

CHEMICAL BASIS OF HOST PLANT SELECTION BY
EASTERN SPRUCE BUDWORM, CHORISTONEURA

FUMIFERANA CLEM. (LEPIDOPTERA:

TORTRICIDAE)

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The epicuticular waxes from four host plants of the eastern spruce budworm are examined with respect to their influence on the feeding behavior of the sixth-instar larva. Both current and one-year old needles contain stimulating chemicals in their epicuticular wax layer. Some pure fatty acids known to occur in balsam fir wax are stimulatory, and may serve to enhance the already strong feeding response to sucrose.

Introduction

The feeding responses of eastern spruce budworm larvae toward various pure chemicals and some extracts from white spruce were first studied by Heron (1965). Their responses to polar and non-polar compounds from a number of evergreen plants were studied by Albert and Jerrett (1981) who showed that the sugar/glycoside fraction from balsam fir was more stimulating than either amino acids or organic acids from the same host. Subsequently, Albert et al. (1982) characterized the feeding responses to pure carbohydrates, and reported that sucrose was the most stimulating of the sugars tested. Sucrose is known to play an important role in the feeding behavior of many insects (Dethier, 1966). In spruce budworm larvae, the peak response to sucrose is in the range from 0.01 to 0.05M, with a behavioral threshold at 10^{-4} to 10^{-3} M, indicating a high degree of sensitivity to this chemical (Albert et al., 1982).

Using four of the more common host species in eastern North America (balsam fir, white, black and red spruces), Albert (1983) found that the sugars/glycosides from all four hosts were highly stimulating to spruce budworm larvae. Amino acids were only slightly stimulating while organic acids were neutral or deterrent. Lipids from white and red spruces were more stimulating than those

from balsam fir and black spruce. This fraction from white spruce was found to be even more stimulating than the sugars/glycosides.

The first stage in the feeding behavior of many phytophagous insects involves the "test biting" of the leaf surface, a process which may be initiated as a result of the stimulation of peripheral sensilla by material contained in the epicuticular wax layer of the plant leaves (Bernays et al., 1975). Simple observations in our laboratory showed that a larva crawling on the surface of a fresh shoot of balsam fir would palpate the surface of the foliage with its maxillae, and bite into the leaf within a few seconds. Larvae given foliage treated with hexane to remove the epicuticular waxes spent considerable time palpating the surface without biting. The present study examines the feeding responses of spruce budworm larvae to surface chemicals from four host plants and to some pure fatty acids known to occur in balsam fir wax (Beri and Lemon, 1970).

Materials and Methods

Sixth-instar larvae were used in two-choice tests as described previously (Albert et al., 1982). Control discs were impregnated with 15µl of hexane which was allowed to evaporate. They were then wetted with 15µl of distilled water. Test discs were impregnated with 15µl of a solution of epicuticular waxes in hexane. The solvent was allowed to evaporate and the discs were then wetted with 15µl of distilled water.

Extracts of epicuticular waxes were obtained from balsam fir (Abies balsamea), white spruce (Picea glauca), black spruce (Picea mariana), and red spruce (Picea rubens). These were prepared by dipping 10g of "fresh" needles (from frozen samples which were collected on June 14, 1982 from the Acadia Forest Experiment Station, N.B.) in 200 ml of glass-distilled hexane for 30 sec. The solvent was evaporated and the extracted material was re-dissolved in 3 ml of hexane to provide the stock solution for the behavior tests.

Some fatty acids were tested at a concentration of 10^{-3} M. Those which proved stimulating were further tested in combination with sucrose (0.025M), using 0.025M sucrose on the control discs.

Data are presented either as mean % consumption ($\pm$ S.E.) of test versus control discs, or as a preference index ($PI = (T - C)/H$), where PI is the preference index, T is the

amount eaten of the test discs, C is the amount eaten of the control discs, and H is the time in hours). Multiplying the PI by 33.183 yields the difference in disc area (mm^2) eaten between test and control discs per hour.

Results and Discussion

The feeding preferences for discs containing extracts of epicuticular waxes from host plants versus control discs are shown in Figure 1. All four hosts' waxes contain stimulatory chemicals which presumably serve as biting stimuli.

FIGURE 1. MEAN $\bar{X}$ CONSUMPTION ($\pm$ S.E.) OF DISCS TREATED WITH HEXANE EXTRACT AND WITH WATER IN TWO-CHOICE TESTS.

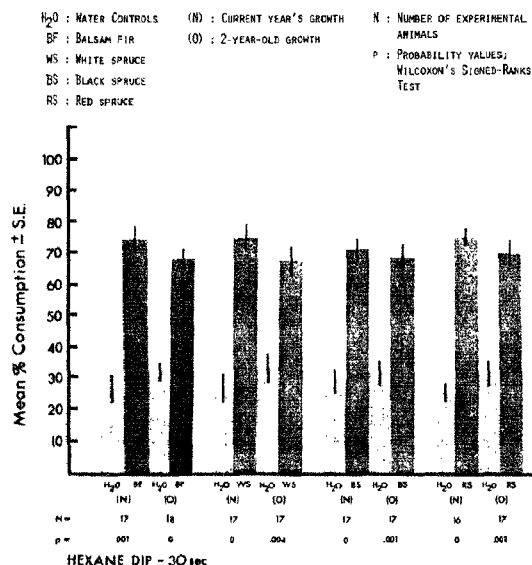
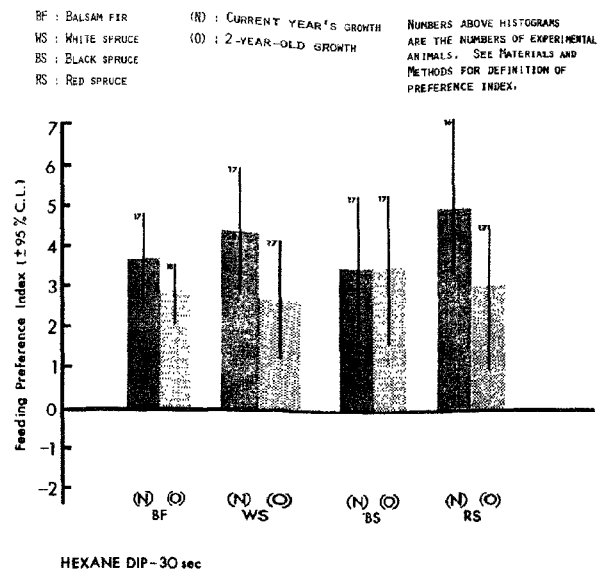


Figure 2 shows the Preference Index values for each host. In general, greater feeding occurs on discs treated with epicuticular waxes from the current year's growth from all hosts except black spruce where new and old needle waxes produce the same results. Red spruce shows the highest Preference Index followed by white spruce, then balsam fir and black spruce. The most significant finding for these tests is that the epicuticular waxes from all hosts, for both old and new needles, not only induce biting by larvae, but they also contribute to the maintenance of feeding though at a reduced level compared to sugars and glycosides (Albert, 1983; Fig. 2).

FIGURE 2. FEEDING PREFERENCE INDEX FOR ANIMALS IN TWO-CHOICE TESTS USING HEXANE EXTRACTS FROM NEW OR OLD FOLIAGE FROM FOUR HOST PLANTS.



A preliminary separation of the epicuticular waxes from balsam fir and white spruce current year's foliage was performed using gas chromatography and mass spectrometry techniques by Dr. A.P. Tulloch (Prairie Regional Lab, N.R.C., Saskatoon). The weights of purified materials recovered for each host are presented in Table 1. To date, the first three fractions from balsam fir have been tested, and each shows strong feeding stimulant activity. These contain hydrocarbons as well as esters.

Since pure fatty acids contained in the epicuticular wax from balsam fir are known (Beri and Lemon, 1970), we tested these at a concentration of 10^{-3} M. The results are shown in Table 2. Lauric, myristic, oleic, linoleic, linolenic, lignoceric, and palmitoleic acids all stimulate feeding. To test whether these could synergize with sucrose, a known pol. feeding stimulant, tests with fatty acid and sucrose combinations were performed. Results are shown in Table 3. Myristic, oleic, and palmitoleic acids all enhance the feeding response to sucrose alone, with oleic acid having the greatest effect.

Some fatty acids which were not stimulating by themselves were also tested in combination with sucrose. Palmitic and heptadecanoic acids were shown to also increase the feeding response to sucrose (Table 4).

TABLE 1. CHEMICAL FRACTIONS RECOVERED FROM EPICUTICULAR WAXES OF BALSAM FIR AND WHITE SPRUCE, HEXANE DIP, 30 SEC, CURRENT YEAR NEEDLES.

Balsam fir.		
Sample weight: 0.340 g		
No.	Weight	Comments on composition
1	0.0113	Hydrocarbons (considerably contaminated)
3	0.0500	Methyl esters C ₂₂ -C ₃₄ ; Esters of hexanol (and octanol) C ₃₂ -C ₄₄ . Numerous unidentified components but <u>no aldehydes</u> detected (difference from white spruce)
4	0.0720	Mostly di(2-ethylhexyl) phthalate (contaminant) and some esters
5	0.0110	Phthalate ?
7	0.0450	10-nonacosanol
8	0.0460	Some free acids C ₂₂ -C ₃₀ and small amount of C ₃₂ -C ₃₆ triacyl glycerols
9	0.0210	Small free alcohols C ₁₈ -C ₃₀
10	0.0700	
11	0.0800	Small diols (C ₂₉ like those in Juniper wax)

Fractions 8 to 11 also contain numerous unidentified components, possibly diterpene acids and probably appreciable amounts of polyesters (estolides) which are always found in conifer waxes.

White spruce.
Sample weight: 0.196 g

1	0.0060	Hydrocarbons (considerably contaminated)
3	0.0210	Methyl esters C ₂₂ -C ₃₂ . Esters of hexanol and octanol C ₃₆ -C ₄₂ . <u>Aldehydes</u> C ₂₂ -C ₃₂ . Numerous unidentified components
4	0.0080	Phthalate ?
5	0.0050	
6	0.0120	
7	0.0450	10-nonacosanol
9	0.0110	Free acids C ₂₂ -C ₃₂
10	0.0730	Acids C ₁₆ -C ₃₀ . Alkanols C ₂₂ -C ₂₈ . Diols

See footnote to balsam fir composition.

Data compliments of Dr. A.P. Tulloch, NRC Prairie Regional Lab, Saskatoon, Saskatchewan. GC-Mass Spec. separations.

TABLE 2. MEAN % CONSUMPTION OF DISCS TREATED WITH WATER (CONTROL) AND WITH FATTY ACIDS IN TWO-CHOICE TESTS

MEAN % CONSUMPTION (±S.E.)				
CONTROL (H ₂ O)	FATTY ACID (10 ⁻³ M)		N	P
58.5 (3.7)	41.5 (3.7)	CAPRIC	17	0.026
33.7 (3.7)	66.3 (3.7)	LAURIC	14	0.002
36.4 (2.9)	63.6 (2.9)	MYRISTIC	18	0
54.2 (5.0)	45.8 (5.0)	PENTADECANOIC	14	0.510
58.4 (4.6)	41.6 (4.6)	PALMITIC	15	0.045
56.6 (5.4)	43.4 (5.4)	STEARIC	17	0.266
34.7 (2.8)	65.3 (2.8)	OLEIC	17	0.001
41.4 (3.0)	58.6 (3.0)	LINOLEIC	18	0.019
38.6 (3.5)	61.4 (3.6)	LINOLENIC	17	0.015
46.3 (2.9)	53.7 (2.9)	ARACHIDIC	16	0.211
48.7 (3.4)	51.3 (3.4)	BEHENIC	18	0.542
35.4 (3.5)	64.6 (3.5)	LIGNOCERIC	18	0.002
35.6 (3.0)	64.4 (3.0)	PALMITOLEIC	17	0.002
44.7 (3.8)	55.3 (3.8)	HEPTADECANOIC	18	0.136

N= Number of experimental animals

P= probability values, Wilcoxon's Signed-Ranks Test

TABLE 3. MEAN % CONSUMPTION OF DISCS TREATED WITH SUCROSE (0.025M) AND WITH A COMBINATION OF SUCROSE AND FATTY ACID IN TWO-CHOICE TESTS

MEAN % CONSUMPTION (±S.E.)				
CONTROL SUCROSE (0.025M)	0.025M SUCROSE + FATTY ACID (10 ⁻³ M)		N	P
45.1 (2.6)	54.9 (2.6)	LAURIC	19	0.084
38.8 (2.7)	61.2 (2.7)	MYRISTIC	20	0.002
32.4 (3.0)	67.6 (3.0)	OLEIC	16	0.001
48.6 (2.3)	51.4 (2.3)	LINOLEIC	17	0.813
46.7 (2.6)	53.3 (2.6)	LINOLENIC	18	0.246
46.5 (3.2)	53.5 (3.2)	LIGNOCERIC	16	0.281
40.8 (3.1)	59.2 (3.1)	PALMITOLEIC	18	0.012

N= Number of experimental animals

P= probability values, Wilcoxon's Signed-Ranks Test

TABLE 4. MEAN % CONSUMPTION OF DISCS TREATED WITH SUCROSE (0.025M) AND WITH A COMBINATION OF SUCROSE AND A FATTY ACID IN TWO-CHOICE TESTS

MEAN % CONSUMPTION (±S.E.)				
CONTROL SUCROSE (0.025M)	0.025M SUCROSE + FATTY ACID (10 ⁻³ M)		N	P
47.9 (3.9)	52.1 (3.9)	CAPRIC	16	0.638
42.7 (3.2)	57.3 (3.2)	PENTADECANOIC	14	0.056
41.3 (2.6)	58.7 (2.6)	PALMITIC	20	0.002
46.9 (2.9)	53.1 (2.9)	ARACHIDIC	19	0.420
55.2 (3.2)	44.8 (3.2)	BEHENIC	17	0.136
39.9 (3.5)	60.1 (3.5)	HEPTADECANOIC	16	0.005

N= Number of experimental animals

P= probability values, Wilcoxon's Signed-Ranks Test

Epicuticular waxes are obviously of some importance in the feeding behavior of eastern spruce budworm larvae. They are the first gustatory chemicals which the insect encounters while palpating the surface of the leaf prior to biting and feeding. It is reasonable to assume that some of the chemicals serve to trigger the "test bite" response. However, their role may be much more important in serving to enhance the feeding response to the polar and non-polar compounds present within the leaf sap. The exact nature of this effect remains to be examined in greater detail both by behavioral and by electro-physiological techniques. More importantly, a study of the effects of epicuticular waxes on the feeding behavior of second- and third-instar larvae may shed some light on their possible role in stimulating these early instars to establish feeding sites on the developing buds. It is at this stage that a feeding or biting deterrent would likely be most effective in preventing larvae from establishing themselves on a host plant.

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