Influence of epicuticular-wax composition on the feeding pattern of a phytophagous insect: implications for host resistance

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Abstract—A white spruce, *Picea glauca* (Moench) Voss (Pinaceae), plantation in southern Quebec was found to contain two distinct types of trees, the first resistant and the second susceptible to attack by spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). To identify the mechanisms of white spruce resistance to spruce budworm, we studied the role of epicuticular waxes, comparing (*i*) the foliar chemistry of susceptible and resistant trees and (*ii*) the feeding pattern of larvae at first contact with the foliage. Needles collected from resistant trees contained concentrations of the monoterpenes α-pinene and myrcene that were 307% and 476%, respectively, above those found in needles collected from susceptible trees. Although there were no significant differences in probing behaviour, significantly fewer larvae transitioned from probing to feeding on resistant needles; this led to fewer feeding bouts as well as a significantly shorter first meal. Removal of waxes increased the number of individuals transitioning from probing to feeding on resistant needles; this led to more feeding bouts. Our results demonstrate that monoterpenes influence the pattern of feeding of spruce budworm larvae as well as playing an important role in white spruce resistance.

Résumé—Une plantation d'épinette blanche, *Picea glauca* (Moech) Voss (*Pinaceae*), composée d'arbres résistants et susceptibles à la tordeuse de bourgeons d'épinette, *Choristoneura fumiferana* (Clemens) (Lepidoptera : Tortricidae) a été utilisée comme modèle pour investiguer le rôle des cires épicuticulaires dans les mécanismes de résistance des arbres hôtes à la tordeuse. Ainsi, cette approche nous a permis (*i*) d'étudier les relations entre la composition chimique des cires épicuticulaires des aiguilles et le comportement de palpage et d'ingestion des larves de tordeuse ainsi (*ii*) que d'analyser le patron d'alimentation de la tordeuse sur le foliage. Les aiguilles provenant d'arbres résistants contenaient respectivement 307 % et 476 % plus d'α-pinene et de myrcene que celles provenant des arbres susceptibles. Aucune différence significative dans le comportement de palpage des aiguilles n'a été détectée. Par contre, moins d'insectes, et cela de manière significative, ont passé de la phase de palpage à la phase d'ingestion lorsqu'en présence d'aiguilles provenant d'arbres résistants. Ce phénomène s'est traduit par une réduction du nombre de périodes d'ingestion et une réduction de la durée du premier repas dans le cas des insectes en présence d'aiguille d'arbres résistants. Lorsque les cires épicuticulaires ont été enlevées, le nombre de tordeuse qui ont passé de la phase de palpage à la phase d'ingestion a augmenté sur les aiguilles provenant d'arbres résistants. Nos résultats démontrent que les monoterpènes semblent influencer le patron d'alimentation de la tordeuse ainsi que jouer un rôle important dans la résistance de l'épinette blanche à la tordeuse.

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Introduction

Plant-surface waxes can play an important role in defense against herbivores (Dussourd 1993); indeed, they are the first point of contact with a host plant by a herbivore and can form the basis for acceptance or rejection. These waxes are complex mixtures of fatty acids, esters, and alkanes, with varying quantities of different secondary metabolites dissolved in the wax (Bernays and Chapman 1994). Many secondary metabolites form an important component of plant defense against herbivores (Dussourd 1993). Monoterpenes, for example, are secondary plant compounds found in the epicuticular wax layer of needles (Städler 1986; Fischer *et al*. 1994; Muller and Riederer 2005) and the resin canals of needles and stems of most conifers (Bernays and Chapman 1994). They deter feeding and oviposition by a variety of herbivores, including various species of bark beetles (Coleoptera: Curculionidae: Scolytinae), lepidopteran defoliators, and mammals (Bauce *et al*. 1994; Litvak and Monson 1998; Chen *et al*. 2002). The concentration and composition of monoterpenes in coniferous trees are influenced by tree genotype (von Rudloff and Rehfeldt 1980; Gershenzon and Croteau 1991; Hanover 1992; Gershenzon 1994) and by microsite, and can vary from year to year (Sturgeon 1979; Chen *et al*. 2002). We examined whether surface waxes play a role in the observed differences in the frequency of attack by spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (hereinafter budworm), between neighbouring white spruce, *Picea glauca* (Moench) Voss (Pinaceae), trees.

Every step in the behavioural chain of events leading to and including plant ingestion is potentially influenced by the chemical composition of plant tissues (Mitchell 1981; Städler 1992; Bernays and Chapman 1994; Frazier and Chyb 1995; Chapman 2003; Wright *et al*. 2003). At first contact with a plant, an insect samples the surface chemistry via probing behaviour; received stimuli then determine whether or not the insect proceeds to biting. If biting occurs, the internal chemical composition of the foliage stimulates or deters feeding and dictates the duration of the feeding bout. It is therefore possible to determine the location of deterrent compounds by examining insect feeding.

White spruce trees ''susceptible'' and ''resistant'' (Clancy *et al*. 1991) to budworm attack grow together in a plantation in southern Quebec, Canada. Resistant trees have a history of light defoliation, whereas neighbouring susceptible trees have been much more heavily defoliated (Bauce and Kumbasli 2007). We compared (*i*) the foliar chemistry of resistant and susceptible trees and (*ii*) the feeding pattern of budworm on the two foliage types to identify the underlying mechanism of white spruce resistance in this system.

Materials and methods

Insects

Second-instar budworm larvae emerging from diapause were obtained from the Great Lakes Forest Research Centre, Sault Ste Marie, Ontario. Larvae were reared on artificial diet (Grisdale and Wilson 1988) in an incubator under a 16L:8D photoperiod at 22 °C and 60% relative humidity. Freshly moulted (within the last 24 h) sixth-instar larvae were used for the behavioural experiments.

Study site and tree types

Field studies conducted in the summers of 2002–2004 within a fast-growing white spruce plantation near Drummondville, Quebec, Canada (45°53′0″N, 72°29′0″W), in a zone of severe spruce budworm infestation (>50 larvae per 45 cm branch length) revealed the presence of two distinct types of white spruce trees (termed ''resistant'' and ''susceptible'' as defined by Clancy *et al*. 1991) (Bauce and Kumbasli 2007).

Foliar chemistry

Current-year foliage was collected on 10 June 2005 from 3 highly resistant (R) and 3 highly susceptible (S) trees randomly selected from 50 susceptible and 50 resistant trees in the Drummondville plantation (Table 1). Foliage samples were packed on dry ice and taken to the laboratory. Twenty needles were

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Table 1. Performance of spruce budworm (*Choristoneura fumiferana*) larvae on susceptible and resistant white spruce (*Picea glauca*) trees.

Performance	Susceptible trees	Resistant trees
Larval mortality, % ($\chi^2 = 4.6$, df = 1, $P = 0.03$)	42 ± 8	80 ± 5
No. of eggs laid ($F_{1,4} = 0.16$, $P = 0.03$)	333 ± 33	322 ± 35
Defoliation, % ($F_{1,4} = 72.06$, $P = 0.001$)	65 ± 5	8 ± 6

Note: Performance was assessed on the basis of field rearing of insects. Two groups of 20 second-instar larvae were each placed in a sleeve cage on a 45 cm long branch. Two cages were placed on three host trees per resistance class (susceptible and resistant). Data were subjected to two-stage nested analysis of variance with individual trees nested within resistance class. Larval mortality was analyzed using χ^2 analysis. Values are given as the mean ± 2 SE.

removed from each sample, sealed in vials, and preserved at −80 °C until analyzed for monoterpene content. Another 20 needles were collected and weighed to determine foliage wet mass. The remaining foliage was frozen using liquid nitrogen, freeze-dried, and ground using a 20-mesh Wiley Mill (Cylcotec 1093 sample mill, Foss Tecator, Hoganas, Sweden). Ground foliage was stored in a freezer at −20 °C prior to chemical analysis.

Chemical analysis of foliage followed Bauce *et al.* (1994) and Bauce (1996). To determine mineral content (nitrogen, phosphorus, potassium, calcium, magnesium), 500 mg of ground foliage was digested in concentrated sulphuric acid and assessed in a multichannel flow-injection analyzer (QuickChem, Lachat, Loveland, Colorado, United States of America). Soluble sugars were analyzed by extracting 100 mL of ground foliage three times in a 12:5:3 solution of methanol, chloroform, and water and allowing the resulting polar phase to react with a phenol − sulphuric acid solution (Dubois *et al.* 1956). Total phenolics were assessed by extracting 300 mg of ground foliage three times with 70% acetone containing 0.1% ascorbic acid and analyzing the resulting solution using the Folin−Denis method (Swain and Hillis 1959). Total tannin concentrations were determined by means of radial diffusion (Dement and Mooney 1974; Wisdom *et al.* 1987), which relies on the formation of binding complexes between tannins and bovine serum albumin protein in agar medium (Hagerman 1987). A known quantity of foliage extract is deposited in a well cut into agar containing bovine serum albumin, and the area of radial diffusion is measured. Thus, total tannin concentrations are expressed in cubic centimetres per milligram dry mass of foliage. Monoterpenes were assessed by grinding 20 needles from each sample in liquid nitrogen and then extracting the contents with a 2:3 methanol:hexane solution. Using tetradecane as an internal standard, the organic phase of the solution was analyzed using a Varian model 3900 gas chromatograph equipped with a flame-ionization detector and a SPB-5 fused-silica capillary column (30 m × 0.25 mm) (Varian, Inc., Palo Alto, California, United States of America).

Foliage

Needles from the three R and three S white spruce trees were randomly sampled at the study site on 10 June 2005. This coincided with the presence of sixth-instar budworm larvae on R and S trees. Samples were coded as soon as they were received so that all experimentation was done under blind conditions. Current-year needles were stored at −20 °C for the duration of the experiments.

Behaviour

Budworm larvae were starved for 4 h at room temperature in 3.5 cm Petri dishes prior to experimentation. The Petri dishes were then placed on a 27 cm × 22 cm Styrofoam board. Styrofoam strips placed between the Petri dishes isolated insects from each other. Larvae were then placed in a 40 cm × 40 cm × 40 cm digital photo box, which served as an isolation chamber. The first 0.5 cm of each test needle was placed in a plastic pipette tip filled with water and sealed with Parafilm®; this allowed

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the needles to remain moist for the duration of the experiment. Larvae were viewed using a Canon GL2 Video Camcorder 3CCD Camera System, 20X/100X professional fluorite lens, 1.7 megapixels, and recorded onto a computer using Virtual Dub software (1.5.10, 1998–2003, Avery Lee, http://www.virtualdub.org/) set at 1 frame / 2 s. Behavioural experiments took place from 1400 to 0900 the following morning. Lights remained on throughout each experiment.

A needle from each sample tree was placed in a different Petri dish containing starved larvae. Five behavioural indices were monitored: first contact with the food item, initiation and cessation of a probing event, and initiation and cessation of a feeding bout. A probing event consisted of any contact of the palps and (or) mandibles of a larva with the spruce needle but no ingestion of plant material. For the purpose of this experiment we did not differentiate between probing with the palps and probing with the mandibles. A feeding bout occurred when plant material was ingested. A meal was defined as a sequence of feeding bouts separated by probing or resting and exploration events of less than 10 min duration. Preliminary studies revealed that pauses between budworm meals on white spruce exceeded 10 min. Behaviour was monitored for the duration of the first meal, therefore total observation time varied among individuals. Ten replicates were used for each sample tree. Treatments were designated S or R according to needle type.

The above experimental protocol was duplicated using dewaxed (DW) needles. Spruce needles were individually dipped in 15 mL of hexane for 10 s; this allowed the epicuticular hydrophobic wax layer to be removed without dissolving any internal hydrophobic compounds (Maloney *et al.* 1988; Rivet and Albert 1990). These two treatments were designated susceptible-dewaxed (S-DW) and resistant-dewaxed (R-DW).

Statistical analyses

Foliar chemistry

Analyses were conducted using the Statistical Analysis System (SAS Institute Inc. 2003). Normality and variance homogeneity tests were performed before data were subjected to a two-stage nested analysis of variance (ANOVA) with individual trees nested within tree type.

Behaviour

All data were analyzed using SPSS® (SPSS Inc. 1999). The number of probing or feeding bouts satisfied conditions of normality (Sokal and Rohlf, 1995); nested ANOVAs, where individual trees were nested within tree type, were used to analyze these.

To avoid pseudoreplication favouring budworm larvae that probed or fed significantly more often than others, the median duration of individual probing or feeding bouts was calculated for each insect. These data did not satisfy conditions of normality and were therefore rank-transformed (Sokal and Rohlf 1995). Total time spent probing and feeding as well as the duration of the first meal did not satisfy the conditions of normality (Sokal and Rohlf 1995) and were also rank-transformed. Nested ANOVAs, where individual trees were nested within tree type, were used to analyze these data once the conditions of normality were met. Lastly, the numbers of budworm that proceeded from probing to feeding were analyzed using Fisher's exact test for $R \times K$.

For each ANOVA, the following *a-priori* pairs of treatments were compared: S *vs.* R, S-DW *vs.* R-DW, S *vs.* S-DW, and R *vs.* R-DW. These planned comparisons allowed us to examine (*i*) the difference in budworm behaviour between R and S trees and (*ii*) the role played by epicuticular waxes. *A-priori* comparisons were performed only when ANOVAs revealed significant differences between treatments.

Results

Foliar chemistry

The chemical profiles of needles from the three S and three R trees are summarized in Table 2. There did not appear to be any notable differences between tree types in concentrations of phosphorus (ANOVA, $F_{[1,4]} = 2.20$, $P = 0.212$), potassium (ANOVA, $F_{[1,4]} = 0.00$, $P = 0.940$), calcium (ANOVA, $F_{[1,4]} = 0.26$, $P = 0.636$), magnesium (ANOVA, $F_{[1,4]} = 3.52$, $P = 0.134$), camphene (ANOVA, $F_{[1,4]} = 2.09$,

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Table 2. Summary of the chemical profiles of needles from three susceptible and three resistant white spruce (*Picea glauca*) trees.

Chemical	Susceptible trees (S)	Resistant trees (R)		(R-S/S) × 100[a]
α-Pinene (ng/mg)	13432.73 ± 8827.20	54665.40 ± 10742.67	**	307
Camphene (ng/mg)	20355.04 ± 7375.44	37640.90 ± 15236.18	ns	85
β-Pinene (ng/mg)	8148.19 ± 5450.43	13568.70 ± 2123.04	ns	67
Myrcene (ng/mg)	1490.34 ± 896.79	8591.25 ± 610.11	**	476
Limonene (ng/mg)	82343.16 ± 29745.73	107946.92 ± 8189.39	ns	31
Bornyl acetate (ng/mg)	76926.22 ± 1285.20	112730.81 ± 1040.91	**	47
Total monoterpenes (ng/mg)	202695.67 ± 52676.93	335143.96 ± 45653.42	**	65
Phenols (%)	9.15 ± 0.63	11.03 ± 0.38	**	20
Total tannins (cm^2 of radial diffusion)	0.24 ± 0.01	0.31 ± 0.002	**	27
Soluble sugars (% dry mass)	8.54 ± 0.37	7.167 ± 0.60	**	−16
Nitrogen (%)	1.22 ± 0.04	1.372 ± 0.08	**	12
Phosphorus (ppm)	1643.33 ± 116.10	1931.00 ± 248.22	ns	18
Potassium (ppm)	9498.33 ± 611.97	9543 ± 684.26	ns	0
Calcium (ppm)	3293.33 ± 762.64	3004.33 ± 236.33	ns	−9
Magnesium (ppm)	880.33 ± 59.65	777.67 ± 49.26	ns	−12

Note: Values are given as the mean ± 2 SE (**, significant difference at $\alpha = 0.01$; ns, nonsignificant difference).
[a]Percent increase from susceptible to resistant white spruce trees.

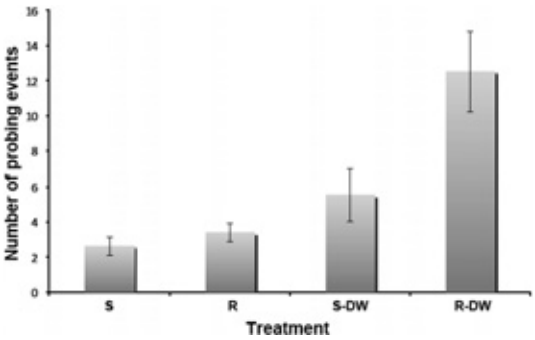
$P = 0.222$), β-pinene (ANOVA, $F_{[1,4]} = 1.72$, $P = 0.260$), or limonene (ANOVA, $F_{[1,4]} = 1.38$, $P = 0.306$). Needles from the sampled R trees contained higher amounts of phenolics (ANOVA, $F_{[1,4]} = 15.03$, $P = 0.018$), tannins (ANOVA, $F_{[1,4]} = 160.00$, $P = 0.0.0002$), and nitrogen (ANOVA, $F_{[1,4]} = 43.87$, $P = 0.0027$) but slightly lower amounts of soluble sugars (ANOVA, $F_{[1,4]} = 45.33$, $P = 0.0025$). Interestingly, R needles contained 65% more total monoterpenes (ANOVA, $F_{[1,4]} = 45.3$, $P = 0.0025$), mainly resulting from 307% more α-pinene (ANOVA, $F_{[1,4]} = 17.6$, $P = 0.014$) and 476% more myrcene (ANOVA, $F_{[1,4]} = 169.2$, $P = 0.0002$).

Behaviour

No significant effect of individual tree nested within tree type was detected in any ANOVA. Therefore, only tree-type results are presented here. Time elapsed before the first contact of budworm larvae with needles did not differ between treatments (ANOVA, $F_{[3,119]} = 1.854$, $P = 0.141$).

The number of probing events differed significantly between treatments (ANOVA, $F_{[3,119]} = 9.322$, $P < 0.0001$) (Fig. 1). Budworm larvae probed almost twice as much on R-DW needles as on S-DW needles ($P <$ 0.0001) and also probed almost six times as much on R-DW needles as on R needles ($P <$ 0.0001). No significant difference in number of probing events was detected for either S *vs.* R ($P > 0.1$) or S *vs.* S-DW ($P > 0.1$).

Fig. 1. Numbers of probing events (mean ± 2 SE) by sixth-instar spruce budworm (*Choristoneura fumiferana*) larvae on susceptible (S), resistant (R), susceptible dewaxed (S-DW), and resistant dewaxed (R-DW) white spruce (*Picea glauca*) needles ($n = 30$ per treatment).



The duration of individual probing events also differed significantly between treatments (ANOVA, $F_{[3,115]} = 4.94$, $P = 0.003$). How-

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Fig. 2. Numbers of feeding bouts (mean ± 2 SE) by sixth-instar spruce budworm (*Choristoneura fumiferana*) larvae on susceptible (S), resistant (R), susceptible dewaxed (S-DW), and resistant dewaxed (R-DW) white spruce (*Picea glauca*) needles ($n = 30$ per treatment).

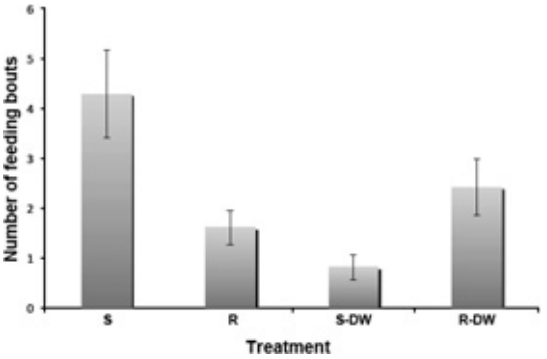
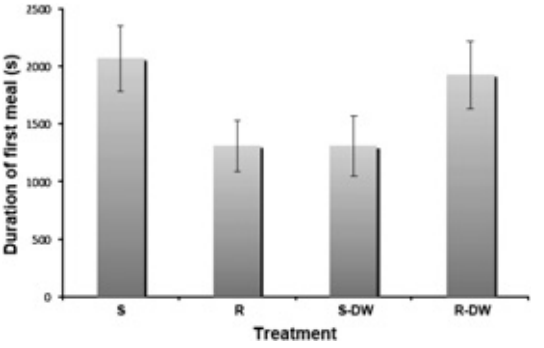


Fig. 3. Duration of the first meal (mean ± 2 SE) of sixth-instar spruce budworm (*Choristoneura fumiferana*) larvae on susceptible (S), resistant (R), susceptible dewaxed (S-DW), and resistant dewaxed (R-DW) white spruce (*Picea glauca*) needles ($n = 30$ per treatment).



ever, no significant differences were observed in any of the *a priori* treatment comparisons. Total time allocated to probing did not differ between treatments (ANOVA, $F_{[3,115]} = 2.464$, $P = 0.066$).

There were significant differences in the number of budworm larvae that successfully transitioned from probing to feeding between treatments (Fisher's exact test for $R \times K$, $P < 0.0001$). Indeed, 79.3% of budworm had at least one feeding bout following a probing event on S needles, but only 34.1% on R needles. When waxes were removed, the number of budworm larvae that fed following a probing event decreased to 31.4% on S-DW needles but increased to 50% on R-DW needles.

The mean number of feeding bouts differed significantly between treatments (ANOVA, $F_{[3,119]} = 7.377$, $P < 0.0001$) (Fig. 2). *A priori* treatment comparisons showed that the budworm larvae had almost three times as many feeding events on S as on R needles ($P = 0.006$). Additionally, insects fed five times more often on S needles than on S-DW needles ($P < 0.0001$). Other comparisons showed no significant differences (S-DW *vs.* R-DW: $P > 0.1$; R *vs.* R-DW: $P > 0.1$).

There was no significant difference in duration of individual feeding bouts (ANOVA, $F_{[3,56]} = 0.539$, $P = 0.657$).

Lastly, the duration of the first meal differed significantly between treatments (ANOVA, $F_{[3,119]} = 3.056$, $P = 0.031$) (Fig. 3). Treatment comparisons demonstrated that total durations of meals were significantly greater on S needles than on R needles ($P = 0.033$). Furthermore, larvae also fed significantly longer on intact S needles than on S-DW needles ($P = 0.028$). Other comparisons showed no significant differences (S-DW *vs.* R-DW: $P > 0.1$; R *vs.* R-DW: $P > 0.1$).

Discussion

Our results show that S and R foliage differ in their secondary chemistry, notably in concentrations of monoterpenes and tannins, and that they elicit different behaviours in budworm at first contact. Larvae were more likely to transition from probing to feeding on S foliage than on R foliage, but this difference disappeared when waxes were removed, suggesting that waxes play a role in feeding deterrence by R foliage. The number of probing events on both foliage types increased when waxes were removed, confirming that waxes are a factor in the decision to begin feeding. Finally, the number of feeding bouts within the first meal, and the duration of the first meal, were greater on intact S foliage than on R foliage. This suggests that on R foliage,

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insects initiate fewer feeding bouts after the first one, and that the deterrent effect of the waxes persists even after the insects have fed on the needle contents. Fewer feeding bouts within a meal lead to a shorter meal overall. Again, this difference disappears with wax removal, confirming the role of the waxes.

Foliar chemistry

Although there were some quantitative differences in concentrations of primary nutrients between needles from R and S host trees, they were not large enough to reflect any significant biological effect on the insect (Bauce *et al.* 2002). There were, however, strong quantitative differences in the concentrations of secondary compounds, especially monoterpenes. On average, needles collected from the three sampled R trees contained 65% more monoterpenes than those from S trees. Furthermore, the concentrations of the monoterpenes α-pinene and myrcene from R trees were 307% and 476%, respectively, above those found in needles collected from S trees. Because of their extremely volatile nature, monoterpenes are capable of crossing the cuticle and being absorbed into the epicuticular wax layer of the needle (Städler 1986; Fischer *et al.* 1994; Muller and Riederer 2005). Indeed, monoterpenes have been detected in spruce epicuticular waxes (Pruegel and Lognay 1996).

It has been shown that the concentration and composition of monoterpenes in coniferous trees are influenced by tree genotype (von Rudloff and Rehfeldt 1980) and environmental factors such as availability of nitrogen (McKinnon *et al.* 1998), water, and sunlight (Johnson *et al.* 1997).

Bauce and Kumbasli (2007) examined the concentrations of tannins in the same tree populations and found that resistant trees contained, on average, 110% more tannins than did susceptible trees, a greater difference than that seen in our study, which suggests that there is considerable variation among individuals and possibly among years. Condensed tannins are water-soluble and therefore are not expected to be present in waxes.

In several insect defoliator + tree systems (including spruce budworm + conifers), past defoliation has caused a reduction in concentrations of foliar nutrients, an increase in concentrations of secondary foliar chemical compounds such as phenolics, and a reduction in performance of insect larvae (Bauce and Hardy 1988; Roitto *et al.* 2009). In our study, because the S trees, unlike the R trees, had a history of defoliation but exhibited lower concentrations of foliar secondary compounds, it is unlikely that the past defoliation could have been the cause of the differences in foliar chemistry between the S and R trees. This suggests that in our study, the documented resistance phenomenon is constitutive rather than inducible. This is supported by the fact that the R trees had no detectable indigenous larval population but did have high levels of defensive foliar chemicals.

Behaviour

Palpation of potential food items prior to ingestion appears to play a key role in decision-making by budworm larvae, as has been observed in many other phytophagous insects (Woodhead and Chapman 1986; Chapman and Sword 1993). Our observations revealed that budworm larvae did not begin a feeding bout without first probing the surface of the food item.

Ascoli and Albert (1985) demonstrated that second-instar spruce budworm larvae orient themselves towards their host plants by means of odours, primarily volatile monoterpenes. Needles collected from resistant trees contain 307% and 476% more α-pinene and myrcene, respectively, than those collected from susceptible trees; at lower concentrations, these monoterpenes are attractive to spruce budworm moths (Hanover 1975). We had therefore anticipated that there would be an olfactory component to the behavioural responses of the larvae in our study. However, the time taken to make the first contact with the needle did not differ significantly between R and S needles or, surprisingly, between needles with or without waxes. It is possible that the arenas used were too small, resulting in probable saturation of the Petri dish with plant volatiles, and were therefore not appropriate for measuring insect responses to volatile chemicals.

Almost 80% of budworm larvae on S needles successfully transitioned from probing to feeding, whereas only 35% of those on R

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needles fed following a probing event. Thus, budworm larvae appeared to distinguish between needle types by means of sensory input received as a result of needle probing, accepting needles and initiating feeding on S trees more often than on R trees. This decision-making is likely based on the chemical content of a needle's epicuticular wax; this is the only source of information available to an insect that has not yet pierced the needle's cuticle. That the waxes are involved in this feeding decision is confirmed by the fact that the difference in initiating feeding disappears once they are removed. To initiate feeding, many phytophagous insects require positive information gathered from probing the external wax layer of the host plant (Woodhead and Chapman 1986).

Indeed, eastern spruce budworm fed on cellulose discs treated with lipid extracts from the host leaf surface in preference to control discs (Maloney *et al.* 1988). Our work confirms the important role played by waxes in decision-making by spruce budworm larvae and, in addition, shows that waxes can be used to discriminate between hosts that vary in quality within the same species.

We also demonstrated significant differences in the chemical composition of susceptible and resistant foliage. Of the different compounds detected, monoterpenes are the most likely candidates for the observed wax-based feeding deterrence, as they are volatile and lipid-soluble. Several other studies have demonstrated that monoterpenes provide an important mechanism of resistance to insects in conifers (McClure and Hare 1984; Redak and Cates 1984; Cates *et al.* 1987; Bauce *et al.* 1994; Litvak and Monson 1998; Chen *et al.* 2002). Interestingly, Redak and Cates (1984) showed that high concentrations of α-pinene and myrcene in Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae), are also linked to high levels of mortality in the western spruce budworm, *Choristoneura occidentalis* Freeman. Those authors found that growth rates of insects in sleeve cages on trees with higher monoterpene concentrations were lower (though they did not examine the mechanisms involved). Chen *et al* (2002) reported differences in the monoterpene profiles of Douglas-fir susceptible and resistant to budworm defoliation, but Palermo *et al.* (2003) reported no difference in taste responses to the two foliage types and concluded that resistance was linked to differences in phenology. Bauce *et al.* (1994) suggested that terpenes could be responsible, at least in part, for lower observed budworm feeding rates on young balsam fir, *Abies balsamea* (L.) Mill. (Pinaceae). The results of our study suggest a feeding-deterrent role for monoterpenes, either present in the epicuticular wax or trapped by it and hence prevented from volatilizing too rapidly.

In phytophagous insects, a meal usually consists of several feeding bouts separated by short pauses (Simpson 1995). In our experiment the first meal consisted of two food sources novel to the test insect and showed that initiation not only of the first feeding bout but also of subsequent bouts within the meal was influenced by needle waxes. Thus, the number of bouts, and food consumption, within the first meal are higher for insects on susceptible foliage than for those on resistant foliage. A decrease in food consumption could explain the lower growth rate observed by Redak and Cates (1984) and Bauce *et al.* (1994) on trees that were richer in monoterpenes.

Conclusions

The primary objective of this study was to characterize host acceptance behaviour and the microstructure of the feeding pattern of budworm larvae on needles collected from white spruce trees known to be susceptible or resistant to attack, in order to elucidate the role of surface waxes in host resistance. A minority of budworm larvae on needles collected from resistant trees successfully transitioned from probing to feeding, resulting in fewer feeding bouts as well as a significantly shorter first meal. Chemical analyses of foliage showed monoterpene concentrations to be higher in resistant foliage and suggested that they could be the basis for the deterrent effect of epicuticular waxes.

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