AN INVESTIGATION OF THE EFFECTS OF SOME CHEMICAL CONSTITUENTS OF THE HOST PLANTS AND RELATED COMPOUNDS ON THE FEEDING RESPONSES OF LARVAE OF THE SPRUCE BUDWORM, *Choristoneura fumiferana* (Clem.) AND THE JACK-PINE BUDWORM, *Choristoneura pinus* Free.

A Thesis

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ABSTRACT

by

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AN INVESTIGATION OF THE EFFECTS OF SOME CHEMICAL CONSTITUENTS OF THE HOST PLANTS AND RELATED COMPOUNDS ON THE FEEDING RESPONSES OF LARVAE OF THE SPRUCE BUDWORM, Choristoneura fumiferana (Clem.) AND THE JACK-PINE BUDWORM, Choristoneura pinus Free.

The feeding responses of penultimate-instar larvae of the spruce budworm, Choristoneura fumiferana (Clem.) and the jack-pine budworm, C. pinus Free. to certain chemical constituents of the host plants and some related compounds were investigated. Particular attention was given to the relative acceptability of staminate flowers, developing vegetative shoots and mature needles.

Substances were tested by incorporating them into thin discs of Japanese elder pith upon which larvae were permitted to feed. Responses were determined quantitatively by measuring the areas of the discs consumed by the larvae.

Feeding responses were tested to host plant extracts prepared with different solvents and to a number of pure chemicals known to be present in the extracts, as well as to some compounds related to these chemicals. Of the solvents tested, 70 per cent ethanol was the most effective in extracting stimulating components.

A number of sugars stimulated feeding. The most effective were

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CHAPTER I

INTRODUCTION

The spruce budworm (Choristoneura fumiferana (Clem.)) and the jack-pine budworm (C. pinus Free.) are closely related North American species of the subfamily Archipinae whose larvae are important defoliators of conifers (Freeman, 1958). The spruce budworm is one of the most destructive forest insects in North America (Craighead, 1950). Recently it has been the subject of intensive ecological investigations and large-scale chemical control operations in the predominantly balsam-fir[1] forests of New Brunswick (Morris, in press). The jack-pine budworm is of less economic concern and has not been studied as intensively. It has nevertheless caused considerable damage in native jack pine stands and Scots pine (Pinus sylvestris L.) plantings in areas of southern Manitoba, northwestern Ontario and the neighboring Lake States.

The life history of the spruce budworm is briefly as follows: The eggs are laid on the needles of the host tree in July and hatch in about eight to twelve days. Shortly after hatching the first-instar larva spins a hibernaculum in a protected spot on the host tree. It molts to the second instar in the hibernaculum and enters diapause.

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[1] Native host trees are referred to by the accepted common name as given in "Native Trees of Canada" 6th Edition, Bulletin #61, Canada Department of Forestry, Ottawa, 1961. These are listed together with their scientific names in Appendix A.

Next spring the larva emerges from its hibernaculum and begins to feed either by mining the old needles or entering staminate flowers or vegetative buds. In later larval stages feeding is confined primarily to the new vegetative shoots. After about three to five weeks the larva pupates on the host tree and the adult emerges in about nine days.

The life history of the jack-pine budworm is similar but emergence in the spring is usually about two weeks later and this developmental difference is normally maintained throughout the season. This temporal differential is considered by Smith (1953) to be the most effective isolating mechanism maintaining the integrity of the two species, as they are sympatric in part of their range and will hybridize to produce fertile progeny.

As their common names imply, the spruce and jack-pine budworms differ in their host tree relationships. The spruce budworm occurs on a variety of conifers. In eastern and central North America the principal hosts are balsam fir and the native spruces. In the northwestern states and British Columbia, Douglas fir and the true firs are the primary hosts. In addition other conifers are occasionally attacked where these occur in mixture with the respective preferred hosts. The jack-pine budworm is primarily a pest of jack pine but occurs on other pines and sometimes on tamarack. In the laboratory spruce budworm larvae can be reared readily on vegetative shoots of jack pine and the jack-pine budworm on spruce or balsam fir terminals. As opposed to probable host-specific ovipositional responses of the

adults, host favorability to the feeding larvae appears to be determined primarily by the phenological relationships of the insects and their principal host trees. Emergence of spruce budworm larvae in the spring coincides, within fairly close limits, with the renewal of bud development of balsam fir and white spruce, while the later emergence of jack-pine budworm larvae occurs at the time when jack pine staminate flowers are nearing full development and the vegetative shoots are beginning to elongate. Blais (1957) has demonstrated that the relative immunity of black spruce to spruce budworm attack is attributable to the delayed development of the buds of this tree. When, due to certain weather conditions, the staminate flowers or vegetative buds of black spruce are available to the newly emerged larvae, they feed on these and develop normally.

Other aspects of the feeding behavior of budworm larvae in addition to inter-specific host tree relationships have received considerable attention from ecologists. Blais (1952) in his study of spruce budworm populations on balsam fir noted that development of the staminate flowers preceded swelling of the vegetative buds by one to two weeks. When staminate flowers were available larvae fed in these and did not mine old needles. Feeding in the flowers continued until the pollen was shed after which the larvae migrated to the newly developing vegetative shoots. It was noted that when a larva had completely consumed the new shoot on which it had been feeding it would search for new growth rather than feed on the readily available needles of previous years' growth. When larvae were forced to feed on

old needles due to exhaustion of current shoots their development was greatly retarded. The adults developing from such larvae were undersized and their fecundity was considerably reduced (Blais, 1953). Despite these effects on development and growth, Blais found that fifth-instar larvae reared in the laboratory on old foliage experienced no greater mortality than larvae reared on new shoots. Greenbank (1956) noted that larvae which fed on current year's needles that had hardened gave rise to abnormally small adults. The old needles and mature new growth of white spruce appear to be even less acceptable to the larvae than those of balsam fir and similar effects on larval growth have been recorded by Swaine et al (1924).

In the case of the jack-pine budworm, the ecological importance of staminate flowers was first recognized by Graham (1935). High population levels of this insect are almost invariably associated with an abundance of staminate flowers (Hodson and Zehngraff, 1946; Lejeune and Black, 1950). Newly-emerged larvae of the jack-pine budworm become established in the male flowers just before pollen shedding commences and they remain feeding in the flowers as long as part of the pollen is retained in them, usually until the larvae are in the fourth or fifth stadium. They then commence to feed on the new terminal shoots. Some larvae complete their entire larval development feeding on the staminate flowers. Jack-pine budworm larvae usually continue to feed in the staminate flowers for a longer period than do spruce budworm larvae. This is undoubtedly due to the fact that the staminate cones of jack pine occur in larger clusters than those of balsam fir or spruce and,

after ripening, retain a portion of their pollen for a longer period.

The Problem

It is evident that the food plant relationships of budworm larvae are complex, concerned as they are with phenological aspects of host tree development as well as behavioral responses of the insects. Orientation of larvae to suitable feeding sites involves the reactions of the larvae to tactile stimuli, light and humidity. These aspects of behavior have been studied intensively by W. G. Wellington (1948, 1949, 1950). The role of chemical stimuli in budworm larval feeding behavior has not been investigated. Their importance is indicated by the findings of E. F. Wellington (1949) who, in attempts to develop a suitable artificial medium for rearing budworm larvae, found it necessary to incorporate host plant foliage in the diet to make it acceptable.

This thesis reports on an investigation of the role of chemical constituents of the host plants as gustatory stimuli for spruce and jack-pine budworm larvae. As has been noted, these larvae will feed on a wide variety of conifers. Although these are not necessarily all equally acceptable it indicates that at least some of the stimulating components are present in most conifers. Especial consideration was given to the relative acceptability of staminate flowers, developing vegetative shoots and mature needles.

Most of the experiments concerned the feeding responses of larvae of the spruce budworm as the population decline of the jack-pine budworm in 1958 made it impossible to secure sufficient numbers of the latter insect. As more information was available on the chem-

istry of the foliage of white spruce than the other host trees, primary attention was given to this host.

Organization of the Thesis

The feeding responses of penultimate-instar spruce budworm and jack-pine budworm larvae were investigated in tests with host plant extracts and certain pure chemicals. Two general types of feeding tests were utilized. The first of these dealt with on the following pages were of the no-choice type. In these tests the various host plant extracts and chemicals were presented to the larvae individually in replicated series. These tests are designated (Chapter IV) as "single-disc" tests. 'Disc' has reference to the Japanese elder pith discs which served as the inert substrate carrying the test substance. Tests of this type provided a means of screening various plant extracts and a number of pure chemicals, including sugars, amino acids and certain known plant constituents, for their effect on larval feeding response. They also provided some indication of the relative effectiveness of the different extracts and chemicals.

The other type of test was the choice test. Choice tests permitted the comparison of response to two or more substances or mixtures of substances and to the same substances at different concentrations. Most of the choice tests were of the alternate-choice type where comparison was made between two substances each represented by one test disc.

By means of choice tests the following aspects of budworm larval feeding behavior were investigated:

1. The relative responses to extracts of host plant tissues prepared with various solvents.
2. The relative effectiveness of host plant extracts and pure sucrose as feeding stimuli.
3. The relative acceptability by the larvae of extracts prepared from staminate flowers, new vegetative shoots and mature needles of white spruce.
4. The relative stimulating effectiveness of the principal host plant sugars.
5. The effect of sucrose concentration on feeding response.
6. Comparison of the response to mixtures of certain individual chemical constituents of the host plants with sucrose to that to sucrose alone to determine whether the substances in question act as feeding stimulants or feeding deterrents.

The stimulant or deterrent effects of the various substances tested are related to their distribution in the various tissues of the host plants and to the relative acceptability of these plant tissues by the feeding larvae.

In the literature review general references on chemoreception in insects are mentioned. This is followed by a consideration of the pertinent literature on the role of the chemical senses in food-plant acceptance by insects. Most of this work is of recent date which is indicative of the current interest in this area of investigation.

CHAPTER II

REVIEW OF THE LITERATURE

In general the chemical senses of insects are highly developed and chemical stimulation plays an important role in initiating various types of behavioral responses. The most intensive studies on the physiology of chemoreception in insects have been those of von Frisch and his students on the honey bee (von Frisch 1919, 1934, 1950) and of Dethier and his students and colleagues on the blowfly, Phormia regina Meigen (Dethier 1955, 1956; Hodgson 1958). Recent comprehensive literature reviews are by Dethier and Chadwick (1948), Dethier (1953, 1954, 1956) and Hodgson (1958).

The role of the chemical senses in food plant recognition by an oligophagous insect was first effectively demonstrated by the classical researches of Verschaeffelt (1910). He showed that larvae of Pieris rapae L. and P. brassicae (L.), that feed almost exclusively on cruciferous plants, are stimulated by the mustard oil glucosides which they contain. These findings have been further elucidated by Thorsteinson (1953) in studies of another crucifer feeder, the diamondback moth, Plutella maculipennis (Curt.). He showed that the mustard oil glucosides, sinigrin, sinalbin and glucoheirolin, in the presence of nutrients, induced continuous feeding. The potency of these compounds is indicated by the finding that a concentration of sinigrin as low as two parts per million was sufficient to produce a feeding response.

There are a few other instances where it has been demonstrated

that the food plant specificity of an insect is associated with the presence of a chemical (or related chemicals) of limited botanical distribution. Dethier (1941, 1947) found that the food plants (mainly Umbelliferae) of the butterfly, Papilio ajax L., contain certain distinctive essential oils. He noted that the larvae would feed on filter paper treated with these oils or pure constituents of the oils, including carvone, methyl chavicol, or coriandrol. Filter paper treated with methyl chavicol was preferred to fresh carrot leaves, a natural food. These volatile plant constituents presumably act through the olfactory receptors. Yamamoto and Fraenkel (1960a) have reported the isolation of a specific feeding stimulant for the larva of the tobacco hornworm, Protoparce sexta (Johan.). Oviposition and feeding of this insect is confined almost exclusively to plants of the family Solanaceae (Yamamoto and Fraenkel, 1960b). The feeding stimulant was isolated in pure form and was said to have the characteristic properties of a glycoside but was not further identified. Its stimulating effectiveness is dependent on the presence of nutrients particularly sugars. Lippold (1957) investigated the host specificity of the Mexican bean beetle, Epilachna varivestis Muls., which feeds on the genus Phaseolus and to some extent on the soybean. He found that a plant extract fraction containing a glycoside of a triterpenoid saponin stimulated feeding by this insect. However, this material was not isolated in pure form.

In contradistinction to the role of host plant chemicals as discussed in the preceding paragraphs rejection of potential host

plants has been shown to be caused, in some instances, by compounds of restricted occurrence which act as feeding deterrents. This has been established most effectively for the Colorado potato beetle, Leptinotarsa decemlineata Say, which feeds only on certain Solanaceae. Plants of this family contain a variety of glycoalkaloids, some that act as feeding deterrents and others that do not influence feeding behavior but may be toxic (Kuhn and Löw, 1955).

On the basis of relationships such as those discussed above and other considerations, Fraenkel (1953, 1959a, 1959b) has proposed that the presence or absence of so-called "secondary" plant substances (glucosides, saponins, tannins, alkaloids, etc.), provides the sole basis for food plant specificity of insects. Criticisms of this viewpoint have been presented by Beck (1956b) and Thorsteinson (1960).

The complex nature of chemical stimuli that may be involved in orientation on the host plant and in feeding reactions has been indicated by studies on larvae of the silkworm Bombyx mori (L.). These larvae can be successfully reared only on a few plants other than their normal host, mulberry, Morus alba. Watanabe (1958) demonstrated that two volatile compounds isolated from mulberry leaves by steam distillation, β-γ-hexenol and α-β-hexenal, were strongly attractive to larvae. Hamamura et al (1961) discovered that citral, linalyl acetate and linalol, which are present in an ether-soluble fraction of mulberry leaves, were even more attractive to the larvae than hexanol. Terpinyl acetate was also present and slightly attractive. These investigators also found that β-sitosterol stimulates the larvae to bite. This com-

pound was found in high concentrations on the surface of mulberry leaves. Continuous ingestion however was dependent on an unidentified factor which Hamamura (1959) found was soluble in water and insoluble in methanol. Ito (1960) tested a variety of carbohydrates and related compounds for their effect on the feeding responses of silkworm larvae. He noted that a number of sugars induced feeding and that sucrose was fed on even in the absence of mulberry leaf powder. However, maximum feeding occurred when sucrose and leaf powder were both present. High concentrations of casein also stimulated feeding.

In contrast to the situation with the silkworm, Beck (1956a) found that sugar alone was adequate to induce normal feeding by larvae of the European corn borer, Pyrausta nubilalis (Hubn.). Under natural conditions this insect feeds on a wide variety of plants. The three principal sugars of the corn plant, sucrose, fructose and glucose, all stimulated feeding. In choice experiments with agar base artificial media, the larvae aggregated at the highest sugar concentration up to an optimum value that was different for each sugar.

Frings (1946) pointed out that sucrose appears to be almost universally acceptable to animals. As plants contain appreciable quantities of sucrose and other sugars, it is not surprising that a number of phytophagous insects have been shown to feed readily on certain sugars. Specific references to such responses will be made elsewhere in this thesis. Instances of feeding responses to other compounds of general or sporadic distribution in plants have been discussed by Thorsteinson (1960).

Polyphagous insects may feed readily on artificial diets that lack any substances that can be demonstrated to have significant stimulating effects when tested individually. Dadd (1960), in his studies on locust nutrition, found that sugar, yeast and wheatgerm oil were the only substances in his artificial diets that appreciably stimulated feeding. Nevertheless, when these three items were eliminated from the diet the nymphs would feed and grow on it. Similarly Beck (1956b) discovered that European corn borer larvae would feed and grow on diets containing no sugar.

The results of ablation experiments with oligophagous insects indicate that spontaneous feeding responses may be restrained to a large extent by inhibitory stimuli originating in the peripheral sense organs. Chin (1950) showed that, following removal of the antennae and maxillary and labial palpi, Colorado potato beetle larvae exhibited biting responses to odorless and tasteless substrates as well as to normally unacceptable plants. Waldbauer and Fraenkel (1961) found that maxillectomized larvae of the tobacco hornworm fed readily on some plants that normal larvae rejected. These larvae also fed to a greater extent than normal larvae on such bland media as filter paper and agar.

CHAPTER III

MATERIALS AND METHODS

Collection, Rearing and Handling of Experimental Insects

Test larvae were obtained from the field during the normal feeding period and were reared from stocks of diapausing larvae maintained in the laboratory at other times of the year.

Spruce budworm material originated mainly from two active infestations, one in northwestern Manitoba near Namew Lake, the other on the western edge of Lake Winnipeg near Loon Straits. Both these infestations are in mixed stands of balsam fir and white spruce.

Jack-pine budworm larvae were obtained during the early stages of this study from localized infestations in southeastern Manitoba and the interlake district. With the decline of this insect to very low population levels throughout the Province by 1958 it became impossible to obtain adequate numbers of this insect for experimental purposes.

Field-collected larvae were reared on developing shoots of the normal host foliage in glass jelly jars with tight-fitting lids. The period during which the feeding larvae were available for test purposes was extended to approximately five weeks by adjusting rearing temperatures.

It was necessary to make special provision to have larvae available for study during those periods of the year when they could not be obtained in the field. Mass collections of ultimate-instar larvae were brought to the laboratory and reared to the adult stage.

The moths were placed in screen cages in groups of four or five pairs each and were provided with branches of the host tree on which to oviposit. Eggs were handled by the method described by Stehr (1954) to provide stocks of diapausing second-instar larvae. After four to five weeks at room temperature (ca. 23° C.) the larvae were stored at 3° C. for a minimum of ten weeks. Larvae were returned to room temperature as required and exposed to high humidity and continuous illumination. These conditions are known to favor survival and early termination of diapause (Harvey, 1958).

In the early stages of this investigation, the newly-emerged larvae were reared on frozen terminal-shoots of spruce or balsam fir as recommended by Stehr (1954). Difficulties were encountered, however, due to the formation of mold and drying of the plant material, which necessitated frequent handling and transferring of the larvae. This caused considerable mortality and was very laborious. A more satisfactory method of rearing was developed which utilized the vegetative shoots of tamarack. These are readily acceptable as food by larvae of both species of budworm.

To provide a source of tamarack shoots, small trees, three feet to four feet in height, were planted in boxes in the autumn and placed in the greenhouse. In the greenhouse, illumination from fluorescent lamps was provided from 8:00 a.m. to 8:00 p.m. and from 12:00 p.m. to 1:00 a.m. This lighting regimen was found by Vaartaja (1957) to be most favorable for shoot growth. Once well established in the greenhouse the trees produced shoots in considerable numbers and pruning

of the trees to provide shoots for feeding purposes further stimulated their production.

The rearing containers consisted of plastic petri dishes with tight-fitting lids.[1] They were 2 3/4 inches in diameter and 3/4 inch in depth. Ventilation was provided by 1/2 inch ports drilled in both the lid and bottom sections. The ports were covered with squares of fine-mesh silk bolting cloth cemented over their inner surfaces. A notch of 3/16 inches depth was cut in the rim of each section to accommodate the branchlets.

Twigs with shoots having partially expanded needles were cut to a length of about four inches. Needle fascicles along the lower portion of the stems were removed and three or four shoots were bound together with a twist of cotton wool. These shoots were then placed in the rearing container with the cotton wool fitting in the notch in the dish rim. A newly-emerged second-instar larva was placed on each shoot with a maximum of four larvae to a container. Two such assemblies with their lids in place were supported upright on a jelly tumbler partially filled with tap water (Figure 1).

The rearing was done in a room maintained at approximately 23° C. and equipped with overhead fluorescent lights. The room was illuminated from 8:00 a.m. to 8:00 p.m. C.S.T. daily. The shoots remained fresh in appearance and nutritionally adequate for two to

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[1] General Biological Supply House, Inc., Chicago, Illinois.

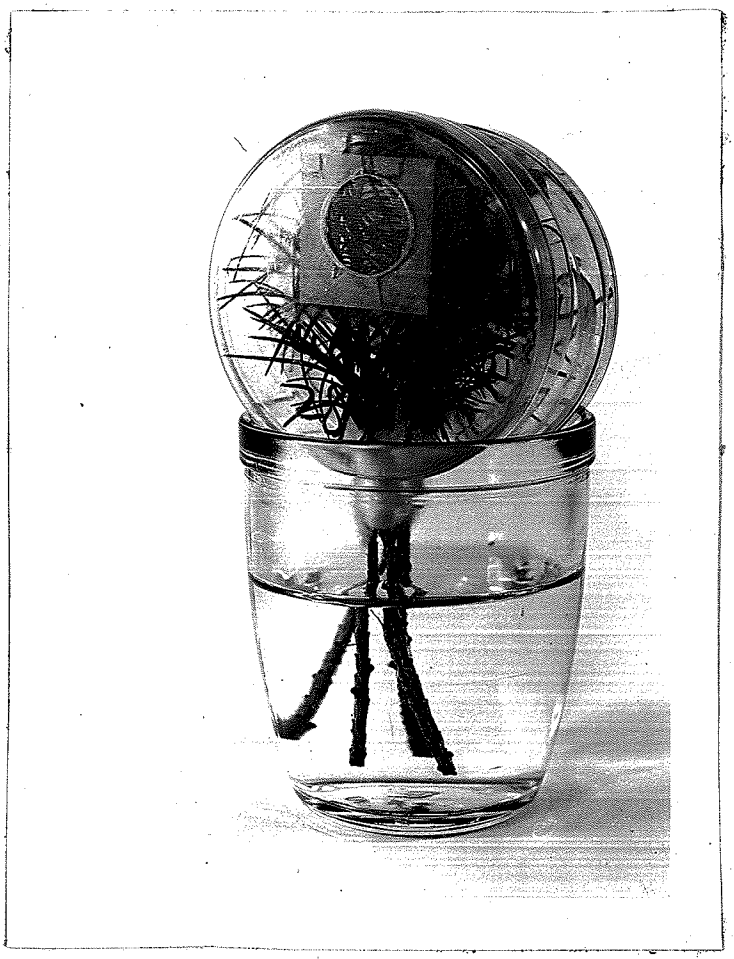

FIGURE 1

CONTAINERS USED TO REAR BUDWORM LARVAE ON TAMARACK SHOOTS

three weeks. In most instances it was only necessary to transfer the larvae to fresh food once prior to their molt to the fifth instar.

Penultimate-instar larvae were selected for the feeding tests because the limited feeding of earlier stage larvae did not permit quantitative evaluation by the technique used in these tests. Ultimate-instar larvae, on the other hand, are less sensitive test subjects because they consume relatively large amounts of food and tend to be less discriminate in their biting reactions. Penultimate-instar (fifth-instar) spruce budworm larvae were identified by their color and by head capsule measurements (McGugan, 1954; Bean and Batzer, 1957). A complication arose in the case of the jack-pine budworm due to the occurrence of a seventh instar in about half the larvae (Lejeune, 1950). The larvae used in these tests were those with penultimate head-capsule widths within the range reported for the seven-instar group.

Larvae were removed from their rearing containers approximately six hours before the commencement of a test and were placed in plastic petri dishes containing discs of filter paper moistened with distilled water. The primary purpose of this pre-conditioning treatment was to allow for evacuation of the gut and thus prevent fouling of the test discs with faecal material that might influence feeding responses.

Methods Used in Testing and Measuring Feeding Responses

Several techniques have been devised for testing the effects of chemicals on the feeding responses of mandibulate phytophagous insects (Thorsteinson, 1955). Basically, the techniques are of two types. In one the substance to be tested is either coated on the sur-

face of the host plant leaf or infiltrated into it. In the second the test material is incorporated into a non-stimulating medium such as filter paper, pith or agar gel. The amount of feeding is determined either directly by measuring the quantity of material eaten or indirectly by counting or weighing the number of frass pellets produced. A novel variation of the filter paper technique was recently used by Davis (1961) in feeding tests with wireworm larvae. He applied the test materials to Unidisks (Difco Laboratories Inc., Detroit, Michigan) and measured the amount of feeding in terms of light transmission values measured with a Photovolt densitometer. Beck (1956a), in his investigations of the feeding reactions of newly-hatched European corn borer larvae, used agar-based diets and expressed results in terms of the quantitative distribution of groups of established feeding larvae in choice tests.

In the present investigation a modification of the pith-disc technique originated by Raucourt and Trouvelot (1936) was utilized. Japanese elder pith has certain advantages over the other two commonly used media, filter paper and agar, for use with budworm larvae. Filter paper was found to be unsatisfactory as it is too tough and fibrous for the larvae to feed on readily. Agar proved unsuitable because the habit of the larvae of tunnelling in the medium made it difficult to count frass pellets accurately. The establishment response method of Beck could not be employed because it was not possible to have available sufficient numbers of newly-emerged postdiapause larvae of uniform age.

Slices of Japanese elder pith[2] were cut to a thickness of 200 microns with a sliding microtome and punched into uniform-sized discs of 1/2 inch diameter with a steel cork borer. To obviate differences due to variation in the texture of the pith, the discs used in any given experiment were cut from the same piece of pith. The dry discs were immersed in a solution of the test substance for five minutes, and then were removed, drained of excess solution and air-dried. For testing purposes the discs were mounted on wax-treated paper. The paper was prepared by dipping in molten paraffin and draining off the excess. The discs were then placed in position on the paper and pressed lightly on the partially-congealed wax with a clean cork. Care was taken that the paraffin did not impregnate the discs.

Budworm larvae are strongly thigmotactic and also respond positively to a diffuse light source in all stadia (Wellington, 1948). These behavioural characteristics were taken into account in designing the test chambers and in arranging the lighting conditions under which the tests were conducted. The test chambers were made of acrylic plastic plates 1.5 mm. in thickness which could be quickly assembled in sandwich-like fasion. A circular area was cut from the middle of the centre plate. In the shallow chamber so-formed the larva could maintain simultaneous contact with the dorsal and ventral body surfaces thus favoring the thigmotactic component of its behavioural response. Experiments were carried out on a light table consisting of a box with

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[2] Purchased from General Biological Supply House, Inc., Chicago, Ill.

black walls and top and a floor composed of a sheet of ground glass. The glass was lighted from below by fluorescent tubes. In single-disc tests the light was restricted to the area of the test disc by means of a black paper mask. This served to orient the larva to the disc. Exploded views of the two types of assemblies used in single-disc and choice tests, respectively, are shown in Figures 2 and 3. Test chambers are shown in position on the light table in Figure 4.

In all cases the treated discs were used in the dry state. The larvae fed readily under these circumstances. Moistening of the discs would have introduced an uncertain variable as it would have been difficult or impossible to maintain uniform conditions of moisture throughout the test period.

All tests were of 48 hours duration. This allowed time for the larvae to consume sufficient material to permit quantitative measurement. Normal larvae remained active throughout the test period. In cases where the larvae died, appeared moribund, molted during the test or were in a near-molt condition at the conclusion of the test, the tests were discarded. Experiments were conducted at room temperature which in most cases was in the range 23° C. to 26.5° C.

Time-lapse photography provided information on the frequency and pattern of larval feeding during the test period. This confirmed that the larvae remained more or less uniformly active throughout the test period and showed that the frequency of feeding was approximately the same during the last half of the test as during the first half. There was no indication of a progressive change in feeding response

FIGURE 2

EXPLODED VIEW OF TEST CHAMBER USED IN SINGLE-DISC TESTS

1. Black cardboard light shield.
2. Clear plastic cover.
3. Feeding chamber.
4. Test sheet with pith disc in centre.
5. Black cardboard light shield with hole in centre.
6. Clear plastic bottom piece.

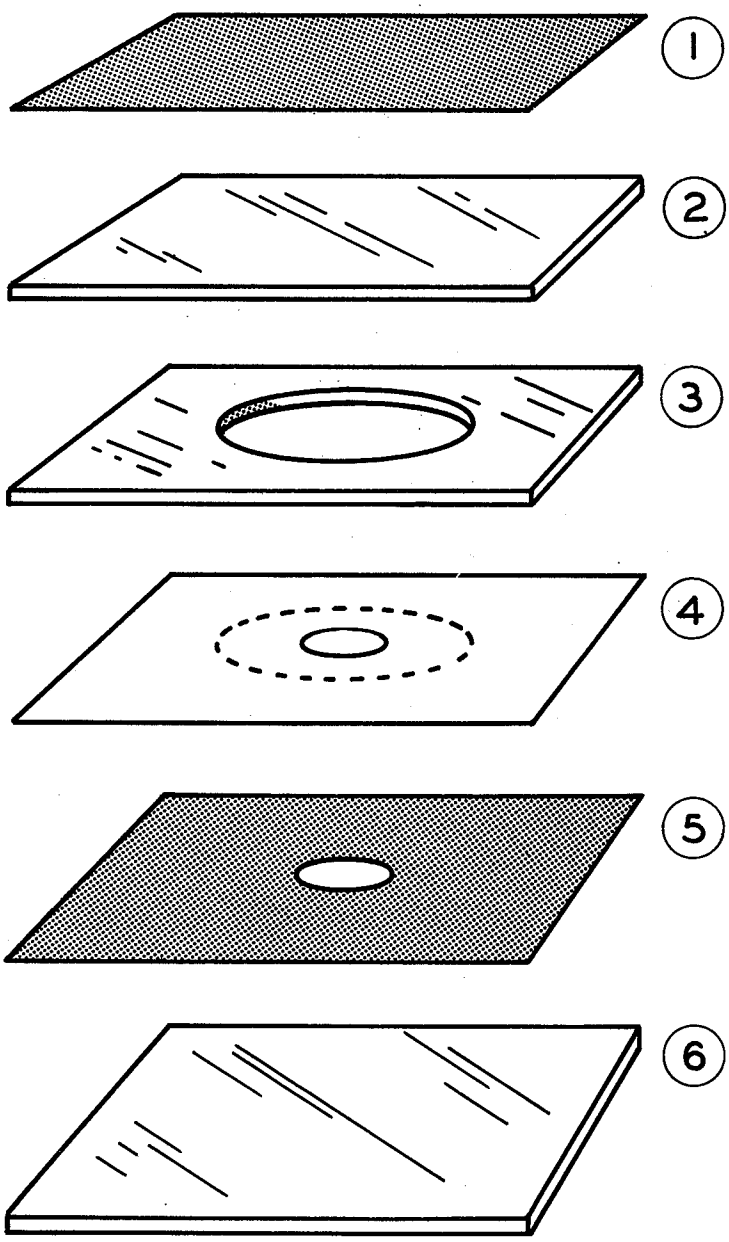
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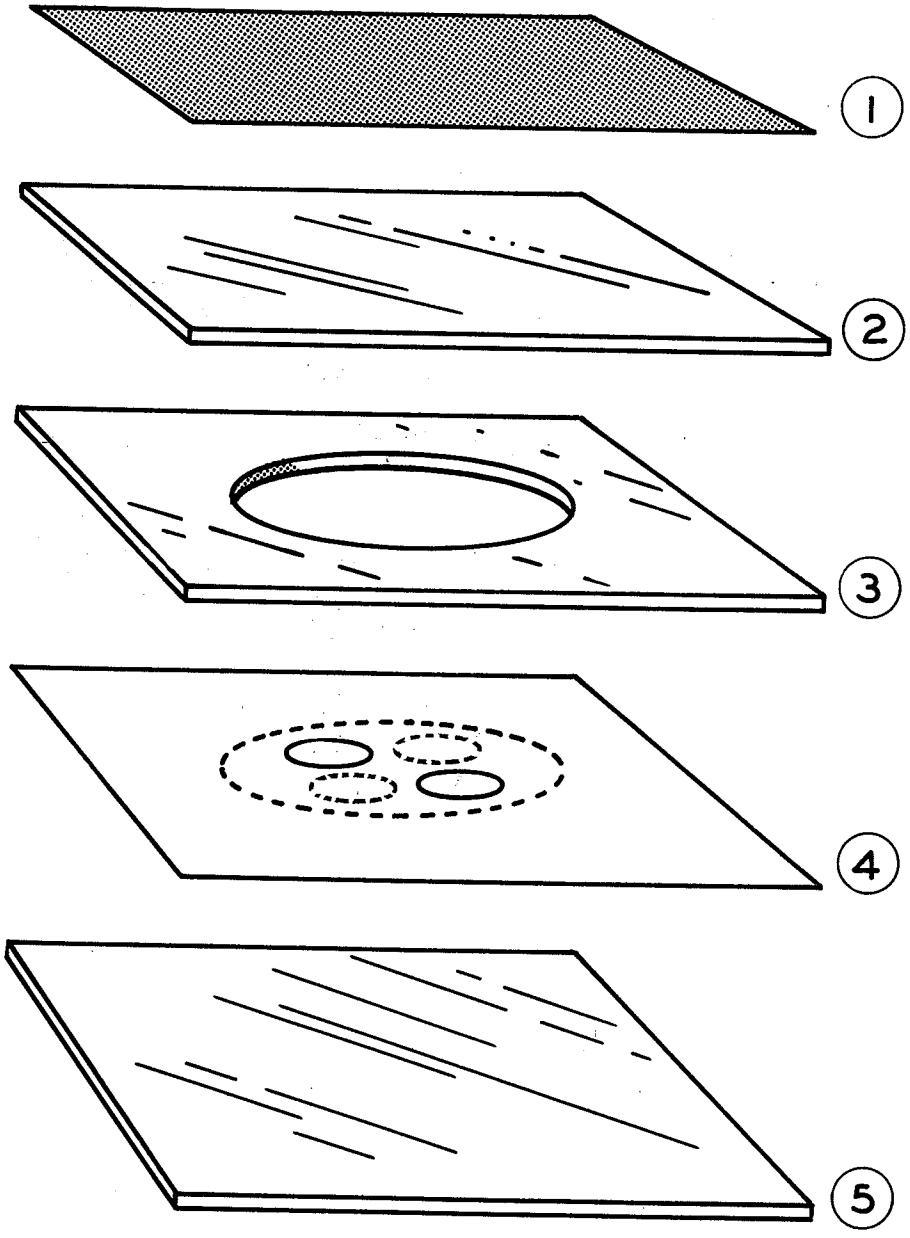
FIGURE 3

EXPLODED VIEW OF TEST CHAMBER USED IN CHOICE TESTS

1. Black cardboard light shield.
2. Clear plastic cover.
3. Feeding chamber.
4. Test sheet with two pith discs in position (small solid circles) Alternate positions indicated by small dotted circles.
5. Clear plastic bottom-piece.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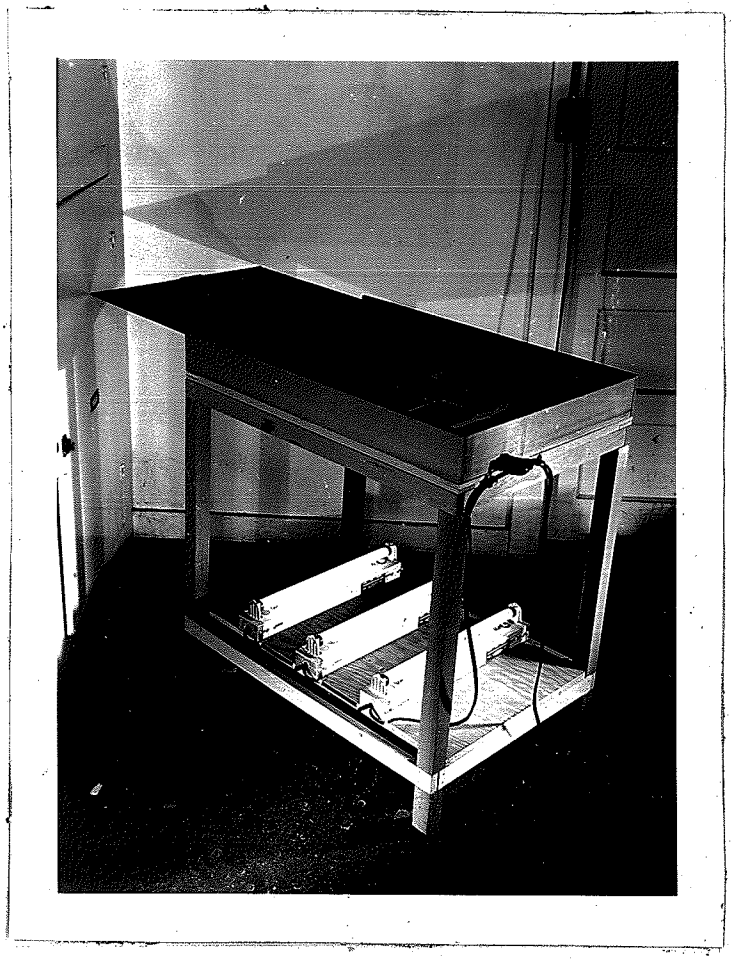

FIGURE 4

LIGHT TABLE WITH SLIDING TOP PARTIALLY OPENED
TO SHOW TEST CHAMBERS IN POSITION

during the test. The time-lapse studies are described and the results summarized in Appendix B.

In order to facilitate measurement of the amount of feeding the pith discs were stained with methylene blue on completion of the test. An enlarged image of the stained disc was projected on an outline form, using a photographic enlarger, and the area eaten was traced on the form and measured with a planimeter. The measurements were expressed in terms of the percentage of the disc area consumed. Figures 5 and 6 illustrate stained discs from a single-disc test and choice test respectively.

Preparation of Host Plant Extracts and Analysis for Sugar Content

Host plant foliage and staminate flowers were collected between the hours of 8:00 a.m. and 10:00 a.m. C.S.T. and immediately deep-frozen. The material was stored at -29° C. and later dried by the freeze-drying process. It was further dried in a vacuum desiccator and then ground in a mechanical mortar until it would pass a 50-mesh sieve. The resulting powdered material was stored in the dark, in tightly stoppered glass bottles, over calcium chloride.

Material for use in feeding tests and sugar analyses was prepared by extracting an accurately weighed portion of the dried powder in a micro-soxhlet apparatus. The extract was then suitably concentrated by means of a rotary vacuum evaporator.

In the analysis of the extracts for sugar concentration they were first clarified by the method of Hassid (1936). Total sugar content of the clarified extract was determined by Dreywood's anthrone

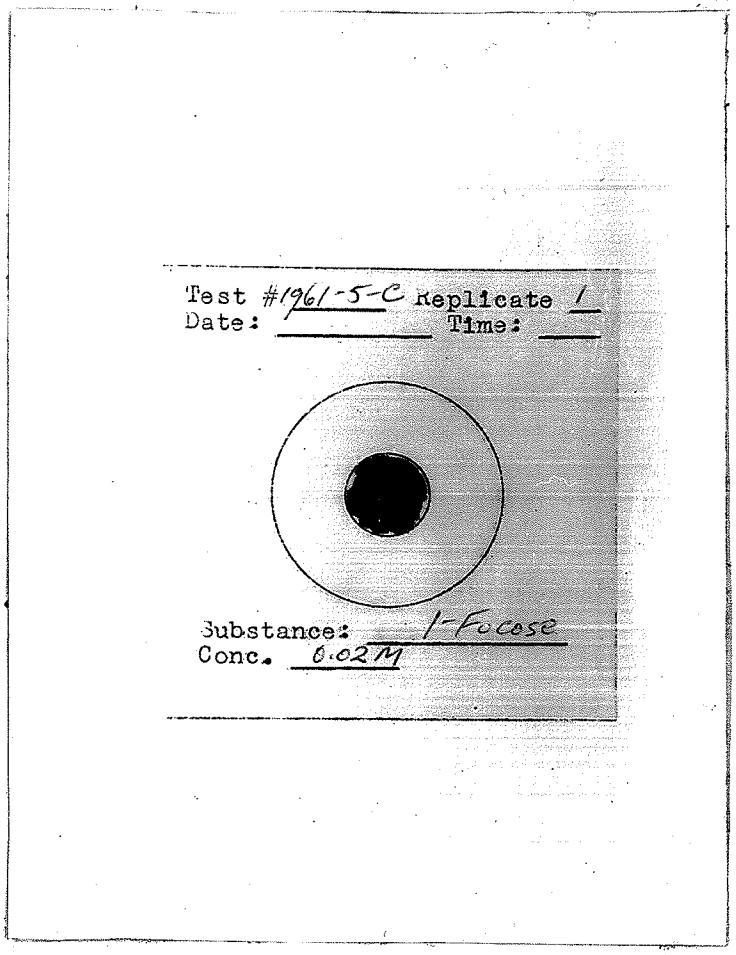

FIGURE 5

AN EXAMPLE OF THE RESPONSE OBTAINED IN A SINGLE-DISC TEST
(the pith disc has been stained with methylene blue)

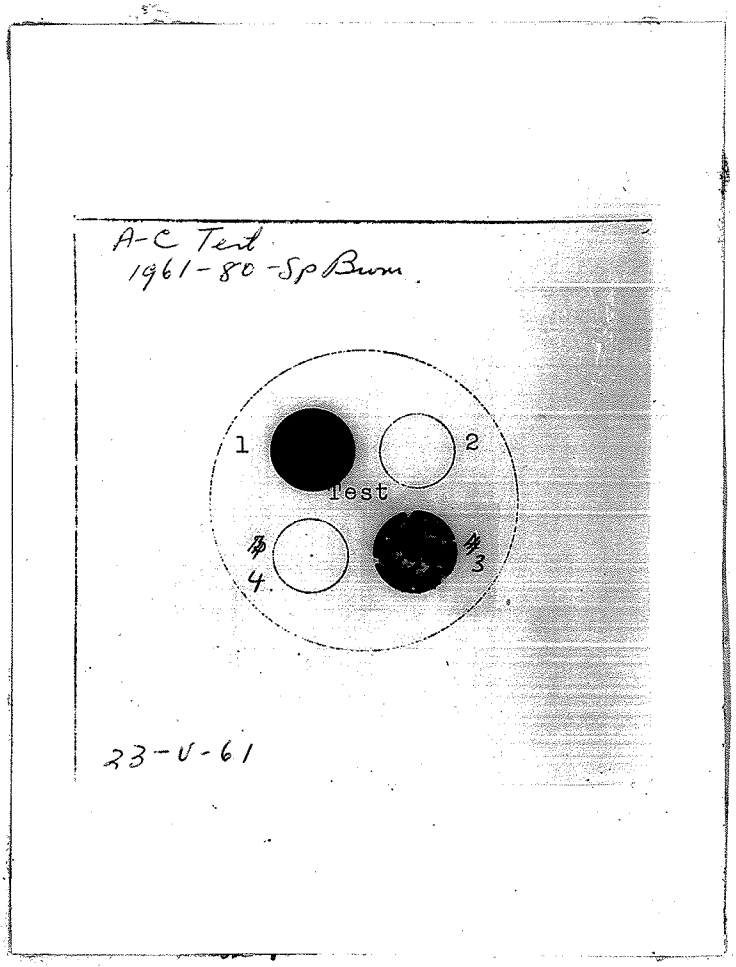

FIGURE 6

AN EXAMPLE OF THE RESPONSE OBTAINED IN AN ALTERNATE-CHOICE TEST

Disc #1. treated with 0.1 M. sucrose.
Disc #3. treated with 0.1 M. sucrose plus 0.02 M. L-proline.
(the pith discs have been stained with methylene blue)

method as described by Morris (1948). The results were expressed as glucose equivalents. Photometric determinations were made with a Model B Beckman spectrophotometer.

Preparation of Solutions used in Feeding Tests

Solutions of all chemicals used in feeding tests were freshly prepared within two to three hours of the commencement of the tests. Except for those cases where the solubility properties of the test chemical did not permit, the solvent used was a 50 per cent solution (by volume) of ethanol in distilled water. Diluted ethanol was preferable to distilled water as a solvent because it accelerated infiltration of the discs and subsequent drying. Concentrations in most cases were expressed as molarities as recommended by Pfaffman et al (1954). Where a number of concentrations were tested, as in the single disc tests, the lower concentrations were prepared by serial dilution. As only a few hundred milligrams of the test substance pungenin and its aglucone were available, solutions of these chemicals were prepared by dissolving a weighed quantity in a small volume (4 or 5 ml.) of solvent delivered from a volumetric pipette. Concentrations were expressed as percentages of weight by volume, i.e. grams of test chemical in 100 ml. of solvent. The concentrations of shikimic acid, D-quinic acid and compounds related to the aglucone, 3, 4-dihydroxyacetophenone, were similarly expressed to facilitate comparison of results.

Sources of Chemicals used in Feeding Tests

Sugars and m-inositol - Nutritional Biochemicals Corporation, Cleveland, Ohio.

Amino acids and amides - L-Asparagine - Distillation Products Industries, Rochester, N. Y.
Glycine and L-glutamic acid (Reagent grade) - Fisher Scientific Co. Ltd., Montreal, P. Q.
All other amino acids and amides - Nutritional Biochemicals Corporation, Cleveland, Ohio.
D-Quinic acid and shikimic acid - K and K Laboratories, Jamaica, N.Y.
3, 4-Dihydroxybenzoic acid, 3, 4-dihydroxycinnamic acid, 2, 6-dihydroxyacetophenone - Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.
2, 4-Dihydroxyacetophenone and catechol - Matheson, Coleman and Bell Division, The Matheson Co., Inc., East Rutherford, New Jersey.
The glucoside pungenin (3 - B-D-glucopyranosyloxy - 4 - hydroxyacetophenone) and its aglucone 3, 4-dihydroxyacetophenone - Dr. A. C. Neish, Atlantic Regional Laboratory, National Research Council, Halifax, N. S.

Rationale of the Alternate-Choice Test Method and Statistical Treatment of Data

Quantitative comparisons of feeding response to two substances, or two concentrations of the same substance, can be obtained by testing the two separately in parallel series of single-disc tests or together in replicated choice tests. The first method has certain advantages but it necessitates the use of considerably more insects especially as there is an appreciable amount of individual variation in the responses of larvae to identically treated pith discs. Availability of adequate numbers of larvae at the proper stage of development was frequently a limiting factor in these experiments and was one of the principal reasons for adopting the choice test method.

The possibility of certain interactions are introduced in the choice test method of testing. Adaptation following feeding on the more stimulating substance in a pair may influence subsequent response to the less stimulating substance. Thus the relative differences in response in such

cases would be greater than when the order of encounter was reversed. However, the interpretation is in no way invalidated by this circumstance since only the more palatable treatments can have this effect. There was no indication in these tests that feeding on one disc influenced response to the other disc in a pair other than with respect to differences in the acceptability of the substances being tested. Errors due to positional effects were largely obviated by the symmetry of the situation (see Fig. 3), the shallowness of the chamber, and the uniform lighting of the floor of the test arena. Movement of the larvae within the arena was unrestricted and they moved about actively throughout most of the test period. Due to the strong thigmotactic responses of the larvae, they tended to wander around the periphery of the chamber but frequent excursions were made across the arena during which contact was made with the test discs. The temporal pattern of feeding was discontinuous (see Appendix B). Feeding on a disc on the second and subsequent encounters by a larva was usually not contiguous with a previous feeding site and there was no evidence of a 'baiting effect' induced by previous feeding. In the rare cases where the larvae regurgitated on a disc this was easily detected and the experiment was discarded.

In some exploratory tests, discs treated with the same sucrose solution were paired together in the alternate-choice situation. There were usually no marked differences in the amount of feeding on the two discs. Occasionally the feeding on one disc was a few times greater than on the other but in a replicated series the mean difference between discs occupying the two positions was always negligible.

There was considerable variability in the total amount of feeding between replicates. Although care was taken to standardize the treatment of the larvae there probably were differences in the nutritional states of the larvae that could have contributed to this variability. Age in relation to the time of the previous and subsequent molts would be expected to influence both locomotor activity and feeding.

The differences in the amounts of feeding on the paired discs in the alternate-choice tests were tested for significance by means of the t test. Due to the wide range of values and the frequence occurrence of zeros, the data were subjected to the transformation $\sqrt{x + \frac{1}{2}}$, as recommended by Cox (1954), before applying the t test. In those cases where no statistically significant differences were demonstrated it is possible that there were real differences in response which were outside the range of sensitivity of the testing technique employed in these experiments.

CHAPTER IV

LARVAL FEEDING RESPONSES TO PLANT EXTRACTS AND PURE CHEMICALS IN SINGLE-DISC TESTS

I. SINGLE-DISC TESTS WITH PLANT EXTRACTS

The feeding reactions of spruce and jack-pine budworm larvae to crude extracts of host plant tissues were investigated in some preliminary experiments. Extracts were prepared from freshly-collected or deep-frozen vegetative shoots of white and black spruce, balsam fir and jack pine and from staminate flowers of white spruce and jack pine using a variety of solvents. The plant tissues were macerated in a Waring blendor with the solvent at the temperature of its boiling point. Ten ml. of solvent were used for each gram of tissue. Following filtration, the extracts were concentrated to about one-eighth their original volume by means of a rotary vacuum evaporator.

Extracts prepared with the following solvents elicited feeding responses: distilled water; 95 per cent and 50 per cent methanol; 80 per cent ethanol; and 90 per cent isopropanol. No appreciable feeding occurred on discs treated with extracts prepared with petroleum ether, chloroform or ethyl acetate. Similar results were obtained when frozen-dried plant tissues were extracted with these solvents in micro-soxhlet extractors.

The results indicated that 80 per cent ethanol was one of the most effective solvents for extraction of active constituents from the host plant tissues. Amongst the variety of substances extractable by

this solvent are sugars and amino acids, two groups of compounds known to induce feeding responses in some insects.

Several sugars and amino acids as well as certain other compounds known to be present in appreciable quantities in ethanol extracts of coniferous foliage were subsequently utilized in single-disc feeding tests.

II. SINGLE-DISC TESTS WITH SUGARS

The principal sugars present in the needles of conifers during the spring and summer months are glucose, fructose and sucrose (Bidwell et al, 1952; Neish, 1958; Parker, 1959). These sugars have also been reported to be present in the pollen of various species of pine (Todd and Bretherick, 1942; Nilsson, 1956; Havivi and Leibowitz, 1960). In the present study their occurrence in extracts of newly-developing vegetative shoots and staminate flowers of white spruce and jack pine was confirmed by paper chromatography using the developing solvent system, n-butanol: acetic acid: water (4:1:5 volumes). Assarsson and Theander (1958) reported that small amounts of arabinose, xylose, galactose and melibiose were present in Scots pine (Pinus sylvestris L.) needles collected in October. Neish (op. cit.) and Parker (op. cit.) found appreciable quantities of raffinose in spruce and pine needles in the fall and winter but none in the late spring and summer.

The above-mentioned sugars, as well as some others of interest due to their reported influence on the feeding activities of other insects, were used in a series of single-disc tests. Test replicates

usually consisted of four concentrations of the test sugar ranging from 0.004 M. to 0.5 M. together with 0.1 M. and/or 0.5 M. sucrose and a disc treated with the solvent, 50 per cent ethanol, alone. Preliminary tests had established that larvae fed readily on discs treated with 0.1 M. or 0.5 M. sucrose. Therefore such discs served as effective positive controls and provided a standard basis for comparison of feeding responses. Discs treated with the solvent alone served as negative controls. A total of fifteen sugars were used in tests with spruce budworm larvae and six of these were also tested with jack-pine budworm larvae.

The results of tests with spruce budworm larvae are summarized in Table I and those with jack-pine budworm larvae in Table II. The numbers of positive and negative feeding responses for each compound and concentration tested are given. The average amount of feeding at each concentration of the test compounds and controls was evaluated semi-quantitatively. The maximum average response in each test series was rated as + + + + + and lesser responses were rated proportionately. A negative sign indicates no feeding response and (+) indicates that the amount of feeding was less than one-fifth the maximum.

D-Arabinose** and L-Arabinose*[1]

Over 50 per cent of the larvae of both species exhibited responses to D-arabinose at concentrations of 0.1 M., 0.02 M. and 0.004 M.

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1 ** Denotes compounds that were tested with both the spruce budworm and jack-pine budworm.
* Denotes compounds tested with the spruce budworm only.

TABLE I

SINGLE-DISC TESTS WITH SUGARS - SPRUCE BUDWORM

Test sugar	Molar concentration / Feeding response	Test sugar				Controls		
		0.5	0.1	0.02	0.004	0.5 M. sucrose	0.1 M. sucrose	Negative control
D-Arabinose	Number positive	0	4	5	3		5	1
	Number negative	5	2	2	2		0	2
	Relative mean response:	-	+	++	(+)		+++++	(+)
L-Arabinose	Number positive	4	5	3	1		9	2
	Number negative	8	6	6	9		0	8
	Relative mean response:	(+)	+	(+)	(+)		+++++	(+)
D-Xylose	Number positive	1	3	3	1		6	2
	Number negative	4	2	1	2		0	5
	Relative mean response:	(+)	+	(+)	(+)		+++++	(+)
L-Rhamnose	Number positive	0	0	0	0	3	4	0
	Number negative	3	3	3	3	0	0	4
	Relative mean response:	-	-	-	-	+++	+++++	-
L-Fucose	Number positive	5	3	4	2		4	1
	Number negative	0	0	0	1		0	4
	Relative mean response:	++++	++++	++++	+++		+++++	(+)

TABLE I (Cont'd)

Test sugar	Molar concentration / Feeding response	Test sugar				Controls		
		0.5	0.1	0.02	0.004	0.5 M. sucrose	0.1 M. sucrose	Negative control
D-Glucose	Number positive	3	2	3	1	5		1
	Number negative	2	3	2	4	0		4
	Relative mean response:	++	+	+	(+)	+++++		(+)
D-Mannose	Number positive	1	2	1	1		4	0
	Number negative	3	4	2	6		0	3
	Relative mean response:	(+)	(+)	(+)	(+)		+++++	-
D-Galactose	Number positive	4	6	1	2		7	0
	Number negative	5	5	4	2		0	10
	Relative mean response:	(+)	+	(+)	(+)		+++++	-
D-Fructose	Number positive	3	3	1	0		2	0
	Number negative	0	0	2	3		0	3
	Relative mean response:	++	++++	(+)	-		+++++	-
Maltose	Number positive	10	7	7	6	3	10	3
	Number negative	1	3	4	4	0	0	5
	Relative mean response:	+++	++	++	++	+++	+++++	(+)
Melibiose	Number positive	1	2	1	2		4	1
	Number negative	3	2	3	4		0	6
	Relative mean response:	(+)	++	(+)	+		+++++	(+)

TABLE I (Cont'd)

Test sugar	Molar concentration / Feeding response	Test sugar				Controls		
		0.5	0.1	0.02	0.004	0.5 M. sucrose	0.1 M. sucrose	Negative control
Sucrose	Number positive	7	8	9	6			1
	Number negative	0	0	1	0			8
	Relative mean response:	+++++	+++++	+++	++			(+)
Trehalose	Number positive	2	3	6	4		7	3
	Number negative	3	3	1	1		0	0
	Relative mean response:	(+)	+	++	++		+++++	(+)
Raffinose	Number positive		8	5	4	5	7	2
	Number negative		4	6	4	0	0	11
	Relative mean response:		+++	++	+	++++	+++++	(+)
Melezitose	Number positive	2	3	2	0	6	3	0
	Number negative	5	4	1	3	0	0	7
	Relative mean response:	(+)	+	+	-	+++	+++++	-

TABLE II

SINGLE-DISC TESTS WITH SUGARS - JACK-PINE BUDWORM

Test sugar	Molar concentration / Feeding response	Test sugar				Controls		
		0.5	0.1	0.02	0.004	0.5 M. sucrose	0.1 M. sucrose	Negative control
D-Arabinose	Number positive	2	2	3	0	4	3	0
	Number negative	2	1	0	3	0	0	4
	Relative mean response:	(+)	(+)	+	-	+++	+++++	-
L-Fucose	Number positive	6	6	6	5		6	1
	Number negative	0	0	0	0		0	6
	Relative mean response:	++++	++++	++	(+)		+++++	(+)
D-Glucose	Number positive	2	2	2	1	2		0
	Number negative	0	0	0	1	0		2
	Relative mean response:	+++++	++++	++++	(+)	+++++		-
D-Mannose	Number positive	4	0	3	1	2	3	0
	Number negative	1	5	2	4	0	0	4
	Relative mean response:	+	-	(+)	(+)	+++++	+++++	-
D-Fructose	Number positive	2	2		1	2		0
	Number negative	0	0		1	0		2
	Relative mean response:	+++	+++++		(+)	+++++		-
Sucrose	Number positive	5	5	4	5			2
	Number negative	0	0	0	0			3
	Relative mean response:	+++++	+++++	+++++	+++			(+)

Jack-pine budworm larvae exhibited some response at a concentration of 0.5 M. but there was no feeding by spruce budworm larvae at this concentration. This sugar was considerably less stimulating than 0.1 M. sucrose. There was only a very slight response to L-arabinose and at no concentration did over 50 per cent of the larvae feed on the discs.

A similar difference in the relative effectiveness of D- and L-arabinose was noted by Dethier (1955) for the blowfly, Phormia regina. The acceptance threshold for the tarsal chemoreceptors was 0.144 M. for D-arabinose compared to 0.536 M. for L-arabinose. Ito (1960), in studies with silkworm larvae, obtained a significant feeding response to L-arabinose at a concentration of ca. 0.05 M. He did not test D-arabinose. Ohnesorge (1953) found that arabinose (isomer ?) induced feeding by adults of the root weevil, Hylobius abietis L. Arabinose is non-stimulating to the honey bee (von Frisch, 1934).

D-Xylose*

Feeding responses occurred in over 50 per cent of the tests at concentrations of 0.1 M. and 0.02 M. The amount of feeding was considerably less than on discs treated with 0.1 M. sucrose.

Among insects that show a feeding response to xylose are the blowfly, P. regina (op. cit.) and the black-mound termite, Amitermes atlanticus Fuller (Skaife, 1955). No stimulating effect was found for the silkworm larva (op. cit.), nymphs of the desert locust, Schistocerca gregaria (Forsk.) (Dadd, 1960) or the honey bee (op. cit.). Ohnesorge (op. cit.) found that xylose acted as an olfactory attractant

for adults of H. abietis.

L-Rhamnose*

No feeding occurred on discs treated with L-rhamnose. Skaife (op. cit.) obtained a feeding response to this sugar with the black-mound termite and it was weakly stimulating for silkworm larvae. No behavioural response was noted with the blowfly, P. regina or the honey bee.

L-Fucose**

This was the only pentose tested on which the larvae fed to almost as great an extent as they did on 0.1 M. sucrose. This is in agreement with results obtained with P. regina adults. Fucose also stimulates feeding by the honey bee but it is not accepted by nymphs of the clear-winged grasshopper, Camnula pellucida (Scudder) (Thorsteinson, 1960).

D-Glucose**

Pronounced feeding responses were obtained with D-glucose especially at a concentration of 0.5 M. This sugar was considerably less stimulating to the spruce budworm than was sucrose. Ito (op. cit.) found the same relationship with silkworm larvae. Glucose has been demonstrated to be an effective feeding stimulant for many insects but is generally less effective than sucrose or fructose.

D-Mannose**

No appreciable feeding occurred on discs treated with

D-mannose at the four concentrations tested.

Mannose was reported to be slightly stimulating to adults of the root weevil H. abietis, nymphs of the desert locust, and the blowfly P. regina. It is non-stimulating to the silkworm larva and the honey bee and in fact is toxic to the latter (Sols et al, 1960).

D-Galactose*

D-Galactose induced a slight feeding response at all concentrations tested. Only at a concentration of 0.1 M. did over 50 per cent of the larvae exhibit a feeding response.

Galactose is acceptable to P. regina (op. cit.) and stimulates feeding to a slight extent in silkworm larvae (op. cit.) and nymphs of the clear-winged grasshopper (Thorsteinson and Jay, unpublished data). It is unacceptable to the honey bee (op. cit.).

D-Fructose**

Pronounced feeding responses were obtained with this sugar at the two highest concentrations tested. The average amount of feeding on discs treated with 0.1 M. fructose was only slightly less than that on discs treated with 0.5 M. sucrose. This sugar is acceptable to most insects that respond to sucrose but an exception in the case of the Colorado potato beetle, Leptinotarsa decemlineata (Say) was noted by Proctor (1961).

Maltose*

Maltose evoked feeding responses at all concentrations tested. It was most effective at 0.5 M. at which concentration the response

approximated that to 0.5 M. sucrose but was not as great as the response to 0.1 M. sucrose.

Melibiose*

Variable responses were obtained with this sugar. It was only weakly if at all stimulating. This compound is non-stimulating to silkworm larvae (op. cit.), the blowfly, P. regina (op. cit.) and the honey bee (op. cit.).

Sucrose**

Sucrose induced marked feeding responses at all concentrations tested. The two highest concentrations were the most effective. As has been noted earlier, this sugar is acceptable to most animals.

Trehalose*

Trehalose induced an appreciable amount of feeding at concentrations of 0.02 M. and 0.004 M. but was less acceptable at the two higher concentrations. This compound evoked positive feeding responses in P. regina (op. cit.) and the honey bee (op. cit.) but does not stimulate feeding of silkworm larvae (op. cit.) at a concentration of about 0.05 M.

Raffinose*

The larvae exhibited a marked feeding response at the highest concentration tested (0.1 M.) and a lesser response at the lower concentrations. Several insects including silkworm larvae, nymphs of the clear-winged grasshopper and P. regina adults will accept this sugar

but it does not evoke a feeding response in the honey bee.

Melezitose*

Only a very slight feeding response was obtained with this trisaccharide as was noted with silkworm larvae. It is acceptable to P. regina and the honey bee.

The most effective of the sugars tested were fucose, fructose, maltose, sucrose and raffinose. Of these only sucrose and fructose occur in appreciable quantities, in the free state, in the plant tissues on which the larvae normally feed. Maltose and raffinose have proven effective feeding stimulants for a number of insects which have been tested. The effectiveness of fucose does not appear to have been extensively investigated. It is very stimulating to the blowfly, Phormia regina, the tarsal threshold in behavioural tests being 0.087 M. (op. cit.). However, it cannot be utilized by this insect and probably is poorly utilized if at all by most insects. Glucose is only slightly stimulating to budworm larvae although it occurs in appreciable quantities in the free state in some parts of the host plant on which the larvae feed.

III. SINGLE-DISC TESTS WITH AMINO ACIDS AND AMIDES

Amino acids and amides occur in the free state in appreciable quantities in growing plant tissues. Some have been reported to be present in especially high concentration in anemophilous pollens. Virtanen and Kari (1955) reported that free proline is present in much greater amounts in such pollens than in the green parts of these plants.

They also found that the amides asparagine and glutamine were often present in appreciable quantities. Hatano (1955), who investigated the amino acids of the staminate flowers of Japanese black pine (Pinus Thunbergii Parl.) and Japanese red pine (P. densiflora Sieb. & Succ.), reported that proline, arginine and glutamic acid were those present in highest concentration in the free state. Havivi and Leibowitz (1960) found that proline, arginine and histidine were the most abundant free amino acids in the pollen of Pinus canariensis. Free amino acids and sugars constituted 5.3 per cent and 11.2 per cent, respectively, of the fresh weight of pollen of P. canariensis.

The effects of amino acids and amides on behavioural responses have been investigated with various insects. Beck and Hanec (1958) tested the effects of several amino acids on aggregation responses of first-instar European corn borer larvae. They found that L-alanine, D, L-α-amino-n-butyric acid, L-serine and L-threonine evoked positive responses while L-tryptophane, L-arginine and β-alanine had deterrent effects. The other amino acids tested had no significant influence. Tauber (1959) used 24 L-amino acids in feeding tests with first-instar nymphs of the clear-winged grasshopper. Slight positive feeding responses were noted for L-alanine, γ-amino butyric acid, L-cystine, glycine, hydroxy-L-proline and L-serine. A mixture of L-alanine, γ-amino butyric acid and L-serine evoked very active feeding responses. These three amino acids occurred in combination in a host plant extract fraction which was very stimulating to the nymphs.

In tests with larvae of the wireworms, Agriotes lineatus,

A. obscurus, A. sputor (Thorpe et al, 1946) and Ctenicera aeripennis destructor (Davis, 1961) none of the amino acids tested evoked feeding responses. Skaife (1955) obtained no positive behavioural responses to amino acids with the black-mound termite but inhibiting effects were noted at high concentrations. Dethier (1955) failed to obtain any positive feeding responses to 53 amino acids tested with the blowfly, P. regina.

The importance of certain amino acids and amides on the orientation of insects has been demonstrated by the studies of Thorpe et al (op. cit.) on wireworm larvae and of Brown and Carmichael (1961) on adults of Aedes aegypti (L.).

The selection of amino acids used in tests with spruce budworm larvae was based on the published results of analyses of coniferous pollens and the feeding responses of other insects as discussed above. The results of the tests are summarized in Table III.

Of the eight compounds tested only L-proline consistently evoked feeding responses. The amount of feeding was much less than that induced by sucrose. The stimulating effect of L-proline was of particular interest in view of its reported presence in relatively high concentration in pine pollens. In the tests with European corn borer larvae and clear-winged grasshopper nymphs referred to above this amino acid was found to be inactive.

IV. SINGLE-DISC TESTS WITH OTHER COMPOUNDS

In addition to the above-mentioned sugars and amino acids a few

other compounds were investigated in single-disc tests with the spruce budworm. Pungenin, a glucoside which occurs in mature spruce needles (Neish, 1957) was tested at concentrations of 0.2, 1.0 and 5.0 per cent. No feeding responses were obtained. Shikimic acid, another compound which is known to be present in spruce foliage in appreciable amounts, was tested at concentrations of 0.5 and 1.0 per cent and no feeding responses were noted. Meso-Inositol was tested at a concentration of 0.1 M. At this concentration it evokes feeding responses by several phytophagous insects including the silkworm (Ito, 1960), the tobacco hornworm, Protoparce sexta (Johannson) (Yamamoto and Fraenkel, 1960 (a)) and the Mexican bean beetle, Epilachna varivestis Mulsant (Lippold, 1957). No feeding responses were obtained with fifth-instar spruce budworm larvae.

TABLE III

SINGLE-DISC TESTS WITH AMINO ACIDS AND AMIDES - SPRUCE BUDWORM

Test compound	Molar concentration / Feeding response	Test compound					Controls		
		0.5	0.1	0.02	0.004	0.0008	0.5 M. sucrose	0.1 M. sucrose	Negative control
Glycine	Number positive	1	1	1	3	0	2	6	2
	Number negative	5	5	4	3	2	0	0	2
	Relative mean response:	(+)	(+)	(+)	+	-	+++	+++++	(+)
L-Alanine	Number positive		0	1	1	1	6	2	0
	Number negative		6	9	9	4	1	0	9
	Relative mean response:		-	(+)	(+)	(+)	+++++	+++++	-
L-Serine	Number positive		1	2	4	0	5	6	1
	Number negative		7	8	7	3	0	1	10
	Relative mean response:		(+)	(+)	+	-	++++	+++++	(+)
L-Arginine	Number positive	0	0	1	0			3	0
	Number negative	4	4	3	5			0	4
	Relative mean response:	-	-	(+)	-			+++++	-
γ-Amino-butyric acid	Number positive	1	2	1	3	1	3	5	2
	Number negative	5	4	6	5	2	0	0	5
	Relative mean response:	(+)	(+)	(+)	(+)	(+)	++++	+++++	(+)

TABLE III (Concl.)

Test compound	Molar concentration / Feeding response	Test compound					Controls		
		0.5	0.1	0.02	0.004	0.0008	0.5 M. sucrose	0.1 M. sucrose	Negative control
L-Proline	Number positive		4	3	0	1		2	1
	Number negative		0	1	3	0		0	2
	Relative mean response:		+	+	-	(+)		+++++	(+)
L-Asparagine	Number positive			0				5	0
	Number negative			5				0	5
	Relative mean response:			-				+++++	-
			(0.05M.)	(0.01M.)					
L-Glutamic acid	Number positive		0	0				5	0
	Number negative		4	6				0	1
	Relative mean response:		-	-				+++++	-

CHAPTER V

LARVAL FEEDING RESPONSES IN CHOICE TESTS

I. CHOICE TESTS WITH PLANT EXTRACTS

Choice Tests with Host Plant Extracts Prepared with Different Solvents

In preliminary tests discussed in the previous chapter it was established that aqueous and alcoholic host plant extracts induced larval feeding while no response was obtained with extracts prepared with certain fat solvents. Further information concerning the role of fat-soluble constituents and the relative effectiveness of aqueous and alcoholic extracts was obtained by means of choice tests.

The plant material from which the extracts were prepared was partially expanded white spruce vegetative shoots approximately one inch in length collected June 4, 1959. These were processed as described in Chapter III to yield a dry powder.

The effect of petroleum ether soluble constituents. Two 500 mg. portions of desiccated white spruce buds were extracted in micro-soxhlet extractors on water baths. One was extracted for 6 hours with 100 ml. petroleum ether and the residue was re-extracted with 100 ml. of 80 per cent ethanol for 18 hours. The other was extracted with 80 per cent ethanol only. The two ethanol extracts were concentrated to 10 ml. volume using a rotary vacuum evaporator and suspended material was removed by centrifugation.

The results of alternate-choice tests with these two extracts are summarized in Table IV. There was no significant difference in the

amount of feeding on the two sets of discs. It can be concluded that the plastid pigments and other constituents soluble in petroleum ether had neither a stimulating nor a deterrent effect.

Comparison of ethanol and aqueous extracts of white spruce buds. A 500 mg. portion of the desiccated plant material was extracted with 100 ml. of distilled water in a micro-soxhlet extractor for 24 hours. The extract was concentrated to 10 ml. by evaporation on a hot plate at low heat and suspended material was removed by centrifugation. Response to this extract and the 80 per cent ethanol extract used in the previous experiment (A of Table IV) was compared in a series of alternate choice tests.

Both extracts were analyzed for total sugars by the anthrone method. The alcoholic extract contained 4.3 grams glucose equivalents per litre and the aqueous extract 4.8 grams glucose equivalents per litre.

The results of the feeding tests are summarized in Table V. There was a considerably greater response to the alcoholic extract than to the aqueous extract and the difference was significant at the 1 per cent level. Although there was little difference in the total sugar concentration of the extracts, it is probable that hydrolysis of the sucrose occurred to a greater extent in the course of extraction with water. This qualitative difference in the sugars might have been responsible for the difference in response to the two extracts. From what is known of the relative stimulating effectiveness of sucrose and invert sugar from other studies this does not seem likely. Probably

TABLE IV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (A)
VS.
80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUD RESIDUE FOLLOWING PETROLEUM ETHER EXTRACTION (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	11	20	-9	-1.137
2	30	16	14	1.461
3	20	18	2	0.227
4	14	5	9	1.463
5	18	13	5	0.627
6	16	18	-2	-0.239
7	20	40	-20	-1.836
8	30	22	8	0.780
9	34	10	24	2.634
10	3	19	-16	-2.545
11	12	24	-12	-1.414
Σx	208	205	3	0.021
$\bar{x}$	18.909	18.636	0.273	0.002
Σx^2				25.463
				t = 0.004
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE V

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (A)
VS.
AQUEOUS EXTRACT OF WHITE SPRUCE BUDS (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	53	2	51	5.733
2	13	0	13	2.967
3	7	0	7	2.032
4	17	0	17	3.476
5	7	3	4	0.868
6	16	1	15	2.837
Σx	113	6	107	17.913
$\bar{x}$	18.833	1.000	17.833	2.985
Σx^2				66.684
				$t = 4.502^{**}$
			$t_{01}=4.032$	$t_{05}=2.571$

differences in the two extracts other than in their sugar content were involved.

Choice Tests with a Plant Extract and Sucrose

One gram of desiccated white spruce buds was extracted in a micro-soxhlet extractor with 100 ml. of 80 per cent ethanol for 8 hours. This extract was concentrated to 10 ml. and on analysis was found to contain 8.4 grams glucose equivalents per litre or slightly less than 0.05 M. in terms of glucose equivalents. The feeding response to this extract was compared with that to 0.1 M. sucrose in a series of alternate-choice tests. The results are given in Table VI.

A greater response was obtained to the extract-treated discs than to the sucrose-treated discs and the difference was significant at the 5 per cent level. In experiments to be described later, larvae permitted a choice were found to respond to the greatest extent to the highest concentration of sucrose present up to at least 0.5 M. The preference for the plant extract, although its sugar concentration was considerably lower than that of the sucrose solution, indicates that substances other than sugar are involved.

Further evidence of the possible presence in the extracts of active substances other than sugars was indicated by the influence on the effectiveness of the extracts of treatment with activated carbon. A 1.5 gram portion of activated carbon (Norit) was added to 10 ml. of an 80 per cent ethanol extract of white spruce buds. These were shaken together in a flask intermittently over a half-hour period and then the carbon was removed by centrifugation. Another 10 ml. of the extract

TABLE VI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (A)
VS.
0.1 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	14	23	-9	-1.039
2	10	5	5	0.895
3	4	1	3	0.897
4	1	3	-2	-0.646
5	8	0	8	2.208
6	30	5	25	3.178
7	16	3	13	2.191
8	5	1	4	1.120
9	23	12	11	1.312
Σx	111	53	58	10.116
$\bar{x}$	12.333	5.889	6.444	1.124
Σx^2				25.856
				$t = 2.503^*$
			$t_{01}=3.355$	$t_{05}=2.306$

was similarly treated except that activated carbon was not added. Feeding responses to the treated and untreated extracts were then determined in a series of choice tests. The results are given in Table VII.

Considerably less feeding occurred on discs treated with the extract processed with activated carbon than on those discs treated with the unprocessed extract. This suggested that some active constituents of the extract were adsorbed by the carbon. The activity could not be recovered from the carbon by elution with water.

A 0.05 M. solution of sucrose in 80 per cent ethanol was similarly treated with activated carbon and response to this was compared to that to an untreated solution (Table VIII). There was no significant difference in response to the two solutions. It seems likely, therefore, that loss of activity of the white spruce extract following treatment with carbon was due to adsorption of some active constituent(s) other than sugars.

Choice Tests with Extracts of Buds and Year-old Needles of White Spruce

As has been noted in the Introduction, spruce budworm larvae feed primarily on the newly developing foliage and staminate flowers and only to a limited extent on the previous years' growth. Physical differences between the succulent new growth and the old needles with respect to moisture content and degree of lignification may make the latter less acceptable to the larvae. The possibility that chemical differences in these tissues were involved in their relative acceptability was investigated by comparing responses to extracts of each. In these tests possible differences in response due to the physical

TABLE VII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (A)
VS.
80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS TREATED WITH ACTIVATED CARBON (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	22	0	22	4.036
2	4	0	4	1.414
3	7	0	7	2.032
4	7	4	3	0.617
5	26	1	25	3.923
6	0	0	0	0.000
7	19	14	5	0.608
8	6	0	6	1.842
9	0	0	0	0.000
10	15	5	10	1.592
11	20	4	16	2.406
12	3	1	2	0.646
Σx	129	29	100	19.117
$\bar{x}$	10.750	2.417	8.333	1.593
Σx^2				50.697
				$t = 4.074^{**}$
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE VIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE SOLUTION IN 80 PER CENT ETHANOL TREATED WITH ACTIVATED CARBON (A)
VS.
0.05 M. SUCROSE SOLUTION IN 80 PER CENT ETHANOL (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	5	8	-3	-0.571
2	21	13	8	0.963
3	6	5	1	0.204
4	5	11	-6	-1.046
5	10	1	9	1.015
6	26	12	14	1.612
7	12	8	4	0.620
Σx	85	58	27	2.797
$\bar{x}$	12.143	8.286	3.857	0.399
Σx^2				6.402
				t = 1.124
			$t_{01}=3.707$	$t_{05}=2.447$

nature of the needles were obviated.

Newly developing white spruce shoots ranging from 3/4 inch to 1 inch in length and year-old needles from the same tree were collected May 27, 1960. These were processed to give desiccated powders as described in Chapter III. A 1 gram portion of each of the desiccated powders was extracted with 80 per cent ethanol in a soxhlet extractor and each was concentrated to a final volume of 25 ml.

The total sugar concentrations of the two extracts were 2.8 grams glucose equivalents per litre for the bud extract and 1.9 grams glucose equivalents per litre for the needle extract.

The results of choice tests with these two extracts are given in Table IX. The larvae fed almost exclusively on the bud extract to the exclusion of the needle extract. As the former contained a higher concentration of total sugars this may have accounted for the difference in response. To investigate this possibility the bud extract was diluted 1:1 with 80 per cent ethanol and this diluted extract was tested against the original needle extract. The results are given in Table X.

The larvae showed a considerably greater response to the diluted bud extract than to the needle extract. The difference was significant at the 1 per cent level.

It is apparent that the relative sugar concentration of the extracts was not the primary factor determing their acceptability by the larvae. Other differences in the chemical constituents of the two extracts apparently are involved. Such chemical differences must

TABLE IX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (A)
VS.
80 PER CENT ETHANOL EXTRACT OF YEAR-OLD WHITE SPRUCE NEEDLES (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	3	0	3	1.164
2	25	4	21	2.928
3	2	0	2	0.874
4	10	0	10	2.533
5	67	7	60	5.477
6	4	1	3	0.896
7	23	0	23	4.141
Σx	134	12	122	18.013
$\bar{x}$	19.143	1.714	17.428	2.573
Σx^2				65.056
				$t = 3.858^{**}$
			$t_{01}=3.707$	$t_{05}=2.447$

TABLE X

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (DILUTED) (A)
VS.
80 PER CENT ETHANOL EXTRACT OF YEAR-OLD WHITE SPRUCE NEEDLES (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	6	0	6	1.842
2	20	1	19	3.303
3	1	0	1	0.518
4	7	3	4	0.868
5	12	7	5	0.796
6	4	0	4	1.414
7	13	0	13	2.967
8	1	0	1	0.518
9	25	5	20	2.704
10	4	0	4	1.414
11	17	3	14	2.312
12	2	0	2	0.874
13	14	0	14	3.101
14	14	6	8	1.259
15	8	8	0	0.000
16	8	1	7	1.690
Σx	156	34	122	25.580
$\bar{x}$	9.750	2.125	7.625	1.599
Σx^2				56.507
				$t = 6.271^{**}$
			$t_{01}=2.947$	$t_{05}=2.131$

account in part for the preference of the larvae for the new growth over the old needles.

Choice Tests with Extracts of Buds and Staminate Flowers of White Spruce

The importance of staminate flowers as feeding sites for larvae of the two species of budworm has been discussed in the Introduction. The relative acceptability of extracts of staminate flowers and newly developing vegetative shoots was investigated by means of choice tests similar to those described in the previous section.

Fully-developed white spruce staminate flowers and developing white spruce vegetative buds 3/4 inch to 1 inch in length were collected May 24, 1960. They were immediately deep frozen and subsequently processed to yield a dry powder as described in Chapter III.

One gram of each of the desiccated staminate flowers and white spruce buds was extracted with 80 per cent ethanol and suitably concentrated. On analysis for total free sugars the staminate flower extract was found to contain 5.4 grams glucose equivalents per litre and the bud extract 3.6 grams glucose equivalents per litre.

The results of choice tests with these two extracts are given in Table XI. Much more feeding occurred on the staminate flower extract than on the bud extract. This difference in feeding response was significant at the 1 per cent level.

As the staminate flower extract contained a higher concentration of total sugars than the bud extract it was again possible that the difference in response was due to this factor. This was investigated by diluting the staminate flower extract with an equal volume of 80

TABLE XI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE STAMINATE FLOWERS (A)
VS.
80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	61	1	60	6.617
2	29	0	29	4.724
3	50	1	49	5.881
4	26	1	25	3.923
5	54	2	52	5.801
6	25	4	21	2.928
7	12	0	12	2.828
8	25	0	25	4.342
9	65	5	60	5.748
Σx	347	14	333	42.792
$\bar{x}$	38.555	1.555	37.000	4.755
Σx^2				218.192
				$t = 6.861^{**}$
			$t_{01}=3.355$	$t_{05}=2.306$

per cent ethanol and testing this against the original vegetative bud extract. The results of this series of tests are given in Table XII.

Although the diluted staminate flower extract now had a lower total sugar concentration than the bud extract it was still preferred. The difference was again significant at the 1 per cent level.

It is apparent that there is a chemosensory basis for the preference of feeding larvae for staminate flowers over vegetative shoots. The higher concentration of sugar in the staminate flowers would be expected to make the former more acceptable. However, this is evidently not the only chemical factor involved.

II. CHOICE TESTS WITH PURE SUGAR SOLUTIONS

The Relative Effectiveness of the Principal Host Plant Sugars as Feeding Stimuli

In single-disc tests with the principal host plant sugars, D-glucose, D-fructose and sucrose, they were all found to be capable of evoking feeding responses. Their relative effectiveness was further investigated in a series of choice tests with spruce budworm larvae. Each sugar was tested against the others at concentrations of 0.1 M. and 0.5 M. Glucose at a concentration of 0.5 M. was also tested against 0.1 M. D-fructose. The results are given in Tables XIII to XIX.

The order of decreasing effectiveness of the sugars as determined by these tests was: sucrose, D-fructose and D-glucose. Sucrose and D-fructose each induced significantly more feeding than D-glucose at both concentrations. The mean response to sucrose was greater than

TABLE XII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE STAMINATE FLOWERS (DILUTED)
(A)
VS.
80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	14	11	3	0.417
2	16	27	-11	-1.182
3	6	1	5	1.324
4	9	6	3	0.533
5	36	0	36	5.334
6	35	2	33	4.377
7	22	4	18	2.622
8	21	5	16	2.292
9	21	4	17	2.516
10	16	1	15	2.837
11	30	6	24	2.974
$\sum x$	226	67	159	24.044
$\bar{x}$	20.545	6.091	14.454	2.186
$\sum x^2$				86.569
				$t = 3.932^{**}$
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE XIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.5 M. SUCROSE (A) VS. 0.5 M. D-GLUCOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	9	1	8	1.858
2	2	0	2	0.874
3	7	0	7	2.032
4	5	0	5	1.638
5	14	1	13	2.583
6	8	0	8	2.208
7	15	0	15	2.229
8	5	0	5	1.638
9	6	0	6	1.842
10	13	0	13	2.967
11	19	0	19	3.709
12	10	10	0	0.000
13	9	0	9	2.375
Σx	122	12	110	26.954
$\bar{x}$	9.385	0.923	8.462	2.073
Σx^2				67.285
				$t = 7.677^{**}$
			$t_{01}=3.055$	$t_{05}=2.179$

TABLE XIV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE (A) VS. 0.1 M. D-GLUCOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	12	0	12	2.828
2	46	1	45	5.794
3	49	6	43	4.486
4	8	0	8	2.208
5	28	0	28	4.631
6	8	0	8	2.208
7	18	5	13	1.956
8	42	4	38	4.386
9	13	5	8	1.329
Σx	224	21	203	29.828
$\bar{x}$	24.889	2.333	22.555	3.314
Σx^2				117.734
				$t = 6.473^{**}$
			$t_{01}=3.355$	$t_{05}=2.306$

TABLE XV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.5 M. SUCROSE (A) VS. 0.5 M. D-FRUCTOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	0	0	0	0.000
2	20	20	0	0.000
3	28	18	10	1.038
4	15	3	12	2.066
5	8	8	0	0.000
6	17	6	11	1.634
7	10	4	6	0.691
8	1	2	-1	-0.356
9	8	10	-2	-0.324
10	15	0	15	3.230
Σx	122	71	51	7.979
$\bar{x}$	12.200	7.100	5.100	0.798
Σx^2				19.158
				t = 2.117
			t_{01}=3.169	t_{05}=2.228

TABLE XVI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE (A) VS. 0.1 M. D-FRUCTOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	32	55	-23	-1.748
2	21	3	18	2.766
3	15	10	5	0.697
4	21	6	15	2.088
5	3	19	-16	-2.545
6	82	0	82	8.376
7	14	8	6	0.971
8	63	3	60	6.098
9	7	6	1	0.190
10	9	2	7	1.501
Σx	267	112	155	18.394
$\bar{x}$	26.700	11.200	15.500	1.839
Σx^2				132.604
				t = 1.756
			$t_{01}=3.250$	$t_{05}=2.262$

TABLE XVII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.5 M. D-FRUCTOSE (A) VS. 0.5 M. D-GLUCOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	15	8	7	1.022
2	0	0	0	0.000
3	2	0	2	0.874
4	4	4	0	0.000
5	8	0	8	2.208
6	4	0	4	1.414
7	7	1	6	1.714
8	34	0	34	5.167
9	26	0	26	4.441
10	20	3	17	2.657
11	8	1	7	1.690
Σx	128	17	111	21.187
$\bar{x}$	11.636	1.545	10.091	1.926
Σx^2				67.957
				$t = 3.883^{**}$
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE XVIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. D-FRUCTOSE (A) VS. 0.1 M. D-GLUCOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	6	1	5	1.324
2	2	0	2	0.874
3	7	2	5	1.158
4	11	12	-1	-0.145
5	4	0	4	1.414
6	49	3	46	5.165
7	9	0	9	2.375
8	16	5	11	1.717
9	5	0	5	1.638
10	7	4	3	0.618
11	20	2	18	2.947
Σx	136	29	107	19.085
$\bar{x}$	12.363	2.636	9.727	1.735
Σx^2				52.894
				$t = 4.102^{**}$
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE XIX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. D-FRUCTOSE (A) VS. 0.5 M. D-GLUCOSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	5	15	-10	-1.592
2	15	12	3	0.402
3	7	1	6	1.714
4	1	9	-8	-2.057
5	33	0	33	5.081
6	4	0	4	1.414
7	6	12	-6	-0.986
8	10	2	8	1.659
9	1	1	0	0.000
10	1	12	-11	-2.510
11	2	0	2	0.874
12	4	5	-1	-0.224
Σx	89	69	20	3.775
$\bar{x}$	7.417	5.750	1.667	0.315
Σx^2				48.519
				$t = 0.527$
			$t_{01}=3.101$	$t_{05}=2.201$

that to D-fructose at both molarities. The differences however were not significant at the 5 per cent level in either case. The difference in response to 0.5 M. D-glucose and 0.1 M. D-fructose was not significant at the 5 per cent level, indicating the considerable disparity in the relative effectiveness of these two sugars.

In investigations of feeding responses of larvae of various phytophagous Lepidoptera, the European corn borer (Beck, 1956a), the tobacco hornworm (Yamamoto and Fraenkel, 1960a) and Apamea velata Wlk. (Dethier, 1939), the same order of effectiveness of these sugars was noted. Ito (1960) found that D-glucose had little or no influence on the feeding behavior of silkworm larvae. Amongst insects in general there is considerable variation in the relative sweetness of the various sugars. The tarsi of the blowfly P. regina are more sensitive to fructose than to sucrose (Dethier, 1955). For the honey bee glucose is as stimulating as fructose (von Frisch, 1935).

As glucose, fructose and sucrose occur in combination in the host plant tissues on which the larvae feed, it was of interest to compare the feeding response to a mixture of these three sugars with that to sucrose, the most stimulating individual sugar. An equimolar mixture containing each of the sugars at a concentration of 0.03 M. was prepared and discs treated with this solution were paired with discs treated with a solution of 0.09 M. sucrose in a series of choice tests. The results are given in Table XX.

The average amount of feeding on the discs treated with the sugar mixture was approximately three times that on the sucrose-treated

TABLE XX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.03 M. D-GLUCOSE + 0.03 M. D-FRUCTOSE + 0.03 M. SUCROSE (A)
VS.
0.09 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	9	1	8	1.857
2	24	8	16	2.033
3	3	5	-2	-0.474
4	3	2	1	0.290
5	9	0	9	2.375
6	1	0	1	0.518
7	8	5	3	0.571
8	16	4	12	1.941
9	36	8	28	3.126
10	22	3	19	2.872
11	14	10	4	0.568
12	4	6	-2	-0.428
13	10	8	2	0.324
14	33	4	29	3.667
15	11	3	8	1.520
Σx	203	67	136	20.760
$\bar{x}$	13.533	4.467	9.067	1.384
Σx^2				52.281
				$t = 4.131^{**}$
			$t_{01}=2.977$	$t_{05}=2.145$

discs. This difference was significant at the 1 per cent level.

It is obvious that the effectiveness of the three sugars in mixture was not what would be expected if the effects of the individual components were merely additive. A similar synergic effect of a mixture of these three sugars on the feeding response of the honey bee was noted by Wykes (1952). Dethier et al (1956) studied the effects of various sugars, mixed together in pairs, on the tarsal threshold of the blowfly, P. regina. They found that synergism occurred with some sugar combinations and inhibition in others. An equimolar mixture of glucose and fructose was found to have a lower tarsal threshold than either of the two individual components. Ohnesorge (1953), in his investigation of the feeding behavior of the weevil, Hylobius abietis, compared the response to a mixture of glucose and fructose with that to an equivalent concentration of sucrose. The response to the mixture was as great as that to the sucrose solution, indicating that the stimulating effect of the mixture was not simply due to the additive influences of the individual components. Ito (1960) tested feeding responses of silkworm larvae to mixtures of sucrose and glucose in varying concentrations. He found that the responses to the mixtures were very similar to those to sucrose alone. The weakly-stimulating glucose was no more effective in mixture with sucrose than it was alone.

Apparently it is impossible to predict with precision the response to a sugar mixture simply on the basis of the effectiveness of the individual components. There is a considerable variation in the relative amounts of glucose, fructose and sucrose in the various tissues

of a plant at a given time. In June the leaves of various species of spruce and pine analyzed by Parker (1959) contained relatively uniform amounts of each sugar or a slightly higher proportion of sucrose than of each of glucose and fructose. By contrast Todd and Bretherick (1942) found that lodgepole pine pollen contained over 10 per cent dry weight of sucrose and no glucose or fructose. Pollen of other species of pine analyzed by these authors and by Havivi and Leibowitz (1960) and Nilsson (1956) contained much higher concentrations of sucrose than glucose or fructose. These differences in the relative proportions of sugars in the various plant tissues probably influence the acceptability of these tissues by feeding larvae provided that the effect is not masked by the presence of a more potent feeding stimulant as yet unidentified.

The Effect of Sucrose Concentration on Feeding Response

In single-disc tests both spruce and jack-pine budworm larvae exhibited feeding responses at all concentrations of sucrose tested within the range 0.004 M. to 0.5 M. Comparisons of feeding response in relation to sucrose concentration were determined in two series of choice tests. The responses of spruce budworm larvae in alternate-choice tests to 0.5 M. sucrose and 0.1 M. sucrose were compared. The results are presented in Table XXI. The average amount of feeding on discs treated with 0.5 M. sucrose was twice that on those treated with 0.1 M. This difference was significant at the 5 per cent level.

In a series of multiple-choice tests the responses of jack-pine budworm larvae to sucrose at concentrations of 0.5 M., 0.1 M., 0.02 M.

TABLE XXI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.5 M. SUCROSE (A) VS. 0.1 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	13	9	4	0.592
2	7	0	7	0.032
3	8	1	7	1.691
4	2	4	-2	-0.540
5	2	0	2	0.874
6	3	2	1	0.290
7	5	0	5	1.638
8	2	2	0	0.000
9	0	2	-2	-0.874
10	6	1	5	1.324
11	7	2	5	1.158
12	4	6	-2	-0.428
13	6	4	2	0.428
14	6	0	6	1.842
15	4	1	3	0.896
16	5	5	0	0.000
17	2	2	0	0.000
Σx	82	41	41	8.923
$\bar{x}$	4.824	2.412	2.412	0.525
Σx^2				15.454
				$t = 2.665^*$
			$t_{01}=2.921$	$t_{05}=2.120$

and 0.004 M. were compared. Numerical data are given in Table XXII. The maximum mean response occurred at the highest concentration and a progressive decrease in response was exhibited at successively lower concentrations.

The relationship between sucrose concentration and percentage of larvae exhibiting feeding responses at these concentrations is shown graphically in Figures 7 and 8. Below the level of maximum response there was a linear relationship between the logarithm of sucrose concentration and the percentage of the larvae responding at these concentrations.

III. FEEDING RESPONSES TO SUCROSE IN MIXTURE WITH CERTAIN HOST PLANT CHEMICALS AND RELATED COMPOUNDS

While it is evident that sugars play an important role in the sensory control of feeding responses of budworm larvae, the exhibited preferences are not determined solely by sugar concentration. In the following experiments the responses to various chemicals in combination with sucrose were compared with that to sucrose alone in alternate-choice tests. It was possible in this way to determine whether these compounds functioned as feeding stimulants or deterrents or were inactive. The compounds investigated were known to be present in host plant tissues or were chemically related to known plant constituents.

Feeding Responses to Sucrose-Amino Acid Mixtures

Four amino acids were tested individually in mixtures with sucrose: L-proline, L-glutamic acid, L-arginine and hydroxy-L-proline.

FIGURE 7

THE RELATIONSHIP BETWEEN THE PER CENT JACK-PINE BUDWORM LARVAE EXHIBITING A FEEDING RESPONSE AND THE MOLAR CONCENTRATION OF SUCROSE IN A SERIES OF MULTIPLE-CHOICE TESTS

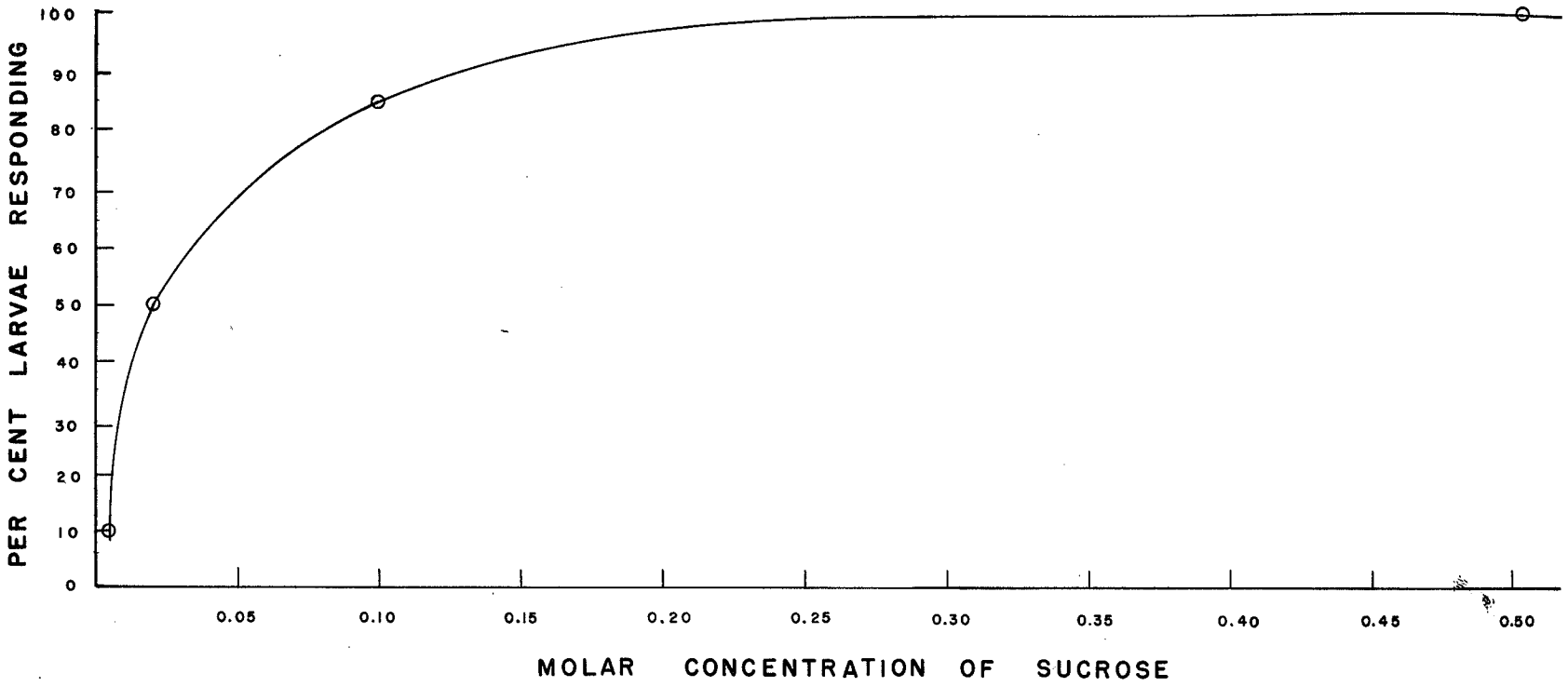
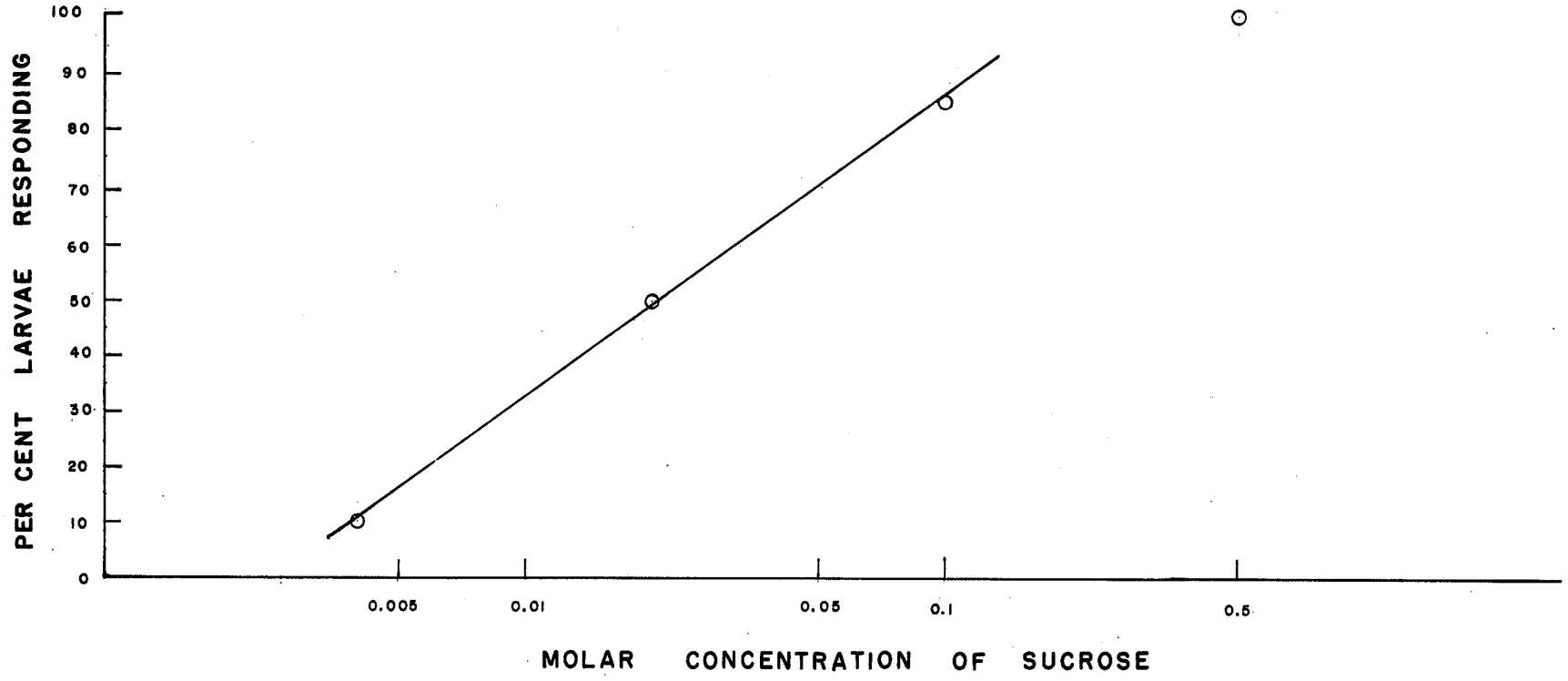

PER CENT LARVAE RESPONDING
100
90
80
70
60
50
40
30
20
10
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
MOLAR CONCENTRATION OF SUCROSE

FIGURE 8

THE RELATIONSHIP BETWEEN THE PER CENT JACK-PINE BUDWORM LARVAE EXHIBITING A FEEDING RESPONSE AND THE MOLAR CONCENTRATION OF SUCROSE (PLOTTED ON A LOG SCALE) IN A SERIES OF MULTIPLE CHOICE TESTS


PER CENT LARVAE RESPONDING
100
90
80
70
60
50
40
30
20
10
0
0.005
0.01
0.05
0.1
0.5
MOLAR CONCENTRATION OF SUCROSE

The first three have been reported to be present in appreciable quantities in pine pollen. In the single-disc tests, L-proline was the only amino acid tested which evoked consistent feeding responses. This compound was tested with both spruce and jack-pine budworm larvae and the remaining compounds with the spruce budworm only. The ratio of molar concentrations of amino acid to sucrose in the mixtures was 1 to 5. Available data from the literature (viz. Havivi and Leibowitz, 1960) indicate this proportion may closely approximate that occurring in pine pollen. The results of these tests are given in Tables XXIII to XXVIII.

There was a markedly greater response to the sucrose-proline mixtures than to sucrose alone at both concentrations tested and similar effects were noted with both budworm species. The differences in response were significant at the 1 per cent level. As sucrose and L-proline are two of the principal compounds present in the pollen of conifers the stimulating effectiveness of this mixture would appear to be of considerable significance in relation to the feeding behavior of budworm larvae.

The mixtures containing hydroxy-L-proline and L-glutamic acid also evoked greater responses than sucrose alone and the differences were significant at the 1 per cent and 5 per cent levels respectively. The sucrose - L-arginine mixture was less acceptable than sucrose alone, indicating a possible deterrent effect of this amino acid at the concentration tested. Statistically the difference was not significant at the 5 per cent level.

TABLE XXIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 0.01 M. L-PROLINE (A) VS. 0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	9	0	9	2.375
2	13	11	2	0.283
3	10	5	5	0.895
4	31	2	29	4.032
5	7	11	-4	-0.652
6	35	3	32	4.087
7	16	3	13	2.191
8	38	5	33	3.860
9	3	0	3	1.164
10	15	10	5	0.697
11	9	1	8	1.857
12	12	1	11	2.310
13	15	2	13	2.356
14	31	0	31	4.906
Σx	244	54	190	30.361
$\bar{x}$	17.429	3.857	13.571	2.169
Σx^2				99.852
				$t = 5.021^{**}$
			$t_{01}=3.012$	$t_{05}=2.160$

TABLE XXIV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE + 0.02 M. L-PROLINE (A) VS. 0.1 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	43	10	33	3.355
2	12	0	12	2.828
3	54	0	54	6.675
4	29	1	28	4.406
5	24	0	24	4.242
6	18	0	18	3.594
7	37	1	36	4.899
8	29	0	29	4.724
9	40	1	39	5.139
10	2	1	1	0.356
11	4	1	3	0.896
12	15	1	14	2.712
Σx	307	16	291	43.826
$\bar{x}$	25.583	1.333	24.250	3.652
Σx^2				195.144
				$t = 7.077^{**}$
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE XXV

ALTERNATE-CHOICE TESTS - JACK-PINE BUDWORM

0.1 M. D-SUCROSE + 0.02 M. L-PROLINE (A)
VS.
0.1 M. D-SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	63	5	58	5.624
2	31	8	23	2.698
3	10	20	-10	-1.288
4	37	1	36	4.899
5	9	5	4	0.737
6	21	29	-8	-0.794
7	52	19	33	2.830
8	72	4	68	6.394
9	62	10	52	4.666
10	54	10	44	4.142
11	6	16	-10	-1.513
12	7	0	7	2.032
13	56	7	49	4.778
14	61	8	53	4.927
15	6	8	-2	-0.366
Σx	547	150	397	39.766
$\bar{x}$	36.467	10.000	26.467	2.651
Σx^2				207.218
				$t = 3.809^{**}$
			$t_{01}=2.977$	$t_{05}=2.145$

TABLE XXVI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE + 0.02 M. HYDROXY-L-PROLINE (A)
VS.
0.1 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	7	3	4	0.868
2	21	7	14	1.898
3	12	0	12	2.829
4	51	9	42	4.094
5	11	0	11	2.684
6	22	2	20	3.162
7	1	0	1	0.518
8	22	0	22	4.036
9	7	1	6	1.514
10	11	1	10	2.166
11	5	1	4	1.120
12	10	1	9	2.015
13	1	1	0	0.000
14	3	2	1	0.290
15	11	1	10	2.166
16	27	5	22	2.899
17	11	1	10	2.166
Σx	233	35	198	34.425
$\bar{x}$	13.706	2.059	11.647	2.025
Σx^2				93.049
				$t = 6.911^{**}$
			$t_{01}=2.921$	$t_{05}=2.120$

TABLE XXVII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE + 0.02 M. L-GLUTAMIC ACID (A)
VS.
0.1 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	9	13	-4	-0.592
2	13	3	10	1.803
3	5	1	4	1.120
4	7	2	5	1.158
5	2	5	-3	-0.764
6	5	2	3	0.764
7	13	5	8	1.329
8	8	1	7	1.691
9	2	1	1	0.356
Σx	64	33	31	6.865
$\bar{x}$	7.111	3.667	3.444	0.763
Σx^2				12.116
				$t = 2.461^*$
			$t_{01}=3.355$	$t_{05}=2.306$

TABLE XXVIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.1 M. SUCROSE (A)
VS.
0.1 M. SUCROSE + 0.02 M. L-ARGININE (B)

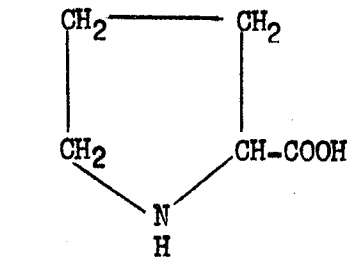
Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	5	2	3	0.764
2	2	7	-5	-1.158
3	29	0	29	4.724
4	4	0	4	1.414
5	1	0	1	0.518
6	7	1	6	1.514
7	2	3	-1	-0.290
8	0	9	-9	-2.375
9	7	1	6	1.514
10	2	2	0	0.000
11	1	0	1	0.518
12	9	2	7	1.501
13	6	2	4	0.968
14	51	0	51	6.469
15	1	2	-1	-0.356
16	1	1	0	0.000
17	0	4	-4	-1.414
Σx	128	36	92	14.311
$\bar{x}$	7.529	2.118	5.411	0.842
Σx^2				84.250
				t = 1.635
			$t_{01}=2.921$	$t_{05}=2.120$

Whereas L-glutamic acid in combination with sucrose was more effective than sucrose alone this amino acid was inactive in itself in single-disc tests. L-Proline which was active in single-disc tests was relatively more effective in combination with sucrose than was L-glutamic acid.

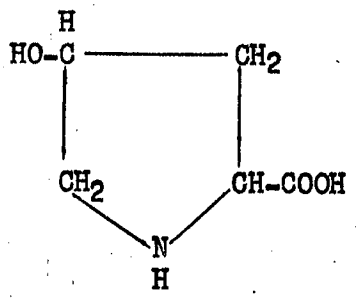
The similarities in the responses to L-proline, hydroxy-L-proline and L-glutamic acid are of interest in view of the structural affinities of these compounds (Fig. 9). Both hydroxy - proline and glutamic acid can be considered to be derived from proline by oxidation and metabolic pathways for such transformations have been established for some organisms.

An enhanced response to amino acids and sucrose similar to that noted in these experiments was reported by Tauber (1959) in his investigations with the clear-winged grasshopper. He found that a mixture of alanine, serine, γ-amino-butyric acid and sucrose was more stimulating to nymphs than any one of the three amino acids in mixture with sucrose. Beck and Hanec (1958) in studies with European corn borer larvae tested certain stimulating amino acids in individual combinations with glucose. They concluded that the responses to the mixtures were simply due to the additive effects of the components and that no synergistic effects or other interactions were involved. On this basis they postulated the existence of separate sense receptors which are stimulated by the two types of compounds.

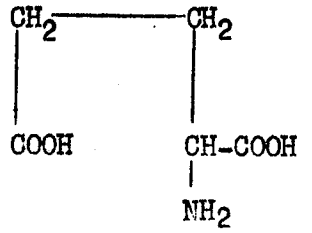
Feeding Responses to Sucrose - Shikimic Acid and Sucrose - D-Quinic Acid Mixtures



Proline



Hydroxy-proline



Glutamic acid

FIGURE 9

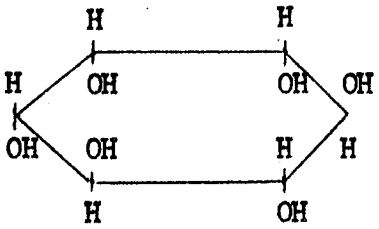
STRUCTURAL FORMULAE FOR PROLINE, HYDROXY-PROLINE, AND GLUTAMIC ACID

Shikimic acid has been detected in the foliage of a wide variety of gymnosperms (Hattori et al, 1954). Manskaja and Kodina (1958) reported it to be more abundant in newly developing tissues than in lignifying tissues. Neish (1958) found it to be the major organic acid in the needles of white and Colorado spruce. It was present in both new growth and mature needles and was readily extractable by 80 per cent ethanol. In early new growth of Colorado spruce it constituted 1.3 per cent of the dry weight compared to 2 per cent for sucrose. Neish (l.c.) was unable to isolate quinic acid from spruce needles but he detected a compound with similar properties on paper chromatograms.

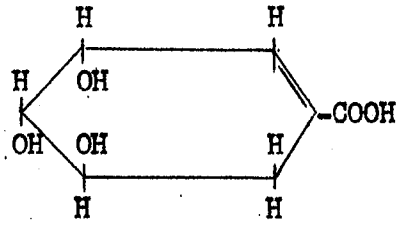
Both of these compounds are structural derivatives of the six-carbon cyclic sugar alcohol, m-inositol (Fig. 10). The chemical relationship between these compounds is of interest because several insects, including some leaf-eating larvae, have been demonstrated to exhibit feeding responses to m-inositol at a concentration of 0.1 M. No such response was obtained with spruce budworm larvae in single-disc tests, nor did they respond to shikimic acid.

In choice tests with spruce budworm larvae shikimic acid was tested in combination with 0.05 M. sucrose at concentrations of 0.2, 0.4, 0.8 and 1 per cent. D-Quinic acid was similarly tested at concentrations of 0.4 and 1 per cent. The results are presented in Tables XXIX to XXXIV.

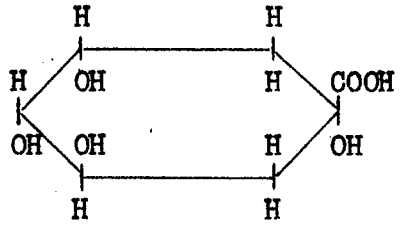
A significant stimulating effect was detected with shikimic acid at a concentration of 1 per cent but not at the lower concentrations. Quinic acid appeared to enhance the response slightly at a concentration



m-Inositol



Shikimic acid



D-Quinic acid

FIGURE 10

STRUCTURAL FORMULAE FOR M-INOSITOL, SHIKIMIC ACID AND D-QUINIC ACID

TABLE XXIX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 0.2% SHIKIMIC ACID (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	26	32	-6	-0.553
2	32	14	18	1.893
3	2	38	-36	-4.624
4	7	4	3	0.618
5	27	11	16	1.853
6	4	4	0	0.000
7	12	3	9	1.665
Σx	110	106	4	0.852
$\bar{x}$	15.714	15.143	0.571	0.122
Σx^2				31.858
				t = 0.140
			$t_{01}=3.707$	$t_{05}=2.447$

TABLE XXX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A) VS. 0.05 M. SUCROSE + 0.4% SHIKIMIC ACID (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	18	7	11	1.562
2	6	18	-12	-1.752
3	20	8	12	1.612
4	27	40	-13	-1.120
5	31	14	17	1.805
6	1	6	-5	-1.324
7	30	7	23	2.784
8	23	34	-11	-1.026
9	0	0	0	0.000
10	4	16	-12	-1.941
11	0	2	-2	-0.874
12	14	3	11	1.937
13	8	10	-2	-0.324
14	15	6	9	1.378
15	1	2	-1	-0.356
16	13	17	-4	-0.509
17	16	21	-5	-0.575
Σx	227	211	16	1.277
$\bar{x}$	13.353	12.412	0.941	0.075
Σx^2				34.180
				t = 0.212
			$t_{01}=2.921$	$t_{05}=2.120$

TABLE XXXI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 0.8% SHIKIMIC ACID (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	7	15	-8	-1.198
2	36	20	16	1.514
3	55	3	52	5.578
4	7	6	1	0.190
5	51	14	37	3.368
6	0	9	-9	-2.375
7	12	0	12	2.829
8	15	20	-5	-0.591
9	18	27	-9	-0.943
10	8	13	-5	-0.758
Σx	209	127	82	7.614
$\bar{x}$	20.900	12.700	8.200	0.761
Σx^2				61.678
				t = 0.966
			$t_{01}=3.250$	$t_{05}=2.262$

TABLE XXXII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 1% SHIKIMIC ACID (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	25	4	21	2.928
2	7	6	1	0.190
3	3	1	2	0.646
4	24	7	17	2.210
5	4	1	3	0.896
6	22	10	12	1.503
7	33	8	25	2.873
8	14	1	13	2.583
9	3	2	1	0.290
10	8	0	8	2.208
11	3	3	0	0.000
Σx	146	43	103	16.327
$\bar{x}$	13.273	3.909	9.364	1.484
Σx^2				36.858
				$t = 4.377^{**}$
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE XXXIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 0.4% D-QUINIC ACID (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	22	14	8	0.935
2	28	20	8	0.811
3	6	2	4	0.968
4	5	7	-2	-0.394
5	34	34	0	0.000
6	21	15	6	0.700
7	23	22	1	0.105
8	36	10	26	2.802
9	2	2	0	0.000
Σx	177	126	51	5.927
$\bar{x}$	19.667	14.000	5.667	0.659
Σx^2				10.976
				t = 2.105
			$t_{01}=3.355$	$t_{05}=2.306$

TABLE XXXIV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A) VS. 0.05 M. SUCROSE + 1% D-QUINIC ACID (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	15	9	6	0.855
2	23	11	12	1.457
3	1	0	1	0.518
4	0	2	-2	-0.874
5	8	12	-4	-0.620
6	2	3	-1	-0.290
7	15	2	13	2.356
8	19	4	15	2.295
9	7	5	2	0.394
10	2	1	1	0.356
11	2	5	-3	-0.764
12	5	4	1	0.224
Σx	99	58	41	5.907
$\bar{x}$	8.250	4.833	3.417	0.917
Σx^2				16.088
				t = 1.198
			$t_{01}=3.106$	$t_{05}=2.201$

of 0.4 per cent and to deter response at 1 per cent. However, in neither case were these differences statistically significant at the 5 per cent level.

The ratio of shikimic acid to sucrose at the concentrations found effective in these tests is only slightly greater than that in the new growth of Colorado spruce as reported by Neish (l.c.). Although the concentration of shikimic acid in the new growth is somewhat higher than in the previous year's needles it is doubtful that this difference in itself is sufficient to account for any differential feeding response. The dissimilarity in the response to shikimic and quinic acids, despite their structural affinity, indicates the high degree of specificity involved.

The Effects of Pungenin, Its Aglucone and Related Compounds on Feeding Response

Tests with pungenin. Neish (1957, 1958) isolated and identified a glycoside from the leafy twigs of white spruce and Colorado spruce to which he gave the name pungenin. Subsequent investigations by von Rudolff (Neish, 1961) demonstrated that it was present in black spruce but not in jack pine or Scots pine. It is not known whether it occurs in balsam fir.

This water-soluble compound is a monoglucoside of 3, 4-dihydroxy-acetophenone (3 - glucopyranosyloxy - 4 - hydroxyacetophenone). It constitutes about five per cent of the dry weight of winter needles and decreases to about half this concentration in midsummer. Neish was unable to detect its presence in newly-developing spruce needles but it

appeared as lignification proceeded and the needles matured.

Spruce budworm larvae showed no feeding response when pungenin was tested alone in concentrations ranging from 0.2 per cent to 5 per cent in single-disc tests. The effect of pungenin on response to sucrose was investigated in alternate-choice tests with both spruce and jack-pine budworm larvae.

With 0.05 M. sucrose and 0.2 per cent pungenin (Table XXXV) the average feeding response of spruce budworm larvae was greater on the discs containing pungenin. However this difference was not significant at the 5 per cent level. At the same sucrose concentration, 1 per cent pungenin had a significant deterrent effect on the responses of both spruce and jack-pine budworm larvae (Tables XXXVI and XXXVII). The effect on jack-pine budworm larvae is of interest in view of the fact that pungenin does not occur in jack pine. This reaction to pungenin may be a residual trait derived from a common ancestry with the spruce budworm. It is also possible that a related glucoside occurs in jack pine foliage. At a lower concentration of sucrose, 0.02 M., 0.4 per cent pungenin had no significant influence on the feeding behavior of spruce budworm larvae (Table XXXVIII) but the deterrent effect of 0.8 per cent pungenin was significant at the 1 per cent level (Table XXXIX).

Figures on the relative amounts of pungenin and sucrose in the various test solutions and in year-old spruce needles (Neish, 1958), at the time of larval feeding, are given in Table XL. Although the ratios of pungenin to sucrose were identical in the tests recorded in Tables XXXVI and XXXVIII the effects on feeding response were not the

TABLE XXXV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 0.2% PUNGENIN (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	14	2	12	2.227
2	3	3	0	0.000
3	6	10	-4	-0.691
4	17	6	11	1.634
5	28	39	-11	-0.946
6	3	8	-5	-1.045
7	19	6	13	1.867
8	34	23	11	1.026
9	80	17	63	4.789
10	15	14	1	0.129
11	39	16	23	2.223
12	15	5	10	1.592
Σx	273	149	124	12.805
$\bar{x}$	22.750	12.417	10.333	1.067
Σx^2				45.059
				t = 2.186
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE XXXVI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A)
VS.
0.05 M. SUCROSE + 1% PUNGENIN (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	22	6	16	2.194
2	33	7	26	3.049
3	35	5	30	3.613
4	22	3	19	2.872
5	44	15	29	2.734
6	54	9	45	4.300
7	40	15	25	2.427
8	16	5	11	1.717
9	12	29	-17	-1.895
10	15	2	13	2.356
11	32	2	30	4.120
12	28	5	23	2.994
13	47	20	27	2.364
14	50	19	31	2.690
15	6	0	6	1.842
16	9	2	7	1.501
17	10	0	10	2.533
18	26	0	26	4.441
Σx	501	144	357	45.852
$\bar{x}$	27.833	8.000	19.833	2.547
Σx^2				149.905
				$t = 7.765^{**}$
			$t_{01}=2.898$	$t_{05}=2.110$

TABLE XXXVII

ALTERNATE-CHOICE TESTS - JACK-PINE BUDWORM

0.05 M. SUCROSE (A) VS. 0.05 M. SUCROSE + 1% PUNGENIN (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	47	9	38	3.810
2	25	8	17	2.134
3	18	1	17	3.076
4	7	0	7	2.032
5	0	2	-2	-0.874
6	21	0	21	3.930
7	16	4	12	1.941
8	24	6	18	2.400
9	1	0	1	0.518
10	22	4	18	2.622
11	9	13	-4	-0.592
12	0	2	-2	-0.874
Σx	190	49	141	20.123
$\bar{x}$	15.833	4.083	11.750	1.677
Σx^2				66.655
				$t = 3.361^{**}$
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE XXXVIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.02 M. SUCROSE (A)
VS.
0.02 M. SUCROSE + 0.4% PUNGENIN (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	4	24	-20	-2.828
2	11	4	7	1.270
3	18	11	7	0.910
4	6	19	-13	-1.867
5	8	2	6	1.334
6	7	12	-5	-0.796
7	18	2	16	2.720
8	21	12	9	1.102
9	6	2	4	0.968
10	9	16	-7	-0.980
11	10	3	7	1.369
Σx	118	107	11	3.202
$\bar{x}$	10.727	9.727	1.000	0.291
Σx^2				28.722
				t = 0.579
			$t_{01}=3.169$	$t_{05}=2.228$

TABLE XXXIX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.02 M. SUCROSE (A)
VS.
0.02 M. SUCROSE + 0.8% PUNGENIN (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	23	3	20	2.977
2	7	0	7	2.032
3	5	0	5	1.638
4	4	0	4	1.414
5	12	0	12	2.829
6	6	0	6	1.842
7	5	0	5	1.638
8	15	4	11	1.816
9	25	5	20	2.704
10	12	1	11	2.310
11	9	1	8	1.857
12	6	0	6	1.842
13	7	0	7	2.032
Σx	136	14	122	26.931
$\bar{x}$	10.461	1.077	9.384	2.072
Σx^2				58.669
				$t = 15.463^{**}$
			$t_{01}=3.055$	$t_{05}=2.179$

TABLE XL

THE RELATIVE AMOUNTS OF PUNGENIN AND SUCROSE IN VARIOUS TEST SOLUTIONS AND IN YEAR-OLD COLORADO SPRUCE NEEDLES

	Per cent concentration of pungenin	Molar concentration of sucrose	Ratio Pungenin:Sucrose in terms of weight per unit volume of solution
Choice Tests Table XXXV	0.2	0.05	1 : 8.5
Choice Tests Table XXXVI	1	0.05	1 : 1.7
Choice Tests Table XXXVIII	0.4	0.02	1 : 1.7
Choice Tests Table XXXIX	0.8	0.02	1 : 0.9
	Per cent dry weight of needles		Ratio by weight
Neish's analysis of year-old Colorado spruce needles, collected June 11, 1956	1.8	3.3	1 : 1.8

same. It would appear that the deterrent effect of pungenin is not determined simply by its concentration relative to that of sucrose but also by the absolute quantity of pungenin present. As the moisture content of mature spruce needles is quite low it can be deduced from Neish's data that the concentration of pungenin in the fresh year-old spruce needles would be of the order of that causing a deterrent effect in these tests.

Tests with the aglucone. In view of the results obtained with pungenin it was of interest to investigate the influence of the aglucone on feeding response. The products of hydrolysis of pungenin are 3, 4-dihydroxyacetophenone and D-glucose and the aglucone constitutes slightly less than half the weight of the intact molecule. To compare its effectiveness on the response of spruce budworm larvae to 0.05 M. sucrose with that of 1 per cent pungenin it was tested at a concentration of 0.5 per cent (Table XLI). At this concentration there was some evidence of a deterrent effect but the difference was not significant at the 5 per cent level. This is in decided contrast to the results obtained with 1 per cent pungenin and indicates that the intact glucoside molecule was a more effective deterrent than its aglucone. The effects of the aglucone at a concentration of 1 per cent were investigated and the results are presented in Table XLII. At this concentration the compound had a very marked deterrent influence.

Tests with compounds structurally related to the aglucone. To investigate the specificity of the effect of 3, 4-dihydroxyacetophenone some related compounds were tested in the same manner at a concentration of 1 per cent. The structural relationships of these compounds are in-

TABLE XLI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A)
VS.
0.05 M. SUCROSE + 0.5% 3, 4-DIHYDROXYACETOPHENONE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	19	3	16	2.545
2	4	8	-4	-0.794
3	17	7	10	1.444
4	9	10	-1	-0.158
5	10	1	9	2.015
6	3	3	0	0.000
7	2	0	2	0.874
8	14	18	-4	-0.493
9	19	4	15	2.295
10	13	8	5	0.759
11	0	2	-2	-0.874
12	3	1	2	0.646
$\sum x$	113	65	48	8.259
$\bar{x}$	9.417	5.417	4.000	0.688
$\sum x^2$				21.309
				t = 2.012
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE XLII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A) VS. 0.05 M. SUCROSE + 1% 3, 4-DIHYDROXYACETOPHENONE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	2	0	2	0.874
2	1	0	1	0.518
3	12	0	12	2.828
4	3	0	3	1.164
5	23	1	22	3.623
6	46	0	46	6.112
7	2	0	2	0.874
8	14	0	14	3.101
9	17	1	16	2.958
10	32	0	32	4.994
11	33	1	32	4.563
12	5	0	5	1.638
13	25	1	24	3.824
14	10	0	10	2.533
15	23	0	23	4.141
16	17	1	16	2.958
17	9	2	7	1.501
18	5	0	5	1.638
19	1	0	1	0.518
20	15	0	15	3.230
21	15	0	15	3.230
22	6	0	6	1.842
Σx	316	7	309	58.662
$\bar{x}$	14.364	0.318	14.046	2.666
Σx^2				204.841
				$t = 8.228^{**}$
			$t_{01}=2.831$	$t_{05}=2.080$

dicated in Fig. 11.

1. 2, 4-Dihydroxyacetophenone (Table XLIII). Structurally this compound differs from the aglucone only in the relative positions of the hydroxyl groups. A deterrent effect was apparent with this compound and the difference in response was significant at the 5 per cent level. The compound was considerably less effective than the aglucone.

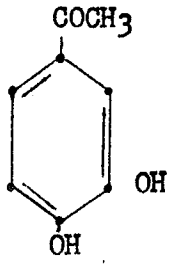
2. 2, 6-Dihydroxyacetophenone (Table XLIV). This compound also differs from the aglucone only in the position of the hydroxyl groups. It had a greater deterrent effect than the preceding compound. The difference in response to the discs containing this compound and those containing sucrose alone was significant at the 1 per cent level.

The relative deterrency of these two compounds and the aglucone indicates that the location of the hydroxyl groups in the benzene ring influences the effectiveness of the molecule. Other aromatic compounds possessing the ortho-dihydroxy grouping but lacking the carbonyl group were also tested to determine their effects on feeding response.

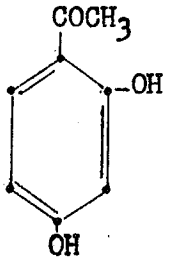
3. 3, 4-Dihydroxybenzoic acid (= Protocatechuic acid) (Table XLV). This compound differs from the aglucone in that the carbonyl group is replaced by a carboxyl group. It had a significant deterrent effect but was somewhat less effective than 3, 4-dihydroxyacetophenone.

Protocatechuic acid is commonly found in plants (Bonner, 1950) in both the free and combined states. No references to its occurrence in the foliage of conifers were found.

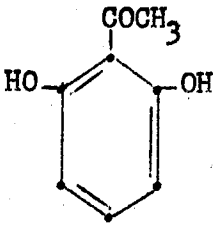
4. Catechol (= Pyrocatechol) (Table XLVI). This orthodihydroxy derivative of benzene had no significant effect on the response to



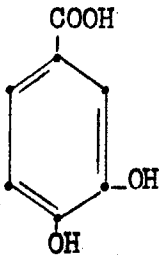
3, 4-Dihydroxyacetophenone



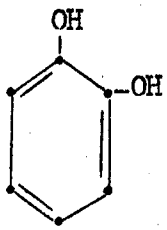
2, 4-Dihydroxyacetophenone



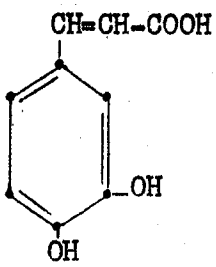
2, 6-Dihydroxyacetophenone



3, 4-Dihydroxybenzoic acid
(protocatechuic acid)



Pyrocatechol



3, 4-Dihydroxycinnamic acid
(Caffeic acid)

FIGURE 11

STRUCTURAL FORMULAE FOR THE AGLUCONE OF PUNGENIN
AND SOME RELATED COMPOUNDS

TABLE XLIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A)
VS.
0.05 M. SUCROSE + 1% 2, 4-DIHYDROXYACETOPHENONE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	20	16	4	0.466
2	11	3	8	1.520
3	20	8	12	1.613
4	3	1	2	0.646
5	3	6	-3	-0.678
6	1	3	-2	-0.646
7	3	3	0	0.000
8	9	0	9	2.375
9	6	4	2	0.428
10	35	6	29	3.409
11	5	0	5	1.638
12	6	7	-1	-0.190
13	13	20	-7	-0.854
14	20	7	13	1.789
15	10	9	1	0.158
Σx	165	93	72	11.674
$\bar{x}$	11.000	6.200	4.800	0.778
Σx^2				30.543
				$t = 2.439^*$
			$t_{01}=2.977$	$t_{05}=2.145$

TABLE XLIV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A)
VS.
0.05 M. SUCROSE + 1% 2, 6-DIHYDROXYACETOPHENONE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	8	0	8	2.208
2	37	1	36	4.899
3	21	0	21	3.930
4	2	0	2	0.874
5	25	3	22	3.178
6	61	2	59	6.261
7	14	1	13	2.583
8	14	9	5	0.726
9	14	2	12	2.227
10	13	1	12	2.449
11	16	0	16	3.355
12	8	1	7	1.690
Σx	233	20	213	34.380
$\bar{x}$	19.417	1.667	17.750	2.865
Σx^2				126.652
				$t = 6.215^{**}$
			$t_{01}=3.106$	$t_{05}=2.201$

TABLE XLV

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE (A)
VS.
0.05 M. SUCROSE + 1% 3, 4-DIHYDROXYBENZOIC ACID (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	10	1	9	2.015
2	11	3	8	1.520
3	5	8	-3	-0.571
4	3	0	3	1.164
5	8	1	7	1.691
6	1	1	0	0.000
7	3	0	3	1.164
Σx	41	14	27	6.983
$\bar{x}$	5.857	2.000	3.857	0.998
Σx^2				12.266
				$t = 2.811^*$
			$t_{01}=3.707$	$t_{05}=2.447$

TABLE XLVI

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 1% CATECHOL (A) VS. 0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	0	1	-1	-0.518
2	26	32	-6	-0.553
3	15	23	-8	-0.911
4	14	0	14	3.101
5	0	3	-3	-1.164
6	29	0	29	4.724
7	0	19	-19	-3.709
8	0	6	-6	-1.842
9	3	2	1	0.290
10	6	3	3	0.678
11	22	2	20	3.162
Σx	115	91	24	3.258
$\bar{x}$	10.455	8.273	2.182	0.296
Σx^2				62.383
				t = 0.396
			t_{01}=3.169	t_{05}=2.228

sucrose. Thus, while the positioning of the hydroxyl groups had an influence on the relative effectiveness of the acetophenone derivatives, the deterrent effect is not specifically due to the ortho-dihydroxy group.

5. 3, 4-Dihydroxycinnamic acid (= Caffeic acid) (Table XLVII). This aromatic acid was found to have a stimulating effect on response at the concentration used in these tests. The difference in response due to this compound was significant at the 1 per cent level.

Neish (1959), in a study of the biosynthesis of pungenin in Colorado spruce, found that caffeic acid was one of the best precursors of pungenin of the various compounds investigated. As pungenin is formed during the course of growth and lignification of the needles it is possible that caffeic acid may be present in appreciable quantities in the new vegetative shoots. If this is the case it would be expected to contribute to the stimulating effectiveness of the extracts prepared from this material.

IV. A FURTHER EXPERIMENT ON THE ROLE OF PUNGENIN

In the tests with host-plant extracts described earlier it was demonstrated that an extract of partially expanded vegetative white spruce buds evoked a greater feeding response than an extract of year-old needles. This was true even when the bud extract was diluted to reduce its sugar content to slightly less than that of the needle extract. A significant difference in the chemistry of the buds and the needles, which has been previously noted, is the relatively high concentration of

TABLE XLVII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

0.05 M. SUCROSE + 1% CAFFEIC ACID (A)
VS.
0.05 M. SUCROSE (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	18	14	4	0.493
2	13	2	11	2.093
3	5	0	5	1.638
4	50	11	39	3.715
5	16	7	9	1.323
6	3	4	-1	-0.250
7	6	0	6	1.842
8	32	1	31	4.476
9	8	14	-6	-0.893
10	29	16	13	1.369
11	24	9	15	1.867
Σx	204	78	126	17.673
$\bar{x}$	18.545	7.091	11.454	1.606
Σx^2				52.506
				$t = 3.432^{**}$
			$t_{01}=3.169$	$t_{05}=2.228$

pungenin in the latter. In view of the demonstrated deterrent effect of pungenin on the response to sucrose it was of interest to investigate the relation of this compound to the effectiveness of the extracts of the two tissues.

Extracts of partially expanded white spruce shoots and year-old needles were prepared from the same desiccated plant material and in the same manner as described on page 56. The sugar concentrations of the bud and needle extracts were 2.8 and 1.9 grams glucose equivalents per litre respectively.

The bud extract was diluted 1:1 with 80 per cent ethanol and 30 mg. of pungenin were added to 3 ml. of this diluted extract. The response to discs treated with this supplemented extract was then compared to the response to discs treated with the needle extract in a series of alternate choice tests. In a parallel set of tests the needle extract was tested against the diluted bud extract to which no pungenin had been added. The results of these two series of tests are given in Tables XLVIII and XLVIX respectively.

Comparison of the results reveals that the addition of pungenin to the bud extract considerably reduced its acceptance by the larvae. However the amount of pungenin added to the bud extract to produce this deterrent effect was over ten times that calculated to be present in the needle extract on the basis of published data (Neish, 1958). Therefore a greater difference in the response to the two extracts than that noted in Table XLVIII would be expected if pungenin content was the only factor influencing the relative acceptability of the two extracts. It is

TABLE XLVIII

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

DILUTED ETHANOL EXTRACT OF WHITE SPRUCE BUDS + 1% PUNGENIN (A)
VS.
ETHANOL EXTRACT OF YEAR-OLD WHITE SPRUCE NEEDLES (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	0	9	-9	-2.375
2	9	25	-16	-1.967
3	6	6	0	0.000
4	11	10	1	0.151
5	20	8	12	1.612
6	14	20	-6	-0.720
7	2	2	0	0.000
8	3	9	-6	-1.211
9	56	40	16	1.153
10	29	20	9	0.903
11	21	36	-15	-1.404
12	7	4	3	0.618
13	9	18	-9	-1.219
14	38	12	26	2.670
15	17	8	9	1.268
16	41	7	34	3.703
Σx	283	234	49	3.182
$\bar{x}$	17.688	14.625	3.063	0.199
Σx^2				42.549
				t = 0.476
			$t_{01}=2.947$	$t_{05}=2.131$

TABLE XLIX

ALTERNATE-CHOICE TESTS - SPRUCE BUDWORM

80 PER CENT ETHANOL EXTRACT OF WHITE SPRUCE BUDS (DILUTED) (A)
VS.
80 PER CENT ETHANOL EXTRACT OF YEAR-OLD WHITE SPRUCE NEEDLES (B)

Replicate	Per cent disc area eaten		Difference	Transformed difference
	A	B	A - B	$\sqrt{A+\frac{1}{2}} - \sqrt{B+\frac{1}{2}}$
1	48	14	34	3.156
2	17	0	17	3.476
3	18	3	15	2.430
4	11	5	6	1.046
Σx	94	22	72	10.108
$\bar{x}$	23.500	5.500	18.000	2.527
Σx^2				29.042
				$t = 4.688^*$
			$t_{01}=5.841$	$t_{05}=3.182$

possible that there is more than one deterrent factor in the old needles. Also the buds may contain stimulating constituents that are absent from or present in lower concentration in the old needles. It was suggested earlier that caffeic acid may be a factor that fits in the latter category.

CHAPTER VI

DISCUSSION

The Ecological Background of the Problem

Various aspects of the feeding behavior of spruce budworm and jack-pine budworm larvae have received attention from ecologists. As referred to in the introduction, these studies have revealed a number of significant relationships between the larvae and their hosts. These include: the correlation between host development and host susceptibility, the importance of staminate flowers as sites for larval establishment, and the differences in feeding responses of late-instar larvae to new vegetative shoots as compared to mature needles.

Some additional observations, by McGugan (1954) and Webb and McLeod (1954), on the behavior of early-instar larvae during the needle-mining stages, are also pertinent. Spruce budworm larvae frequently emerge from their hibernacula before the development of staminate flowers or vegetative buds has proceeded sufficiently to permit establishment of the larvae. Under these circumstances most of the population normally feeds by mining the mature needles. The duration of the needle-mining period on the various hosts is determined primarily by their phenology, as once the buds have expanded sufficiently the larvae abandon the needles and enter the buds. The foliage development of white spruce parallels that of balsam fir quite closely although the opening of white spruce vegetative buds is normally slightly in advance of that of balsam fir and growth proceeds somewhat more rapidly. Bud development of black spruce,

as has been mentioned before, is considerably retarded. McGugan noted that whereas larvae feeding on balsam fir normally mined only one needle before entering the newly-developing buds, larvae on white spruce frequently mined as many as five or six needles each of which was abandoned after a short period of occupancy. Some related observations were noted by Webb and McLeod. They made counts of the larvae on various hosts from the time of emergence from the hibernacula to the time of establishment in the expanding vegetative buds. Larvae were recorded as: mining in old needles, mining in unexpanded buds, feeding in partially expanded shoots or wandering on the foliage. Higher proportions of the larval population were found wandering on the foliage of white and black spruce than on balsam fir. Larvae on white spruce exhibited a sharp decline in wandering once the new buds became suitable for larval establishment. These observations indicate that the mature needles of black and white spruce are unfavorable sites for early-instar feeding. Thus there is a similarity in the behavior of both early-instar and late-instar larvae with respect to feeding on mature spruce needles.

Many of the aforementioned host relationships of budworm larvae can be rationalized on the basis of differences in host phenology and larval responses to various physical stimuli. Thus, staminate flowers mature at a time coincident with or following shortly after emergence of larvae from the hibernacula and hence are available when the vegetative shoots are seldom in an optimal condition for larval establishment. The ecoclimate within the flowers is considered to favor rapid growth of the larvae once they are established, as the internal temperatures, when in

direct spring sunlight, are higher than those in adjacent vegetative buds (Wellington, 1950b). The unsuitability of mature spruce needles as sites for needle mining by early-instar spruce budworm larvae has been attributed by McGugan (op. cit.) to the fact that they dry out very quickly once the mines are formed and become unfavorable as food. The reluctance of late-instar larvae to feed on mature needles, has been ascribed to the toughness of the needles as contrasted to the succulence of the new growth.

Although these various ecological factors and physical characteristics of the host plant tissues are of undoubted significance in the responses exhibited by the larvae they do not provide an entirely satisfactory explanation of the feeding behavior. Jack-pine budworm larvae exhibit a strong propensity for feeding within the senescent staminate flowers even though food which is more succulent is available in the adjacent vegetative shoots. Lejeune (1950) found that jack-pine budworm larvae feeding in staminate flowers attain a greater head capsule size than larvae feeding in vegetative shoots. Such disparities in growth imply that there are differences in either nutritional adequacy or intake of the two foods.

Some Considerations of the Experimental Methods

The present study was undertaken in order to investigate the role of chemical constituents of the host plants on feeding behavior and to determine whether there was any relationship between the chemosensory responses of the larvae and their feeding habits. In the testing technique used the larvae were, of necessity, subjected to physical conditions

quite different from their normal feeding environment. The test discs were dry, which is in contrast to the normal succulent state of the preferred host plant tissues. Also, the air in the test chamber was drier than that in the normal feeding environment.

The dry state of the test discs may have had a limiting effect on the responses of the larvae. In normal feeding, water in the plant juices is probably an important source of stimulation. Some observations made during the experiments support this conclusion. Larvae were preconditioned by being held for six hours in petri dishes containing a circle of filter paper moistened with distilled water. It was frequently noted that larvae, almost immediately after being placed in the dishes, flexed the head in the feeding attitude and began to imbibe water from the moistened fibres of the filter paper. This suggests that water is an important source of sensory input in the feeding reaction. The responses obtained to substances in the dry state may therefore be intensified in feeding on the succulent tissues of the host plant. It is possible that specific water receptors are involved. Such receptors are known in some vertebrates and have recently been demonstrated in electrophysiological studies with Phormia regina (Evans and Mellon, 1962).

The discontinuous pattern of feeding behavior that is evident from the graphs of feeding activity given in Appendix B are probably, in part at least, a consequence of the suboptimal physical conditions of the test environment, especially the rather low humidity. It is known that such high evaporation rates stimulate the larvae to greater locomotor activity (Wellington, 1950).

In spite of the limitations of the technique, the larvae fed quite readily under these conditions. Very often it was observed that larvae began feeding on discs treated with plant extracts or sugar solutions immediately after making initial contact with them. In view of the error inherent in the fecal count method of measuring food intake, as discussed by Kasting and McGinnis (1962), the direct measurement afforded by the use of pith discs was a distinct advantage. Some factors considered in the adoption of this technique have been discussed in Chapter III.

In the present investigation the emphasis has been on the role of substances that act as contact chemical stimuli. Although olfactory stimuli are considered to be important in food plant recognition and as factors involved in orientation and aggregation, convincing evidence is lacking for a direct function of odors as feeding stimuli for insects (Dethier, 1961a; Evans, 1961). In the case of spruce budworm larvae some evidence can be cited to suggest that olfactory stimuli do not play an important role in food acceptance: (1) Sucrose has a negligible vapor pressure and is generally conceded to be odorless. Nevertheless it serves as an adequate stimulus for larval feeding. (2) Lipoid solubility is considered to be an essential property of odorous substances (Moncrieff, 1951) yet when desiccated white spruce shoots were extracted with petroleum ether and this was followed by extraction with 80 per cent ethanol, the feeding response in choice tests to this alcoholic extract was no different than to one prepared from material which had not been pre-extracted with petroleum ether.

Feeding Responses to Single Compounds and Mixtures in Relation to the Effectiveness of Host Plant Extracts

Of the various host plant chemicals and related compounds that were utilised in single-disc tests only certain sugars and the amino acid L-proline elicited consistent feeding responses. The most effective compound tested was sucrose, a major component of the host plant tissues. In choice tests with this sugar, feeding response increased with concentration up to the highest concentration tested, 0.5 M.

Choice tests with host plant extracts indicated that, while the sugars constituted major stimulating components, feeding response could not be entirely attributed to these compounds. Discs treated with 0.1 M. sucrose induced less feeding than discs treated with an extract of white spruce shoots having a total sugar concentration of less than half this amount. Similarly the preferential response to an extract of white spruce staminate flowers over one of white spruce shoots or to the shoot extract over a mature needle extract cannot be accounted for entirely on the basis of differences in their sugar concentrations.

The results suggest that one or more components of the extracts acts in conjunction with sugar to influence the feeding response. Choice tests in which responses to sucrose were compared to responses to mixtures of sucrose and other host plant constituents have aided in identifying at least some of the factors involved in the responses to the host plant extracts. Most of the compounds, utilized in these experiments were known host plant constituents that had been previously investigated in single-disc tests. Of these, the sugars were the only substances that

evoked strong feeding responses. The remaining substances either produced only weak reactions, e.g., L-proline, or were ineffective in eliciting feeding, e.g., shikimic acid and L-glutamic acid.

The simplest of the mixtures tested, in terms of diversity of molecular structure, consisted of the three sugars common to most of the host plant tissues on which the larvae normally feed. This mixture proved more stimulating than an equivalent concentration of the most stimulating single component of the mixture, sucrose. Thus even within a single modality of taste the stimulating capacity of the mixture was not simply the resultant of the additive effects of its components. Similar synergic effects of this and other sugar mixtures have been noted with other insects as discussed earlier. No satisfactory physiological explanation for the synergism of sugar mixtures has been proposed and the data obtained in these experiments do not provide an adequate basis for such speculation. The effect noted here would be of adaptive significance to the larvae as the threshold of response to the mixture would be expected to be lower than that to any of its individual components.

In the tests with mixtures of sucrose and certain non-sugar compounds, the most pronounced differential responses were obtained with certain amino acids. The three effective compounds tested have definite structural affinities. In view of the very slight but consistent responses obtained to L-proline when tested alone, the responses to the mixtures were much greater than would be expected on the basis of a simple additive effect. Synergism is again indicated. An interesting comparison concerns the relative differences in response in choice tests between

sucrose and sucrose plus proline (Tables XXIII and XXIV) and those to sucrose compared at concentrations of 0.1 M. and 0.5 M. (Table XXI). L-Glutamic acid failed to induce feeding when tested alone but effectively increased the response when in mixture with sucrose.

Very little is known concerning the stimulating effects of amino acids or the nature of the receptors involved. In humans amino acids produce various taste sensations depending on optical isomerism and configuration of the amino group. A pronounced electrophysiological response following stimulation of the dactyl chemoreceptors of the decapod crustacean, Carcinides maenas, with glutamic acid has recently been demonstrated (Case and Gwilliam, 1961). Other amino acids and related compounds that were tested with this organism were much less effective although amino acids as a group were considerably more effective than sucrose. In Phormia regina, the only insect in which detailed electrophysiological studies of the contact chemoreceptors have been made, amino acids do not elicit a feeding response (Dethier, 1955). However, proteins are acceptable to this fly and apparently there is a peripheral sensory mechanism for discriminating between protein and carbohydrate although no specific protein receptor has been identified (Dethier, 1961). On the basis of behavioral responses, Beck and Hanec (1958) have postulated the existence of separate receptors for amino acids and sugars in European corn borer larvae. It is possible therefore that the responses observed in the present study involve a dual sensory mechanism for detection of carbohydrates and amino acids based on separate receptors or receptors with differential sensitivities. More detailed study of this response would

be of interest especially in relation to response thresholds and the quantitative relationship between concentration and feeding response.

The observed responses to sucrose-proline mixtures are of particular interest as these two chemicals are the principal simple compounds present in coniferous staminate flowers. Although nothing is known of the specific amino acid requirements of budworm larvae, the adaptive significance of the responses to amino acids seem unlikely to bear any relation to specific nutritional needs. The amino acids found effective in these tests, proline, hydroxyproline, and glutamic acid, are non-essential for the growth of most insects that have been studied. On the other hand, L-arginine, which was shown to be non-stimulating or possibly slightly deterrent in effect, at the concentration tested, is required in the diet of all insects that have been investigated (Gilmour, 1961).

In choice tests with sucrose and certain sucrose plus non-sugar combinations, with spruce budworm larvae, some significant relationships between the chemistry of white spruce foliage and the feeding responses of the larvae were demonstrated. Shikimic acid is the major organic acid in spruce foliage and is present in somewhat higher concentration in new growth than in mature needles. While it did not stimulate feeding when tested alone it had a significant effect when in combination with sucrose in relative concentrations similar to those in the host plant tissues. The effect appears to be rather specific as the closely related D-quinic acid did not induce similar responses.

Another organic acid that effectively increased response when combined with sucrose was caffeic acid. Again the effect of this com-

pound was quite specific as closely related substances such as protocatechuic acid and pyrocatechol did not enhance the feeding response. It is possible that shikimic acid and caffeic acid are present in combined form in the host plant as a compound of similar composition, chlorogenic acid, which is formed by the union of caffeic and quinic acids, is known to be present in some conifers (Gortner and Gortner, 1953, page 756).

In contrast to the stimulating effects of the above-mentioned compounds, the glucoside pungenin, a major component of mature spruce needles, has a pronounced deterrent effect on the response to sucrose. Choice tests between an extract of new shoots supplemented with pungenin and an extract of mature needles indicated that differences in the pungenin and sugar content of the two tissues could not account entirely for the preference exhibited for extracts of the new shoots. Aside from the deterrent effects of pungenin, the mature needles are probably deficient in certain stimulating components that are present in the new shoots other than sugars. The proportion of shikimic acid is known to be somewhat lower in mature needles and it is likely that there is a smaller quantity of caffeic acid present. Such differences may account for at least part of the demonstrated differences in feeding response to the extracts of the two plant tissues.

Concluding Remarks

It is apparent that the feeding relationships of budworm larvae are influenced by responses to certain characteristic chemical components of the host plant tissues. The sources of stimulation are complex as several chemical factors have been identified and it is likely that

others remain undetected. There appears to be no single dominant constituent of high specificity. This is not surprising as the acceptable host plants include species of several genera of conifers. The primary effective chemicals vary according to the plant tissues and also may change with maturation and growth of the tissues. There is a positive correlation between the feeding behavior of the larvae relative to the various host plant tissues and the responses to the extracts of these tissues and some of their principal chemical components. The favorability for larval growth of the various parts of the plants also shows a relationship to the acceptability of extracts prepared from them. This relationship seems unlikely to be simply fortuitous and, although possible nutritional effects per se cannot be ignored, the influences on growth may be a direct result of the amounts of the different plant tissues consumed. There seems to be little doubt that the dwarfing effect of feeding on mature needles is due to the restricted amount of feeding. Spruce budworm larvae never feed extensively on mature white spruce needles and this has an important bearing on the tolerance of this host to attack. The physical characteristics of the mature needles undoubtedly have a significant effect on the feeding response but the present studies have demonstrated that the acceptability of the needles is influenced by their content of certain chemical constituents and these may be the primary determinants of the extent of feeding.

CHAPTER VII

SUMMARY

Larvae of the spruce budworm (Choristoneura fumiferana (Clem.)) and the jack-pine budworm (Choristoneura pinus Free.) will feed on the staminate flowers and foliage of a wide variety of conifers. Host availability in a given geographic location is determined primarily by the synchronization of host tree development and emergence of larvae from their hibernacula in the spring. Staminate flowers are favored sites of larval establishment. After the flowers have shed their pollen the larvae migrate to the newly-developing shoots and feed on these. Following destruction of the new shoots the larvae will feed on the mature needles but these are much less acceptable to them.

The purpose of this study was to determine the effects of various chemical constituents of the host plants on the feeding responses of the larvae. Particular attention was given to the relative acceptability of the staminate flowers, developing vegetative shoots and mature needles of white spruce in relation to the occurrence in these plant tissues of substances which stimulated or deterred feeding responses.

Discs of Japanese elder pith were treated with the extracts and chemicals to be tested. Feeding responses were evaluated by measuring the areas of the discs consumed by ultimate instar larvae over a 48 hour period.

Active constituents could be effectively extracted from the host plant tissues with 80 per cent ethanol. Extracts prepared with fat sol-

vents were inactive.

Several sugars were tested at concentrations of 0.004 M., 0.02 M., 0.1 M. and 0.5 M. The compounds most effective in eliciting feeding responses were, L-fucose, D-fructose, maltose, sucrose and raffinose.

Eight amino acids were selected for testing on the basis of their known occurrence in the pollen of conifers and their influence on the feeding behavior of other insects. Of these, only L-proline induced consistent feeding responses.

Other compounds, including shikimic acid and the glucoside pungenin, that were known to be present in the host plant in appreciable quantities were investigated but none of these induced feeding when tested alone.

In choice tests it was found that an extract of developing white spruce shoots was more acceptable than one of mature needles. An extract of staminate flowers was more acceptable than one prepared from the new shoots.

The principal host plant sugars are not equally effective in inducing feeding responses. Sucrose and D-fructose are much more stimulating than D-glucose. An equimolar mixture of the three sugars induced more feeding than an equivalent concentration of sucrose when these were paired in choice tests. Response to sucrose in choice tests increased with concentration up to the maximum concentration tested, 0.5 M.

When the amino acids, L-proline, hydroxy-L-proline and L-glutamic acid were mixed individually with sucrose these mixtures were preferred to sucrose alone. The difference in response indicated a synergic effect. The response to a mixture of L-arginine and sucrose was not significantly

different than that to sucrose alone. As free proline is known to be present in appreciable amounts in the pollen of conifers, its effect on feeding is probably of significance in relation to the known pattern of larval behavior.

Shikimic acid is present in appreciable amounts in spruce foliage and in mixture with sucrose it was found to enhance the feeding response of spruce budworm larvae. The closely related D-quinic acid did not have this effect.

The glucoside pungenin is a major component of mature spruce needles but is absent from the new shoots in the early stages of development. It had a significant deterrent effect on the response to sucrose at a concentration approximating that in the needles. The aglucone, 3, 4-dihydroxyacetophenone, was less effective than the glucoside but it did deter feeding.

Several compounds structurally related to the aglucone had significant deterrent effects. These included: 2, 4-dihydroxyacetophenone, 2, 6-dihydroxyacetophenone and 3, 4-dihydroxybenzoic acid. Pyrocatechol had no apparent effect on response at the concentration tested while caffeic acid enhanced the feeding response.

The effect of caffeic acid was of particular interest because it has been demonstrated to function as an effective precursor in the synthesis of pungenin and therefore may be present in the developing vegetative shoots in appreciable quantities.

The difference in the acceptability of the new vegetative shoots and mature needles of white spruce cannot be explained solely on the

basis of the occurrence of pungenin in the latter even when the difference in sugar concentration is taken into account. Apparently differences in both stimulant and deterrent factors are involved in the relative responses to these two extracts.

The experiments described, have demonstrated that there is a chemosensory basis for the selective feeding behavior of spruce budworm larvae and indicate that this also applies in the case of jack-pine budworm larvae. Several host plant chemicals act as feeding stimulants. Except in the case of L-proline, stimulation by the non-sugar constituents was detectable only when they were combined with sugar. The mature needles of white spruce contain a glucoside, pungenin, which exerts a deterrent effect.

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APPENDIX A

A LIST OF THE COMMON NAMES OF NATIVE TREES USED, WITH THEIR SCIENTIFIC EQUIVALENTS[1]

Balsam fir	-	*Abies balsamea* (L.) Mill.
Tamarack	-	*Larix laricina* (Du Roi) K. Koch
White spruce	-	*Picea glauca* (Moench.) Voss
Black spruce	-	*Picea mariana* (Mill.) BSP.
Red spruce	-	*Picea rubens* Sarg.
Jack pine	-	*Pinus Banksiana* Lamb.
Douglas fir	-	*Pseudotsuga menziesii* (Mirb.)

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1 As given in "Native Trees of Canada" 6th Edition, Bulletin #61, Canada Department of Forestry, Ottawa, 1961.

APPENDIX B

TIME-LAPSE PHOTOGRAPHY STUDIES OF LARVAL FEEDING BEHAVIOR

As has been described in Chapter IV, the larval feeding tests were of 48 hours duration. Although the larvae usually appeared normal and active at the end of this time, it was desirable to determine whether there was any progressive or abrupt change in the frequency or amount of feeding during the tests. The technique of time-lapse photography was made use of to determine this.

The equipment consisted of a Kodak Cine-Special Mark II, 16 mm. camera with which was associated a Stevens Timer[1] to control the lights and shutter. The camera was fitted with a Cine Ektar 25 mm. f/1.4 lens with an extension ring and spacer added to reduce the field size. The film used was Kodachrome type A. Illumination was provided by two 100 watt photoflood lamps. Single-frame exposures were made at six-minute intervals. The timer activated the light circuit before tripping the single-frame button.

Each test was of 45 hours duration making it possible to record eight such tests on each 100 foot roll of film.

The test chamber was of the same type used in the single-disc tests except that the light masks were omitted and the pith disc was mounted on a light blue paper which provided a suitable degree of color contrast. The experimental set-up (Fig. 1) was enclosed in a frame

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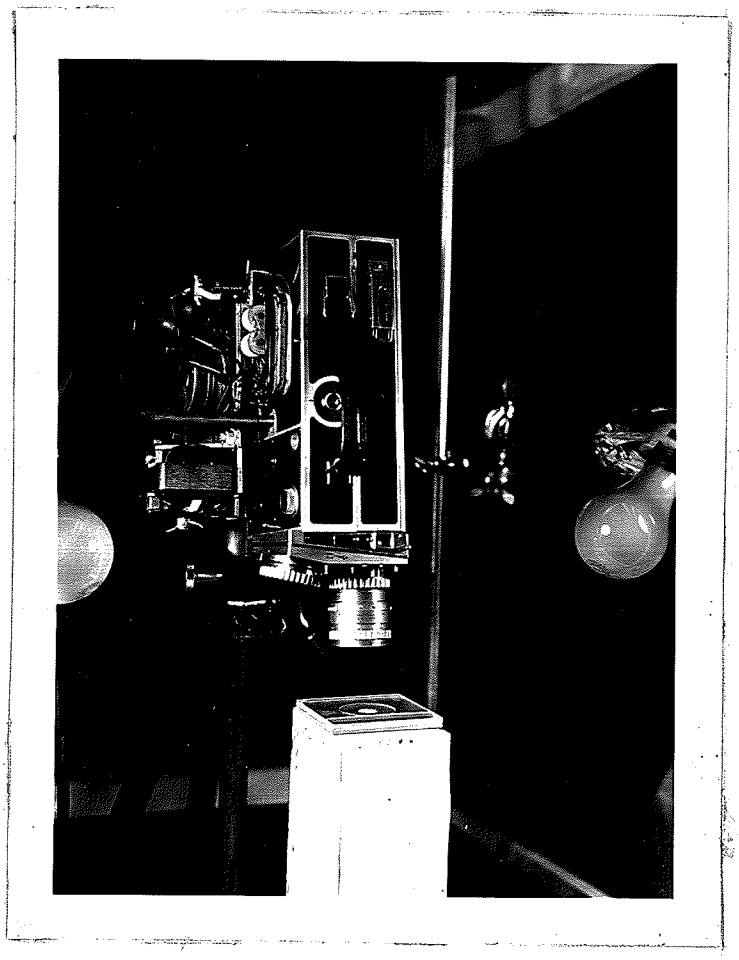
[1] Stevens Engineering Co., 2421 Military Ave., Los Angeles 64, California.



APPENDIX FIGURE 1

ARRANGEMENT OF PHOTOGRAPHIC APPARATUS AND FEEDING CHAMBER AS USED IN TIME-LAPSE RECORDING OF FEEDING ACTIVITY

covered with heavy black cotton and the room was kept darkened throughout the test. Temperature was maintained at approximately 23° C.

Ten feeding tests were made with discs treated with 0.1 M. sucrose and ten with discs treated with 0.5 M. sucrose. The larvae used were fifth-instar spruce budworm larvae which had been reared on tamarack.

Frame-by-frame analysis of the films was carried out using a Kodak analysis projector. The image of the disc was projected on an outline form and a tracing was made of each picture which showed a feeding increment. Each tracing was then measured with a planimeter.

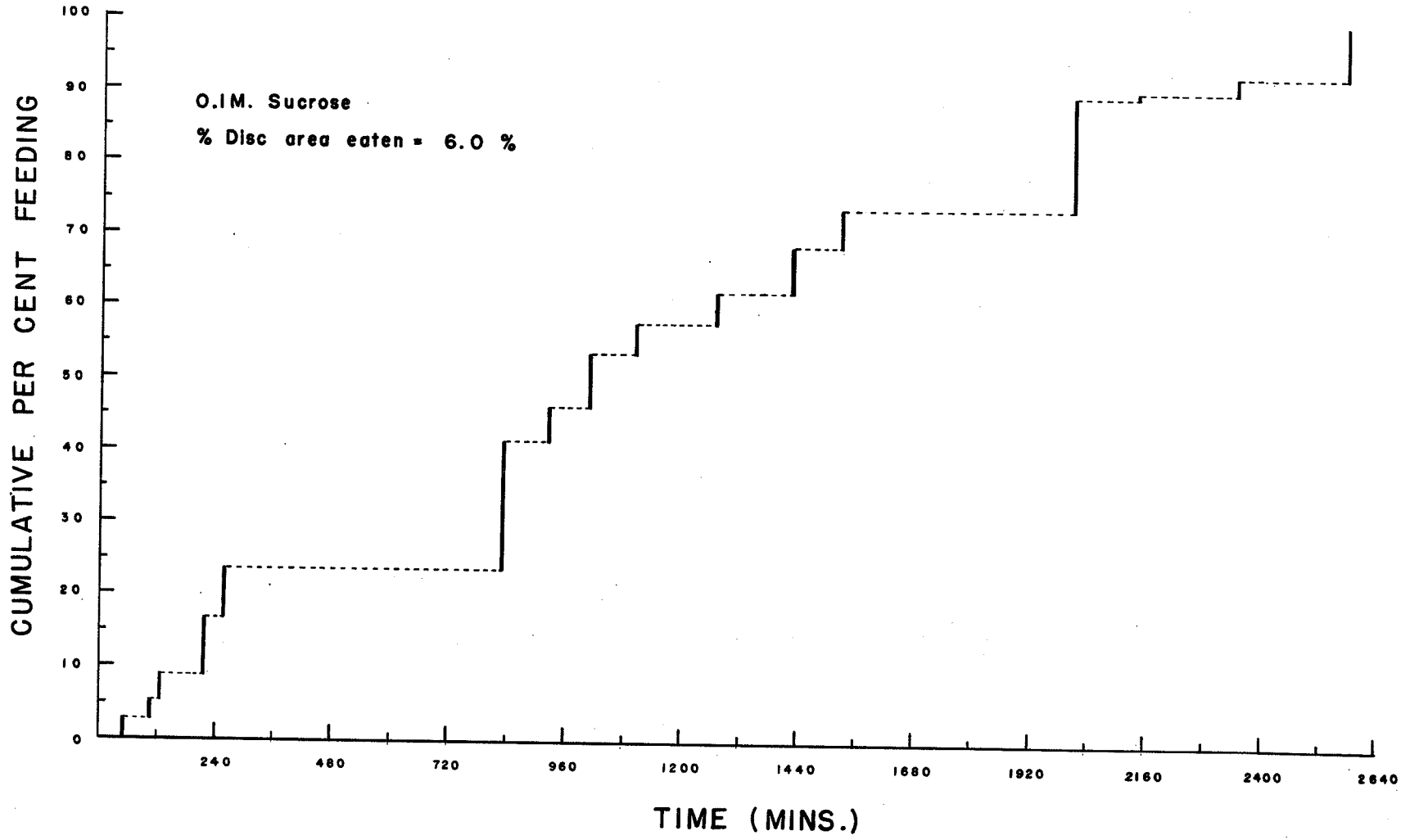
The data thus obtained from six representative tests, three with 0.1 M. sucrose and three with 0.5 M. sucrose, are presented graphically in Figures 2 to 7. These graphs depict the feeding increments expressed as cumulative percentages of the disc area consumed. The upright solid bars represent the feeding increments and the horizontal dotted lines depict the intervals between feeding periods. Departure of the upright bars from the vertical, which occurs only once in these graphs, indicates that the feeding period extended over a longer interval than the six minutes between successive frames.

From these graphs it can be seen that the inception of feeding usually occurred shortly after the commencement of the test but was sometimes delayed. In all cases a somewhat greater amount of feeding took place during the first half of the test period than the last half. However, there was no marked drop-off or significant change in frequency of feeding in the latter part of the test period.

These results indicated that during a test period of 48 hours the feeding responses of the larvae could be expected to remain relatively uniform.

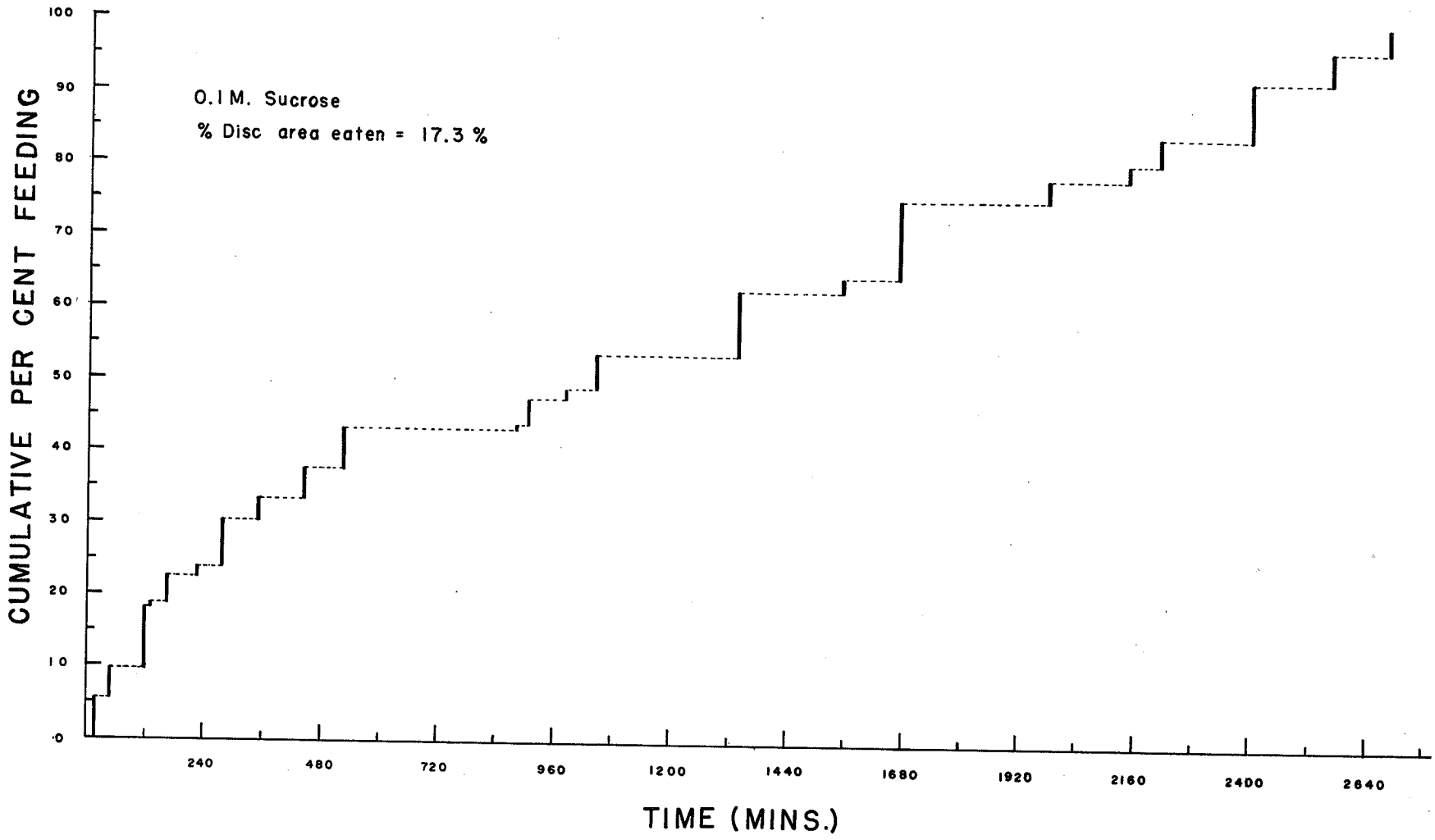
APPENDIX FIGURE 2

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.1 M. SUCROSE


O.1M. Sucrose
% Disc area eaten = 6.0 %
CUMULATIVE PER CENT FEEDING
0
10
20
30
40
50
60
70
80
90
100
240
480
720
960
1200
1440
1680
1920
2160
2400
2640
TIME (MINS.)

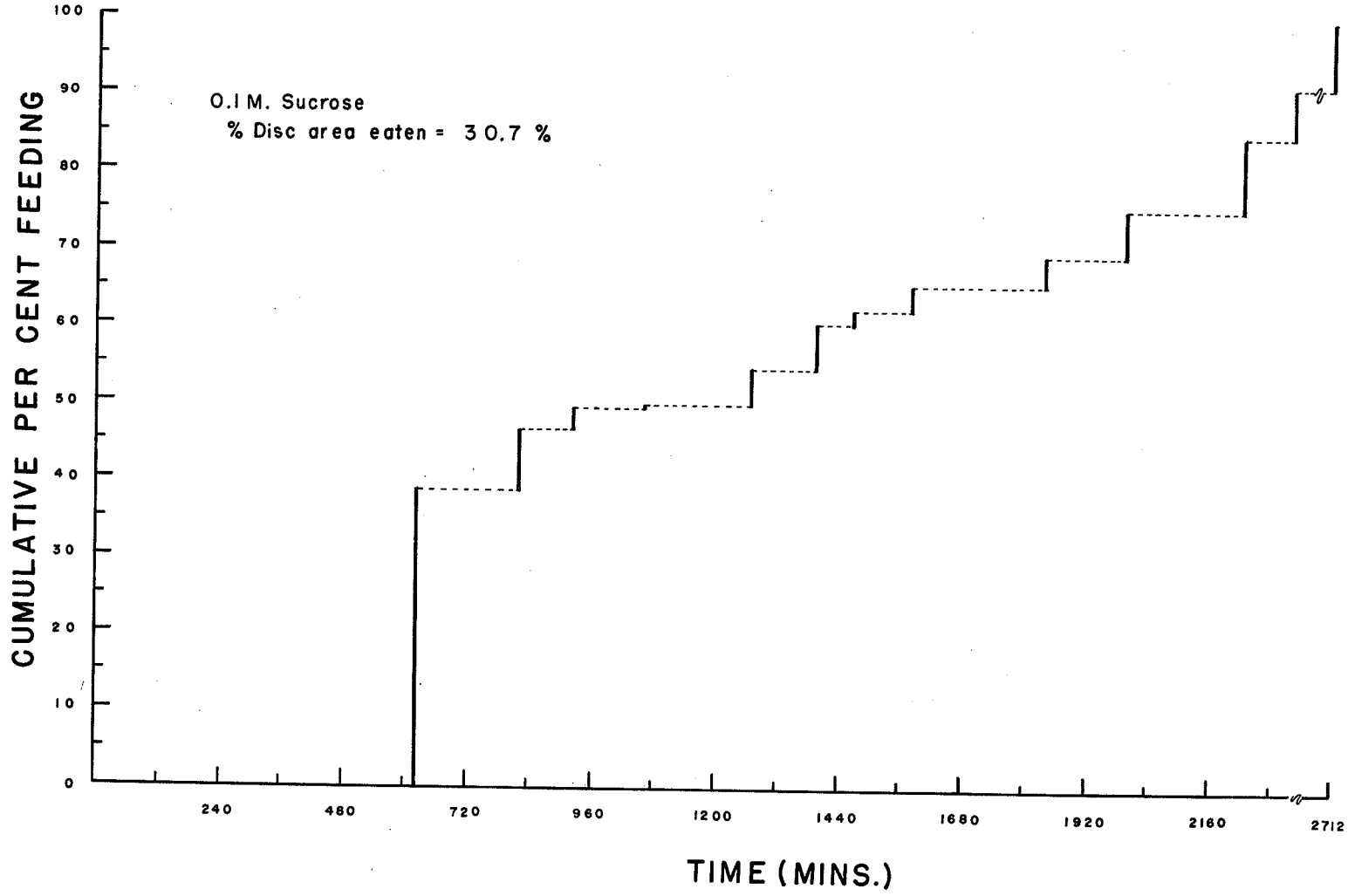
APPENDIX FIGURE 3

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.1 M. SUCROSE


0.1 M. Sucrose
% Disc area eaten = 17.3 %
CUMULATIVE PER CENT FEEDING
100
90
80
70
60
50
40
30
20
10
0
240
480
720
960
1200
1440
1680
1920
2160
2400
2640
TIME (MINS.)

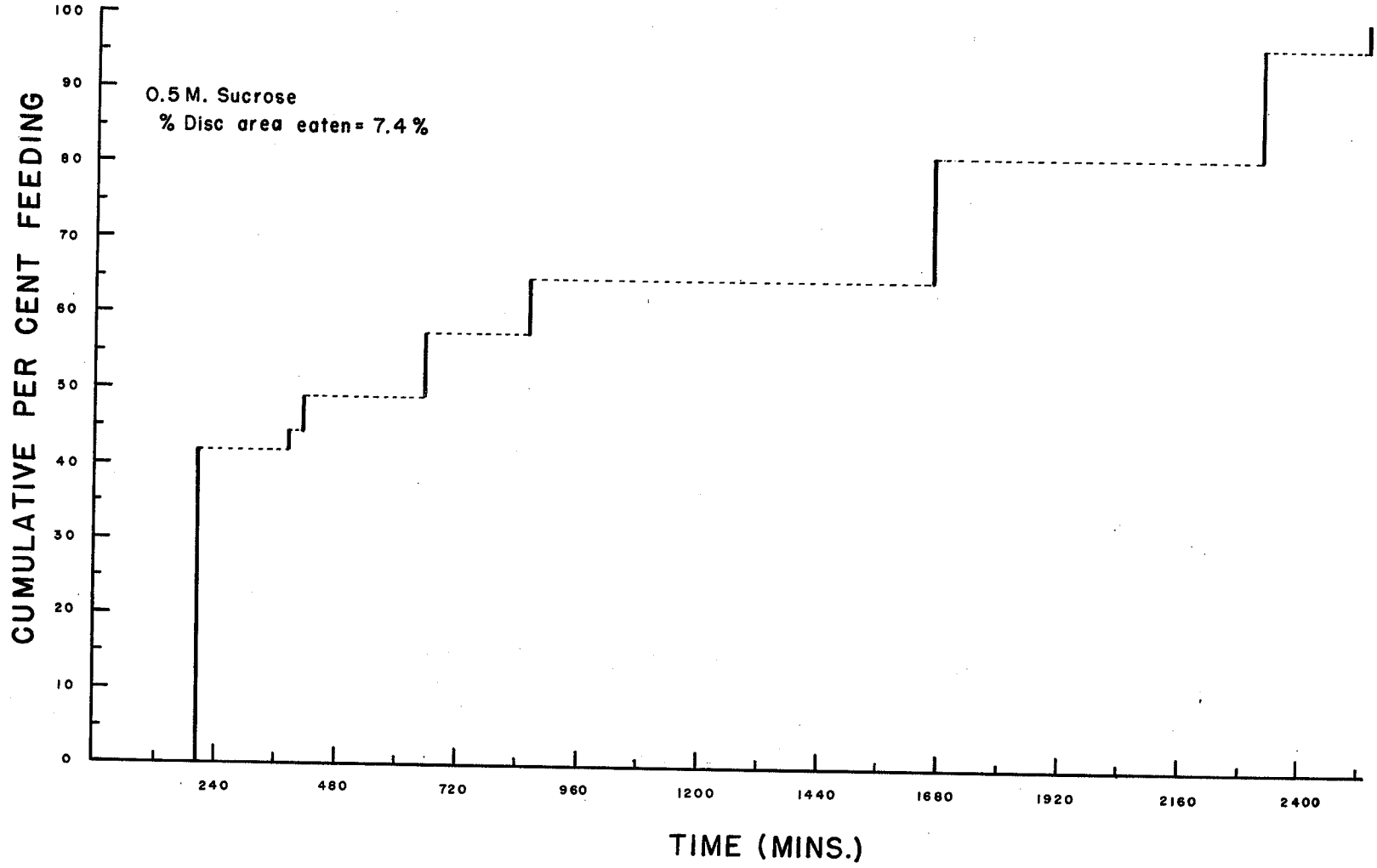
APPENDIX FIGURE 4

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.1 M. SUCROSE


0.1 M. Sucrose
% Disc area eaten = 30.7 %
CUMULATIVE PER CENT FEEDING
0
10
20
30
40
50
60
70
80
90
100
240
480
720
960
1200
1440
1680
1920
2160
2712
TIME (MINS.)

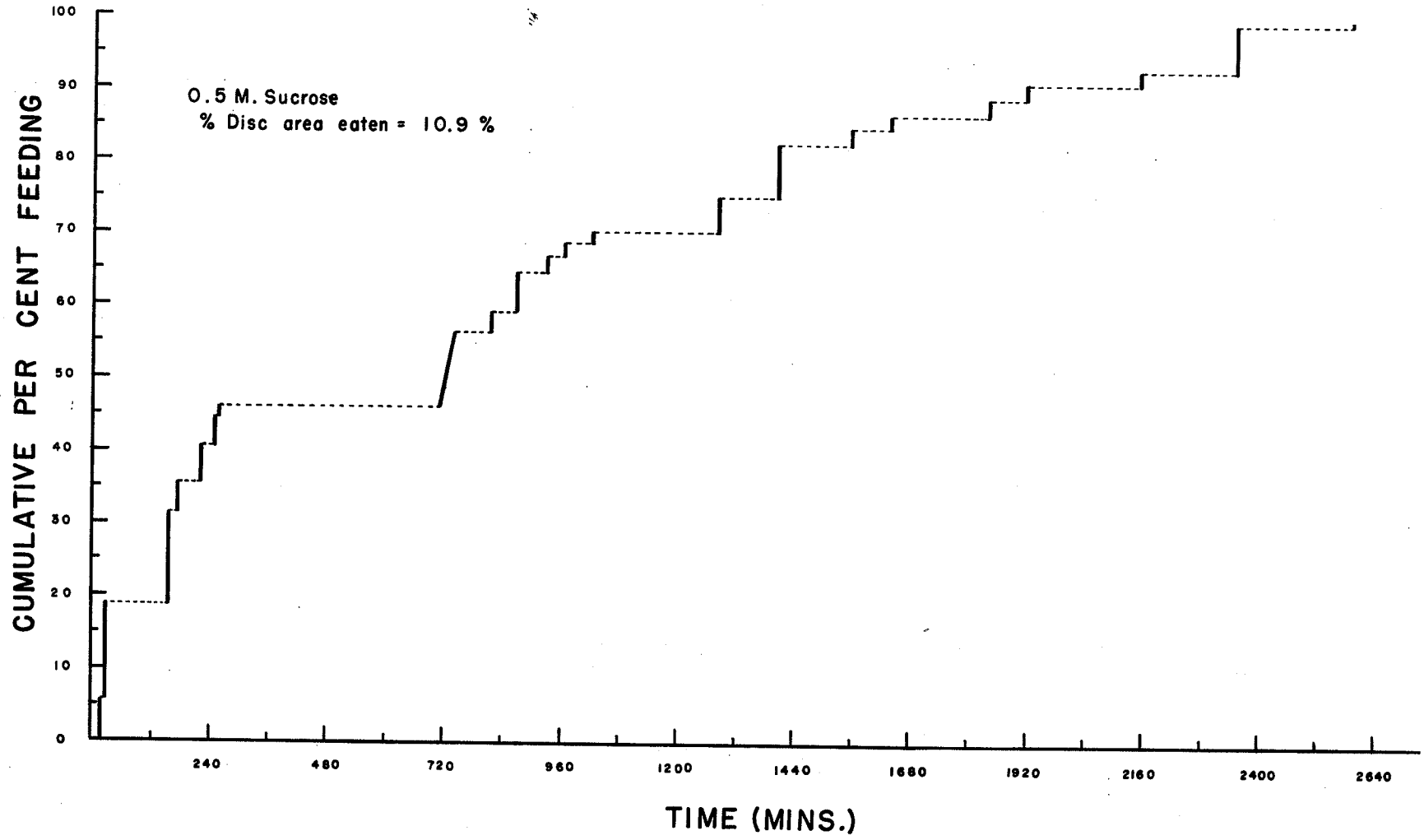
APPENDIX FIGURE 5

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.5 M. SUCROSE


0.5 M. Sucrose
% Disc area eaten = 7.4 %
CUMULATIVE PER CENT FEEDING
0
10
20
30
40
50
60
70
80
90
100
240
480
720
960
1200
1440
1680
1920
2160
2400
TIME (MINS.)

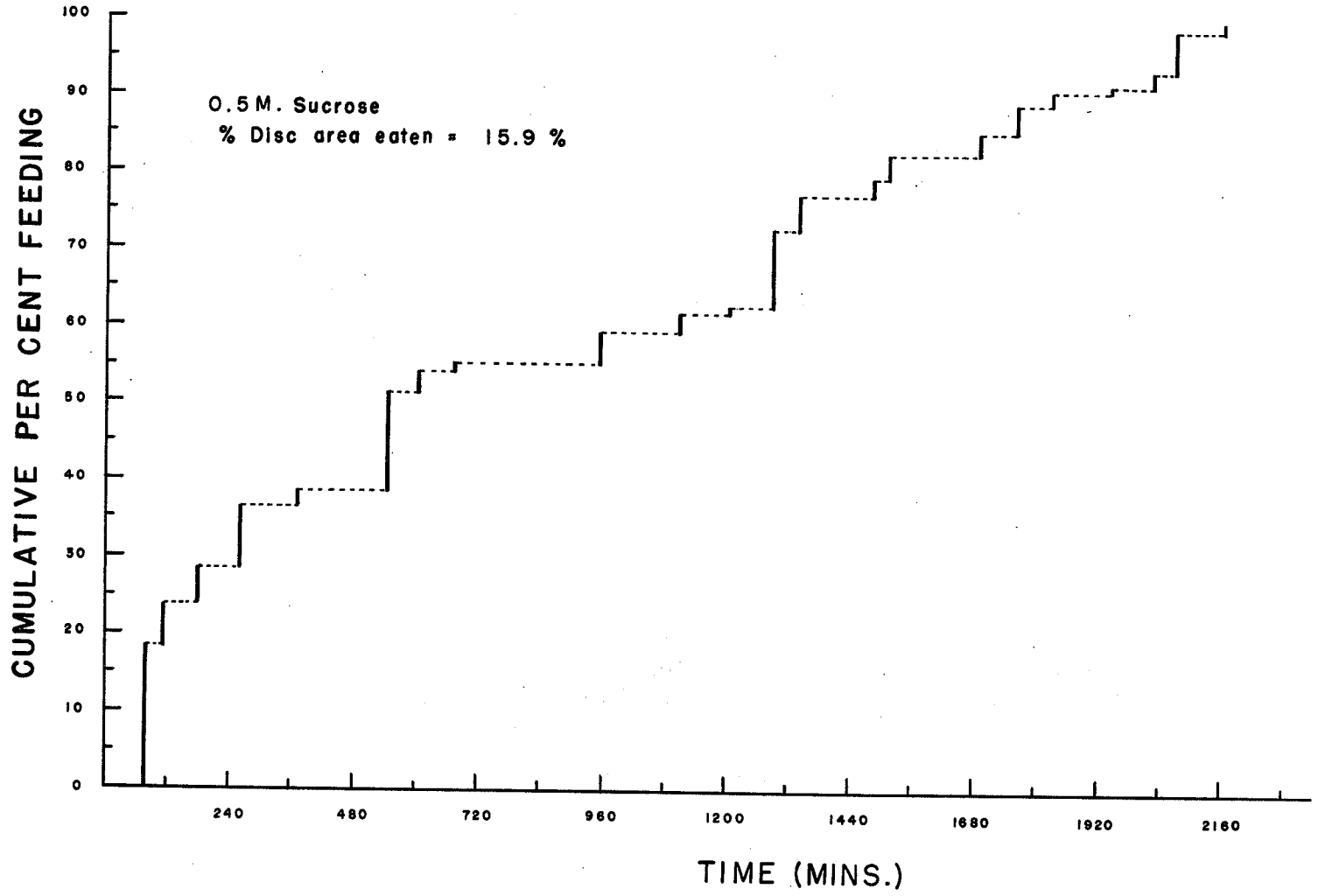
APPENDIX FIGURE 6

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.5 M. SUCROSE


0.5 M. Sucrose
% Disc area eaten = 10.9 %
CUMULATIVE PER CENT FEEDING
0
10
20
30
40
50
60
70
80
90
100
240
480
720
960
1200
1440
1680
1920
2160
2400
2640
TIME (MINS.)

APPENDIX FIGURE 7

GRAPH OF TIME-LAPSE PHOTOGRAPHY RECORD OF FEEDING OF A FIFTH-INSTAR SPRUCE BUDWORM LARVA ON A PITH DISC TREATED WITH 0.5 M. SUCROSE


0.5 M. Sucrose
% Disc area eaten = 15.9 %
CUMULATIVE PER CENT FEEDING
0
10
20
30
40
50
60
70
80
90
100
240
480
720
960
1200
1440
1680
1920
2160
TIME (MINS.)