ficiently rapid to equalize the concentrations. Soluble carbohydrate content of the tops was not affected significantly by the partial stress, but the initial (24- and 48-hour) carbohydrate content of both roots of the partially stressed plant was increased over that of the control. Subsequently it was the same as the controls. These results suggest that utilization of carbohydrate was reduced by a stress on any part of the root system before translocation from the top was affected.

A gross measurement of all soluble ninhydrin-positive material (α-amino groups, primarily amino acids and peptides) indicated that this fraction was greatly reduced in the shoots subjected to partial stress at 24 and 48 hours, but this fraction subsequently increased. The content of this fraction in the roots was also reduced below that of the control. This would indicate that syntheses of these compounds may be reduced by a stress on any portion of the plant and could be a reflection of the reduced nitrogen uptake indicated earlier.

The results obtained are likely due to some specific effect occurring at the root, resulting in subsequent disturbance of metabolism throughout the plant. That the results were not due to changes in the turgidity of the plant or in the energy status of the water in the plant was indicated by relative turgidity measurements and the appearance of the plants.

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Chromic Oxide Indicator Method for Measuring Food Utilization in a Plant-Feeding Insect

Abstract. *The chromic oxide indicator method was used to determine percentage utilization of three diets prepared from lyophilized plant tissues for fifth-instar larvae of the pale western cutworm. Because of the simplicity of the method and uniformity of results within each of the diets, the procedure can be used routinely in feeding trials with this insect, and perhaps others with biting and chewing mouth parts.*

The quantitative aspects of food consumption and utilization have frequently been neglected in previous studies of insect nutrition (*1*). Many phytophagous insects feed readily only on fresh material, making it difficult to determine food consumption accurately. Moreover, determination of food utilization by the classical gravimetric procedure requires that excreta must be quantitatively collected and weighed. The indicator technique originally described by Bergeim (*2*) does not require quantitative measurements of consumption and excretion and has been used extensively with laboratory and farm animals, Cr_2O_3 being used as the index compound. In this report we describe the application of this method to a plant-feeding insect.

This method requires that the indicator compound incorporated in the food be neither absorbed from the gut nor toxic at the concentrations used; under these circumstances, percentage utilization is given by [1 − (concn of indicator in dry matter of food/concn of indicator in dry matter of excreta)] × 100. The method has not previously been applied to insects, probably because of difficulty in distributing the index compound uniformly in their diets. Larvae of the pale western cutworm, *Agrotis orthogonia* Morr., can be reared on plant tissues that have been lyophilized and ground (*3*), and therefore the indicator method was practical. Moreover, availability of Cr_2O_3 paper (*4*), which can be more easily distributed in the diet than Cr_2O_3 itself, contributed to the feasibility of this approach.

Table 1. Utilization of dry matter in diets by fifth-instar larvae of the pale western cutworm as determined by the Cr_2O_3 indicator method.

Diet	Mean wt. of larvae (mg)		Percentage utilization*
	Initial wt.	Gain in wt.	
Sprouts	65 ± 21	159	41 ± 2
Sprouts and cellulose	62 ± 21	155	21 ± 2
Pith	66 ± 23	46	16 ± 1

* $\left(1 - \frac{\mu g\ Cr_2O_3/mg\ dry\ matter\ in\ food}{\mu g\ Cr_2O_3/mg\ dry\ matter\ in\ excreta}\right) \times 100$

Three diets known to vary in nutritional value were prepared from (i) sprouts of Thatcher wheat; (ii) equal parts of sprouts and cellulose powder (wt/wt); (iii) pith from stems of solid-stemmed Rescue wheat. The first two diets are excellent foods for the cutworm, whereas pith is nutritionally unsatisfactory (*5*). The wheat plant tissues were grown as described (*6*), lyophilized, and ground to pass a 40-mesh screen. Chromic oxide paper (*4*), also ground to pass a 40-mesh screen, was added to each of the dry plant materials to give a concentration of Cr_2O_3 near 4 percent. This concentration was not toxic to the cutworm, had no apparent effect on consumption, and was not absorbed from the gut. Uniform distribution of Cr_2O_3 was accomplished by tumble mixing for 48 hours. Distilled water was added to each dry meal in quantities sufficient to provide a medium that was acceptable to the cutworm. The diets were packaged in approximately 1-g portions in aluminum foil and stored at −30°C until fed.

Percentage utilization of each diet was determined with 10 newly molted fifth-instar larvae of the cutworm. Individual larvae were maintained in 60 × 15-mm petri dishes and had unrestricted access to a single diet in a humid atmosphere in the dark. The larvae were transferred to clean dishes and provided with fresh food each day. Excreta were separated daily from residual food and were combined into a single collection per insect. Larvae that were fed the diets containing sprouts were maintained on these foods until they molted into sixth instar, about 5 days; those fed the pith diet were retained on test for 7 days, but none molted. The excreta and samples of the diets were dried at 120°C for 2 hours. After the dry material was pulverized and mixed in a mortar, samples were analyzed in triplicate for Cr_2O_3 by a micro-method in which perchloric acid digestion was followed by colorimetric determination of chromium with diphenylcarbazide (*7*).

The percentages of dry matter utilized by individual larvae within a diet were similar (Table 1) but the percent-

age utilization of dry matter among the diets differed significantly ($p < .01$). Although the percentage utilization of the three diets had not been determined previously, the values we obtained are acceptable. Our utilization value of 41 percent is similar to the value of 48 percent determined by the gravimetric method when fresh sprouts were fed to fifth-instar larvae of the cutworm (*8*). A further indication of the reliability of the method is that the percentage utilization of the sprout : cellulose diet (50 : 50 wt/wt) was about half of that found for the sprout diet. Such a result would be expected if it is assumed that little or no cellulose is digested by cutworm larvae. The percentage utilization of pith determined by the Cr_2O_3 procedure was low and is compatible with the slower rate of larval growth (Table 1).

The simplicity of the Cr_2O_3 procedure indicates that it should be useful for studies of food utilization by other insect species with biting and chewing mouth parts provided they will feed on artificial diets in which Cr_2O_3 has been incorporated. Because utilization values determined by the Cr_2O_3 method are computed from the concentrations of the indicator compound in food and excreta, quantitative separation and recovery of all of the excreta is not necessary, whereas the gravimetric method requires quantitative measurement of all food consumed and excreta produced. Although the Cr_2O_3 procedure was used here to determine dry matter utilization it can be extended to measure utilization of other dietary components. The gravimetric and Cr_2O_3 procedures are being compared with both the pale western cutworm and a grasshopper, *Melanoplus bivittatus* Say, and preliminary results support the usefulness of the indicator method for studies of food utilization.

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Insect Tissue Culture: Use of Blastokinetic Stage of Leafhopper Embryo

Abstract. *To find the proper material for cultivation in vitro of cells of leafhopper vectors of plant viruses, embryonic tissues of the six-spotted aster leafhopper* (Macrosteles fascifrons) *were tested during early developmental stages, during blastokinetic movement, and in late developmental stages. Growing cells were only obtained from the stage of blastokinetic movement. This stage can be determined visually in leafhopper eggs.*

Several species of leafhoppers (Homoptera, Cicadellidae) are capable of harboring and transmitting pathogenic plant viruses that multiply in both plants and insect vectors (*1*). Repeated attempts to cultivate leafhopper tissues in vitro have been made, since tissue cultures would provide a useful tool for the study of the interactions of plant viruses and insect cells. Although various organs of nymphs and of adult leafhoppers had been maintained in vitro for several weeks (*2*), no growing cells were obtained in these earlier experiments. Until now, successful cultivations of arthropod cells had been carried out mainly with ovarian tissues of Lepidoptera (*3*), larval and pupal tissues of Diptera (*4*), nymphal tissues of Ixodina (*5*), and embryonic tissues of Orthoptera (*6*).

Tissues of the regenerating cockroach leg have been used successfully by Marks and Reinecke for tissue culture purposes (*7*). Cells and tissues of holometabolous insects are grown fairly easily in vitro, but those of paurometabolous insects can be cultivated only with considerable difficulty. These difficulties have been partly overcome in the cultivation of embryonic tissues of the six-spotted aster leafhopper, *Macrosteles fascifrons* Stål (*8*). After the initial success in growing such cells, it was expected that any undifferentiated embryonic cells obtained from leafhopper eggs would prove suitable for cultivation. However, this expectation was not fulfilled. It seemed that the stage of embryonic development was of critical importance for the attempted tissue culture. Experiments were therefore conducted to find the developmental stage of the leafhopper embryo which would grow in vitro.

For oviposition, 200 adult six-spotted leafhoppers were caged on young rye plants (*Secale cereale* L.) grown in 10-cm pots. Insects were transferred to groups of fresh plants every day. Rye plants with deposited eggs were maintained at a constant temperature of 25°C; standard fluorescent tubes provided 6600 lu/m^2 of light during a 16-hour day. Under these controlled conditions, first-instar nymphs hatched 11 days after oviposition. Daily transfers of insects to fresh plants facilitated the collection for each test of large numbers of embryos at the same stage of embryonic development. On the 1st, 3rd, 5th, 7th, 8th, 10th, and 11th day

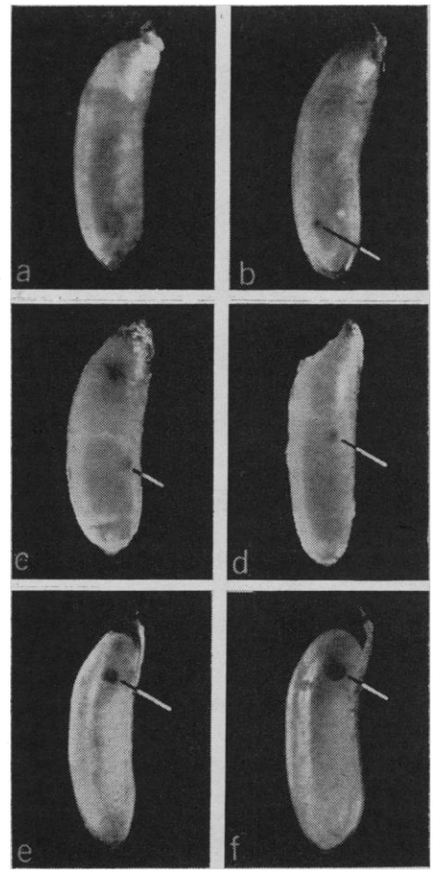

Fig. 1. Developmental stages of the embryo of the six-spotted aster leafhopper (× 30). *A*, One of the early development stages, 5-day-old egg. The pigmentation of eye discs is not yet visible. *B–D*, The eggs in the stage of blastokinetic movement during the 7th and 8th day. Slightly pigmented eye discs arise in the posterior position of the egg (*B*), then gradually move to the anterior position (*C* and *D*). Eggs in late development stages: *E*, 10-day-old egg; *F*, 11-day-old egg, just before hatching.