The spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is considered the most important pest threatening coniferous forests of eastern North America. Its major hosts are balsam fir (*Abies balsamea* (L.)), white spruce (*Picea glauca* (Moench) Voss), red spruce (*Picea rubens* Sarg.), and black spruce (*Picea mariana* (Mill.) Britton, Sterns and Poggenb.), all of which contain antitherbivore defensive compounds, including tannins. Tannins are known to act as feeding deterrents, digestibility reducers, and toxins in numerous insects and specifically to reduce growth and survival in the spruce budworm. Using two-choice feeding assays, we show that tannic acid deters spruce budworm feeding, whereas tannins extracted from white spruce and containing 97.8% condensed tannins stimulate it. Moreover, a dose–response relationship was demonstrated for both types of tannin. To our knowledge, this is the first report of a phagostimulatory role of condensed tannins in any insect. Tannic acid only deterred feeding when in solution with sucrose but remained undetected when in solution alone, whereas the condensed spruce tannins produced a positive response alone, and this response was increased by the addition of sucrose. Based on these results, we speculate that both types of tannins produce their respective effects through different mechanisms: tannic acid seems to interfere with sucrose detection, whereas spruce tannins can evidently stimulate feeding directly in this insect. For both types of tannins, the insects do not seem to be able to detect the 0.75% tannin concentration, suggesting a possible response threshold. Rearing insects on diets containing tannic acid revealed possible effects of aversion learning and of induction when tested in feeding assays with tannic acid and spruce tannins, respectively.

**KEY WORDS** condensed tannins, hydrolysable tannins, *Choristoneura fumiferana*, spruce budworm, insect herbivore

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No generalizations regarding the effects of tannins on insect herbivores can yet be made (Bernays et al. 1989, Bautio et al. 2007). Tannins have been described as digestibility reducers (Feeny 1968, 1969; Swain 1977, Nomura and Itioka 2002), toxins (Steinly and Berenbaum 1985), and feeding deterrents (Bennett 1965, Muir et al. 1999), or in some cases, as having no significant affect (Lawson et al. 1984, Ayres et al. 1997). However, some hydrolysable tannins apparently produce a phagostimulatory response in some insects (Foss and Rieske 2003). The vast diversity of phytophagous insects and their myriad feeding habits has resulted in an unpredictable array of adaptations and counteradaptations to specific host tannins and perhaps to some classes of tannins in general. Furthermore, the tremendous structural diversity of the tannins reflects qualitative and quantitative functional differences with regards to their deterrent, toxic, and digestibility reducing effects. Therefore, the only way to assess with certainty the effect of a given tannin on a particular insect is to test that specific tannin on that specific insect.

The focus of prior research has been on postigestive mechanisms, such as decreased food nutritional quality, whereas the preigestive gustatory mecha-
nisms have been largely ignored. It is necessary to combine information on both pre- and postigestive effects of tannins to completely understand the role these compounds play in insect-plant interactions. Through a dual-choice feeding assay design, we tested whether the spruce budworm was capable of preigestive detection of tannins. We compared the effects of two different tannins on spruce budworm feeding: condensed tannins from the insect’s host plant, *P. glauca*, and tannic acid, with which the spruce budworm has no evolutionary history of interaction. Insects were reared on diets containing various concentrations of tannic acid to determine whether this would subsequently affect their preference in the feeding assay through habituation or aversion learning mechanisms. Plant material is chemically complex and several studies have shown that combinations of chemicals can produce unexpected results compared with responses to individual components (Chapman 2003). Thus, we combined the tannins with sucrose, a strong phagostimulant for the spruce budworm, to determine any interactions between these compounds. A mixture of two compounds is a considerable oversimplification of the host plant’s chemical composition but is a necessary step toward beginning to tease apart the individual roles of the various compounds involved.

**Materials and Methods**

A dual-choice feeding assay was used to study the preigestive detection of tannins by the spruce budworm and to examine the effect of these compounds on the insect’s feeding behavior. Feeding assays were performed as described previously by Albert and Parisella (1988). Insects were placed individually into a feeding arena in which they could choose between four control and four test discs wetted with 15 μl of a control or test solution, respectively. The amount (area) of each disc consumed was then measured and preference indices (mean percentage of consumption, feeding rate) calculated. In the first series of experiments, tannic acid and white spruce condensed tannin were each dissolved alone in distilled water to assess whether the spruce budworm was capable of detecting these compounds. In the second series of experiments, TA and SP were each dissolved in 25 mmol liter⁻¹ sucrose to determine the result of interactions between the two compounds on spruce budworm feeding. The third series of experiments proceeded as the second but tested insects reared on diets containing different amounts of tannic acid to determine the effect this might have on disc preference. Table 1 summarizes the combinations of diet, control solution, and test solution investigated during the dual-choice feeding assays.

| Diet          | Control solution | Test solution |
|---------------|------------------|---------------|
| Normal (0%)   | 25 mmol liter⁻¹ sucrose | 8% TA alone |
| dH₂O          | 8% ST alone      |               |
| 25 mmol liter⁻¹ sucrose | 0.75% ST | 8% ST |
|               | 8% ST alone      |               |
| 8% TA diet    | 0.75% ST         | 8% ST         |
|               | 15% TA           | 0.75% TA      |
| 15% TA diet   | 0.75% TA         | 15% TA        |
|               | 30% TA           |               |
| 8% TA         | 3% TA            | 8% TA         |
|               | 15% TA           | 3% TA         |
| 15% ST        | 0.75% TA         | 15% ST        |
|               | 30% TA           |               |

**Table 1. Combinations of diet, control solution, and test solution used in two-choice feeding tests**

Discs (surface area = 31.74 ± 0.05 mm²; 0.45-μm Millipore cellulose nitrate filter paper, Sartorius AG, Goettingen, Germany) were pinned with stainless steel 2.5-cm sewing pins ~5 mm above the floor of a Styrofoam sheet and arranged in a circle of 21 mm in diameter (feeding arena). Each feeding arena contained eight discs, four control and four test arranged in an A-B-A-B manner. Colored pins were used to distinguish between solutions (i.e., red = test, yellow = control) and colors were alternated between control and test solutions to control for possible color and position preferences. Each arena was covered by a 3.5-cm-diameter by 1-cm-high petri dish. The discs were wetted with a 15-μl aliquot of the appropriate solution (Table 1) just before placing the insect in the feeding arena. Larvae were starved for 24 h before being individually placed into a feeding arena for 24 h. In most cases, a minimum of 20 insects was tested. Insects that ate >3.75 discs or <0.25 discs of either control or test were discarded, along with insects that pupated or died during the experiment. Approximately 5% of test insects were discarded for the above-mentioned reasons.

**Larva Weighing.** Because tannins in the diet can affect larval weight and larval weight affects consumption (Kumbali 2005), insects reared on the three different diets were weighed. Twenty-six instars reared respectively on each of the three different diets (i.e., 0, 8, and 15% tannic acid diet) were dried in an oven at 75°C until the weight of the larvae remained constant between weighings (~72 h). Larvae were weighed individually, and mean weights of larvae reared on the different diets were compared by analysis of variance (ANOVA).

**Insect Rearing.** Insects were received as postdiapause second instars from the Forest Pest Management Institute (Sault Ste Marie, ON, Canada). Insects were reared according to the methods of Wilson and Grisdale (1988) and were maintained in an incubator at 22°C and 60% humidity with a photoperiod of 16:8 (L:D) h. Subsequent to emergence from the hiber-
Tannic Acid. Tannic acid was purchased from Sigma Chemie (Deisenhofen, Germany). The tested tannic acid (C_{76}H_{52}O_{46}) is a mix of gallotannins. The molecular weight of the tannic acid used in the current experiment was 1,294 g/mol.

Spruce Tree Tannins. Current-year foliage of white spruce was collected during the period of maximal budworm feeding (21 June 2001) in a 50-yr old white spruce stand in the Forêt Montmorency (47°19'N, 79°09'W), an experimental forest managed by Laval University (Ste-Foy, QC, Canada). The foliage was quick-frozen in liquid nitrogen before being brought to the laboratory. The foliage was freeze-dried to prevent oxidation of tannin. The dried foliage was ground in a Wiley mill and passed through a 20-mesh sieve. The foliage was ground in small batches (0.5 g) to avoid increase of temperature >40°C and enzymatic changes in phenolics. Foliage samples were then preserved at −20°C until use. Four kilograms of dried foliage was processed to obtain 320 g of purified tannins, indicating that white spruce current-year foliage contains ~8% tannins. Tannins were extracted per batch of 300 mg of dried foliage. Extraction was performed using the technique of Hagerman (1988) in a 70% aqueous-acetone, containing 1 g of ascorbic acid liter⁻¹ solution to limit oxidation of the tannins. Tissue homogenization in the solvent was performed with an Ultra-Turrax homogenizer, followed by filtration over Whatman no. 41 filter paper (Whatman, Maidstone, United Kingdom). The crude tannin extracts were purified on a column (22.8 cm by 1.7 cm in diameter) packed with Sephadex LH-20 gel (Hagerman and Butler 1980). Aliquots of the extracts (2 ml) were added to the column and rinsed with 120 ml of 95% ethanol, followed by elution of the tannins by 70 ml of 1:1 acetone-water solution. Purified extracts were freeze-dried to obtain the final tannin powder used in the insect rearing bioassays. The quality of the powder was tested by using the radial diffusion assay of Hagerman (1987) to evaluate its capacity to precipitate proteins. Also, a subsample of purified extract was separated using Kramer and Singleton (1969) methodology to document the proportion of hydrolysable tannin (2.2% dry weight basis) and the proportion of condensed tannin (97.8% dry weight basis) present in the extract. The content of spruce foliage in tannin also was analyzed using the chemical methodology described by Makkar et al. (1993). According to this method, spruce foliage contains 7.97% of tannin in tannic acid equivalent. This result is similar to the one that we obtained (8%) with the Hagerman (1988) and Hagerman and Butler (1980) methodology. The purified tannin extract used for the insect bioassays was also analyzed using the chemical methodology described by Porter et al. (1986). The results obtained using this chemical technique indicate that the purified tannin extract contained 95% condensed tannin in leucocyanidin equivalent which is consistent with what was found using the Kramer and Singleton (1969) method (97.8% dry weight basis).

Solutions. Concentrations were expressed as percent dry weight and were calculated to equal the corresponding concentration in the diet. A concentration of 0.75% therefore corresponds to 1.88 mg/ml, that of 8% to 0.020 g/ml, 15% to 0.0375 g/ml, and 30% to 0.075 g/ml. A 30% solution of spruce tannins was not used because the tannins did not completely dissolve at that concentration. The concentration normally found in white spruce foliage is ~8% dry weight (Bauce et al. 2006).

Analysis. After completion of the test, the remains of the discs were removed, glued to a black background and photographed, along with a ruler for scale. The area of each disc was calculated using ImageJ 1.36b software (Wayne Rasband, National Institutes of Health, Bethesda, MD). Mean percentage of consumption control and test discs was calculated by dividing the amount (area) of control or test discs eaten by the total amount of discs consumed. Feeding rate was calculated by dividing the total amount of discs consumed by the duration of the test (i.e., 24 h). The values obtained for mean percentage of consumption were arcsine transformed before analysis, as prescribed by Sokal and Rohlf (1995) for percentage of data. Normality was assessed by the Kolmogorov-Smirnov test. A paired t-test or Wilcoxon’s signed rank test was performed on all sets of mean percentage of consumption data. Two-way ANOVA was performed with diet and concentration as treatments, once for tests with spruce tannins and once for tests with tannic acid. Feeding rates were similarly analyzed by two-way ANOVA but not arcsine transformed. Linear regression was performed on arcsine transformed mean percentage of consumption data for test discs for each of the six diet-tannin/tannic acid combinations (i.e., three diets × 2 types of tannin).

During the two-choice feeding tests with larvae reared on normal (i.e., 0%) and 8% tannic acid diets, larvae were sexed by presence or absence of testes that look like two dark kidney-shaped spots visible in the posterior abdomen of males. Data were analyzed by three-way ANOVA, adding sex as a treatment along with diet and tannin concentration. Sex had no significant effect (i.e., \( P > 0.05 \)) on larval preference or feeding rate (Table 2) for larvae.
reared on these diets. No sex-related differences have ever been reported for feeding preferences in larval spruce budworm.

**Results**

Dual Choice Feeding Assays with Spruce Tannins and Tannic Acid. In the first series of experiments, TA and ST were dissolved alone (i.e., no added sucrose) in distilled water and tested against either water or a 25 mmol l⁻¹ sucrose solution (Table 1; Fig. 1). Tannic acid and spruce tannins produced opposite effects. Tannic acid deterred feeding, whereas the spruce tannins stimulated feeding enough to compete with the 25 mmol l⁻¹ sucrose solution. In all cases, the feeding rate was low (Table 3), about half that of control insects reared on normal diet and tested with 25 mmol l⁻¹ sucrose (Table 4), 4 mm²/h compared with 8 mm²/h.

In the second series of experiments, various concentrations of TA and ST were prepared in a solution of 25 mmol l⁻¹ sucrose. In these experiments, the test solution always consisted of a mixture of either TA or ST with 25 mmol liter⁻¹ sucrose. A solution of 25 mmol l⁻¹ sucrose was always used as the control solution. These solutions were tested on insects reared on either 0.8 or 15% TA diet. In every case, a significant effect was detected above the 0.75% concentration (Tables 5 and 6). A dose–response trend was apparent for tests with ST (Fig. 2) and TA (Fig. 3). The two-way ANOVA confirmed there was a significant effect of

| Compound         | Test factor | Treatment | df | $F$     | $P$     |
|------------------|-------------|-----------|----|---------|---------|
| Spruce tannins   | MPCT        | A: Diet   | 1  | 12.19   | <0.001  |
|                  |             | B: Concen | 2  | 0.32    | 0.72    |
|                  |             | A × B     | 2  | 7.46    | <0.001  |
|                  |             | A × C     | 1  | 0.15    | 0.70    |
|                  |             | B × C     | 2  | 0.97    | 0.38    |
|                  |             | A × B × C | 2  | 1.02    | 0.36    |
| FR               |             | A: Diet   | 1  | 8.67    | 0.004   |
|                  |             | B: Concen | 2  | 10.48   | <0.001  |
|                  |             | A × B     | 2  | 6.54    | 0.002   |
|                  |             | C: Sex    | 1  | 1.94    | 0.17    |
|                  |             | A × C     | 1  | 1.84    | 0.18    |
|                  |             | B × C     | 2  | 0.49    | 0.61    |
|                  |             | A × B × C | 2  | 0.47    | 0.63    |

| Tannic acid      | MPCT        | A: Diet   | 1  | 2.55    | 0.11    |
|                  |             | B: Concen | 3  | 14.32   | <0.001  |
|                  |             | A × B     | 3  | 0.36    | 0.78    |
|                  |             | C: Sex    | 1  | 3.97    | 0.05    |
|                  |             | A × C     | 1  | 0.33    | 0.57    |
|                  |             | B × C     | 3  | 0.14    | 0.93    |
|                  |             | A × B × C | 3  | 0.60    | 0.61    |
| FR               |             | A: Diet   | 1  | 1.91    | 0.17    |
|                  |             | B: Concen | 3  | 2.15    | 0.09    |
|                  |             | A × B     | 3  | 0.99    | 0.97    |
|                  |             | C: Sex    | 1  | 0.11    | 0.74    |
|                  |             | A × C     | 1  | 1.03    | 0.31    |
|                  |             | B × C     | 3  | 0.26    | 0.85    |
|                  |             | A × B × C | 3  | 2.96    | 0.03    |

FR, feeding rate; MPCT, mean percentage of consumption test. Although $P = 0.05$ for MPCT on tannic acid, a post hoc Bonferroni correction indicated no significant difference between male and female consumption. A significant interaction between concentration, diet, and sex was detected for FR tested with tannic acid.

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**Table 2.** Sex has no significant effect on larval preference ($\alpha = 0.05$)

| Compound   | Test factor | Treatment | df | $F$     | $P$     |
|------------|-------------|-----------|----|---------|---------|
| Spruce tannins | MPCT       | A: Diet   | 1  | 12.19   | <0.001  |
|             |             | B: Concen | 2  | 0.32    | 0.72    |
|             |             | A × B     | 2  | 7.46    | <0.001  |
|             |             | A × C     | 1  | 0.15    | 0.70    |
|             |             | B × C     | 2  | 0.97    | 0.38    |
|             |             | A × B × C | 2  | 1.02    | 0.36    |

| Tannic acid | MPCT       | A: Diet   | 1  | 8.67    | 0.004   |
|             |             | B: Concen | 2  | 10.48   | <0.001  |
|             |             | A × B     | 2  | 6.54    | 0.002   |
|             |             | C: Sex    | 1  | 1.94    | 0.17    |
|             |             | A × C     | 1  | 1.84    | 0.18    |
|             |             | B × C     | 2  | 0.49    | 0.61    |
|             |             | A × B × C | 2  | 0.47    | 0.63    |

**Table 3.** Feeding rates (mean ± SEM) of insects reared on 0% tannic acid diet and tested with discs treated with solutions of either tannic acid or spruce tannins alone (i.e., in distilled water)

| Diet          | Control solution | Test solution | Feeding rate (mm²/h) |
|---------------|------------------|---------------|----------------------|
| 0% TA (normal)| Sucrose          | 8% TA alone   | 2.3 ± 0.3            |
|               | Distilled water  | 8% ST alone   | 2.6 ± 0.4            |

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Fig. 1. Mean percentage of consumption (mean ± SEM) of discs treated with solutions of either TA alone (i.e., in distilled water) or ST alone by insects reared on 0% TA (i.e., normal) diet. n, sample size. *$P < 0.05$. NS, not significant.
concentration on MPC in both cases (Table 7). The linear regressions support the trend of the dose–response relationships and indicate that tannin concentration accounted for ≈20–30% of the variability in the data (Table 8). Insects responded similarly to the 0 and 0.75% concentrations of both types of tannin (i.e., no significant difference between MPC for test and control discs) (Tables 5 and 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6). After a post hoc Bonferroni correction it seemed that insects ate more of the test discs wetted with the higher concentrations of ST (8 and 15%; Table 6).

To summarize, feeding was consistently decreased and as ST concentrations increased, MPC of the test discs decreased and as ST concentrations increased, MPC of test discs increased.

Diet had a slight effect on disc selection (Figs. 2 and 3; Table 7). A post hoc Bonferroni correction revealed that the MPC on test discs was slightly higher on 15% TA diet than on 8% TA diet when tested with solutions of ST (P < 0.05; Fig. 2). For TA solutions, the MPC of test discs was slightly lower on 8% TA diet compared with 0% TA diet (Fig. 3). A significant interaction between diet and concentration was found for both types of tannin (Table 5).

Feeding rate was significantly affected by diet and concentration and a significant interaction was found for both types of tannin (Table 9). A post hoc Bonferroni correction showed that, for tests with TA, the 15% TA diet was significantly different than the other two (P < 0.01) and that the 30% TA solution was significantly different than all the other concentrations (P < 0.001). Feeding rates were significantly reduced for insects tested with the 30% TA solution, regardless of diet. At this high concentration deterrence might be stronger and toxic effects more rapidly induced, resulting in severely reduced feeding rates. For tests with ST all diets differed significantly from each other; feeding rate decreased with increasing tannic acid content of the diet. Mean feeding rate of insects reared on 8% TA diet and tested with 15% ST decreased for insects tested with the 30% TA solution, regardless of diet. At this high concentration deterrence might be stronger and toxic effects more rapidly induced, resulting in severely reduced feeding rates.

**Table 4.** Feeding rates (mean ± SEM) of insects reared on different tannic acid-containing diets and tested on discs treated with solutions of either tannic acid or spruce tannins

| Diet          | Control solution | Test solution | Mean feeding rate (mm²/h) |
|---------------|------------------|---------------|--------------------------|
| 0% TA diet    | 25 mmol liter⁻¹  | 25 mmol liter⁻¹ | 4.3 ± 0.3                |
|               | sucrose          | sucrose       |                          |
|               | 0.75% ST         | 3.9 ± 0.3     |                          |
|               | 8% ST            | 4.4 ± 0.5     |                          |
|               | 15% ST           | 4.7 ± 0.5     |                          |
|               | 0.75% TA         | 3.7 ± 0.3     |                          |
|               | 8% TA            | 3.2 ± 0.3     |                          |
|               | 15% TA           | 3.5 ± 0.4     |                          |
|               | 30% TA           | 2.5 ± 0.3     |                          |
|               | 25 mmol liter⁻¹  | 3.9 ± 0.3     |                          |
|               | sucrose          |               |                          |
| 8% TA diet    | 0.75% ST         | 4.0 ± 0.5     |                          |
|               | 8% ST            | 3.7 ± 0.4     |                          |
|               | 15% ST           | 2.0 ± 0.3     |                          |
|               | 0.75% TA         | 4.1 ± 0.3     |                          |
|               | 8% TA            | 3.8 ± 0.3     |                          |
|               | 15% TA           | 3.6 ± 0.4     |                          |
|               | 30% TA           | 3.1 ± 0.4     |                          |
|               | 25 mmol liter⁻¹  | 2.9 ± 0.3     |                          |
|               | sucrose          |               |                          |
| 15% TA diet   | 0.75% ST         | 1.8 ± 0.2     |                          |
|               | 8% ST            | 2.7 ± 0.3     |                          |
|               | 15% ST           | 2.9 ± 0.4     |                          |
|               | 0.75% TA         | 2.1 ± 0.4     |                          |
|               | 8% TA            | 1.9 ± 0.2     |                          |
|               | 15% TA           | 2.0 ± 0.2     |                          |
|               | 30% TA           | 1.4 ± 0.2     |                          |

**Table 5.** Pairwise comparisons of mean percentage of consumption of control discs and test discs treated with TA by insects reared on different TA-containing diets

| Diet | Concentration | Test statistica | P     |
|------|---------------|-----------------|-------|
| 0% TA| 0%            | t = -1.71       | 0.10  |
|      | 0.75%         | t = 3.19        | 0.04  |
|      | 8%            | t = 4.82        | <0.001|
|      | 15%           | t = 4.48        | <0.001|
|      | 30%           | t = 0.84        | <0.001|
| 8% TA| 0%            | t = -1.66       | 0.32  |
|      | 0.75%         | t = 3.43        | 0.002 |
|      | 8%            | t = 4.88        | <0.001|
|      | 15%           | t = 7.85        | <0.001|
|      | 30%           | Z = 4.01        | <0.001|
| 15% TA| 0%           | t = 1.33        | 0.27  |
|      | 0.75%         | Z = 1.08        | 0.28  |
|      | 8%            | t = 8.15        | <0.001|
|      | 15%           | t = 9.65        | <0.001|
|      | 30%           | t = 3.99        | <0.001|

a t for paired t-test and Z for Wilcoxon’s signed rank test.

**Table 6.** Pairwise comparisons of mean percentage of consumption of control discs and test discs treated with solutions of spruce tannins by insects reared on different tannic acid-containing diets

| Diet | Concentration | Test statistica | P     |
|------|---------------|-----------------|-------|
| 0% TA| 0%            | t = -1.71       | 0.10  |
|      | 0.75%         | t = 1.62        | 0.08  |
|      | 8%            | t = -3.81       | 0.002 |
|      | 15%           | t = -4.55       | <0.001|
| 8% TA| 0%            | t = -1.66       | 0.32  |
|      | 0.75%         | t = -0.62       | 0.55  |
|      | 8%            | t = -2.90       | 0.009 |
|      | 15%           | t = -3.42       | 0.005 |
| 15% TA| 0%           | t = 1.33        | 0.27  |
|      | 0.75%         | t = -0.77       | 0.45  |
|      | 8%            | Z = 3.25        | 0.001 |
|      | 15%           | t = -7.17       | <0.001|

a t for paired t-test and Z for Wilcoxon’s signed rank test.
indicated a significant difference between treatments (diets) \((df = 2; F = 48.52; P < 0.0001)\) and a post hoc Bonferroni correction revealed that the mean weight of insects reared on the 0% diet differed significantly from the mean weight of those reared on both the eight and 15% TA diets which, however, did not differ significantly from each other.

**Discussion**

Tannins are known to affect the feeding behavior and physiology of insect herbivores, both positively and negatively, through several different mechanisms. The range of effects that tannins produce on a variety of insects has led to their classification as general purpose digestibility reducers (Feeny 1968, 1969; Swain 1977, Nomura and Itioka 2002); toxins (Bernays 1978, Bernays et al. 1980, Steinvly and Berenbaum 1985); phagodeterrents (Bennett 1965, Schoonhoven and Derksen-Koopers 1973, Chapman and Bernays 1977, Muir et al. 1999); and more recently, as oxidative stressors (Barbehenn et al. 2006). It also seems that certain hydrolysable tannins can act as phagostimulants to some insects (Foss and Rieske 2003). Still other insects seem to be unaffected by the presence of tannins in their diet (Lawson et al. 1984, Ayres et al.

![Fig. 2. Mean percentage of consumption (mean ± SEM) of discs treated with solutions of ST dissolved in 25 mmol liter\(^{-1}\) sucrose by insects reared on 0% TA (i.e., normal), 8% TA, and 15% TA diet. N, sample size. *P < 0.01; **P < 0.001.](image2)

![Fig. 3. Mean percentage of consumption (mean ± SEM) of discs treated with solutions of TA dissolved in 25 mmol liter\(^{-1}\) sucrose by insects reared on 0% TA (i.e., normal), 8% TA, and 15% TA diet. N, sample size. *P < 0.01; **P < 0.001.](image3)
1997). These several postulated modes of action of tannins—digestibility reducers, toxins, oxidative stressors, phagostimulants, phagodeterrents—are not mutually exclusive and are likely found acting in various combinations for different insects and for different tannins.

The focus of most prior research into insect herbivore-tannin interactions has been on postingestive mechanisms, such as decreased food nutritional quality, whereas the preingestive gustatory mechanisms have been largely ignored. Pre- and postingestive mechanisms must both be studied to gain a complete understanding of the effects of secondary plant compounds such as tannins and of the feeding strategies of insect herbivores. Our results clearly demonstrate preingestive effects of both tannic acid and spruce tannins on the spruce budworm, implying that this insect was somehow able to detect these compounds before ingestion. Feeding was consistently deterred by tannic acid and stimulated by spruce tannins and a dose–response relationship was demonstrated in both cases. To our knowledge, this is the first time that condensed tannins have been reported to produce a positive (phagostimulatory) effect on an insect herbivore (Bernays 1981, Ayres et al. 1997).

Tannic acid deterred feeding despite the addition of 25 mmol liter$^{-1}$ sucrose, a common phagostimulant for all phytophagous insects studied (Chapman 2003), including the spruce budworm (Heron 1965, Albert et al. 1982). Panzuto et al. (2002) demonstrated a similar feeding deterrent effect of a solution of tannic acid and glucose in the related species, Choristoneura rosacea (Harris). The phagostimulatory effect of the spruce tannins was unexpected, but the numerous tests performed and the consistent responses observed strongly confirm the validity of the results. It should be noted that the purified tannin extracts contained $\approx$97% condensed tannins and $\approx$3% hydrolysable tannins (Bauce et al. 2006). Although potential effects of the hydrolysable tannins cannot be completely excluded, the highest concentration used represents an $\approx$0.45% (dry weight) solution of hydrolysable tannins, well below the detectable limit of the spruce budworm, as discussed below. The absence of any account of such phagostimulatory effect in the literature might be partially explained by the fact that most previous studies with condensed tannins have been conducted with quebracho, derived from Schinopsis sp., rather than with tannins extracted from the host plant of the insect under investigation (e.g., Roehrig and Capinera 1983). Most previous studies of tannins and insect herbivores have focused on one type of tannin and those that have tested both hydrolysable and condensed tannins on the same insect have reported similar effects for both (e.g., Manuwoto and Scriber 1986). Even in studies where condensed tannins were extracted from the insect’s host plant, a deterrent effect was still found (e.g., Reese et al. 1982). However, these studies were not specifically investigating preingestive gustatory effects and the deterrence reported might be attributed to other factors such as water content and leaf toughness (Feeny 1969, Forkner et al. 2004). Interestingly, in a recent study by Bauce et al. (2006) using the same white spruce condensed tannins incorporated into the diet of the spruce budworm, mortality was found to be highest at 8% (dry weight), a level found normally in white spruce trees and that we report as phagostimulatory.

Not only do the responses to the two types of tannin differ, apparently so do their modes of action. The condensed tannins tested alone stimulated feeding even enough to compete with a solution of sucrose, implying that the spruce budworm is capable of detecting condensed tannins directly and independently and that these are strong phagostimulants. Conversely, solutions of combined tannic acid and sucrose deterred feeding. Feeding deterreants can act on the sensory system in two ways: by stimulating a specialized deterrent cell or by modifying the activity of cells responding to feeding stimulants (Schoonhoven and Jermy 1977). We speculate that tannic acid acts via the latter mechanism, at least in the case of the spruce budworm; it cannot be detected alone but seems somehow to inhibit the positive signal when mixed

| Test compound | Factor | df | $F$ | $P$ |
|---------------|--------|----|-----|-----|
| TA            | Diet   | 2  | 4.27| 0.01|
|               | Conc.  | 4  | 31.54| <0.0001|
|               | Inter. | 5  | 2.37 | 0.01|
| ST            | Diet   | 2  | 9.80| <0.0001|
|               | Conc.  | 3  | 30.53| <0.0001|
|               | Inter. | 6  | 4.33 | <0.0001|

Table 8. Linear regressions for mean percentage of consumption of test discs treated with either TA or ST by insects reared on different TA-containing diets

$$y = -0.171x + 0.795$$

$R^2 = 0.305$

$$y = -0.137x + 0.714$$

$R^2 = 0.206$

$$y = -0.113x + 0.761$$

$R^2 = 0.145$

$$y = 0.177x + 0.773$$

$R^2 = 0.304$

$$y = 0.209x + 0.726$$

$R^2 = 0.223$

$$y = 0.208x + 0.789$$

$R^2 = 0.242$

$x$, concentration of TA or ST; $y$, mean percentage of consumption of test discs.

Table 9. Two-way ANOVA of feeding rates of insects reared on different tannic acid-containing diets and tested with discs treated with solutions of either tannic acid or spruce tannins

| Test compound | Factor | df | $F$ | $P$ |
|---------------|--------|----|-----|-----|
| TA            | Diet   | 2  | 37.76 | <0.001|
|               | Conc.  | 4  | 17.16 | <0.001|
|               | Inter. | 8  | 2.61  | 0.0085|
| ST            | Diet   | 2  | 31.07 | <0.001|
|               | Conc.  | 3  | 10.67 | 0.018 |
|               | Inter. | 6  | 4.98  | <0.001|
with sucrose. Similar results were obtained by Albert and Parisella (1988) for glutamic acid, which is not detected in solution alone but produces a deterrent effect on the spruce budworm when mixed with 25 mmol liter\(^{-1}\) sucrose. Furthermore, Panzuto et al. (2002) observed that tannic acid interfered with the response of the sugar-sensitive cell of the lateral sensillum styloconica in C. rosaceana, and Dethier (1982) reports similar results for two other phytophagous caterpillars. Although tannic acid deterred feeding, the suggestion of Chapman (2003) that the spruce budworm possesses a deterrent cell remains unproved. We managed to reduce the duration of the feeding assay from 24 h to 1 h to rule out slow acting feedback mechanisms on feeding preference and obtained similar results, with regard to preferences, as with the 24-h tests (P.J.A., unpublished data).

Although feeding preference increases for spruce tannins (with sucrose) and decreases for tannic acid (with sucrose) with increasing concentration as confirmed by linear regression analysis, the relationship is not necessarily a linear one. The results indicate that, for both types of tannin, the 0 and 0.75% concentrations group together, as do the 8, 15 (for both types of tannin), and 30% (for tannic acid only) concentrations with respect to mean percentage of consumption. This suggests the possibility that the response varies in a stepwise manner as the concentration changes such that each response is characteristic of a certain range of concentrations. This also reveals a minimum response threshold somewhere between 0.75 and 8% tannin below which the insect does not respond, though this does not automatically imply the inability to detect these lower concentrations. Recent evidence supports an approximate threshold of around 2% tannin (P.J.A., unpublished data).

Spruce budworm larvae reared on 8% tannic acid diet and tested with tannic acid showed an increased preference for control (25 mmol liter\(^{-1}\) sucrose) discs over those reared on 0% tannic acid diet. This is possibly a form of aversion learning. When the spruce budworm is forced to feed on the tannic acid containing diet, the deterrent properties of the tannic acid solution are enhanced, meaning there is no shift toward a preference for tannic acid, but rather an increase in its distastefulness. The effect was not enhanced by the increased tannic acid concentration of the 15% tannic acid diet indicating a potential response plateau. When tested with spruce tannins a different effect was produced. Larvae had an increased preference for spruce tannins when reared on the 15% tannic acid diet than when reared on the 8% tannic acid diet. This is certainly not a case of habituation, because the compounds are chemically distinct, but rather induction. As discussed, the preingestive effects and mechanisms of tannic acid and spruce tannins are unrelated; spruce tannins actually possess phagostimulant qualities. When reared on a diet of sufficiently high concentration of tannic acid, the insect’s preexisting preference for the spruce tannin-sucrose solution is enhanced.

Results for feeding rates corroborate the trends observed for mean percentage of consumption. As a phagostimulant, the white spruce condensed tannins have a comparable feeding rate to that of 25 mmol liter\(^{-1}\) sucrose, whereas the tannic acid, as a phagodeterrent, tended to decrease the feeding rate. Tannic acid in the diet had a noticeable effect on feeding rates and tended to decrease the mean feeding rate regardless of the type of tannin being tested in the feeding assays. Decreased larval weight does not explain the lower feeding rates. Rearing spruce budworm on tannin-containing diet has been shown to reduce larval weight and smaller insects generally consume less food (Bauce et al. 2006). However, larval weights were similarly lower for both the eight and 15% tannic acid diets whereas feeding rates were significantly lower for only the 15% tannic acid diet. Therefore, it is likely that toxic effects of tannic acid in the diet are responsible for the weight reduction and become pronounced with increased concentration.

The vast diversity of phytophagous insects and their myriad feeding habits in combination with the tremendous diversity of tannins and their various effects on insect herbivores, means the only way to assess with certainty the effect of a given tannin on a particular insect is to test that specific tannin on that specific insect. Therefore, we compared the effects of both hydrolyzable and condensed tannins on feeding behavior of the spruce budworm. To ensure biological relevance and applicability, we used condensed tannins extracted from the insect’s host plant. Tannin concentrations were chosen to represent those found in white spruce and to correspond to the previous work of Bauce et al. (2006). The spruce budworm has managed to adapt to its hosts by evolving to respond positively to certain plant chemicals (e.g., spruce tannins) and negatively to others (e.g., tannic acid, although not a host plant compound), while limiting deleterious effects by slight physiological modifications. Despite never naturally encountering tannic acid, the spruce budworm responds negatively to this plant chemical through its interactions with sucrose (or the sucrose receptor). However, it seems to deter feeding by the same mechanism as some host plant chemicals, such as glutamic acid, which similarly deters feeding only when combined with sucrose but not alone. Perhaps this is a more widespread phenomenon than currently thought. Clearly, the interactions of plant compounds can produce seemingly unpredictable effects on insect feeding behavior and physiology. The white spruce condensed tannins were found to stimulate feeding, the first time such a phagostimulatory effect of condensed tannins has been reported. Previously, however, Kumbasli (2005) demonstrated that these very same phagostimulant condensed tannins produce negative effects on the spruce budworm in terms of growth, survival, and digestion despite the insect’s limited ability to compensate by increasing its approximate digestibility. It therefore seems that the positive preingestive (gustatory) effects of tannins from C. fumiferana’s host plant increase consumption...
and thereby eventually lead to negative postdigestive effects on growth and survival.

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