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INTERACTION BETWEEN THE EASTERN SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) [LEPIDOPTERA: TORTRICIDAE], AND WHITE SPRUCE, *PICEA GLAUCA* (MOENCH) VOSS, ITS HOST PLANT: VARIATIONS IN FOLIAR CARBOHYDRATE AND AMINO ACID CONTENT AND INSECT FEEDING BEHAVIOUR

Claude Guertin

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ABSTRACT

INTERACTION BETWEEN THE EASTERN SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) [LEPIDOPTERA: TORTRICIDAE], AND WHITE SPRUCE, *PICEA GLAUCA* (MOENCH) VOSS, ITS HOST PLANT: VARIATIONS IN FOLIAR CARBOHYDRATE AND AMINO ACID CONTENT AND INSECT FEEDING BEHAVIOUR

Claude Guertin, Ph.D.

Concordia University, 1991

Feeding Preferences and Feeding Rates of third- and sixth-instar larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), exposed to carbohydrate and amino acid fractions from currently defoliated and undamaged white spruce, *Picea glauca* (Moench) Voss, needles were measured to determine whether changes in feeding behaviour occurred during the period of insect larval development. In a first experiment, the insects were exposed to carbohydrate fractions of white spruce needles. No significant difference in Feeding Preferences of third- and sixth-instar larvae was found between currently defoliated and undamaged trees. Sugar fractions extracted from current- and one-year-old foliage have the same stimulating effect on the

Feeding Preferences for the two larval stages. On the other hand, significant differences were found between the relative Feeding Rates of early and late instar larvae. No significant difference was observed between the Feeding Preferences of early and late instar larvae. However, the relative amounts of discs eaten by the third-instar larvae were significantly higher compared to sixth-instar larvae. The second experiment was designed to test the feeding behaviour of the larvae exposed to amino acid fractions of white spruce needles. No significant difference was observed between the Feeding Preferences of early and late instar larvae. However, the relative amounts of discs eaten by the third-instar larvae were significantly higher compared to sixth-instar larvae. The possible role of feedback mechanisms regulating food intake is discussed.

Variations in concentrations of carbohydrates and amino acids in white spruce needles were monitored during the larval development period of the eastern spruce budworm. Results show that the total amount of carbohydrates found in the current-year needles increases during this period, and decreases in one-year-old needles. The common sugars found in the foliage were sucrose, glucose, fructose and raffinose. Finally, during the period of larval development, carbohydrate distribution did not seem to be affected by the current-year defoliation from the insect. However, the concentration of foliar amino acids was greater in current-year needles than in one-year-old needles.

This result is in accordance with the known suitability of young foliage for the spruce budworm larvae. The total amount of amino acids decreases in current- and one-year-old needles during the period of spruce budworm larval development. As previously observed with carbohydrates, no significant differences were found between currently defoliated and undamaged trees during this period. Variations in carbohydrates and amino acids seem associated to natural growth processes of the plant.

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INTRODUCTION

A. Project summary

The aim of this research is to investigate the feeding behaviour of early and late instar larvae of the eastern spruce budworm in relation to chemical changes in white spruce needles that can occur during regular plant growth or under current-year defoliation by insects. The specific objectives are **(1)** to establish whether the feeding behaviour of the immature stages of the spruce budworm changes during their development; **(2)** to follow modifications in some foliar constituents of white spruce needles during the period corresponding to the budworm's development; **(3)** to determine if these changes in the host plant provoke any changes in the feeding behaviour of the insect; **(4)** to identify variations in the phytochemistry of white spruce needles that may result from the current-year defoliation by the insect. It has been proposed that carbohydrate, amino acid and organic acid fractions of some spruce budworm host species might influence the feeding preference of the ultimate instar larvae. An understanding of the effect of these chemical products on younger instar larvae is essential considering that it is the second and third instar larvae which must choose whether to remain on the host after coming out of diapause.

B. General

The variety of life styles displayed by insects depends, to a large extent, on the co-evolution of insects and their food sources. The sensory perception of the chemical composition of a food source is a dominant factor influencing the insect's choice of food and its feeding behaviour (Albert 1980, Alfaro *et al.* 1980, Blaney and Simmonds 1988, Scriber 1984, Städler 1984, 1986). Phytophagous insects can be separated into different groups, according to the type of food plants they consume in their natural environment. Monophagy refers to insects that feed on plants of a single species. Other plant consuming insect are oligophagous, limiting themselves more to leaves from plants of the same family or closely related families. Finally, polyphagy refers to insects which consume material from several plant families.

The basis of food selection by plant feeding insects is largely dependent on the capacities of the herbivore to detect phytochemical compounds. Several reviews exist on insects' abilities to evaluate their food sources (see Schoonhoven 1969, Chapman and Blaney 1979, Dethier 1970, Städler 1980). Their chemosensory capabilities can alter different aspects of the feeding behaviour of an insect, and this can lead to a modification in the consumption and utilization of nutrients. With regard to the insect's evaluation of foods, four different behavioural feeding strategies can be distinguished. First, an

insect may continue its feeding behaviour in its current mode, rather than search for another food source. This decision is normally associated with the presence of continued favourable feeding stimuli. Second, an insect may initiate a compensatory response. Some insect species increase their feeding rate in response to a decline in the nutrient quality and quantity of the food sources (see review of Slansky and Rodriguez 1987). This process is intended to maintain the insect's growth rate. Third, an insect may respond to poor food quality or low food availability by searching for another source of nutrient. Finally, an insect may begin genetic processes that are triggered by environmental conditions. These processes are induced to maintain the optimal performance of the insect. The initiation of facultative diapause is one of the best examples (see Tauber and Tauber 1976).

Preferential feeding selection by phytophagous insects is influenced by the presence of plant nutritional compounds as well as secondary phytochemical products, as was earlier suggested by Kennedy and Booth (1951). Compounds such as alkaloids, flavonoids, volatile oils and glycosides were defined as secondary plant metabolites, because they were considered to have no specific physiological function for the plant, but to act as potent insecticides or insect repellents. Fraenkel (1959) was the first to suggest that secondary metabolites were responsible for the degree of feeding specificity of foliage-consuming insects. There is growing evidence that suggests a primary

role of plant secondary metabolites in host selection by the insect (see Rosenthal and Janzen 1979, Slansky and Rodriguez 1987, Barbosa and Letourneau 1987).

Secondary compounds are not the only factors to consider when studying insect feeding behaviour. Products of the plant primary processes provide essential nutritional materials for the insect. Moreover, the nutritional requirements of a given insect species may seriously affect predation on plants. Nutrient compounds such as amino acids (e.g., Bryant *et al.* 1987, Hollinger 1986, Cates *et al.* 1987, Karowe and Martin 1989) and carbohydrates (Capinera 1981, Albert *et al.* 1982, Ladd 1986, Honda and Matsumoto 1987, Adler 1989, Schiff *et al.* 1989) are found to influence the feeding behaviour of several insect species. The present thesis will focus on the relations between these groups of compounds - carbohydrates and amino acids - and the feeding behaviour of a defoliator insect species. The pairwise model used to describe this relation is the system of interactions existing between white spruce and the eastern spruce budworm.

C. Review of Literature

Biology of the Spruce Budworm

The eastern spruce budworm was officially named *Choristoneura fumiferana* by Clemens in 1895. The geographical distribution of this indigenous lepidopteran species (Miller 1963) in Eastern North America is delimited by Labrador, Virginia State, and the Mackenzie river and Yukon territories (Stehr 1967). In eastern Canada, the spruce budworm is principally associated with the boreal forest, the St-Lawrence and Acadian coniferous forest (Rowe, 1972). This insect is considered as a pest, because of its impacts on wood supply and its periodic eruptive type of outbreak (Berryman 1986). During an epidemic period, important damages are observed on its host plants. Balsam fir, *Abies balsamea* (L.) Mill., and white spruce, *Picea glauca* (Moench) Voss, are recognized as the preferential host plants for the spruce budworm larvae. To a lesser extent, the insect also feeds on red spruce, *P. rubens* Sarg., and black spruce *P. mariana* (Mill.) B.S.P. Occasionally, the species attacked include eastern hemlock, *Tsuga canadensis* (L.) Carr., tamarak, *Larix laricina* (Du Roi) K. Koch and white pine, *Pinus strobus* L. Usually, mortality of these plants occurs following five years of successive severe attacks (Belyea, 1952).

The life cycle of the spruce budworm begins with the oviposition of eggs in elongate masses in late July and early August. The oviposition rate is

approximately 180 eggs per female (Talerico 1984). A large proportion of egg masses are found on needles near the periphery of the crown. All larvae progress through a series of developmental stages that are referred to as larval instars. Emergence of first-instar larvae is usually completed by mid-August. An important dispersion (ballooning) of the larvae occurs at about the same time. Larvae that remain on, or disperse to host material, spin hibernacula (cocoon-like shelters) and moult to the second-instar. Usually, no feeding activity occurs between the emergence of larvae and the hibernation of the second larval stage. In spring, the overwintering larvae emerge. At this time, a second important dispersion of the young immature stages takes place. This period also corresponds to the initiation of larval feeding activities. At the beginning, as the new plant material is not available, most second-instars mine needles of one-year-old foliage, while few attack the vegetative buds. Later, the second-instar larvae moult, and third-instar larvae begin to feed on new foliage. The third-instar to sixth-instar larval period persists from early June to early July, and most of the feeding takes place during the sixth-instar. The pupal stage, which varies from 8 to 12 days, occurs about mid-July. Following this period emergence of the adults occurs.

Feeding Behaviour of the Spruce Budworm

Numerous investigations of the behavioural and physiological feeding mechanisms of spruce budworm larvae have been conducted over the past decades. Feeding preferences of larvae for their common host plants were first studied by Heron (1965). More recently, feeding responses of sixth-instar larvae for polar phytochemical extracts from evergreen host plants have been reported (Albert and Jerrett 1981, Albert 1982, Albert and Parisella 1985ab, 1988a). The general conclusion from these studies is that the sugar extracts of the host plants seem to be the most stimulating (Albert and Parisella 1985b). Albert *et al.* (1982), using twelve pure carbohydrates, also observed a strong and significant preference for sucrose. Albert and Parisella (1988b) found that the behavioural responses of the sixth-instar larva to sucrose stimuli were closely correlated with its electrophysiological responses.

Amino acids are also know to have significant effects on the feeding behaviour of the spruce budworm larvae. Phagostimulant characteristics of proline (Heron 1965), arginine and glutamic acid (Albert and Jerrett 1981), alanine, lysine, serine and valine (Albert and Parisella 1988a) have been determined for sixth-instar larvae. Albert (1982) also indicated that amino acid fractions extracted from common host plants have significant phagostimulant properties for the late spruce budworm larvae. However, the general preference patterns demonstrate that the amino acid fractions show

less phagostimulant properties than carbohydrate extracts. Albert and Parisella (1985b) showed that the organic acid fractions of different host plants are either neutral and deterrent, but that this effect can be masked by the presence of other phagostimulant products.

White spruce is considered the most suitable host for the spruce budworm larva (Koller and Leonard 1981, Lavallée and Hardy 1988). This species is also more desirable for the larvae because of the abundance of the foliar biomass (Greenbank 1963), and the quality of the food its provides (Koller and Leonard 1981). Albert and Guertin (1991) indicated that the larval feeding preference for this host can be associated with the stimulating properties of the sugars and amino acids and the less deterrent property of the organic acids found in white spruce. Durzan and Lopushanski (1968) observed that the proportion of amino acids in tissues of the fifth-instar larvae was greater when the insects were fed white spruce needles. When fed with white spruce foliage, insects exhibit faster growth and greater fecundity than when fed with balsam fir foliage (Koller and Leonard 1981, Lavallée and Hardy 1988).

D. Goals and Objectives

The nutritional quality of a host plant is considered a major factor in the

population dynamics of defoliator insects (House 1969, McClure 1980). However, the composition of nutrient compounds found in a plant can be substantially modified during the course of its development (Kramer and Kozlowski 1979) and following defoliation stress (e.g., Feeny 1970, Ericsson *et al.* 1985, Niemela *et al.* 1984, Baldwin and Schultz 1983, Haukioja and Neuvonen 1985, Mattson *et al.* 1988). These variations in phytocomposition may have outcomes favourable or unfavourable to the feeding responses of the insect larvae.

The goal of this study is to determine how variations in the phytochemistry of white spruce host plants can influence the feeding behaviour of the spruce budworm larvae during their development. Two main assumptions are associated with this approach. First, since the chemical composition of the plant changes during its period of growth, it would be logical to assume that the insect's feeding preferences would change also over its larval instars to accommodate to this. Second, insect defoliation should provoke a stress on the plant, that may change the specific composition of the foliage. The specific objectives are (**1**) to establish whether the feeding behaviour of the immature stages of the spruce budworm changes during their development; (**2**) to follow modifications in some phytochemical constituents of the needles during the period corresponding to the insect's larval development; based on the above, (**3**) to determine if these plant modifications provoke any

changes in the feeding behaviour of the larvae; and finally, (**4**) to identify variations in phytochemistry of white spruce needles that may result from the cuurent-year defoliation by the insect.

CHAPTER 1

Feeding Preferences and Feeding Rates of Two Larval Instars of Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), for Pure Sucrose and Sugar Extracts of White Spruce

A. Abstract

Feeding behaviour of two larval instars of the spruce budworm, *Choristoneura fumiferana* (Clem.), was studied using sucrose and white spruce sugar extracts. Feeding Preferences were similar for third- and sixth-instar larvae. Relative Feeding Rates on plant sugar extracts were higher for third- than for sixth-instar larvae. When sugar fractions from early and late growth current-year foliage were used, Relative Feeding Rates were the greatest for third- and sixth-instar larvae respectively.

B. Introduction

The eastern spruce budworm, *Choristoneura fumiferana* (Clem.), is a major pest of several coniferous trees in eastern Canada. Larval development occurs on current year foliage of balsam fir, *Abies balsamea* (L.) Mill., white

spruce, *Picea glauca* (Moench) Voss, and to a lesser extent other spruce species.

Numerous investigations of the behavioural and physiological feeding mechanisms of spruce budworm larvae have been conducted over the past decades. Feeding preferences of larvae for their common host plants were first studied by Heron (1965). More recently, feeding responses of sixth-instar larvae for phytochemical extracts from evergreen host plants have been reported (Albert and Jerrett 1981, Albert 1982, Albert and Parisella 1985ab, 1988a). The general conclusion from these studies is that the sugars extracts %' 0 ^[ost plants seem to be the most stimulating. Albert *et al.* (1982), using twelve pure carbohydrates, also observed a strong and significant preference for sucrose.

White spruce is considered the most suitable host for the spruce budworm larvae (Koller and Leonard 1981, Lavallée and Hardy 1988). Albert and Guertin (1991) indicated that the larval feeding preference for this host can be associated with the stimulating properties of the sugars and the less deterrent properties of the organic acids found in white spruce. Albert and Parisella (1988b) found that the physiological responses of the sixth-instar larva to sucrose stimuli were closely correlated with its behavioural responses.

The chemical composition of a plant changes during the course of its growth. The important utilization of carbohydrates in the production of new plant tissues (Kramer and Kozlowski 1979) should considerably influence the phytocomposition of the sugar fraction of the current year's needles. The effects of these changes on the feeding behaviour of an insect are unknown. The purpose of the present study was to evaluate and compare the feeding preferences and feeding rates of third- and sixth-instar larvae of the spruce budworm for the sugar fraction of white spruce. First, we wanted to determine if the feeding behaviour of these immature stages changed during their development and, secondly, to determine if the modifications in their host plant provoked any changes in their feeding behaviour.

C. Materials and Methods

Insects

Unfed second-instar larvae of *C. fumiferana* were obtained from the Forest Pest Management Institute, Sault Ste Marie, Ontario. They were reared on McMorran's (1965) artificial diet, and placed in an incubator with 16:8 light:dark (L:D) photoperiod, 25 °C and 60 % relative humidity (R.H.). Third- and sixth-instar larvae were identified by head capsule width (McGugan 1954). The insects were starved 24 h prior to tests, and were used only once. Any larva which moulted during the experiment was rejected.

Plants

Three-year-old white spruce plants were obtained from the Laurentian Forestry Centre, Ste Foy, Québec. In 1988 and 1989, 12 trees were placed outside in cages (30 x 30 x 45 cm, covered with 60-mesh nylon) to prevent any external contamination by insects, birds, etc. During the period corresponding to the spruce budworm larval development, a weekly sampling of the foliage was done. Needles of the current year's growth of three plants were removed each week and kept at -15 °C until used in extractions.

Extraction of Sugars

The foliage was analyzed separately for each tree at each collecting period. The needles were freeze-dried and ground into a fine powder using a Wiley Mill (40 mesh). The percentage moisture content of each sample was evaluated as the difference between the fresh and the dry weights.

The sugar fraction was recovered using the procedures described by Dickson (1979). The freeze-dried needles were placed in a solution of methanol, chloroform, and water (12:5:3) and the polar extracts were recovered. The sugar fractions were then separated by ion-exchange chromatography on AG50-X8 resin (Bio-Rad Lab.) followed by AG1-X8 resin. Based on the weight, and the percentage moisture content of the foliage

collected, the sugar extracts were redissolved in a proper volume of distilled water to reach a final concentration equal to that found in each plant.

Bioassay

The two-choice feeding test developed by Jermy *et al.* (1968), and modified by Maloney *et al.* (1988) for the fourth-instar larvae, and by Albert *et al.* (1982) for the sixth-instar larvae, was used to evaluate the feeding preferences and feeding rates of the third- and sixth-instar larvae. Cellulose nitrate discs were punched from 0.45 μm pore size filter paper strips (Sartorius). Each feeding arena consisted of four discs of 3.3 mm diameter for the third-instar larvae, and eight discs of 6.5 mm diameter for the sixth-instar larvae placed by alternating control and test discs in a circular fashion (Appendix 1). Test discs in an arena were impregnated respectively with a 3 or 8 μl aliquot of 25 m*M* sucrose (Aldrich Chemical Co.) or with the same volume of extracted sugar fractions. Every control disc in all experiments was impregnated with distilled water.

During each trial, one larva was placed in each arena and allowed to feed for 24 h at 25 °C, 60% R.H. and 16:8 (L:D) photoperiod. After the test period, the amount of each disc eaten was visually estimated. For each experiment, the Feeding Preference, corresponding to the Mean Percentage Consumption of test discs (MPC_t) versus control discs (MPC_c), the Feeding

Rates (FR), and the Relative Preference Index (RPI) were calculated as follows:

$$MPC_t = [T / (T + C)] \times 100$$

$$MPC_c = 100 - MPC_t$$

$$FR = [(T + C) \times sdisc / 100] / h$$

$$RPI = (T - C) / (T + C),$$

where T is the proportion eaten on the test discs, C is the proportion eaten on control discs, *sdisc* is the surface area of discs (mm^2), and h is the time in hours. A Feeding Rate Index was developed to minimize this effect of budworm instar size dimorphism using the following formulae:

$$FRI_3 = (FR + 1.49) / 1.55$$

$$FRI_6 = (FR - 1.49) / 1.55$$

where FRI_3, and FRI_6 are the Feeding Rate Indices of third- and sixth-instar larvae, and FR is the feeding rate previously calculated. This index was based on the mean FR results obtained with 25 m*M* sucrose by the third- and sixth-instar larvae. After transformation, Feeding Rate Indices of the two larval stages were equal to 1 when exposed to sucrose.

Statistical Methods

Since no differences were found between trees from the same collecting period, data were pooled. The Mean Percent Consumption data did not conform to a normal distribution, and results were analyzed with Wilcoxon's Signed-Ranks test (Sokal and Rohlf 1969). The Relative Preference Index and

Feeding Rate Index were used to compare the preferences of the early- and late-larval instars for a tree's extract. The results were normally distributed, and individual *t*-tests on means of each feeding estimator measured were performed on the results of third- versus sixth-instar larvae using the SAS TTEST procedure (SAS Institute Inc. 1985). Waller-Duncan's multiple-range tests from GLM procedure (SAS Institute Inc. 1985) were performed to determine if each and every pair of mean values corresponding to a larval instar did not differ significantly. For all statistical tests, the level of rejection was set at $\alpha = 0.05$.

D. Results and Discussion

Control Experiment

The initial step in this study was to test and compare the feeding responses of spruce budworm larvae for 25 m*M* sucrose. Larvae showed a significant preference for test discs containing sucrose over discs wetted with only distilled water (*Figure 1*). The strong response of larvae for this carbohydrate was consistent with the findings of Albert *et al.* (1982) for the sixth-instar and Maloney *et al.* (1988) for the fourth-instar larvae. No significant differences were found between the Relative Preference Index of the third- (0.84 ± 0.46, $n = 61$) and sixth-instar larvae (0.76 ± 0.21, $n = 56$) for

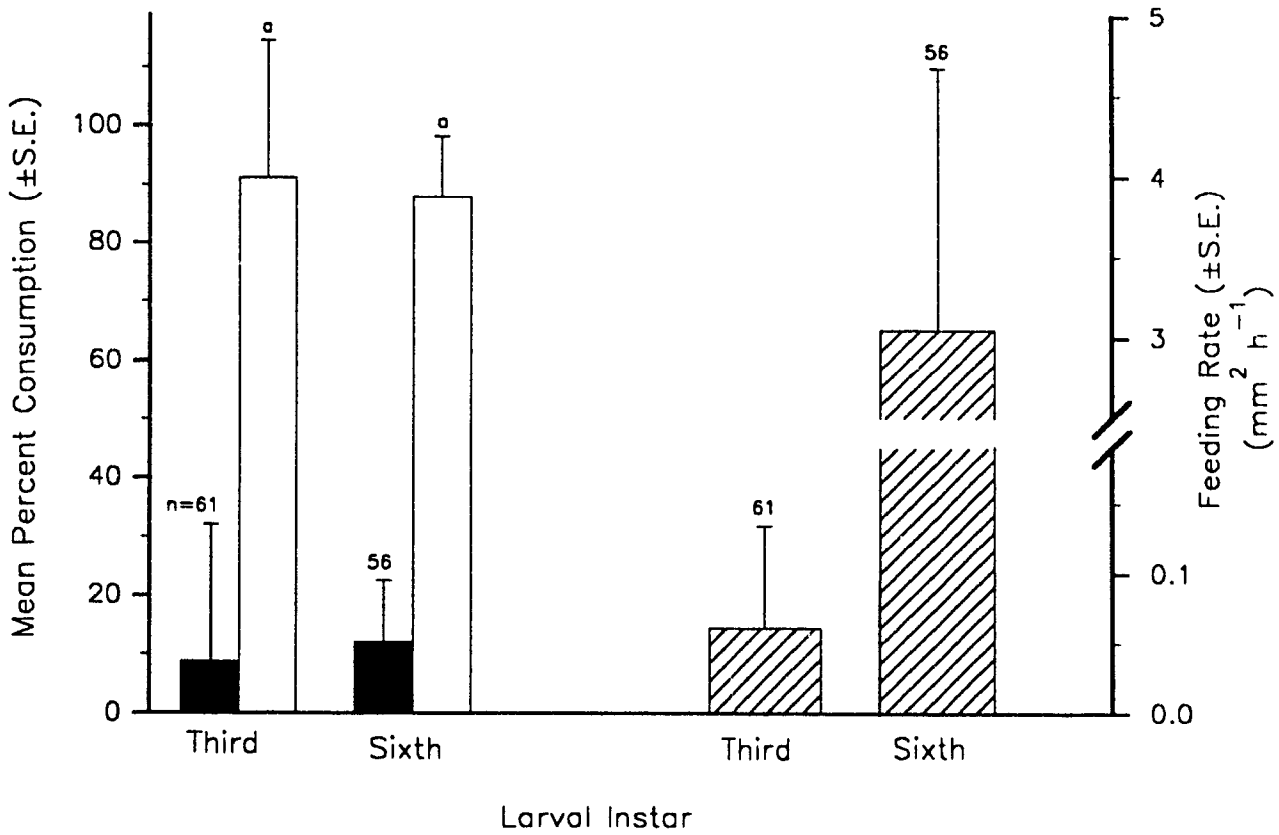

Figure 1. Percent Total Consumption and Feeding Rates of third- and sixth-instar larvae of eastern spruce budworm for control (distilled water) and test (25 m*M* sucrose) discs: ■ consumption on control discs; □ consumption on test discs; ▨ Feeding Rates; a, significant difference between control and test disc ($\alpha < 0.001$, Wilcoxon's Signed-Ranks test).

sucrose ($p > 0.05$, t-test), underscoring the strong feeding stimulant effect of this compound on the eastern spruce budworm larvae.

Feeding Rates were significantly lower for third- than for sixth-instar larvae ($p < 0.001$, t-test). The body size of each larval instar, 3.0 to 5.0 mm for the third- and 15 to 24 mm for the sixth-instar larvae (Titus 1977), could be the principal factor explaining this significant difference.

White Spruce Sugar Extract Experiments

In 1988 and 1989, for the early- and late-larval instars of the spruce budworm, the Mean Percent Consumption of control and test discs were characterized by a distinct and significant preference for the white spruce sugar extracts (*Table 1*). Sugars, which occur in appreciable quantities in plant tissues, are an important group of compounds that generally stimulate feeding in many phytophagous insect species (e.g., Heron 1965, Dethier 1966, Schoonhoven 1969, Albert *et al.* 1982, Ladd 1986, Adler 1989). The phagostimulant effect of sugars is characterized by a dose-dependent correlation between the physiological (Albert and Parisella 1988a) and behavioural (Heron 1965) responses of spruce budworm larvae.

For each year and each collecting period, no difference was found between the Relative Preference Index of third- and sixth-instar larvae for

Table 1. Percent consumption of control and test discs of third- and sixth-instar larvae of *Choristoneura fumiferana* for white spruce sugar fractions.

Year	Instar	Period[a]	Mean % Consumption Control[b]	Test[c]	±S.E.	n	p[d]
1988	3	138	10.00	89.92	±2.91	45	0.0000
		146	7.05	92.95	±1.92	50	0.0000
		152	12.03	87.97	±2.99	41	0.0000
		159	4.49	95.51	±2.27	23	0.0000
	6	138	10.17	89.83	±1.59	49	0.0000
		146	10.32	89.68	±1.65	45	0.0000
		152	12.36	87.64	±1.61	49	0.0000
		159	10.39	89.62	±2.74	38	0.0000
1989	3	144	20.40	79.56	±5.80	19	0.0016
		151	17.68	82.32	±5.19	34	0.0001
		158	15.85	84.15	±2.85	53	0.0000
		165	9.50	90.50	±2.79	50	0.0000
	6	144	11.18	88.82	±2.58	19	0.0001
		151	8.36	91.64	±1.10	37	0.0000
		158	8.52	91.48	±1.54	51	0.0000
		165	11.40	86.07	±1.30	49	0.0000

[a] Julian date.
[b] Distilled water for all experiments.
[c] Sugar fractions.
[d] Wilcoxon's Signed-Ranks test, probability value.

sugar extracts with the exception of period 158, which indicated a significant difference between the two larval stages (p = 0.0272) (*Table 2*). Moreover, an analysis of variance indicated that this index was not affected by the time of tree sampling (*Table 3*). This is an agreement with the previous results obtained with 25 m*M* sucrose (*Figure 1*). Results suggest that the capacities of perception of the larvae were similar during their larval development. For several insect species, the perception of chemical compounds synthesized by plants is known to form the basis of the larval feeding behaviour (Dethier 1970, Albert 1980). The reduction in sugar content that occurs during the production of new plant tissues (Kramer and Kozlowski 1979) seems, in this case, to never reach a particular level where modifications of the feeding preference of the larvae can be observed.

The Feeding Rate Index data were significantly influenced by the larval stage of the insect (*Table 4*). The results show that relative responses of the sixth-instar larvae were significantly lower then those observed for third-instar larvae. On the other hand, the relative amount of test discs eaten by each larval stage was influenced by the plant collecting period, except for the sixth-instar of 1988 which was nonsignificant (p = 0.2046) (*Table 5*). Without exception, the Feeding Rate Index data differed between discs imbibed with sugar extracts from plants collected at the beginning and those collected at the end of the larval development period (*Figure 2*). The relative amount of discs

Table 2. Relative Preference Indices of third- and sixth-instar larvae of *Choristoneura fumiferana* for white spruce sugar extracts.

Year	Period[a]	Relative Preference Index (±S.D.)						p^b
		n	Third		n	Sixth		
1988	138	45	0.798	(±0.391)	49	0.797	(±0.223)	0.9780
	146	50	0.859	(±0.273)	45	0.794	(±0.222)	0.2005
	152	41	0.760	(±0.384)	49	0.753	(±0.225)	0.9183
	159	23	0.910	(±0.218)	38	0.792	(±0.337)	0.1404
1989	144	19	0.591	(±0.504)	19	0.776	(±0.225)	0.1533
	151	34	0.646	(±0.606)	37	0.833	(±0.134)	0.0722
	158	53	0.683	(±0.415)	51	0.830	(±0.219)	0.0272
	165	50	0.810	(±0.394)	49	0.772	(±0.182)	0.5419

[a] Julian date.
[b] t-Test, probability value.

Table 3. Analysis of variance of Relative Preference Indices of third- and sixth-instar larvae of eastern spruce budworm for plant collecting period factor.

Year	Instar	Source of Variation	df	SS	MS	F	*p*
1988	3	Collecting Period	3	0.432	0.144	1.29	0.2790
		Error	155	17.274	0.111		
		Total	158	17.706			
	6	Collecting Period	3	0.062	0.021	0.33	0.8059
		Error	177	11.201	0.063		
		Total	180	11.264			
1989	3	Collecting Period	3	0.940	0.313	1.43	0.2356
		Error	152	33.248	0.219		
		Total	155	34.188			
	6	Collecting Period	3	0.128	0.04	1.17	0.3244
		Error	152	5.557	0.037		
		Total	155	5.685			

Table 4. Feeding Rate Indices of third- and sixth-instar larvae of *Choristoneura fumiferana* for white spruce sugar extracts.

		Feeding Rate Index (±S.D.)						
Year	Period[a]	n	Third		n	Sixth		p[b]
1988	138	45	1.178	(±0.182)	49	-0.518	(±0.299)	0.0001
	146	50	1.120	(±0.121)	45	-0.413	(±0.306)	0.0001
	152	41	1.203	(±0.172)	49	-0.413	(±0.314)	0.0001
	159	23	1.086	(±0.094)	38	-0.409	(±0.251)	0.0001
1989	144	19	1.165	(±0.141)	19	-0.515	(±0.271)	0.0001
	151	34	1.132	(±0.103)	37	-0.423	(±0.226)	0.0001
	158	53	1.163	(±0.121)	51	-0.480	(±0.276)	0.0001
	165	50	1.086	(±0.076)	49	-0.334	(±0.253)	0.0001

[a] Julian date.
[b] t-Test, probability value.

Table 5. Analysis of variance of Feeding Rate Indices of third- and sixth-instar larvae of eastern spruce budworm for plant collecting period factor.

Year	Instar	Source of Variation	df	SS	MS	F	*p*
1988	3	Collecting Period	3	0.289	0.097	4.21	0.0068
		Error	155	3.553	0.023		
		Total	158	3.843			
	6	Collecting Period	3	0.405	0.135	1.54	0.2046
		Error	177	15.467	0.087		
		Total	180	15.872			
1989	3	Collecting Period	3	0.178	0.059	5.15	0.0020
		Error	152	1.750	0.012		
		Total	155	1.927			
	6	Collecting Period	3	0.717	0.239	3.61	0.0148
		Error	152	10.059	0.066		
		Total	155	10.776			

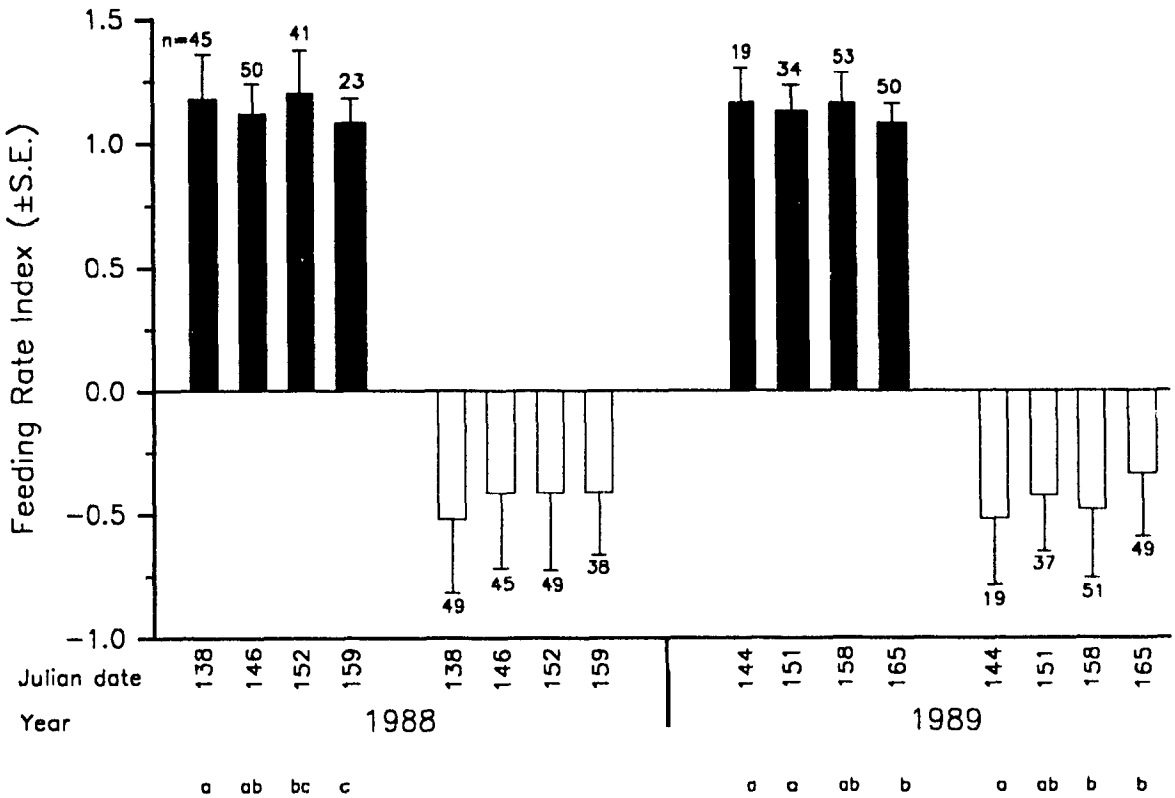

Figure 2. Feeding Rate Indices related to the plant collecting period: ■ third-instar larvae; □ sixth-instar larvae. For each year and instar larvae, data with same letter are not different ($p < 0.05$, Waller-Duncan's test).

eaten was greater on those discs wetted with early-plant extracts for third-instar larvae, and greater on discs wetted with late-plant extracts for sixth-instar larvae. It is generally assumed that food consumption by late-larval instars normally represents a large proportion of the total consumption for the whole larval stage (see Scriber and Slansky 1981). However, the relative performance rate tends to decline during larval development (Slansky and Scriber 1985). This reduction seems to be associated with a decrease in the assimilation rate, which in turn is directly associated with the relative consumption rate. The real implication of this and other factors on the performance rate are not understood.

Differences observed between the Relative Feeding Rate on the sugar fractions of early- and late-growth current year foliage may reflect modifications in the feeding habits that can be associated with changes in the food quality. During plant growth, modifications of the quantity and quality of carbohydrates should be normally observed in the foliage, because they are required for the production of new plant tissues (Kramer and Kozlowski 1979). As previously indicated, the quantity of carbohydrates found in early- and late-growth white spruce foliage seems not to influence the feeding preference of the early- and late-larval instars, and may not contribute to explain differences in Relative Feeding Rate. However, the specific composition of the white spruce sugar fraction can be a hypothetical factor that may explain these

differences. In this sense, the carbohydrate composition of the early-growth foliage can be more suitable for the young-instar larvae, and late-growth white spruce needles can represent a more appropriate source of food for the late-instar. Therefore, more investigations should be made to verify if any change in the chemical composition of the sugar fraction occurs during plant development, and to estimate how this variation can affect the feeding rate of larval instars.

CHAPTER 2

Variation in the Foliar Carbohydrate Composition of White Spruce, *Picea glauca* (Moench) Voss, in Relation to Current-Year Defoliation by the Spruce Budworm, *Choristoneura fumiferana* (Clem.)

A. Abstract

Variations in foliar carbohydrates of white spruce, *Picea glauca* (Moench) Voss, were monitored during the larval development period of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). Results show that the total amount of carbohydrates found in the current-year needles increases during this period, and decreases in one-year-old needles. The common sugars found in the foliage were sucrose, glucose, fructose and raffinose. Finally, during larval development, carbohydrate distribution seems not to be affected by the insect's current-year defoliation.

B. Introduction

During the process of growth, changes in the phytochemical compounds occur in a plant. Seasonal changes in carbohydrates of coniferous trees have been shown in various tissues and organs (Kozlowski and Keller 1966, Kruger

1967, Little 1970). During summer, an important decrease in foliar carbohydrates is expected. This change is mainly due to the utilization of a substantial amount of carbohydrates for the intensive growth process of the plant that occurs during this period (Kramer and Kozlowski 1979). Also, defoliation has been described as a factor that can affect the chemical characteristics of coniferous leaves (Ericsson et al. 1980, 1985). Considering that evergreen species retain a large fraction of the reserves in leaves rather than stems and roots (Kozlowski and Keller 1966), herbivory on foliage should result in an important loss in nutrient and carbon resources, as well as an inherent loss in photosynthesic capacity of the plant (Mooney and Gulmon 1982). In fact, defoliation should normally induce an important depletion of the carbohydrate reserves. Such changes in the sugar composition should modify the resistance and the palatability of the foliage as a food source for leaf-feeding insects.

White spruce, *Picea glauca* (Moench) Voss, is a common host of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). This host-plant is recognized to be the most suitable for budworm larval development (Koller and Leonard 1981, Lavallée and Hardy 1988). Carbohydrates are common constituents of white spruce needles, and are known to strongly influence the feeding preferences of larval instars (Chapter 1). Albert and Guertin (1991) suggest that the specific phagostimulant property of sugars may be an

important factor explaining the feeding preference of budworm larvae for this host. Since carbohydrates found in white spruce needles provide important stimuli to spruce budworm feeding behaviour, experiments were first designed to follow changes in the carbohydrate pattern during the period corresponding to the spruce budworm's larval development. Secondly, this study was conducted to identify variations in the sugar constituents that may result from current-year defoliation by the insect.

C. Materials and Methods

Plant material and experimental design

Three-year-old white spruce plants were obtained from the Laurentian Forestry Centre, Sainte Foy, Québec. In 1988 and 1989, 24 trees were placed outside in insectarium cages (30 x 30 x 45 cm, shielded with 60-mesh nylon) to prevent any external contamination and predation. The experimental design used to compare the variation in carbohydrates between currently defoliated and undamaged white spruce was a two-factor randomized block design with three replicates. The density of insects found on a plant was the first factor considered in this experiment. This factor occurred at two different levels, with or without larvae. Fifteen second-instar spruce budworm larvae per plant were introduced on 50 % of the trees shortly after budbreak, and these plants were identified as treated plants. The insects were obtained from

the Forest Pest Management Institute, Sault Sainte Marie, Ontario. The control trees were remaining plants which developed under the same conditions, but without the presence of insects. The second factor considered was the number of weeks during which development of the insect larvae took place. In 1988 and 1989, four levels were associated with this factor. The level of defoliation for each period are presented in Appendix 2.

In each block, 8 white spruce trees which corresponded to a specific combination of each factor, were randomly placed. During the period corresponding to the spruce budworm larval development, one control and one treated tree from each block were collected each week, and all current-year and one-year-old needles were removed from about 50 % tree height, frozen in liquid nitrogen and then stored at -15 °C until used in extractions.

Carbohydrate analysis

The technique used for the extraction of carbohydrates has been previously described in Chapter 1. Freeze-dried needles collected on defoliated and undamaged plants were extracted using a solution of methanol, chloroform, water (12:5:3), according to the method of Dickson (1979). The carbohydrates were separated from amino and organic acids by chromatography on ion-exchange columns containing AG50-X8 resin (Bio-Rad

Lab.) and AG1-X8 resin. The sugars were recovered by further elution with water.

Carbohydrates were separated, identified and quantified by injecting a diluted sample into a HPLC (Waters) interfaced with a DIGITAL computer. Chromatography was done on a Waters 10 mm Sugar-Pack (6.5 x 300 mm) column kept at 90 °C. Ethylene-diaminetetraacetic acid (EDTA, 50 mg/l of water) was the mobile phase; the pump gradient was set at 0.5 ml/min, and the resolution time was fixed at 25 min. One injection of 25 μl was made for each sample from a 1·10 dilution to get all peaks on the scale. A separate standard was prepared consisting of a solution of different carbohydrates (1 mg/ml of water). The quantification of plant sugars was obtained by comparison with this standard.

Statistical Methods

Analyses of variance (PROC GLM; SAS Institute Inc 1985) were performed to measure effects of the blocks, treatments and time, and interactions between these two last factors on the carbohydrate content of white spruce needles. To test for significant differences between each and every pair of means, Waller-Duncan's multiple-range tests were performed. For all statistical tests, the level of rejection was set at $\alpha = 0.05$.

D. Results

Total Carbohydrate Content

To measure the effect of different parameters on the total carbohydrate content, an analysis of variance was done for each year and for current- and one-year-old foliage (*Table 6*). In all cases, the total sugar found in the plant was significantly influenced by the collecting period factor. Moreover, the analysis indicated that the presence or absence of insects on leaves was not affecting the proportion of carbohydrates found in the tree, with the exception of current-year foliage of 1988 (p = 0.0032). No interaction was observed between the insect density factor and collecting period factor. Total sugar concentrations found in current-year needles increased during the period of larval development (*Figure 3*). Total sugars also decreased during the same period in one-year-old foliage.

Carbohydrate Specific Composition

In 1988 and 1989, fructose, glucose, raffinose, and sucrose were the principal carbohydrates found in white spruce needles. More specifically, the sucrose content of the expanded shoot increased slightly during budworm larval development (*Figure 4*). On the other hand, it strongly decreased in the one-year-old foliage. The collecting period factor significantly affected the

Table 6. Analysis of variance of total carbohydrate found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	1	Block	2	2569958.27	0.89	0.4356
		Treatment	1	37825520.27	13.05	0.0032
		Collecting Period	3	34793281.88	12.00	0.0005
		Treatment x Period[b]	3	2842538.94	0.98	0.4320
		Error	13	2899192.25		
1988	2	Block	2	8000.26	0.02	0.9812
		Treatment	1	144082.95	0.34	0.5696
		Collecting Period	3	11219913.51	26.62	0.0001
		Treatment x Period	3	455999.45	1.08	0.3938
		Error	13	421477.08		
1989	1	Block	2	1306212.15	0.57	0.5860
		Treatment	1	951308.82	0.41	0.5364
		Collecting Period	3	22760616.24	9.89	0.0033
		Treatment x Period	3	516940.70	0.22	0.8032
		Error	13	2302185.00		
1989	2	Block	2	234273.31	0.61	0.5601
		Treatment	1	980049.63	2.56	0.1381
		Collecting Period	3	9552713.83	24.93	0.0001
		Treatment x Period	3	443730.18	1.16	0.3694
		Error	13	383251.31		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor.

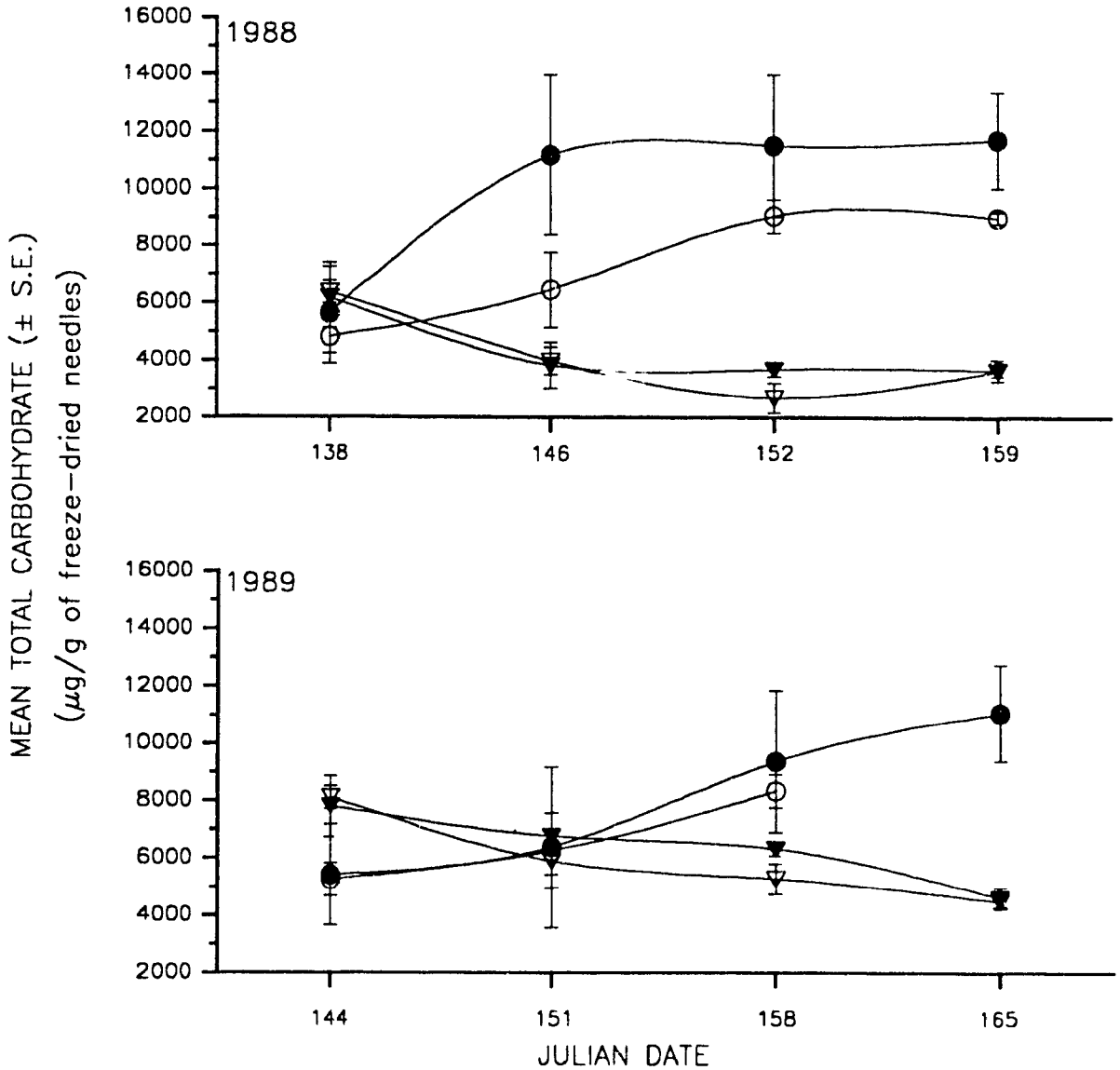

Figure 3. Mean total carbohydrate content found in 1988 and 1989 in current- and one-year-old foliage of white spruce: —●—●—● control and —○—○—○ defoliated current-year needles; —▼—▼—▼ control and —▽—▽—▽ defoliated one-year-old needles.

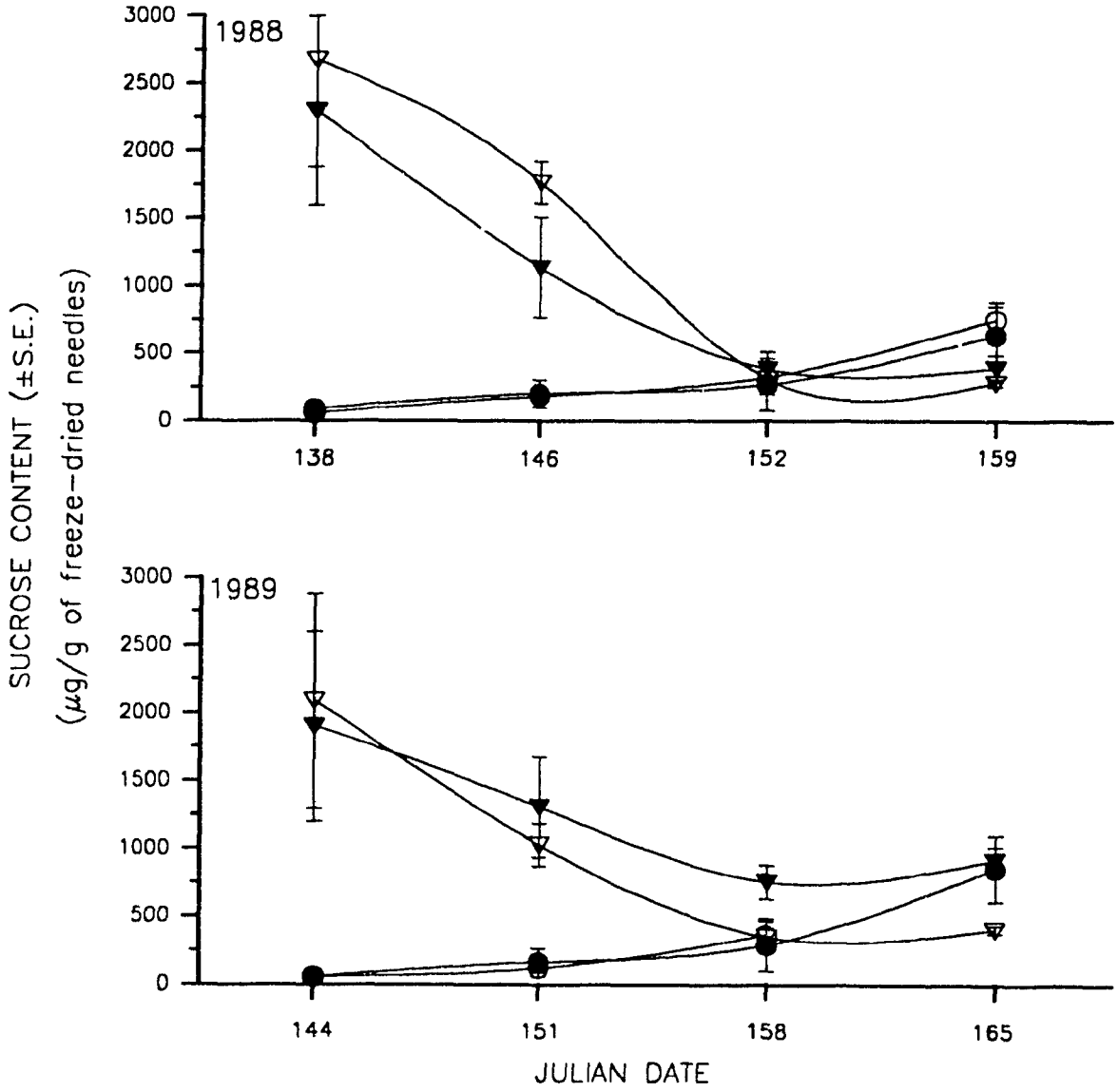

Figure 4. Sucrose content found in current- and one-year-old foliage of white spruce. See *Figure 3* for legend.

sucrose content of young and mature needles (*Table 7*). The general distribution patterns of glucose in current- and one-year-old leaves (*Figure 5*) were similar to those observed for total carbohydrate. Variance analysis showed that collecting period had a significant effect on the glucose concentration of the new shoots (1988, $p = 0.007$; 1989, $p = 0.0392$), and did not seem to influence the glucose concentration of the old foliage (*Table 8*). Raffinose was detected only in one-year-old needles (*Figure 6*). Analysis of variance indicated that raffinose concentration was not affected by the measured factors during the period of spruce budworm larval development (*Table 9*). Finally, the analysis of variance indicated that the collecting period as well as the treatment influenced the distribution of fructose in current-year needles of 1988 and 1989 (*Table 10*). No interaction between these two factors had been observed in these cases. The measured parameters did not seem to influence the concentration of fructose in one-year-old needles of 1988. However, there was evidence for a small interaction between the collecting period and the treatment for old foliage of 1989 ($p = 0.0433$).

E. Discussion

During the period corresponding to the spruce budworm larval development, variations in total carbohydrates were found in current- and one-year-old needles (*Figure 3*). The concentration of carbohydrates found in

Table 7. Analysis of variance of sucrose found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	1	Block	2	3436.84	0.19	0.8315
		Treatment	1	4322.13	0.24	0.6357
		Collecting Period	3	425401.21	23.16	0.0001
		Treatment x Period[b]	3	7125.75	0.39	0.7636
		Error	13	18364.98		
1988	2	Block	2	237744.76	0.11	0.8955
		Treatment	1	243176.55	1.14	0.3068
		Collecting Period	3	5981102.56	28.03	0.0001
		Treatment x Period	3	156388.16	0.73	0.5521
		Error	13	213408.05		
1989	1	Block	2	1479.96	0.11	0.8956
		Treatment	1	178.02	0.01	0.9103
		Collecting Period	3	384571.09	29.01	0.0001
		Treatment x Period	3	3610.06	0.27	0.7676
		Error	13	13254.50		
1989	2	Block	2	512775.73	1.70	0.2265
		Treatment	1	131505.76	0.44	0.5221
		Collecting Period	3	2236905.18	7.44	0.0054
		Treatment x Period	3	133599.81	0.44	0.7262
		Error	13	300779.69		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor.

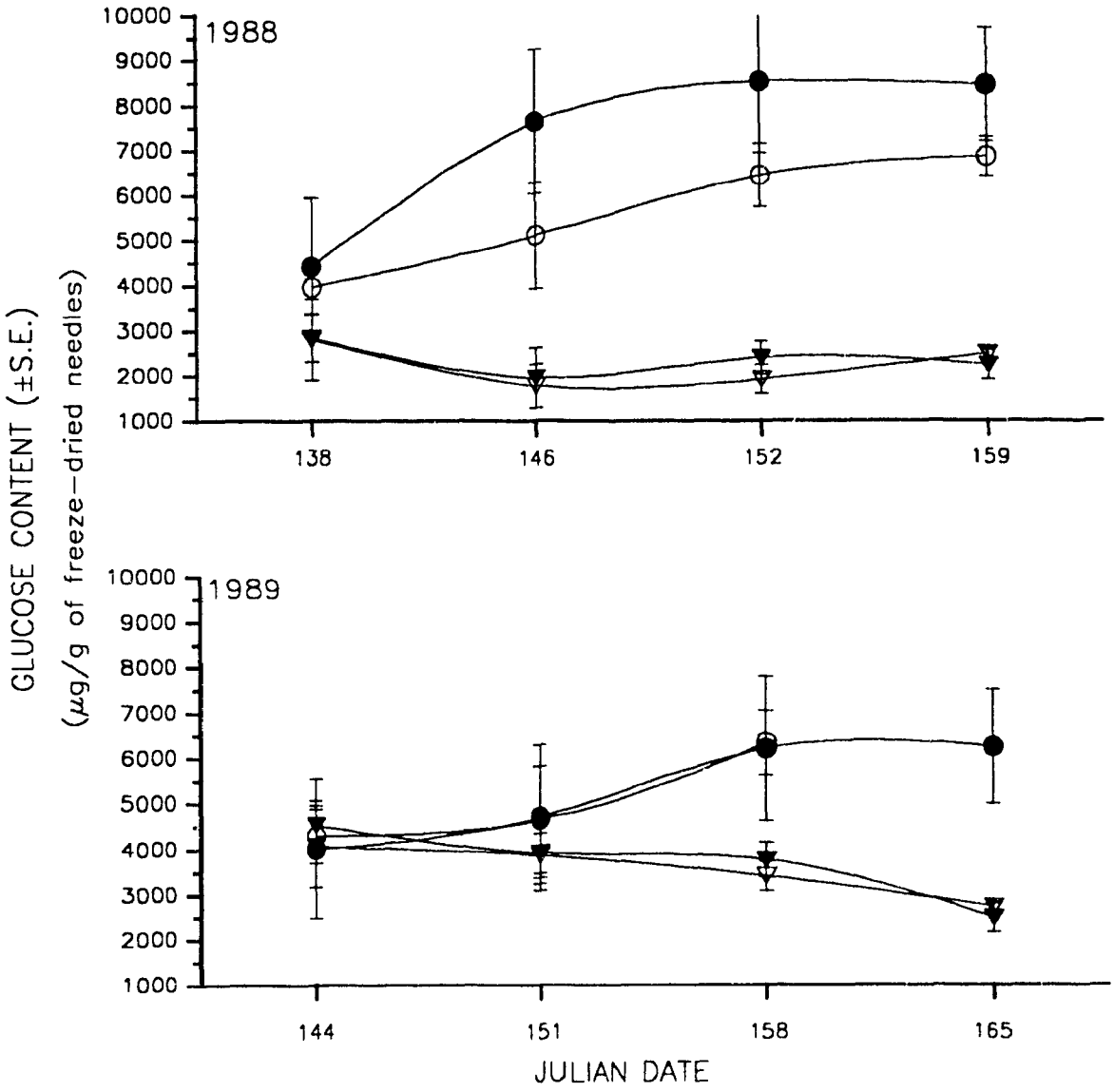

Figure 5. Glucose content found in current- and one-year-old foliage of white spruce. See *Figure 3* for legend.

Table 8. Analysis of variance of glucose found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	1	Block	2	1601377.72	1.13	0.3515
		Treatment	1	14516536.03	10.28	0.0069
		Collecting Period	3	15488479.25	10.97	0.0007
		Treatment x Period[b]	3	959385.35	0.68	0.5800
		Error	13	1411890.37		
1988	2	Block	2	26591.91	0.08	0.9233
		Treatment	1	75771.67	0.23	0.6410
		Collecting Period	3	911684.07	2.75	0.0886
		Treatment x Period	3	124099.70	0.37	0.7728
		Error	13	331086.81		
1989	1	Block	2	46734.76	0.04	0.9576
		Treatment	1	47755.46	0.04	0.8377
		Collecting Period	3	4582862.01	4.27	0.0392
		Treatment x Period	3	31736.12	0.03	0.9710
		Error	13	1073905.77		
1989	2	Block	2	176599.24	0.19	0.8299
		Treatment	1	191066.36	0.21	0.6594
		Collecting Period	3	2563954.69	2.75	0.0930
		Treatment x Period	3	117435.16	0.13	0.9427
		Error	3	931381.73		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor that can explain variation in the results.

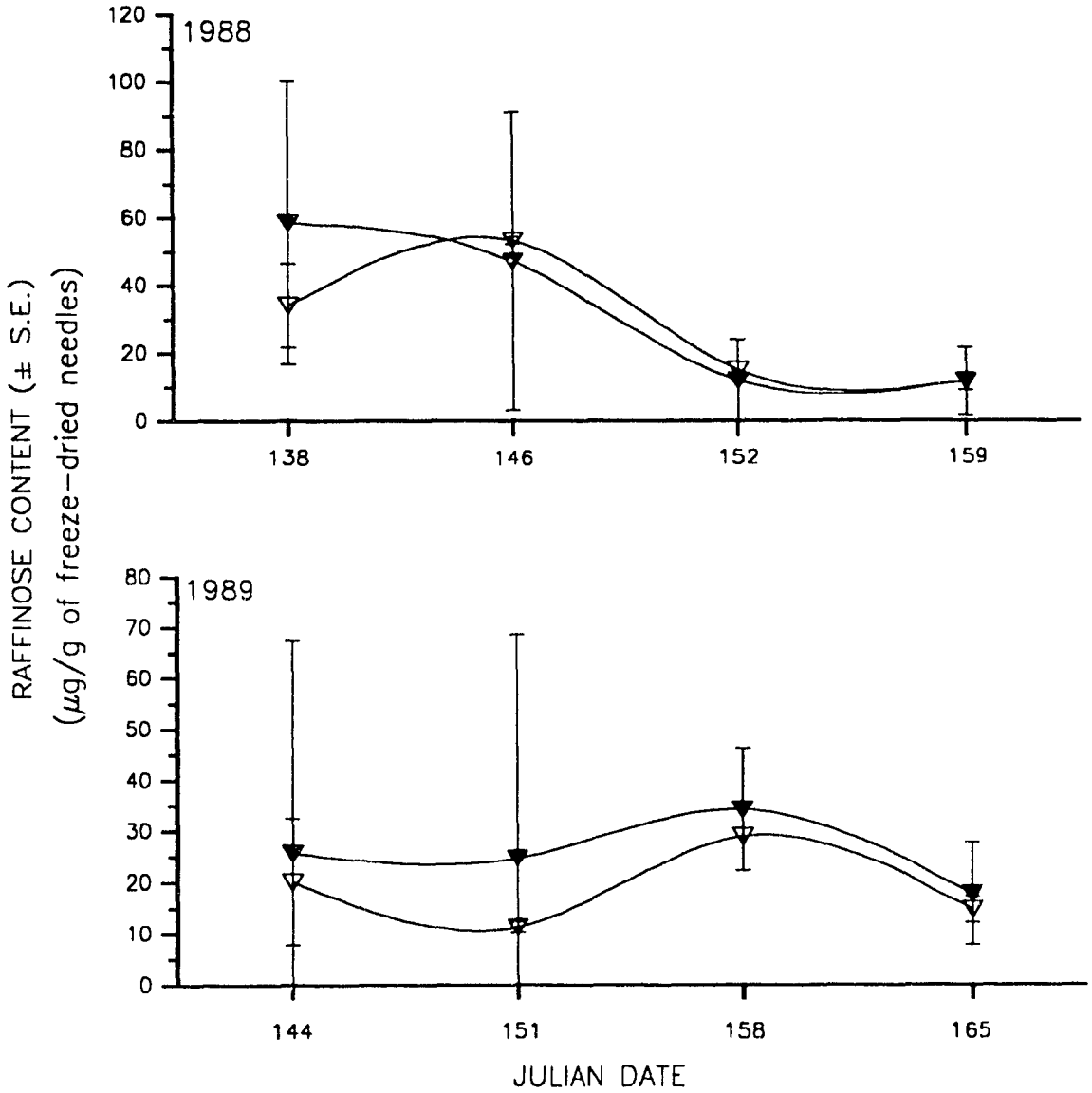

Figure 6. Raffinose content found in one-year-old foliage of white spruce. See *Figure 3* for legend.

Table 9. Analysis of variance of raffinose found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	2	Block	2	1061.67	1.48	0.3136
		Treatment	1	67.11	0.09	0.7724
		Collecting Period	3	1734.82	2.41	0.1828
		Treatment x Period[b]	3	474.53	0.66	0.6114
		Error	13	719.62		
1989	2	Block	2	478.21	5.60	0.0693
		Treatment	1	187.50	2.20	0.2126
		Collecting Period	3	229.91	2.69	0.1814
		Treatment x Period	3	85.48		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor that can explain variation in the results.

Table 10. Analysis of variance of fructose found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	1	Block	2	110859.93	0.22	0.8020
		Treatment	1	4012327.62	8.12	0.0136
		Collecting Period	3	3002433.31	6.08	0.0081
		Treatment x Period[b]	3	887038.87	1.80	0.1975
		Error	13	493853.04		
1988	2	Block	2	77313.12	1.13	0.3558
		Treatment	1	145522.66	2.12	0.1708
		Collecting Period	3	127570.70	1.86	0.1900
		Treatment x Period	3	92050.93	1.34	0.3068
		Error	13	68553.98		
1989	1	Block	2	268957.94	0.85	0.4573
		Treatment	1	5587867.01	17.76	0.0023
		Collecting Period	3	4698455.54	14.93	0.0008
		Treatment x Period	3	668344.79	2.12	0.1756
		Error	13	314713.92		
1989	2	Block	2	249095.72	3.93	0.0515
		Treatment	1	36186.04	0.57	0.4656
		Collecting Period	3	244863.06	3.87	0.0412
		Treatment x Period	3	240337.52	3.79	0.0433
		Error	3	63346.46		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor that can explain variation in the results.

current-year needles increased during this period but remained relatively low compared to the amount found in one-year-old needles. The increase in the total carbohydrate content in current-year needles during the spruce budworm larval development is in agreement with results observed in white spruce (Harvey 1974), and balsam fir (*Abies balsamea* (L.) Mill.) (Edel'man 1963, Durzan and Lopushanski 1968, Little 1970, Harvey 1974).

The low concentration of total carbohydrates in current-year leaves might suggest that the process of sugar transfer occurs in expanding white spruce shoots. The intensive growth process associated with shoot elongation should involve the utilization of a substantial amount of sugars (Kramer and Kozlowski 1979). The decrease in total sugar content in one-year-old foliage suggests that these needles may be an important source of carbohydrates that are allocated for the growth of current-year leaves. Clark (1961) indicated that the photosynthesic process in expanding needles should be negative during the first weeks of development as a result of a high rate of dark respiration (in Little 1970). Results using $^{14}CO_2$ in *Pinus* proposed that carbohydrates are imported during the rapid elongation of the shoots, and that the one-year-old needles are the major source of sugars (Dickmann and Kozlowski 1968, Ursino *et al.* 1968).

The principal soluble sugars found in current- and one-year-old white spruce needles were fructose, glucose, raffinose and sucrose. The presence of these carbohydrates was reported previously by Chapula and Fraser (1968). The general distribution of sucrose during larval development was similar to that previously observed for total carbohydrates. The sucrose is utilized in plants to synthesize the structural polysaccharides used in the formation of cell walls (Goodwin and Mercer 1986) which occurs during shoot elongation. This sucrose utilization should provide an explanation for the moderated increase of sucrose content of the young foliage (*Figure 2*). In spring polysaccharide food stores found in the one-year-old needles are converted into sucrose and then translocated to the rapidly developing shoots (Goodwin and Mercer 1986), explaining the important decrease observed in this foliage.

Glucose and fructose are important elements of the photosynthesic process. In daylight situations, they are involved in the Crassulacean Acid Metabolism (CAM) which is responsible for the production of sucrose (Goodwin and Mercer 1986). The glucose distribution observed during the larval development can be associated with the biosynthetic activities of the plant. However, no hypothesis can be proposed to explain the variation of the fructose concentration.

Raffinose that was found only in one-year-old foliage (*Figure 4*) is normally associated with cold hardiness protection of evergreen trees. Chapula and Fraser (1968) indicated that the biochemical process that converts sucrose to raffinose was promoted by low temperature, explaining the absence in current-year foliage.

An important result of this study is that current-year defoliation seems to not modify the general distribution of the sugars during the period of budworm larval development. Despite many observed changes in carbohydrate content following defoliation of evergreen trees (see Tuomi *et al.* 1988), little information is available on the short term effects of defoliation on foliar sugar composition of evergreen trees. It is generally assumed that defoliation of these plant species, which typically store carbon reserves in foliage, should, over a long term, provoke a reduction in the carbohydrates found in the leaves. For example, Niemela *et al.* (1984) indicated that defoliation of current-year shoots of Scots pine by the European pine sawfly, *Neodiprion sertifer* (Geoff.), provokes an improvement in the quality of the mature foliage during subsequent years.

In conclusion, the present work indicates that statistically significant differences in the carbohydrate pattern exist between early and late foliage of current- and one-year-old needles during the larval development period.

Sugars are recognized to influence several physiological and behavioural traits of lepidopterous species. These compounds are known to stimulate feeding behaviour in several phytophagous insects (e.g., Thorsteinson 1960, Dethier 1966, Schoonhoven 1969, Hsiao 1972, Albert *et al.* 1982, Ladd 1986, Adler 1989). Sugar compounds are also known to be an important dietary substrate for the spruce budworm (Miller 1963, Harvey 1974, Shaw and Little 1977). Normally, one-year-old foliage is avoided by spruce budworm larvae. The lower water content (Albert and Parisella 1988a) and the hardness (Heron 1965) of these needles compared to young foliage have been proposed to explain this avoidance by the late instar larvae. The differences in sugar concentration of current- and one-year-old needles can also be an important determinant of the feeding preference of the spruce budworm larvae for the newly extended shoots. Future investigations must be completed to understand the effect of these variations on the spruce budworm feeding behaviour.

CHAPTER 3

Effects of Sugar Extracts from Currently Defoliated and Undamaged White Spruce on the Feeding Behaviour of the Third- and Sixth-Instar Larvae of the Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae).

A. Abstract

To examine the feeding behaviour of the spruce budworm, *Choristoneura fumiferana* (Clem.) larvae during their development, we measured the Feeding Preferences and Feeding Rates of early and late instar larvae exposed to foliar carbohydrate fractions of currently defoliated and undamaged white spruce, *Picea glauca* (Moench) Voss. No significant differences in Feeding Preferences of third- and sixth-instar larvae were found between currently defoliated and undamaged trees. Sugar fractions extracted from current- and one-year-old foliage seem to have the same stimulating effect on the Feeding Preference for the two larval stages. On the other hand, significant differences were found between the relative Feeding Rates of early and late instar larvae. The role of a possible feedback mechanism to explain this difference is discussed.

B. Introduction

Plant chemicals are known to play an important role in the regulation of insect feeding behaviour. The selection of a food source by a phytophagous insect can be affected by the presence of plant nutritional compounds. Carbohydrates stimulate feeding behaviour in several insect species (Hsiao 1972, Albert and Jerrett 1981, Capinera 1981, Albert *et al.* 1982, Ladd 1986, Honda and Matsumoto 1987, Adler 1989, Schiff *et al.* 1989). Variations in the concentration of these phagostimulants can affect the consumption and utilization of a food source (Hsiao and Fraenkel 1968, Ladd 1986).

The feeding preference of sixth-instar larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), has been investigated for pure carbohydrates (Albert *et al.* 1982), and for sugar extracts of several host species (Albert and Jerret 1981, Albert and Parisella 1985ab, see also Chapter 1). As previously described in Chapter 2, during the period corresponding to the spruce budworm larval development, the total amount of carbohydrates found in white spruce, *Picea glauca* (Moench) Voss, increases in current-year needles, and decreases in one-year-old needles. These modifications in the carbohydrate concentration are related to the plant growth processes which occur at this time. The goal of this study was to determine whether these variations in sugar concentration associated with plant physiological activities

provoked changes in the feeding response during larval somatic growth of the eastern spruce budworm. The objective was to examine the feeding preferences and feeding rates of early and late instar larvae of the spruce budworm in relation to chemical changes in carbohydrate fractions extracted from currently defoliated and undamaged white spruce trees.

C. Materials and Methods

Insects

Post-diapause second instar larvae of the spruce budworm were provided by the Forest Pest Management Institute, Sault Ste Marie, Ontario. The insects were subsequently reared on an artificial diet (McMorran 1965), and placed in an incubator with a 16:8 (L:D) photoperiod, 25 °C and 60 % R.H. Larval stages of the insects used in all experiments were determined by measurement of the head capsule width (McGugan 1954). Prior to any test, third- and sixth-instar larvae were starved 24 hours. The insects were used for one experiment only, and those which moulted during a test were discarded.

Plants

Three-year-old white spruce trees were obtained from the Laurentian Forestry Centre, Sainte-Foy, Québec. In 1988 and 1989, 24 trees were placed outside in individual cages (30 x 30 x 45 cm covered with 40 mesh nylon) to

prevent contamination and predation by birds and insects. Fifteen second-instar spruce budworm larvae per tree were introduced on 50 % of the plants after the beginning of bud-burst, and these were identified as defoliated plants. The control or undamaged trees were plants on which no insects had been placed.

During the period corresponding to spruce budworm development, current- and one-year-old needles were collected each week from three defoliated and three undamaged trees, and foliage was kept at -15 °C until used for extraction.

Carbohydrate Extraction

Polar compounds of each freeze-dried sample were extracted in a solution of methanol, chloroform and water, according to the methods of Dickson (1979) previously described in Chapter 1. The carbohydrates were then separated by ion-exchange chromatography on cation resin (AG50-X8, Bio-Rad Lab.) followed by anion resin (AG1-X8, Bio-Rad Lab.), and recovered by subsequent elution with distilled water.

The moisture content of each sample was evaluated as the difference between the fresh and the freeze-dried weights. Based on the freeze-dried weight and the percentage of moisture, carbohydrate fractions were redissolved

in a proper volume of water to reach the initial concentration found in the needles.

Bioassays

The two-choice feeding test developed by Jermy *et al.* (1968) and modified by Maloney *et al.* (1988) for the early instar larvae, and Albert *et al.* (1982) for the late instar larvae was used to evaluate the feeding behaviour of the third- and sixth-instar larvae. Four cellulose discs of 3.3 mm diameter and eight discs of 6.5 mm diameter for the third- and sixth-instar larvae respectively were punched from 0.45 μm pore size filter paper (Sartorius). Test discs were wetted with a 3 μl aliquot of extracted sugar fraction for the small discs and a 8 μl aliquot for large discs, or with similar volumes of distilled water for control discs. For each arena, test and control discs were alternated in a circular fashion to prevent bias due to a common orientation for all arenas, and the position of the discs was randomly marked (Appendix 1).

For third- and sixth-instar larvae, a freshly moulted insect, starved for 24 h after the moult, was placed in the center of the test arena and was allowed to feed for 24 h. The arenas were covered to prevent water loss, and were then placed at 25 °C, 16:8 h (L:D) photoperiod and 60 % R.H. For each larval stage, tests with a particular carbohydrate extract were repeated using 20 different larvae.

The Feeding Preference and the Feeding Rate were different parameters considered during this experiment. The Feeding Preference between test and control discs was estimated as the difference between the Mean Percent Consumption of test discs and that of control discs. The Feeding Rate was also measured, and it corresponded to the average total consumption per hour of a particular combination of test and control discs. Since the control discs of all experiments were wetted with distilled water, the Feeding Rate was used to evaluate the larva's interest for a particular carbohydrate extract.

In order to compare the feeding responses of the two instars, data were transformed using correction factors developed following several behaviour experiments with pure sucrose. These correction factors were previously described in Chapter 1. The Relative Preference Index and the Feeding Rate Index were employed to compare the Mean Percent Consumption and the Feeding Rate of third- and sixth-instar larvae. The Feeding Rate Index is based on feeding behaviour tests using 25 m*M* sucrose, where the Feeding Rates of the third- and sixth-instar larvae after transformation were equal to 1 unit.

Statistical Methods

Since no differences were found between the feeding responses of larvae exposed to carbohydrate fractions of current- and one-year-old needles from

trees of a same collecting period, data were pooled. Because the mean consumption scores were not normally distributed, the Wilcoxon's Signed-Rank test (NPAR module; Wilkinson 1989) was used to determine the significant level of the difference in response between test and control discs (Sokal and Rohlf 1969). As data of the Relative Preference Index and Feeding Rate Index were normally distributed, individual *t*-tests were performed on results of third- versus sixth-instar larvae (SAS TTEST procedure; SAS Institute Inc. 1985). To determine the effect of the treatments applied to the trees and the effect of the collecting period on the feeding response, an analysis of variance was performed (SAS GLM procedure; SAS Institute Inc. 1985). The level of rejection for all statistical analyses was set at $\alpha = 0.05$.

D. Results

Feeding Preference

By using the feeding bioassays, the first objective was to follow the Feeding Preference of the third- and sixth-instar larvae of the spruce budworm toward carbohydrate fractions from currently defoliated and undamaged white spruce host plants collected during the period of larval development. In 1988 and 1989, results showed that discs wetted with the sugar extracts were

strongly preferred by the early and late instar larvae, than those impregnated with distilled water (*Table 11* and *12*). In one case, the feeding response of the third-instar larvae for carbohydrate fractions did not conform to these previous observations ($p = 0.4143$; t-test). The small number of test insects appears to be the main factor explaining this non conformity.

The Feeding Preference Index was used to compare the feeding response of the two larval stages. Based on this relative index, in most cases no differences were found between the Feeding Preferences of the third- and sixth-instar larvae exposed to current-year foliage of currently defoliated and undamaged trees (*Figure 7*). Similar results were observed with one-year-old foliage (*Figure 8*). In fact, the feeding preferences of the two larval instars exposed to carbohydrate fractions of current-year needles were almost identical to those of larvae exposed to one-year old needle extracts. Finally, no correlation was detected between results of Feeding Preference Index and the total carbohydrate concentration found in different foliages during the period corresponding to the spruce budworm's larval development (*Table 13*).

Feeding Rate

In 1988 and 1989, for all white spruce sugar extracts, the Feeding Rates of the third-instar larvae were significantly lower than those of sixth-instar larvae ($p < 0.001$; t-test) (*Figure 9*). However, these result cannot be used for

Table 11. Percent consumption of control and test discs of third-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from currently defoliated and undamaged white spruce trees.

Year	Needle[a]	Treatment[b]	Period[c]	n	Mean % Consumption Control[d]	Test	±S.E.	p[e]
1988	1	1	138	35	5.72	94.28	3.83	0.0000
	1	1	146	53	5.10	94.90	1.46	0.0000
	1	1	152	45	7.89	92.11	3.00	0.0000
	1	1	159	53	5.85	94.15	2.49	0.0000
	1	2	138	45	10.05	89.92	2.91	0.0000
	1	2	146	50	7.05	92.95	1.92	0.0000
	1	2	152	41	12.01	87.97	2.99	0.0000
	1	2	159	23	4.49	95.51	2.27	0.0000
	2	1	138	54	9.83	90.17	3.05	0.0000
	2	1	146	52	11.82	88.11	3.05	0.0000
	2	1	152	38	3.36	96.64	2.66	0.0000
	2	1	159	36	13.36	86.67	3.81	0.0000
	2	2	138	51	8.76	91.24	3.13	0.0000
	2	2	146	49	6.70	93.30	1.53	0.0000
	2	2	152	45	3.43	96.57	1.20	0.0000
	2	2	159	35	2.10	97.90	1.08	0.0000
1989	1	1	144	12	4.44	95.56	3.11	0.0011
	1	1	151	45	18.85	81.16	3.69	0.0000
	1	1	158	25	6.86	93.14	2.36	0.0000
	1	1	165	n.a.	.	.	.	
	1	2	144	19	20.49	79.56	5.80	0.0016
	1	2	151	34	17.64	82.32	5.19	0.0001
	1	2	158	53	15.83	84.15	2.85	0.0000
	1	2	165	24	17.38	82.62	5.25	0.0000
	2	1	144	16	7.92	92.08	4.46	0.0003
	2	1	151	15	22.65	77.39	7.58	0.0085
	2	1	158	41	4.37	95.63	1.98	0.0000
	2	1	165	24	17.34	82.62	5.25	0.0002
	2	2	144	17	6.37	93.63	3.36	0.0001
	2	2	151	6	3.33	66.67	1.08	0.4142
	2	2	158	28	18.08	81.99	6.25	0.0003
	2	2	165	47	16.47	83.53	3.26	0.0000

[a] 1= Current-year needles; 2 = One-year-old needles.
[b] 1 = Defoliated trees; 2 = Undamaged trees.
[c] Julian Date.
[d] Distilled water for all experiments.
[e] Wilcoxon's Signed-Ranks test, probability value.

Table 12. Percent Consumption of control and test discs of sixth-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from currently defoliated and undamaged white spruce trees.

Year	Needle[a]	Treatment[b]	Period[c]	n	Control[d]	Test	±S.E.	p[e]
1988	1	1	138	51	5.89	94.11	2.84	0.0000
	1	1	146	45	8.03	91.97	1.20	0.0000
	1	1	152	52	10.42	89.59	1.40	0.0000
	1	1	159	57	10.27	89.71	1.33	0.0000
	1	2	138	49	10.19	89.83	1.59	0.0000
	1	2	146	45	10.35	89.68	1.65	0.0000
	1	2	152	49	12.39	87.64	1.61	0.0000
	1	2	159	38	10.38	89.62	2.74	0.0000
	2	1	138	35	6.00	94.01	1.22	0.0000
	2	1	146	44	10.14	89.81	1.59	0.0000
	2	1	152	53	12.53	87.46	1.61	0.0000
	2	1	159	29	7.58	92.42	1.65	0.0000
	2	2	138	32	8.73	91.27	1.66	0.0000
	2	2	146	47	9.94	90.06	1.41	0.0000
	2	2	152	51	11.21	88.72	1.63	0.0000
	2	2	159	20	10.60	89.33	2.65	0.0001
1989	1	1	144	19	4.45	95.55	1.74	0.0001
	1	1	151	53	7.02	92.98	1.01	0.0000
	1	1	158	19	11 02	88.28	3.89	0.0001
	1	1	165	n.a.	.	.	.	
	1	2	144	19	11.19	88.82	2.58	0.0001
	1	2	151	37	8.36	91.64	1.10	0.0000
	1	2	158	51	8.52	91.48	1.54	0.0000
	1	2	165	49	11.49	88.60	1.30	0.0000
	2	1	144	14	3.56	96.44	1.04	0.0010
	2	1	151	33	15.03	84.97	2.28	0.0000
	2	1	158	51	11.01	88.93	1.72	0.0000
	2	1	165	37	11.17	88.89	1.72	0.0000
	2	2	144	15	8.28	91.72	2.53	0.0006
	2	2	151	17	11.17	88.81	3.02	0.0003
	2	2	158	49	9.43	90.57	1.66	0.0000
	2	2	165	49	12.49	87.52	2.66	0.0000

[a] 1= Current-year needles; 2 = One-year-old needles.
[b] 1 = Defoliated trees; 2 = Undamaged trees.
[c] Julian Date.
[d] Distilled water for all experiments.
[e] Wilcoxon's Signed-Ranks test, probability value.

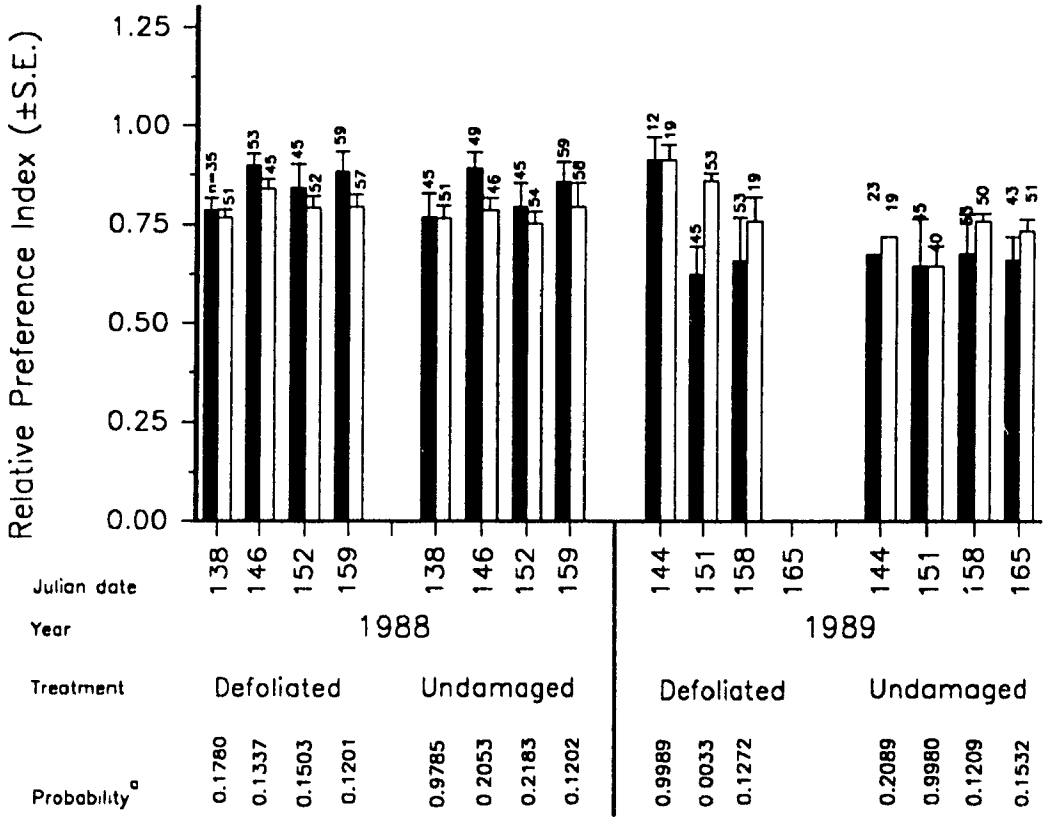

Figure 7. Relative Preference Indices of the third- and sixth-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from current-year needles of currently defoliated and undamaged white spruce trees. ■ Third-instar larvae; □ Sixth-instar larvae; [a] *t*-test probability.

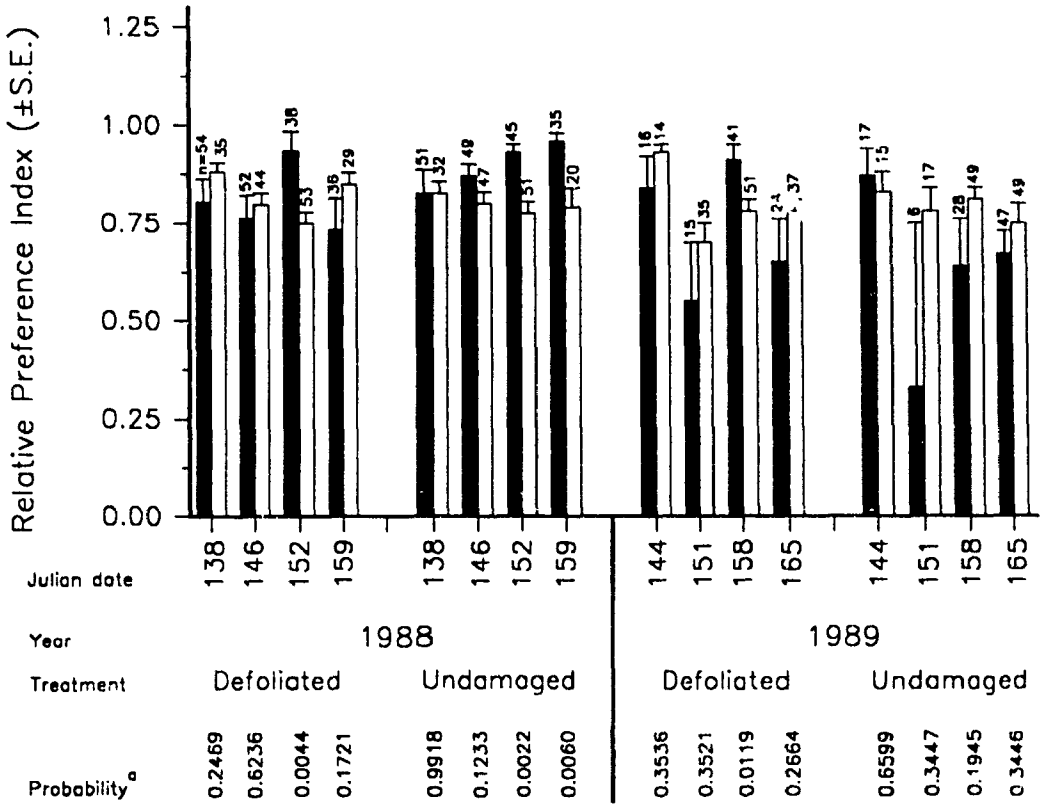

Figure 8. Relative Preference Indices of the third- and sixth-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from one-year-old needles of currently defoliated and undamaged white spruce trees. See *Figure 7* for the legend.

Table 13. Correlations between the Relative Preference Index of the third- and sixth-instar larvae of the eastern spruce budworm and the total amount of carbohydrates found in current and one-year-old needles of white spruce.

	Instar Larvae	
	3rd	6th
Current-Year Needles (1988)		
Total Carbohydrates	-0.0169 (0.7725)[a] 310[b]	-0.0382 (0.0023) 321
One-Year-Old Needles (1988)		
Total Carbohydrates	-0.0515 (0.3433) 341	0.0144 (0.8138) 292
Current-Year Needles (1989)		
Total Carbohydrates	0.1233 (0.0687) 219	-0.2135 (0.0017) 213
One-Year-Old Needles (1989)		
Total Carbohydrates	0.0172 (0.8196) 178	0.0271 (0.6694) 251

[a] Probability.
[b] Number of observations.

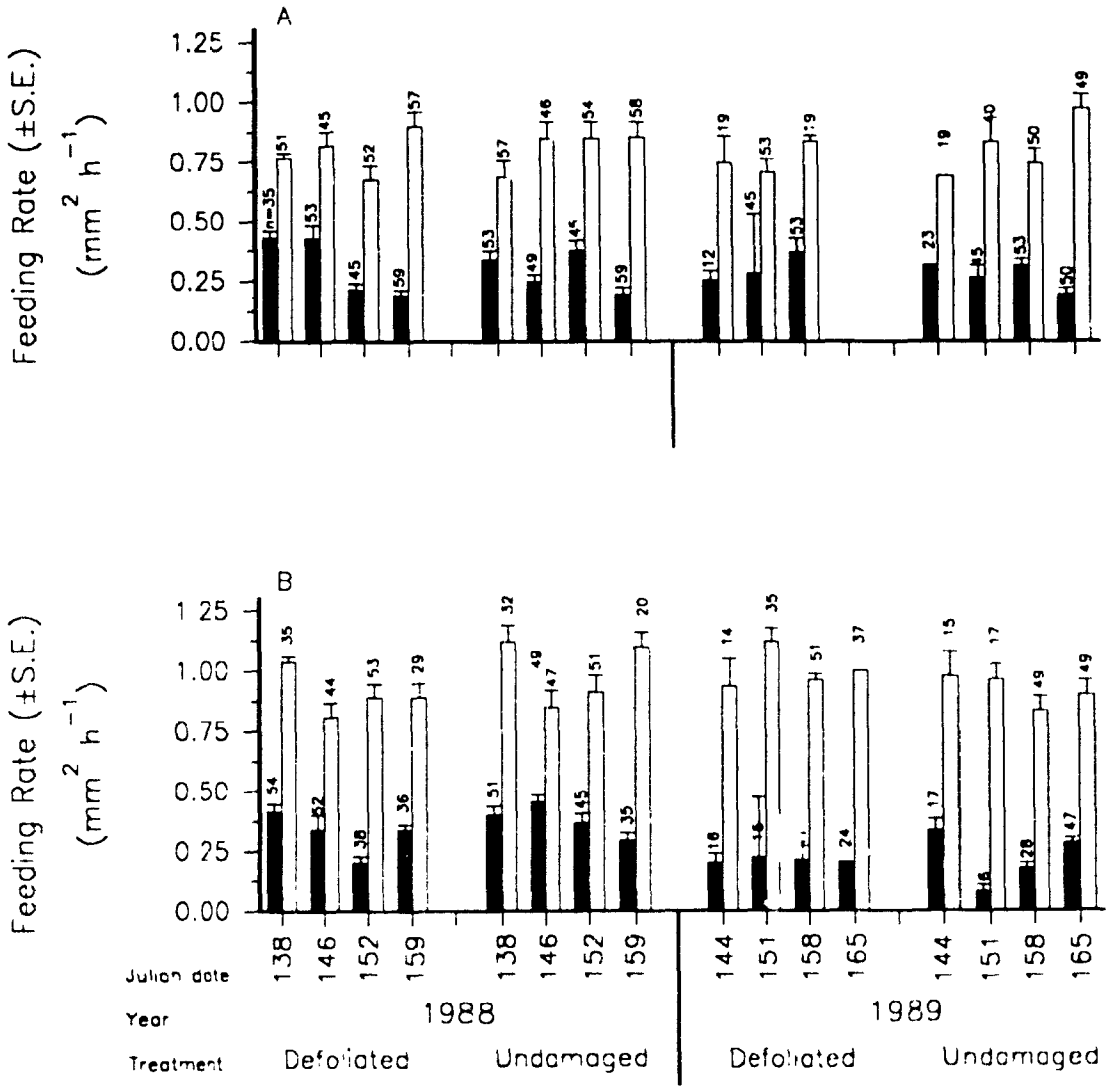

Figure 9. Feeding Rates of third- and sixth-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from currently defoliated and undamaged white spruce trees. **A** Current-year foliage; **B** One-year-old foliage; See *Figure 7* for the legend.

further analysis because of the bias due to the dimorphism in size between the third- (3.0 to 5.0 mm) and the sixth-instar larvae (15 to 24 mm) (Titus 1977). In order to minimize this larval size factor, the Feeding Rate Index was used to compare the total disc consumption between the two larval stages. Results show that in each test, the third-instar larvae consumed significantly more than the sixth-instar larvae ($p < 0.001$; *t*-test) (*Figure 10*). Moreover, comparisons with pure sucrose indicated that plant sugar extracts were more stimulating for early instar larvae than pure sucrose, and the inverse was the case for the late instar larvae. Significant correlations were found between the Feeding Rate Indices of third- and sixth-instar larvae and total carbohydrate concentration found in current-year and one-year-old needles with the exception of results for one-year-old needles of 1989 (*Table 14*).

E. Discussion

The significant preference of sixth-instar larvae for carbohydrate extracts is consistent with previous observations with current-year needles (Albert and Jerrett 1981, Albert 1982) and one-year-old foliage (Albert and Parisella 1988a). The strong response of early instar larvae for carbohydrate fractions from young shoots was similar to those observed in Chapter 1. On the other hand, it is the first mention of a preference of the third-instar larvae

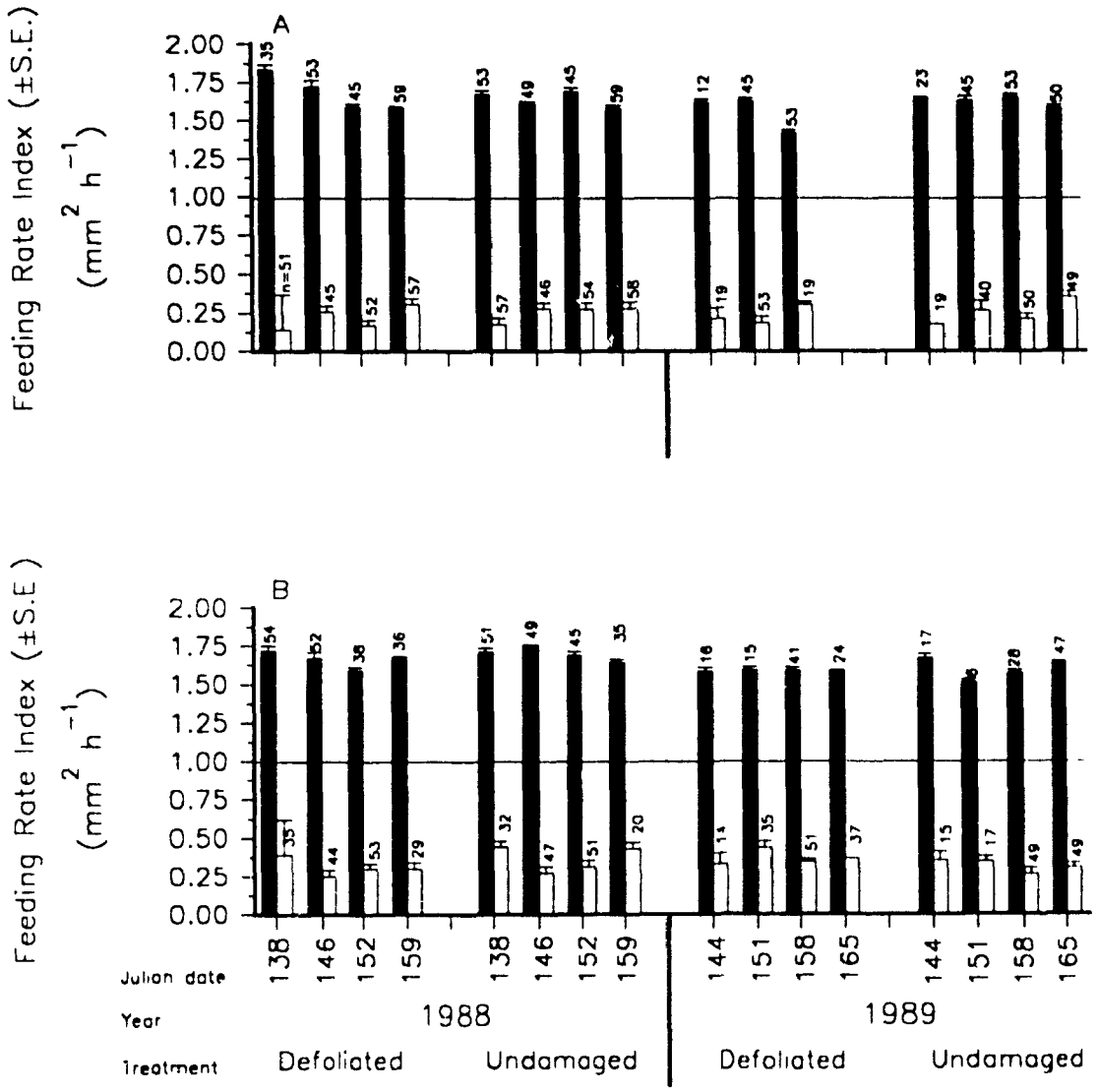

Figure 10. Feeding Rate Indices of third- and sixth-instar larvae of the eastern spruce budworm for carbohydrate fractions extracted from currently defoliated and undamaged white spruce trees. **A** Current-year foliage; **B** One-year-old foliage; See *Figure 7* for the legend.

Table 14. Correlations between the Feeding Rate Index of the third- and sixth-instar larvae of the eastern spruce budworm and the total amount of carbohydrates found in current and one-year-old needles of white spruce.

	Instar Larvae	
	3rd	6th
Current-Year Needles (1988)		
Total Carbohydrates	0.1771 (0.0023)[a] 310[b]	0.1342 (0.0161) 321
One-Year-Old Needles (1988)		
Total Carbohydrates	0.1390 (0.0102) 341	0.1604 (0.0062) 292
Current-Year Needles (1989)		
Total Carbohydrates	0.2626 (0.0023) 219	0.1783 (0.0091) 213
One-Year-Old Needles (1989)		
Total Carbohydrates	0.0357 (0.6365) 178	0.0873 (0.1251) 251

[a] Probability.
[b] Number of observations.

for carbohydrate fractions extracted from one-year old foliage. The results confirm the importance of carbohydrates as a major feeding stimulant for the spruce budworm larvae.

The variation in carbohydrate concentration between current and one-year-old foliage (see Chapter 2) seems to have no significant effect on the spruce budworm's feeding preference. This observation was corroborated by the weak of correlations between the Feeding Preference Index and the amount of carbohydrates found in different foliages. Considering that one-year-old foliage is normally avoided by the spruce budworm, these results support the hypothesis which suggests that their utilization is restricted by factors other than the lack of feeding stimulant properties. Heron (1965) suggested that differences in feeding preferences of the spruce budworm larvae for various plant tissues were not attributable simply to differences in the sugar concentration. Other reasons have been proposed to explain the avoidance of old foliage by the budworm larvae. First is the higher degree of lignification of the one-year old needles (Heron 1965, Albert and Parisella 1988a). A high level of fiber in artificial diet decreases the digestibility and the suitability of the food source (Peterson *et al.* 1988). The water concentration of the old foliage is lower compared to young foliage (approximately 50 % for one-year old needles and 80 % for current-year needles, see Appendix 3), and this is probably another factor that can affect the utilization of old leaves by the

larvae (Albert and Parisella 1988a). Finally, Maloney *et al.* (1988) showed that epicuticular waxes found on the needle surface of certain host plants had significant effects on the budworm's feeding preferences. Changes associated with needle development processes may induce modifications in the structure and composition of this wax layer, and thus may play an important role in the budworm's avoidance of one-year-old foliage.

The differences observed between the Feeding Rates of third- and sixth-instar larvae exposed to carbohydrate extracts were in conformity with those observed in previous experiments with young white spruce shoots (see Chapter 1), suggesting that a modification of the feeding rate occurs during somatic larval development. Considering that the preference of early and late spruce budworm instar larvae remain relatively similar, these results clearly suggest that for a typical feeding stimulus no correlation exist between the feeding preference and the level of consumption or feeding rate. I propose that the differences in relative feeding rates between the two larval instars arises from a possible feedback mechanism that could regulate nutrient intake during the period of larval growth. Modifications in nutrient demands of each larval instar may influence the levels of a particular nutrient in the haemolymph, which in turn may modify peripheral sensitivity to that nutrient and so may modify the insect's feeding behaviour. Electrophysiological studies on nymphs (Abisgold and Simpson 1987, 1988) and adults (Simpson *et al.* 1990) of the

migratory locust, *Locusta migratoria* L., have shown that levels of free amino acids in haemolymph influence the feeding behaviour and the physiological sensitivity to an amino acid solution. Moreover, modification in carbohydrate ingestion can be related to changes in chemosensitivity to sucrose (Simpson *et al.* 1990), and these compounds are known to have physiological stimulating properties on the sixth-instar larvae of the spruce budworm (Albert and Parisella 1988b). Electrophysiological studies are needed to compare the responses of young and late instar larvae for carbohydrates, and to investigate the relationship which might exist between these peripheral responses and the feeding behaviour.

CHAPTER 4

Amino Acid Variations in Current- and One-Year-Old Foliage of White Spruce, *Picea glauca* (Moench) Voss, During the Period of the Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.) Larval Development.

A. Abstract

The amino acids of new and old foliage of white spruce, *Picea glauca* (Moench) Voss were investigated during the period of larval development of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.). The data were analyzed for variation associated with current-year defoliation by the insect. The concentration of foliar amino acids was greater in current-year needles than in one-year-old needles. Results were in accordance with the known suitability of young foliage for the spruce budworm larvae. No significant difference was found between currently defoliated and undamaged trees during this period.

B. Introduction

Several studies have shown that plant defensive chemicals are important determinants of growth in insect defoliators (Dethier 1954, Fraenkel

1959, Ehrlich and Raven 1964, Whittaker and Feeny 1971). However, other factors such as nitrogen level, moisture content, and leaf toughness appear to be as important as defensive compounds in influencing leaf feeder growth (Feeny 1970, Slansky and Feeny 1977, White 1974, 1978, Scriber 1978, 1979, Mattson 1980). Larval development of the gypsy moth, *Lymantria dispar* (L.), is greater when the insects have access to a nitrogen rich diet (Hough and Pimentel 1977). Montgomery (1982) pointed out that gypsy moth larvae required a significant amount of nitrogen, especially during the first larval stages. A similar observation had been made by Harvey (1974) in a study on the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) larvae. Shaw *et al.* (1978) reported an improvement of the development of spruce budworm larvae when insects fed on fertilized balsam fir, *Abies balsamea* (L.). These results suggest that variations in foliar nitrogen can directly influence the performance of a herbivorous insect.

White spruce, *Picea glauca* (Moench) Voss is an important host of the eastern spruce budworm larvae. Studies on the quality of the foliage of the two most common budworm host plants indicated that larval growth was improved when larvae were fed white spruce foliage rather than balsam fir (Koller and Leonard 1981, Lavallée and Hardy 1988). Durzan and Lopushanski (1968) showed that the relative proportion of amino acids in fifth-instar larval tissues of the eastern spruce budworm was significantly higher

when the insects fed on white spruce. A significant feeding preference of the sixth-instar larvae of *C. fumiferana* for the current-year white spruce foliage over four common hosts has also been reported by Albert and Parisella (1985a).

Larvae normally feed on the expanding new needles of their conifer hosts (Blais 1958). However, when new foliage is entirely consumed, the insect will be constrained to feed on older foliage (McGugan 1954, Blais 1952, 1979). Consuming old needles results in reduced fecundity, retarded development, and increased mortality (Blais 1952, 1953, Miller 1957, Heron 1965). Kimmins (1971) reported that the concentration of foliar amino acids was greater in current-year foliage than in old foliage. The author suggests that this difference in amino acid concentration may be an important factor that could explain the utilization of new expanding needles by the spruce budworm larvae. However, experiments on the feeding preference of the sixth-instar larvae suggested that the chemical composition of old needles, in this case amino acid fractions, does not account for avoidance of the old needles (Albert and Parisella 1988a).

Although nitrogen compounds represent only 2% of the dry weight of a plant, there are numerous nitrogen-containing organic substances in plants. Amino acids are very important plant constituents that are involved in the biosynthesis of several nitrogenous plant compounds such as proteins,

alkaloids, amides, cyanogenic glycosides, etc. (Harborne 1988). However, considerable variations in concentration and composition of amino acids can be found between species, individuals, and tissues of a same plant (Goodwin and Mercer 1986). These differences can be related to the metabolic status of the plant or tissues (Harborne 1988).

Defoliation has been shown to induce changes in trees that may have adverse effects on the growth, reproduction, and survival of lepidopteran defoliators (Haukioja and Niemela 1977, Wallner and Walton 1979, Werner 1979, Haukioja 1980). However, other studies showed that defoliation may cause no observable effect on the insect's performance (Myers 1981), and it may provoke improvement in the quality of the foliage that favours the development of the insect (Niemela *et al.* 1984). In evergreen conifers, the leaves form the principal source of stored reserves to support growth (Bryant *et al.* 1983). Consequently, herbivory by folivorous insects should result in important modifications in concentration of phytochemical constituents such as carbohydrates and nutrient elements.

The aim of the study was to monitor the changes in amino acids during the period of spruce budworm larval development, and to detect variations in this group of compounds that may result from current-year defoliation by the insect.

C. Materials and Methods

Biological Materials

The trees used in this experiment were healthy three-year-old white spruce obtained from the Laurentian Forestry Centre, Sainte-Foy, Québec. In 1988 and 1989, 24 plants were placed outside in individual cages to prevent external damage by birds and insects. Post-diapause second-instar larvae were obtained from the Forest Pest Management Institute, Sault-Ste-Marie, Ontario. A two-factor randomized block design with three replicates was used in this study. The insect density was the first factor considered. To measure the action of an insect's defoliation on the amino acid composition of current- and one-year-old needles, 15 second-instar spruce budworm larvae per tree were introduced on 50 % of the trees, and these plants were identified as defoliated trees. The introduction was accomplished shortly after bud-burst. The remaining plants on which no insects were introduced were identified as undamaged trees.

The effect of time on the variation of amino acids was the second factor examined in this analysis. The period corresponding to that of the spruce budworm larval development was associated with this factor. In 1988 and 1989, insect development took placed during four weeks. Each week, three defoliated and three undamaged white spruce plants were collected, and

current- and one-year-old needles were separated and frozen in liquid nitrogen. Samples were then freeze-dried and stored at -15 °C until used in extractions.

Chemical Analysis

The extraction and analysis of amino acids were done for each collecting period on current- and one-year-old needles of each tree and for each collecting period. The extraction procedures were modifications of those described by Dickson (1979). Freeze-dried needles were reduced to a fine powder using a Wiley Mill (40 mesh). The powder was then placed in a solution of methanol, chloroform, water (12:5:3) in order to extract all polar compounds. The solution was passed through a column filled with a cation exchange resin (AG50-X8 resin; Bio-Rad Lab.). Adhering nitrogenous compounds were eluted with 2 *N* ammonium hydroxide, dried, and dissolved in a known volume of distilled water.

The amino acid composition was determined using a Waters HPLC, interfaced with a Digital computer. Chromatography of amino acids was performed on a Waters Radial-Pak Resolve C_{18} 5 µm cartridge (8 x 100 mm) column kept at 90 °C, using two buffers: Buffer A, which was a solution of methanol, tetrahydrofuran and water (2:2:96); and Buffer B, which was a solution of methanol and water (65:35) (see Appendix 4 for more details). The maximum flow rate during the elution of the amino acids was set at 2.5

ml/min, and the time of the elution was set at 27 min. The amount of each amino acid was estimated by comparison with a known standard (Appendix 4). The total amount of amino acids was determined by summation of all determined amino acids. In order to compare the specific amino acid composition of each sample, estimation of the relative contribution of each element to total amino acids was performed.

Statistical Methods

To measure the effect of the blocks, the insect density, and the collecting period on the amino acid content of current- and one-year-old white spruce needles, analyses of variance (PROC GLM, SAS Institute Inc. 1985) were performed on the amount of total amino acids and on each amino acid component.

D. Results

For each year, and each plant treatment, the total amount of amino acids was higher in current-year needles compared to one-year-old foliage (*Figure 11*). Total amounts of amino acids in new and old needles decrease during the period of spruce budworm larval development. Analyses of variance indicated that the amount of nitrogenous compounds was significantly affected by the time of tree sampling (*Table 15*). No evidence was

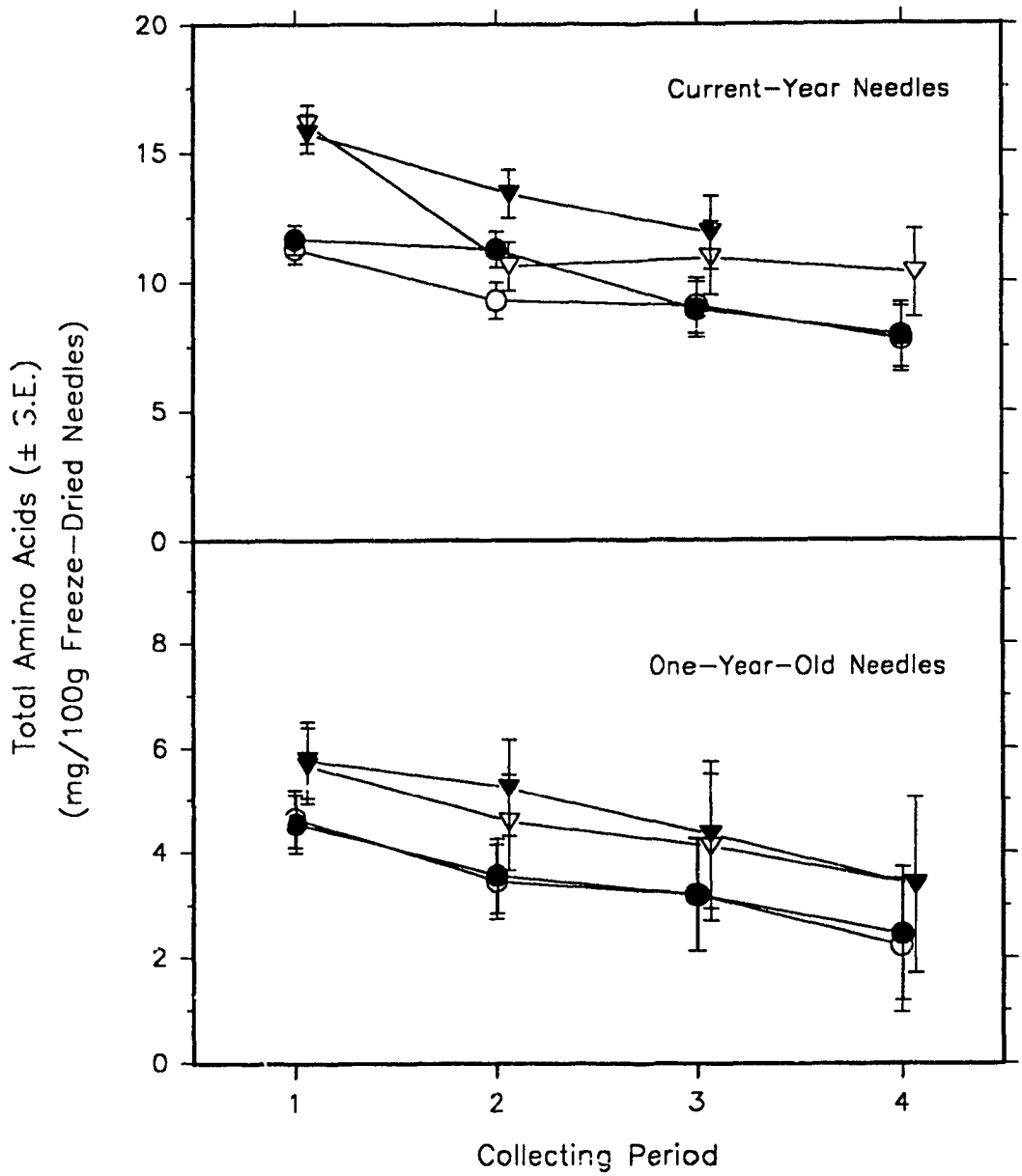

Figure 11. Variations in total amino acids in current- and one-year-old needles of white spruce during the period of spruce budworm larval development. —●—●—● Currently defoliated trees, 1988; —○—○—○ Undamaged, trees 1988; —▼—▼—▼ Currently defoliated trees, 1989; —▽—▽—▽ Undamaged trees, 1989.

Table 15. Analysis of variance of total amount of amino acids found in current- and one-year-old foliage of white spruce among different factors.

Year	Needle[a]	Source of Variation	df	MS	F	*p*
1988	1	Block	2	0.1117	0.15	0.8597
		Treatment	1	1.7517	2.41	0.1519
		Collecting Period	3	10.5340	14.47	0.0006
		Treatment x Period[b]	3	3.3456	1.53	0.2662
		Error	10	0.7280		
1988	2	Block	2	0.0145	0.10	0.9044
		Treatment	1	0.0148	0.10	0.7534
		Collecting Period	3	4.7354	33.22	0.0001
		Treatment x Period	3	0.0310	0.22	0.8824
		Error	11	0.1425		
1989	1	Block	2	1.8671	1.89	0.2067
		Treatment	1	2.0637	2.09	0.1825
		Collecting Period	3	10.7865	10.91	0.0024
		Treatment x Period	2	2.2966	2.33	0.1524
		Error	9	0.9890		
1989	2	Block	2	0.3025	3.89	0.0529
		Treatment	1	0.2672	3.43	0.0909
		Collecting Period	3	3.1403	40.34	0.0001
		Treatment x Period	3	0.0659	0.85	0.4968
		Error	11	0.0779		

[a] 1 = Current-Year Needles; 2 = One-Year-Old Needles.
[b] Interaction between the Treatment factor and the Collecting Period factor.

found for inducible variations of the total amount of amino acids associated with current-year defoliation by the insect.

In undamaged white spruce collected in 1988, the proportion of glutamic acid found in current-year needles was nearly twice the amount observed for lysine, arginine, threonine, alanine, phenylalanine and valine (*Figure 12*). Leucine, isoleucine, glutamine, asparagine, serine, methionine, aspartic acid, glycine and α-aminobutyric acid were also found in needles but their proportions were smaller compared to the percentage of the previous main amino acids. During the period corresponding to the spruce budworm development, the proportion of each amino acid remained relatively constant with the exception of the relative amount of α-aminobutyric acid that increased during this interval and the proportion of lysine that decreased. The proportion of serine found in one-year-old needles was higher than that seen in new shoots. This increase of the relative amount of serine was associated with an important reduction in glutamic acid. Variations of the proportion of each amino acid during the insect development period were similar to those observed in new shoots. The previous patterns of amino acid distribution in current- and one-year-old needles were also similar to those observed on currently defoliated trees collected in 1988 (*Figure 13*), and on undamaged and currently defoliated trees collected in 1989 (*Figure 14* and *15*).

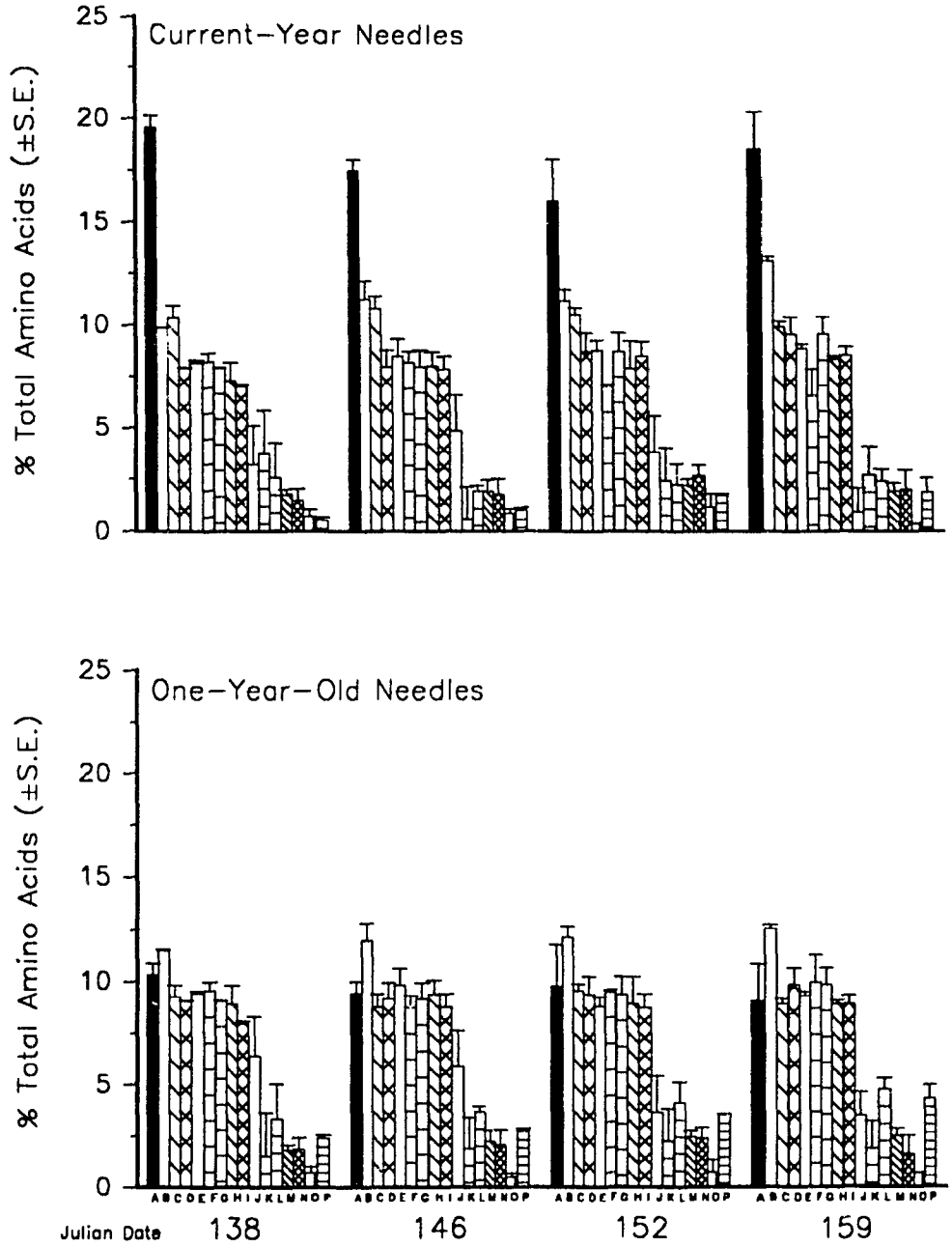

Figure 12. Variations in specific amino acids (% Total Amino Acids) in current- and one-year-old needles of 1988 undamaged white spruce during the period of spruce budworm larval development. (A) Glutamic acid; (B) Lysine; (C) Arginine; (D) Threonine; (E) Alanine; (F) Phenylalanine; (G) Valine; (H) Leucine; (I) Isoleucine; (J) Glutamine; (K) Asparagine; (L) Serine; (M) Methionine; (N) Aspartic acid; (O) Glycine; (P) α-Aminobutyric acid.

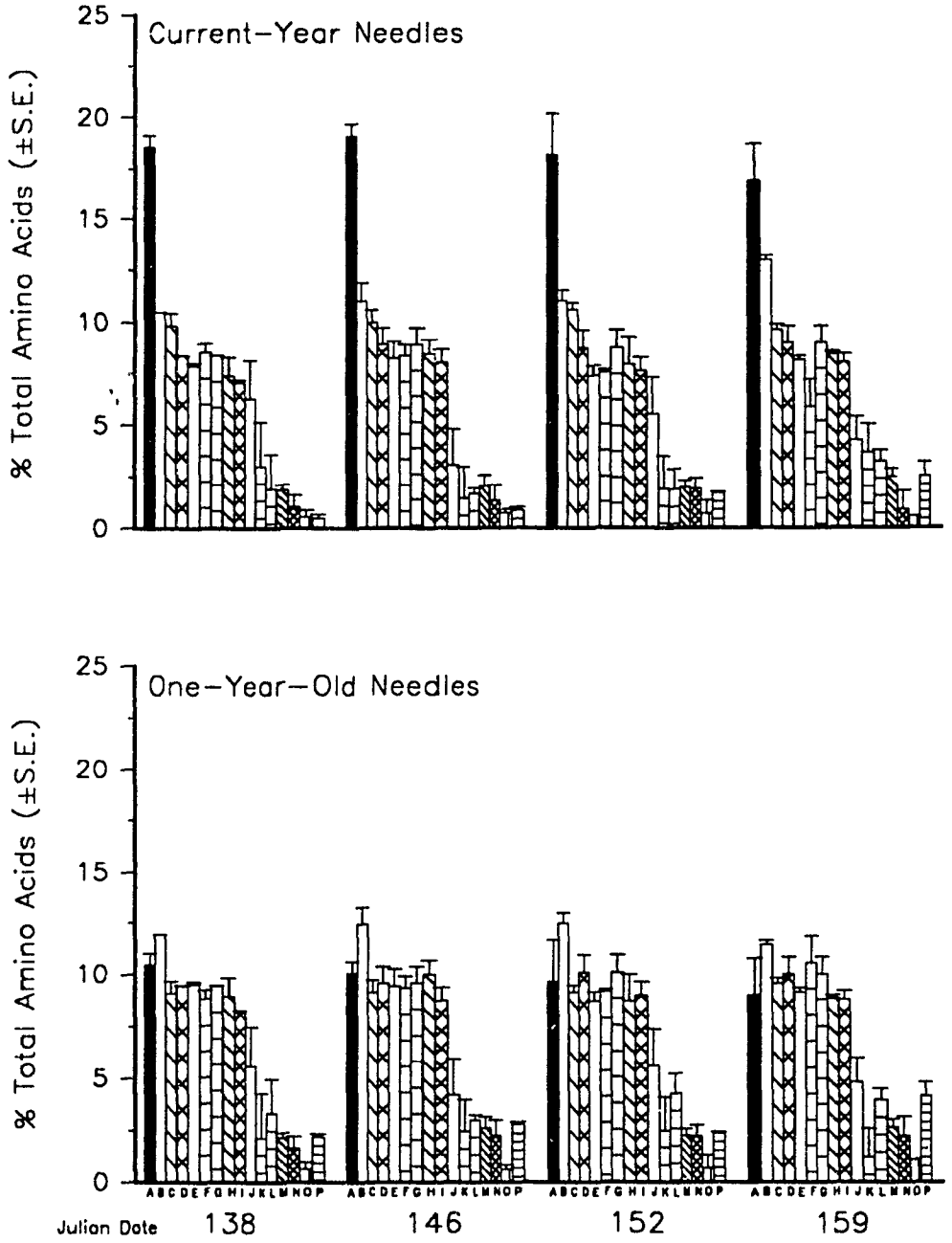

Figure 13. Variations in specific amino acids (% Total Amino Acids) in current- and one-year-old needles of 1988 currently defoliated white spruce during the period of spruce budworm larval development. See *Figure 12* for identification of the amino acids.

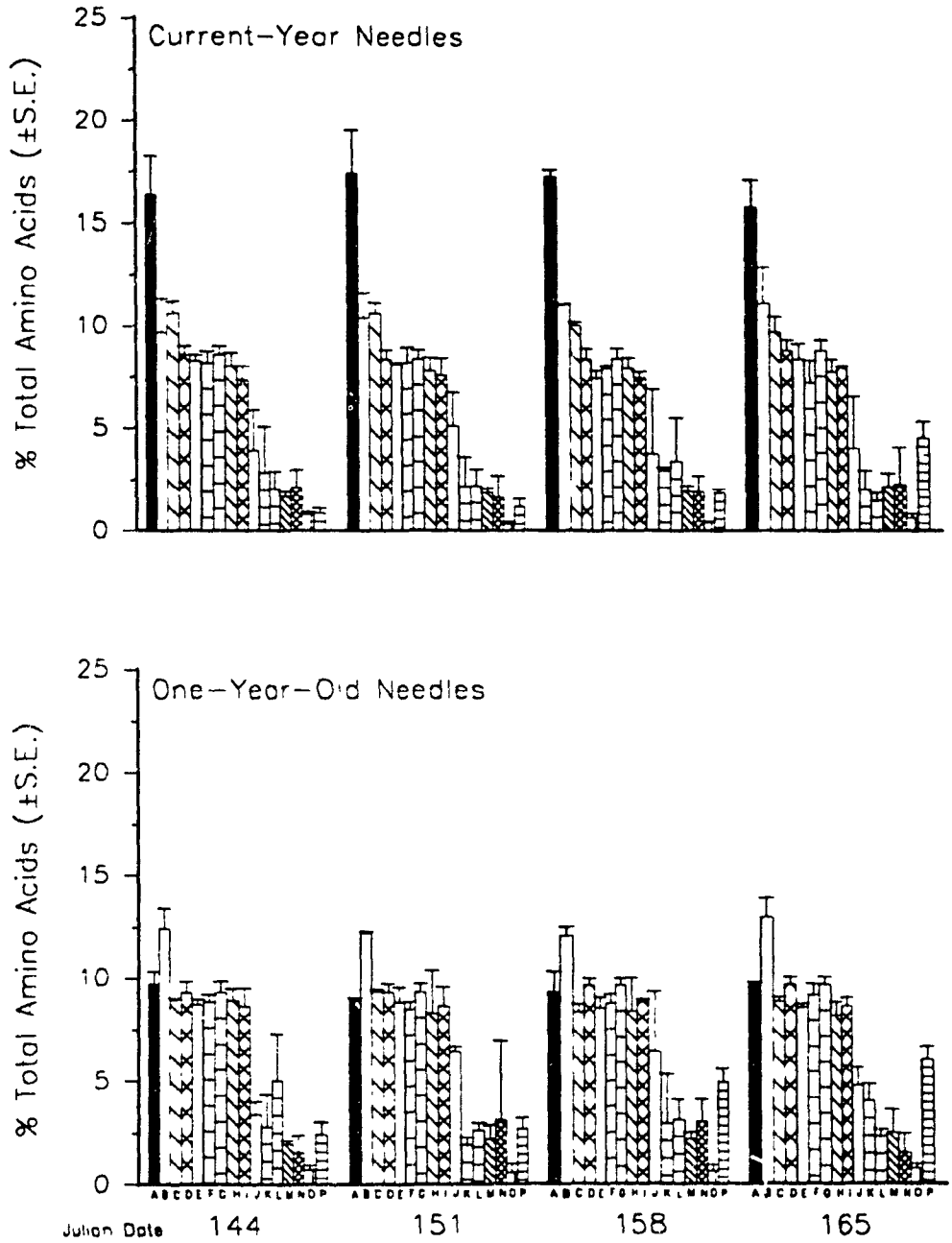

Figure 14. Variations in specific amino acids (% Total Amino Acids) in current- and one-year-old needles of 1989 undamaged white spruce during the spruce budworm larval development. See *Figure 12* for identification of the amino acids.

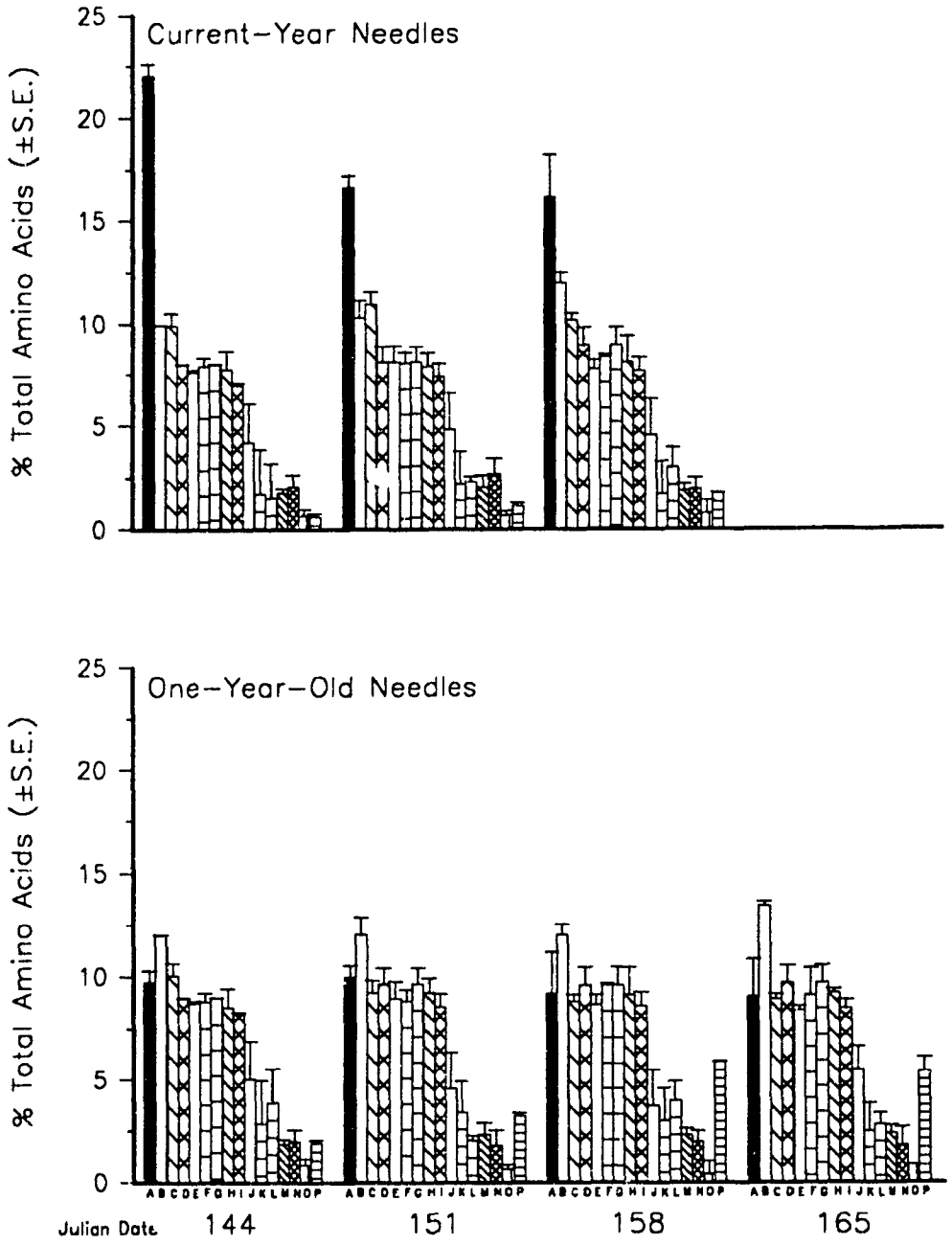

Figure 15. Variations in specific amino acids (% Total Amino Acids) in current- and one-year-old needles of 1989 currently defoliated white spruce during the period of spruce budworm larval development. See *Figure 12* for identification of the amino acids.

Variance analyses of the effect of insect density and time of tree sampling on the foliar concentration of each amino acid are presented in *Table 16*. In 1988 and 1989, in new shoot and old leaves, the total amount of each main amino acid was significantly affected by the collecting period. Current-year defoliation by the spruce budworm did not seem to affect the concentrations of these nitrogenous compounds, with a marginal exception for the amounts of arginine found in one-year-old needles of the trees collected in 1989, which were significantly affected by the insect density factor ($p = 0.0079$) and by a block effect ($p = 0.0108$). Concentrations of leucine and isoleucine found in one-year-old needles, and concentrations of α-aminobutyric acid detected in new shoots were also influenced by the time of tree sampling.

E. Discussion

Decreases of the total amino acid concentration in current- and one-year-old needles of young white spruce during the period when spruce budworm larval development occurs, were also reported by Durzan (1968). The author pointed out an important depletion in the nitrogen content of new and old leaves after emergence of the shoots. He also observed that an increase in nitrogenous compounds took place when the shoot elongation process ceased. Diminutions in total amount of amino acids and concentrations of specific amino acids found in current-year needles can be associated with plant

Table 16. Analysis of variance for specific foliar amino acid concentration of current- and one-year-old needles each with repeated measures for insect density and collecting period.

	1988								1989							
	Current Year Needles				One-Year-Old Needles				Current Year Needles				One-Year-Old Needles			
Source of Variation	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
Glutamic Acid																
Block	2	0.0188	0.38	0.6957	2	0.0000	0.00	0.9980	2	0.0645	1.07	0.3527	2	0.0020	1.32	0.3063
Treatment	1	0.0972	1.95	0.1933	1	0.0003	0.10	0.7556	1	0.3285	5.45	0.0444	1	0.0027	1.74	0.2145
Collecting Period	3	0.5617	11.24	0.0015	3	0.0665	22.12	0.0001	3	0.7108	11.79	0.0018	3	0.0334	21.60	0.0001
Treatment x Period*	3	0.0897	1.80	0.21[illegible]	3	0.0003	0.09	0.9646	2	0.0870	1.44	0.2860	3	0.0020	1.32	0.3181
Error	10	0.0500			11	0.0030			9	0.0603			11	0.0016		
Lysine																
Block	2	0.0015	0.07	0.9286	2	0.0009	0.44	0.6525	2	0.0588	5.89	0.0232	2	0.0036	3.23	0.0788
Treatment	1	0.0003	0.02	0.9047	1	0.0012	0.56	0.4693	1	0.0021	0.21	0.6591	1	0.0010	0.85	0.3758
Collecting Period	3	0.2347	11.68	0.0013	3	0.0429	20.19	0.0001	3	0.1743	17.46	0.0004	3	0.0585	52.41	0.0001
Treatment x Period	3	0.0080	0.40	0.7587	3	0.0005	0.22	0.8803	2	0.0329	3.29	0.0845	3	0.0002	0.15	0.9296
Error	10	0.0201			11	0.0021			9	0.0100			11	0.0011		
Arginine																
Block	2	0.0089	0.81	0.4735	2	0.0005	0.23	0.8000	2	0.0093	0.88	[illegible].4489	2	0.0047	7.04	0.0108
Treatment	1	0.0035	0.32	0.5848	1	0.0002	0.10	0.7623	1	0.0171	1.61	0.2359	1	0.0069	10.50	0.0079
Collecting Period	3	0.1116	10.10	0.0023	3	0.0391	17.42	0.0002	3	0.1294	12.24	0.0016	3	0.0363	54.95	0.0001
Treatment x Period	3	0.0071	0.64	0.6036	3	0.0009	0.40	0.7535	2	0.0513	4.85	0.0372	3	0.0011	1.69	0.2264
Error	10	0.0111			11	0.0022			9	0.0106			11	0.0007		
Threonine																
Block	2	0.0096	0.72	0.5090	2	0.0000	0.01	0.9855	2	0.0144	1.98	0.1940	2	0.0018	1.90	0.1962
Treatment	1	0.0002	0.01	0.9141	1	0.0000	0.03	0.8738	1	0.0052	0.72	0.4183	1	0.0015	1.53	0.2418
Collecting Period	3	0.0591	4.44	0.0314	3	0.0479	16.28	0.0002	3	0.0659	9.04	0.0044	3	0.0269	28.22	0.0001
Treatment x Period	3	0.0251	1.54	0.2639	3	0.0002	0.05	0.9828	2	0.0282	3.87	0.0613	3	0.0005	0.49	0.6968
Error	10	0.0133			11	0.0029			9	0.0073			11	0.0010		
Alanine																
Block	2	0.0042	0.31	0.7437	2	0.0004	0.17	0.8486	2	0.0431	4.87	0.0368	2	0.0033	2.95	0.0944
Treatment	1	0.0154	1.12	0.3147	1	0.0000	0.02	0.8960	1	0.0083	0.94	0.3584	1	0.0046	4.09	0.0683
Collecting Period	3	0.1919	13.95	0.0007	3	0.0326	14.47	0.0004	3	0.0799	9.04	0.0044	3	0.0212	19.06	0.0001
Treatment x Period	3	0.0086	0.62	0.615[illegible]	3	0.0019	0.85	0.4973	2	0.0199	2.26	0.1606	3	0.0010	0.91	0.4683
Error	10	0.0138			11	0.0023			9	0.0088			11	0.0011		
Phenylalanine																
Block	2	0.0071	0.58	0.5769	2	0.0008	0.37	0.6979	2	0.0091	1.35	0.3067	2	0.0013	0.88	0.4418
Treatment	1	0.0029	0.24	0.6348	1	0.0006	0.28	0.6079	1	0.0070	1.05	0.3326	1	0.0003	0.21	0.6592
Collecting Period	3	0.0268	2.21	0.1503	3	0.2551	12.52	0.0007	3	0.0386	5.75	0.0178	3	0.0213	14.77	0.0004
Treatment x Period	3	0.0190	1.57	0.2584	3	0.0001	0.06	0.9804	2	0.0163	2.42	0.1438	3	0.0005	0.37	0.7782
Error	10	0.0122			11	0.0020			9	0.0067			11	0.0014		
Valine																
Block	2	0.0040	0.25	0.7815	2	0.0007	0.29	0.7509	2	0.0114	1.00	0.4041	2	0.0021	1.95	0.1886
Treatment	1	0.0298	1.87	0.2013	1	0.0024	1.01	0.3375	1	0.0075	0.66	0.4376	1	0.0017	1.54	0.2408
Collecting Period	3	0.0349	2.19	0.1525	3	0.0356	15.24	0.0003	3	0.0668	5.88	0.0167	3	0.0223	20.80	0.0001
Treatment x Period	3	0.0239	1.50	0.2743	3	0.0008	0.07	0.9747	2	0.0242	2.13	0.1753	3	0.0007	0.63	0.6109
Error	10	0.0159			11	0.0023			9	0.0114			11	0.0011		
Leucine																
Block	2	0.0213	1.45	0.2791	2	0.0005	0.12	0.8892	2	0.0276	1.24	0.3335	2	0.0044	1.62	0.2417
Treatment	1	0.0309	2.12	0.1765	1	0.0005	0.12	0.7394	1	0.0457	2.06	0.1854	1	0.0029	1.08	0.3215
Collecting Period	3	0.0294	2.01	0.1767	3	0.0630	14.75	0.0004	3	0.0524	2.36	0.1399	3	0.0387	14.31	0.0004
Treatment x Period	3	0.0125	0.86	0.4939	3	0.0001	0.02	0.9956	2	0.0201	0.90	0.4386	3	0.0006	0.24	0.8695
Error	10	0.0146			11	0.0043			9	0.0222			11	0.0027		

Table 16. (continued)

Source of Variation	1988 Current-Year Needles df	MS	F	p	1988 One-Year Old Needles df	MS	F	p	1989 Current Year Needles df	MS	F	p	1989 One-Year Old Needles df	MS	F	p
Isoleucine																
Block	2	0.0009	0.08	0.9281	2	0.0009	0.34	0.7197	2	0.0343	5.07	0.0336	2	0.0058	2.91	0.0966
Treatment	1	0.0195	1.58	0.2380	1	0.0003	0.11	0.7429	1	0.0088	1.31	0.2827	1	0.0069	3.44	0.0908
Collecting Period	3	0.0389	3.15	0.0734	3	0.0404	15.70	0.0003	3	0.0639	9.44	0.0039	3	0.0248	12.40	0.0007
Treatment x Period	3	0.0133	1.07	0.4039	3	0.0005	0.19	0.9039	2	0.0240	3.54	0.0733	3	0.0013	0.62	0.6139
Error	10	0.0124			11	0.0026			9	0.0068			11	0.0020		
Glutamine																
Block	2	0.0141	0.85	0.4575	2	0.0031	1.02	0.3915	2	0.0031	0.08	0.9228	2	0.0019	0.97	0.4094
Treatment	1	0.1178	7.06	0.0240	1	0.0000	0.00	0.9702	1	0.0188	0.50	0.4985	1	0.0000	0.00	0.9807
Collecting Period	3	0.0816	4.89	0.0241	3	0.0289	9.60	0.0021	3	0.0163	0.43	0.7350	3	0.0049	2.56	0.1086
Treatment x Period	3	0.0544	3.26	0.0677	3	0.0041	1.35	0.3096	2	0.0014	0.04	0.9632	3	0.0051	2.70	0.0970
Error	10	0.0167			11	0.0030			9	0.0378			11	0.0019		
Asparagine																
Block	2	0.0136	0.71	0.5157	2	0.0007	0.37	0.6990	2	0.0225	0.81	0.4757	2	0.0052	1.51	0.2635
Treatment	1	0.0001	0.01	0.9337	1	0.0013	0.65	0.4384	1	0.0235	0.85	0.3816	1	0.0010	0.30	0.5964
Collecting Period	3	0.0677	3.53	0.0563	3	0.0024	1.21	0.3529	3	0.0107	0.38	0.7670	3	0.0020	0.58	0.6385
Treatment x Period	3	0.0046	0.23	0.8753	3	0.0004	0.22	0.8800	2	0.0156	0.56	0.5896	3	0.0025	0.71	0.5678
Error	10	0.0192			11	0.0020			9	0.0278			11	0.0035		
Serine																
Block	2	0.0129	0.92	0.4306	2	0.0002	0.12	0.8885	2	0.0144	2.49	0.1376	2	0.0007	0.45	0.6507
Treatment	1	0.0010	0.07	0.7911	1	0.0002	0.12	0.7345	1	0.0002	0.04	0.8524	1	0.0001	0.03	0.8583
Collecting Period	3	0.0065	0.46	0.7164	3	0.0030	1.78	0.2086	3	0.0092	1.60	0.2565	3	0.0155	9.31	0.0024
Treatment x Period	3	0.0005	0.03	0.9920	3	0.0001	0.03	0.9924	2	0.0053	0.92	0.4334	3	0.0013	0.77	0.5335
Error	10	0.0141			11	0.0017			9	0.0058			11	0.0017		
Methionine																
Block	2	0.0005	0.24	0.7905	2	0.0001	0.65	0.5397	2	0.0008	0.66	0.5386	2	0.0004	1.39	0.2905
Treatment	1	0.0024	1.31	0.2791	1	0.0003	1.28	0.2820	1	0.0017	1.45	0.2592	1	0.0002	0.75	0.4043
Collecting Period	3	0.0010	0.52	0.6801	3	0.0009	4.25	0.0319	3	0.0009	0.80	0.5233	3	0.0005	1.83	0.1995
Treatment x Period	3	0.0015	0.82	0.5127	3	0.0002	0.70	0.5716	2	0.0015	1.28	0.3243	3	0.0002	0.71	0.5651
Error	10	0.0019			11	0.0002			9	0.0012			11	0.0003		
Aspartic Acid																
Block	2	0.0068	1.34	0.3043	2	0.0001	0.06	0.9421	2	0.0015	0.16	0.8530	2	0.0014	0.67	0.5309
Treatment	1	0.0113	2.23	0.1660	1	0.0000	0.00	0.9515	1	0.0100	1.09	0.3230	1	0.0004	0.18	0.6828
Collecting Period	3	0.0091	1.80	0.2104	3	0.0009	0.57	0.6477	3	0.0057	0.62	0.6180	3	0.0021	1.04	0.4111
Treatment x Period	3	0.0018	0.35	0.7900	3	0.0002	0.13	0.9417	2	0.0075	0.82	0.4708	3	0.0006	0.27	0.8449
Error	10	0.0051			11	0.0016			9	0.0091			11	0.0020		
Glycine																
Block	2	0.0016	0.91	0.4321	2	0.0003	2.23	0.1534	2	0.0013	3.22	0.0881	2	0.0002	1.41	0.2852
Treatment	1	0.0002	0.09	0.7657	1	0.0001	0.56	0.4707	1	0.0009	2.39	0.1564	1	0.0000	0.00	0.9724
Collecting Period	3	0.0020	1.14	0.3804	3	0.0001	1.19	0.3568	3	0.0013	3.42	0.0660	3	0.0002	2.08	0.1610
Treatment x Period	3	0.0011	0.67	0.5916	3	0.0001	0.59	0.6325	2	0.0016	4.07	0.0550	3	0.0000	0.26	0.8601
Error	10	0.0017			11	0.0001			9	0.0004			11	0.0001		
α-Aminobutyric Acid																
Block	2	0.0010	0.99	0.4051	2	0.0013	1.07	0.3759	2	0.0019	1.74	0.2300	2	0.0003	0.32	0.7316
Treatment	1	0.0007	0.72	0.4169	1	0.0012	1.02	0.3347	1	0.0000	0.02	0.8914	1	0.0008	0.91	0.3599
Collecting Period	3	0.0134	12.91	0.0009	3	0.0002	0.12	0.9435	3	0.0439	40.84	0.0001	3	0.0067	7.99	0.0042
Treatment x Period	3	0.0009	0.85	0.4981	3	0.0003	0.24	0.8688	2	0.0007	0.62	0.5615	3	0.0015	1.72	0.2206
Error	10	0.0010			11	0.0012			9	0.0011			11	0.0008		

[a] Interaction between the Treatment factor and the Collecting Period factor that can explain variation in the results.

growing processes that occur after bud-break. During shoot elongation important quantities of amino acids are required for the biosynthesis of several nitrogenous plant constituents (Harborne 1988), which are fundamental for the formation of protoplasm and production of chlorophyll (Kramer and Kozlowski 1979). Reductions of the total amount of amino acids in one-year-old foliage may also suggest that these nitrogenous elements are allocated for the growth activity of current-year needles. Loach and Little (1973) mentioned that an important transfer of nitrogen from old leaves to young shoots occurs in balsam fir early in the plant growth season. Although the experiment was not designed to determine the suitability of current- and one-year-old white spruce needles for the spruce budworm, results support the hypothesis of Kimmins (1971), which suggests that differences in foliar amino acid concentrations between new and old foliage may explain plain the utilization of new expanding shoots by the larvae.

Glutamic acid was the dominant amino acid found in current-year needles. Important quantities of this amino acid were observed by Durzan (1968) before bud-break. The technique we used to identify the amino acids composition did not allow for the detection of proline. Proline is known to be an important amino acids of the white spruce foliage (Durzan 1968). The absence of information about the proline concentration of our foliage may explain the importance taken by other amino acids, e.g., arginine, aspartic

acid, asparagine, alanine, etc., which are normally minimal compared to the large amount glutamic acid and proline (Durzan 1968).

Of the parameters measured in this investigation, the effect of herbivory by the spruce budworm larvae on the phytochemical composition of amino acids was an important objective. Contrarily to expected results, it was found that variations in total amino acids and concentrations of main amino acids could not be attributed to an effect of current-year defoliation by the spruce budworm larvae. Defoliation is known to reduce the photosynthetic capacity by removing photosynthetic leaf area (Harper 1977). In evergreen coniferous species, herbivory is also recognized to affect the formation of subsequent shoots (Ericsson *et al.* 1985). Plant response to defoliation should occur after defoliation by herbivores. For example, in an experiment with Scots pine, *Pinus sylvestris* L., Ericsson *et al.* (1985) observed an increase of the foliar concentration of nitrogenous compounds after several defoliations. Piene (1980) observed the same increment in balsam fir needles one year after defoliation by the eastern spruce budworm. Niemela *et al.* (1984) observed on Scots pine, an improvement of the quality of the mature foliage for the European pine sawfly, *Neodiprion sertifer* (Geoff.), several years after previous defoliation of new shoots. Despite the fact that the present results show no significant difference between currently defoliated and undamaged trees during the period of spruce budworm larval development, we believe that possible

modifications in the amino acid composition following defoliation should take place later during the plant's growing season and during subsequent years.

In conclusion, the present work shows that natural variations exist in the amino acid composition of white spruce foliage during the period of spruce budworm larval development, and that these changes are associated with plant phenology. No variations in these phytochemical compounds during this period can be associated to current-year defoliation by the insects. More extensive analyses must be done to establish long term effects of defoliation on the chemical composition of white spruce, and their possible influence on the interaction between the insect and its host plant.

CHAPTER 5

Feeding Behaviour of Early and Late Instar Larvae of the Eastern Spruce Budworm, *Choristoneura fumiferana* (Clem.) [Lepidoptera: Tortricidae], Exposed to Amino Acid Fractions of White Spruce, *Picea glauca* (Moench) Voss.

A. Abstract

Feeding Preferences and Feeding Rates of third- and sixth-instar larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) exposed to amino acid fractions from currently defoliated and undamaged white spruce foliage were measured to determine whether changes in feeding behaviour occurred during the period of insect larval development. No significant differences were observed between the Feeding Preferences of early and late instar larvae. However, the relative amounts of discs eaten by the third-instar larvae were significantly higher compared to sixth-instar larvae. The possible role of feedback mechanisms regulating food intake is discussed.

B. Introduction

Variations in foliar nitrogen can directly influence the performance of phytophagous insects. Harvey (1974) pointed out that the larval development

of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.), required an important amount of nitrogen, especially during the first larval stages. Similar observations were made by Montgomery (1982) on gypsy moth, *Lymantria dispar* (L.), larvae. An acceleration of the larval development was also shown when spruce budworm fed on fertilized balsam fir, *Abies balsamea* (L.) (Shaw and Little 1977). Variations in nitrogen availability are considered as an important factor affecting the population size of several phytophagous insects (White 1976, 1978), and can be a limiting factor for certain herbivore populations Brewer *et al.* 1985). In a study on the effects of nitrogen concentration on performance of the western spruce budworm, *C. occidentalis* Freeman, Brewer *et al.* (1985) indicated that extreme surplus or deficits of nitrogen have significant detrimental effects on the survival and reproduction of this insect.

Of the common hosts used by the eastern spruce budworm, white spruce, *Picea glauca* (Moench) Voss is the most suitable for larval development (Koller and Leonard 1981, Lavallée and Hardy 1988). Results on feeding preferences have shown that sixth-instar larvae should prefer white spruce to all other hosts when given a choice (Albert and Parisella 1985a). Durzan and Lopushanski (1968) reported that the levels of amino acids in spruce budworm larvae varied with food source. Larvae which fed on white spruce had significantly higher relative levels of free amino acids than larvae which fed

on other host plants. Amino acids are known to have significant effects on certain physiological and behavioural processes of many defoliator insects (Feeny 1970, White 1974, 1978, Scriber 1978, Slansky and Feeny 1977, Mattson 1980, Montgomery 1982). Certain amino acids have been shown to play a particular role on the feeding behaviour of the eastern spruce budworm. Peripheral chemoreceptors of the sixth-instar larvae are sensitive to some pure amino acids (Albert pers. comm.). Proline (Heron 1965, Albert and Parisella 1988a), alanine, lysine, serine and valine (Albert and Parisella 1988a) are known to stimulate feeding in sixth-instar larvae. These larvae also show a preference for amino acid fractions extracted from the host plants (Albert and Parisella 1985ab, 1988a). However, little is known on the feeding preferences of young instar larvae for these amino acid fractions.

Evidence suggests that the amino acid requirement can be modified during the insect's development (Hill *et al.* 1968). The present work describes and compares the feeding behaviour of third- and sixth-instar spruce budworm larvae exposed to amino acid fractions extracted from young white spruce trees. The first objective was to establish whether, during the larval growth period, modifications in the feeding response would occur in response to changes in amino acid concentrations associated with plant physiological activities, and in response to current-year defoliation by budworm larvae.

C. Materials and Methods

Three-year-old white spruce trees used in the study were provided by the Laurentian Forestry Centre, Sainte-Foy, Québec. Amino acid fractions from the current- and one-year-old needles used in the feeding bioassays were obtained from trees placed outside in individual cages. Twenty-four trees were used in a one 2 x 3 factorial design: two densities of spruce budworm larvae, and four collecting periods corresponding to the number of weeks during which spruce budworm larval development occurs; with 3 replicates per treatment combination. Fifty percent of the trees were considered as defoliated trees, and were characterized by the introduction on each plant, just after the beginning of shoot elongation, of 15 second-instar spruce budworm larvae obtained from the Forest Pest Management Institute, Sault Ste Marie, Ontario. The control or undamaged trees were plants reared under the same conditions, but on which no insects had been added.

In 1988 and 1989, during the period of development of the spruce budworm larvae, current- and one-year-old foliage samples were collected each week. These were stored at -15 °C, lyophilized and powdered, and then stored until used for chemical extraction. Methods of amino acid extraction were similar to those described in Chapter 4. For young and old foliage collected on each tree during each sample period, amino acid extractions were done using

a methanol, chloroform, water solution. The suspension of extracted polar compounds was passed through a column filled with a cation exchange resin (Ag50-x8, Bio-Rad Lab.), and the adhering amino acids were eluted using a 2 *N* ammonium hydroxide solution. Based on the freeze-dried weights of the sample and the percentages of moisture, the extracts were dried and dissolved in a proper volume of water to reach the amino acid concentrations found in each age category of foliage.

The two-choice disc bioassay design used in this experiment was based on that of Albert *et al.* (1982) for the sixth-instar larvae, and on that of Maloney *et al.* (1988) for the third-instar larvae, and was described in Chapter 3. Eight large discs (6.5 mm diameter) and four small discs (3.3 mm diameter) were cut from cellulose nitrate filter paper (0.45 μm pore size; Sartorius), and were arranged in a circular fashion in individual test arenas (Appendix 1). Test discs were wetted with an 8 μl aliquot of extracted amino acid fraction for large discs and a 3 μl aliquot for small discs. The control discs were impregnated with the same volume of distilled water respectively. For each test arena, test and control discs were alternated and the positions of the discs were randomly determined.

Third- and sixth-instar spruce budworm larvae were used to measure the feeding preferences and the feeding rates of the insect for each amino acid

extract. Prior to any tests, larvae were starved 24 h after the moult, and each insect was used only once in each experiment. Larval stages of the spruce budworm were determined by measurement of the head capsule width (McGugan 1954). Data from tests in which the insect moulted during the experiment were discarded. A single larva was placed in the center of each arena, and covered with a lid to prevent water loss. The arenas were placed in a controlled environment chamber [25 °C, 60 % R.H., 16:8 (L:D) photoperiod] and the insects were allowed to feed for 24 h. For each larval stage and each amino acid extract, the experiment was repeated using 20 different insects.

The percentages of total area consumed were visually determined for control and treated discs. The Feeding Preference was estimated as the difference between the mean percent consumption of test discs and that of control discs. Preference data were analyzed non-parametrically using Wilcoxon's Signed-Rank test (NPAR module; Wilkinson 1989). The Feeding Rate was measured and corresponded to the average total consumption per hour of test and control discs. Since the control discs in all experiments contained distilled water, the Feeding Rate was utilized to evaluate the preference of a larva for each amino acid extract.

In order to compare the feeding responses of the third- and sixth-instar larvae for each amino acid extract, data were transformed using correction

factors previously described in Chapter 1. The Relative Preference Index and Feeding Rate Index calculated from these transformations were used to compare the Feeding Preference and the Feeding Rate of each larval instar for a specific amino acid fraction. Since no differences were observed between feeding responses of larvae exposed to foliage collected the same week for each age category, data were pooled. Individual *t*-tests were performed on data of each measured feeding parameter for third- versus sixth-instar larvae (TTEST procedure; SAS Institute Inc. 1985).

D. Results and Discussion

Feeding Preference

Feeding Preference data of third-instar larvae of the spruce budworm show that discs wetted with amino acid fractions extracted from currently defoliated and undamaged white spruce foliage were significantly preferred over those impregnated with distilled water (*Table 17*). However, no significant difference was found between control and test discs in three cases, and these responses can be explained by a low number of replicates for these plant extracts. Feeding Preferences were similar for the sixth-instar larvae exposed to amino acid extracts (*Table 18*). The Feeding Preference Index, which was used to compare the feeding preferences of the two larval stages, indicated that in most cases, no significant differences were found between the

Table 17. Percent consumption of control and test discs of third-instar larvae of the eastern spruce budworm for amino acid fractions from currently defoliated and undamaged white spruce foliage.

Year	Needle[a]	Treatment[b]	Period[c]	n	Mean % Consumption Control[d]	Test	±S.E.	p[e]
1988	1	1	138	25	31.84	68.16	7.80	0.0322
	1	1	146	21	12.70	87.30	6.70	0.0005
	1	1	152	32	34.68	65.32	6.40	0.0352
	1	1	159	36	25.38	74.62	6.19	0.0012
	1	2	138	41	7.55	92.45	3.19	0.0000
	1	2	146	50	35.63	64.37	6.02	0.0210
	1	2	152	38	20.54	79.46	6.00	0.0002
	1	2	159	32	28.30	71.70	6.60	0.0048
	2	1	138	33	31.29	68.71	7.18	0.0120
	2	1	146	24	22.40	77.60	6.92	0.0029
	2	1	152	28	8.33	91.67	5.04	0.0000
	2	1	159	26	26.76	73.24	8.03	0.0099
	2	2	138	7	28.57	71.43	18.44	0.2568
	2	2	146	34	32.11	67.89	5.76	0.0124
	2	2	152	31	27.46	72.54	6.45	0.0029
	2	2	159	21	25.60	74.40	8.89	0.0178
1989	1	1	144	9	24.44	75.56	11.44	0.0495
	1	1	151	34	22.74	77.26	5.36	0.0002
	1	1	158	15	32.22	67.78	8.83	0.0732
	1	1	165	n.a.	.	.	.	
	1	2	144	13	24.51	75.49	10.03	0.0260
	1	2	151	10	46.17	53.83	13.92	0.8516
	1	2	158	32	28.98	71.02	5.24	0.0006
	1	2	165	30	23.06	76.95	5.77	0.0007
	2	1	144	11	11.19	88.81	9.12	0.0100
	2	1	151	16	31.41	68.59	8.40	0.0427
	2	1	158	43	31.70	68.30	5.42	0.0026
	2	1	165	17	25.39	74.61	9.29	0.0298
	2	2	144	11	29.54	70.46	10.74	0.0948
	2	2	151	17	19.61	80.39	8.21	0.0081
	2	2	158	31	22.35	77.65	6.32	0.0007
	2	2	165	42	19.19	80.81	4.85	0.0000

[a] 1= Current-year needles; 2 = One-year-old needles.
[b] 1 = Defoliated trees; 2 = Undamaged trees.
[c] Julian Date.
[d] Distilled water for all experiments.
[e] Wilcoxon's Signed-Ranks test, probability value.

Table 18. Percent consumption of control and test discs of sixth-instar larvae of the eastern spruce budworm for amino acid fractions from currently defoliated and undamaged white spruce foliage.

Year	Needle[a]	Treatment[b]	Period[c]	n	Mean % Consumption Control[d]	Test	±S.E.	p[e]
1988	1	1	138	46	26.40	73.60	2.61	0.0000
	1	1	146	33	36.76	63.21	3.86	0.0009
	1	1	152	53	38.53	61.47	3.25	0.0002
	1	1	159	55	37.31	62.69	3.35	0.0010
	1	2	138	57	32.94	67.06	3.02	0.0000
	1	2	146	32	33.13	66.87	2.69	0.0000
	1	2	152	53	40.45	59.55	3.51	0.0080
	1	2	159	54	39.95	60.05	3.17	0.0039
	2	1	138	54	24.51	75.49	2.56	0.0000
	2	1	146	49	42.39	57.61	3.51	0.0379
	2	1	152	51	65.70	34.30	3.78	0.0003
	2	1	159	35	30.82	69.18	3.52	0.0000
	2	2	138	37	33.30	66.70	3.28	0.0001
	2	2	146	49	32.37	67.33	2.82	0.0000
	2	2	152	54	37.62	62.38	3.42	0.0007
	2	2	159	37	30.35	69.65	3.81	0.0001
1989	1	1	144	18	17.35	82.15	8.03	0.0003
	1	1	151	55	30.91	69.09	3.16	0.0000
	1	1	158	18	33.48	66.52	5.90	0.0228
	1	1	165	n.a.	.	.	.	
	1	2	144	19	27.43	72.57	4.50	0.0008
	1	2	151	33	36.01	63.99	3.47	0.0010
	1	2	158	52	28.81	71.19	3.22	0.0000
	1	2	165	57	31.67	68.33	2.48	0.0000
	2	1	144	18	31.25	68.75	6.00	0.0156
	2	1	151	38	34.48	65.52	2.81	0.0000
	2	1	158	43	33.61	66.39	3.90	0.0002
	2	1	165	36	35.94	64.06	4.12	0.0018
	2	2	144	17	26.40	73.60	5.09	0.0027
	2	2	151	18	26.68	73.32	5.57	0.0006
	2	2	158	55	37.32	62 .68	3.25	0.0002
	2	2	165	48	29.59	70.41	2.87	0.0002

[a] 1= Current-year needles; 2 = One-year-old needles.
[b] 1 = Defoliated trees; 2 = Undamaged trees.
[c] Julian Date.
[d] Distilled water for all experiments.
[e] Wilcoxon's Signed-Ranks test, probability value.

preference of third- and sixth-instar larvae for amino acid fractions extracted from current-year needles of currently defoliated and undamaged white spruce (*Figure 16*). Similar results were observed when amino acid extracts obtained from one-year-old foliage were presented to the larvae (*Figure 17*). It is interesting to note that preferences of third-instar larvae were in several cases stronger than those of sixth-instar larvae. The feeding preferences of third- and sixth-instar larvae exposed to current-year amino acid fractions were relatively similar to those of larvae exposed to foliar extracts of one-year-old needles. No significant correlation was found between the Feeding Preference Index and the total amount of amino acids (previously determined in Chapter 4) found in current-year needles (*Table 19*).

With the exceptions note above, preferences of spruce budworm larvae for amino acid extracts were consistent with observations on the sixth-instar larvae exposed to current-year foliage (Albert and Jerrett 1981, Albert 1982) and one-year-old foliage (Albert and Parisella 1988a), suggesting that new and old leaves provide similar feeding stimuli. Our previous work showed that current-year needles were constantly higher in total amino acid concentration (Chapter 4). Normally, spruce budworm larvae feed on new expanding shoots of their host plants (Blais 1958). The consumption of old needles can occur when new foliage is unavailable (McGugan 1954, Blais 1952, 1979). Kimmins

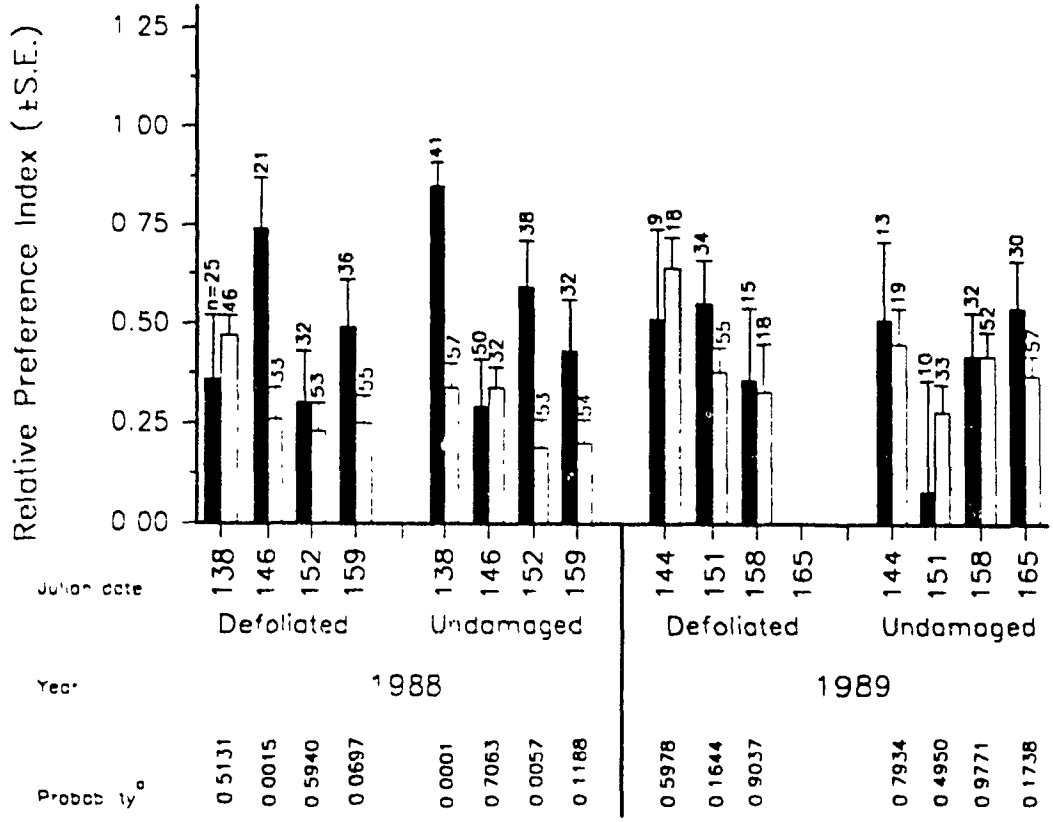

Figure 16. Relative Preference Indices of third- and sixth-instar larvae of the eastern spruce budworm for amino acid fractions from current-year needles of defoliated and undamaged white spruce trees. ■ Third-instar larvae; □ Sixth-instar larvae; [a] *t*-test probability.

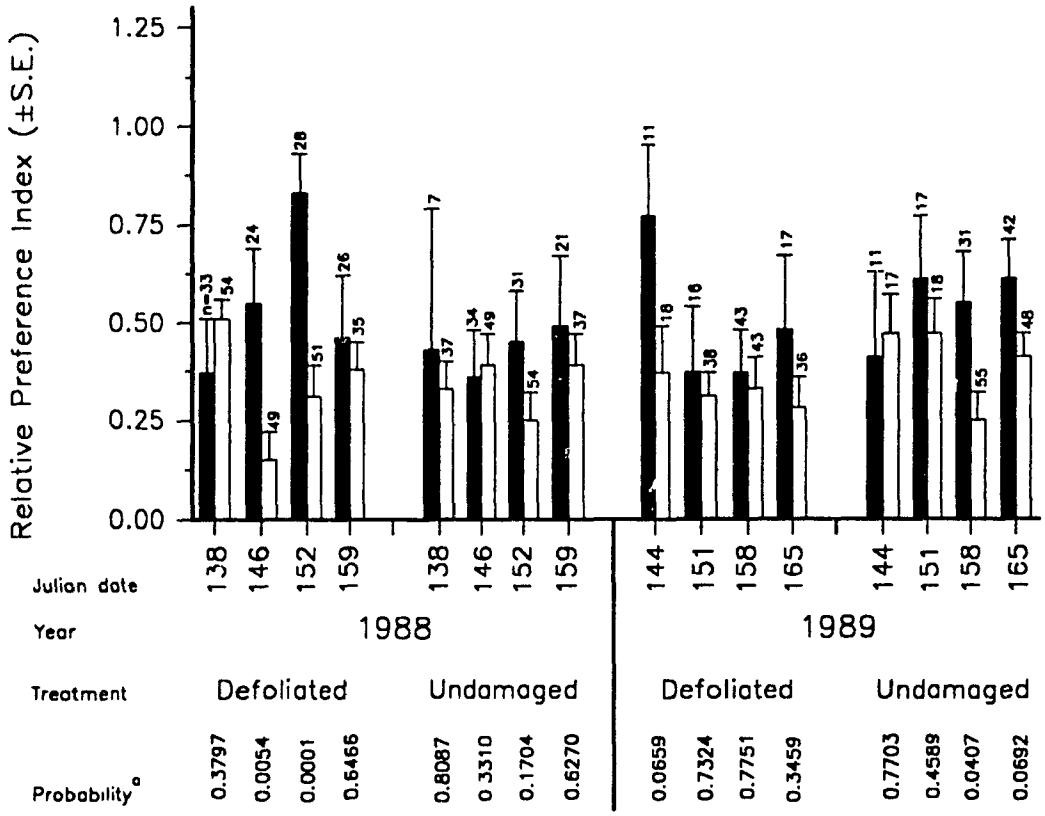

Figure 17. Relative Preference Indices of third- and sixth-instar larvae of the eastern spruce budworm for amino acid fractions from one-year-old needles of currently defoliated and undamaged white spruce trees. See *Figure 16* for the legend.

Table 19. Correlations between the Relative Preference Indices of the third- and sixth-instar larvae of the eastern spruce budworm and the total amount of amino acids found in current and one-year-old needles of white spruce.

	Instar Larvae	
	3rd	6th
Current-Year Needles (1988)		
Total Amino Acids	0.0932 (0.1231)[a] 275[b]	0.0551 (0.2825) 383
One-Year-Old Needles (1988)		
Total Amino Acids	0.0527 (0.4544) 204	0.0457 (0.3834) 366
Current-Year Needles (1989)		
Total Amino Acids	0.0695 (0.4096) 143	-0.0131 (0.8365) 252
One-Year-Old Needles (1989)		
Total Amino Acids	0.0550 (0.2825) 188	0.0050 (0.9350) 273

[a] Probability.
[b] Number of observations.

(1971) reported that differences in amino acid concentrations between current- and one-year-old needles should be an important factor to explain the preferred utilization of young foliage. However, the results on feeding preferences suggest that, in most cases, amino acid concentrations may not account for the avoidance of old foliage. The absence of correlations between the concentrations of amino acids and the relative feeding preferences of each instar larva is in accordance with this inference. These results reinforce the hypothesis that the spruce budworm's avoidance of the old needles may be the result of their high degree of lignification (Heron 1965, Albert and Parisella 1988a), the low concentration of water (Albert and Parisella 1988a), and the structure and composition of their wax layers (Chapter 3). The similarity in the feeding preferences between fractions from currently defoliated and undamaged white spruce foliage suggests that the chemical composition of the amino acid extracts provides the same, or similar stimuli to each instar. This deduction is reinforced by our previous chemical analyses which showed that current-year defoliation by the spruce budworm did not induce variations in the amino acid composition of current- and one-year-old needles during the period of insect larval development (Chapter 4).

Feeding Rate

In 1988 and 1989, the Feeding Rate Index shows that significant differences ($p < 0.05$; t-test) were observed between third- and sixth-instar

larvae exposed to amino acid fractions (*Figure 18*). In all cases, the relative disc consumption indicates that third-instar larvae consumed more than sixth-instar larvae. Moreover, amounts of discs eaten were more important when young instar larvae were exposed to discs impregnated with amino acids extracts rather then those wetted with 25 m*M* sucrose, and the inverse was true for the late instar larvae. No significant correlations were found between the Feeding Rate Indices of the third-instar larvae and the total concentration of amino acids previously established in current- and one-year-old needles (*Table 20*). However, significant correlations exist between the relative consumption of sixth-instar larvae and the amino acid concentration of new and old foliage.

Differences in the relative consumption of third- and sixth-instar larvae compared to the similarities in feeding preferences clearly demonstrate that no association can be made between these two measures of feeding behaviour when insects are exposed to amino acid extracts. As proposed previously in Chapter 3, the dissimilarity in results of Feeding Rate Indices between young and late instar larvae reinforces the hypothesis that some feedback mechanism could be present which regulates food intake during the period of growth of the larvae. Such feedback mechanism has been proposed to explain the variations in feeding behaviour of the desert locust, *Locusta migratoria* L. (Abisgold and Simpson, 1987, 1988, Simpson *et al.* 1990). For the spruce budworm, demands

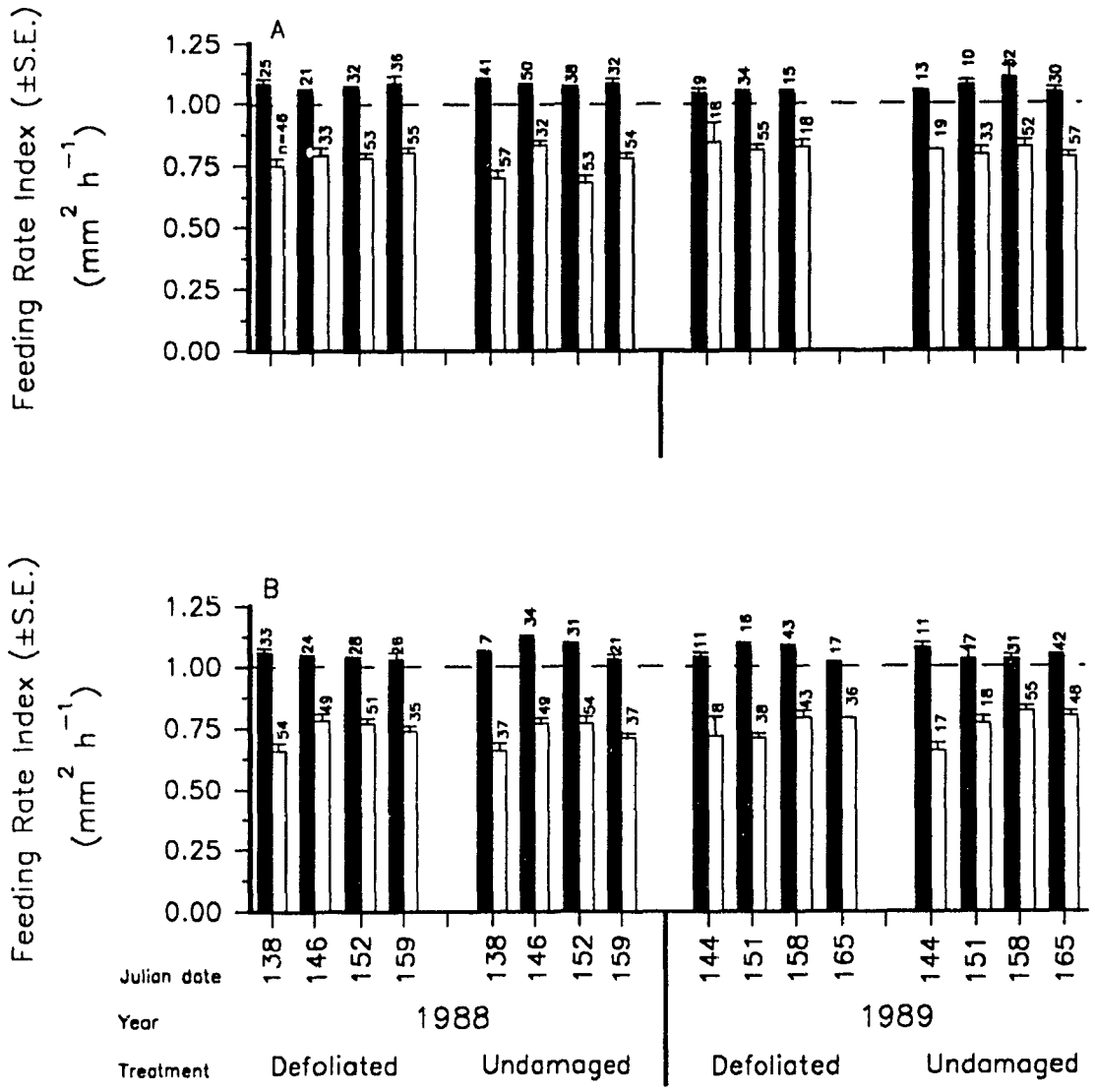

Figure 18. Feeding Rate Indices of third- and sixth-instar larvae of the eastern spruce budworm for amino acid fractions from currently defoliated and undamaged white spruce trees. **A** Current-year foliage; **B** One-year-old foliage. See *Figure 16* for the legend.

Table 20. Correlations between the Feeding Rate Indices of the third- and sixth-instar larvae of the eastern spruce budworm and the total amount of amino acids found in current and one-year-old needles of white spruce.

	Instar Larvae	
	3rd	6th
Current-Year Needles (1988)		
Total Amino Acids	-0.0335 (0.5804)[a] 275[b]	0.1636 (0.0013) 383
One-Year-Old Needles (1988)		
Total Amino Acids	0.0055 (0.9376) 204	0.1189 (0.0228) 366
Current-Year Needles (1989)		
Total Amino Acids	0.0003 (0.9960) 143	0.1778 (0.0337) 252
One-Year-Old Needles (1989)		
Total Amino Acids	0.1137 (0.1204) 188	0.1971 (0.0011) 273

[a] Probability.
[b] Number of observations.

of nitrogenous elements are very important during the development of the young instar larvae (Harvey 1974). These may influence the levels of amino acid in haemolymph. Variations in the relative proportion of amino acids in spruce budworm larval tissues were previously reported by Durzan and Lopushanski (1968). The change in the level of nitrogenous compounds in blood may in turn modify the peripheral sensitivity to that group of compounds, and so may influence the feeding behaviour for a specific food source.

The present work shows the complexity of the relationship which exists between the nutritional status of a food source and the feeding behaviour of a growing insect. More investigations are needed to compare the electrophysiological responses of young and late instar larvae of the spruce budworm to amino acids, in order to get a more complete picture of the interaction between the peripheral response and the feeding behaviour of this insect.

CONCLUSION

This study is a part of a global research program to understand the bases of food selection by phytophagous insects in terms of behavioural responses to food plant chemicals. The phytochemical products present in plants are a priori assumed to play an important role in influencing the feeding behaviour of insects. The results of this study using third- and sixth-instar larvae of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) are in accordance with this assumption. Two main groups of compounds - carbohydrates and amino acids -were investigated for their potential influence on the feeding behaviour of this insect. These chemical products, extracted from foliage of white spruce, *Picea glauca* (Moench) Voss, a common host of the spruce budworm, are known to be important for the insect's larval development. Based on the relationships between these plant chemical compounds and the insect feeding behaviour, the following questions were addressed during this study: (1) How different are the feeding behaviours of third- and sixth-instar larvae of the spruce budworm exposed to carbohydrate and amino acid extracts of white spruce foliage? (2) What are the modifications in the phytochemical fractions that should influence the feeding behaviour of each larval instar? (3) What are the short term effects of defoliation by the spruce budworm on the chemical composition of the two foliar fractions? and related to the above (4) what are the implications of these variations on the feeding responses of each larval instar?

To answer the questions, feeding preferences and feeding rates of early and late instar larvae were determined. Larvae were offered a choice between test discs and control discs, using distilled water as a control in all experiments, and plant extracts as test discs. The results indicated that third- and sixth-instar larvae exposed to carbohydrate fractions (Chapter 1 and 3) and to amino acid fractions (Chapter 5) show significant p·eferences for these extracts. No significant differences were found between feeding preferences of the two larval stages for each chemical extract. However, among these two group of compounds, carbohydrate fractions appear to be the most stimulating compared to amino acid fractions, and these results were consistent with the previous work of Albert and Jerrett (1981).

Relative feeding rates were calculated in order to compare the feeding preferences of each larval stage for the plant extracts. In relation to responses using pure sucrose, the results suggest that preferences of third-instar larvae exposed to carbohydrate fractions were more important than those of larvae exposed to sucrose, and inversely for the sixth-instar larvae (Chapter 3). For the amino acid extracts, preferences of the young instar were almost identical to sucrose, and preferences for this pure sugar remained important for the sixth-instar larvae (Chapter 5). These variations in relative amount of discs eaten between young and late instar larvae clearly demonstrate that chemical fractions may play different roles in each larval stage. A feedback mechanism

was proposed to explain the variations in consumption of third- and sixth-instar larvae. Future behavioural and electrophysiological studies should be done to understand this model, and should focus on the relations that can exist between the chemical extracts, the feeding behaviour and the specific larval instar requirements and their potential roles on the insect's food selection.

An important aspect of this research was to evaluate the chemical composition of the foliage. Several chemical differences were observed between fractions of the current- and one-year-old foliage (Chapter 2 and 4). However, evidence suggests that these differences did not influence the feeding behaviours of each larval instar (Chapter 3 and 4). The results suggest that the variations in chemical composition of the two age categories of needles does not account for the known avoidance of the old foliage by the spruce budworm, and that current-year defoliation by the insects did not affect the chemical composition of the host during the period corresponding to the budworm's development. These results are measured over a relatively short period of time, and following our observation, we believe that variations in chemical composition of defoliated trees should take place later during the plant's growing season and during subsequent years. Future studies should be done to establish the long term effects of defoliation by the spruce budworm on the chemical composition of white spruce. Electrophysiological experiments should be done to assess the sensitivity of the peripheral chemosensilla of the two

larval instars to the plant's phytochemicals, in order to determine whether there is a physiological basis for the differences in Feeding Rate Indices observed in the present study.

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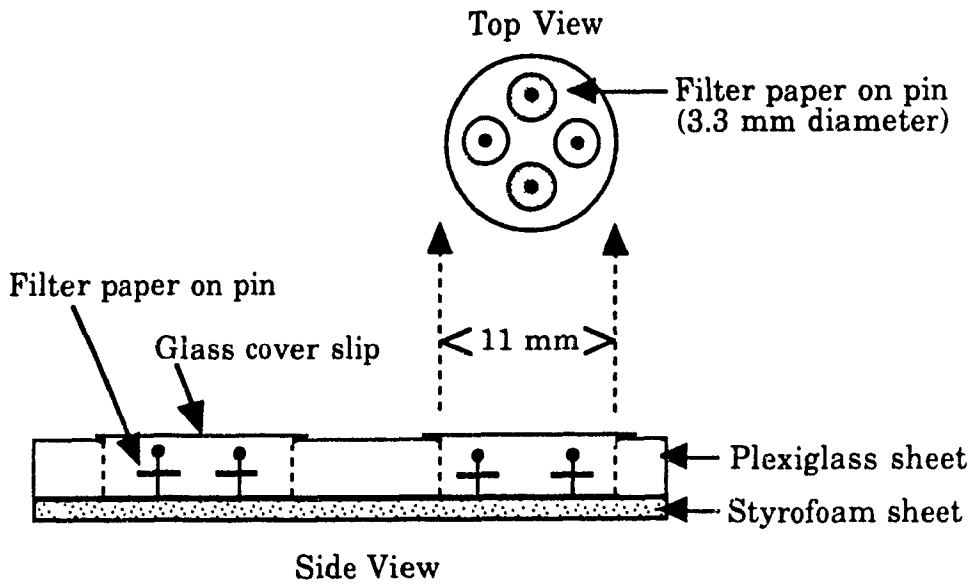
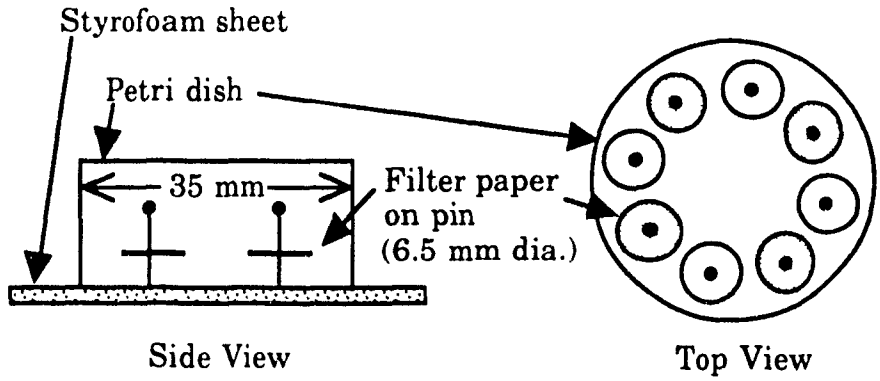
APPENDICES

Appendix 1. Two-choice feeding arena used in the bioassays to evaluate the Feeding Preference and Feeding Rate of third- (**A**) and sixth-instar larvae (**B**) of the eastern spruce budworm.

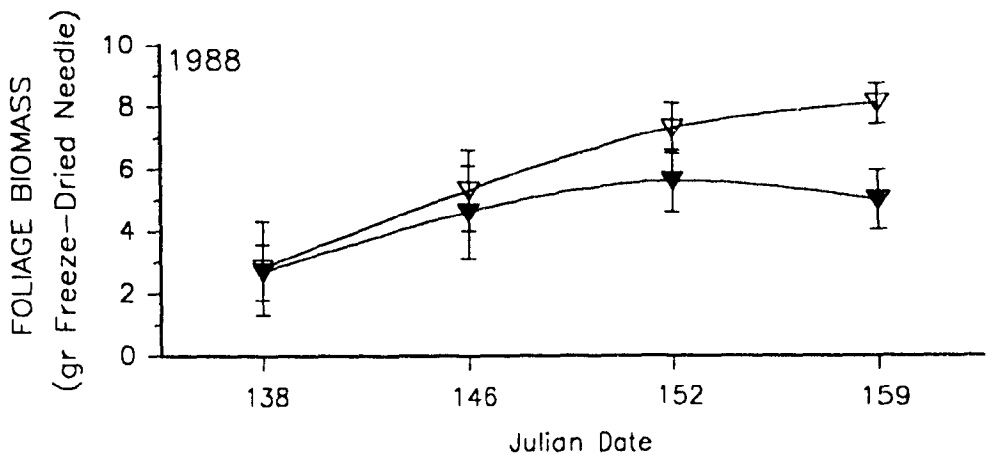
A

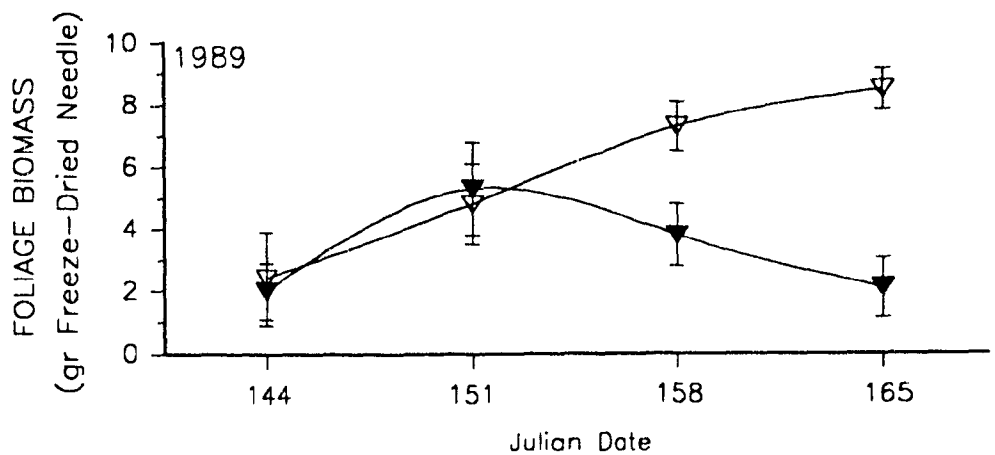


B

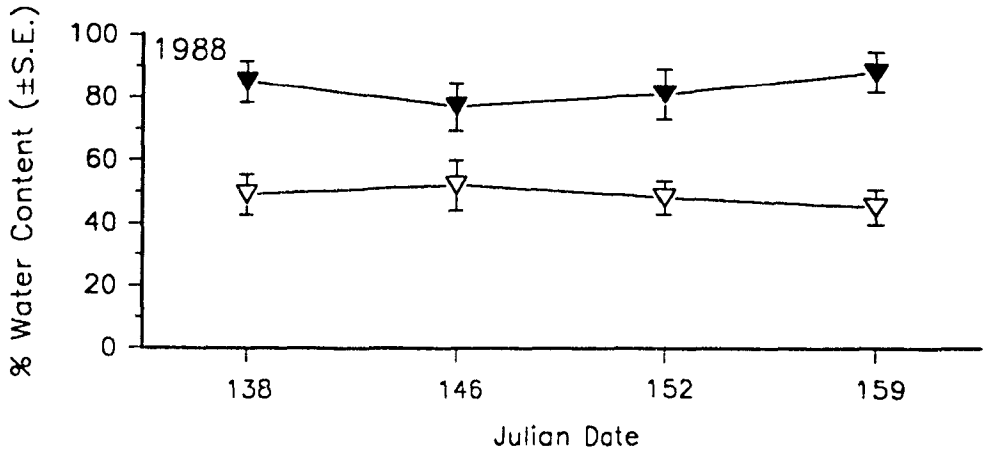


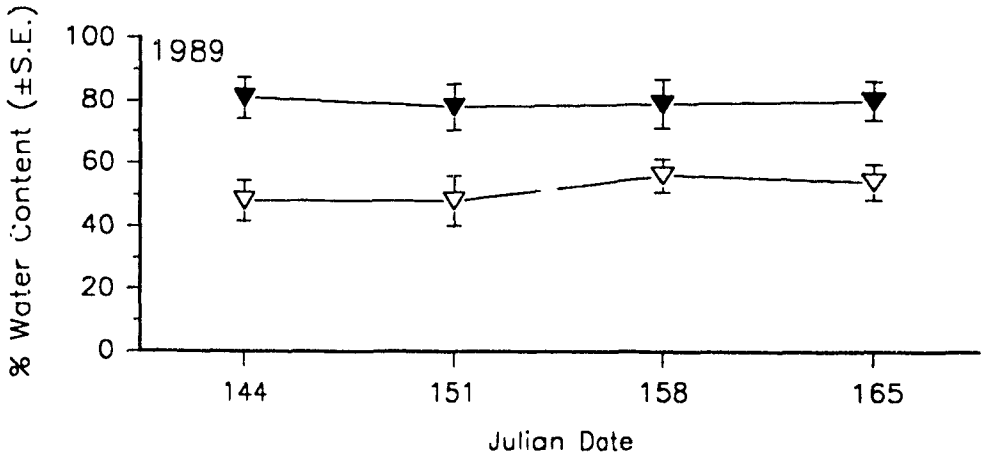
Appendix 2. Biomass of the current-year foliage (gr of freeze-dried needle) collected on currently defoliated —▼—▼—▼— and undamaged trees —▽—▽—▽—, during the period corresponding to the eastern spruce budworm larval development (n = 3).





Appendix 3. Percentage of water (gr/gr freeze-dried needle x 100) found in current- ▼—▼—▼ and one-year-old ▽—▽—▽ foliage of white spruce, *Picea glauca* (Moench) Voss, during the period corresponding to the eastern spruce budworm larval development (n = 6).





Appendix 4. Technical information of amino acid analysis by High Pressure Liquid Chromatography.

Column: Water Radial-Pak Resolve C_{18} (80 x 100 mm)

Buffers:

A- Solution of methanol, tetrahydrofuran and water in a ratio of 2:2:96, in which 0.05 M of disodium phosphate and 0.05 M of sodium acetate have been add and equilibrated at pH 7.5 with acetic acid.

B- Solution of methanol and water in a ratio of 65:35.

Reagent: o-phtalaldehyde 5 mg/ml in buffer of 0.5 M Sodium Borate equilibrated at pH 10.0.

Elution Gradient:

Time	Flow	% A	% B
0.0	0.1	70	30
2.0	0.1	70	30
2.5	2.5	70	30
6.5	2.5	70	30
14.0	2.5	38	62
19.0	2.5	15	85
20.0	2.5	0	100
21.0	2.5	70	30
26.5	2.5	70	30
27.0	0.0	70	30

Appendix 5. Standard used in the determination of total amount and concentration of each amino acid found in white spruce foliage.

Standard was prepared from a solution stock using 10 mM in a 10% methanol solution.

Solution stock:

Amino Acid	mg/10 ml
Aspartic Acid	13.31
Glutamic Acid	14.71
Asparagine	13.21
Serine	10.51
Glutamine	14.61
Glycine	7.51
Threonine	11.91
Arginine	21.07
Alanine	8.90
Tyrosine	18.12
α-Aminobutyric Acid	10.30
τ-Aminobutyric Acid	10.30
Methionine	14.92
Valine	11.72
Phenylalanine	16.50
Leucine	13.12
Isoleucine	13.12
Lysine	18.20
Tryptophane	20.40
Histidine	20.96