (Tables 7 and 8). The effect of tannic acid was always to deter while spruce tannin stimulated feeding. The 2-way ANOVA confirms that there is a significant effect of concentration on MPC in both cases (Table 9). Insects respond similarly to the 0% and 0.75% concentrations of both types of tannin (i.e. there is no significant difference between MPC for test and control discs) (Bonferroni multiple comparison). Insects ate more of the test discs wetted with the higher concentrations of ST (8% and 15%) and less of the test discs with higher concentrations of TA (8%, 15%, and 30%) (Bonferroni multiple comparison). The linear regressions support the dose-response relationships and indicate that tannin concentration accounts for about 20% to 30% of the variance in the data (Table 10). Although the correlation coefficients (R^2) improve slightly with increasing order of the equation, the number of points used to determine the equations is too few to conclude the precise nature of the relation. The first order equations summarize the results clearly and simply. As TA concentrations increase, MPC of the test discs decreases (negative slope) and as ST concentrations increase, mean percent consumption of test discs increases (positive slope) (Table 10).

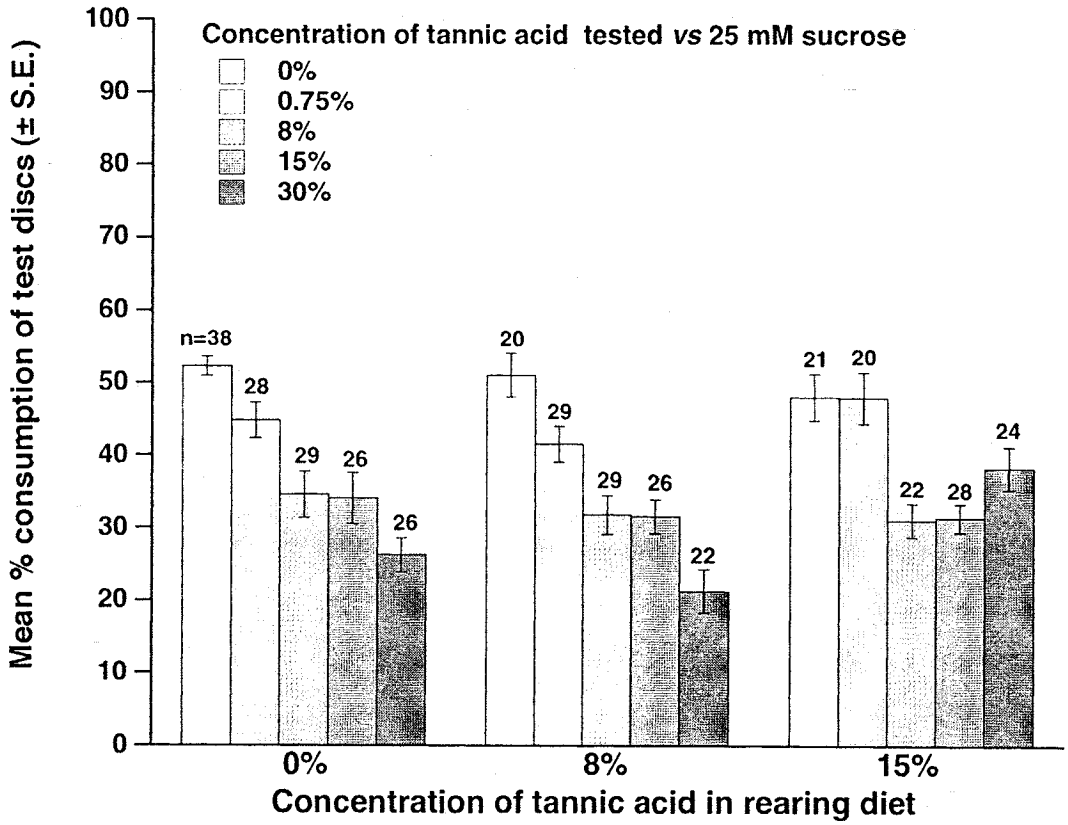

Fig. 7. Mean percent consumption (±S.E.) for insects reared on 0% TA (normal), 8% TA, and 15% TA diet and tested with solutions of tannic acid (TA) dissolved in 25 mM sucrose. n=sample size.

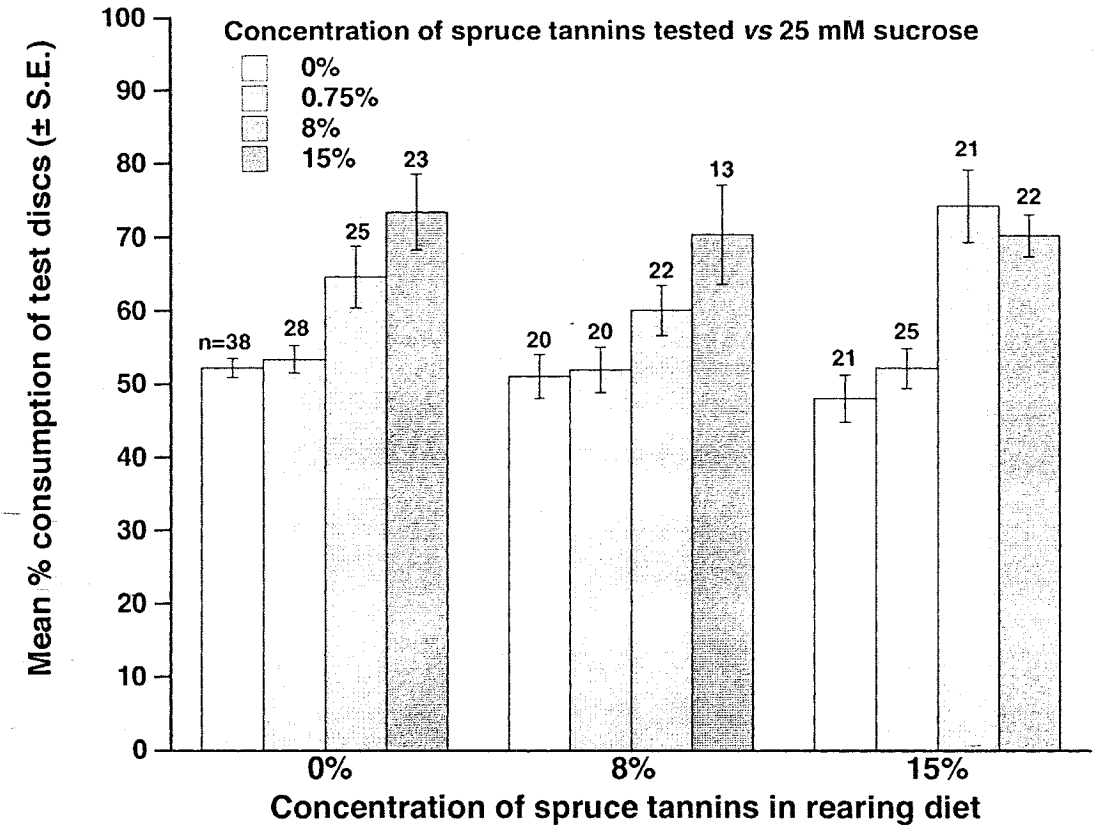

Fig. 8. Mean percent consumption (±S.E.) for insects reared on 0% TA (normal), 8% TA, and 15% TA diet and tested with solutions of spruce tannin (ST) dissolved in 25 mM sucrose. n=sample size.

Table 7. Pairwise comparisons of MPC of control and test discs tested with TA. α=0.05.

Diet	Concentration	Test statistic*	P
0% TA	0%	t=-1.71	0.10
	0.75%	t=2.19	0.04
	8%	t=4.82	<0.001
	15%	t=4.48	<0.001
	30%	t=9.84	<0.001
8% TA	0%	t=-1.66	0.32
	0.75%	t=3.43	0.002
	8%	t=6.88	<0.001
	15%	t=7.85	<0.001
	30%	Z=4.01	<0.001
15% TA	0%	t=1.33	0.27
	0.75%	Z=1.08	0.28
	8%	t=8.15	<0.001
	15%	t=9.65	<0.001
	30%	t=3.99	<0.001

*t for Student's t-test and Z for Wilcoxon's signed rank test.

Table 8. Pairwise comparisons of MPC of control and test discs tested with ST. α=0.05.

Diet	Concentration	Test statistic*	P
0% TA	0%	t=-1.71	0.10
	0.75%	t=1.82	0.08
	8%	t=-3.51	0.002
	15%	t=-4.55	<0.001
8% TA	0%	t=-1.66	0.32
	0.75%	t=-0.62	0.55
	8%	t=-2.90	0.009
	15%	t=-3.42	0.005
15% TA	0%	t=1.33	0.27
	0.75%	t=-0.77	0.45
	8%	Z=3.25	0.001
	15%	t=-7.17	<0.001

*t for Student's t-test and Z for Wilcoxon's signed rank test.

Table 9. 2-way ANOVA results for mean percent consumption on test discs of insects reared on different diets and tested with TA or ST.

Test compound	Factor	df	F	P
TA	Diet	2	4.27	0.01
	Concentration	4	31.84	<0.0001
	Interaction	8	2.37	0.01
ST	Diet	2	9.80	<0.0001
	Concentration	3	30.53	<0.0001
	Interaction	6	4.33	<0.0001

Table 10. Linear regressions for MPC on test discs. y = mean percent consumption of test discs; x = concentration of TA or ST.

Compound Tested	Diet	Equation	R^2
TA	0% TA	y = -0.171x + 0.795	0.305
	8% TA	y = -0.137x + 0.714	0.206
	15% TA	y = -0.113x + 0.761	0.145
ST	0% TA	y = 0.177x + 0.773	0.294
	8% TA	y = 0.209x + 0.726	0.223
	15% TA	y = 0.208x + 0.789	0.242

The effect of diet on disc selection is not as obvious as the effect of tannin concentration but is revealed by the 2-way ANOVA (Table 6) and is discernible from the graphs (Figures 7 and 8). The *post hoc* Bonferroni correction determined that the MPC on test discs was slightly higher on the 15% TA diet as compared to the 8% TA diet when tested with solutions of ST (Figure 8). When tested with TA solutions, the MPC of test discs was slightly lower on the 8% TA diet compared with the normal (0% TA) diet

(Figure 7). A significant interaction between diet and concentration was found for both types of tannin (2-way ANOVA; Table 7).

Feeding rate was significantly affected by diet and concentration and a significant interaction was found for both types of tannin (Tables 6 and 11). A *post hoc* Bonferonni correction showed that, for tests with TA, the 15% TA diet is significantly different than the other two and that the 30% TA solution is significantly different than all the other concentrations (Table 6). For tests with ST it revealed that all diets differed significantly from each other; feeding rate decreased with increasing tannic acid content of the diet. Mean feeding rate of insects reared on 8% TA diet and tested with 15% ST was significantly reduced as well.

Table 11. 2-way ANOVA results for feeding rates for insects reared on different diets and tested with TA or ST.

Test compound	Factor	df	F	P
TA	Diet	2	37.76	<0.001
	Concentration	4	17.16	<0.001
	Interaction	8	2.61	0.0085
ST	Diet	2	31.07	<0.001
	Concentration	3	10.67	0.018
	Interaction	6	4.98	<0.001

Caterpillar weighing

Caterpillars reared on 0% TA (normal) had a mean weight of 0.0089 ± 0.0005 g, on 8% TA of 0.0031 ± 0.0005 g, and on 15% TA diet of 0.0022 ± 0.0005 g. An ANOVA indicated that there is a significant difference between treatments (diets) (df=2; F=48.52;

p<0.0001) and a *post hoc* Bonferroni correction revealed that the 0% diet differed significantly from both the 8% and 15% diets which, however, did not differ significantly from each other.

Foliar extracts

Feeding rates for insects tested with white spruce and balsam fir foliar extracts did not differ significantly from each other or from those of control insects tested with a 25 mM sucrose solution (ANOVA, df=12, F=17.24, p=0.65) and were around 4.5 ± 0.4 mm^2/h. No trend is visible from the graphs of MPC for insects tested with balsam fir or white spruce foliar extracts (Figures 9 and 10, respectively). The direction of preference and the significance of the results vary unpredictably between tests and trees (Table 12).

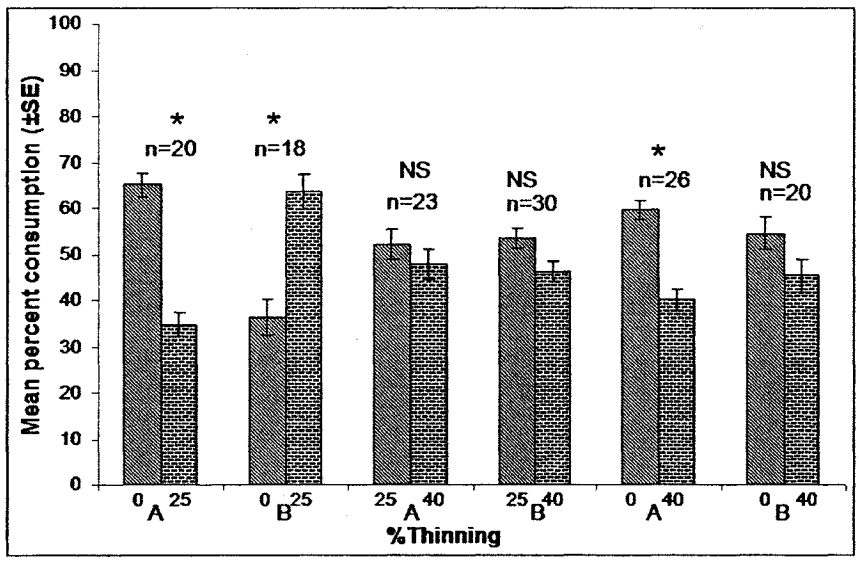

Figure 9. Mean percent consumption (±SE) for insects tested with balsam fir extracts from different thinning regimes. 0=0% thinning (i.e. control stand), 25=25% thinning, and 40=40% thinning; n=sample size; * = p<0.05; NS = p>0.05; A=tree 1; B=tree 2.

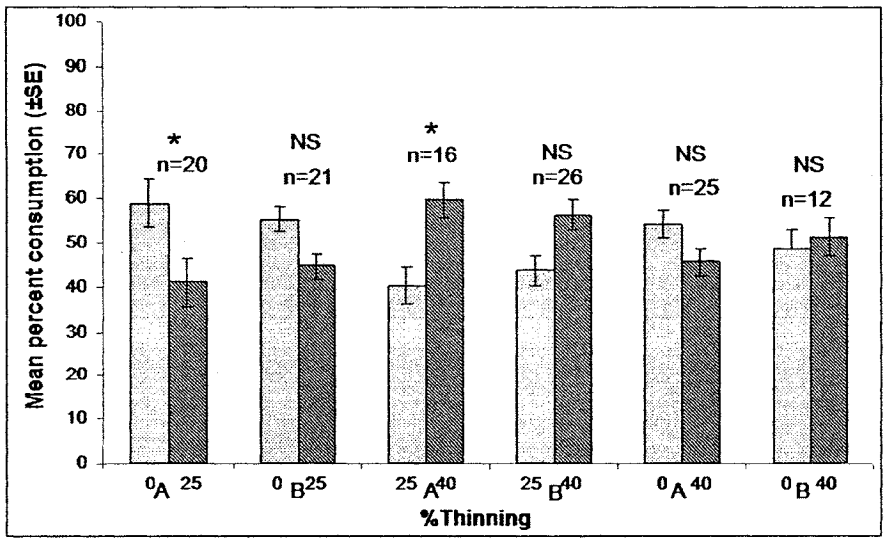

Figure 10. Mean percent consumption (±SE) for insects tested with white spruce extracts from different thinning regimes. 0=0% thinning (i.e. control stand), 25=25% thinning, and 40=40% thinning; n=sample size; * = $p<0.05$; NS = $p>0.05$; A=tree 1; B=tree 2.

Table 12. Pairwise comparisons for tests with foliar extracts. $\alpha=0.05$.

Species	Test	Sample	Test statistic*	P
Balsam fir	0 vs. 25	A	t=5.86	<0.001
		B	t=-3.45	0.003
	25 vs. 40	A	Z=1.34	0.18
		B	t=1.66	0.11
	0 vs. 40	A	t=4.21	<0.001
		B	Z=1.83	0.07
White spruce	0 vs. 25	A	Z=2.46	0.01
		B	t=1.95	0.07
	25 vs. 40	A	t=-2.36	0.03
		B	Z=1.54	0.12
	0 vs. 40	A	t=1.39	0.18
		B	t=-0.33	0.75

**t* for Student's *t*-test and Z for Wilcoxon's signed rank test.

It is immediately apparent, in fact, that some of the results are contradictory. For example, when tested with balsam fir extracts, insects consumed about 65% of the discs wetted with the control stand extracts and 35% of those wetted with extracts from the 25% thinned stand for test A and the opposite is true for B (Figure 9). Unfortunately, owing to time constraints and limited sample size, no further practical or useful analysis is possible considering the nature of the data collected.

Discussion

The results of the two-choice feeding tests clearly demonstrate that the spruce budworm is capable of detecting tannins in its diet. Briefly, the insects respond negatively to tannic acid, a hydrolysable tannin, and positively to the condensed spruce tannins. A dose-response relationship is apparent in both cases. For both hydrolysable and condensed tannins, diet and concentration had a significant effect on the response of the insect and, again in both cases, a significant interaction was found between diet and tannin concentration. For both types of tannins, the insects do not appear to be able to detect the 0.75% tannin concentration. Interestingly, Kumbaşli (2005) demonstrated that these very same phagostimulant condensed tannins produce negative effects on the spruce budworm with regards to growth, survival, and digestion despite the insect's ability to compensate by increasing its approximate digestibility.

Most previous studies of tannins and insect herbivores have focused on one type of tannin (Appendix 1) and those that have tested both hydrolysable and condensed tannins on the same insect have reported similar effects for both (e.g. Manuwoto and Scriber, 1986). To my knowledge, this is the first time that condensed tannins have been reported to produce a positive effect (i.e. phagostimulatory) on an insect herbivore (Bernays, 1981 and Appendix 1). It is highly unlikely that this preference was due to the presence of impurities in the spruce tannin because any impurities are proteins and it is known that insects do not have taste receptors that respond to this class of molecule (Chapman, 2003). The absence of any account of such an effect in the literature might be partially explained by the fact that most previous studies with condensed tannins have been conducted with quebracho from the tree *Schinopsis* sp. and not from the host plant

of the insect under investigation. Even in studies where condensed tannins were extracted from the insect's host plant, a deterrent effect was still found. However, these studies were not specifically investigating pre-ingestive gustatory effects and the deterrence reported might be attributable to other factors such as water content and leaf toughness (Feeny, 1970; Forkner *et al.*, 2004).

Another advantage of using tannins extracted from the insect's host plant, besides obtaining interesting results as presented above, is obtaining more meaningful and applicable data. It is a much easier task to extrapolate results from laboratory experiments employing compounds that the insect naturally comes into contact with. Insights into the insect's biology based on these sorts of tests are rendered much more ecologically relevant. However, it should be mentioned that there exists intrinsic theoretical value in testing other, non host plant compounds in order to increase one's knowledge of the organism and perhaps reveal or explain aspects which might otherwise remain elusive. The concentrations used in the experiments described herein were chosen to represent the wide range of tannin concentrations found naturally in the host plant and induced after certain sylvicultural methods. The concentrations were chosen to correspond to those studied by Kumbaşli (2005). He found that white spruce normally contained around 7.5% to 8% dry weight tannins and that after various thinning regimes these concentrations can increase to nearly 30%. Therefore, concentrations were also selected to emulate certain natural (or anthropogenically induced) phenomena.

The fact that the spruce budworm responds differently to the two types of tannins is perhaps an indication of an evolutionary adaptation to its host plants' chemistry. While condensed tannins are present in the spruce budworm's common hosts, hydrolysable

tannins are not and are therefore completely foreign. The precise mixture of white spruce condensed tannins may represent the chemical fingerprint of the insect's host plant, act as a sign stimulus and therefore stimulate feeding. Secondary compounds may act as phagostimulants and particular secondary compounds act as sign stimuli (Chapman, 2003). As Chapman (2003) suggested for sign stimuli, the spruce budworm might be expressing the appropriate receptor, in this case for tannins, on a phagostimulatory cell instead of a deterrent cell. A negative response to hydrolysable tannins could represent a specific response to tannic acid or a broader response to foreign tannins in general. This can easily be determined by performing similar two-choice feeding tests with other non-host tannins, such as quebracho.

Tannic acid clearly inhibited feeding of the spruce budworm larvae. In order to demonstrate feeding deterrent properties using behavioural tests, one can combine the substance (i.e. tannic acid) with some acceptable substrate and observe whether or not the acceptability changes (Schoonhoven and Jermy, 1977). Not only was the tannic acid combined with an 'acceptable substrate', it was combined with sucrose. For phytophagous insects, sugars are commonly, and perhaps usually, the key phagostimulatory component of the food (Chapman, 2003). Moreover, sugars, usually sucrose, are phagostimulants for all the phytophagous insects studied (Chapman, 2003) and sucrose is known to be a strong feeding stimulant in the spruce budworm (Heron, 1965; Albert *et al.*, 1982). Carbohydrates can 'mask' the unpleasant taste of some secondary plant chemicals, rendering them more palatable (Glendinning, 2002). Yet even after combining it with 25 mM sucrose, the most stimulating concentration for the spruce budworm (Albert *et al.*, 1982), the tannic acid-sucrose solution deters feeding.

Not only do the responses to the two types of tannin differ, apparently so do their modes of action. When tested alone (i.e. dissolved in water alone) the hydrolysable tannins are not detected by the insect (Figure 6). However, the condensed tannins tested alone stimulated feeding even enough to compete with a solution of 25 mM sucrose (Figure 6). This leads to the conclusion that the spruce budworm is capable of detecting condensed tannins independently and that these are strong phagostimulants. The hydrolysable tannins cannot be detected alone but seem somehow to inhibit the positive signal when mixed with sucrose (e.g. Figure 7). Similar results were obtained for glutamic acid which is not detected in solution alone but produces a deterrent effect on the spruce budworm when mixed with 25 mM sucrose (Albert and Parisella, 1988). Likewise, Panzuto *et al.* (2002) observed that tannic acid interfered with the response of the sugar-sensitive cell of the lateral sensillum styloconica in the oblique-banded leafroller, *Choristoneura rosaceana*, and Dethier (1982) reports similar results for two other phytophagous caterpillars. Feeding deterrents can act on the sensory system in two ways: by stimulating a specialized deterrent cell or by modifying the activity of cells responding to feeding stimulants (Schoonhoven and Jermy, 1977). Tannic acid appears to act via the latter mechanism, at least in the case of the spruce budworm. Glendinning (1996, 2002) has proposed a fast-acting post-ingestive mechanism as mediating the rapid (e.g. 30 s) rejection response of some insects to certain chemicals (e.g. nicotine), but this does not apply to tannic acid and the spruce budworm. The spruce budworm is not deterred by a solution of tannic acid alone and, therefore, there is no need to explain rejection through pre- or post-ingestive effects since rejection does not occur.

Feeding preference increases for spruce tannins and decreases for tannic acid with increasing concentration. This is nicely summarized by the linear regressions yielding a positive slope for spruce tannins and a negative slope for tannic acid plotted against concentration (Table 7). Tannin concentration accounts for around 20% to 30% of the variance in the data. The interaction between a phagostimulant and a deterrent typically results in some concentration-dependent inhibition of the response (Chapman, 2003) as described here for sucrose and tannic acid (Table 7). Although experimental conditions were controlled to reduce fluctuations in environmental factors such as temperature and humidity, individual variability between insects can still be important, especially in behavioural experiments, since behaviour, by its very nature, is a more or less plastic response. Behavioural methods combine the advantage of dealing with total responses of the intact animal and the disadvantage of high inter-individual variability to many variables of various sorts (Schoonhoven and Jermy, 1977). However, the numerous tests performed and the consistent responses observed solidly confirm the validity of the results. Most likely, much of the variance is due to the fact that the data are not properly represented by a linear equation.

Despite increasingly significant correlation coefficients, the value of the quadratic and cubic equations in describing the relation of mean percent consumption to tannin concentration is minimal. Because of the limited number of points used to determine the equations, their biological relevance is much reduced. The first order equation summarizes the trends in the data clearly and succinctly. That is not to say that this is the best mathematical description of the biological phenomena, but rather that it is the most parsimonious explanation of the available data. In order to determine the precise nature

of the relation, more data need to be collected at intermediate tannin concentrations. This work was begun by Thomson (2007) who investigated various concentrations of spruce tannins with insects reared on 0% tannic acid diet. He determined a threshold of around 2%, below which insects do not respond and above which, insects show preference for the spruce tannin-containing solution. Further analysis of his data, in combination with my own, is required to elucidate the exact character of the relation. From this, I would argue that the relationship between tannin concentration and larval feeding preference more closely resembles a sigmoid or step function, since certain ranges in concentration seem to produce one response which then switches abruptly to another response over the next range of values. This seems to be supported by the *post hoc* Bonferroni tests of the 2-way ANOVAs that indicate that, for both types of tannin, the 0% and 0.75% concentrations group together, as do the 8%, 15%, and 30% (for tannic acid only) concentrations with respect to mean percent consumption.

Spruce budworm larvae reared on 8% tannic acid diet and tested with tannic acid showed an increased preference for control (25 mM sucrose) discs than when reared on 0% tannic acid diet. This is possibly a form of aversion learning. When the spruce budworm is forced to feed on the tannic acid containing diet, the deterrent properties of the tannic acid solution are enhanced. Albert and Parisella (1985a) failed to observe any induction effects in their study of spruce budworm feeding, but noted possible effects of habituation and avoidance learning. There is no change of preference for tannic acid; there is only an increase in its distastefulness. An analogy can be drawn to a child who is forced to feed on broccoli for several consecutive days; after several days the child's original dislike for broccoli has been enhanced to downright loathing for the vegetable.

The effect was not enhanced by the increased tannic acid concentration of the 15% tannic acid diet indicating a potential response plateau or that a more complicated set of interactions are involved between concentration and diet.

When tested with spruce tannins a different effect was produced. Larvae had an increased preference for spruce tannins when reared on the 15% tannic acid diet than when reared on the 8% tannic acid diet. This is certainly not a case of habituation since the compounds are chemically distinct. Rearing an insect on caffeine, for example, should not reduce the deterrent effects of food items containing azadirachtin and neither should rearing on hydrolysable tannin produce increased preference for condensed tannins. It is more likely due to induction which can also be explained using the broccoli diet analogy. When fed numerous heads of broccoli for several consecutive days, a child's preference for ice cream increases significantly. As discussed above, the pre-ingestive effects and mechanisms of tannic acid and spruce tannins are quite unrelated; spruce tannins actually possess phagostimulant qualities. When reared on a diet of sufficiently high concentration of tannic acid, the insect's preexisting preference for the spruce tannin-sucrose solution is enhanced.

Albert and Parisella (1988) stress that there is no direct correlation between the two measures of feeding behaviour (i.e. mean percent consumption and feeding rate), thus care must be taken in interpreting the results of feeding preference tests. Mean percent consumption and feeding rate represent two different aspects of feeding and can be affected differently and by different factors. For example, glutamic acid and serine were determined to be deterrent from mean percent consumption data but their feeding rates were similar to control insects (Albert and Parisella, 1988). Comparing the feeding

rates from the tests with solutions of tannins alone to those with solutions of tannins plus sucrose, it is clear that the presence of sucrose increased the feeding rate. This is not surprising since sucrose is a known phagostimulant for the spruce budworm (Albert *et al.*, 1982). Also, the effect of adding sucrose (in fact, specifically 25 mM) to solutions of some pure amino acids has been reported to increase feeding rates in the spruce budworm (Albert and Parisella, 1988).

Larval feeding rate was significantly affected by diet and tannin concentration (Table 9). Although there was a slight trend toward decreasing mean feeding rate with increased tannic acid content of the diet for insects tested with tannic acid, it was only significant at the 15% tannic acid concentration. The trend was slightly stronger for insects tested with spruce tannins. Rearing spruce budworm on tannin-containing diet has previously been shown to reduce larval weight and smaller insects generally consume less food (Kumbaşli, 2005). It is unlikely that larval weight is responsible for the reduced feeding rate since weights were equally decreased for both the 8% and 15% tannic acid diets whereas feeding rates were especially low on the 15% tannic acid diet. It is more likely that toxic effects of tannic acid in the diet, which may be more pronounced with increasing concentration, are responsible. Tannin concentration was reported as significantly affecting feeding rate because of the reduced feeding rates of larvae tested at the 30% tannic acid concentration, regardless of diet. At this high concentration, aversion might be stronger and toxic effects more rapidly induced, resulting in severely reduced feeding rates. When reared on 8% tannic acid diet and tested with 15% spruce tannin, feeding rate was rather anomalously reduced. This effect was not produced on the

0% or 15% diets and therefore further explanation must await supplementary investigations.

Application of a dual choice, as opposed to no-choice, experimental design increases the sensitivity of the test (Schoonhoven and Jermy, 1977). However, such research should preferably combine behavioural and electrophysiological methods, because both methods have their advantages as well as their intrinsic limitations and thus complement each other (Schoonhoven and Jermy, 1977). The electrophysiological research investigating insect responses to tannins is limited. Dethier (1982) proposed that tannins do not act on a specific tannin cell but rather modulate the response of a sugar cell. Panzuto *et al.* (2002) confirmed this for *Choristoneura rosaceana* by testing solutions of glucose alone and glucose plus tannin on the galeal lateral sensillum styloconica. The same inhibitory effect of tannic acid on a sugar-cell appears to apply to the spruce budworm as well. Future electrophysiological experiments can use this information as a starting point. A cell responding specifically to either type of tannins has yet to be reported in any insect (Panzuto *et al.*, 2002). If spruce tannins do indeed stimulate a phagostimulatory neuron, electrophysiological characterization of this cell's response to these compounds will prove useful in more fully understanding natural feeding patterns in the spruce budworm and in illuminating theories of plant anti-herbivore chemistry. Ablation studies, whereby specific mouthparts are removed and the effect(s) on larval feeding observed, might be useful in creating a more structured approach to electrophysiological tests by providing areas and sensilla on which to focus.

Results from the tests involving foliar extracts were less informative for several reasons. From a purely biological standpoint, foliar extracts are incredibly chemically

complex and their effects on insect feeding remain unclear. The mechanisms involved in the interactions between chemicals at the periphery, i.e. at the level of the gustatory sensilla, are not understood (Chapman, 2003). Aside from the inherent difficulties involved in testing plant extracts, the situation was compounded by time constraints and a restricted sample size. Foliage samples were extracted and tested before any chemical analysis was available. The *a posteriori* chemical analysis of the foliage performed by Dr. Éric Bauce (Université Laval) displays little difference of levels of total phenols or total tannins between treatments. In fact, the more significant differences in chemical composition appear in the non-polar extracts. These extracts were saved and should be tested against a 25 mM sucrose control rather than against other extracts. The experiments were originally designed with more samples in mind, without which analysis is nigh impossible. With only 2 replicates, the statistical power of any conclusions is tremendously limited. From the meager analysis performed it seems that any differences in mean percent consumption between treatments are overshadowed by differences between individual trees. In fact, these differences probably account for the contradictory results obtained. A more appropriate test would be to test individual extracts against a 25 mM control as suggested for the non-polar extracts.

Even though, at this point, information from the tests with foliar extracts cannot be used to predict the effects of certain forestry thinning regimes on spruce budworm dynamics, the results presented herein can provide some instruction. Young balsam fir (30 years old) was found to contain a lower nitrogen:tannin ratio and a higher concentration of monoterpenes compared with old balsam fir (70 years old) (Bauce *et al.*, 1994). These differences are directly related to increased mortality, decreased pupal

weight, and longer larval development of the spruce budworm (Bauce *et al.*, 1994). The feeding preference of sixth instars for young trees seems to correlate with its increased sucrose content (Albert and Bauce, 1994) but might also reflect higher tannin concentrations. Albert and Bauce (1994) also found that, unlike sixth instars, feeding preferences and feeding rates of fourth instars correlated with foliar nitrogen and nitrogen:tannin ratio, respectively. It is therefore likely that younger insects will respond differently to variations in tannin concentrations. Age-related differences in responses to various chemicals and extracts have been observed in the spruce budworm (Toufexis *et al.*, 1996; Sandoval and Albert, 2007) and future studies can more precisely determine the effects of tannins on young larvae.

Furthermore, one year after a selective cut, the remaining trees experience a decrease in concentrations of defensive compounds such as monoterpenes and tannins (Bauce, 1995). However, several years after the treatment (i.e. 2 or 3 years) the trees react by increasing the production of foliage which gradually compensates for any prior positive effects to the insect and presumably results in increased resistance (Bauce *et al.*, 2001). It has recently been shown that tannins (specifically white spruce condensed tannins), at levels found in thinned spruce stands, negatively affect the growth and survival of the spruce budworm and that the decreased levels of tannins observed in white spruce during precommercial thinning likely translate into positive effects on the spruce budworm (Kumbaşli, 2005). Considering the spruce budworm's preference for increased host tannin concentrations, a decrease in feeding should be expected during the first year following a selective cut, but this will be accompanied by an increase in growth and survival. Application of *Bacillus thuringiensis* toxin can be applied during this time to

limit the potential of a serious outbreak. In the next 2 or 3 years, as tannin concentrations increase in the plant, spruce budworm can be expected to feed more vigorously but experience more pronounced negative effects from tannin consumption. Spraying of *B. thuringiensis* toxin should cease at this point. Kumbaşli (2005) demonstrated that when employed separately, tannins and *B. thuringiensis* toxin have deleterious effects on the spruce budworm, but that when applied together, antagonistic effects are produced that reduce the efficiency of the combined treatments. Tannins must be considered in forest management aimed at controlling spruce budworm populations.

There exists a great inter-specific variability in responses towards secondary plant substances and, in general, Schoonhoven and Jermy (1977) estimate that "no two insect species react identically to all plant substances or, in other words, that no single secondary plant substance will elicit the same reaction in all insects." The same holds true within the group of tannins (Appendix 1). However, some generalizations may yet emerge from the complex details. It is clear that tannins can affect insect herbivores in several ways including stimulating and inhibiting feeding, as seen in the spruce budworm for spruce tannins and tannic acid respectively. Experimental evidence from the food choices of such herbivorous wild animals as giant tortoises, deer, *Colobus* monkeys, gorillas, and insects indicate that tannin concentrations higher than 2% deter feeding (Swain, 1979). A 2% concentration threshold has also been suggested for the positive effects of spruce tannins on the spruce budworm (Thomson, 2007). Although the spruce tannins were found to stimulate feeding, Kumbaşli (2005) demonstrated that these very same phagostimulant condensed tannins produce negative effects on the spruce budworm with regards to growth, survival, and digestion despite the insect's limited ability to

compensate by increasing its approximate digestibility. It appears therefore that the positive pre-ingestive (gustatory) effects of tannins to its host plant inevitably lead to negative post-ingestive effects on growth and survival.

The spruce budworm has somewhat successfully adapted to its hosts by evolving to respond positively to certain plant chemicals (e.g. spruce tannins) and negatively to others (e.g. tannic acid) while limiting deleterious effects by slight physiological modifications. Despite never naturally encountering tannic acid, the spruce budworm was shown to respond negatively to this plant chemical. However, it appears to deter feeding by the same mechanism as some host plant chemicals such as glutamic acid and might therefore represent the evolutionary consequence of such adaptations. Schowalter (2006) describes physiology as representing "fixed" adaptations to predictable variation in environmental conditions and behavior as representing a more flexible means of adjusting to unpredictable variation (Schowalter, 2006). Unfortunately, there is still only a very limited understanding of how the genetically based (fixed) physiological responses of sensory neurons translates into (more or less plastic) behaviour. Such is the case for the spruce budworm that likes to eat what is not good for it. Truly a bizarre predicament.

Appendix 1

Tannin-insect herbivore research arranged chronologically.

Reference	Insect(s)			Type of tannin[b]	Approximate concentration[c]	Effect reported
	Species	Family	Order[a]			
Grevillius, 1905	*Euproctis chrysorrhea*	Lymantriidae	Lep.	H	1.5-3.5% d.w.	Stimulated feeding
Görnitz, 1954	*Lymantria dispar*	Lymantriidae	Lep.	H	1.2-10% d.w.	Increased feeding and stimulated feeding on non-host plants; dose effect
	Orgyia antiqua	Lymantriidae				
	Euproctis chrysorrhea	Lymantriidae				
	Malacosoma neustria	Lasiocampidae				
	Operophthera brumata	Geometridae				
	Amphidasis betularia	Geometridae				
	Acronycta aceras	Noctuidae				
	Phalera bucephala	Notodontidae				
Bennett, 1965	*Hypera postica*	Curculionidae	Col.	H	1.2% (w/v)	Deterrent
Feeny, 1968	*Operophthera brumata*	Geometridae	Lep.	Oak (*Quercus robur*) leaf tannins	1-10% d.w.	Decreased growth and survival
Feeny, 1970	*Operophthera brumata*	Geometridae	Lep.	Oak (*Quercus robur*) leaf tannins	-	Decreased growth

Todd *et al.*, 1971	*Schizaphis graminum*	Aphididae	Hem.	H	3.75×10^{-4} M	Decreased growth, soon died; possible deterrent effect
Schoonhoven and Derksen-Koppers, 1973	*Dysdercus koenigii*	Pyrrhocoridae	Hem.	H	0.01 M	Deterrent
	D. fulviniger					
	D. völkeri					
	Spilostethus pandurus	Lygaeidae				
Meisner and Skatula, 1975	*Lymantria dispar*	Lymantriidae	Lep.	H	0.2% d.w.	Increased feeding
Fox and Macauley, 1977	*Paropsis atomaria*	Chrysomelidae	Col.	*Eucalyptus* tannins	-	No effect
Chapman and Bernays, 1977	*Schistocerca gregaria*	Acrididae	Ortho.	H & C	5% d.w.	Deterrent
Bernays and Chapman, 1978						
Bernays, 1978	*Schistocerca gregaria*	Acrididae	Ortho.	H	10% and 20% d.w.	Lesions in midgut and caeca of *Locusta*; No effect on *Schistocerca* spp. or *Z. variegatus*
	S. americana					
	S. cancellata					
	Locusta migratoria			C	10% and 20% d.w.	No effect on digestion
	Zonocerus variegatus					
Chan *et al.*, 1978	*Heliothis armigera*	Noctuidae	Lep.	C	0.1% d.w.	Restricted growth

Haukioja *et al.*, 1978	*Trichiosoma lucorum*	Cimbicidae	Hym.	Birch (*Betula pubescens*) tannins	-	Decreased growth; deterrent
	Dineura viridiversata	Tenthredinidae	Hym.			
Jones and Firn, 1979	*Pieris brassicae*	Pieridae	Lep.	C; Bracken fern (*Pteridium aquilinum*) tannins	-	Deterrent
Bernays *et al.*, 1980	*Locusta migratoria*	Acrididae	Ortho.	H	20% d.w.	Reduced consumption; some midgut lesions; increased mortality
	Locustana pardalina					Increased mortality
	Gastrimargus africanus					Midgut lesions; increased mortality
	Oedaleus senegalensis					Reduced consumption
	Chortoicetes terminifera					Increased mortality
	Heteropternis obscurella					Increased mortality
	Acrida conica					Increased mortality
	Chorthippus brunneus					Increased mortality; digestibility not

						affected
	Melanoplus sanguinipes					No effect; digestibility not affected
	Atractomorpha crenaticeps					Increased survival
	Zonocerus variegatus					No effect
	Schistocerca gregaria					Digestibility not affected
	S. americana					No effect
	S. cancellata					No effect
	Anacridium melanorhodon					Increase in weight and digestion indices and increased survival
Bernays *et al.*, 1981	*Schistocerca gregaria*	Acrididae	Ortho.	C	4% and 10% d.w.	No effect on growth or survival
	Locusta migratoria					
	Chortoicetes terminifera					
	Zonocerus variegatus					
Bernays and Woodhead, 1982	*Anacridium melanorhodon*	Acrididae	Ortho.	H	0.2% d.w.	Increased survival and growth
Klocke and Chan, 1982	*Heliothis zea*	Noctuidae	Lep.	C	0.1%, 0.15%, 0.2% d.w.	Decreased ingestion; deterrent
Reese *et al.*, 1982						

Berenbaum, 1983	*Papilio polyxenes*	Papilionidae	Lep.	Tuliptree (*Liridiodendron tulipifera*) tannins	0.66% d.w.	Increased mortality of *P. polyxenes* only; no effect on growth rate, digestion or N utilization
	P. glaucus					
Roehrig and Capinera, 1983	*Hemileuca oliviae*	Saturniidae	Lep.	C	0.1%, 0.5%, 1%, 5% w.w.	Deterrent; antimetabolite; decreased growth and survival
Lawson *et al.*, 1984	*Alsophila pometaria*	Geometridae	Lep.	C	-	No effect
	Anisota senatoria	Saturniidae	Lep.			
Steinly and Berenbaum, 1985	*Papilio polyxenes*	Papilionidae	Lep.	Tuliptree (*Liridiodendron tulipifera*) tannins	0.66% d.w.	Midgut lesions and necrosis (more severe in *P. polyxenes*)
	P. glauca					
Manuwoto and Scriber, 1986	*Spodoptera eridania*	Noctuidae	Lep.	H and C	0.5 to 3% w.w.	Decreased growth rate; no dose effect
	Callosamia promethea	Saturniidae	Lep.			
Karowe, 1989	*Malacosoma disstria*	Lasiocampidae	Lep.	H	0-8%	Increased mortality; decreased growth; decreased digestion efficiency
	Orgyia	Lymantriidae	Lep.			No effect

	leucostigma					
Raubenheimer and Simpson, 1990	*Schistocerca gregaria*	Acrididae	Ortho.	H	10% d.w.	Increased consumption
	Locusta migratoria					Deterrent
Raubenheimer, 1992	*Locusta migratoria*	Acrididae	Ortho.	H	10%	Decreased consumption (on low protein diet); midgut lesions; restricted compensatory feeding
Zou and Cates, 1997	*Choristoneura occidentalis*	Tortricidae	Lep.	Douglas fir (*Pseudotsuga menziesii*) phenolics	2.5% and 5% d.w.	No effect on larval growth
Muir *et al.*, 1999	*Melanoplus sanguinipes*	Acrididae	Ortho.	C	1-20% w.w.	Deterred at high concentrations
	Mamestra configurata	Noctuidae	Lep.			Deterred at high concentrations
	Plutella xylostella	Plutellidae	Lep.			Deterred at intermediate concentrations
	Phyllotreta cruciferae	Chrysomelidae	Col.			Deterred at intermediate concentrations
Simpson and Raubenheimer, 2001	*Locusta migratoria*	Acrididae	Ortho.	H	3.3% to 10% d.w.	Decreased ingestion on foods with low P:C ratio;

						decreased N utilization efficiency on foods with excess protein
Nomura and Itioka, 2002	*Spodoptera litura*	Noctuidae	Lep.	H	2 mg/10 g diet to 40 mg/10 g diet	Inhibited growth, dose effect; decreased survival, pupal mass and extended larval period
Panzuto *et al.*, 2002	*Choristoneura rosaceana*	Tortricidae	Lep.	H	0.5% by volume	Not phagostimulant; performed better on tannic acid-enriched diet; reduced development time
Salminen and Lempa, 2002	*Epirrita autumnata*	Geometridae	Lep.	H	5 mg/g (H_2O) and 20 mg/g (H_2O)	Reduced leaf consumption of 2^{nd} and 4^{th} instars; no effect on 5^{th} instar feeding
Foss and Rieske, 2003	*Lymantria dispar*	Lymantriidae	Lep.	Oak (*Quercus* sp.) tannins	-	Increased consumption and growth with increased tannins
Forkner *et al.*, 2004	Survey of oak-visiting phytophagous	-	-	-	-	Specialists more than generalists correlate with

	insects					negative effects of condensed tannins
Park *et al.*, 2004	*Riptortus clavatus*	Alydidae	Hem.	Persimmon (*Diospyros kaki*) tannins and tannic acid (H)	1%, 3% by volume	Decreased ingestion; decreased reproduction
Kumbaşli, 2005	*Choristoneura fumiferana*	Tortricidae	Lep.	C; white spruce (*Picea glauca*) tannins	0%-30%	Midgut lesions; increased mortality; decreased digestion; increased development time (30%)

[a]Lep.=Lepidoptera; Col.=Coleoptera; Hem.=Hemiptera; Ortho.=Orthoptera; Hym.=Hymenoptera
[b]H=hydrolysable (usually tannic acid); C=condensed (usually quebracho).
[c]Concentrations as reported by the authors; d.w.=dry weight; w.w.=wet weight

Appendix 2

Extraction and purification of spruce tannins

The method of tannin extraction and purification described below is taken from Kumbaşli (2005).

Current year white spruce (*Picea glauca*) foliage was collected 21 June 2001 (corresponding to spruce budworm 6^{th} instar) from a 50-yr-old stand in the experimental Montmorency forest of the Université Laval. Tannins were extracted in 70% acetone (containing 1 g ascorbic acid per litre) which is the best solvent for this task (Hagerman 1988). 5 mL of solvent were added to centrifuge tubes containing 300 mg of dry spruce foliage and homogenized for 10 sec in an ULTRA-TURRAX T25 at 24 000 rpm and this mixture was then centrifuged for 5 min at 3000 rpm. The supernatant was removed and the process repeated 2 more times by homogenizing with a VORTEX. The supernatant (15 mL) was finally filtered using WHATMAN #41 filter paper.

Before proceeding to the isolation and purification of tannins, the acetone was evaporated by roto-evaporator (YAMATO RE200). A SEPHADEX LH-20 gel was used to purify the tannins according to the methods described by Hagerman and Butler (1980). A column 22.8 cm high and 1.7 cm diameter was filled with the SPHADEX LH-20 gel and 2 mL of extract were washed with 120 mL 95% ethanol. This was followed by rinsing everything with 70 mL 50% acetone to recover the tannins. After evaporation of the acetone by roto-evaporator and freeze-drying the mixture, the tannins are obtained as a powder.

The protein precipitating capacity of the final product was tested by the radial diffusion method described by Hagerman (1987) consisting of visual observation of the capacity of the tannins to precipitate the protein bovine serum albumin (BSA).

References

Albert, P. J. 1982. Host plant preferences in larvae of the eastern spruce budworm, *Choristoneura fumiferana*. *Proc. 5th int. Symp. Insect-Plant Relationships, Wageningen*: 19-24.

Albert, P. J. and Bauce, E. 1994. Feeding preferences of fourth- and sixth-instar spruce budworm larvae for foliage extracts from young and old balsam fir hosts. *Environmental Entomology* **23**: 645-653.

Albert, P. J., Cearley, C., Hanson, F., and Parisella, S. 1982. Feeding responses of eastern spruce budworm larvae to sucrose and other carbohydrates. *Journal of Chemical Ecology* **8**: 233-239.

Albert, P. J. and Jerrett, P. A. 1981. Feeding preferences of spruce budworm (*Choristoneura fumiferana* Clem.) larvae to some host-plant chemicals. *Journal of Chemical Ecology* **7**: 391-402.

Albert, P. J. and Parisella, S. 1985a. Tests for induction of feeding preferences in larvae of eastern spruce budworm using extracts from three host plants. *Journal of Chemical Ecology* **11**: 809-817.

Albert, P. J. and Parisella, S. 1985b. Feeding preferences of eastern spruce budworm larvae in two-choice tests with combinations of host-plant extracts. *Entomologia Experimentalis et Applicata* **38**: 221-225.

Albert, P. J. and Parisella, S. 1988. Feeding preferences of the eastern spruce budworm larvae in two-choice tests with extracts of mature foliage and with pure amino acids. *Journal of Chemical Ecology* **14**: 1649-1656.

Bate-Smith, E. C. 1973. Haemanalysis of tannins: The concept of relative astringency. *Phytochemistry* **12**: 907-912.

Bate-Smith, E. C. and Swain, T. 1962. Flavonoid compounds. in Comparative Biochemistry, Vol. 3A. edited by H.S. Mason and A.M. Florkin, Academic Press, New York: pp. 705–809.

Barbehem, R. V., Walker, A. C., and Uddin, F. 2003. Antioxidants in the midgut fluids of a tannin-tolerant and a tannin-sensitive caterpillar: effects of seasonal changes in tree leaves. *Journal of Chemical Ecology* **29**: 1099-1116.

Baskerville, G. L. 1975. Spruce budworm – super sylviculturist. in The Spruce budworm. *The Forestry Chronicle* **51:** 138-140.

Bauce, E. 1996. One and two year impact of commercial thinning on spruce budworm feeding ecology and host tree foliage production and chemistry. *The Forestry Chronicle* **72**: 393-398.

Bauce, E., Carisey, N., and Dupont, A. 2001. Implications des relations alimentaires plante-insecte dans la lutte contre la tordeuse des bourgeons de l'épinette. *Actes du colloque «Tordeuse des bourgeons de l'épinette : l'apprivoiser dans nos stratégies d'aménagement »* tenu à Shawinigan, 27-29 mars 2001 : pp.27-32.

Bauce, E., Crépin, M., and Carisey, N. 1994. Spruce budworm growth, development and food utilization on young and old balsam fir trees. *Oecologia* **97**: 499-507.

Bennett, S. E. 1965. Tannic acid as a repellent and toxicant to alfafa weevil larvae. *Journal of Economic Entomology* **58**: 372.

Berenbaum, M. R. 1980. Adaptive significance of midgut pH in larval Lepidoptera. *American Naturalist* **115**: 138-146.

Berenbaum, M. R. 1983. Effects of tannins on growth and digestion of two species of papilionids. *Entomologia Experimentalis et Applicata* **34**: 245-250.

Bernays, E. A. 1978. Tannins: an alternative viewpoint. *Entomologia Experimentalis et Applicata* **24**: 44-53.

Bernays, E. A. 1981. Plant tannins and insect herbivores: an appraisal. *Ecological Entomology* **6**: 353-360.

Bernays, E. A. and Chapman, R. F. 1978. Plant chemistry and acridoid feeding behavior. in Coevolution of Plants and Animals edited by Harborne, J. B. Academic Press, London: pp. 99–141.

Bernays, E. A. and Chapman, R. F. 2001. Taste cell responses in the polyphagous arctiid, *Grammia geneura*: towards a general pattern for caterpillars. *Journal of Insect Physiology* **47**: 1029–1043.

Bernays, E. A., Chamberlain, D., and Leather, E. M. 1981. Tolerance of acridids to ingested condensed tannins. *Journal of Chemical Ecology* **7**: 247-256.

Bernays, E. A., Chamberlain, D., and McCarthy, P. 1980. The differential effects of ingested tannic acid on different species of acridoidea. *Entomologia Experimentalis et Applicata* **28**: 158-166.

Bernays, E. A. and Woodhead, S. 1982. Plant polyphenols utilized as nutrients by a phytophagous insect. *Science* **216**: 201-203.

Carisey, N. and Bauce, E. 1997. Balsam fir foliar chemistry in middle and lower crowns and spruce budworm growth, development, food and nitrogen utilization. *Journal of Chemical Ecology* **23**: 1963-1978.

Chafe, S. C. and Durzan, D. J. 1973. Tannin inclusions in cell suspension cultures of white spruce. *Planta* **113**: 251-262.

Chan, B. C., Waiss, A. C., and Lukefahr, M. M. 1978. Condensed tannin, an antibiotic chemical from *Gossypium hirstum*. *Journal of Insect Physiology* **24**: 113-118.

Chapman, R. F. 2003. Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology* **48**: 455-484.

Chapman, R. F. and Bernays, E. A. 1977. The chemical resistance of plants to insect attack. *Scripta Varia* **41**: 603–633.

Dethier, V. G. 1980. Evolution of receptor sensitivity to secondary plant substances with special references to deterrents. *American Naturalist* **115**: 45-66.

Dethier, V. G. 1982. Mechanism of host-plant recognition. *Entomoogia Experimentalis et Applicata* **31**: 49–56.

Dethier, V. G. and Kuch, J. H. 1971. Electrophysiological studies of gestation in lepidopterous larvae. *Zeitschrift fur vergleichende Physiologie.* **72**: 343-363

Feeny, P. 1968. Effect of oak leaf tannins of larval growth of the winter moth, *Operopthera brumata. Journal of Insect Physiology* **14**: 805-807.

Feeny, P. 1969. Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. *Phytochemistry* **8**: 2119-2126.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**: 565-581.

Feeny, P. 1975. Biochemical coevolution between plants and their insect herbivores. in Coevolution of Plants and Animals edited by Gilbert, L. E. and Raven, P. H. Texas University Press, Austin, Texas: pp. 3–19.

Feeny, P. 1976. Plant apparency and chemical defense. *Recent Advances in Phytochemistry* **10**: 1-40.

Forkner, R. E., Marquis, R. J., and Lill, J. T. 2004. Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. *Ecological Entomology* **29**: 174-187.

Foss, L. K. and Rieske, L. K. 2003. Species-specific differences in oak foliage affect preferences and performance of gypsy moth caterpillars. *Entomologia Experimentalis et Applicata* **108**: 87-93.

Fox, L. R. and Macauley, B. J. 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia* **29**: 145-162.

Fraenkel, G. S. 1959. The raison d'être of secondary plant substances. *Science* **129**: 1466-1470.

Freudenberg 1920. Die Chemie der natürlichen Gerbstoffe. *Berichte* **53**: 1416.

Glendinning, J. I. 1996. Is chemosensory input essential for the rapid rejection of toxic foods? *Journal of Experimental Biology* **199**: 1523-1534.

Glendinning, J. I. 2002. How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata* **104**: 15-25.

Gledndinning, J. I., Domdom, S., and Long, E. 2001. Selective adaptation to noxious foods by a herbivorous insect. *Journal of Experimental Biology* **204**: 3355-3367.

Görnitz, K. 1954. Fraβauslösende Stoffe für polyphag an Holzgewäschen fressended Raupen. *Verhanlungen der Deutschen Geselschaft für Angewandte Entomologies E. V.* **6**: 39-47 (Translated by Virginia Hock).

Grevillius, A. Y. 1905. Zur Kenntnis der Biologie des Goldafters (*Euproctis chrysorrhoea* L.) und der durch denselben verursachten Beschädigungen. *Beih. Bot. Zbl.* **18**: 222-322.

Grisdale, D. 1970. An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Canadian Entomologist* **102**: 1111-1117.

Hagerman, A. E. 1987. Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology* **13**: 437-449.

Hagerman, A. E. 1988. Extraction of tannins from fresh and preserved leaves. *Journal of Chemical Ecology* **14**: 453-461.

Hagerman, A. E. and Butler, L. G. 1980. Condensed tannin purification and characterization of tannin associated proteins. *Journal of Agricultural and Food Chemistry* **28**:947-952.

Hallem, E. A., Dahanukar, A., and Carlson, J. R. 2006. Insect odor and taste receptors. *Annual Review of Entomology* **51**: 113-135.

Hansen, K. 1978. Insect Chemoreception. in Taxis and Behavior (Receptors and Recognition, Series B, Volume 5). edited by Hazelbauer, G. L., Chapman and Hall, London: pp. 233-239.

Haslam, E. 1988. Plant polyphenols (*syn.* Vegetable tannins) and chemical defense – a reappraisal. *Journal of Chemical Ecology* **14**: 1789-1805.

Haslam, E. 1989. Plant Polyphenols; Vegetable tannins revisited. Cambridge University Press, Cambridge, 230 pp.

Haslam, E. 1999. Che farò senza polifenoli? in Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology. edited by Gross, G. G., Hemingway, R. W., Yoshida, T., and Branham, S. J., Kluwer Academic / Plenum Publishers, New York: pp. 15-40.

Haukioja, E., Niemelä, P., Iso-Iivari, L., Ojala, H., Aro, E.-M. 1978. Birch leaves as a resource for herbivores. I. Variation in the suitability of leaves. *Reports from the Kevo Subarctic Research Station* **14:** 5–12.

Heron, R. J. 1965. The role of chemotactic stimuli in the feeding behavior of the spruce budworm larvae on white spruce. *Canadian Journal of Zoology* **43**: 247-269.

Jermy, T. 1961. On the nature of oligophagy in *Leptinotarsa decimlineata* Say (Coleoptera: Chrysomelidae). *Acta Zoologica Academiae Scientiarum Hungaricae* **7**: 119-132.

Jones, C. G. and Firn, R. D. 1979. Resistance of *Pteridium aquilinum* to attack by non-adapted phytophagous insects. *Biochememical Systematics and Ecology* **7**: 95–101.

Karowe, D. N. 1989. Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for theories of plant anti-herbivore chemistry. *Oecologia* **80**: 507-512.

Klocke, J. A. and Chan, B. G. 1982. Effects of cotton condensed tannin on feeding and digestion in the cotton pest, *Heliothis zea*. *Journal of Insect Physiology* **28**: 911-915.

Kraus, T.E.C., Dahlgren, R.A., and Zasoski, R.J. 2003. Tannins in nutrient dynamics of forest ecosystems – a review. *Plant and Soil* **256**: 41-66.

Kumbaşli, M. 2005. Études sur les composés polyphénoliques en relation avec l'alimentation de la tordeuse des bourgeons d'épinette. Ph. D. thesis presented to the Faculté de Foresterie et de Géomatique de l'Université Laval, Québec.

Lagerheim, G. 1900. Zur Frage der Schutzmittel der Pflanzen gegen Raupenfraβ. *Entomologisk Tidskrift* **21:** 209-232.

Lamontagne, M., Bauce, E., and Margolis, H. A. 2002. Testing the ecophysiological basis for the control of monoterpene concentrations in thinned and unthinned balsam fir stands across different drainage classes. *Oecologia* **130**: 15-24.

Lawson, D. L., Merritt, R. W., Martin, M. M., Martin, J. S., and Kukor, J. J. 1984. The nutritional ecology of larvae of *Alsophila pometria* and *Anisota senatoria* feeding on early- and late-season oak foliage. *Entomologia Experimentalis et Applicata* **35**: 105-114.

Little, C. H. 1970. Seasonal changes in carbohydrate and moisture content in needles of balsam fir (*Abies balsamea*). *Canadian Journal of Botany* **48**: 2021-2028.

Manuwoto, S. and Scriber J. M. 1986. Effects of hydrolysable and condensed tannin on growth and development of two species of polyphagous lepidoptera: *Spodoptera eridania* and *Callosomia promethea*. *Oecologia* **69**: 225-230.

Mattson, W. J., Haack, R. A., Lawrence, R. K., and Slocum, S. S. 1991. Considering the nutritional ecology of the spruce budworm in its management. *Forest Ecology and Management* **39**: 183-210.

Martin, M. M. and Martin, J. S. 1984. Surfactants: their role in preventing the precipitation of proteins by tannins in insect guts. *Oecologia* **61**: 342-345.

Martin, M. M., Martin, J. S., and Bernays, E. A. 1987. Failure of tannic acid to inhibit digestion or reduce digestibility of plant protein in gut fluids of insect herbivores: implications for theories of plant defense. *Journal of Chemical Ecology* **13**: 605-621.

Martin, M. M., Rockholm, D. C., and Martin, J. S. 1985. Effects of surfactants, pH, and certain cations on precipitation of proteins by tannins. *Journal of Chemical Ecology* **11**: 485-494.

McKey, D. 1979. Chapter 2: The distribution of secondary metabolites within plants. In: Rosenthal, G. A. and Janzen, D. H. (eds.) Herbivores, their interaction with secondary plant metabolites. Academic Press, New York; pp 55-133.

McMorran, A. 1965. A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *Canadian Entomologist* **97**: 58.62.

Meisner, J. and Skatulla, U. 1975. Phagostimulation and phagodeterrency in the larva of the gypsy moth, *Porthetria dispar* L. *Phytoparsitica* **3**: 19-26.

Miller, C. A. 1963. The spruce budworm. in Spruce Budworm Dynamics edited by Morris, R., *Memoirs of the Entomological Society of Canada no. 31.*

Miller, A. and Rusnock, P. 1993. The rise and fall of the silvicultural hypothesis in spruce budworm (Choristoneura fumiferana) management in eastern Canada. *Forest Ecology and Management* **61:** 171-189.

Morris, R. 1963. Spruce Budworm Dynamics *Memoirs of the Entomological Society of Canada no. 31.*

Muir, A. D., Gruber, M. Y., Hinks, C. F., Lees, G. L., Onyilagha, J., Soroka, J., and Erlandson, M. 1999. Effect of condensed tannins in the diets of major crop insects. In Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology. edited by Gross *et al.*, Kluwer Acdemic/Plenum Publishers, New York: pp. 867-881.

Nealis, V. G. and Régnière, J. 2004. Insect-host relationships influencing disturbance by the spruce budworm in a boreal mixedwood forest. *Canadian Journal of Forest Research* **34**: 1870-1882.

Nierenstein, M. 1934. The Natural Organic Tannins: History, Chemistry, Distribution. J. & A. Churchill Ltd., London: 319 pp.

Nomura, M. and Itioka, T. 2002. Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Applied Entomological Zoology* **37**: 285-289.

Park, C. G., Lee, K. C., Lee, D. W., Choo, H. Y., and Albert, P. J. 2004. Effects of purified persimmon tannin and tannic acid on survival and reproduction of bean bug, *Riptortus clavatus*. *Journal of Chemical Ecology* **30**: 2269-2283.

Panzuto, M. and Albert, P. J. 1998. Chemoreception of amino acids by female fourth- and sixth-instar larvae of the spruce budworm. *Entomologia Experimentalis et Applicata* **86**: 89-96.

Panzuto, M., Mauffette, Y., and Albert, P. J. 2002. Developmental, gustatory, and behavioral responses of leafroller larvae, *Choristoneura rosaceana*, to tannic acid and glucose. *Journal of Chemical Ecology* **28**: 145-160.

Prebble, M. L. 1975. Spruce budworm, *Choristoneura fumiferana* Clem., Introduction. in Aerial Control of Forest Insects in Canada: a view of control projects

employing chemical and biologiocal insecticides. edited by Prebble, M. L., Department of the Environment, Ottawa, Canada: pp. 76-84.

Raubenheimer, D. 1992. Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in locusts. *Ecology* **73**: 1012-1027.

Raubenheimer, D. and Simpson, S. J. 1990. The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behavior of larval *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskal). I. Short-term studies. *Physiological Entomology* **15**: 219-233.

Reese, J. C., Chan, B. G., and Waiss Jr., A. C. 1982. Effects of cotton condensed tannin, maysin (corn) and pinitol (soybeans) on *Heliothis zea* growth and development. *Journal of Chemical Ecology* **8**: 1429-1436.

Roehrig, N. E. and Capinera, J. L. 1983. Behavioral and developmental responses of range caterpillar larvae, *Hemileuca oliviae*, to condensed tannin. *Journal of Insect Physiology* **29**: 901-906.

Ribéreau-Gayon, P. 1972. Plant Phenolics (University Reviews in Botany, 3). Oliver and Boyd, Edinburgh: 254 pp.

Salminen, J. and Lempa, K. 2002 Effects of hydrolysable tannins on a herbivorous insect: fate of individual tannins in insect digestive tract. *Chemoecology* **12**: 203-211.

Sandoval, M. and Albert, P. J. 2007. Chemoreception of sucrose and amino acids in second and fourth instars of the spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *Journal of Insect Physiology* **53**: 84–92.

Schnuch, M. and Hansen, K. 1990. Sugar sensitivity of a labellar salt receptor of the blowfly *Protophormia terranovae*. *Insect Physiology* **36**: 409-417.

Schoonhoven, L. M. and Derksen-Koppers, I. 1973. Effects of secondary plant substances on drinking behavior in some Heteroptera. *Entomoogia Experimentalis et Appicata* **16**: 141–145.

Schoonhoven, L. M. 1977. Insect chemosensory responses to plant and animal hosts. in Chemical control of insect behavior. edited by Shorey, H. H. and McKelvey, J. J., John Wiley, New York: pp. 7-15.

Schoonhoven, L. M., W. M. Blaney, and Simmonds, M. S. J. 1992. Sensory coding of feeding deterrents in phytophagous insects. in Insect-Plant Interactions Vol. 4. edited by E. A. Bernays, CRC Press, Boca Raton: pp. 59–79.

Schoonhoven, L. M. and Jermy, T. 1977. A behavioural and elctrophysiological analysis of insect feeding deterrents. In Crop protection agents – their biological evaluation edited by McFarlane, N. R., Academic Press: pp. 133-146.

Schoonhoven, L. M. and van Loon, J. J. A. 2002. An inventory of taste in caterpillars: each species its own key. *Acta Zoologica Academiae Scientiarum Hungaricae* **48** (Suppl. 1): 215-263.

Schwowalter, T. D. 2006. Insect Ecology, An Ecosystem Approach 2nd ed. Academic Press, New York: 572 pp.

Simmonds, M. S. J. and Blaney, W. M. 1990. Gustatory codes in lepidopterous larvae. *Symposia Biologica Hungarica* **39**: 17-27.

Simpson, S. J. and Raubenheimer, D. 2001. The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. *Ecology* **82**: 422-439.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry 3rd ed. W. H. Freeman and Company, New York; 887 pp.

Steinly, B. A. and Berenbaum, M. 1985. Histophathological effects of tannins on the midgut epithelium of *Papilio polyxenes* and *Papilio glaucus*. *Entomologia experimentalis et applicata* **39**: 3-9.

Swain, T. 1977. Secondary plant substances as protective agents. *Annual Review of Plant Physioiology.* **28**: 479–501.

Swain, T. 1979. Chapter 19: Tannins and Lignins. In: Rosenthal, G. A. and Janzen, D. H. (eds.) Herbivores, their interaction with secondary plant metabolites. Academic Press, New York; pp 657-682.

Thomson, C. 2007. Tannins and the spruce budworm, *Choristoneura fumiferana* (Clemens). Biol 490 Independent Project Thesis, Concordia University, Montreal, Canada.

Todd, G. W., Getahun, A., and Cress, D. C. 1971. Resistance in barley to the greenbug, *Schizaphis graminum* 1, Toxicity of phenolic and flavonoid compounds and related substances. *Annals of the Entomological Society of America.* **64**: 718–722.

Toufexis, D. J., Albert, P. J., and Bauce, E. 1996. Age-related responses from the maxillary sensilla styloconica of *Choristoneura fumiferana* larvae to foliage extracts from balsam fir hosts. *Entomologia Experimentalis et Applicata* **78**: 129-133.

Triplehorn, C. A. and Johnson, N. F. 2005. Borror and Delong's Introduction to the Study of Insects 7th ed., Thomson Brooks/Cole Publishers: p. 85.

Loon, J. J. A. van 1996. Chemosensory basis of feeding and oviposition behaviour in herbivorous insects: a glance at the periphery. *Entomologia Experimentalis et Applicata* **80**: 7–13.

Wellington, W. G. 1948. The light reactions of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). *Canadian Entomologist* **80**: 56-82A.

Wilson, G. G. and D. G. Grisdale 1988. *Guidelines for rearing spruce budworm received from the Forest Pest Management Institute.* Forestry Canada: Ottawa, Canada.

Zou, J. and Cates, R. G. 1997. Effects of terpenes and phenolic and flavonoid glycosides from douglas fir on western spruce budworm larval growth, pupal weight, and adult weight. *Journal of Chemical Ecology* **23**: 2313-2326.