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HOST PLANT NUTRITIVE VALUE AND VARIABLE NUMBER OF INSTARS IN A SAWFLY, *DIPRION SIMMS*

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ABSTRACT

In measurements of the utilization of dry matter and nitrogen by fifth-instar penultimate females of *Diprion similis* (Hartig), leaves of *Pinus banksiana* Lamb. *(=P. divaricata* (Ait.) Dumont) and *P. sylvestris* L. were found to be nutritionally superior to those of *P. resinosa* Ait. and *P. strobus* L. When larvae were fed on leaves of low nutritive value and low nitrogen digestibility, some feeding sixth instars (supernumerary larvae) were obtained but only five feeding instars occurred on leaves with high nutritive value and high nitrogen digestibility. Topical application of a juvenile hormone preparation to 3-day-old fifth instars prolonged the stadium without introducing an additional instar.

INTRODUCTION

There is evidence that some insects which feed on leaves of forest trees can be controlled by fertilizing the forests (see review by Stark, 1965), but the changes in composition of the leaves are likely complex and explanations for the effect on the growth and reproduction of the insects may reside in deficiencies of, or imbalances among nutrients, changes in levels of secondary metabolites acting as deterrents or stimulants, or in quantities of poorly utilized constituents such as fibre. The spectrum of change may be detected by studies of food utilization, such as those outlined by Waldbauer (1968) which measure the consumption, digestibility and efficiency of conversion of the food. As a prelude to further work to explain the effects of mineral fertilization of host trees on diprionid sawflies, measurements of utilization of dry matter and nitrogen by the fifth-instar female of *Diprion similis* (Hartig) were initiated to assess and compare the nutritive value of 1-year-old foliage from four species of pine which are common to Ontario: jack pine, *Pinus banksiana* Lamb. (= *P. divaricata* (Ait.) Dumont); Scots pine, *P. sylvestris* L.; red pine, *P. resinosa* Ait.; and white pine, *P. strobus* L. tests to substantiate the reliability of the measurements are also presented.

MATERIALS AND METHODS

Insect rearing:

D. similis collected in a Scots pine plantation in Simcoe County, Ontario had been reared in the laboratory for several generations as follows: mating pairs were set up on 25- to 50-cm-long Scots pine branch tips containing current- and previous-year foliage. The cut ends of the branches were inserted through a hole in a styrofoam cylinder (2.5 cm thick) which served as a plug in the top of a water-filled jar. A muslin bag placed over the foliage and tied around the top of the jar served as a cage. The progeny were allowed to feed on the oviposition foliage and at the third to fourth instar were transferred by colony to glass jars (15 cm by 8 cm diam.) and given fresh foliage as required. Cocoons obtained from these larvae were incubated singly in plastic vials (5 cm by 2.8 cm diam.) in the dark. All rearings and experiments were done in a room kept at 21°C and 70 percent RH with a 16-h photophase.

Foliage:

Collections were made during August and early September from plantations of 25-year-old red pine and 13-year-old jack and Scots pine, and from white pine trees ranging in age from 19 to 32 years. Branches were taken from the mid- and lower crown 2 to 3 times weekly and stored at 5°C with their cut ends in water.

Analytical methods:

Dry weights of foliage, faeces and insects were obtained after drying at 80°C for 16 hours. Nitrogen contents[1] of the dried ground food and faeces and dried whole larvae were determined by a micro-Kjeldahl method (Horwitz, 1970).

EXPERIMENTS AND RESULTS

1. Nutritive value of foliage from jack, Scots, red and white pine for female fifth-instar larvae of *D. similis.*

The following account describes the utilization of 1-year-old foliage of jack, Scots, red and white pine by the female fifth-instar larvae of *D. similis* following the methods outlined by Waldbauer (1968) and assesses the nutritive value of the foods.

Females were chosen for these tests because of our interest in possible relationships between food utilization and egg production. The fifth instar was selected because this stage consumes the whole pine leaf and because the sexes can be separated; females are larger and they ecdyse 1 to 2 days later than males. Female larvae which had ecdysed no more than 24 hours previously were starved for 6 hours at which time dissections revealed little food remaining in the gut. To ensure the selection of females, all the larvae were weighed and only those above or close to the median weight of the sample were used in the tests. The initial dry weights of larvae selected were calculated from their wet weights by using the mean percent dry matter of an aliquot of 20 larvae.

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1 Nitrogen determinations made by J. Ramakers.

The dry weight of food was calculated from the wet weight and the percent dry matter of a suitable sample. For the two-needle pines, jack, Scots and red pine, needles of the fascicle pairs were compared by classifying them as left- or right-handed when looking from the base to the apex of the branch. The mean dry matter contents of 20 pairs from each species were:

Pine species	Percent dry matter in-	
	Left hand needle	Right hand needle
jack	47.6±0.4*	47.6±0.4
Scots	46.1±0.2	46.3±0.2
red	48.4±0.2	48.2±0.2

* standard error

Since there is no difference in dry matter between left- and right-hand needles, one from each pair was used as food and the other was used to measure the percentage of dry matter.

For the five-needle white pine, the basal-most needle of each fascicle was compared clockwise with the four other needles of the fascicle. The results for 20 fascicles follow.

Percent dry matter in—				
Basal needle	2nd needle	3rd needle	4th needle	5th needle
50.2±0.2*	50.4±0.2	50.2±0.2	50.4±0.2	50.3±0.2

*Standard error

Each needle in the fascicle had the same dry matter content.,therefore, three needles were used for feeding and the other two for dry matter determinations.

Single larvae were confined to aerated plastic vials (5 cm by 2.8 cm diam.), rubber-stoppered at the open end. The pine needles cut into lengths to fit the tubes were held upright by inserting one end into slits in the rubber stopper. Food was replaced every 24 hours and residual food and faeces were collected, oven dried, weighed, and stored for analyses. The final weight of each larva after ecdysis was taken as the dry weight of the larva plus the exuvium.

The utilization indices, approximate digestibility (AD), efficiency of conversion of ingested food to body (ECI) and the efficiency of conversion of digested food

(ECD) were computed as described by Waldbauer (1968). In determining the consumption index (CI) which considers the rate of feeding in terms of the size and rate of development of the insect, the mean weight was obtained by averaging the initial and final dry weights.

In this experiment there was complete survival of larvae on jack pine; some mortality occurred on each of the other species, with the highest on red pine (Table I).[2] The weight gains were significantly higher on jack and Scots pine than on red and white pine and the duration of the instar was also longer in the latter two. Food intake was significantly higher on Scots and red pine than on jack and white pine. The digestibility of dry matter was highest for jack pine and lowest for red pine with intermediate values on Scots and white pine. The pattern of digestibility of nitrogen is closely related to the pattern of weight gain, whereas the efficiency of conversion of digested food is not. The amount of digested food converted to body was highest for jack pine but there was no significant difference among the other three. The highest ECI occurred with jack pine and the values decreased significantly through Scots, white to red pine. The consumption index was highest for larvae fed on Scots and red pine and lowest for those fed jack pine. An intermediate value was obtained with white pine. Larvae on Scots pine ate more than those on jack pine thereby compensating for the reduced digestibility and permitting equivalent weight gains. The high consumption index on red pine was a result of high food intake and small mean body size.

The nitrogen gained during feeding should equal the difference between total nitrogen consumed and total faecal nitrogen giving an ECD (nitrogen) of 100 percent. As a test of the reliability of our measurements of food intake, excreta output and weight gain, the ECD's (nitrogen) for five larvae feeding on Scots pine were determined. The mean and standard error was 101±3; this suggests a high degree of precision in the measurements obtained.

All foods tested in this experiment were consumed and utilized, but to varying degrees. The weight gains, AD's, ECD's and ECI's all indicated that jack pine had the highest nutritive value and red pine the lowest; the value of Scots pine was close to jack pine and white pine approached the value of red pine. Mortality was highest on red pine and lowest on jack pine, suggesting a nutritional basis for death. However, mortality did not exceed 12 percent on any diet and factors other than nutritional quality could be involved.

2. Effects of the food plant consumed in early instars on the responses to different food plants in the fifth instar.

The results of the food utilization experiment indicated significant differences among the food plants in their nutritive value for the fifth-instar sawfly. Perhaps the food supplied in earlier instars affected the assessments. If so, appropriate food should be provided in the earlier stages if growth response in the fifth instar is to serve as a reliable indicator of nutritive value. The following experiment was designed to determine if the pattern of response to the food plants in terms of net weight gain in the fifth instar is similar when younger larvae are fed on foliage of high (jack pine) or low (red pine) nutritive value.

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2 Dead Larvae were found free of nuclear polyhedrosis virus by J.M. Burke of the Insect Pathology Research Institute, Canadian Forestry Service, Sault Ste. Marie.

TABLE I

CONSUMPTION AND UTILIZATION OF FOLIAGE
FROM FOUR SPECIES OF PINE TREES
BY THE FEMALE FIFTH-INSTAR LARVA OF *D. SIMILIS*

	jack pine	Scots pine	red pine	white pine
% larval survival	100	93	88	93
No. larvae surviving	13	14	11	12
Mean duration of instar (days)	9	9	11	10
Weight gain (mg)	42.6 ±1.2*[a]	42.2 ±1.3[a]	30.5 ±1.0[b]	33.5 ±0.7[b]
Food eaten (mg)	543.6 ±4.1[b]	612.9 ±19.0[a]	595 ±10.3[a]	538.8 ±7.6[b]
Approximate digestibility of dry matter (%)	19.4 ±0.8[a]	18.0 ±0.3[b]	14.2 ±0.2[c]	16.9 ±0.4[b]
Approximate digestibility of nitrogen (%)	47.3 ±0.5[a]	46.5 ±0.2[a]	40.4 ±0.2[b]	39.6 ±0.3[b]
Efficiency of conversion of digested food (%)	41.2 ±1.9[a]	38.3 ±0.9[ab]	36.2 ±1.2[b]	36.9 ±1.1[b]
Efficiency of conversion of ingested food (%)	7.9 ±0.03[a]	6.9 ±0.14[b]	5.1 ±0.15[d]	6.2 ±0.07[c]
Consumption index (%)	1.84 ±0.04[c]	2.04 ±0.03[a]	1.98 ±0.03[ab]	1.92 ±0.03[bc]

*Standard error

NOTE: Means not bearing the same letter are significantly different ($P<0.05$). Analysis of variance; Duncan's multiple range test. Percentage values were transformed to the Arc Sine Square Root before analysis.

TABLE II

NET WEIGHT GAINS ON JACK, SCOTS, WHITE AND RED PINE IN FIFTH-INSTAR LARVAE OF *D. SIMILIS* FED IN EARLY INSTARS ON (A) JACK PINE OF HIGH NUTRITIVE VALUE OR (B) RED PINE OF LOW NUTRITIVE VALUE

Food plant in 5th instar	Initial wt of test larvae (mg)	% survival of test larvae	No. of last-instar larvae weighed	Net wt gain (mg)	Mean duration of feeding (days)	No. of feeding 6th instars
(a) Jack pine	15.3±0.8*	85	11	42.0±1.1*	8.6	0
Scots pine	15.3±0.9	100	13	37.9±1.9	8.5	0
White pine	14.8±0.8	100	13	32.0±2.2	9.7	0
Red pine	14.5±0.8	100	13	28.3±1.8	10.0	0
(b) Jack pine	9.2±0.4	92	11	46.0±4.0	12.7	5
Scots pine	9.0±0.5	92	11	33.8±5.6	11.0	2
White pine	9.2±0.5	90	10	31.7±4.1	12.8	2
Red pine	9.3±0.3	75	9	26.6±3.1	12.1	0

* Standard error

Freshly hatched larvae from 20 egg colonies on Scots pine were transferred to fresh shoots of jack and red pine. To randomize the larvae, one was transferred from each colony to give a total of 20 larvae on each fresh shoot. At ecdysis to the third instar, the larvae were transferred by groups of five to screen-capped jars (9 cm by 7 cm diam.), checked daily for ecdysis and given fresh foliage every second day. At ecdysis to the fifth instar, all the female larvae from each type of foliage were divided into four lots and transferred to the four host plants. Fresh weights of the larvae were taken and dry matter content was determined on samples of larvae from both types of food. The test larvae were fed in groups of five per jar and given fresh food daily. Net weight gains were calculated from the dry weights (exuvia excluded) of the freshly ecdysed last instar. Dead larvae were all virus-free.

Regardless of the nutritive value of the food in the early instars, the net weight gains show similar patterns (Table II). However, a noteworthy feature of the response of larvae transferred from red pine to food of higher nutritive value was the occurrence of feeding sixth-instar larvae, the number being highest in those transferred to the most nutritious food, jack pine. By comparison with the fifth instar, these larvae had larger bodies and head capsules. In larvae left on the same host or transferred from jack pine to food of lower nutritive value, no additional instar occurred. In additional experiments, feeding sixth instars occurred when larvae were left on white pine or transferred to the three other foods but only nonfeeding last-instar larvae were obtained by transferring larvae from Scots pine to any of the other foods. The average period of development increased with the decrease in nutritive value of the food when the larvae were transferred from jack pine. This pattern did not prevail when the transfer was from red pine because of the additional instar The data were insufficient to make comparisons of the response to the four food plants in terms of weight gain and therefore no firm conclusions with respect to conditioning by the food supplied in the earlier instars could be drawn. Finally, variability in the number of instars is apparently sex-limited since no additional instar was seen in males fed similar food regimes.

3. Effects of topical application of a synthetic mixture of juvenile hormones to fifth instar larvae of *D. similis.*

The extra instar encountered when larvae were reared on a poor food suggested interference in the endocrine activities that regulate ecdysis to the last instar. The possibility that the additional instar resulted from a higher than normal titre of juvenile hormone was tested by topical application of a juvenile hormone preparation to female fifth-instar larvae.

A large number of freshly ecdysed female larvae were collected from 20 separate colonies feeding on Scots pine. Three-day-old larvae were treated with doses of 0.1, 1, and 10 μg of a synthetic hormone preparation[3] applied topically in 1 μl of acetone to the dorsum of the thorax. These were compared to untreated and acetone controls by recording the number of days to spinning of the cocoon, plus the numbers of cocoons,

3 The juvenile hormone preparation, a mixture of the eight possible geometric isomers containing 17.5 percent of the authentic *Cecropia* hormone (trans-trans-cis configuration), possessing about one-tenth the activity of the pure hormone, was a gift from the Ayerst Research Laboratories, Montreal.

the average width and diameter of cocoons, and adults obtained. The results are shown in Table III. With increasing dose of hormone, there was an increase in the duration of the instar; feeding was prolonged and more growth occurred as shown by the larger dimensions of the cocoons. Some treated larvae failed to spin cocoons, but no delay in the onset of cocoon spinning was evident in those that did form cocoons following ecdysis to the last instar. Larvae failing to spin cocoons were typically last instar in appearance and the few that did spin out the silk did so in a completely unorganized manner on the bottom of the container. Two of the larvae treated with the highest dose were unable to shed the exuvium but apolysis was complete. Adults emerging from the cocoons were normal in appearance and dissection of those failing to emerge revealed dead larvae or pupae but no intermediates.

TABLE III

THE EFFECTS OF TOPICAL APPLICATION OF A JUVENILE HORMONE PREPARATION TO 3-DAY-OLD PENULTIMATE INSTAR LARVAE OF *D. SIMILIS*

	Untreated control	Acetone control	Treatments (μg per larva)		
			.1	1	10
No. females treated	19	20	20	19	20
Days from treatment to cocoon spinning	5.7	5.7	9.3	11.2	15.0
No. cocoons	18	19	18	12	9
Cocoon length (mm)	9.9±0.09	9.7±0.08	10.5±0.08	11.4±0.14	12.0±0.05
Cocoon diameter (mm)	5.0±0.10	5.0±0.08	5.5±0.07	5.8±0.12	6.3±0.09
No. adults	16	13	15	7	5

Treatment of 3-day-old larvae with this juvenile hormone preparation did not produce supernumerary larvae or retention of visible characteristics of the feeding larva in the nonfeeding last instar nor did it give larva-pupa or pupa-adult intermediates. It increased the duration of feeding and interfered with the formation of cocoons; this might be due to a delay in the development of the behavioral pattern for cocoon spinning or to a reduced synthesis of silk.

DISCUSSION

In a recent discussion of the role of secondary plant substances in food selection, Fraenkel (1969) pointed out that an insect does not necessarily choose food with the highest nutritive value and that the quantitative composition of a plant may vary considerably without greatly affecting host selection. The present studies on food utilization by *D. similis* support this view. White pine and Scots pine are favored hosts for oviposition (Tsao and Hodson, 1956; Wallace, 1961) but the white pine foliage is low in nutritive value. Conversely, jack pine which is not a favored host has high nutritive value. Similar experiments with *Neodiprion lecontei* and *N. sertifer* add further support to the concept enunciated by Fraenkel. Jack pine is highly nutritious for *N. lecontei* but is not preferred by this sawfly and red pine is a common host for *N. sertifer* yet the leaves of this tree are low in nutritive value.

Early studies on *D. similis* (Middleton, 1923) indicated that there were five male and six female feeding instars. The *D. similis* female used in this study ordinarily has five feeding instars; however, depending on the food, an additional instar can occur. Variability in the number of instars in insects is common (Hoskins and Craig, 1935) and in the grasshopper, *Melanoplus bilituratus* (Walker), extra instars appear more frequently when nymphs are raised from hatching to adult on a poor food compared to when they are fed on foods of higher nutritive value (Smith, 1959). In *D. similis* an extra instar occurs when larvae are kept on food of low nutritive value and if the larvae are transferred from a poor food to a better food. A juvenilizing factor in the more nutritious foods is unlikely because continuous feeding on jack pine which has high food value does not produce the extra instar. Perhaps such a factor exists in the poor foods. However, an extra instar was not obtained by topical application of a juvenile hormone preparation. Moreover, injections of juvenile hormone into the last instar larvae of *Bombyx mori* L. (Akai and Kobayashi, 1971) and into *Sarcophaga bullata* Parker (Srivastava and Gilbert, 1969) at different times prolonged the larval life without producing supernumerary larvae. Evidently juvenile hormone applied by injection to those insects is not sufficient stimulus to produce an additional instar either.

In the sawfly the extra instar occurred when larvae were kept on a diet of low nitrogen digestibility or when they were transferred from food with a low digestibility of nitrogen to food from which nitrogen is more readily utilized. It is interesting to compare this to experiments where rats are fed an adequate protein diet following a low protein diet; hepatic protein is synthesized more rapidly than in rats fed an adequate diet throughout (Horie and Ashida, 1971). If synthesis of protein in the sawfly is affected by nutrition in a comparable manner, an alteration in the action of the hormones which control ecdyses may occur because protein synthesis has a central role in the growth-regulating activities of ecdysone (Karlson and Sekeris, 1966) and juvenile hormone (Ilan *et al.*, 1970). It is also possible that sustained nutrient deprivation in early instars in some way alters the output and hence the level of endocrine factors.

Our aim is to use the fifth instar to attempt to explain the effects of alterations in the composition of the leaves on the growth of the insect, and an additional instar can interfere where critical comparisons are to be made. This can be overcome by feeding the larvae on food of high nutritive value in the earlier instars.

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REFERENCES

Akai, Hiromu and M. Kobayashi. 1971. Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L. (Lepidoptera: Bombycidae). Appl. Ent. Zool. *6*:138-139.

Fraenkel, G. 1969. Evaluation of our thoughts on secondary plant substances. Ent. exp. appl. *12*:473-486.

Horie, Yoshimitsu and K. Ashida. 1971. Stimulation of hepatic protein synthesis in rats fed an adequate protein diet after a low protein diet. J. Nutrition *101*:1319-1326.

Horwitz, William. 1970. Official Methods of Analysis of the Association of Official Analytical Chemists. Eleventh Edition. Association of Official Analytical Chemists, Washington.

Hoskins, W.M. and R. Craig. 1935. Recent progress in insect physiology. Physiological Reviews *15*:525-596.

Ilan, Joseph, Judith Ilan and N. Patel. 1970. Mechanism of gene expression in *Tenebrio molitor*. Juvenile hormone determination of translational control through transfer ribonucleic acid and enzyme. J. Biol. Chem. *245*:1275-1281.

Karlson, P. and C.E. Sekeris. 1966. Ecdysone, an insect steroid hormone, and its mode of action. Recent Progr. Horm. Res. *22*:473-502.

Middleton, William. 1923. The imported pine sawfly. United States Department of Agriculture. Bull. No. 1182.

Smith, D.S. 1959. Utilization of food plants by the migratory grasshopper, *Melanoplus bilituratus* (Walker) (Orthoptera: Acrididae), with some observations on the nutritional value of the plants. Ann. Entomol. Soc. Am. *52*:674-680.

Srivastava, U.S. and L.I. Gilbert. 1969. The influence of juvenile hormone on the metamorphosis of *Sarcophaga bullata*. J. Insect. Physiol. *15*:177-189.

Stark, R.W. 1965. Recent trends in forest entomology. Annu. Rev. Entomol. *10*:303-324.

Tsao, C.H. and A.C. Hodson. 1956. The effect of different host species on the oviposition and survival of the introduced pine sawfly. J. Econ. Entomol. *49*:400-401.

Waldbauer, G.P. 1968. The consumption and utilization of food by insects. Adv. Insect. Physiol. *5*:229-288.

Wallace, D.R. 1961. Pine sawflies. *In*: "White and Red Pine – Ecology Silviculture and Management", (K.W. Horton and G.H.D. Bedell) pp. 140-143. Forestry Bull. 124. Canada Department of Northern Affairs and National Resources. Resources.