Association of within-tree jack pine budworm feeding patterns with canopy level and within-needle variation of water, nutrient, and monoterpane concentrations

Kimberly F. Wallin and Kenneth F. Raffa

Abstract: The possibility that uneven within-tree feeding patterns by jack pine budworm (Choristoneura pinus pinus Freeman) larvae could be related to underlying variation in host jack pine (Pinus banksiana Lamb.) foliar water, nutrient, and monoterpenes was examined. Choristoneura pinus pinus feeds disproportionately in the upper portion of the canopy and almost exclusively on the basal portions of needles. Within needles, the distribution of water, several nutrients, and monoterpenes varied significantly between the distal and basal sections. Water, nitrogen, sulfur, manganese, and zinc levels occurred in higher concentrations in the distal section of the needle, which would not be predicted based on C. pinus pinus feeding patterns. Phosphorus, potassium, calcium, iron, and copper were significantly higher in the basal section. Although the latter differences might be predicted based on C. pinus pinus feeding patterns, they were not strong (ranging from 7.8% to 36.4% relative differences, as compared with 10.7%–50.0% relative differences in the former group). By contrast, concentrations of foliar monoterpenes were more strongly associated with known feeding patterns of C. pinus pinus. These relative differences ranged from myrcene (26.8%) to limonene (44.7%). Thus, foliar differences affecting within-needle feeding selection appear more associated with allelochemicals than nutrients or water. In contrast with the within-needle associations between larval feeding and foliar chemistry, no associations between upper and lower canopy foliage were observed. Water, nutrients, and monoterpenes were evenly distributed across the upper and lower canopy locations. Thus, differential feeding between canopy locations cannot be explained by foliar constituents. Rather, it is more likely explained by other environmental factors, such as proximity to and density of reproductive and vegetative shoots.

Résumé : La possibilité que les différents patrons d’alimentation observés chez les larves de la tordeuse du pin gris (Choristoneura pinus pinus Freeman) à l’intérieur de la cime du pin gris (Pinus banksiana Lamb.) soient reliés à des variations sous-jacentes des contenus foliaires en eau, éléments nutritifs et composés monoterpéniques a été examinée. Choristoneura pinus pinus s’alimente principalement dans la partie supérieure du houppier, et presque exclusivement de la section proximale des aiguilles. La répartition de l’eau, de plusieurs éléments nutritifs et des monoterpènes variait de façon significative entre les sections proximales et distales des aiguilles. Les concentrations en eau, azote, souffre, manganèse et zinc étaient plus élevées dans la section distale de l’aiguille, ce qui apparaissait inconsistant avec les patrons d’alimentation observés chez C. pinus pinus. Les concentrations foliaires en phosphore, potassium, calcium, fer et cuivre étaient significativement plus élevées dans la section proximale. Bien que ces dernières différences aient pu être prédites par les patrons alimentaires de C. pinus pinus, elles n’étaient pas très marquées (différences relatives allant de 7.8 à 36.4%, comparativement à celles du premier groupe, s’étendant de 10.7 à 50.0%). À l’opposé, les concentrations monoterpéniques foliaires se sont avérées plus fortement associées aux patrons d’alimentation connus chez C. pinus pinus. Ces différences relatives variaient du myrcène (26.8%) au limonène (44.7%). Ainsi, les différences foliaires affectant le choix de la section d’aiguille à consommer semblent plus relever des composés monoterpéniques que des éléments nutritifs ou de l’eau. Contrairement aux associations intra-aiguilles observées entre les patrons alimentaires des larves et la chimie foliaire, aucune association de ce type n’a pu être détectée entre les feuillages provenant du haut et du bas du houppier. L’eau, les éléments nutritifs et les composés monoterpéniques étaient également répartis à travers les parties supérieures et inférieures de la cime. Ainsi, les différents patrons d’alimentation selon l’emplacement dans la cime, ne peuvent être expliqués par les composantes foliaires. Ce phénomène serait vraisemblablement mieux expliqué par d’autres facteurs environnementaux, tels que la proximité et la densité des bourreles floraux et végétatif.

Introduction

A wide variety of ecological and physiological factors can contribute to the selection by phytophagous insects of certain plants within a host species population, and specific tissues within an individual host plant. Variation in host phenologies (Holdren and Ehrlich 1982; Wood and Keese 1990), nutrients (Zou and Cates 1994; Wagner and Evana 1985; Clancy et al. 1980), and allelochemicals (Scriber and Slansky 1981; Cates 1980).
and Zou 1990) have all been shown to affect individual plant and tissue selection by insects. Differential feeding can occur across multiple levels of scale: plant communities, individuals within a population, plant parts within an individual, and specific portions of plant parts (Kulman et al. 1963; Clancy et al. 1980; Moran 1984; Volney and McCullough 1994).

Variation in plant quality within a population can result in differential feeding on particular individuals (Holdren and Ehrlich 1982; Schlichting and Levin 1984; Städler 1992; Slansky 1992). Expression of traits such as allelochemicals and availability of nutrients (Harvey 1974; Mattson and Scriber 1987; Karban and Myers 1989; Slansky 1992) that affect feeding patterns by phytophagous insects may be under genetic (Whitman 1989) and environmental (Rhodes 1977; Gershenzon 1984; Scriber 1984) control. Differences among plant parts contribute further to variation in patterns of insect feeding (Moran 1981; Slansky 1992). For example, cottonwood leaf beetle adults (Coleoptera: Chrysomelidae) and Lepidoptera larvae display distinct preferences for leaves of specific development ages on Populus (Meyer and Montgomery 1987; Bingaham and Hart 1992; Bauce and Carisey 1996). Likewise, several sawfly species feed almost exclusively on the mature but not juvenile foliage of confiers (Ikeda et al. 1977). Differential feeding within individual leaves has been observed in several insects, but within-leaf variation in substrate quality has rarely been quantified (Krause and Raffa 1995; Trier and Mattson 1997).

These varying levels of herbivory suggest that either foliar constituents are not homogeneous between and within leaves (Trier and Mattson 1997) or that there are other factors influencing foliage selection. Associations of foliar constituents within a naturally occurring host and their impacts on known insect feeding patterns remain poorly understood.

Jack pine budworm (Choristoneura pinus pinus Freeman) is a needle-feeding caterpillar found throughout the Great Lakes Region of North America. Differential feeding patterns of C. pinus pinus at the landscape level have been documented (Kulman et al. 1963; Clancy et al. 1980; Volney and McCullough 1994). Periodic outbreaks also generate uneven distributions of defoliated trees within forests, as well as uneven defoliation levels within the canopy and within needles (Volney 1992). Choristoneura pinus initially feed on the nitrogen-rich pollen cones and then proceed to current-year foliage (Nealis 1990, 1995). Larvae rarely eat the entire needle, but rather consume the needle base, proceed to adjacent foliage, and form shelter with the clipped needles as they continue. Underlying reasons for observed differential feeding by C. pinus pinus within canopies and within needles in relatively homogeneous stands remain unknown.

The objective of this study was to identify possible associations between known C. pinus pinus feeding patterns and the water, nutrient, and monoterpane content of jack pine (Pinus banksiana Lamb.) foliage.

Materials and methods

Plot description

Three plots of 66 trees each were established in mid-August 1994 from a young naturally growing P. banksiana stand in Jackson Co., Wisconsin (sec. 17, tp. 20N, rge. 2W). The plots are relatively homogeneous with regard to soil, slope, and ground cover, and selected trees were similar in size (DBH 11.43 (1.27) cm (mean (SE))) and phenotypic characteristics. Within each plot, trees had been naturally defoliated to varying extents by C. pinus pinus during late July – early August 1994.

Canopy-level defoliation

A modified method described by Gower (1993) was used to quantify within-canopy defoliation. Canopies of 12 randomly selected trees were vertically divided into equal upper and lower sections. Each section was delimbed, and a subsample, consisting of four representative branches, was collected from each canopy location. These subsamples were immediately weighed, placed in plastic bags on ice, and transported to the laboratory. The needles and branches were separated, weighed, dried at 57°C for 72 h, and reweighed. Needle and branch moisture and needle to branch ratios were calculated for each canopy subsample.

Foliar nutrient composition

Four to 6 shoots each from the upper and lower canopy sections of 12 trees (3 trees/defoliation level) were collected in July 1995. Shoots were immediately placed in liquid nitrogen, returned to the laboratory, and kept at –30°C until analysis. The current-year needles were removed from the shoots and cut into basal one-third and distal two-thirds sections. Approximately one-half of the needles from each subsample were removed for mineral analysis. These were weighed, dried for 94 h at 70°C, reweighed, and sent to the Wisconsin–Madison Soil & Plant Analysis Laboratory for mineral analysis. Analyses were performed using an inductively coupled plasma emission spectrophotometer, model 34000 ARL (Applied Research Labs) coupled with a DEC (Digital Equipment Corporation) PDP 11/34 computer (Schulte et al. 1987). Percent nitrogen was analyzed using a semi-micro Kjeldahl procedure (Schulte et al. 1987). Micronutrients were expressed as parts per million, and macronutrients, as percent element on a tissue-weight basis. The remaining half of the needles from each subsample was used for monoterpane analysis.

Foliar monoterpane composition

Foliar levels of monoterpenes were analyzed by gas liquid chromatography using the conditions of Raffa and Steffeck (1988). Foliar samples were finely chopped and extracted in a 10-ml pentane 0.1% para-cymene solution for 24 h. Para-cymene was used as an internal standard because it is not present in P. banksiana foliage and is easily separated from the naturally present monoterpenes. The extracts were separated from the foliage by vacuum filtration and dried over calcium chloride for approximately 1 h. Concentrations of each monoterpane were determined by integrating peak areas with a Shimadzu GLC 9A (Shimadzu Scientific Instruments, Inc., Columbia, Md.). Separations were performed on a 25 m x 0.25 mm bonded fused silica open tubular polyethylene glycol column (Alltech Assoc., Deerfield, Ill.). The oven temperature was 60°C for 10 min and increased to 160°C by 10°C/min increments. Helium was used as the carrier gas at 30 cm per second, with a methane retention time of 83.8 s. Monoterpane compositions were determined by integrating peak areas and comparing each peak area with reference monoterpane (Aldrich Chemical Co., Milwaukee, Wis.) standard areas (Wallin 1996). Following analysis, each foliage sample was oven-dried and weighed.

Data analysis

The experiment was conducted as a nested design. Effects of defoliation level, canopy location, needle section, and interactions, on water, nutrients, and concentrations of individual monoterpenes were analyzed using split split plot (Snedecor and Cochran 1989), with tree or defoliation level as the whole plot error, canopy location within the tree as the split-plot error, and needle section within the canopy location as the split split plot error. Results of this analysis indicated that defoliation level did not interact with tree, canopy location, or needle
section and therefore was removed. Canopy location, needle section, and interactions on water, nutrients, and concentrations of individual monoterpenes were subsequently analyzed using split plot, with tree or canopy as the whole plot error, and needle section within the canopy location as the split-plot error. Homogeneity of variance was tested with Levene’s test. Variances of individual monoterpenes that were not homogeneous were log transformed prior to analysis. Non-transformed values are given in all tables. Analyses were conducted using SAS Institute Inc. (1989).

**Results**

**Feeding patterns of *C. pinus pinus***

Defoliation had no to minimal effects on percent moisture content of either the remaining needles ($\bar{x} = 0.19, \text{SE} = 0.02; df = 3, F = 0.448, p = 0.09$) or remaining branches ($\bar{x} = 0.34, \text{SE} = 0.09; df = 3, F = 0.678, p = 0.95$). Therefore, the allometric equation of needle to branch mass can be used effectively to quantify defoliation intensity.

Defoliation was concentrated in the upper portion of the canopy (df = 1, $F = 6.39, p = 0.007$). The ratio of needle to branch dry weights averaged 0.75 and 1.27 for the upper and lower canopy locations, respectively (Fig. 1).

**Effects of canopy location and leaf section on foliar nutrients***

Foliar nutrient concentrations, and their sources of variation, are shown in Table 1. Foliar water content did not differ ($F = 1.38, p = 0.2739, df = 1$) between the upper ($\bar{x} = 1.539, \text{SE} = 0.65$) and lower ($\bar{x} = 1.69, \text{SE} = 0.49$) canopy locations (Table 1). However, there were significant differences in needle sections within canopy locations. The basal section of the needle had only about half the water content as the distal section (Table 1). There was no interaction between canopy location and needle section.

Lower canopy foliage had higher concentrations of three nutrients than lower canopy foliage ($p < 0.05$). Concentrations of K in the lower ($\bar{x} = 0.366, \text{SE} = 0.002$) canopy foliage were significantly greater than those in the upper ($\bar{x} = 0.32, \text{SE} = 0.001$) canopy foliage. Concentrations P in the lower ($\bar{x} = 0.126, \text{SE} = 0.003$) canopy were greater than those in the upper ($\bar{x} = 0.117, \text{SE} = 0.002$) canopy foliage. Concentrations of Zn in the lower ($\bar{x} = 60.70, \text{SE} = 4.8$) canopy were significantly greater than those in the upper ($\bar{x} = 52.4, \text{SE} = 3.9$) canopy foliage. The remaining nine nutrient concentrations did not differ significantly between the lower canopy and upper canopy locations.

Differences within sections of individual needles were more common and pronounced than those between canopy locations. There was significantly higher N, S, Mn, and Zn in the distal sections, and significantly higher P, K, Ca, Fe, and Cu in the basal sections, of the needles (Table 1). There were no canopy location by needle section interactions (Table 1).

These patterns were consistent across canopy locations, needle sections, and defoliation levels. That is, there were no significant interactions between canopy location and needle section, or among defoliation intensity, canopy location, and needle section, for any nutrient concentration.

**Effects of canopy location and leaf section on monoterpane composition and concentration***

Foliar monoterpane concentrations, and their sources of variation, are shown in Table 2. Five monoterpenes were identified in *P. banksiana* foliage. In order of decreasing abundance, these are α-pinene, myrcene, carene, β-pinene, and limonene. The concentrations and compositions of monoterpenes did not differ between upper and lower canopy locations (Table 2). Monoterpene concentrations varied considerably within needle sections and were significantly different for all major compounds except α-pinene. Myrcene, carene, limonene, and total monoterpenes were higher in the distal than the basal sections of the needles. β-Pinene was higher in the basal portion of the needles.

Monoterpane concentrations did not differ between the upper and lower canopy locations within needle sections (Table 2).

There were no significant interactions between canopy location and needle section, or among defoliation intensity, canopy location, and needle section, for any monoterpane concentration.

**Discussion***

Foliar nutrients and monoterpenes are not evenly distributed among the potential food substrate of *C. pinus pinus*. Nutrients varied primarily between the basal and distal sections of individual needles. This general trend, and the patterns of several micronutrients, agree with Krause and Raffa (1995). Likewise, monoterpenes varied between the basal and distal sections of individual needles. However, they did not vary between canopy locations. Thus, overall variation was higher within than between needles.

*Choristoneura pinus pinus* feeding was associated more with low monoterpane concentration than high water and nutrient content. Based on higher nutrient and water content, *C. pinus pinus* would be expected to consume entire needles.

© 1998 NRC Canada
Table 1. Split-plot analysis of variance of nutrients found in jack pine foliage (mean nutrient quantity and standard error of each needle section (distal and basal) are given).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Needle section</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Distal</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Water (%)</td>
<td>0.62</td>
<td>0.23</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.96</td>
<td>0.31</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.129</td>
<td>0.0004</td>
</tr>
<tr>
<td>K (%)</td>
<td>0.45</td>
<td>0.022</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.222</td>
<td>0.017</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.1</td>
<td>0.004</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.075</td>
<td>0.002</td>
</tr>
<tr>
<td>B (ppm)</td>
<td>16.36</td>
<td>0.757</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>259.18</td>
<td>23.46</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>68.65</td>
<td>5.47</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>5.15</td>
<td>0.092</td>
</tr>
<tr>
<td>Al (ppm)</td>
<td>499.35</td>
<td>205.5</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>46.5</td>
<td>2.21</td>
</tr>
<tr>
<td>Na (ppm)</td>
<td>59.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Split-plot analysis of variance of monoterpenes found in jack pine foliage (mean monoterpene (mg/g) quantity and standard error of each needle section (distal and basal) are given).

<table>
<thead>
<tr>
<th>Monoterpene (mg/g)</th>
<th>Needle section</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Distal</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>368.14</td>
<td>51.30</td>
</tr>
<tr>
<td>Myrcene</td>
<td>61.26</td>
<td>7.71</td>
</tr>
<tr>
<td>Carene</td>
<td>53.49</td>
<td>7.9</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>56.97</td>
<td>8.9</td>
</tr>
<tr>
<td>Limonene</td>
<td>19.50</td>
<td>2.55</td>
</tr>
<tr>
<td>Total</td>
<td>529.4</td>
<td>70.3</td>
</tr>
</tbody>
</table>

or perhaps only the distal portions. The habit of feeding on only basal sections of the needles, however, greatly reduces consumption of monoterpenes (Table 2). The behavior of feeding disproportionately in the upper canopy does not appear to offer any benefit in terms of either elevated nutrient or reduced monoterpene content. This suggests that factors other than variation in foliar substrate quality affect larval distribution and feeding across the canopy. One possibility is the proximity to pollen cones. Early instar *C. pinus pinus* feed on pollen cones, which provide a source of nitrogen for this (Nealis 1990) and other *Choristoneura* (Albert and Bauce 1994; Zou and Cates 1994) species. Although pollen cones occur throughout the upper and lower canopy, they tend to be concentrated in the upper portion (Lejeune 1950; Bauce and Carisey 1996). For example, *C. pinus pinus* feed in the pollen cones and first-year foliage of *P. banksiana*, which is generally more concentrated in the upper canopy (Nealis 1990). Additional nutritional factors in new foliage, such as carbohydrate content, may also affect feeding (Scriber and Slansky 1981; McCullough and Kulman 1991; Zou and Cates 1994; Rieske and Raffa 1998).

Previous studies with *P. banksiana* have produced mixed results on the influence of foliar percent N on *Choristoneura* spp. success (Montgomery 1983; Brewer et al. 1985; McCullough and Kulman 1991; Guetrin and Albert 1994). In this study, percent N in the selected host foliage, regardless of canopy location, is significantly lower in the nonselected tissue. Thus, percent foliar N may not be a primary factor influencing *C. pinus pinus* feeding selection. This is consistent with observations of McCullough and Kulman (1991), indicating that lower levels of N result in increased larval feeding due to compensation for lower N availability (Scriber and Slansky 1981) and thus increase defoliation intensity. Although nutrients can have a major influence on feeding patterns in some systems (Albert and Bauce 1994; Zou and Cates 1994), allelochemicals seem more consistently associated with *C. pinus pinus* feeding patterns. Concentrations of myrcene, the second most abundant monoterpene, are lowest in the tissues most preferred by *C. pinus pinus*. Likewise, carene, limonene, and the total monoterpene concentration were lower in the preferred basal section of the needle. Conversely, α-pinene was evenly distributed within the canopy and within the needle, suggesting that it is not a primary factor influencing *C. pinus pinus* feeding. These within-needle patterns agree with several studies on between-tree variation (McCullough and Kulman 1991). McCullough and Kulman (1991) further demonstrated the adverse effects of monoterpenes on *C. pinus pinus* larval success. Our results suggest that the possible deleterious
effects of feeding on the distal section of the needle with elevated monoterpene concentrations may outweigh the possible benefits of increased water, percent N, and substrate quantity gained from consuming the entire needle. That is, consumption of the entire needle would have minimal nutritional advantages compared with the possible disadvantages of consuming greater amounts of monoterpenes.

The jack pine budworm’s habit of preferentially feeding on needle bases provides an interesting contrast with conifer sawflies (Hymenoptera: Tenthredinoidea), which are also confronted with uneven distributions of nutrients and allelochemicals in red pine (Pinus resinosa Ait.) needles, but prefer the distal portions (Krause and Raffa 1995). Differences in feeding behavior may relate to aspects of their overall life histories. For example, sawfly larvae must obtain all of their nutrients from host foliage, whereas early instar C. pinus pinus obtain a substantial portion of their nutrients from staminate flowers, increasing survival and the likelihood of subsequent feeding (Nealis 1990). Moreover, sawfly larvae sequester host terpenes in specialized diverticulate pouches and exploit them as defensive allomones (Codella and Raffa 1995). Conversely, C. pinus pinus are not known to possess chemical defences, but rely partially on the shelters constructed from discarded distal needle tissue for protection. The disproportionately high concentrations of terpenes in this portion of the tissue could conceivably augment this defense.

Acknowledgements

This study was funded by the USDA Forest Service, McIntire–Stennis, and the University of Wisconsin–Madison College of Agricultural and Life Sciences. Field assistance by R. Hofstetter, T. Lanigan, and T. Trowbridge, and statistical advice by Dr. R. Nordheim are gratefully acknowledged. We thank Dr. D. Mahr, Dr. D. Hogg, Dr. E. Kruger, and two anonymous reviewers for reviewing earlier versions of this manuscript. The Wisconsin Department of Natural Resources kindly provided study sites.

References


