SPECTROPHOTOMETRIC DETERMINATION OF LARVAL INGESTION RATES IN THE SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE)

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Abstract

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The food intake of fourth, fifth, and sixth instar spruce budworm, *Choristoneura fumiferana* Clemens, was investigated by feeding larvae for 24 h artificial diet incorporating amaranth dye. Amaranth was selected because it followed Beer's law over a wide concentration range, mixed well with the meridic diet, could be extracted in ice-cold water, was not absorbed by larval tissue, had minimal feeding deterrence as well as marginal adverse chronic effects on the larvae, and finally had negligible effect on ingestion rate over a 24 h period. The results indicated that 6th instars consumed 20 times and 5th instars 3 times as much as 4th instars, but when compared on a body weight basis the ingestion rate was similar in all three instars. Temperature and photoperiod influenced the rate of food intake. The application of these findings in assessing defoliation to the forests as well as estimating dosage of pesticides for control of this species are discussed.

Résumé

On a étudié la quantité de nourriture ingérée par les larves de la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* [Clem.] des quatrième, cinquième et sixième stades soumises pendant 24 heures à un régime artificiel coloré à l'amarante. On a choisi l'amarante parce que c'est un colorant qui obéit à la loi de Beer dans un large éventail de concentrations, qu'il se mélange bien avec les constituants du régime, qu'il peut être extrait à l'eau glacée, mais n'est pas absorbé par les tissus des larves, qu'il ne dissuade que très peu les larves et ne produit sur elles qu'un faible effet chronique nuisible et, enfin, qu'il n'a qu'un effet négligeable sur la qu antité de nourriture ingérée en 24 heures. D'après les résultats, les larves des sixième et cinquième ont ingéré respectivement 20 et 3 fois plus de nourriture que les larves du quatrième stade, mais, en fonction du poids corporel, la proportion ingérée était semblable dans les trois cas. La température et la photopériode ont influé sur la quantité de nourriture ingérée. On discute de l'application de ces résultats à l'évaluation de la défoliation des forêts ainsi qu'à l'estimation de la dose de pesticides à utiliser contre la tordeuse.

Introduction

A study of food intake by phytophagous insect pests is not only helpful in understanding quantitative utilization (Parra and Kogan 1981; Waldbauer 1968) but also useful in predicting potential damage to the host plant. For instance, the spruce budworm *Choristoneura fumiferana* Clemens, feeds on the current year's foliage of balsam fir, *Abies balsamea* L., and white spruce, *Picea glauca* Moench causing considerable damage (Rose and Lindquist 1977). The last three of the six larval instars feed on flushed buds and are usually the targets for control (Prebble 1976). The contribution of each of these instars to defoliation could be estimated by determining its ingestion rate.

Another reason for studying food intake concerns the use of biorational control agents (Chock and Dover 1980) such as microbial insecticides (Burges 1981) and insect growth regulators (Maas *et al.* 1980; Retnakaran 1981) that have to be ingested in order to be effective. A lethal dose of the control agent must be consumed before the material is degraded or washed off. With knowledge of half life of the material as well as the lethal dose and feeding rates of the insect pest the quantity of biorational insecticide to be applied over a given area to produce the desired effect can be logically predicted with reasonable accuracy. The ingestion rate of the 4th, 5th, and 6th instar spruce budworm was studied using artificial diet containing amaranth and the results are presented in this report.

Materials and Methods

Insect culture. Spruce budworm larvae were reared on a meridic diet (McMorran 1965) after the method of Stehr (1954) modified by Grisdale (1970) at 22°C, 70% r.h., and a photoperiod of 16 h light and 8 h darkness. Precisely aged 4th, 5th, and 6th instars were used in all the experiments. The age of the larvae was determined by selecting moulting 3rd, 4th, and 5th instars identified by the head capsule slippage as indicated by a white band behind the head capsule (Retnakaran 1973). These larvae were considered to be on "day 0" of the 4th, 5th, and 6th instar and in 24 h when they had completed the moult they were on "day 1" of the respective instars. On "day 4", 4th instar larvae showed head capsule slippage and therefore could be considered "day 0" of the next instar. The same was true for "day 5" of 5th instar which could be "day 0" of 6th instar. The values for the overlapping days were averaged and reported as "day 0" values in the graphic presentations.

Amaranth estimation. Incremental concentrations of amaranth (or Acid red 27) were dissolved in water and the absorbance of 5 ml aliquots were measured in a Gilford 250 spectrophotometer at 522.5 nm to obtain an absorption curve.

Amaranth was incorporated in molten diet, dispensed into coffee creamer cups (29 ml capacity), allowed to gel and stored in plastic bags in the refrigerator (Retnakaran 1979). Four concentrations of approximately 0.1%, 0.5%, 1.0%, and 2.0% (w/w) were prepared. Concentrations used for studying the ingestion rates were 0.5% for 6th instar, 1.0% for 5th instar, and 2.0% for 4th instar.

The relationship between weight of amaranth diet and the optical density was determined by weighing various quantities of diet, extracting the amaranth, and measuring its absorbance. To each weighed piece of amaranth diet sufficient ice-cold water was added to make exactly 5 ml and the diet was then homogenized at 0°C. The homogenate was centrifuged for 1 h at 15,000 rpm and filtered through a 0.45 μ pore size millipore filter. The absorbance of the filtrate was measured as before in a spectrophotometer.

To determine the ingestion rate, 20 larvae for each day of the stadium were selected, weighed, and placed individually in coffee creamer cups. A piece of amaranth diet approximately 1 cm^3 was placed in each cup and covered with a lid. Each larva was allowed to feed on the amaranth diet for 24 h after which it was transferred to either a cup (4th instar) or a 10 cm petri dish (5th and 6th instars) containing a piece of amaranth-free diet for 48 h. All the red frass from both the cups were collected in a tube. Any area of amaranth-free diet in the second cup that had been stained red by contact with red frass was excised and included with the frass. As before ice-cold water was added to make 5 ml, the mixture was processed at 0°C, and the absorbance was measured.

Tissue absorption of amaranth. Twelve, 2-day-old 6th instar larvae (6 male and 6 female) were allowed to feed on 2.0% amaranth diet for 24 h and then transferred to amaranth-free diet for 48 h. The larvae were dissected under a microscope in water and the wall of the alimentary tract, fat body, ovaries, salivary glands, Malpighian tubules, tracheae, body wall, and musculature were examined for the presence of amaranth. An equal number of larvae reared on amaranth-free diet were used as controls.

The absorption of amaranth into the hemolymph was studied by collecting hemolymph from 40, 2-day-old 6th instar larvae that had fed on 2% amaranth diet for 24 h. To prevent melanization a few crystals of ascorbic acid were added to the hemolymph and the test tube was placed in an ice bucket. It was then diluted 1:1 with ice-cold water, mixed, and centrifuged at 3000 rpm for 30 min at 0°C to remove hemocytes. The optical density of the supernatant was measured at 522.5 nm. Hemolymph from an equal number of larvae reared on amaranth-free diet served as the control.

Diet preference. Two-day-old 6th instars were offered a choice of amaranth-containing diet or amaranth-free diet in a 10 cm petri dish. Four diet cylinders (1.0 cm diam × 0.5 cm high), two with and two without amaranth, were punched-out with a cork borer, and placed equidistant from each other in the dish. Ten larvae were allowed to make a choice and the distribution recorded over a 24 h period. Ten replicates were used for each concentration (0.1, 0.5, 1.0, 2.0%) of amaranth.

Development on amaranth diet. One-day-old 5th instars were individually reared on amaranth-containing diet and amaranth-free diet until they pupated. Twenty larvae were used for each concentration of amaranth as well as 20 for controls. The weight gain and time to pupation were determined.

Weight loss of diet. Pieces of 2% amaranth diet, approximately 1 cm^3 were placed individually in coffee creamer cups with and without lids and incubated at 22°C and 32°C in environmental chambers. The relative humidity was about 70%. Each of the four treatments was replicated 20 times. The cups with diet were weighed at the beginning of the experiment and on each successive day for 4 days and the loss of weight recorded.

Temperature and photoperiod effects on ingestion rate. The ingestion rate of 2-day-old 6th instars maintained on 0.5% amaranth diet at 12°, 17°, 22°, and 27°C for 24 h was determined as described earlier. Twenty larvae were used for each temperature regime and the r.h. and photoperiod were maintained at 70% and 16 h light and 8 h darkness respectively in all cases.

The effect of photoperiod on ingestion rate was investigated using 2-day-old 6th instars. The temperature and r.h. were maintained at 22°C and 70% respectively. The food intake for 24 h, 16 h and 8 h under different photoperiods was determined. Twenty larvae were used for each photoperiodic regime.

Analysis of data. All values reported were statistically analyzed wherever necessary using a *t*-test for comparing two means and by analysis of variance for more than two means. Duncan's new multiple-range test was used to determine significantly different means in the latter use. The level of probability was 0.05.

Results

Amaranth absorption. The relationship between the concentration of Amaranth (Fig. 1) in water and its optical density at 522.5 nm was linear up to a concentration of 500 μg/5 ml thereby satisfying Beer's law (Fig. 2).

Amaranth diet and optical density. The relationship between the weight of amaranth-containing diet and the optical density of the amaranth extracted from it for the 0.5% amaranth diet was measured up to 80 mg of diet (Fig. 3); for the 1.0% diet up to 40 mg of diet (Fig. 4); and for the 2.0% diet up to 15 mg of diet (Fig. 5). The linear relationships of weight versus absorbance of these three diets were well within the estimated range of 24 h food intake or 6th, 5th, and 4th instars respectively.

Tissue absorbance of amaranth. When 6th instars that had fed on amaranth diet were dissected, none of the tissues showed any evidence for the presence of amaranth. A few

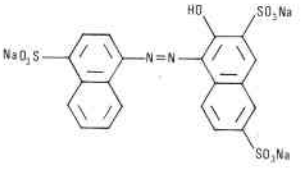

FIG. 1. Structure of the dye amaranth, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-2, 7-naphthalenedisulfonic acid trisodium salt.

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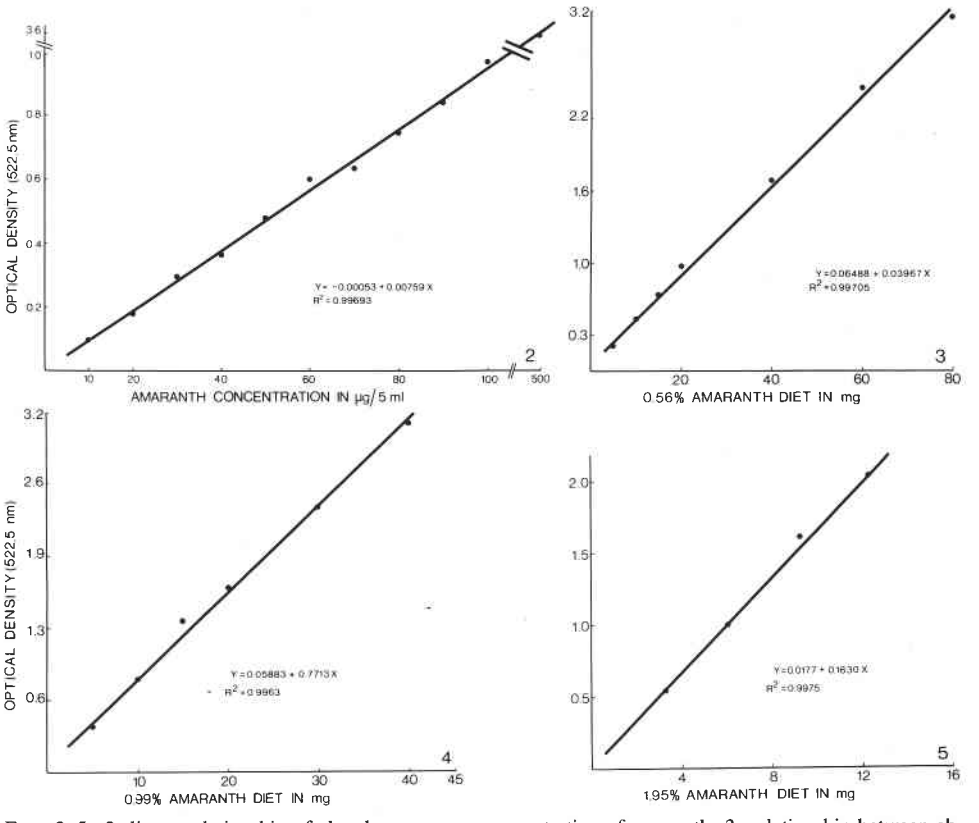

FIGS. 2–5. 2, linear relationship of absorbance versus concentration of amaranth. 3, relationship between absorbance and weight of diet containing 0.56% (w/w) amaranth used in determining food intake of 6th instar spruce budworm. 4, relationship between absorbance and weight of diet containing 0.99% (w/w) amaranth used in determining food intake of 5th instar spruce budworm. 5, relationship between absorbance and weight of diet containing 1.95% (w/w) amaranth used in determining food intake of 4th instar spruce budworm.

Table I. Absence of amaranth in hemolymph of 6th instar spruce budworm that had ingested 2% amaranth diet

Source	Dilution of hemolymph:water	O.D. at 522.5 nm
Larvae reared on 2% amaranth diet for 4 days	1:1	0.206
Larvae reared on amaranth-free diet	1:1	0.292

preparations of fat body had reddish-brown granules but these did not dissolve in the water. Also, similar granules were found in some of the controls. Thus the granules in the treated larvae were not considered to be amaranth.

When the hemolymph of 6th instars that had fed on amaranth diet was examined for absorption at 522.5 nm and compared with hemolymph from similar larvae reared on amaranth-free diet, no difference was observed indicating absence of amaranth in the hemolymph (Table I).

Feeding preference of amaranth and amaranth-free diet. In these tests, 6th instar larvae preferred the amaranth-free diet over treated diet at each concentration (Table II). About 50% of the feeding larvae, however, did feed on the amaranth diet and hence the feeding deterrent effect was considered marginal.

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Table II. Feeding preference of 6th instar spruce budworm offered synthetic diets with and without amaranth[a]

Elapsed time (h)	Number of larvae feeding on: Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	0.1% amaranth	Unt.[b]	0.5% amaranth	Unt.	1.0% amaranth	Unt.	2.0% amaranth	Unt.
0	21	33	21	27	23	34	14	30
2	25	47	17	34	23	37	15	37
4	26	45	16	34	13	35	16	37
6	26	42	17	32	17	36	20	43
24	20	31	20	44	23	40	18	45

[a]Each experiment was conducted with 10 replicate of 10 larvae each. Values in each row within an experiment when added to the number of larvae not feeding total 100. The 24 h observations in each experiment are significantly different at the 0.05 level.
[b]Unt., untreated.

Development on amaranth diet. The development in terms of weight gain and days to pupation of 5th instars on the 0.1% amaranth concentration was normal. However, on the 0.5%, 1.0%, and 2.0% amaranth diets, the time to pupation was prolonged, and for the 2.0% diet the weight gain after 11 days was lower (Table III). While there may be long-term chronic effects at higher concentrations, the effect of amaranth on feeding over a 24 h study was considered to be marginal.

24 h feeding rate on three different concentrations of amaranth in diet. The food intake of 6th instar larvae over a 24 h period was not different between 0.5% and 1.0% diet nor between 1.0% and 2.0% amaranth diet. However, the ingestion rates of 0.5% and 2.0% were different (Table IV).

Since the food consumption of 6th and 5th instars was determined using 0.5% and 1.0% amaranth diet respectively, the results obtained should be reasonably close to the actual rate. Since food intake of 4th instars was determined using 2.0% amaranth diet the results might slightly underestimate the actual value.

Weight loss of diet. The loss of weight of diet, in closed cups after 24 h, due to evaporation of water was 6.5% at 22°C and 37.3% at 32°C. When the cups were left open, the loss of weight was 43% at 22°C and 53.9% at 32°C. The weight loss after 48 and 72 h was more severe (Table V). This loss in weight would tend to concentrate the amaranth in the diet and overestimate the actual food intake especially at the highest concentration, namely 2% diet.

Daily food intake of larvae. The ingestion rate of 4th, 5th, and 6th instars over a 24 h period was determined. Each instar showed peak feeding at mid stadium and this occurred

Table III. Growth and development of 5th instar spruce budworm reared on amaranth diet

Experiment	Amaranth in diet (% w/w)	Increase in weight (mg/larvae ± SE) on: Day 1	Day 6	Day 11	Time to pupation (days ± SE)
1	0.10	5.08 ± 0.33	36.71 ± 4.20	69.26 ± 8.29	14.0 ± 0.5
	–	5.62 ± 0.30	53.80 ± 5.80	80.58 ± 10.52	13.3 ± 0.8
2	0.50	5.13 ± 0.44	36.17 ± 4.82	79.22 ± 11.54	15.9 ± 1.0[a]
	–	5.33 ± 0.34	36.19 ± 2.43	78.26 ± 6.52	13.3 ± 0.5
3	0.99	5.69 ± 0.31	27.78 ± 2.35	69.20 ± 7.33	15.5 ± 0.5[a]
	–	5.33 ± 0.34	36.19 ± 2.43	78.36 ± 6.52	13.3 ± 0.5
4	1.98	4.84 ± 0.28	17.43 ± 1.91	37.86 ± 5.19	19.5 ± 1.0[a]
	–	5.51 ± 0.32	48.81 ± 4.24	79.32 ± 8.59	13.4 ± 0.6

[a]Significantly different from their respective controls at the 0.05 level.

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Table IV. Effect of amaranth concentration on diet consumption by 6th instar spruce budworm

Amaranth in diet (% w/w)	Diet ingested in 24 h	
	mg ± SE/larva	mg ± SE/mg body weight
0.50	54.23 ± 5.13 b	1.72 ± 0.14 b
0.94	53.81 ± 6.44 ab	1.48 ± 0.18 ab
1.98	43.93 ± 4.49 a	1.11 ± 0.10 a

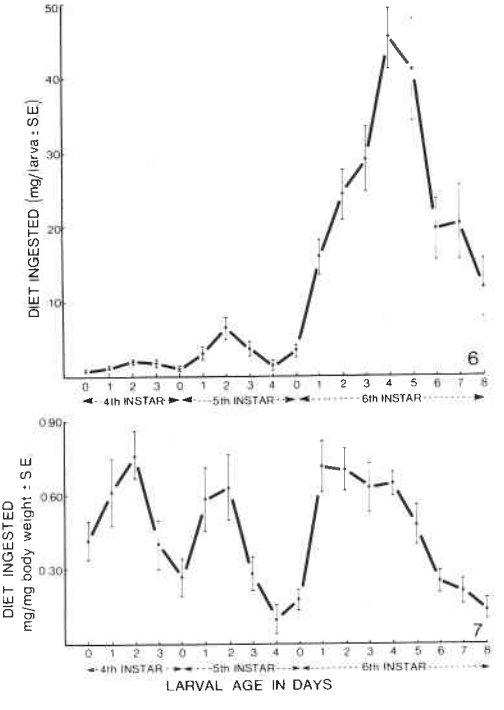
NOTE: Values in each column followed by the same letter are not significantly different at the 0.05 level.

Table V. Weight loss of diet over time

Temp. (°C)	Diet cup (closed/ open)	Weight in g ± SE (%) on:			
		Day 1	Day 2	Day 3	Day 4
22	Closed	3.007 ± 0.059 (100)	2.813 ± 0.056 (93.5)	2.551 ± 0.052 (84.8)	2.309 ± 0.018 (76.8)
22	Open	2.105 ± 0.053 (100)	1.202 ± 0.049 (57.0)	0.971 ± 0.014 (46.3)	0.950 ± 0.009 (45.3)
32	Closed	2.878 ± 0.061 (100)	2.312 ± 0.050 (62.7)	1.763 ± 0.016 (61.4)	1.761 ± 0.016 (61.4)
32	Open	2.305 ± 0.064 (100)	1.057 ± 0.014 (46.1)	0.982 ± 0.010 (42.8)	0.976 ± 0.010 (42.6)

on "day 2" of 4th and 5th instars and "day 4" of 6th instar (Fig. 6). The maximum ingestion rate of 5th instars was 3 times and that of 6th instars was 20 times that of 4th instars. The maximum feeding rate of 6th instars was 7 times that of 5th instars.

When the daily rate of diet consumption was plotted in terms of larval body weight, the peak feeding rate for all three instars was similar (Fig. 7). Such a relationship indirectly suggests that the 24 h feeding rate of the three instars was as close to the actual value as



FIGS. 6–7. Daily intake of diet (6) per larva and (7) per mg body weight of 4th, 5th, and 6th instar spruce budworm.

possible. Any possible deterrence of the 2% amaranth diet to 4th instars was probably compensated by the amaranth concentrating effect of evaporation on the diet.

When the growth rate was plotted against larval age, a characteristic sigmoid relationship was observed (Fig. 8). The increase in weight of each instar was proportional to the feeding rate of each larva suggesting a direct relationship which is not unexpected.

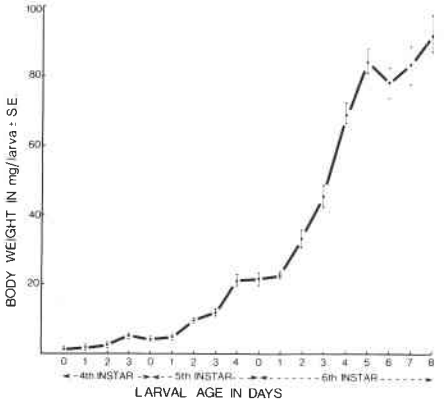


FIG. 8. Growth curve of 4th, 5th, and 6th instar spruce budworm.

Effect of temperature on food intake. The daily ingestion rate of 6th instars rose by a factor of 1.4 from 12° to 17°C, by a factor of 2 from 12° to 22°C, and by a factor of 5 from 12° to 27°C (Table VI). On a larval body weight basis the ingestion rate increased by a factor of 1.5, 1.8, and 4 respectively. The relationship appears to be exponential.

Effect of photoperiod on food intake. The diet ingestion rate of 6th instars was determined for a 24 h period consisting of 16 h light and 8 h darkness, for a 16 h period under continuous light, and for an 8 h period under darkness (Table VII). The food intake for 8 h darkness was significantly less than that for 16 h light even when the 8 h value was doubled indicating that night feeding was less. However, when the sum of the 16 h light feeding and 8 h darkness feeding was compared with the 24 h feeding (16 h light and 8 h darkness), the latter was significantly greater. The difference can be explained by examining the feeding behavior of the spruce budworm. As soon as a larva is placed on a piece of diet it actively feeds and at the same time spins a web and establishes a feeding niche. After this initial activity which can be referred to as "disturbance feeding", the larva settles down and feeds at a slower pace. In the present experiment when the larva was placed on a piece of diet, regardless of the photoperiodic regime (16 h light or 8 h darkness) it engaged in "disturbance feeding". And hence when the two values were added it not only included feeding for 16 h light and 8 h darkness but also included the added effect of two "disturbance feeding" periods. When such a value (16 h light feeding + 8 h dark feeding) was compared with the continuous 24 h feeding under 16 h light and

Table VI. Effect of temperature on diet consumption by 6th instar spruce budworm

	Diet ingested in 24 h	
(°C)	mg ± SE/larva	mg ± SE/mg body weight
12	12.25 ± 2.04	0.39 ± 0.05
17	17.25 ± 2.27	0.60 ± 0.08
22	24.43 ± 3.31	0.70 ± 0.09
27	60.31 ± 9.20	1.62 ± 0.23

NOTE: Values in each column of diet ingested are significantly different from each other at the 0.05 level.

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Table VII. Effect of photoperiod on diet ingestion by 6th instar spruce budworm

Item No.	Photoperiod	Diet ingested	
		mg ± SE/larva	mg ± SE/mg body weight
1	16 hr light and 8 h darkness	26.59 ± 2.65	0.68 ± 0.06
2	16 h light	28.38 ± 2.02	0.72 ± 0.05
3	8 h darkness	10.12 ± 3.10	0.27 ± 0.02
4	Sum of 16 h light and 8 h darkness (items 2 & 3)	38.50 ± 3.10	0.96 ± 0.08
5	Twice 8 h darkness (items 3 & 2)	20.24 ± 2.17	0.55 ± 0.04

NOTE: Diet ingested in either of the columns of items 1 and 4, 2 and 3, and 2 and 5 are significantly different from each other at the 0.05 level.

8 h darkness, which had only the initial "disturbance feeding", the latter value was significantly lower than the former additive rate.

If indeed "disturbance feeding" can obscure the effect of photoperiod, then the feeding rate under a short photoperiod regime of either 8 h light or 8 h darkness should not be different. When such a study was conducted, no significant difference was observed (Table VIII). Thus, photoperiod appears to have a marginal effect as indicated by the slightly lower feeding rate at night.

Table VIII. Effect of 8 h photoperiod on diet ingestion by 6th instar spruce budworm

Photoperiod	Diet ingested	
	mg ± SE/larva	mg ± SE/mg body weight
8 h light	7.10 ± 0.92	0.18 ± 0.02
8 h darkness	5.39 ± 1.47	0.17 ± 0.03

NOTE: Diet ingested in either of the columns are not significantly different from each other at the 0.05 level.

Discussion

Amaranth as a marker for diet ingestion studies. Food intake in insects has been investigated using different methods, both direct and indirect (Parra and Kogan 1981; Waldbauer 1968). The most common direct procedure has been the gravimetric method of weighing the frass. This method is suitable regardless of whether artificial diet is available for the insect or the insect has to be fed its natural food. Loss of weight due to evaporation of water and residual food in the gut are two of the major shortcomings of this method.

Indirect methods using radioactive markers like cesium-137 (Crossley 1966), sucrose-u-^{14}C or cellulose-u-^{14}C (Kasting and McGinnis 1965), and sodium^{22} (Buscarlet 1974) have been utilized in determining food intake. In addition to being expensive, special facilities are required especially when high energy nuclides are used. For example when ^{14}C-materials are used, a special trapping system for $^{14}CO_2$ is necessary.

Indirect colorimetric methods using non-digestible dyes such as chronic oxide (McGinnis and Kasting 1964) and calco oil red (Daum *et al.* 1969) have been used in some studies but these studies have shown that there is a significant reduction in food intake (Parra and Kogan 1981).

Although amaranth has not been used previously in an indirect colorimetric method for determining the food intake of any phytophagous insect, it has been used in studying aspects of feeding of the marine nematode, *Enoplus brevis* Bastian (Atkinson 1977), the meal size of the horn fly, *Haematobia irritans* L. (Kuramochi and Nishijima 1980), ingestion and metabolic studies of bugs such as *Creontiades dilutus* (Miles and Hori

1977) and *Lygus disponsi* (Hori and Endo 1977), and finally in the study of the secretion of organic anions and alkaloids from the bathing medium by isolated Malpighian tubules of *Rhodnius prolixus* (Maddrell and Gardiner 1975, 1976). The putative non-toxicity of amaranth to man has led to its use as food coloring since 1907 (Palmer *et al.* 1979). More recently genetic toxicology studies using Ames test have shown it to be non-mutagenic (LeConte and Lesca 1978); however, chronic studies at relatively high doses have indicated certain morphological effects on rat liver (Gales *et al.* 1972). It is of interest to note that at higher doses chronic effects resulting in growth retardation of the spruce budworm larvae were observed in the present study.

Several factors were considered in selecting amaranth for determining the ingestion rate of the spruce budworm. It is water soluble and readily miscible in the synthetic diet. It is non-absorbable and assumed to be non-digestible although the possibility of intestinal breakdown of amaranth into products such as sodium α-naphthylamine-4-sulfonate and 1-amino-2-naphthol-3, 6-disulfonic acid sodium salt could not be ruled out (Palmer *et al.* 1979). The absorbance versus concentration is linear (Beer's law) over the range used in this study. Although there are minor adverse effects, on a 24 h basis these appear to be minimal. Although extraction was not possible with most organic solvents, it could be extracted in water but agar in the aqueous extracts increased the turbidity and seriously affected absorbance. However, this was overcome by using ice-cold water and millipore filtration.

When all facts are considered, 24 h appears to be the ideal length of time for studying food intake with amaranth. Anything less than that would increase biological variation and anything more than that would affect the growth rate which in turn may be due to poor food intake. Besides, at greater than 24 h weight loss of diet due to evaporation might become a serious complication (Table V).

Ingestion rate and defoliation appraisal. The spruce budworm overwinters as a 2nd instar and the 3rd instars either mine needles or buds but the 4th, 5th, and 6th instars feed on the bud. For this reason, these latter three instars have been the target for control to protect the foliage (Morris 1963). The present study indicates that the major damage to the new shoot is done by the 6th instars.

Temperature and photoperiod may also influence defoliation by the larvae. By the time the larva reaches the 6th instar in late June, the temperature is relatively high (Retnakaran 1981) and this will increase the feeding rate (Table VI). Night feeding will be less not only because of photoperiod (Table VII) but also due to the added effect of lower temperatures at night (Table VI).

Food intake and insecticide dosage. Ingestion of a lethal dose of toxicant by a larva is a function of the rate of feeding. A high dose of a labile material is required if the rate of food intake by the insect is relatively small. One of the insect growth regulators tested against the spruce budworm, Diflubenzuron (Dimilin®), illustrates the need for understanding the food intake in formulating dosages for controlling the pest in question. Unlike most insecticides, this material shows stadial susceptibility, the 6th instars being more susceptible and the 5th and 4th instars being relatively refractory (Granett and Retnakaran 1977). Experimental aerial applications of Diflubenzuron against 4th and 5th instar budworm resulted in poor control of this pest (Retnakaran 1981). It is quite conceivable that the low food intake of the 4th and 5th instars resulted in a much higher dose requirement than that used in the field trials.

Another factor is the time of spraying of control agents. Night feeding is less due to photoperiod and temperature effects on the larvae than day feeding. Consequently, spraying at dark calls for a higher dosage than at dawn if the material is relatively labile.

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