NUTRITIONAL STUDIES OF EASTERN SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE)
II. STARCHES

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Abstract

Amylopectin added to a sugar-free wheat-germ diet was equal to or better than sucrose as a carbohydrate source, and appears to be readily utilized by the eastern spruce budworm (*Choristoneura fumiferana* (Clem.)). Larval growth on diets containing dextrins or potato starch shows that they are partly utilized. Starches from other sources, including those isolated from mature balsam fir (*Abies balsamea* (L.) Mill.) or white spruce (*Picea glauca* (Moench) Voss) needles, are not utilized to any extent, on the basis of larval growth on diets to which they have been added.

Sixth-instar budworm reared on artificial diets contain amylase(s) in midgut and salivary gland homogenates, which show a low rate of digestion of starches from host foliage. However, the presence of appreciable starch in frass from foliage-fed insects and the apparent low utilizability of foliar starch indicate that the latter is not an important nutrient for the budworm under natural conditions.

Résumé

Ajoutée à une diète de germe de blé sans sucre, l'amylopectine s'est avérée égale ou supérieure au sucrose en tant que source d'oses, et semble être aisément utilisée par la Tordeuse des bourgeons de l'Épinette, *Choristoneura fumiferana* (Clem.). La croissance larvaire obtenue sur des diètes contenant des dextrines ou de la fécule de pomme de terre montre que ces substances sont partiellement utilisées. D'après la croissance larvaire observée sur les diètes auxquelles ils ont été ajoutés, les amidons d'autres provenances y compris ceux isolés des aiguilles du Sapin baumier (*Abies balsamea* (L.) Mill.) ou de l'Épinette blanche (*Picea glauca* (Moench) Voss) à maturité ne sont nullement utilisés.

Les intestins et les glandes salivaires de la Tordeuse au sixième stade élevée sur diète artificielle contiennent des amylases, ce qui indique un faible taux de digestion des amidons du feuillage de l'arbre-hôte. Il appert cependant de la présence d'amidon en quantité considérable dans les chiures des insectes nourris au feuillage, ainsi que de la médiocre capacité évidente de l'amidon des feuilles à être utilisé, que ce dernier ne constitue pas une nourriture importante pour la Tordeuse dans les conditions naturelles.

Introduction

The principal soluble sugars of developing needles and shoots of balsam fir and white spruce, i.e., fructose, glucose, sucrose, and raffinose (Little 1970; Neish 1958), have been shown to be utilizable by the eastern spruce budworm (*Choristoneura fumiferana* (Clem.)) (Harvey 1974), and in artificial diets the concentration of dietary sugars influences mature insect size. In order to relate carbohydrate levels in developing shoots to development of insects feeding on those shoots, other substances in the plant tissues that can serve as dietary carbohydrates must be identified, and their nutritive value for the insect determined.

The presence of starch in developing needles of both principal host trees for the spruce budworm during at least part of the larval feeding period has been demonstrated (Little 1970; Krueger 1967). Starches are utilized by some insects (Altman and Dittmer 1968, pp. 154–155), although very few leaf-eating insects have been shown to digest them (Waldbauer 1968). If usable by the spruce budworm, even to a small degree, needle starches could serve as additional carbohydrate to augment the soluble sugars and permit greater increases in larval size. Shaw (1973) reported that several commercial starches were less nutritious than sucrose in an artificial diet fed to late-instar larvae, and he inferred that balsam fir starch was probably not utilized. In the present paper results are given of several experiments to determine if the starch present in developing needles of both hosts of this insect can be utilized under natural conditions.

Materials and Methods

Insect stocks consisted of first-laboratory-generation families produced by insects field-collected as late-instar larvae from several locations in eastern Canada. They were reared through all feeding stages on a meridic diet at constant $21°C \pm 1.5°C$ and $70 \pm 3\%$ R.H. with a 17-h photophase, following the general procedure of Stehr (1954) and McMorran (1965) with modifications as described previously (Harvey 1974). Concentrations of salt mixture W (0.5%) and wheat germ (1.75%) in the control diets were reduced from the standard recipe for reasons discussed previously, and wheat germ (Nu-Age Biorganic Products of Canada, Ltd., Toronto) was replaced by an extracted residue free of soluble sugars and fats plus wheat-germ oil to yield the ''basic'' diet (Harvey 1974). Apart from the added starch or sucrose these diets contained virtually no soluble carbohydrate, but did contain small amounts of starch still present in the wheat germ (up to 14% dry wt. (Fraser and Holmes 1959)), probably less than 0.2% of the fresh diet.

Nutritive values of different starches were tested by adding them to the 'basic' diet (Harvey 1974); the control diet contained sucrose which is readily used by this insect. Arrowroot starch was obtained from International Health Foods Ltd., Thornhill, Ont., corn starch from Best Foods Div., Canada Starch Co. Ltd., and dextrins (C grade) from Calbiochem. All other starches were obtained from ICN Nutritional Biochemicals Corporation. Generally the starches were added to the diet as dry powder during the mixing of the other ingredients. Cooked starch was prepared by adding a paste of starch to boiling water, boiling for 1 min, then cooling before adding to the rest of the diet mixture. The final level of sucrose and added starches was 1.75 g/100 g diet; this level of usable sugars was found to support rapid growth of the insect to average natural size (Harvey 1974). Percentage survival of second-instar larvae to the pupal stage, mean weight of pupae, and larval development time (Harvey 1974) have been used as measures of insect performance on the various diets. Although in some experiments no breeding or fecundity measurements were attempted, on all of the test diets the majority of pupae became healthy, apparently fertile adults.

Needle starches for use in the feeding experiments were extracted from 1-year-old balsam fir (*Abies balsamea* (L.) Mill.) and white spruce (*Picea glauca* (Moench) Voss) needles collected in mid-May from full-grown trees near Sault Ste. Marie, Ont. Starch concentration in 1-year-old balsam fir needles has been reported to increase through April and May and to reach a peak just preceding or at the time of the flush of current shoots (Little 1970). This developmental condition is normally reached about mid-May in the Sault Ste. Marie area; the shoots collected for starch extraction at this time had very early flushed buds, and were considered to be approximately at the point of peak starch content. Needles were clipped from the shoots and stored frozen. Starch was extracted from both species using the general method of Badenhuizen (1964, pp. 14–15). Starch preparations were mounted in water or glycerol and studied or photographed using interference contrast microscopy. Confirmation of starch identification was obtained by staining with iodine.

Preparation of Extracts and Assay of Enzyme Activity

The intact gut, its contents, and the salivary glands including reservoirs were removed from chilled sixth-instar larvae actively feeding on artificial diet. After removal of fat-body remnants the insect tissue and a small volume of chilled McIlvaine buffer, pH 7.0, were homogenized in an all-glass homogenizer. The homogenate was centrifuged at 7700 R.C.F. for 15 min. The precipitate was washed with two more portions of the buffer and resuspended in buffer (1 ml/insect) for enzyme tests. The supernatants were pooled and buffer was added to a final volume of 1 ml/insect. Clear,

cell-free extracts prepared in this way contained the major part of the enzyme activity. They were used immediately, or stored frozen until required. Enzyme activity remained stable for several months in the frozen extracts. This same procedure was used to prepare extracts of frass samples for tests of enzyme activity.

Frass was collected from mid-sixth-instar larvae actively feeding on the artificial diets, and represented a 4-h feeding period following removal of all previously accumulated frass. Silk and cast head capsules were removed from the frass samples, which were then frozen until needed. About 13 June, frass was also collected over two successive 12-h periods from larvae feeding on fresh foliage of both hosts. Frass pellets were macerated in saline for microscopic examination.

Assays to determine activity of enzyme preparations with various polysaccharides were carried out in test tubes in a water bath held at 25° and 37°C. Tubes contained 1 ml McIlvaine buffer (ph 7.0), 1 ml of enzyme preparation in buffer, and 1 ml of 3% solution of the substrate; a small drop of toluene was added to the surface of the liquid. After incubation for 20 h, with occasional agitation, the presence of reducing sugars was shown with a Modified Benedict's test (Hawk *et al*. 1949, p. 57). Similar tubes without substrate or with boiled extract were tested and showed negligible levels of reaction products.

Products of enzyme action were identified in centrifuged tube contents by descending paper chromatography of 10 λ spots (Whatman 3M paper) at room temperature (Whelan and Roberts 1953). The solvent system was ethyl acetate – pyridine – water (6:2:1) as in Little (1970). Spots were made visible by spraying the dried chromatograms with aniline – phosphoric acid and heating at 100°C for 5–8 min; they were then identified by comparison with purified sugars on the same sheet, and by co-chromatography.

Results

Foliage Starch

Starch grains isolated from both balsam fir and white spruce (Fig. 1a, b) were near-white in color, contained minimal amounts of cell debris and only 0.3% nitrogen (Kjeldahl). Grain sizes for white spruce starch averaged about 7 μm, ranging from 0.8 to 11.5 μm. Balsam fir grains averaged about 6 μm and ranged from 0.3 to 14.6 μm. Additional purification tended to remove the smaller grains which during processing appeared grey-green, and yielded purer preparations with slightly greater average grain sizes. Yields amounted to 3–9% of the dry weight of the starting foliage, with both starch content of the foliage and efficiency of isolation undoubtedly contributing to the variation. Starch in 1-year-old balsam-fir needles at the time of flushing can attain 14% of needle dry weight (Little 1970). Starch was isolated also from immature current balsam fir foliage collected on 11 June 1970. Because of the smaller size of the grains in this foliage (Fig. 1c) extraction was less efficient, the yield lower (1.0%) and the final buff-colored product less pure (Fig. 1c). A small but undetermined proportion of the starch grains were smaller than 0.4 μm and not readily measured; the starch grains larger than this minimum averaged 1.31$\pm$0.08 μm, with a maximum of 3.5 μm. Because of the lower yield there was insufficient starch for diet experiments. Little (1970) found approximately 5% starch in foliage at about the same developmental stage.

Feeding Experiments

Rearing survival in these experiments was generally somewhat lower (Table I) than the 48.8% reported for budworm reared on artificial diet (McMorran 1965); much of this difference is attributable to the difference in nutrient level and treatment of the
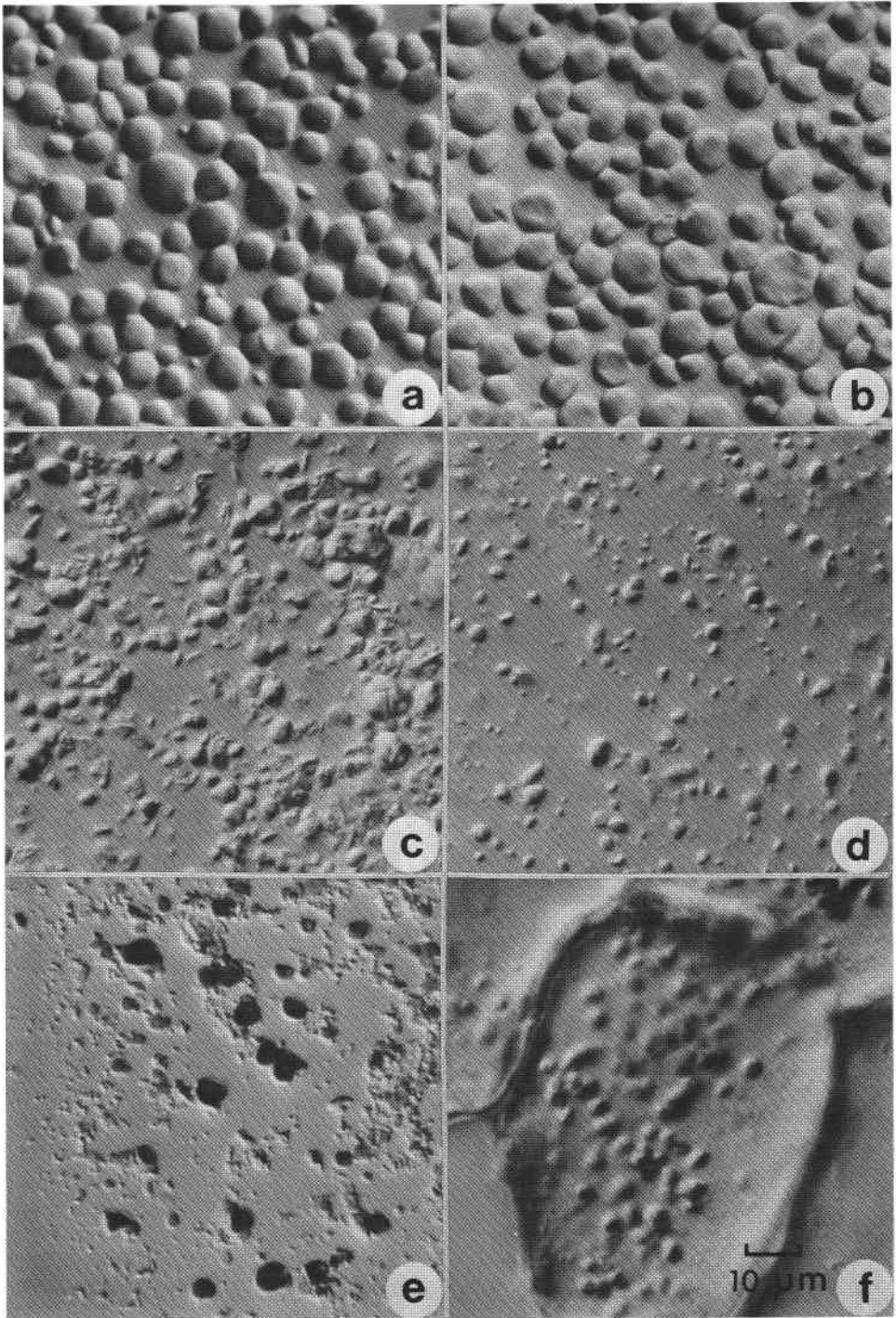
FIG. 1. (a) Starch grains from mature balsam fir needles, ×820. (b) Starch grains from mature white spruce foliage, ×820. (c) Starch grains from immature (13 June) balsam fir foliage, ×820. (d) Starch grains from frass of balsam fir fed spruce budworm, ×820. (e) As in (d), showing iodine stain of some of the starch grains, ×820. (f) Macerated frass of spruce budworm fed immature white spruce needles showing partially digested cell contents, including small starch grains, ×820.

Table I.   The effects on insect performance of additions of different starches to the basic diet

Diet	No. of exp.	Survival (%)	Females			Males		
			No.	Pupal[a] wt. (mg)	Dev. time[a] (½ days)	No.	Pupal[a] wt. (mg)	Dev. time[a] (½ days)
Control[b]	6	36.0	334	99.4±1.6*b*	58±0.6*bdg*	174[f]	67.8±1.5*c*	55±0.7*cde*
Sucrose	6	35.4	337	96.9±1.5*bc*	57±0.5*bch*	209[f]	71.7±1.3*b*	52±0.7*b*
Amylopectin	2	13.6[d]	43	109.9±4.2*a*	60±1.7*c,e–h*	28	81.1±3.8*a*	56±2.2*c–f*
Dextrins	2	30.9	70	91.8±3.0*cd*	58±0.9*bcegh*	97	67.2±1.7*cd*	53±0.8*bce*
Potato starch	2	28.9	83	88.0±2.8*de*	60±0.8*c,e–h*	76	66.4±1.7*cde*	53±0.9*bce*
Arrowroot starch	1	13.6[d]	17	86.5±7.8*b–f*	64±1.9*h*	18	63.3±3.3*b–f*	61±2.5*fgh*
Amylose[c]	1	–	88	80.9±2.5*efg*	56±0.7*bc*	0	–	–
Corn starch[e]	1	25.4	26	80.2±4.0*d–i*	51±1.7*a*	38	56.9±2.7*fgh*	47±1.1*a*
Balsam fir starch	2	22.9	186	78.3±1.8*f–j*	61±0.8*fgh*	14	42.9±3.9*i*	66±2.4*ghi*
α–Methyl glucoside	2	16.8[d]	45	78.2±4.0*e–k*	70±1.4*jk*	37	60.0±2.1*e–h*	61±1.2*fg*
Soluble starch	4	25.1	170	76.9±1.6*f–m*	61±0.8*fgh*	87	57.9±1.6*fgh*	60±1.3*f*
Cooked soluble starch	2	37.3	48	80.5±3.0*e–h*	62±1.1*gh*	50	61.5±1.8*d–g*	57±0.9*def*
Spruce starch	2	24.6	96	77.2±2.3*f–l*	61±1.1*e–h*	18[f]	44.7±2.6*i*	67±2.5*hi*
Basic diet	5	17.9	191	71.6±1.5*hikln*	66±1.1*i*	94[f]	48.8±1.3*i*	68±1.6*i*
Rice starch	1	16.8[d]	8	65.9±7.9*f–n*	75±4.7*ik*	16	50.5±2.2*fhi*	58±1.8*ef*

[a]Values are means ± 1 S.E. Values followed by different letters (*a*, *b*, ...) differ significantly at 5% probability (Duncan's New Multiple Range Test).
[b]Control diet containing sucrose 1.75%; all other diets based on basic recipe.
[c]Larvae established on basic + sucrose diet, females transferred to basic + amylose at instar III and IV, males discarded.
[d]Low survival values partially due to a high incidence of second diapause (treated as mortality) in two experiments.
[e]Corn starch added to standard rather than control diet (except wheat germ 2% instead of 3%).
[f]Males discarded at establishment in some experiments.

wheat germ. Survival, pupal weights, and developmental times on the basic diet were substantially below those on the control diet (Harvey 1974). Only a few of the starches added to the basic diet substantially improved developmental performance; none of the additions produced a diet on which performance equalled that of insects reared on the control diet or the basic diet containing sucrose.

The addition to the basic diet of arrowroot or potato starch, dextrins or amylopectin produced significant improvements in insect performance. Survival on diets containing potato starch or dextrins was close to that on the diet containing sucrose. Survival was notably lower on diets containing amylopectin or arrowroot starch but this result is not strictly comparable because of the high incidence in these experiments of larvae entering second diapause (Harvey 1961). Arrowroot starch and potato starch, though apparently utilized to an appreciable extent as evidenced by the pupal weights, were definitely inferior to sucrose, although this was not significant in all comparisons. Dextrins were almost as good a carbohydrate source as sucrose. Amylopectin added to the basic diet produced higher pupal weights in both sexes than the sucrose-supplemented basic diet or the control diet, although there were no significant differences in development times.

Of the remaining starches only amylose and corn starch produced female pupal weights and development rates significantly greater than those produced by the basic diet alone, according to the statistical tests. However, female pupal weights of insects fed on these diets were not significantly better than those of insects fed on the diets containing the other 'poor' starches. Amylose apparently effected a significant improvement in development time but because initial development of these insects was on the basic + sucrose diet, the data are not strictly comparable. The performance on corn starch, tested as a substitute for sucrose in the standard diet (McMorran 1965) in a separate experiment, was no better than that on the standard diet without sugar; it is probable that corn starch is of very little or no nutritional value to the budworm, as Shaw (1973) found.

The rest of the starches tested produced more or less insignificant improvements in insect performance compared with the basic diet and appear to have little or no nutritive value for this insect. Spruce starch, soluble starch (cooked or uncooked), and balsam fir starch produced slightly greater survival and female pupal weights than the basic diet, though not significantly so; the slight improvement in female development times compared with that produced by the basic diet was significant. Although α-methyl glucoside produced slightly greater pupal weights than the basic diet alone, its presence prolonged development. Rice starch had no significant effect on survival, pupal weight, or development time of females compared with the basic diet alone.

The response of male budworm to the 'poor' starches was not the same as that of the females; such sex differences in response to dietary components have been reported previously (Shaw 1973; Harvey 1974). All starches except spruce, balsam fir, and rice produce significant increases in male pupal weights compared with the basic diet. Development times were shorter on all starches, including rice, except those derived from balsam fir and white spruce needles. However, male performance was still poorer on the 'poor' starches than on the control diet, or on the basic diet containing the better starches.

These results indicate that both amylopectin and dextrins appear to be highly utilizable, in that they are essentially equivalent to sucrose; however, the increase in mean female pupal weight resulting from the addition of dextrins to the basic diet was only about 80% of that produced by sucrose. Potato and arrowroot starches produced increases of only 65 and 59%, respectively, which indicates that they were not as utilizable. All other starches, including those isolated from needles of the budworm's host trees, produced improvements of 35% or less, and most were not significantly better than the basic diet without additions.

Enzyme Tests

Further evidence of the possible place of starch in budworm nutrition was sought in a brief study of the activity of the carbohydrases in midgut and salivary gland homogenates against a number of carbohydrates at 37°C (Table II). Tests at 25°C gave generally similar results although reaction rates were noticeably slower. Most of the tests utilized extracts from actively feeding larvae; however, similar though somewhat reduced enzyme activities were demonstrated in extracts of larvae starved for 24 h. The optimum pH for activity of these preparations with soluble starch as substrate was 7.6–8.0; the reaction rate was only slightly lower at pH 7.0, the pH used for tests of substrates (Table II). The addition of Cl⁻ caused no detectable activation at any pH up to 8.5. Although the method was not quantitative it did provide evidence of the presence of several enzymes, including amylase(s), in cell-free suspensions and gave a rough measure of the level of activity. The di- and trisaccharides readily utilized by the budworm, sucrose, maltose, trehalose, and raffinose (Harvey 1974), as well as dextrins were readily split enzymatically, yielding the expected products (Table II). There was no evidence of enzyme action against melezitose or lactose; however, the latter conclusion is based only on the apparent absence of the expected products. Although there was evidence of splitting of melibiose, the rate appeared to be very low, in agreement with the apparent low nutritive value of this sugar (Harvey 1974).

The presence of amylase(s) in the enzyme preparations was shown by the production of reducing sugars from soluble starch and amylopectin, although the rate against the former was low. This enzyme, however, was inactive against most of the starch substrates tested. Some activity occurred with rice starch as substrate, and also

Table II. Evidence of carbohydrase activity in extracts of midgut and salivary glands from sixth-instar budworm larvae after incubation for 24 h at 37°C

Substrate	Reducing sugar		Products detected[a]	Enzyme[a] activity
	Initial	Final		
Sucrose	−	+ + +	Glucose, fructose	+
Maltose	+	+ +	Glucose (maltose, maltotriose, maltotetrose[b])	+
Melibiose	+	+ +	(Glucose, galactose)	±
Lactose	+	+	Lactose	−
Trehalose	−	+ +	Glucose	+
Raffinose	−	+ +	Melibiose, sucrose, fructose (glucose, galactose)	+
Melezitose	−	−	—	−
Inulin	−	−	None	−
Stachyose	−	+ +	Raffinose, melibiose, galactose, fructose (glucose, sucrose, other)	+
Dextrins	+	+ +	Maltotetrose, maltotriose, maltose, glucose	+
Amylose	−	−	None	−
Amylopectin	−	+	Maltotetrose, maltotriose, maltose, glucose	+
Soluble starch	−	+	None	(+)
Rice starch	−	±	None	(+)
Corn starch	−	−	—	−
Potato starch	−	−	None	−
Spruce starch	−	±	None	(+)
Balsam starch	−	+	Maltotetrose, maltotriose, maltose, glucose	+

[a]Parentheses indicate where only traces of products or enzymatic activity were found.
[b]These products represent impurities in this substrate.

with needle starch from white spruce; that only a limited amount of digestion took place is indicated by our inability to detect the expected end products. Balsam fir needle starch appeared more readily digested than the other starches. All of the starch substrates were more readily hydrolyzed after cooking. Consequently, there remains some question as to whether the apparent digestibility of spruce starch and particularly of balsam fir starch was to some extent attributable to the treatment used for their isolation.

With the notable exception of amylopectin, there seems to be little relationship between the susceptibility of the starches tested to attack by budworm gut enzymes under the conditions of our experiments, and their utilizability in feeding experiments. The two tests do agree in indicating that balsam fir starch appears more readily digested and better utilized by the spruce budworm than does spruce starch.

Both soluble and particulate fractions of homogenates of frass from budworm fed on the artificial diet with or without added starch contained some amylase activity when tested with soluble starch. Sucrose-splitting (invertase) activity was also present, but at low levels in the soluble portion only.

Starch in Frass

Microscopic examination of frass pellets from budworm fed on diets containing starch isolated from balsam fir and white spruce showed the presence of some apparently unaltered starch grains. Frass from foliage-fed insects also contained starch grains (Fig. 1d, e). The latter were all small starch grains, many of them in unbroken cells but some also outside the cell remnants. The starch grains appeared to be all that remained of the contents of many of the unbroken cells (Fig. 1f). However, in no case was there any appreciable change in shape or surface texture of the starch grains which, in fact, appeared no different from those isolated from intact foliage. The procedure used to extract starch from foliage was used to extract it from frass samples representing both host species. Some of the same problems were encountered as with the extraction of the immature foliage samples, and no yield data were obtained. Many of the grains were very small (Fig. 1e). Excluding all grains smaller than 0.4 μm, starch from balsam fir frass averaged 1.05 ± 0.10 μm, that from white spruce 0.95 ± 0.08 μm. The average size of starch grains from balsam fir frass was not significantly different from that of starch from immature foliage of about the same age; the largest grains were about the same size in both samples.

Discussion

Spruce budworm larvae enter current buds during the early stages of flushing and remain there feeding until they pupate near the end of the period of shoot elongation. Evidence from several plant species indicates that starch grains are normal constituents of most photosynthesizing tissue from the very early stages of its development (Badenhuizen 1969) and that the amylose and amylopectin components of starch grains are intimately associated throughout the development of the starch grains (Badenhuizen 1973), although the ratio of the one to the other may change considerably. During most of the period of insect feeding, however, starch levels in balsam fir and white spruce buds are relatively low (Little 1970) and the major carbohydrates are the soluble sugars. During this period the starch grains are small and usually imbedded in plastids, both of which circumstances may affect their accessibility to the insect's digestive enzymes. Nevertheless, some starch appears to be present in all needle tissue consumed by the insect and, if digestible, could contribute to carbohydrate nutrition.

Can the starch present in developing needles at this time be utilized by the insect? We have attempted to assemble sufficient evidence by indirect methods to warrant a conclusion about the importance of starch in budworm nutrition. Results of feeding

experiments often cannot be interpreted as valid measures of nutritional value of the ingredients under study as the latter may affect the insect in several ways. No experimental data are available on the phagostimulatory characteristics of the test starches for the spruce budworm. However, the dietary concentration of the starches tested (1.75%), though adequate to make a measurable difference in insect performance if utilized, was sufficiently low to have minimal effects on overall nutrient or phagostimulant content of the diets. Moreover, throughout the tests there was complete uniformity of composition of all other ingredients of the diets which differed only in the nature of the carbohydrate added. No appreciable differences in diet consumption or frass production were noted among the various diets. Although some of the starches may, in combination with other diet ingredients, have affected consumption or utilization in some way, it is probable that the effects on survival, mature weight, and developmental rates are attributable mainly to the nutritive properties of the starches themselves, and that they are directly related to utilization of these substances, as defined more explicitly elsewhere (Waldbauer 1968).

The feeding experiments demonstrated that the spruce budworm can apparently use, at least partly, some starches and starch products. However, most intact starches are of low nutritional value for this insect. Similar conclusions were drawn from feeding studies of large larvae on diets containing different starches (Shaw 1973). However, because Shaw did not use a basal diet devoid of added carbohydrate he could only conclude that the starches tested were inferior to sucrose. In the present studies all starches added to the basic diet, except rice starch, gave some, though generally insignificant, improvements in percentage survival and female pupal weights compared with those of the basic diet. Such evidence is surely not proof of even a limited degree of utilization, but neither is it evidence against a low level of utilization of some of these starches. Improvements in insect performance indicated that both potato and arrowroot starch were, in fact, utilized, although both were much inferior to sucrose. Shaw's data indicated that arrowroot starch probably was utilized by females but not by males, and that potato starch was of low nutrient value for both sexes (Shaw 1973).

Of the two principal components of intact starches, amylose appeared to be utilized to a limited extent; it is unquestionably inferior as a carbohydrate source to the major component, amylopectin, which was perhaps even better nutritionally than sucrose. In this respect the spruce budworm closely resembles the southwestern corn borer, *Diatraea grandiosella*, which utilizes dietary amylopectin, but not corn starch or amylose (Chippendale and Reddy 1974). However, the amylose and amylopectin molecules have been found to be intermingled throughout the starch grain (Schoch 1969) and access to either molecule appears to be limited. Thus, the demonstration of utilization of purified amylopectin in the diet and its hydrolysis by insect enzymes does not necessarily imply its digestion from starch grains. Nor is it likely that such differences in amylose:amylopectin ratios as occur among starch grains from different species will produce wide differences in susceptibility of the respective grains to hydrolysis by digestive enzymes. The amylose:amylopectin ratios for balsam fir and white spruce needle starches are not known.

Shaw (1973) concluded that utilizability of starches by the spruce budworm was not related to starch grain size. In the present studies, insect performance on the diet containing potato starch equalled that on arrowroot starch. These two starches, with the largest grain size (30–40 μm) (Whistler and Smart 1953) were the best utilized of the natural starches. Other starches had grain sizes ranging from 5 μm (rice starch) to 15 μm (corn starch) and were inferior nutritionally. There is thus some indication of an inverse relationship between grain size and nutritive value for the budworm. However, the differences were not great and other characteristics of the starch grains may be more important.

The purified starches isolated from mature balsam fir and white spruce needles were poorly if at all utilized when incorporated into the artificial diet. However, because of the susceptibility of starch grains and their components to degradation during isolation (Manners 1973), this apparent low utilizability is not necessarily evidence of their value to the insect under natural conditions. The treatments might have increased or decreased their accessibility to digestive enzymes. Bailey and Macrae (1973) report that intact starch grains from white clover leaves are much more readily hydrolyzed by glucamylase than intact grains from potato tubers. Thus, leaf starches may be more susceptible to hydrolysis by the digestive enzymes of insects than storage starches, and the failure to demonstrate utilization of the latter does not prove the inability of the insects to use the former.

Unfortunately, data are not available on the utilizability of the small starch grains present in immature balsam fir or spruce foliage because of the difficulty in extracting a sufficient quantity for feeding experiments. Nevertheless, the presence of apparently unaltered starch grains in frass from insects fed on immature foliage appears to be incontestable evidence that all starch present in fresh foliage is not utilized by feeding budworm. The absence of almost all cell contents except starch grains from the apparently unbroken cells present in frass supports this conclusion. That there were no detectable changes in size or appearance of starch grains after passage through the insect is consistent with the conclusion that there is, in fact, little or no digestion of starch. However, quantitative data on consumption and excretion of dietary starch would be necessary to detect whether or not a low level of utilization of the smaller grains in immature needles does, in fact, occur.

Results of enzyme tests indicate the probable presence in larval gut and salivary glands of amylase(s) capable of digesting starches under the appropriate conditions. Amylases of Lepidoptera are reported to function optimally at about pH 10 (Dadd 1970). Heimpel (1956) found a mean pH of 9.0 in midguts of last-instar spruce budworm larvae feeding on fresh foliage. The pH used for the enzyme assays (7.0), though below the normal gut pH and suboptimal for the amylases of this insect, was not too low for a significant level of activity with suitable substrates. From the results we can conclude the probable presence of some amylase activity under natural conditions. The utilization of amylopectin and potato starch in the feeding experiments supports this conclusion. However, enzyme activity at 37°C was low against most kinds of intact starch grains, including those from mature foliage of this insect's natural hosts, particularly white spruce. The rate of digestion at natural feeding temperatures can be expected to be lower by a considerable factor although the higher midgut pH might offset this reduction. Separate feeding tests with colored artificial diets (20°C) indicate a passage time of about 2 h or less for ingested materials (Harvey unpublished). Although a longer time might be required for a more particulate food, such as needle tissue, it is unlikely to be more than doubled. Thus, the apparent low amount of starch digestion in vivo may be attributed to a low level of enzyme activity coupled with a short exposure time.

The digestion and utilization of starch by insects have recently been reviewed by Waldbauer (1968). Only a few insects that feed on fresh leaf tissue have been studied; of these, less than half utilize starch. Evans (1939) showed that *Malacosoma neustria* fed on willow leaves utilized about 35% of the starch. Utilization of starch by fifth-instar larvae of the silkworm (*Bombyx mori* L.) varies between races and is related to the level of amylase activity in their digestive juices (Makaiyama *et al.* 1964). Although dietary starch was shown to prolong survival of otherwise starved silkworm larvae, starches normally appear to be of small importance; maximum utilization of dextrin plus starch by any race reached about 40%. Very little appears to be known of nutrient utilization by insects feeding on conifers. However, the red-banded leafroller (*Argyrotaenia*

velutinana) which feeds on spruces and other species, though best known as a pest on apple, has recently been shown to use soluble starch and rice, potato and corn starches; the latter two starches have lower nutritive values for this insect (Rock and Sharma 1974).

The inability of several insects to utilize starch has been attributed to the properties of the outer surface of the intact starch grains (Ullman 1932). The starch grains extracted from conifer needles for use in the present feeding experiments were generally intact (Fig. 1). However, starch pretreated to increase its solubility (i.e., soluble starch) was not utilized significantly better than the other starches tested, nor was any increase in utilization apparently effected by cooking this starch during diet preparation. Cooking or abrading the outer surface of the grains made them more susceptible to hydrolysis by *Tenebrio* and several leaf-feeding Lepidoptera (Ullman 1932), and by the bug *Lygus disponsi* (Hori 1972). Cooking starches increased their hydrolysis by budworm gut enzymes in vitro but did not increase apparent utilization of soluble starch.

The presence of some amylase activity in frass from diet-fed budworm raises the possibility of digestion of starch in the frass as suggested for *Chilo suppressalis* (Tsutsui and Sato 1954). However, there is no evidence of coprophagy among budworm and any benefit from starch digestion in the frass appears doubtful. The presence of this enzyme, however, does add to the difficulty of truly quantitative determination of the utilizability of dietary starches.

Finally, some utilization of needle starches by the spruce budworm, particularly when other more readily used carbohydrates are at low levels, must still be considered a possibility. However, in most cases it appears to be of little consequence during insect development under natural conditions, as predicted by Shaw (1973). Soluble sugars in foliage must be considered the principal carbohydrate source for eastern spruce budworm.

Acknowledgments

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