René I. Alfaro

Host selection by Pissodes strobi Peck: chemical interaction with the host plant.

Simon Fraser University

Doctor of Philosophy

1980

Dr. John H. Borden

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HOST SELECTION BY PISSODES STROBI PECK:
CHEMICAL INTERACTION WITH THE HOST PLANT

by

Rene I. Alfaro

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in the Department
of
Biological Sciences

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SIMON FRASER UNIVERSITY
April 1980

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ABSTRACT

A feeding bioassay was developed for the white pine weevil, *Pissodes strobi* Peck, that consisted of plastic petri dishes containing paired agar discs embedded in paraffin wax. Lens paper covering the top surface of the discs provided a surface through which weevils made regular feeding punctures which could be easily counted. Candidate feeding stimulants or deterrents were applied to the paper covering one of the discs, while the other served as a control. Feeding stimulants were tested using discs of pure agar, and deterrents were assayed on agar discs that contained 2% ground, dried Sitka spruce bark, *Picea sitchensis* (Bong) Carr.

The weevils exhibited a concentration dependent response to the amount of Sitka spruce bark in the agar disc; the threshold amount of bark that triggered a response was 60 μg. Chemical feeding stimulants in Sitka spruce were present throughout the phloem of the tree. Needles elicited little feeding, and contained chemicals with feeding deterrent activity. Xylem contained feeding stimulants only in the terminal leaders.

Several active non-volatile chemicals were detected in Sitka spruce bark extracts, and are currently being isolated and identified by research chemists at Simon Fraser University.
Volatile chemicals from Sitka spruce bark and foliage, captured in Porapak-Q, did not attract walking *P. strobi* in 2 olfactometers, nor did they trigger a feeding response when tested on plain agar. However, α-pinene, β-pinene, and β-myrcene acted as synergists to the non-volatile chemicals present in the bark; piperitone had a marked feeding deterrent effect, and (+)-camphor and limonene stimulated the feeding at low concentrations but caused feeding inhibition when the concentration rose above a particular threshold.

Feeding stimulants for *P. strobi* were present in many conifers in addition to Sitka spruce. No non-conifers studied triggered feeding. Feeding response is induced by a complex mixture of chemicals whose optimum blend is present only in Sitka spruce and a few other conifers in the genera *Pinus* and *Picea*. Several non-host conifers, including eastern white pine, *Pinus strobus* L., contained feeding deterrents. *Pinus strobus* is not attacked by *P. strobi* in British Columbia.

Preference for Sitka spruce did not change after the weevils were raised from egg to adult in lodgepole pine, *Pinus contorta* Dougl., indicating that preference for Sitka spruce may be genetically fixed.
A test for feeding deterrents failed to detect major differences in feeding induced by the dried, ground bark of attacked and unattacked trees in 2 plantations, and in 3 clines taken from "resistant" trees. Intra-specific resistance to P. strobi may rely on volatile feeding deterrents, on several characters acting in concert, and/or on the absence of a proper blend of feeding stimulants. The feeding deterrent activity of the foliage of western red cedar, Thuja plicata Donn, was most pronounced in the volatile fraction that comprises the leaf oil. Fractionation of the leaf oil indicated feeding deterrent activity in the monoterpenic hydrocarbon, thujone, and terpene alcohol fractions. When tested alone, both (-)-3-isothujone and (+)-3-thujone, which made up 75-88% and 5-10% of the leaf oil, respectively, deterred feeding by the weevils.

Western red cedar leaf oil also showed antifeedant activity with the alder flea beetle, Altica ambiens (Le Conte), and deterred oviposition by the onion maggot, Hylemya antiqua Meigen, but had no inhibitory effect on feeding the leaf roller, Epinotia solandriana L., and the red-backed sawfly, Eriocampa ovata L.
DEDICATION

TO HILDA AND CLAUDIO
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1. Introduction

The white pine weevil, *Pissodes strobi*, Peck is the most important damaging agent of Sitka spruce, *Picea sitchensis* (Bong.) Carr., in the Pacific Northwest of North America (McMullen 1976). Other important host tree species damaged by this insect are eastern white pine, *Pinus strobus* L., in eastern North America (MacAloney 1930), and Engelmann spruce, *Picea engelmannii* Parry ex Engelm., in Central British Columbia, the Rocky Mountains and Alberta (Stevenson 1967).

The biology of *P. strobi* (MacAloney 1930, Belyea and Sullivan 1956, Silver 1968, McMullen and Condrashoff 1973) may be summarized as follows. The adult weevils overwinter in the duff or on the laterals of host trees, and resume activity in late April or early May. After mating, the females lay their eggs in small punctures cut in the bark of the previous year's host leaders. The eggs hatch in 7 to 10 days. The small, white grubs feed in the inner bark, soon form a feeding ring and move downwards as a group, progressively girdling and killing the leader. The newly formed adults emerge in August and feed on various parts of the tree until they go to their overwintering sites. The crook or fork deformations that result from the attack reduce tree growth and the value of the lumber to the
point that planting of this fast growing species has been seriously questioned (Wright and Baisinger 1956).

The selection of a plant for feeding or oviposition by phytophagous insects is heavily dependent on the presence or absence in the plant of a number of chemical substances that act as permissive or restrictive factors (Thorsteinson 1960, Kennedy 1965, Hsiao 1976). The terminology adopted in this thesis to describe the response of insects to behaviorally active chemicals corresponds to the one proposed by Dethier et al. (1960) as follows:

**Attractant**: chemical stimulus to which the insect responds by orienting movements toward the source.

**Repellent**: chemical stimulus that elicits an oriented response away from the apparent source.

**Arrestant**: Chemical stimulus that causes the insect to reduce locomotion in close contact with the source.

**Feeding stimulant**: chemical stimulus that promotes continuous feeding.
Feeding deterrent: chemical stimulus preventing continuous feeding or hastening its termination.

The identification of plant chemicals that determine the feeding behavior of insects is important because such chemicals could be potentially used as tools in integrated pest management. Research into this area is increasing as a consequence of the trend towards the development of environmentally acceptable methods of insect control.

VanderSar (1977) suggested that host selection by flying P. strobi starts with an initial visual search for objects that have a silhouette similar to an erect Sitka spruce leader. Using visual cues, the weevil would select the thickest and longest leaders in the stand. VanderSar (1977) hypothesized that, upon arrival at the tree, the physical and chemical properties of the leader would determine the ultimate acceptance or rejection of the tree. Feeding stimulatory chemicals would promote the initiation and maintenance of feeding, whereas feeding deterrents and repellents would prompt the weevil to abandon non-host species.

The objectives of this thesis were: to develop the necessary methodology to demonstrate that the feeding behavior
of P. strobi on Sitka spruce is chemically mediated, to assess the relative stimulatory or inhibitory activity of the bark of host and non-host trees, and to gain some insight into the nature and characteristics of the chemicals involved.

This work was carried out in collaboration with Drs. A.C. Oehlschlager and H.D. Pierce Jr. of the Chemistry Department, Simon Fraser University, who did all the work pertaining the preparation and chemical analysis of fractions from host and non-host trees. All biological aspects of the study were designed and carried out by the author.
2. General methods

2.1 Collecting, handling and maintenance of the insects.

Unless otherwise stated, the weevils used in these experiments were obtained as mature larvae infesting Sitka spruce leaders collected in various Vancouver Island and Lower Fraser Valley locations in British Columbia. In the laboratory, the leaders were placed in screened cages at room temperature to allow the larvae to finish their development. The emerging weevils were stored at 3°C in petri dishes containing thin strips of the rearing diet described by Zerillo and Odell (1973), modified as indicated in Table I. Prior to their use in feeding experiments, the weevils were held for 24h at room temperature, without food, on moist filter paper.
Table I. *Pissodes strobi* maintenance diet formula

<table>
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<tr>
<th>Ingredient</th>
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<tr>
<td>Distilled water</td>
<td>790.0 ml</td>
</tr>
<tr>
<td>Sitka spruce bark powder</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Toasted wheat germ</td>
<td>30.0 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>12.0 g</td>
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<tr>
<td>Corn starch</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Salt Wesson</td>
<td>3.6 g</td>
</tr>
<tr>
<td>Casein</td>
<td>24.0 g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>25.0 g</td>
</tr>
<tr>
<td>Vitamin Diet Fortification</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Antimicrobial mixture</td>
<td>15.0 ml</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>10.0 ml</td>
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a Mix agar and sugars in water and bring to boil. Cool to 70 C, pour into a blender, add rest of ingredients, blend for 2 min, and pour into petri dishes.

b Bark is stripped from lateral branches, dried (8h at 60 C) and milled in a rotary mill.

c Made by dissolving 20g of sorbic acid and 15g of methyl p-benzoic acid in 170 ml of 95% ethanol.
3. DEVELOPMENT OF A QUANTITATIVE FEEDING BIOASSAY FOR 
PISSEODES STROBI.

3.1 Introduction

The white pine weevil relies on visual (VanderSar and Borden 1977a) as well as chemical (Anderson and Fisher 1956, 1960; VanderSar and Borden 1977b) cues to find its hosts. VanderSar and Borden (1977b) showed that feeding stimulants present in an ethanolic extract of Sitka spruce bark caused the weevils to feed on extract-impregnated elderberry pith discs. The weevils fed little on Sitka spruce twigs soaked in pressure-extracted fluids of western red cedar, Thuja plicata Donn. They hypothesized that the ultimate step in host selection was mediated by olfaction and contact chemoreception, involving chemical feeding stimulants as well as deterrents.

As a prerequisite to identifying the chemicals involved, it was essential to develop a reliable, quantitative bioassay for the response of P. strobi to feeding stimulants and deterrents. The spruce twig bioassay (VanderSar and Borden 1977b) required extensive preparation, and was subject to genetic variations between individual trees. An alternative inert substrate to elderberry pith discs (VanderSar and Borden 1977b) was also
needed, as the weevils made irregular feeding cavities on them instead of the round, neat punctures that they make in host bark. Agar is a common inert substrate used in feeding bioassays for other coleoptera, including the smaller European elm bark beetle, *Scolytus multistriatus* (Marsham) (Peacock et al. 1967), and the boll weevil, *Anthonomus grandis* Boheman (Keller et al. 1962). This section describes the development and evaluation of an agar-based feeding bioassay for *P. strobi*.

3.2 Methods and materials

3.2.1. Description and standardization of the feeding bioassay

After experimentation with several substrates and designs, a feeding bioassay was developed which explicitied the fact that *P. strobi* will readily feed on agar containing Sitka spruce bark, whereas they will feed very little on agar alone.

Each experimental unit (a replicate) (Fig. 1) consisted of a 5.5 cm diam. plastic petri dish that contained one or two agar discs (1.5 cm diam. x 0.4 cm high). The agar discs were covered on their upper surface with a piece of Fisher standard, lens paper, the most acceptable of a number of coverings tested.
Figs. 1-2. Bioassay apparatus showing *P. strobi* feeding through lens paper into agar disk containing 2% Sitka spruce bark.
After placing the agar discs 2 cm apart in the petri dishes, hot paraffin wax was added to a height equal to that of the discs. Thus, only the upper, paper-covered surface of the agar discs was exposed, retarding dehydration and preventing shrinkage of the agar away from the paper. The paper was kept in contact with the agar by embedding its edge in the hot wax. Insects placed in the dish had ready access to the surface-level test materials as they walked over the discs.

Stimulants were normally applied in solution to the paper surface of the discs or they were incorporated into the agar as finely powdered, oven dried (60°C for 8h) bark from lateral branches of host trees. Feeding deterrents were applied to the paper surface on agar discs that contained the highly stimulatory powder of Sitka spruce bark. Feeding activity was evaluated by comparing the number of feeding punctures made by the test insects in the paper over treated and control discs.

The relationship between the number of feeding punctures and the area and volume of agar ingested was investigated in 10, 20-replicate experiments. Two weevils per replicate were allowed to feed for 24h in bioassay preparations with only one agar disc, containing 2% Sitka spruce bark. An estimate of the volume of each feeding puncture was obtained by multiplying its
area at surface level, calculated by planimeter from a camera lucida drawing, by its depth, measured by carefully introducing an insect-pin depth gauge into each cavity. By addition, a "total volume" and "total area eaten" were obtained for each disc. Correlation coefficients were calculated between the number of feeding punctures produced and 1) surface area, and 2) volume of agar ingested.

The number of weevils to use per replicate was evaluated in a 4-treatment, 15-replicate experiment, in which the number of punctures made in 24h by 1, 2, 3 or 4 weevils on single 2% spruce bark-agar discs were counted.

3.2.2. Test of the bioassay for evaluating Sitka spruce bark and bark extracts as feeding stimulants

An experiment was designed to test whether the weevils would exhibit a concentration-dependent feeding response to different amounts of Sitka spruce bark incorporated into the agar. Each replicate comprised one agar-bark disc plus a plain agar control disc. Eight different concentrations of bark, ranging from 0.01% to 3% were tested; 15 replicates, each with 2 insects, were run for 24h for each treatment and sex of P. strobi.
Two extracts of Sitka spruce bark were tested for feeding stimulatory activity. Dried Sitka spruce bark (30g) obtained from lateral branches collected in the fall, was sequentially extracted for 24h in a soxhlet extractor with pentane and ether. The 2 solvent extracts were concentrated to 25ml by distillation, and then 10 µl of extract (the equivalent of 0.012g of spruce bark) was applied with a micropipette to the paper surface of one of pure agar discs in each dish. The control disc in each dish was treated with 10µl of either ether or pentane. The number of replicates, insects and the experimental duration were the same as in the previous experiment.

3.2.3. Comparison between fresh and dried bark stimuli.

The feeding activity elicited by dry and fresh Sitka spruce bark incorporated into agar discs was compared in 2, 15-replicate experiments. Bark was peeled from 5 Sitka spruce leaders and divided into 2 portions. One portion was oven dried at 60 C and then ground to a powder. The second portion (6g) was ground with distilled water (40ml) to a paste in a Waring blender. Experimental dishes were prepared by adding 1% dry bark powder, or its equivalent in fresh bark paste, into the agar. Two types of experiments were conducted:
a) Single bark stimulus: 3 weevils were given a choice, in each dish, between a disc containing either dry or fresh bark, and a disc of plain agar.

b) Double bark stimulus: 3 weevils were given a choice, in each dish, between an agar disc containing fresh bark and an agar disc with dried bark.

3.2.4. Test of the bioassay with feeding deterrents

Branchlets of western red cedar (200g) were macerated in a Waring blender with water (1750ml). The mixture was transferred to a 3 l distilling flask fitted with a modified Nielsen-Kryger condenser (Veith and Kivus 1977), and steam-distilled for 3.5h. The continuous extraction section of the condenser was charged with approximately 5ml of doubly-distilled pentane. The pentane solution of cedar leaf oil was washed with 2% NaHCO₃ solution and water, dried (Na₂SO₄), and concentrated by distillation. Residual solvent was removed by brief vacuum pumping.

The cedar leaf oil was tested for feeding deterrent activity in an experiment in which both agar discs in a dish contained 2% Sitka spruce bark. A pentane solution of the cedar leaf oil was applied to the paper surface covering one of the discs in each
replicate. The control disc received an equal volume of distilled pentane. The amounts of extract applied were: 1, 10, 50 and 100µg. Fifteen replicates, each with 2 weevils, were run for 24h for treatment and sex of P. strobii.

3.3. Results and discussion.

The weevils readily fed on agar containing Sitka spruce bark. The response of the insect was caused by chemicals that eluted out of the bark, into the agar and impregnated the paper cover of the discs. After introduction into the dishes, the weevils wandered about for 2-3h before starting to feed. They discovered the bark-containing disc apparently by close range chemoreception. The feeding punctures made in the lens paper were very distinct and easy to count (Fig. 2).

High correlation coefficients were obtained between the number of feeding punctures produced and the area and volume of agar ingested by the insects (Table II). Therefore, the number of punctures is a good indicator of the amount of feeding by the test insects, justifying their use as the sole measure of evaluating subsequent experiments. If poor correlations were found, the number of feeding punctures would be indicative of weevil biting response rather than feeding activity.
Table II. Linear correlation coefficients between number of feeding punctures produced and the volume and area of agar ingested by 2 *P. strobi* on Sitka spruce bark (2%)-agar discs. *N*=20 replicates for each experiment.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Punctures/Area</th>
<th>Punctures/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.89</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>0.72</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td>0.85</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>7</td>
<td>0.91</td>
<td>0.84</td>
</tr>
<tr>
<td>8</td>
<td>0.82</td>
<td>0.92</td>
</tr>
<tr>
<td>9</td>
<td>0.86</td>
<td>0.85</td>
</tr>
<tr>
<td>10</td>
<td>0.88</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*All coefficients were statistically significant at *P*<0.01.*
The number of feeding punctures in the agar discs increased linearly with the number of female weevils per replicate. However, when 4 instead of 3 males were included per replicate, feeding did not increase proportionally (Table III). This observation may indicate a behavioral difference between sexes in response to crowding. The differences in feeding between males and females were not significant for any number of weevils. The data in Table III indicate that the use of 2 or 3 weevils of undetermined sex per replicate will result in reliable data. Use of 2 or 3 weevils in each dish eliminates some of the variability in the data that is obtained when only one insect is used per replicate.

There was a concentration-dependent feeding response of *P. strobi* to the various concentrations of Sitka spruce bark in the agar discs (Table IV). Comparison of separate linear regression equations obtained from the data of males and females, transformed to natural logarithms, indicated that the feeding response of the two sexes could be represented by a single equation:

\[ \text{NFP} = 13.8 + 2.8 \times \ln \text{Concentration (\%)} \]

where NFP = number of feeding punctures.
Table III. Mean number of feeding punctures produced on Sitka spruce bark (2%) - agar discs by different numbers of *P. strobi*. N=15 replicates for each mean.

<table>
<thead>
<tr>
<th>Number of weevils</th>
<th>Mean number of feeding punctures&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>1</td>
<td>30.4 a</td>
</tr>
<tr>
<td>2</td>
<td>45.8 a</td>
</tr>
<tr>
<td>3</td>
<td>68.9 b</td>
</tr>
<tr>
<td>4</td>
<td>60.0 b</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within each column followed by the same letter are not significantly different, Newman-Keuls test, p<0.05.
Table IV. Feeding response of *P. strobi* to agar discs containing different concentrations of Sitka spruce bark. N=15 replicates, 2 weevils/replicate.

<table>
<thead>
<tr>
<th>Park concentration (%)</th>
<th>Males Agar-spruce discs</th>
<th>Males Untreated discs</th>
<th>Females Agar-spruce discs</th>
<th>Females Untreated discs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>20.3</td>
<td>0.0**</td>
<td>14.5</td>
<td>0.5**</td>
</tr>
<tr>
<td>1.5</td>
<td>12.1</td>
<td>0.1**</td>
<td>8.8</td>
<td>0.2**</td>
</tr>
<tr>
<td>0.5</td>
<td>11.5</td>
<td>0.1**</td>
<td>20.5</td>
<td>0.1**</td>
</tr>
<tr>
<td>0.25</td>
<td>12.5</td>
<td>0.5**</td>
<td>15.3</td>
<td>0.3**</td>
</tr>
<tr>
<td>0.10</td>
<td>3.1</td>
<td>0.1**</td>
<td>3.7</td>
<td>0.1**</td>
</tr>
<tr>
<td>0.05</td>
<td>1.6</td>
<td>0.4*</td>
<td>9.3</td>
<td>0.4**</td>
</tr>
<tr>
<td>0.02</td>
<td>0.3</td>
<td>0.1</td>
<td>1.8</td>
<td>0.3*</td>
</tr>
<tr>
<td>0.01</td>
<td>1.1</td>
<td>0.3</td>
<td>1.5</td>
<td>0.0*</td>
</tr>
</tbody>
</table>

* t-test significance level on difference between treated and control for each sex indicated by: **=p<0.01; *=p<0.05
Correlation analysis yielded a correlation coefficient of 0.81 (P<0.01). By extrapolation of the fitted curve, the threshold of response to bark in the agar was found to be 0.01% or 60μg of dry bark in the discs (an agar disc weighs about 600mg).

The weevils readily responded to the pentane and ether extracts of spruce bark (Table V). The high level of feeding obtained on the treated discs is in agreement with earlier findings that extractable feeding stimulants are present in Sitka spruce bark (VanderSar and Borden 1977b).

The feeding responses of P. strobi weevils to fresh and dried bark were both significantly different from the feeding on plain agar (ANCOVA, P<0.01). The difference between fresh and dried bark, however, was not significant (Table VI), indicating that the volatile chemicals that are presumably lost during the drying process are not crucial in triggering a feeding response by P. strobi. They could be important, however, as attractants or repellents.

Western red cedar leaf oil proved to be highly deterrent to feeding by P. strobi. The bioassay effectively quantified the
Table V. Feeding response of *P. strobi* to agar discs treated in the surface with pentane and ether extracts of Sitka spruce bark. N=15 replicates, 2 weevils/replicate.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Untreated</td>
<td>Treated</td>
<td>Untreated</td>
</tr>
<tr>
<td>Pentane (10μl)</td>
<td>36.3</td>
<td>2.3**</td>
<td>19.7</td>
<td>1.1**</td>
</tr>
<tr>
<td>Ether (10μl)</td>
<td>19.3</td>
<td>0.1**</td>
<td>46.5</td>
<td>0.4**</td>
</tr>
</tbody>
</table>

* t-test significance level on difference between treated and control for each sex indicated by: **= P<0.01
Table VI. Feeding response of *P. strobi* to agar discs with fresh and dry bark from Sitka spruce terminal leaders. *N*=15 replicates per experiment, 3 weevils/replicate.

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Agar discs with dry bark</th>
<th>Plain agar discs</th>
<th>Agar discs with fresh bark</th>
<th>Plain agar discs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single bark stimulus</td>
<td>9.8</td>
<td>0.1</td>
<td>12.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Double bark stimulus</td>
<td>11.9</td>
<td>---</td>
<td>9.7</td>
<td>---</td>
</tr>
</tbody>
</table>

*Differences in feeding on discs with fresh and dry bark were not statistically significant, t-test.*
increasing deterrent effect of higher stimulus concentrations (Table VII). As the amount of cedar leaf oil increased on the treated side, the weevils fed preferentially on the untreated agar discs.

The experiments reported herein show that an agar disc bioassay can be effectively utilized to evaluate quantitatively the feeding stimulatory and deterrent properties of natural plant products on *P. strobi*. Stimuli can either be incorporated into the agar or applied to an absorbent paper surface atop the disc.

Evaluation of feeding in this bioassay can be done by counting the numbers of feeding punctures, which are highly correlated with the amount of material actually ingested by the insects. The bioassay can be carried out using 1-3 insects per replicate without affecting the behavior of the weevils. Separate tests for each sex are not necessary as both sexes responded equally to feeding stimulants and deterrents (Tables III-V).

The bioassay has been used to confirm that feeding stimulants for *P. strobi* occur in the bark of Sitka spruce, and that chemicals from western red cedar leaf oil inhibit feeding
Table VII. Feeding response of *P. strobi* to agar discs containing 2% Sitka spruce bark and treated on the surface with increasing concentrations of western red cedar leaf oil. N=15 replicates, 2 weevils/replicate.

<table>
<thead>
<tr>
<th>Amount of cedar oil applied (µg)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated discs</td>
<td>Untreated discs</td>
</tr>
<tr>
<td>1</td>
<td>19.7</td>
<td>33.4*</td>
</tr>
<tr>
<td>10</td>
<td>22.2</td>
<td>35.4</td>
</tr>
<tr>
<td>50</td>
<td>6.0</td>
<td>37.7**</td>
</tr>
<tr>
<td>100</td>
<td>3.3</td>
<td>47.7**</td>
</tr>
</tbody>
</table>

*t*-test significance level on difference between treated and control for each sex indicated by: **=P<0.01; *=P<0.05.
on an otherwise acceptable feeding substrate. The large differences between feeding on treated and untreated discs (Table V), make this bioassay particularly suitable for detecting compounds that are even mildly stimulatory to feeding by *P. strobi*.
4. FEEDING STIMULANTS AND DETERRENTS IN SITKA SPRUCE TREES

4.1. Introduction.

Experiments in section 3 indicated that both sexes of *P. strobi* exhibited a feeding response to agar discs containing Sitka spruce bark. The bark used in these experiments was obtained from lateral branches of Sitka spruce and not from the tree leaders which are the preferred food for the weevils when they attack trees in the spring. Therefore, it was of interest to determine whether stimulants from the leader would elicit a stronger feeding response than those from lateral branches. An additional objective was to test the foliage, on which the weevils do not feed, for feeding stimulant or deterrent activity.

4.2. Methods and materials.

Xylem, needles and bark from the leader and lateral branches, collected in the fall, were separately dried (60 C for 8h), ground to a powder and incorporated at 1% w/w into agar discs. The base of each needle was cut to eliminate attached pieces of bark that could contaminate the needle powder. The treatments consisted of 15 dishes, each containing a plain agar disc and an agar disc with 1% test powder.
An experiment was conducted to determine whether the needles contain chemical deterents that prevent feeding or whether the observed absence of feeding is due to a deficiency in feeding stimulants. Experimental dishes were prepared that contained one agar disc each. A control treatment contained 1% dried and ground Sitka spruce bark while experimental treatments contained bark plus dried and ground Sitka spruce needles from the leader incorporated at concentrations of 0.1, 1.0, 2.5, and 10%, into the agar. A decrease in the feeding activity in the agar discs containing the bark-needle mixture with respect to the feeding on the discs with bark alone would demonstrate that chemical feeding deterrents are present in the needles of Sitka spruce. Each experiment was run for 8h with 3 insects in each of 15 replicates.

4.3. Results and discussion.

All parts of the Sitka spruce tree tested elicited a feeding response that was higher than the control stimuli (Table VIII). The strongest response was to the bark of both the leader and the lateral branch. No difference was found between leader and lateral branch bark. Leader xylem elicited a high
Table VIII. Feeding response of *P. strobi* to agar discs containing powdered bark, xylem or needles of the leader or lateral branches of Sitka spruce. *N=15* replicates, 3 weevils/replicate.

<table>
<thead>
<tr>
<th>Part of the tree tested</th>
<th>Mean number of feeding punctures</th>
</tr>
</thead>
</table>
|                         | Plain agar | Agar disc with test powder  
|                         | disc       |                                  |
| Leader xylem            | 0.0        | 19.6 a                             |
| Leader needles          | 0.3        | 4.1 b                              |
| Leader bark             | 0.1        | 26.2 a                             |
| Lateral branch xylem    | 0.1        | 4.2 b                              |
| Lateral branch needles  | 0.1        | 2.4 b                              |
| Lateral branch bark     | 0.0        | 35.1 a                             |

*Feeding on discs with test powder was in all cases significantly different from the feeding on plain agar, t-test, *P*<0.05.*

*b* Means followed by the same letter are not significantly different, *Newman-Keuls* test, *P*<0.05.
degree of feeding stimulation, whereas feeding stimulation by lateral branch xylem was low. Stimulatory chemicals that appear to be present in the xylem of the leader may in part be the stimuli that retain weevils on the leader tips for oviposition. They may also induce mature P. strobi larvae to bore into the xylem shortly before pupation. It is concluded that the 2 types of bark can be equally used in a chemical isolation program for the feeding stimulatory chemicals.

Feeding stimulation caused by needles of both lateral branches and leader was very weak (Table VIII). This finding agrees with the fact that P. strobi does not feed on or puncture the needles. The addition of needle powder to agar discs that contained Sitka spruce bark drastically reduced the feeding response of P. strobi (Fig. 3), demonstrating that chemical feeding deterrents are present in the needles. The presence of these deterrents, perhaps coupled to a relative absence of feeding stimulants, may in part direct the weevils, upon landing on the leaders, away from the needles, toward the appropriate site for feeding and oviposition, i.e., the bark surface.
Fig. 3. Feeding response of *P. strobi* to agar-Sitka spruce discs containing increasing amounts of Sitka spruce needle powder.
Concentration of needle powder (%) in agar-spruce bark discs
5.0. FEEDING OF P. STROBI ON HOST AND NON-HOST TREES.

5.1. Introduction.

The host selection process in *P. strobi* starts early in the spring when adults of both sexes emerge from their overwintering sites in the soil, duff or litter, or on the lateral branches of trees attacked the previous year (Belyea and Sullivan 1956, Silver 1968, McMullen and Condrashoff 1973). Upon emergence, the weevils must reach suitable hosts, which in the Pacific Northwest are Sitka spruce trees having a large dominant leader (Gara *et al.* 1971, VanderSar and Borden 1977a). Movement to the host is accomplished by flying (Harman and Kulman 1967, Overhulser and Gara 1975, Harman 1975) or by crawling (Belyea and Sullivan 1956, Dirks 1964). Dispersing *P. strobi* differentiate the host from several other coincident conifer and non-conifer plants in an apparently very precise manner. The mechanism by which the selection is accomplished is not very well understood. VanderSar and Borden (1977a) showed in laboratory bioassays that overwintered *P. strobi* are attracted to black silhouettes that resemble the leaders of Sitka spruce and postulated that vision could play an important role in selection of the host tree. This visual perception, however, is insufficient to account for the ability of the weevil to
differentiate between conifers that have similar leader characteristics. Silhouette cues can perhaps be more important in differentiating conifers from shrub-like non-conifer plants. Doubtless, a more complex pattern of cues is involved in the food plant recognition process. As indicated by Thorsteinson (1960), the spectral composition of light reflected from surfaces of plants and the chemical constitution of plants, especially the latter, provides the complex patterns of stimuli necessary to explain the discriminatory powers of insects.

This section comprises a series of experiments designed to determine whether the chemicals that incite biting and stimulate feeding by *P. strobi* are also present in other plants, or whether they are unique to Sitka spruce and the other conifer hosts of the weevil.

5.2. Methods and materials.

The agar disc bioassay (Section 3) was used to study the presence of feeding stimulants in several native and exotic conifers. The species tested were collected in plantations or in an arboretum at the University of British Columbia Research Forest, Maple Ridge, B.C., and in nature at Manning Provincial Park, B.C. The experiments also included common non-conifer
plants found in plantations of Sitka spruce in the lower mainland of British Columbia.

For each conifer species (Table IX), a sample of 10g of lateral branch bark from each of 10 trees for field collected bark and 4-10 trees from arboretum species, was collected and pooled. In the laboratory, the needles were removed and the bark oven dried (60°C for 8h) and milled to a powder. Bark or leaves (the latter used for sword fern and salal only) from non-conifers were collected and prepared in a similar manner.

Three types of experiments, each with 10 replicates, and 3 weevils per replicate, were performed:

a) Single stimulus bioassays: weevils given a choice between a plain agar disc and a disc that contained 1% powdered test plant tissue.

b) Double stimulus bioassays: weevils given a choice between one agar disc with 1% powdered Sitka spruce bark and a disc with 1% test plant powder.
Table IX. Conifer and non-conifer species tested in feeding stimulation experiments with *G. strobi*. Species followed by asterisk are sympatric with Sitka or Engelmann spruce in British Columbia.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bishop pine</td>
<td><em>Pinus muricata</em> D. Don.</td>
</tr>
<tr>
<td>Bhutan pine</td>
<td><em>Pinus wallichiana</em> Jackson</td>
</tr>
<tr>
<td>Eastern white pine</td>
<td><em>Pinus strobos</em> L.</td>
</tr>
<tr>
<td>Jack pine</td>
<td><em>Pinus banksiana</em> Lamb.</td>
</tr>
<tr>
<td>Korean pine</td>
<td><em>Pinus koraiensis</em> Sieb. et Zucc.</td>
</tr>
<tr>
<td>Limber pine</td>
<td><em>Pinus flexilis</em> James</td>
</tr>
<tr>
<td>Lodgepole pine</td>
<td><em>Pinus contorta</em> Dougl. *</td>
</tr>
<tr>
<td>Macedonian pine</td>
<td><em>Pinus peuce</em> Griseb.</td>
</tr>
<tr>
<td>Monterey pine</td>
<td><em>Pinus radiata</em> D. Don.</td>
</tr>
<tr>
<td>Ponderosa pine</td>
<td><em>Pinus ponderosa</em> Laws.</td>
</tr>
<tr>
<td>Scotch pine</td>
<td><em>Pinus sylvestris</em> L.</td>
</tr>
<tr>
<td>Western white pine</td>
<td><em>Pinus monticola</em> Dougl. *</td>
</tr>
<tr>
<td>Blue spruce</td>
<td><em>Picea pungens</em> Engel.</td>
</tr>
<tr>
<td>Brewer spruce</td>
<td><em>Picea breweriana</em> Wats.</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td><em>Picea engelmannii</em> Parry ex Engel.</td>
</tr>
<tr>
<td>Norway spruce</td>
<td><em>Picea abies</em> (L.) Karst.</td>
</tr>
<tr>
<td>Red spruce</td>
<td><em>Picea rubens</em> Sarg.</td>
</tr>
<tr>
<td>Species</td>
<td>Scientific Name</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td><em>Picea sitchensis</em> (Bonq.) Carr.</td>
</tr>
<tr>
<td>Serbian spruce</td>
<td><em>Picea omorika</em> (Pancic) Purkyne</td>
</tr>
<tr>
<td>White spruce</td>
<td><em>Picea glauca</em> (Moench) Voss. *</td>
</tr>
<tr>
<td>Alpine fir</td>
<td><em>Abies lasiocarpa</em> (Hook.) Nutt. *</td>
</tr>
<tr>
<td>Amabilis fir</td>
<td><em>Abies amabilis</em> (Dougl.) Forbes *</td>
</tr>
<tr>
<td>Grand fir</td>
<td><em>Abies grandis</em> (Dougl.) Lindl. *</td>
</tr>
<tr>
<td>Greek fir</td>
<td><em>Abies cephalonica</em> Loud.</td>
</tr>
<tr>
<td>Noble fir</td>
<td><em>Abies nobilis</em> Rehder</td>
</tr>
<tr>
<td>Sachalin fir</td>
<td><em>Abies sachalinensis</em> Mast.</td>
</tr>
<tr>
<td>White fir</td>
<td><em>Abies concolor</em> Lindl. ex Hildebr.</td>
</tr>
<tr>
<td>Western hemlock</td>
<td><em>Tsuga heterophylla</em> (Raf.) Sarg. *</td>
</tr>
<tr>
<td>Japanese hemlock</td>
<td><em>Tsuga sieboldi</em> Carr.</td>
</tr>
<tr>
<td>Lawson cypress</td>
<td><em>Chamaecyparis lawsoniana</em> Parl.</td>
</tr>
<tr>
<td>Alaska yellow cedar</td>
<td><em>Chamaecyparis nootkatensis</em> (D. Don) Spach. *</td>
</tr>
<tr>
<td>Western red cedar</td>
<td><em>Thuja plicata</em> Donn. *</td>
</tr>
<tr>
<td>Douglas fir</td>
<td><em>Pseudotsuga menziesii</em> (Mirb.) Franco *</td>
</tr>
<tr>
<td>Broadleaf maple</td>
<td><em>Acer macrophyllum</em> Pursh. *</td>
</tr>
<tr>
<td>Red alder</td>
<td><em>Alnus rubra</em> Bong. *</td>
</tr>
<tr>
<td>Red huckleberry</td>
<td><em>Vaccinium parvifolium</em> Smith *</td>
</tr>
<tr>
<td>Sword fern</td>
<td><em>Polystichum munitum</em> Presl. *</td>
</tr>
<tr>
<td>Salal</td>
<td><em>Gaultheria shallon</em> Pursh *</td>
</tr>
<tr>
<td>Salmonberry</td>
<td><em>Rubus spectabilis</em> Pursh *</td>
</tr>
</tbody>
</table>
c) Test for feeding deterrents: weevils given a choice between one disc with 1% Sitka spruce bark and one with a mixture of Sitka spruce bark (1%) and test plant powder (0.5%), of selected species with low stimulatory activity in the first 2 types of experiments.

Due to the amount of work involved, plants were tested in groups of 7-10 species, each including Sitka spruce. Single stimulus bioassays, in which comparisons between species were sought, were analyzed using analysis of variance and Newman-Keuls multiple range test of means. In double stimulus bioassays and in the test for feeding deterrents, each species was compared with Sitka spruce, within each treatment, by using a standard t-test for paired comparisons.

5.3. Results and discussion.

5.3.1. Single stimulus bioassays.

All conifers tested, with the exception of *Tsuga heterophylla* and *Chamaecyparis nootkatensis*, elicited a significantly higher feeding response in *P. strobi* than the response to plain agar (P<0.05) (Fig. 4). This result indicates that the feeding stimulatory chemicals are not restricted only to host trees, but
Fig. 4. Feeding response of *P. strobi* in single-stimulus bioassays, to agar discs containing 1% powdered bark or leaves of selected conifer and non-conifer plants. Responses expressed as percent of response to Sitka spruce. Feeding on species under horizontal bar was not significantly lower than on Sitka spruce (Newman-Keuls test).
A bar graph showing feeding response (%) for different species of conifers and non-conifers. The graph is divided into two main categories: Pinus and Picea. The species listed include:

**Pinus**:
- Pinus strobus
- Pinus rigida
- Pinus ponderosa
- Pinus bungeana
- Pinus thunbergii
- Pinus lambertiana
- Pinus taeda
- Pinus lambertiana

**Picea**:
- Picea glauca
- Picea engelmannii
- Picea pungens
- Picea rubens
- Picea glauca
- Picea abies
- Picea mariana
- Picea engelmannii

**Abies**:
- Abies alba
- Abies concolor
- Abies lasiocarpa

**Other Conifers**:
- Thuja plicata
- Thuja occidentalis
- Tilia americana
- Tilia tomentosa
- Populus tremuloides
- Populus nitida
- Acer saccharum
- Acer rubrum

**Non Conifers**:
- Vaccinium parvifolium
- Vaccinium angustifolium
- Gaultheria shallon
- Polytrichum commune

The x-axis represents different species as mentioned above, and the y-axis represents the feeding response percentage.
that they are present in a wide variety of species in different conifer genera. The fact that none of the non-conifers tested triggered a feeding response in *P. strobi* (Fig. 4), however, suggests that the distribution of the stimulatory chemicals may be restricted to conifers only. All species tested within the genus *Pinus* and all but one in *Picea* (*P. breweriana*) elicited feeding responses higher or not different from to that of Sitka spruce (Fig. 4). Differences among the three hosts of *P. strobi*, *Pinus strobus*, *Picea sitchensis* and *P. engelmannii*, were not statistically significant (*p > 0.05*). Conifers in genera other than *Pinus* and *Picea* elicited a response that was less than 50% of the response elicited by Sitka spruce, suggesting that feeding stimulants present in *Pinus* and *Picea* are quantitatively and/or qualitatively different from the chemicals present in other genera. These differences may partially account for the non-preference of *P. strobi* for species outside *Pinus* or *Picea*.

*Pseudotsuga menziesii, Abies lasiocarpa, Thuya plicata* and *Tsuga heterophylla* elicited less than 30% of the feeding activity triggered by Sitka spruce (Fig. 4). These species share the range with Sitka and Engelmann spruce and must, therefore, be actively discriminated against by searching *P. strobi*. 
5.3.2. Double stimulus bioassays.

In choice bioassays between Sitka spruce and several conifer spp., *P. strobi* did not discriminate between Sitka spruce, *Pinus banksiana*, *P. sylvestris*, *P. ponderosa*, *P. muricata*, *Picea engelmannii* and *P. glauca* (Fig. 5). These species are probably chemically similar to each other. All, except *Pinus ponderosa* and *P. muricata*, are attacked (at least occasionally) by the weevil (MacAloney 1932, Balyea and Sullivan 1956). Feeding on *Picea breweriana* was marginally lower (*P<0.11*) than the feeding on Sitka spruce. In all other cases there was a significant preference (*P<0.01*) for discs with Sitka spruce (Fig. 5). This result demonstrates that, despite the high feeding on *Pinus* and *Picea* in single stimulus experiments, most non-host species tested were sub-optimal for *P. strobi*. The high feeding rate on non-host species in a more forced feeding situation (Fig. 4), indicates that *P. strobi* can lower its acceptability threshold due to stress and feed on species that have inadequate feeding stimulatory characteristics.

Species sympatric with the weevil's western hosts (Table IX), were readily discriminated against in favor of Sitka spruce (Fig. 5), confirming that the levels and/or combination of feeding stimulants for *P. strobi* from Sitka spruce are optimum
Fig. 5. Feeding response of *P. strobii* when given a choice between agar discs with 1% Sitka spruce bark and discs with 1% bark from other conifers. Responses expressed as percent of response to Sitka spruce. Feeding on species under horizontal bars was not significantly different from feeding on Sitka spruce (t-test).
only in Sitka and Engelmann spruce. The striking preference for Sitka spruce over eastern white pine is different from VanderSar et al.'s (1977) findings that *P. strobi* reared on Sitka spruce did not differentiate cut twigs of Sitka spruce from those of eastern white pine. In view of the disparity of results, the feeding choice bioassays with these 2 species was repeated again, with identical results. These experiments suggest that the complement of feeding stimulants in eastern white pine is of inferior stimulatory quality for *P. strobi* reared on Sitka spruce.

The low feeding on *Picea pungens* and *P. omorika*, in choice feeding experiments, correlates well with the fact that these species are rarely injured by the weevil (MacAloney 1932). The development of hybrids between Sitka spruce and these two *Picea* species, in an attempt to develop less susceptible strains to *P. strobi*, deserves further investigation.

5.3.3. Test for feeding deterrents.

The test for feeding deterrents demonstrated that several conifer and non-conifer species contain chemicals that have a deterrent effect on feeding by *P. strobi* (Fig. 6). The significant (*P<0.01*) deterrenery by *Pseudotsuga menziesii* A.
Fig. 6. Feeding response of P. strobi when given a choice between agar discs with 1% Sitka spruce bark and discs with 1% Sitka spruce bark plus 0.5% bark or leaves of selected conifer and non-conifer plants. Responses expressed as percent of response to Sitka spruce. Feeding on agar discs containing Sitka spruce and any of the species under horizontal bars was not significantly different from feeding on agar discs with Sitka spruce alone (t-test).
lasiocarpa, T. plicata, T. heterophylla and, notably in choice bioassays, by the weevil's eastern host, Pinus strobus (Fig. 6), explains their low feeding stimulatory activity in the other 2 experiments (Figs. 4, 5).

Pinus monticola and P. contorta were readily discriminated against in choice feeding bioassays (Fig. 5), but they did not deter feeding significantly when added to Sitka spruce bark (Fig. 6). Therefore, non-preference for these species in choice feeding bioassays is apparently due to quantitative and/or qualitative deficiency in feeding stimulants rather than to the presence of feeding deterrents.

The non-preference of P. strobi for eastern white pine in choice feeding experiments (Fig. 5), and the finding that it contains chemical feeding deterrents for P. strobi (Fig. 6) contradicts the fact that eastern white pine is the preferred host for the weevil in eastern North America. The results, however, explain the observed non-preference of P. strobi for eastern white pine in British Columbia. At the UBC Research Forest where eastern white pine was collected, there are several clones of this species growing side by side with a Sitka spruce plantation. Although the weevil population in the area is high (up to 80% of the Sitka spruce trees attacked), eastern
white pine has never been attacked. This observation poses 2 hypotheses. Either British Columbia grown eastern white pine is different from white pine from the East, or the eastern and western populations of P. strobi are different. Since the eastern white pine clones sampled were brought directly from the East, it is unlikely that the first hypothesis is correct. Manna and Smith (1959), Smith (1962) and Smith and Sudgen (1969) demonstrated on morphological and serological basis, that P. strobi reared from eastern white pine, Sitka spruce and Engelmann spruce are races of P. strobi and not different species as Hopkins (1911) proposed. However, it is possible that in the long period of isolation, the populations in eastern white pine and Sitka spruce have differentiated to the point at which P. strobi reared from Sitka spruce does not recognize eastern white pine as a host. A definite answer could be obtained by testing white pine collected in the east with western weevils. The study of hybrid weevils would also be pertinent.

In conclusion, the finding that the feeding response of P. strobi to agar discs containing ground up bark or leaves of plants varied gradually from high acceptance to absolute non-preference indicates that the feeding response in this insect is determined by a complex variety of chemicals that are present in different species in mixtures that differ in quality and quantity. Acceptance of certain species by P. strobi is mediated by conifer-borne feeding stimulants. Rejection of some
non-host plants was shown to be due to the presence of feeding deterrents, and of others to inappropriate levels or blends of feeding stimulants. The selection of Sitka and Engelmann spruce over all other non-host species by B.C. weevils is probably determined ultimately by only a few chemicals that are specific to these tree species.
6.0 DIFFERENCES IN HOST SELECTION AND DEVELOPMENT OF PISSODES STROBI REARED ON HOST AND NON-HOST TREES

6.1 Introduction.

The finding that P. strobi can differentiate between Sitka spruce and most other conifer species poses the question of whether the preference is the consequence of a rigid, genetically based behavior, that is transmitted from generation to generation, or whether preference is caused by conditioning of the insect by exposure to that species. Food conditioning in insects, caused by their previous feeding experience has been reported previously. Hopkins host selection principle (Hopkins 1917, Craighead 1921, Graham 1963) stated that an insect species that breeds in 2 or more hosts will prefer to continue to breed in the host to which it has become adapted. This hypothesis, however, has not been supported for certain insect species (Richmond 1933, Wood 1963).

The study of the degree of pre-determination of the feeding behavior in insects is of practical importance because it indicates how easily an insect attacking one species can shift to attack another species or variety, broadening its host range. Modification of feeding preference is possible in some insects
species, and very difficult with others. Ali (1976) demonstrated that the preference of adult alfalfa lady beetle, *Subcoccinella 24-punctata* L., for alfalfa, *Medicago sativa* L., could be changed to prefer *Chenopodium album* L., if the larvae were raised in the latter species. The degree of resistance of the African armyworm moth, *Spodoptera exempta* Wlk. to feed on non-host cassava, *Manihot esculenta* Crantz., was dependent on whether the insect had previously fed on maize, *Zea mays* L., its normal host (Ilope 1975). The resistance to feed on cassava increased with the number of hours the insect had been in contact with maize.

Hanson (1976) investigated whether raising lepidopteran larvae on certain plants enhanced feeding preference for that plant in 2- or 3-choice situations. Responses ranged from strong induction of preference to no detectable effect. He concluded that, in general, more polyphagous species could be more easily induced to change their feeding preferences by previous exposure than less polyphagous species.

Experiments reported in Section 5 indicated that *P. strobi* did not differentiate between Sitka and Engelmann spruce and showed clear non-preference for lodgepole pine. The experiments in this section aimed to test whether the rejection
response for lodgepole pine indicated its unsuitability as a host, and whether the weevil's host selection preference could be modified by raising it in this species.

6.2. Methods and materials.

Two populations of *P. strobi*, collected on either Sitka or Engelmann spruce, were used in this investigation (hereafter referred to as SSW and ESW, respectively). Sitka spruce terminal leaders were collected from a heavily weeviled Sitka spruce plantation in the UBC Research Forest, Maple Ridge, B.C., and terminal leaders of Engelmann spruce and lodgepole pine were collected in the vicinity of Kootenay National Park, B.C. Both types of weevils were hand picked early in the spring of 1979 from the leaders of small trees. Most insects were caught as mating pairs, thus ensuring a supply of inseminated females. The weevils were kept until the start of the experiments in glass containers with fresh Sitka spruce or Engelmann spruce twigs, in the refrigerator (4±2 C).

Feeding preferences of collected adults were tested in a 75-replicate experiment using the agar dish bioassay. The 2 insect populations were given a choice between agar discs that contained 1% Sitka spruce bark and discs that contained the bark
of either Sitka spruce, Engelmann spruce or lodgepole pine. Only one insect was included in each replicate.

After the feeding preferences of the collected adults were tested, the weevils were forced to oviposit on leaders of Sitka spruce, Engelmann spruce and lodgepole pine. This was accomplished by caging 5 insects (2 females and 3 males) of either of the 2 populations, by means of a plastic screen sleeve, to excised leaders maintained with their basal portions inside water filled plastic bottles. Five screened leaders for each species of tree and weevil population were placed in separate cages in a growth chamber at 25°C and a 14h light : 10h dark photoperiod. The sleeves were kept on the leaders for one week. Progeny emerging from the leaders were collected daily and kept in the refrigerator on fresh twigs of the species from which they had emerged. The feeding preferences of the laboratory reared weevils were tested under the same conditions as for the parent populations.

The relative suitability of the 3 conifers was estimated by determining the number of oviposition punctures excavated by weevils of the 2 parent populations, the number of leaders colonized, the weight of the emergent weevils and the time to develop from egg to adult.
6.3. Results and discussion.

Adult SSW and ESW exhibited a clear preference for Sitka spruce when given a choice between this species and lodgepole pine (Table X), confirming the results in Fig. 5. No discrimination was shown between the two hosts.

Progeny of SSW and ESW raised from egg to adult in Sitka spruce, Engelmann spruce and lodgepole pine retained their preference for Sitka spruce over lodgepole pine (Table XI). The fact that P. strobi reared in lodgepole pine did not prefer the species in which it was reared, indicates that feeding preference in this insect may be a rather fixed, genetically determined behavior. Similar evidence was obtained by Graham and Satterlund (1956) who gave P. strobi a choice of either red pine or eastern white pine twigs. Regardless of whether they had emerged from red pine or eastern white pine, the weevils preferred to feed on eastern white pine. These results, however, must be taken with caution until further experimentation with additional tree species is done, and several insect generations are tested.

The degree of plasticity in the feeding behavior of P. strobi is an important consideration in a program leading to the development of trees resistant to P. strobi by manipulating the
Table X. Feeding response of 2 populations of *P. strobi* when given a choice between agar discs with 1% Sitka spruce and discs with 1% Engelmann spruce or lodgepole pine. N = 15 replicates, 1 weevil/replicate.

<table>
<thead>
<tr>
<th>Population Test species</th>
<th>Mean number of feeding punctures</th>
<th>t-test probabilitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agar discs with Sitka spruce bark</td>
<td>Agar discs with test species bark</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>11.5</td>
<td>12.9</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>6.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Lodgepole weevil pine</td>
<td>10.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>13.4</td>
<td>10.3</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>8.3</td>
<td>20.0</td>
</tr>
<tr>
<td>Lodgepole weevil pine</td>
<td>32.0</td>
<td>11.2</td>
</tr>
</tbody>
</table>

a t-test significance level on difference between feeding on agar discs with Sitka spruce and feeding on discs with test species indicated by: **= P<0.01, *= P<0.05, NS= P>0.05.
Table XI. Feeding response of progeny of 2 populations of *P. strobi* reared on Sitka spruce, Engelmann spruce and lodgepole pine. Weevils were given a choice between agar discs containing 1% Sitka spruce and discs with 1% lodgepole pine. *N=15* replicates, 1 weevil/replicate.

<table>
<thead>
<tr>
<th>Population</th>
<th>Species in which weevils reared</th>
<th>Feeding on disc with</th>
<th>Feeding on disc with</th>
<th>t-test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sitka spruce bark</td>
<td>lodgepole pine bark</td>
<td></td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>Sitka spruce</td>
<td>12.9</td>
<td>6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>Engelmann spruce</td>
<td>21.3</td>
<td>5.1</td>
<td>**</td>
</tr>
<tr>
<td>weevil</td>
<td>Lodgepole pine</td>
<td>11.8</td>
<td>4.1</td>
<td>*</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>Sitka spruce</td>
<td>27.7</td>
<td>7.4</td>
<td>**</td>
</tr>
<tr>
<td>spruce</td>
<td>Engelmann spruce</td>
<td>23.8</td>
<td>0.9</td>
<td>**</td>
</tr>
<tr>
<td>weevil</td>
<td>Lodgepole pine</td>
<td>27.9</td>
<td>3.0</td>
<td>**</td>
</tr>
</tbody>
</table>

* t-test significance level on difference between feeding on agar discs with Sitka spruce and feeding on discs with test species indicated by: **= P<0.01, *= P<0.05, NS= P>0.05.

b t-test probability level: P<0.06.
array of feeding stimulants and deterrents. Food preference that is easily changed is less desirable since the insect might readily adapt to feed on a newly developed variety. On the contrary, changes in fixed preference would require possibly several generations of adjustment and adaptation of the insect's gene pool.

Both SSW and ESW laid eggs and produced adults on all 3 conifers (Table XII). However, the number of oviposition punctures by the 2 weevil populations was highest in the species from which they were collected. This result suggests that the oviposition stimuli for each of the 2 weevil populations are not exactly the same. The oviposition stimulus in Sitka spruce is more adequate for SSW than for ESW, whereas the stimulus present in Engelmann spruce is optimum for ESW. This difference in oviposition stimuli between Sitka and Engelmann spruce contrasted with the absence of a difference in feeding stimulation between the 2 hosts indicates that the stimuli triggering oviposition and feeding are different.

Although both populations laid eggs and produced adults in non-host lodgepole pine, the number of oviposition punctures was, for both populations, lower than in their normal hosts.
Table XII. Infestation characteristics of 2 populations of *P. strobi* reared experimentally in leaders of 3 conifer species.\(^a\)

<table>
<thead>
<tr>
<th>Parent population</th>
<th>Species in which weevils reared</th>
<th>Total number of oviposition punctures on 5 leaders</th>
<th>No. of leaders colonized (5=maximum)</th>
<th>Total number of weevils produced on 5 leaders</th>
<th>Mean b weight of progeny (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitka spruce</td>
<td>Sitka spruce</td>
<td>503 a</td>
<td>3</td>
<td>110 a</td>
<td>9.91 a</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>Engelmann spruce</td>
<td>212 b</td>
<td>3</td>
<td>50 a</td>
<td>8.54 b</td>
</tr>
<tr>
<td>Lodgepole pine</td>
<td>Lodgepole pine</td>
<td>183 c</td>
<td>2</td>
<td>27 a</td>
<td>7.58 b</td>
</tr>
<tr>
<td>Sitka spruce</td>
<td>Engelmann spruce</td>
<td>107 d</td>
<td>3</td>
<td>29 a</td>
<td>6.42 b</td>
</tr>
<tr>
<td>Engelmann spruce</td>
<td>Lodgepole pine</td>
<td>237 b</td>
<td>4</td>
<td>101 a</td>
<td>9.08 a</td>
</tr>
<tr>
<td>Lodgepole pine</td>
<td>Lodgepole pine</td>
<td>80 e</td>
<td>4</td>
<td>57 a</td>
<td>8.93 a</td>
</tr>
</tbody>
</table>

\(^a\)Figures within each column, followed by some letter are not significantly different, Newman Keuls test, \(P < 0.05\).  

\(^b\)Each mean calculated from 20 weevils.
These results should encourage further research to elucidate the exact nature of the oviposition stimuli, as they may prove to be chemically mediated, and a source of resistance in species or individual plants that lack the adequate stimuli.

Sitka spruce, Engelmann spruce and lodgepole pine apparently differ in the nutrient quality of the phloem, as reflected by the weight of emergent adults. SSW reared in Sitka spruce were significantly (Newman-Keuls test, P<0.05) heavier than the adults obtained from Engelmann spruce or lodgepole pine (Table XII), indicating the superiority of Sitka spruce phloem for SSW. Similarly, ESW obtained their best weight in Engelmann spruce, although they were not significantly heavier than ESW from lodgepole pine. ESW reared in Sitka spruce, however, produced very small and light weevils, indicating that this species is not very suitable for ESW.

The difference in suitability of lodgepole pine phloem for the 2 weevil populations coincides with differences in the degree to which they accepted lodgepole pine for oviposition. SSW colonized only 2 of 5 lodgepole pine leaders, whereas ESW colonized 4. Also, the length of time from the start of the experiment to peak adult emergence (reflecting developmental
time from egg to adult) of SSW was the longest in lodgepole pine (Fig. 7). For ESW, on the contrary, the developmental time was the same on all 3 host species (Fig. 8).

The observations on the development of P. strobi on host and non-host trees permit the conclusion that the 2 populations differ in their nutritional requirements. Each population is more adapted to the natural host from which it was collected. The finding that the SSW populations did poorly in lodgepole pine, coupled to a "fixed" non-preference for this species, may explain, in part, why SSW seems unable to establish populations in lodgepole pine, despite the fact that this species and Sitka spruce are sympatric in coastal B.C. Genetic manipulation of the nutritional quality of the host (Maxwell 1977) seems to be an interesting possibility for developing varieties of Sitka spruce that are less suitable to P. strobi.

The poor development and rate of colonization of ESW in Sitka spruce strengthen the suggestion (VanderSar et al. 1977) that SSW and ESW are races of P. strobi with a high degree of divergence.
Figs. 7-8. Daily emergence pattern of the progeny of 2 populations of *P. strobi*, collected from Sitka spruce (Fig. 7) and Engelmann spruce (Fig. 8) when reared on Sitka spruce, Engelmann spruce, or lodgepole pine.
Emergence of *P. strobi*

- •• reared on Sitka spruce
- ▲△ reared on Engelmann spruce
- ○○ reared on lodgepole pine
7.0. TEST FOR PRESENCE OF FEEDING DETERBENTS IN UNATTACKED
SITKA SPRUCE TREES.

7.1. Introduction.

Varietal resistance associated with the presence in a plant of chemicals with feeding deterrent action has been reported for some species of vertebrates (Radwan and Ellis 1975, Radwan 1978) and several species of insects, e.g. Hanover (1975), and references therein, Robinson et al. (1978), Russell et al. (1978). The action of feeding deterrents fits Painter's (1951) non-preference type of resistance in plants. Non-preference, according to Painter, "is present when the plant possesses a character or group of characters that cause the insect to move away from the use of that particular plant for oviposition or food".

Most published reports on the resistance to P. strobi came from observations on the eastern host, eastern white pine. The majority deal with non-preference resistance associated with morphological characteristics of the terminal leaders. P. strobi prefers to attack eastern white pines with larger leaders (MacAloney 1930) and thicker bark (Kriebel 1954, Sullivan 1961, Stroh and Gerhold 1965). Stroh and Gerhold (1965) determined
that eastern white pine with shallow cortical resin ducts in the leader were less attacked by P. strobi than leaders with deeper ducts. The reason underlying this resistance was hypothesized to be that feeding P. strobi avoids rupturing resin ducts, and thus cannot excavate feeding and oviposition cavities in the bark.

Resistance of Sitka spruce to P. strobi has not been studied in detail. Silver (1968) mentions the existence of some apparently weevil resistant trees in a plantation established in 1930, in the B.C. Forest Service Green Timbers Nursery, Surrey, B.C.. That plantation has been under heavy weevil attack since 1937, when the trees were about 2m tall. I visited the plantation in the spring of 1979 and found that most weeviled trees had been suppressed by competing vegetation and had died. There are, however, 4 trees (marked 1-4) which reached co-dominant status and that appear to have suffered little or no weevil infestation in the past. Trees 1, 2 and 3 were vegetatively reproduced by the B.C. Forest Service and the clones are presently kept at its North Road Laboratory, Victoria, B.C.

The effectiveness of the agar disc bioassay (Section 3), encouraged its use for detecting differences in feeding stimulatory and deterrent power of bark from attacked and unattacked Sitka spruce trees in nature and in cloned trees 1-3.
7.2. Methods and materials.

The number of attacks per tree was recorded in 2 Sitka spruce plantations (Nos. 1 and 2, established in 1972 and 1967, respectively) at the UBC Research Forest, Maple Ridge, B.C. All undamaged trees were tagged and a sample of lateral branch bark from the previous year's growth was collected. Plantation 1 had 30 unattacked trees (Table XIII), but only 10 were studied. Plantation 2 contained only 9 unattacked trees, all of which were studied. Each unattacked tree was tested in a double-stimulus experiment, using the agar disc bioassay. Three weevils in each of 10 replicates per tree, were given a choice between agar discs containing 1% unattacked, "resistant" tree bark and discs containing 1% pooled, "susceptible" tree bark from 5 trees that had been attacked 5 times or more. Leader bark, although more preferable, was not collected to avoid damaging the trees.

The clones from the resistant trees at Green Timbers, were tested for presence of feeding deterents, in the same manner as above. A bark sample was taken from the previous year's lateral branch growth from the only surviving clone from tree No. 1 and from 5 clones of tree Nos. 2 and 3 (10 clones of each tree are alive). Weevils were given a choice between agar discs containing susceptible bark and discs with resistant clone bark.
Table XIII. Number of weevilings per tree and proportion of trees in each weevil class in 2 plantations at the University of British Columbia Research Forest, Maple Ridge, B.C.

<table>
<thead>
<tr>
<th>No. of weevilings per tree</th>
<th>Plantation 1</th>
<th></th>
<th>Plantation 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Proportion of total No. of trees (%)</td>
<td>Frequency</td>
<td>Proportion of total No. of trees (%)</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>24.6</td>
<td>9</td>
<td>9.2</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>39.3</td>
<td>19</td>
<td>19.4</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>15.6</td>
<td>22</td>
<td>22.4</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>12.3</td>
<td>24</td>
<td>24.5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4.1</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3.2</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.8</td>
<td>4</td>
<td>4.1</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>100.0</td>
<td>98</td>
<td>100.0</td>
</tr>
</tbody>
</table>
7.3. Results and discussion.

The feeding response of *P. strobii* to bark of 10 of the 30 unattacked trees in the less severely attacked plantation 1 (Table XIII) was not significantly different from the response to susceptible tree bark (ANOVA) (Table XIV). Unattacked trees in plantation 2, however, triggered a significantly lower feeding response than on susceptible tree bark (ANOVA, F=8.79, P<0.01), indicating the absence of an optimal stimulus and/or the presence of feeding deterrents in the unattacked trees (Table XIV). However, differences in feeding between attacked and unattacked trees were small. The response to the 3 resistant clones from Green Timbers was not significantly different from the response to susceptible tree bark (Table XV).

The absence of a significant feeding deterrent response in plantation 1 and the 3 clones, and the small deterrenty detected in plantation 2 could have several possible explanations.
Table XIV. Feeding response of *P. strobi* when given a choice between agar discs with 1% pooled bark from susceptible Sitka spruce trees and discs with 1% bark from unattacked trees. Two plantations tested, 10 replicates/tree, 3 insects/replicate.

<table>
<thead>
<tr>
<th>Plantation 1</th>
<th>Unattacked tree</th>
<th>Mean No. of feeding punctures</th>
<th>Disc with 1% susceptible bark</th>
<th>Disc with 1% unattacked bark</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Disc with 1%</td>
<td>Disc with 1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>susceptible bark</td>
<td>unattacked bark</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.7</td>
<td>23.5</td>
<td>+ 5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>26.8</td>
<td>21.0</td>
<td>- 5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23.4</td>
<td>33.6</td>
<td>+10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22.6</td>
<td>25.1</td>
<td>+ 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.2</td>
<td>32.1</td>
<td>+16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21.7</td>
<td>24.0</td>
<td>+ 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12.8</td>
<td>8.9</td>
<td>- 3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>26.9</td>
<td>34.7</td>
<td>+ 7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>22.5</td>
<td>25.7</td>
<td>+ 3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>27.9</td>
<td>19.1</td>
<td>- 8.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plantation 2</th>
<th>Unattacked tree</th>
<th>Mean No. of feeding punctures</th>
<th>Disc with 1% susceptible bark</th>
<th>Disc with 1% unattacked bark</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Disc with 1%</td>
<td>Disc with 1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>susceptible bark</td>
<td>unattacked bark</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.3</td>
<td>14.7</td>
<td>- 5.6 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16.8</td>
<td>9.9</td>
<td>- 6.9 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.6</td>
<td>14.1</td>
<td>- 1.3 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21.6</td>
<td>15.8</td>
<td>- 5.8 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>19.8</td>
<td>6.6</td>
<td>-13.2 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13.2</td>
<td>15.7</td>
<td>+ 2.5 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13.5</td>
<td>7.7</td>
<td>- 5.8 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>22.1</td>
<td>18.1</td>
<td>- 4.0 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>15.6</td>
<td>9.8</td>
<td>- 5.8 *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pooled bark from 5 trees that had been attacked 5 times or more.

* P value for type of bark = 1.35, non-significant (ANOVA).

* P value for type of bark = 8.79, significant at P<0.01 (ANCOVA).

Probability level for difference between susceptible and unattacked bark indicated by: **=P<0.01, *=P<0.05, NS= P>0.05.
Table XV. Feeding response of *P. strobi* when given a choice between agar discs with 1% pooled bark from susceptible Sitka spruce trees and discs with 1% bark from "resistant" clones from the B.C. Forest Service Green Timbers Nursery, Surrey, B.C. N=10 replicates/tree, 3 insects/replicate.

<table>
<thead>
<tr>
<th>Unattacked clone No.</th>
<th>Mean No. of feeding punctures</th>
<th>Disc with 1% susceptible tree bark</th>
<th>Disc with 1% resistant clone bark</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.6</td>
<td>15.1</td>
<td>- 2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.4</td>
<td>20.6</td>
<td>+ 9.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.3</td>
<td>16.8</td>
<td>+ 4.5</td>
<td></td>
</tr>
</tbody>
</table>

*Differences in feeding on susceptible and resistant bark were not significant, ANOVA.*
A) It is possible that feeding deterrents are restricted to the leader, and therefore, are not detected by the bioassay of lateral bark.

B) Unattacked trees may not contain feeding deterrents. The reason for the non-preference for these trees may be the absence of an adequate blend of feeding stimulants, perhaps coupled to other forms of resistance, e.g. morphological. It is possible that the responses observed in plantation 2 may be due to a superior stimulatory activity of the susceptible bark rather than to the presence of feeding deterrents in unattacked trees.

C) Trees in plantation 1 are not different from susceptible trees and are undamaged simply by chance. This alternative is possible, since attack intensity in this plantation was lower than in plantation 2.

D) The dried bark preparation method for the bioassay may have eliminated volatiles which have feeding deterrent activity, alone or in combination with non-volatile compounds.

Investigations on the chemical characteristics of the clones from Green Timbers being carried out at the Chemistry Dept. of Simon Fraser University, indicate that the "resistant" clones
have no different resin acid content in the bark with respect to susceptible trees. Susceptible trees have no detectable amounts of the monoterpenes limonene and a higher content of an unidentified monoterpenes, while the "resistant" clones have up to 13% limonene, a compound that acts as feeding stimulant at low concentrations and as potent feeding deterrent above a certain threshold concentration (Fig. 16).
8. ISOLATION OF PISSODES STROBI FEEDING STIMULANTS

8.1. Role of volatile and non-volatile components of Sitka spruce bark as feeding stimulants.

8.1.1. Introduction

Selection of Sitka spruce by *Pissodes strobi* is apparently mediated by vision (VanderSar and Borden 1977a), and at close range by feeding stimulants in the bark (VanderSar and Borden 1977b). Previous research (Section 3) indicated that the weevils exhibited a highly sensitive, concentration-dependent response to non-volatile chemicals present in Sitka spruce bark. The non-volatile mixture appears to be quantitatively and qualitatively different among various conifer species (Section 5). The role of host volatiles in the feeding behavior of *P. strobi* is poorly understood. Anderson and Fisher (1956, 1960), claimed that ground green bark from weevil-resistant *Picea* species emitted volatiles that had a repellent effect on *P. strobi*. However, the weevils were also repelled by the odors of their eastern host, *P. strobus*.

In a laboratory olfactometer, 5% methanolic solutions of the monoterpens *α*-pinene and myrcene were repellent to *P. strobi*.
whereas limonene was attractive (Carlson 1971). Since the limonene content in susceptible Sitka spruce trees appeared to increase at the time of weevil dispersion, Carlson (1971) postulated that this monoterpene was primarily responsible for host selection by *P. strobi*. Soles (1970), however, found very weak correlations between incidence of weevil attack and the monoterpene composition of the host trees.

This section reports the results of studies designed to examine the feeding stimulatory activity on *P. strobi* adults of various types of extracts containing volatile and non-volatile chemicals from the bark and needles of Sitka spruce.

8.1.2. Methods and materials.

8.1.2.1. Bicassay Procedures.

Feeding stimulation and inhibition were investigated using the agar disc bioassay (Section 3). Candidate chemicals for feeding stimulation were applied to the paper-covered surface, or were mixed into one of 2 paired agar discs. The second disc in each dish was the solvent or blank control. Feeding inhibition was tested by applying chemicals to the surface of agar discs that contained 1% Sitka spruce bark (ground and
dried), a substrate upon which the weevils feed readily. Experiments were run for 24h, using 2 or 3 insects per replicate and 10-15 replicates per treatment.

8.1.2.2. Bioassay of steam-distilled volatiles of Sitka spruce bark.

Finely ground, dry Sitka spruce lateral branch bark (25g in 500ml of water) was steam distilled, and 2 fractions of distillate (112mg and 186mg, respectively) were collected. The fractions were extracted with ether (4 times with 50 ml). The ethereal extracts of each fraction were combined, washed with saturated salt solution, dried (MgSO₄), filtered, and concentrated to 25ml by distillation. The aqueous digest remaining in the still pot was separated from the bark particles by centrifugation. The 3 fractions obtained were bioassayed for feeding stimulatory activity. The equivalent of 12.5mg of bark of each fraction (12.5mg = the weight of bark in a 1% agar-bark disc) was applied to the agar discs.
8.1.2.3. Extraction of Sitka spruce bark with hot water, pentane and ether.

Ground, dried Sitka spruce bark from lateral branches (20g), was boiled for 10 min. in 200 ml of distilled water. After cooling, the mixture was centrifuged for 10 min. at 2000 rpm. The supernatant was removed, and the procedure was repeated 3 more times with the residual bark. The combined supernatants were filtered through Celite to remove particulate matter, and the clear solution was drawn with suction 3 times through Porapak-Q (ca. 10.5g). The Porapak-Q was washed several times with water and the excess water was removed with nitrogen before extraction with 250 ml of 80/20 ether-methanol solution. This extract was concentrated by rotary evaporation, dried and weighed. The residual bark from the water extraction was dried, weighed and extracted in a Soxlet extractor with pentane for 12h and then with ether for 24h.

The various extracts and residues bioassayed for feeding stimulant activity were: 1) unextracted bark, 2) bark after extraction with boiling water, 3) bark after extraction with boiling water, pentane and ether, 4) combined water extracts from boiling water extraction, 5) water extract, Celite filtered, 6) water extract after filtration through Celite and percolation.
through Porapak-Q. 7) Porapak-Q extract (tested at 2 concentrations), 8) pentane extract of water-extracted bark, and 9) ether extract of bark after extraction with water and pentane.

Solid samples were dried, ground, and then mixed at 1% into the agar of the test discs. Aqueous fractions were mixed with distilled water and used to prepare the agar of the corresponding treated discs. The amount of aqueous extract used was adjusted to be the equivalent of 1% bark. In both cases, the control consisted of agar discs prepared with distilled water. Fractions dissolved in volatile solvents, were applied to the paper surface of one of the discs in the dishes; the other disc served as a solvent control. One hundred µg of the pentane and ether extracts, and 10 and 100µg of the Porapak-Q extract were tested.

In the bioassays in which the chemicals were applied to the paper surface of the agar disc, a standard amount of 100µg was selected and served as a basis for further exploration. For comparison, note that the threshold amount of dry bark dispersed in agar that elicits a feeding response by P. strobi is less than 100µg (calculated from dose response equations, page 17).
8.1.2.4. Extraction and bioassay of cuticular constituents of bark and needles.

Five excised, undamaged Sitka spruce terminal leaders (140g) were dipped first in chloroform and then in pentane. Care was taken to avoid contamination of the solvents with materials from the cut end of the twigs. After evaporation of the solvents, the semi-solid extracts weighed 324 and 44mg for chloroform and pentane extracts, respectively. The extracts were dissolved back into their respective solvents and were tested by applying 12.5μl of solution (100μg of extract) to one of 2 paired agar discs.

8.1.2.5. Bioassay of volatiles of bark and needles trapped in Porapak-Q.

Strips of fresh bark (130g) and needles (68g) from lateral branches were placed in separate glass chambers, and for a one week aeration period, the volatiles were captured in and recovered from Porapak Q, as described by Vernon et al. (1977). Pentane extracts containing the host volatiles were bioassayed with groups of 10-15 insects for attractancy in 2 types of olfactometers, one developed for the boll weevil, Anthonomus grandis Boheman (Hardee et al. 1967), and the other for
ambrosia beetles (Borden and Stokkink 1973). The attractiveness of fresh Sitka spruce foliage was also tested in the boll weevil olfactometer. Feeding stimulatory activity of the extracts was tested in the standard paired agar disc bioassay.

8.1.2.6. Test of Sitka spruce needle oil.

Sitka spruce needle oil was obtained by steam distillation. It was tested for feeding stimulation and deterrenncy by applying different amounts (3.5, 17.5, 35.0, and 70.0 μg) of the oil in pentane to the paper surface of plain agar discs.

8.1.2.7. Test of Sitka spruce monoterpenes

Eight of the most common monoterpenes found in Sitka spruce were tested for feeding stimulation and deterrenncy. The chemicals were obtained from commercial suppliers and, with the exception of camphor, were redistilled before use. They were from 87 to 99% pure. In the feeding stimulation experiments, 1 and 100μg stimuli were tested, whereas for feeding inhibition, the amounts tested were 2, 20, 200, and 500μg.

In a separate experiment, a detailed study of the reaction of P. strobi to limonene was made. Agar discs containing Sitka
spruce bark were treated with pentane solutions of limonene at 8
levels ranging from 0.021 to 420µg.

8.1.3. Results and discussion.

The response of P. strobi to the fractions obtained by
steam distillation clearly indicated that the volatile chemicals
extracted in the distillate did not elicit a feeding response,
whereas the non-volatile fraction remaining in the distillation
flask was very active (Table XVI). This result suggests that
non-volatile chemicals are responsible for feeding stimulation
in this insect. The response of males and females was similar.

Activity appeared in all fractions extracted with water,
ether and pentane (Table XVII), indicating that several
chemicals with different properties are involved in feeding
stimulation. The amount of feeding by the test insects on the
discs that contained unextracted bark was significantly lower
than the response to the discs containing the water extract.
The better distribution of the water extract than the bark
granules in the agar may account for this difference. An
alternative explanation is that a feeding inhibitor may have
been destroyed or removed during the extraction procedure. The
chemicals extracted by the Porapak-Q appeared to have a weaker
Table XVI. Feeding response of *P. strobi* to steam distillation products of Sitka spruce bark. N= 15 replicates, 2 weevils/replicate.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>disc</td>
<td>disc</td>
</tr>
<tr>
<td>Steam distillate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fraction 1</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>Steam distillate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fraction 2</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Aqueous digest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in still pot</td>
<td>0.07</td>
<td>17.66 **</td>
</tr>
</tbody>
</table>

* t-test significance level on difference between control and treated indicated by **=P< 0.01
Table XVII. Feeding response of _P. strobi_ to chemical fractions obtained from Sitka spruce bark. N = 10 replicates, 3 insects/replicate.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Control</th>
<th>Treated</th>
<th>t-test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark before extraction</td>
<td>0.8</td>
<td>24.7 a</td>
<td>**</td>
</tr>
<tr>
<td>Bark after extraction with boiling water</td>
<td>0.7</td>
<td>35.2 ab</td>
<td>**</td>
</tr>
<tr>
<td>Bark after extraction with boiling water, pentane and ether</td>
<td>0.9</td>
<td>35.7 ab</td>
<td>**</td>
</tr>
<tr>
<td>Water extract from bark</td>
<td>0.1</td>
<td>66.4 b</td>
<td>**</td>
</tr>
<tr>
<td>Water extract after Celite filtration</td>
<td>0.1</td>
<td>48.4 ab</td>
<td>**</td>
</tr>
<tr>
<td>Water extract after Celite filtration and percolation through Porapak-Q</td>
<td>0.5</td>
<td>50.6 b</td>
<td>**</td>
</tr>
<tr>
<td>Porapak-Q extract (20 μg)</td>
<td>3.5</td>
<td>6.6 c</td>
<td>NS</td>
</tr>
<tr>
<td>Porapak-Q extract (100 μg)</td>
<td>0.9</td>
<td>15.5 a</td>
<td>**</td>
</tr>
<tr>
<td>Pentane extract of boiling water-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extracted bark</td>
<td>2.8</td>
<td>40.6 a</td>
<td>**</td>
</tr>
<tr>
<td>Ether extract of bark after extraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with pentane and boiling water</td>
<td>0.6</td>
<td>41.8 a</td>
<td>**</td>
</tr>
</tbody>
</table>

*a* Means followed by the same letter are not significantly different, *Newman-Keuls test, P*<0.05.

*b* t-test significance level on difference between control and treated indicated by: **= P<0.01, *= P<0.05, and NS= P>0.05.
feeding stimulatory activity, suggesting that this material was a selective separating media and, therefore, the captured mixture did not contain (quantitatively and/or qualitatively) the complete array of feeding stimulants present in the unextracted bark. The fact that the activity of the bark remained high even after extraction with pentane and ether, indicates that the stimulatory chemicals were not completely extracted.

The cuticular components extracted with chloroform and pentane, from the surface of Sitka spruce leaders, had significant feeding stimulatory activity (Table XVIII). It was not possible to extract the leader bark without either including the needles or exposing the internal tissues by needle removal. Thus, the question of whether the active chemicals came from the surface of the leader bark or from the needles is not resolved. However, preliminary studies using Thin Layer Chromatography of needle extracts indicate that the active chemicals are apparently present in the entire bark-needle surface of the leaders. The presence of the chemicals on the needles, on which the weevils do not feed, corroborates the slight feeding stimulatory activity of the needles (Table VIII) and the fact that they also produce a feeding deterrent (Fig. 3) which probably acts to restrict feeding to the leader bark surface.
Table XVIII. Feeding response of *P. strobi* to 2 extracts obtained by dipping (1 min) entire Sitka spruce leaders in chloroform and pentane. *N*=75 replicates, 3 weevils /replicate. 100μg of each extract applied.

<table>
<thead>
<tr>
<th>Stimulus description</th>
<th>Control disc</th>
<th>Treated disc^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>6.2</td>
<td>11.4 *</td>
</tr>
<tr>
<td>Pentane extract</td>
<td>2.6</td>
<td>9.7 *</td>
</tr>
</tbody>
</table>

^a* t-test significance level on difference between control and treated indicated by: * = P<0.05.
The Porapak-Q-trapped volatiles of bark and needles did not elicit a positive reaction in either of the 2 olfactometers. The weevils distributed randomly between the air stream containing the host volatiles and the one containing the solvent. Moreover, when fresh Sitka spruce foliage was tested in the olfactometer, it was not attractive. In addition, neither the Porapak-Q-trapped volatiles nor the Sitka spruce needle oil induced the weevils to feed. In fact, the needle oil was significantly deterrent (t-test, P<0.01) to males at high concentrations (70µg), reducing the feeding activity on treated spruce bark-agar discs by 50%.

None of the monoterpenes induced P. strobi to feed when they were applied to plain agar (Table XIX). However, when tested on the agar-bark mixture, the monoterpenes elicited different responses (Figs. 9-16). α-Pinene, β-pinene and β-myrcene (Figs. 9-11) caused the weevils to feed significantly more on the treated discs (Wilcoxon matched-pairs signed rank test, P<0.001 for α and β-pinene and P<0.06 for β-myrcene). This response indicates an apparent synergistic effect between these volatiles and the non-volatiles present in the agar-bark mixture. Feeding on the treated discs, however, decreased as the concentration of the chemicals increased (Figs. 9-11), suggesting that an inhibitory concentration could be reached.
Table XIX. Feeding response of *P. strobi* to plain agar discs treated on the surface with different monoterpenes. *N* = 10 replicates for each concentration and chemical, 3 insects per replicate.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>1pg stimulus concentration control disc</th>
<th>100pg stimulus concentration control disc</th>
<th>1pg stimulus concentration treated disc</th>
<th>100pg stimulus concentration treated disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>2.7</td>
<td>4.5</td>
<td>4.3</td>
<td>2.3</td>
</tr>
<tr>
<td>β-pinene</td>
<td>6.3</td>
<td>2.2</td>
<td>8.7</td>
<td>3.5</td>
</tr>
<tr>
<td>β-myrcene</td>
<td>6.2</td>
<td>2.4</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>limonene</td>
<td>0.6</td>
<td>0.5</td>
<td>3.0</td>
<td>1.7</td>
</tr>
<tr>
<td>β-phellandrene</td>
<td>1.0</td>
<td>1.9</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>(+) camphor</td>
<td>5.7</td>
<td>5.6</td>
<td>9.4</td>
<td>4.0</td>
</tr>
<tr>
<td>(+) camphor</td>
<td>5.5</td>
<td>4.5</td>
<td>3.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Piperitone</td>
<td>4.6</td>
<td>1.1</td>
<td>2.7</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*All differences between treated and control were statistically non-significant, t-test.*
Figs. 3-15: Feeding response of *P. strobi* to agar-bark discs treated (solid line) and untreated (dotted line) with various concentrations of Sitka spruce monoterpenes.
\( \alpha - \text{PINENE} \)

\( \beta - \text{PINENE} \)

\( \beta - \text{MYRCENE} \)

\( \beta - \text{PHELANDRENE} \)

\( (\pm) - \text{CAMPHOR} \)
B-Pellandrene (Fig. 12) and (1)-camphor (Fig. 13) had virtually no effect, whereas piperitone (Fig. 14) had a potent inhibitory effect. The amount fed by P. strobi at the higher concentrations of piperitone was extremely low.

(1)-Camphor (Fig. 15) and limonene (Fig. 16) proved to be inhibitory to feeding at high concentrations (Wilcoxon test,  \( P<0.05 \) for (1)-camphor and  \( P<0.01 \) for limonene) whereas they appeared to be stimulatory at low concentrations (\( P<0.28 \) for (1)-camphor and \( P<0.01 \) for limonene). The transition concentrations were 20\( \mu \)g and 1\( \mu \)g, respectively. In each case (Figs. 15-16), the straight lines fitted to the mean feeding responses on the control and treated discs were significantly different (ANOVA on regression analysis using dummy variables, \( P<0.01 \)).

The feeding behavior of P. strobi on Sitka spruce appears to be determined primarily by non-volatile chemicals present on the surface of the bark and needles as well as within the phloem of the host tree (Tables XVI-XVIII). They are involved not only as incitants that elicit a feeding response, but also as stimulants that maintain continuous feeding. The active chemicals appear to be several, some water soluble, and others soluble in pentane or ether.
Fig. 16. Feeding response of *P. strobi* to agar-bark discs treated (solid line) and untreated (dotted line) with various concentrations of limonene.
The importance of resin volatiles in the host selection process by conifer feeding insects has been long recognized (Hopkins 1902). Some monoterpenes inhibit feeding whereas others act as attractants (Stark 1965, Rudinsky 1966). Although the captured volatile chemicals from Sitka spruce bark and needles did not attract walking *P. strobi* and did not induce feeding, some monoterpenes appeared to complement or deter the stimulatory action of non-volatile chemicals in the bark (Figs. 9-16). My studies suggest that, like (+)-camphor and limonene (Figs. 15-16), other monoterpenes could act at low concentrations as synergists of non-volatile feeding stimulants, and that at higher concentrations they may cause feeding inhibition, or, as shown by Smith (1966), they may be toxic. Further investigations, however, should include the study of plant volatiles other than monoterpenes (Wood 1972).

The concentration-dependent effect of plant volatiles should be considered in behavioral experiments. When tested at only one concentration, certain monoterpenes might induce responses quite different from those at higher or lower stimulus concentrations. In addition, olfactometric studies in which the length of time taken by the test insects to cross an air-stream containing host volatiles is used as a measure of repellency (Anderson and Fisher 1956, 1960; Bordasch and Berryman 1977), may fail to distinguish between repellency and arrestment (a positive response). My studies reaffirm that experiments on the feeding
inhibitory or stimulatory action of chemicals should be conducted over a wide range of concentrations.

The choice feeding bioassay allows the insects to discriminate effectively between treated and control discs, favouring the most suitable substrate-chemical combination. The weak correlations found by Soles (1970) between several indices of *P. strobi* attack and the monoterpenes composition of the host trees, are probably a result of the forced attack technique used to evaluate resistance. A caged insect confined to a suboptimal host may lower its acceptability threshold, and feed on relatively unsuitable substrates. This point was clearly demonstrated in Figs. 4, 5. *P. strobi* sustained high feeding levels on non-host tree species that were not preferred in subsequent choice feeding experiments.

Carlson (1971) found that the concentration of limonene (the only monoterpenes that was attractive to *P. strobi* in the laboratory) increased in fast growing trees at the time when the weevils were dispersing to find new hosts. He interpreted this observation by postulating that host selection occurred in response to limonene. However, my observations (Fig. 16) indicate that a rise in limonene content in the bark, above a certain threshold, would have a deterrent effect on the feeding
of P. strobi. Therefore, it could be equally hypothesized that Sitka spruce "turns on" its resistance mechanism at the time of weevil dispersal.

Piperitone, which is primarily present in the needles (Hrutfiord et al. 1974), caused a drastic reduction of feeding as the concentration increased (Fig. 14), and may explain the deterreny shown in Fig. 3. This chemical could play an important role in restricting feeding to the bark, as well as in the resistance mechanism of trees with sufficiently high concentrations. The identification of such chemicals and the understanding of their effect on P. strobi may provide the basis for the development of a sound screening program for trees resistant to this insect pest.

The final acceptance or rejection of a tree as a host is probably determined by a complicated profile of volatile and non-volatile compounds. Susceptible trees would be those having an adequate diversity and quantity of feeding stimulants and an absence or a low concentration of feeding deterrents.
8.2 IDENTIFICATION OF CHEMICAL FEEDING STIMULANTS FOR *P. STROBI* FROM THE BARK OF SITKA SPRUCE.

The importance of chemoreception in the final phases of host selection has long been recognized. However, understanding of the host plant chemistry in relation to insect feeding has been neglected in the past mainly because of the complex nature of the chemical message, and technical difficulties in the isolation of plant chemicals.

The chemical bases for host selection is known for a few insects (Hsiao and Fraenkel 1968a, b; Haga, 1970; Numata et al. 1979), all of them non-conifer feeders. This subject was reviewed by Schoonhoven (1968), Hedin et al. (1974) and Jermy (1976).

A program aimed at isolating and identifying chemical feeding stimulants for *P. strobi*, from the bark of Sitka spruce, was started in 1977, in collaboration with Drs. A.C. Oehlenschlager and H.D. Pierce, Jr. of the Chemistry Department, Simon Fraser University. The progress of the program to December 1979, is indicated in Figs. 17 to 19. The various steps in the isolation were monitored using the standard agar disc bioassay, by applying dissolved fractions to the
Fig. 17. Flow chart of extraction of feeding stimulant fractions from Sitka spruce bark with water, pentane and ether, with indication of biological activity in paired agar discs bioassays.
Sitka spruce Bark (dry and ground)

Extd. with hot water

Water extract (active)  Residue (active)

Extd. with pentane

Pentane extract (active)  Residue (active)

Extd. with ether

Ether extract (active)  Residue (active)
Fig. 18. Flow chart of fractionation procedure of the pentane extract of Sitka spruce bark, with indication of biological activity in paired agar discs bioassays.
Column chromatography (Silica gel)

- Sterols and less polar compounds (not active)
  - Resin acids (active)
    - TLC
    - Band 3 (Rf 0.86-1.0)
      - Resin acids (very active)
      - Isopimaric acid? (Rf 0.33-0.86)
  - Fatty acids (active)
    - Band 1 (Rf 0-0.33)
      - (not active)
  - Compounds more polar than fatty acids. (active)

Chemical separation (separation funnel)

- Acidic compounds (active)

Saponification (KOH)

- Non-saponifiable fraction (active)
  - Acidic fraction (active)
    - TLC
    - Fatty and resin acid band (Rf 0.53-0.67)
    - More polar (Rf 0.12-0.49)
      - compounds (active).

*Solvent mixture Ether: Hexane : acetic acid 100 : 97 : 3
Fig. 19. Flow chart of fractionation procedure of the ether extract of Sitka spruce bark, with indication of biological activity in paired agar discs bioassays.
TLC
Ether: Hexane: acetic acid
100: 97: 3

- Band 1 (Rf 0.29-0.50)
  (active) Hydroxy – ester.

- Band 2 (Rf 0.50-0.60)
  (active) Hydroxy – ester

- Band 3 Rf (0.67-0.84)
  (not active) unsaturated ester
  (possibly a fat).

Ether extract

Column Chromatography
(Silica gel)

Fraction eluted with
Ether – Me OH
(95:5, v/v) contained
most activity

NMR
Major compound
probably a wax
Sitka spruce bark, from lateral branches, was dried (60 °C for 24 h), and ground to a fine powder. The bark powder was extracted 5 times with hot water in a Soxhlet extractor. Both the hot water extract and the residual bark contained high feeding stimulatory activity (Fig. 17). The water extract has not been studied further yet. Ten of the most common water soluble chemicals found in plants were tested to determine whether P. strobi would exhibit a feeding response to them. The chemicals were:

- Inositol
- Rhamnose
- Dextrose
- Sucrose
- Citric acid
- L-Ketoglutaric acid
- Malic acid
- Succinic acid
- Sodium chloride
- Potassium chloride
Sucrose was the only chemical that triggered feeding; however, the level of activity was low when compared to unextracted bark.

The hot water extracted bark was further extracted with pentane and ether, and these extracts were further fractionated using different chemical techniques (Figs. 17-19). The fractions obtained are complex mixtures containing several feeding stimulatory chemicals. Thin layer chromatography (TLC), and nuclear magnetic resonance (NMR) analysis indicate that the most active chemicals in the pentane extract are resin acids or closely related chemicals (Fig. 18). A very active compound in the ether extract seems to be a wax (Fig. 19). Both extracts, however, contain several other unidentified chemicals that are also active. The process of separation and identification of stimulatory chemicals is still underway.
9.0. INSECT FEEDING AND OVIPosition DETERRENTS FROM WESTERN RED CEDAR FOLIAGE

9.1. INTRODUCTION

The wood of western red cedar, *Thuja plicata* Donn, is resistant to fungal decay due to toxic substances in the heartwood (Rennerfelt 1948, Erdtmann and Gripenberg 1948, Roff and Whittaker 1959, Van Der Kamp 1975). Insect resistance, however, has been less studied, but Wellington (1969) and Barton et al. (1972) have shown that western red cedar wood contains compounds with juvenile hormone activity. Hach and McDonald (1971) found that the wood extractive thujic acid had a repellent effect on mosquitoes.

Little is known about the susceptibility of *T. plicata* to attack by defoliating insects in its natural habitat. As fewer insects are reported on western red cedar than on other

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Data collected in British Columbia by the Forest Insect and Disease Survey, Canadian Forestry Service, indicates that between 1972 and 1978, only 65 different insect species were collected from *T. plicata* compared to 144 from *Tsuga heterophylla* (Raf.) Sarg. and 235 from *Pseudotsuga menziesii* (Mirbel) Franco (J.E. Harris, pers. com., Pacific Forest Research Centre, Canadian Forestry Service, Victoria, B.C.).
tree species, its foliage may contain chemical feeding deterrents, repellents or substances with insecticidal effect. VanderSar and Borden (1977b), investigating discrimination by P. strobi, between sympatric host species, demonstrated that the weevil would not feed on twigs of its normal host, Sitka spruce, if they had been soaked in a crude extract of western red cedar branchlets. These results were corroborated with T. plicata leaf oil (Table VII).

This section reports a series of experiments designed to elucidate the chemical nature of the antifeedant activity of western red cedar foliage using P. strobi as a test insect.


9.2.1. Instrumentation

A Varian 2100 chromatograph modified for use with glass capillary columns and equipped with a Spectra-Physics Autolab Minigrator was employed for analytical gas-liquid partition chromatography (GLC). Analyses were carried out on the following capillaries: a 33m x 0.25mm ID column coated with OV-101, a 23.4m x 0.27mm ID whisker-walled column coated with
SILAR-10C and a 23.2\texttimes\ 0.28\text{mm} ID whisker-walled column coated with Carbowax 20M \cite{Schieke1975,Sandra1977}. A Varian 700 was used for preparative GLC, and separations were performed on a 152\text{cm} \times 6.35\text{mm} OD stainless steel column packed with 25\% Carbowax 1540 on Chromosorb A 60/80, at 133°C. Combined gas chromatography-mass spectroscopy (GC/MS) was carried out with a HP5985/MS/DS mass spectrometer interfaced through a Pt-Ir capillary to a 30\text{m} \times 0.25\text{mm} ID glass capillary column coated with SP-1000 \cite{J&WScientific}, available from Supelco). Optical rotations of neat liquids were measured with a Rudolph polarimeter.

9.2.2. Extraction of branchlets and preparation of western red cedar leaf oil

Western red cedar foliage was extracted as indicated in Fig. 20. Cedar branchlets (400g) were macerated with 50\% aqueous alcohol in a Waring Blender. The syrupy pulp was allowed to stand overnight at 7°C, filtrated through cheesecloth and the residue discarded. The aqueous alcoholic solvent in the filtrate was removed on a rotary evaporator. A portion of the residue left in the rotary evaporator was separated for testing (Fraction A); the rest was triturated with ethanol, filtrated under suction to give an ethanol insoluble and soluble fraction
Fig. 20. Flow chart of aqueous alcoholic extraction of western red cedar foliage.
Western red cedar branchlets

Macerated 50% ethanol

Aqueous alcoholic pulp

Filtration (cheesecloth)

Extract

Residue

Rotary evaporation

Residue

Condensate

Extraction with ether, concentration by distillation

Total aqueous alcoholic extract (Fraction A)

Trituration with ethanol, filtration

Residue Cedar leaf oil (Fraction D)

Ethanol insoluble residue (Fraction B)

Ethanol soluble filtrate (Fraction C)
(B and C respectively). The condensate in the receiver of the rotary evaporator was diluted with water, and extracted several times with ether. The combined ethereal extracts were washed, dried (MgSO₄) and concentrated by distillation. The residual oil (hereafter referred to as cedar leaf oil) was redistilled in a microdistillation apparatus at reduced pressure (approx. 0.5 mm) to give 0.55 g of a colorless liquid (Fraction D).

9.2.3. Fractionation of cedar leaf oil

Cedar leaf oil obtained from fresh branchlets by steam distillation (von Rudloff 1962) was separated by preparative GLC into fractions as indicated in the analytical chromatogram in Fig. 21. Analysis of the leaf oil by GC/MS (temp. program, 60-160 °C at 4 °C/min) indicated that Fraction I contained, inter alia, α-pinene, myrcene, limonene, γ-terpinene and γ-myrcone. Fraction II comprised (-)-3-isothujone and (+)-3-thujone (M⁺ 152). Terpinen-4-ol was the major component of fraction III. (-)-3-Isothujone was isolated from redistilled cedar leaf oil by preparative GLC, and was 97% pure by analysis on the OV-101 glass capillary column (temp. program, 85 °C at 4 °C/min). (+)-3-Thujone was prepared by the procedure of Hach et al. (1971) and exhibited only one peak on the SILAR-10C capillary column (temp. program, 60-200 °C at 6 °C/min): [α] = +78.6 (Lit.
Fig. 21. Gas chromatogram of cedar leaf oil on the Carbowax 20M capillary column. Temp. prog.: 60 C until elution of solvent, then 4 C/min to 160 C. Fraction I (F-I): monoterpenes hydrocarbons. Fraction II (F-II): thujones. Fraction III (F-III): major peak is terpinen-4-ol.
9.2.4. Bioassays with *P. strobi*.

Feeding deterrence to *P. strobi* was studied with the paired agar disc procedure described previously. One percent Sitka spruce bark (dried and finely powdered) from lateral branches was incorporated into the agar as a feeding stimulant. Candidate antifeedant stimuli were applied in solvent to the lens paper covering one of the paired agar discs while the other served as a solvent control.

An experiment was conducted to determine whether the fractions obtained in the aqueous alcoholic extraction of cedar branchlets exhibited antifeedant activity with *P. strobi*. Extracts were tested at concentrations equivalent to 0.5mg of *T. plicata* foliage. The solvents used were: 50% ethanol for the total extract (Fraction A); distilled water for the ethanol-insoluble fraction (B); pure ethanol for the ethanol-soluble fraction (C); and pentane for the volatile fraction (D). The amount of feeding was evaluated by counting the number of feeding punctures made in 24 h by 2 weevils into the surface of
the treated and untreated discs. The first 3 fractions were tested using 20 replicates each, and the fourth included 15 replicates.

In another experiment, I studied deterrenz to P. strobi of cedar leaf oil and its components when tested at similar concentrations to those which occur in nature (Table XXI). In western red cedar, the leaf oil accounts for about 5.5% of the dry weight of the foliage (von Rudloff 1962). Cedar leaf oil was applied (100µg) to the paper surface of agar-spruce bark discs. Since the weight of this paper surface is about 2.4mg, the resulting concentration on the paper was about 4% of its dry weight. The experiment included 15 replicates per treatment with 3 weevils per replicate, and was run for 24h in a growth chamber at 24 C.

The deterrent effect of cedar leaf oil on feeding by P. strobi on a natural substrate was tested using Sitka spruce twigs. The experimental unit consisted of 2 Sitka spruce twigs (2cm long), from lateral branches, connected end to end by means of a headless entomological pin inserted through the pith. One of the twigs in each couple was dipped for 2 seconds in a 1% pentane solution of cedar leaf oil. The second, control twig,
was dipped in pure pentane. Each replicate consisted of one pair of connected twigs placed with a single weevil under an inverted glass jar (448 ml) over a filter paper floor. The experiment lasted 24h and was evaluated by counting the number of feeding punctures made by each weevil on the twigs. The number of replicates for each sex was 16. Except where otherwise indicated, experiments were run at 20-21°C on a laboratory counter top with a natural photoperiod.

In all experiments, feeding deterrency was calculated as a percentage by subtracting the amount of feeding on the treated discs from the feeding on the control and dividing the difference by the feeding on the control.

9.2.5. Effect of cedar leaf oil on feeding and oviposition of other insects

Insects in 3 species, the alder flea beetle, *Altica ambiens* (Le Conte) (Coleoptera: Chrysomelidae); a leaf roller, *Erinotia solandriana* L. (Lepidoptera: Olethreutidae); and the red-backed sawfly, *Eriocampa ovata* L. (Hymenoptera: Tenthredinidae), were collected on Burnaby Mountain, B.C., where they defoliate red alder, *Alnus rubra* Bong., a species which is sympatric with *T. plicata*. The effect of cedar leaf oil on the
feeding of these phytophagous insects was tested by giving the insects a choice between one half of an alder leaf dipped for 2 seconds in a pentane solution of cedar leaf oil and a solvent-treated half-leaf. The two halves were placed on moist filter paper in a 10 cm glass petri dish. Four concentrations of cedar leaf oil (0.01%, 0.1%, 1%, and 2%) were tested.

The experiment with adult A. ambiguus included 5 replicates with 7 insects each. Feeding was evaluated by counting the number of feeding holes made by the insects in 12h. F. solandriana and F. ovata larvae were tested in separate, 5-replicate experiments using 5 insects per replicate. Feeding was evaluated with the aid of a camera lucida, by drawing on paper the area eaten in 24h. The drawings were then cut and weighed on a balance.

Cedar leaf oil was tested as an oviposition deterrent for the onion root maggot, Hylemya antiqua Meigen (Diptera: Anthomyiidae), using the oviposition bioassay of Vernon et al. (1977), in which H. antiqua is induced to oviposit into notches cut around the lip of an inverted Walgene beaker containing a suspended onion slice. In the oviposition deterrency test reported here, 300μg of cedar leaf oil, dissolved in pentane, were applied to the filter paper under each notch. The control
papers received only pentane. Two treated and two control sets of bioassay apparatus were introduced into each of 3 screen mesh cages that contained 25 gravid female flies. The number of eggs laid under each beaker after 48h were counted. The experiment was repeated 3 times with different groups of insects.

9.3. Results and discussion.

The total aqueous alcoholic extract of western red cedar branchlets had no significant antifeedant effect on P. strobi (Table XX). Surprisingly, the ethanol-soluble and insoluble fractions (fractions C and B, respectively) were stimulatory. Only the fraction containing the volatile leaf oil, showed significant feeding deterrent activity (Table XX). Analysis by gas chromatography indicated that (-)-3-isothujone and (+)-3-thujone accounted for more than 80% of the leaf oil, similar to results reported by Von Rudloff (1962).

Table XXI indicates the relative composition of the components of cedar leaf oil. When tested at similar relative concentrations, all fractions that comprise the leaf oil of western red cedar had a deterrent effect on the feeding of P. strobi. (+)-3-Thujone caused significant inhibition at very low concentrations (5μg) whereas the inhibition caused by
Table XX. Total number of feeding punctures made by *P. strobi* on agar-bark discs treated and untreated with fractions of western red cedar foliage (Fig. 20) tested at concentrations equivalent to 0.5mg of foliage.

<table>
<thead>
<tr>
<th>Stimulus Description</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Percent</td>
<td>Control</td>
</tr>
<tr>
<td>disc deterrenacy</td>
<td></td>
<td></td>
<td></td>
<td>disc deterrenacy</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
<td>---</td>
<td>----------</td>
<td>---</td>
</tr>
<tr>
<td>Total extract</td>
<td>60</td>
<td>57</td>
<td>5.0</td>
<td>85</td>
</tr>
<tr>
<td>50% aqueous, ethanol</td>
<td>27</td>
<td>72</td>
<td>**</td>
<td>--</td>
</tr>
<tr>
<td>soluble</td>
<td>54</td>
<td>87</td>
<td>--</td>
<td>57</td>
</tr>
<tr>
<td>50% aqueous, ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>insoluble</td>
<td>20</td>
<td>1</td>
<td>**   95.0</td>
<td>20</td>
</tr>
</tbody>
</table>

* Each total includes 20 replicates (2 weevils each), except for the volatile fraction which included only 15 replicates.

*t*-test significance level on difference between treated and control, indicated by: *=P<0.05, **=P<0.01.
Table XXI. Feeding response of _P. strobi_ to agar discs containing Sitka spruce and treated on the surface with a solution of cedar leaf oil or its components. N=15 replicates, 3 insects/rep.

<table>
<thead>
<tr>
<th>Stimulus description</th>
<th>Proportion of cedar leaf oil (%)</th>
<th>Amount used in bioassay (ug)</th>
<th>No. of feeding punctures Control disc</th>
<th>Treated disc a</th>
<th>Percent deterrency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated cedar leaf oil</td>
<td>100</td>
<td>100</td>
<td>14.3</td>
<td>7.7**</td>
<td>46.1</td>
</tr>
<tr>
<td>Fraction I, monoterpenes hydrocarbons</td>
<td>15</td>
<td>15</td>
<td>33.7</td>
<td>16.4**</td>
<td>51.3</td>
</tr>
<tr>
<td>Fraction II, (-)-3-isothujone and (+)-3-thujone</td>
<td>80-90</td>
<td>80</td>
<td>28.2</td>
<td>14.1**</td>
<td>50.0</td>
</tr>
<tr>
<td>Fraction III terpene alcohols</td>
<td>5</td>
<td>5</td>
<td>30.0</td>
<td>20.5*</td>
<td>31.7</td>
</tr>
<tr>
<td>(-)-3-Isothujone</td>
<td>75-88</td>
<td>75</td>
<td>21.4</td>
<td>16.3</td>
<td>23.7</td>
</tr>
<tr>
<td>(+)-Thujone</td>
<td>5-10</td>
<td>5</td>
<td>28.7</td>
<td>18.7*</td>
<td>34.8</td>
</tr>
<tr>
<td>Fractions I+II+ III combined</td>
<td>100</td>
<td>100</td>
<td>26.7</td>
<td>5.1**</td>
<td>80.2</td>
</tr>
</tbody>
</table>

a Student t-test on difference between treated and control indicated by * = P < 0.05, and ** = P < 0.01.
(-)-3-Isothujone was lower and not significant. However, additional testing showed that when the concentration of (-)-3-isothujone was increased to 100μg/disc, feeding inhibition was highly significant (t-test, P<0.01).

The experiment in which Sitka spruce twigs were dipped in a cedar leaf oil solution showed that cedar leaf oil depressed significantly the feeding of P. strobi (Table XXII).

Alder flea beetles fed significantly more on solvent-treated alder leaves than on leaves dipped in the cedar leaf oil solution (Table XXIII). The feeding deterrence did not increase proportionately with increasing stimulus concentration, suggesting that the lowest concentration tested may have been above the threshold for deterrence; thus the difference between treatments is probably due to random variation. Cedar leaf oil had no effect on the feeding activity of E. solandriana and E. ovata larvae which fed equally on treated and untreated leaves. The leaf rollers (E. solandriana) exhibited their normal leaf rolling behavior on the treated leaves before starting to feed. Oviposition by the onion root maggot was highly inhibited by the presence of cedar leaf oil in the treated oviposition stations (Table XXIV).
Table XXII. Mean number of feeding punctures made by one *P. strobi* on Sitka spruce twigs dipped for 2 seconds in a 1% pentane solution of cedar leaf oil. Control twigs were dipped in pure pentane. *N*=16 replicates for each sex, 1 weevil/replicate.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control twig</th>
<th>Treated twig</th>
<th>Percent deterrency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>2.5</td>
<td>0.5 **</td>
<td>80.0</td>
</tr>
<tr>
<td>Females</td>
<td>2.3</td>
<td>1.2 *</td>
<td>47.8</td>
</tr>
</tbody>
</table>

*α*-test significance level on difference between treated and control, indicated by: *=P<0.05, **=P<0.01.
Table XXIII. Mean number of feeding holes made by *A. ambiens* on red alder half-leaves treated and untreated with cedar leaf oil.

<table>
<thead>
<tr>
<th>Stimulus concentration (%)</th>
<th>Control leaf</th>
<th>Treated leaf</th>
<th>Percent deterrency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>17.7</td>
<td>6.0 *</td>
<td>66.1</td>
</tr>
<tr>
<td>0.10</td>
<td>26.6</td>
<td>14.4 *</td>
<td>45.9</td>
</tr>
<tr>
<td>1.00</td>
<td>26.2</td>
<td>10.4 *</td>
<td>60.3</td>
</tr>
<tr>
<td>2.00</td>
<td>26.2</td>
<td>7.0 *</td>
<td>73.3</td>
</tr>
</tbody>
</table>

At-test significance level on difference between treated and control, indicated by: *=P<0.05, **=P<0.01.
Table XXIV. Total number of eggs laid by 25 *H. antiqua* on cedar leaf oil-treated and untreated oviposition stations. N = 3 replicates per experiment.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Control stations</th>
<th>Treated stations</th>
<th>Percent deterrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1660</td>
<td>186 *</td>
<td>88.8</td>
</tr>
<tr>
<td>2</td>
<td>965</td>
<td>367 *</td>
<td>62.0</td>
</tr>
<tr>
<td>3</td>
<td>1763</td>
<td>162 **</td>
<td>90.8</td>
</tr>
<tr>
<td>Total</td>
<td>4388</td>
<td>715</td>
<td>83.7</td>
</tr>
</tbody>
</table>

\( t \)-test significance level on difference between treated and control, indicated by: *\( P<0.05 \), **\( P<0.01 \).
My experiments indicate that cedar leaf oil inhibits the feeding of at least 2 phytophagous insects. These insects, P. strobi and A. ambiens, must discriminate between T. plicata and their normal hosts in nature. However, other host discrimination mechanisms must exist for insects such as P. solandriana and P. ovata which are not inhibited by cedar leaf oil but which do not feed on T. plicata in nature. Feeding (and possibly oviposition) deterrency may partially account for the observed resistance of western red cedar to foliage feeders in nature. However, the relatively high concentrations required to elicit feeding inhibition and the lack of absolute deterrency (Tables XXI-XXIV) suggest that applications of cedar leaf oil or its components as commercial feeding deterrents are unlikely.
10.0. CONCLUDING DISCUSSION.

The agar disc bioassay developed during the course of this research was effectively used to study the intimate chemical interactions that determine whether or not a plant is accepted or rejected as food by a phytophagous insect.

Host selection behavior by *P. strobi* can be considered as a series of sequential steps including host tree finding, location of the correct feeding area within the tree, testing and acceptance of the bark surface as a feeding substrate, and maintenance of feeding.

Host finding behavior exhibited by *P. strobi* in the field suggests that olfaction, besides vision (VanderSar and Borden 1977a), may play a role in host finding. However, monoterpene hydrocarbons, the major volatile components in Sitka spruce, failed to attract walking *P. strobi* in olfactometric studies. Detection of olfactory responses in *P. strobi* may need the development of novel olfactometric techniques.

After landing on the tree, the weevils may be oriented to feeding on the bark by tactile stimuli and short-range olfactory stimuli. Feeding deterrents in the needles (Fig. 3) may serve to restrict feeding to the bark surface. Once on the bark surface, the insects must initiate the next step in the host selection sequence: the biting response. Feeding stimulants present in the cuticle of the leader surface may trigger this
crucial step (Table XVIII). Chemical analysis currently being performed indicates that the cuticular feeding stimulant is a wax or a closely related chemical. It is possible that failure to trigger a biting response may be an important factor in determining that some conifer species are not harmed by this insect.

Maintenance of feeding follows a biting response only if the mixture of chemical feeding stimulants in the inner bark of the leader is appropriate for the weevil. Feeding response stimuli for P. strobi on Sitka spruce comprise a complex mixture containing several chemicals with different properties (Tables XVII and XVIII, Figs. 9-19). The distribution of the chemicals within Sitka spruce trees correlated well with the preferred sites for feeding by the weevils (Table VIII), i.e. the leader bark. Chemical feeding stimulants appeared to be present in larger quantities or in better blends in the phloem of the trees. Volatile monoterpenes appear to synergize non-volatile stimulants in the bark (Figs. 9-11). Some volatile chemicals, particularly piperitone, limonene, and (+)-camphor had a deterrent effect on feeding when present above a certain threshold concentration, and may play a role in the resistance
to insect attack.

Extensive testing of conifer and non-conifer species demonstrated that the chemical stimuli triggering feeding vary in intensity among the species tested (Figs. 4-6). The chemical stimuli are optimal only in the host Sitka spruce and a few other conifers (Fig. 5). Since the stimuli in Sitka spruce are made up of a group of chemicals, apparently acting in concert, it is possible to hypothesize that plants eliciting a low feeding response have an incomplete set of stimulatory chemicals. The preferred host is probably selected over other coincident conifer species because it contains a complete array of stimulants in appropriate concentrations. The identification of the various chemicals involved, especially the key chemicals determining host specificity, may provide the basis for developing resistant varieties by removing key chemicals from otherwise susceptible varieties.

This thesis has also shown that *P. strobi* is sensitive to chemicals that have a feeding deterrent action (Table VII). Feeding deterrents exist within Sitka spruce trees (Table XIV, Fig. 3) and in several non-host species (Fig. 6, Tables VII, XX-XXII). Introduction of feeding deterrents into susceptible varieties, by inter-specific hybridization with more resistant
species, (like *Picea omorika*), and perhaps selection for deterrents already present may prove to be productive means of developing resistance for the weevil.

*P. strobi* may also be sensitive to variation in ovipositional stimulants and the nutritional quality of the host plant (Table XII). Ovipositional resistance was postulated by VanderSar (1978) as a possible explanation for the failure of *P. strobi* to colonize *P. monticola* in British Columbia. Removal of ovipositional stimulants or key nutrients from susceptible varieties of Sitka spruce may produce lines that do not support a healthy, fecund weevil population.

The process of host selection by *P. strobi* and of resistance by host and non-host plants is complex and involves physical and morphological factors identified by earlier workers (Stroh and Gerhold 1965, VanderSar and Borden 1977a), and complex biochemical interactions between the plant and the insect. Elucidation of the nature of this interaction may prove to be a landmark in the understanding of host selection by conifer-infesting insects.
LITERATURE CITED


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