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Feeding and Nutrition of Insects Associated with Soybeans: 1. Growth and Development of the Mexican Bean Beetle, *Epilachna varivestis*,[1] on Artificial Media[2]

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ABSTRACT

A semiliquid medium applied to layers of Cellucotton®, was readily accepted by 3rd- and 4th-stage larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae). Larvae reared on this medium completed development and yielded normal adults. Tests were conducted with a total of 19 semiliquid (sol) and agar-base (gel) media differing in sucrose concentration, source of essential amino acids, and *p*H. A sol-casein medium with 0.1M sucrose yielded the best results. This medium was used in routine rearings of the beetle, partially replacing fresh bean leaves. The sol-casein medium was found extremely convenient in studies on host selection and nutrition of the Mexican bean beetle in its associations with soybeans.

A new program on soybean insects initiated by the Illinois Natural History Survey and the University of Illinois at Urbana-Champaign, defined as one of its objectives the search for the behavioral and physiological bases of insect:soybean plant associations. The relative specificity of the Mexican bean beetle, *Epilachna varivestis* Mulsant, to certain genera and species of the Leguminosae has prompted its selection as the first of a series of species to be used in feeding and nutritional studies within the scope of this program. Suitable artificial diets were needed to develop a bioassay for the analysis of these processes.

The difficulty in developing a suitable artificial diet has delayed progress in host selection and nutritional studies with this insect. For example, Gothilf and Waites (1968) discussed the effect of certain antimetabolites on growth and mortality of this beetle but they used green leaves, dipped in various antimetabolite solutions, instead of defined media, possibly introducing uncontrolled variables in the experimental technique. Howard (1941) described the characteristic "scrape and suck" feeding process of the beetle, and Butt (1951) discussed its anatomical bases. These typical feeding patterns were used to advantage in studies of host plant selection by Nayar and Fraenkel (1963) and Augustine et al. (1964). The method used by these researchers consisted of impregnating filter-paper discs with various test solutions and observing or counting the scars left on the paper. Scars on paper discs were similar to those observed on chewed host leaves.

Based on this evidence and on the fact that agar-base diets did not elicit normal continued feeding responses in larvae and adults, other supporting materials were tested. In this study I measured the effect on growth and development of Mexican bean beetle larvae of different medium carriers, as well as sources of essential amino acids, sucrose concentrations, and *p*H.

MATERIALS AND METHODS

Insect Cultures.—Cultures were established with about 80 adult beetles collected on soybeans at Versailles, Indiana, in August 1969.[4] The cultures were maintained in small plastic cages described by Kogan and Goeden (1970) on bouquets of soybean trifoliates (mainly 'Harosoy' and 'Clark' varieties). Eggs were incubated in glass petri dishes on a layer of plaster of paris covered with a disc of Whatman no. 1 filter paper that was kept moist. Immediately after hatching, 60–90 larvae (from 2–3 egg clusters) were transferred to the cages and placed on the bouquets of leaves. The larvae dispersed as they started feeding and bouquets were usually changed every 48 hr. After the 2nd molt the larvae in one cage were divided into groups of 18–20 and placed in separate cages. This procedure permitted maintenance of uniform age groups, prevented overcrowding and excessively rapid consumption of available food. Prepupae were transferred to gallon carton containers where adults emerged. Groups of ovipositing adults, each consisting of 2 ♀ and 1 ♂, were placed in separate cages. The number of groups at a given time was determined by their relative fecundity and the num-

[1] Coleoptera: Coccinellidae.

[2] Received for publication Dec. 10, 1970.

[3] The technical help of Mrs. M. Monkman is gratefully acknowledged.

[4] Thanks are due Dr. Delmar B. Broersma, Purdue University, for collecting the beetles used to start this culture.

Table 1.—Composition of artificial media tested with Mexican bean beetle larvae.

Constituents	Medium			
	Control	Sol-casein	Gel-casein	Sol-soy
Bacto-agar[a]	0.2 g	0.2 g	3 g	0.2 g
Casein hydrolysate[b, c]	0	10 ml	10 ml	0
Soy hydrolysate[b, c]	0	0	0	10 ml
Alphacel[c]	3 g	3 g	3 g	3 g
Casein vit.-free[c]	0	5 g	5 g	5 g
Salt mixture (Wesson's)[c]	0	1 g	1 g	1 g
Choline chloride[d]	0	.05 g	.05 g	.05 g
Meso-inositol[c]	0	.10 g	.10 g	.10 g
Cholesterol acetate[e]	0	.05 g	.05 g	.05 g
Corn oil (Mazola®)	0	1 ml	1 ml	1 ml
B-vitamins (Vanderzant)[c]	0	2 g	2 g	2 g
Ascorbic acid[c]	0	.10 g	.10 g	.10 g
Formaldehyde (10%)	1 ml	1 ml	1 ml	1 ml
Methyl *p*-hydroxybenzoate[f]	.4 g	.4 g	.4 g	.4 g
Aureomycin[g]	.004 g	.004 g	.004 g	.004 g
KOH	—Necessary to adjust to *p*H 7—			
H_2O	100 ml	100 ml	100 ml	100 ml
Sucrose[h]				

[a] Manufacturer: Difco Laboratories.
[b] A suspension of 10 g hydrolysate in 100 ml H_2O.
[c-g] Manufacturers: [c] Nutritional Biochemicals Corp.; [d] Merck & Co.; [e] Baker Chemical Co.; [f] Eastman Organic Chemicals; [g] American Cyanamid Co.
[h] Each medium was used at 4 concentrations of sucrose: 0, 0.01, 0.1, and 1.0M.

ber of larvae needed for experiments and continuity of the culture. Rearings were kept in an insectary room at 27±2°C, 50±4% RH, and 14:10-hr photoperiod.

Composition of the Media.—After initial screening of media used in rearings of other insects, such as the corn earworm medium (Berger 1963), a modified pinto bean medium (based on Shorey and Hale 1965), and variations of them, tests in our laboratory were concentrated around 3 basic types of media using as carriers either Cellucotton® wadding (sol-media) or 3% agar (gel-media). Soy hydrolysate and casein hydrolysate were tested as sources of essential amino acids with 3 concentrations of sucrose (0.01, 0.1, and 1.0M). Basic nonnutritive sol-media containing only 0.2% agar, 3% Alphacel®, the bactericides and fungistats, and with 0, 0.01, 0.1, or 1.0M sucrose were used as controls. The composition of the media is presented in Table 1.

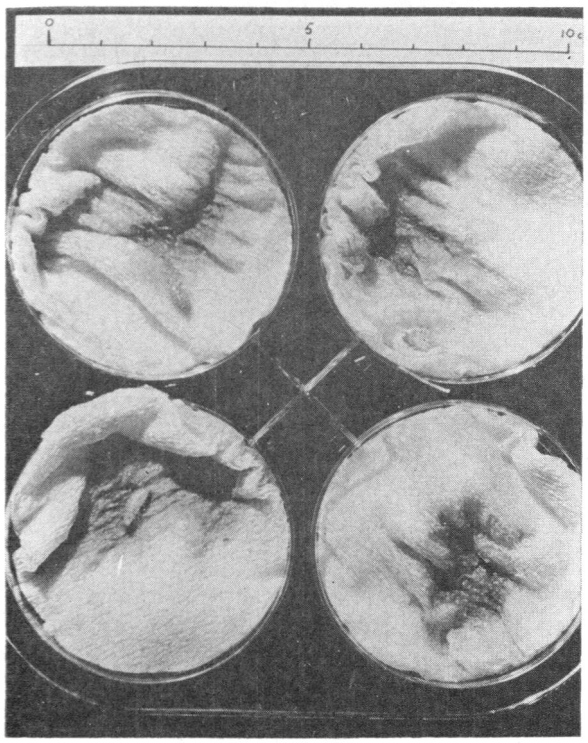

FIG. 1.—Clustered petri dish lined with Cellucotton used to test the sol-media.

Testing Procedure.—Five groups of 4 larvae each were used to evaluate the media. The 4 larvae in each group were confined individually in the 4 compartments of 53×15-mm clustered petri dishes (Fig. 1). The 4 larvae of each group were weighed together and the development of the group was used as the basis for analyses and comparison.

Agar-base media were offered as ca. 4-mm-thick, 16-mm-diam plugs. Semiliquid media were applied with a 5-ml Cornwall® continuous pipet, adjusted to deliver 0.5-ml amounts. The liquid media were applied to 55×55-cm squares of 4 plies of Cellucotton wadding, spread over the cover plate of the clustered dishes. After the 4 larvae were positioned on the medium in individual compartments, the clustered dish was closed snugly with the aid of two 30-mm long "bulldog" clips.

Early 4th-stage larvae were used in tests to evaluate the combined effects of carriers and sucrose concentrations, and the weight of larvae was recorded every 12 hr over a period of 48 hr. Early 3rd-stage larvae were used in experiments on *p*H and they were weighed every 48 hr until they pupated. An

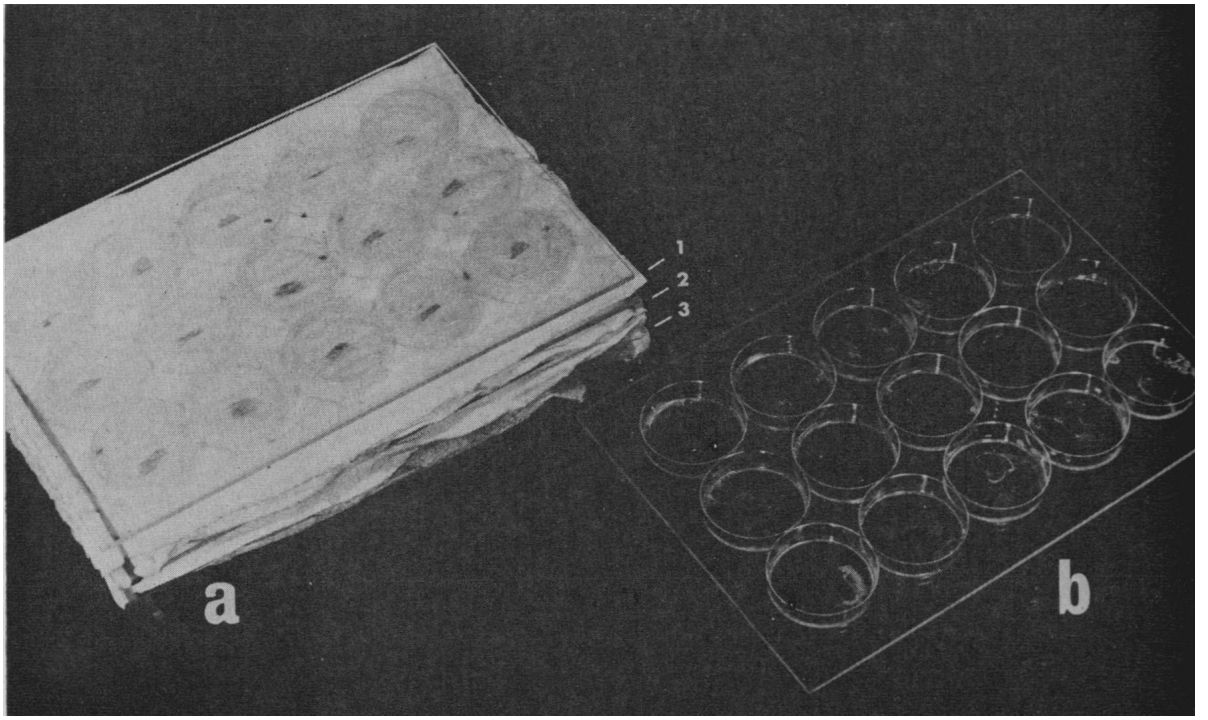

FIG. 2.—Technique used for large-scale rearing of 3rd-stage Mexican bean beetle larvae on the sol-casein (0.1M sucrose) media. (a), Stack of rearing plates, 1–3, with Cellucotton layers between them; (b), rearing plate.

H-20 Mettler® semimicro balance was used in all measurements.

Supplementary Feeding of Cultures with an Artificial Medium.—The sol-casein medium was used to maintain the cultures during winter when leaves of greenhouse-grown soybeans were in short supply. The technique used is illustrated in Fig. 2. Rearing units were prepared by gluing fifteen 55×15-mm plastic petri dishes in 3 rows of 5 dishes each to a 20×30-cm plate of 4-mm-thick Plexiglas® with ethyl acetate. Only covers or bottoms of dishes were used in the preparation of 1 plate unit. These plates were stacked so that a layer of Cellucotton wadding was sandwiched between the rims of the rows of dishes and the flat bottom of an adjacent Plexiglas plate. Drops of the medium were placed on the wadding to the center of the area limited by each of the 15 dishes of a unit, and one 3rd-stage larva was placed in each cell thus formed. Stacks, 4–5 plates high, holding 60–75 larvae, were placed in a plastic refrigerator crisper and reared at 27±2°C. Best results were obtained when relative humidity was maintained at ca. 60%. Larvae were transferred to new cells every 2–3 days when fresh medium was added. As 3rd- and 4th-stage larvae consume ca. 92% of the total fresh weight of leaves consumed during larval development (Kogan, unpublished data), a considerable saving of fresh plant material was achieved when 1st- and 2nd-stage larvae only were maintained on fresh leaves and transferred to the medium after the 2nd molt.

RESULTS AND ANALYSES

Carriers, Amino Acid Sources, and Sucrose Concentrations.—A growth index based on gravimetric records was used to compare the effect of 16 media (Table 1) on 4th-stage larvae of the Mexican bean beetle. This index was adapted from Waldbauer (1968) using the formula:

$$Gr = \frac{W_{max}}{W_o\, t}$$

where W_{max} = maximum weight attained by larvae during the 48-hr period of observation; W_o = initial weight of the larvae; t = time in days after the 1st 24 hr at which W_{max} was reached (t = 1.5 (36 hr) or 2 (48 hr) in this experiment).

A factorial analysis of the calculated growth indices allowed comparison of the interactions of carriers, sucrose concentrations, and sources of amino acid. The calculated mean growth indices for all media, ranked by Duncan's multiple range test, are presented in Table 2. Fig. 3 shows the variation of activity (mean growth index) as a function of sucrose concentration for media differing by carrier and kind of protein hydrolysate (amino acid source), including a growth index obtained with larvae reared on soybean leaves under identical conditions. Fig. 4 compares the 12-hr weight variation of 4th-stage larvae reared on the 4 basic media with 0.1M sucrose, and on soybean leaves.

These tests suggested that the semiliquid medium containing casein hydrolysate (sol-casein) with 0.1M

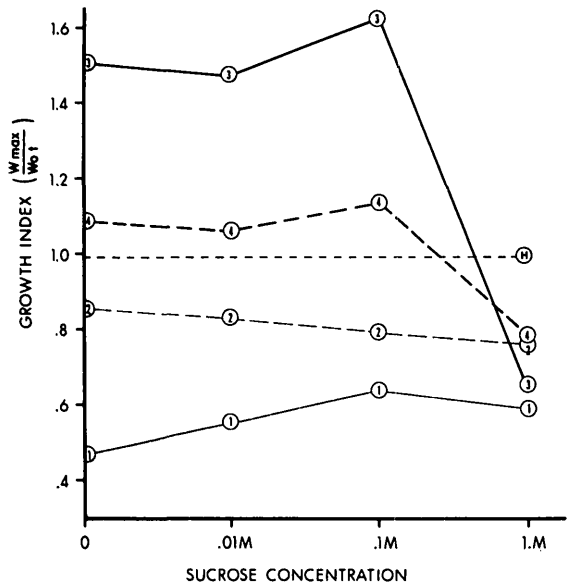

FIG. 3.—Growth index as a function of sucrose concentration. 1, Control, nonnutritive media: 2, sol-soy media; 3, sol-casein media; 4, gel-casein media; H, normal growth index for larvae reared on soybean (Harosoy) leaves.

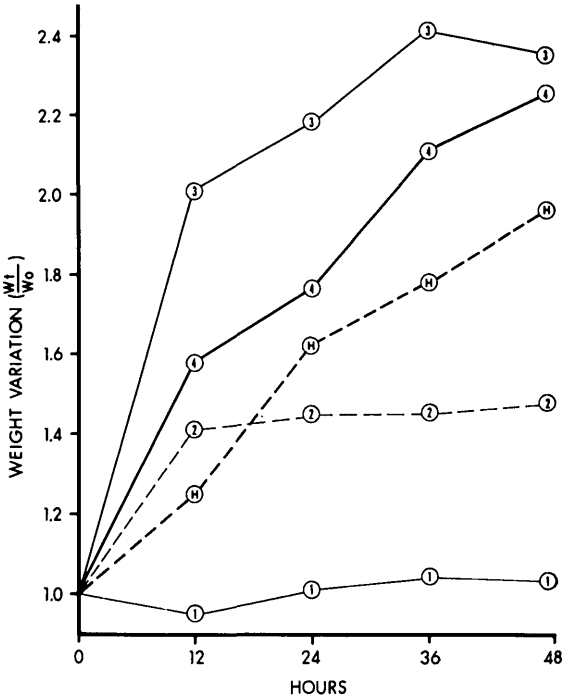

FIG. 4.—Comparison of rates of growth of 4th-stage Mexican bean beetle larvae fed media with 0.1M sucrose, and Harosoy leaves. 1, Control, nonnutritive medium; 2, sol-soy; 3, sol-casein; 4, gel-casein; H, Harosoy leaves.

sucrose applied to Cellucotton wadding afforded the best feeding response. This medium was readily accepted by 4th-stage larvae, it supported normal growth, and yielded normal adults, and it was therefore adopted for further nutritional studies.

Development of 3rd-Stage Larvae on the Sol-Casein Medium.—Forty larvae were reared through the 2nd stadium on soybean leaves and weighed every 24 hr. Immediately after the 2nd molt, 20 larvae were maintained on soybeans and 20 larvae were transferred to the sol-casein (0.1M sucrose) medium. Weight increase of the 2 groups of larvae is shown in Fig. 5. It may be observed that initially the rate of weight increase was slightly slower for larvae on the medium than for those on soybean leaves. However, the final weight of the larvae and prepupae on the medium was higher than the weight of larvae on soybean leaves. Duration of the last 2 larval stadia was similar in the 2 groups.

Table 2.—Growth index of 4th-stage Mexican bean beetle larvae reared on artificial media.

Medium description[a]	Sucrose concn (M)	Growth index[b]
Sol-casein	0.1	1.612 a
Sol-casein	0	1.502 a
Sol-casein	.01	1.471 a
Gel-casein	.1	1.126 b
Gel-casein	0	1.095 b
Gel-casein	.01	1.055 b
Sol-soy	0	0.859 c
Sol-soy	.01	.826 c
Sol-soy	.1	.781 cd
Gel-casein	1.0	.754 cd
Sol-soy	1.0	.745 cd
Control medium	.1	.644 cde
Sol-casein	1.0	.643 cde
Control medium	1.0	.593 de
Control medium	.01	.558 e
Control medium	0	.472 e

[a] See Table 1 for composition of media.
[b] Indices followed by the same letter are not significantly different at the 5% level of probability.

Total Larval Development on the Sol-Casein Medium.—One hundred twenty newly hatched larvae were placed in groups of 5 in clustered petri dishes. After 1 week each surviving larva was isolated. Weights were recorded every 24 hr. Mean weights obtained are shown in Fig. 5 where the percent larval mortality and the duration of the 4 larval stadia are also represented. The weight increase curve can be compared to normal curves of larvae reared on soybean leaves and on the medium after the 2nd molt.

About 25% of the larvae died during the 1st stadium and less than 10% completed larval development. All surviving larvae were underweight, formed abnormal pupae, and adults failed to emerge.

Effect of pH.—Third-stage larvae were reared through pupation on sol-casein (0.1M sucrose) media adjusted to *p*H 5.5, 6.0, 7.0, and 8.0. Five groups of 4 larvae each were tested with each medium. Fig. 6 shows the mean weight gain of 3rd-stage larvae with the 4 media.

The more alkaline media promoted normal develop-

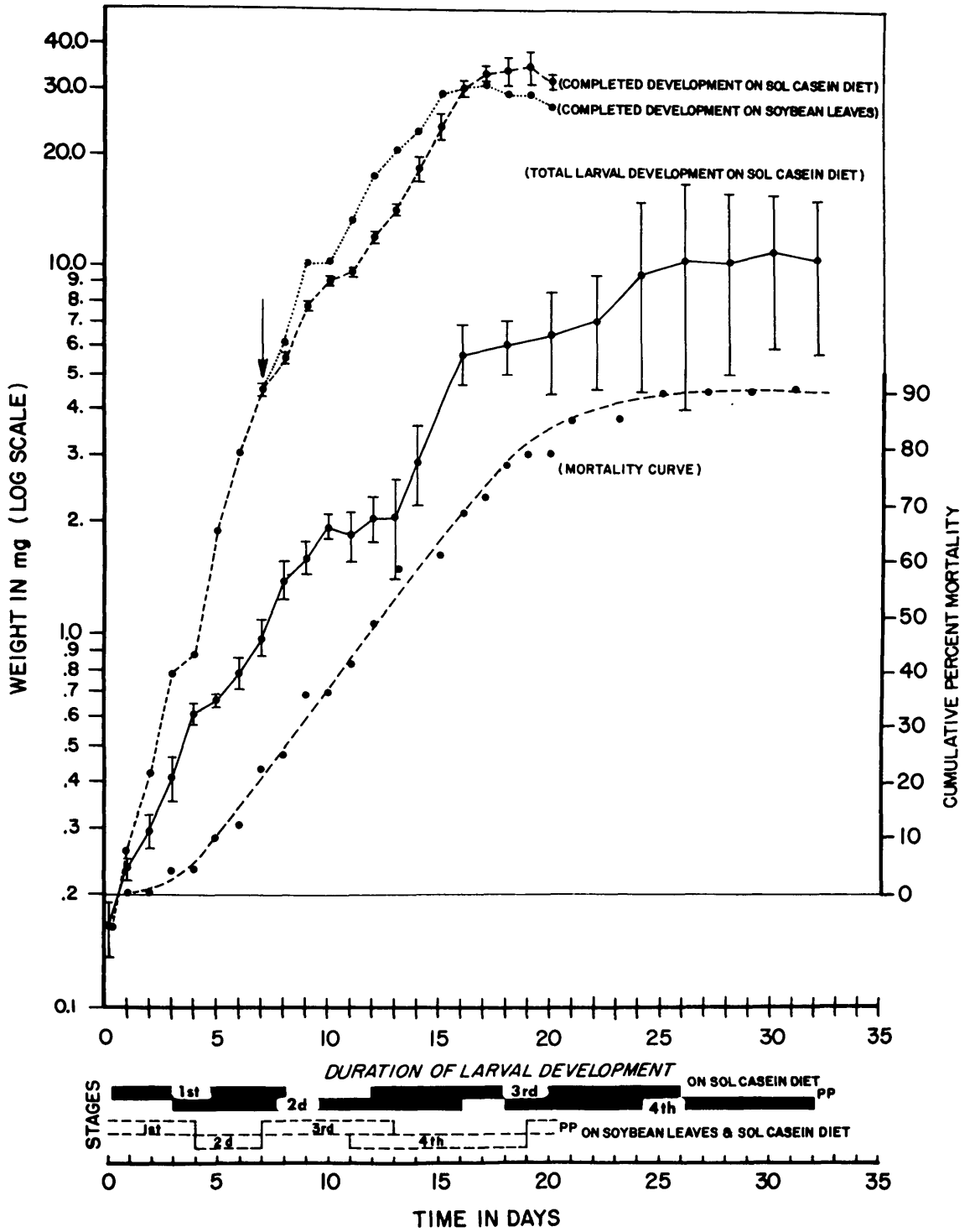

FIG. 5.—Weight gain of 3rd-stage Mexican bean beetle larvae reared on soybean leaves and sol-casein (0.1M sucrose) medium, and total larval development and mortality on the same medium. Arrow indicates date at which the 2 groups of larvae were separated.

ment, but mortality was high at *p*H 8.0. Low *p*H was generally detrimental, causing lower rates of weight increase and higher mortality. On the basis of this experiment, the *p*H of the standard medium was adjusted to 7.2±0.2.

DISCUSSION AND CONCLUSIONS

The first encouraging results of artificially feeding the Mexican bean beetle were obtained with media based on a formulation previously used for some chrysomelid beetles (Kogan 1969[5]). The present sol-casein medium differs from those other media by its semiliquid consistency and the use of a cellulosic carrier. Semiliquid media have been used in rearing *Chrysopa carnea* Stephens (Vanderzant 1969) and in artificial feeding of aphids and certain Heteroptera.

The presence of soy-hydrolysate clearly deterred feeding. Larvae fed these media gained less weight and the larval period was considerably extended. The sol-casein medium proved adequate for rearing larvae after the 2nd molt. Third- and 4th-stage larvae readily accepted the medium and usually gained more fresh weight than larvae fed soybean leaves in the laboratory. Rates of growth were normal. Many newly emerged larvae accepted the diet and successfully completed the 1st molt, but 2nd-stage larvae were usually depigmented, could not shed the exuviae, or frequently did so abnormally. This 2nd molt crisis could not be traced to obvious nutritional imbalances. One initial assumption was made that ascorbic acid could be in short supply. Experiments with media containing 2- and 4-fold concentrations of ascorbic acid did not yield better results than did the standard medium. No improvement was detected when ascorbic acid was added to aliquots of an ascorbic acid-free medium immediately prior to feeding the larvae to prevent possible degradation of the vitamin. The symptoms observed with young larvae on the sol-casein medium suggest deficiencies in systems associated with molting processes. This and related questions are now under investigation.

No attempts were made to optimize the concentration of individual components of the diet, except in the case of sucrose. Concentrations of other ingredients were based on published results obtained with other species of insects. A concentration of 0.1M sucrose was adopted, although formulations without this sugar or with 0.01M sucrose were similarly effective for 4th-stage larvae. Sucrose has been isolated from seeds of *Phaseolus vulgaris* and proposed as the active compound for feeding excitation of Mexican bean beetle (Augustine et al. 1964). My results indicate that high sucrose concentrations were generally detrimental, and also that larvae were excited to feed even in the absence of sucrose. The inert material used with the sol-casein medium (Cellucotton wadding) contains highly purified or bleached celluloses and hemicelluloses.[6] The possibility that traces of

[5] M. Kogan. 1969. Host-selection studies with *Lema trilineata daturaphila* n.n. (Coleoptera: Chrysomelidae). Ph.D. thesis, University of California, Riverside.

[6] Personal communication from technical personnel of Kimberley Clark Corporation.

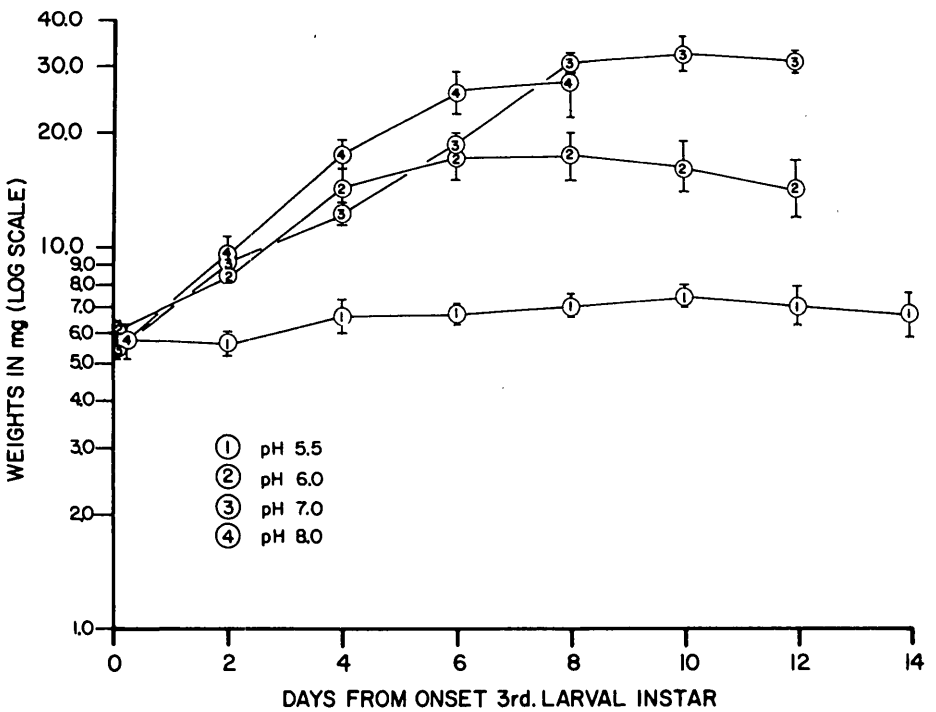

FIG. 6.—Weight gain of 3rd-stage Mexican bean beetle larvae reared on sol-casein (0.1M or sucrose) media adjusted to: *p*H 5.5, 6.0, 7.0, and 8.0.

lower-molecular-weight carbohydrates may be present in this material is not ruled out, and could account for the acceptance of sucrose-free sol-media. However, sucrose-free agar-base media were also similarly active (Fig. 3), therefore suggesting that sucrose was not a prime factor in food acceptance by 4th-stage larvae under the conditions of this experiment.

The main objective in developing this medium was to provide a basis for host selection and nutrition studies with the Mexican bean beetle in its associations with soybeans. In this context, the medium proved extremely convenient. It could be prepared in large volumes and cold-stored for future use, with the degradable ingredients added just prior to use. In comparative studies, a larger volume of the common basic medium was prepared and divided into aliquots to which those ingredients to be tested were incorporated. This procedure assured great uniformity, enhancing confidence in results attributable to the variable components of the media.

This medium so far has been used successfully to test the activity of select soybean leaf powders and specific leaf extracts. It is also being used in preliminary studies of the chemical bases of resistance of certain soybean selections. Adults were maintained on the medium for several weeks but oviposition dropped sharply after a few days. Adults from larvae reared on the medium after the 2nd molt were normal and yielded viable eggs when fed soybean leaves.

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Behavioral Responses of the Gypsy Moth Egg Parasitoid *Ooencyrtus kuwanai*[1] to Abiotic Environmental Factors[2]

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ABSTRACT

The behavioral responses of females of *Ooencyrtus kuwanai* (Howard), an encyrtid egg parasitoid of the gypsy moth, *Porthetria dispar* (L.), to humidity, temperature, gravity, and light were investigated. The parasitoid oriented to the drier of 2 humidity choices at moderate temperatures, but toward higher relative humidities at high temperatures. Its behavior in a temperature gradient indicated that a preferendum between 20–30°C exists, but the broadness of the responses observed and the parasitoid's tendency to accumulate at the cold end made more detailed inferences impossible. Females exhibited negative geotaxis both in the dark and in the light (though to a greater degree in the former) and at different temperatures. They were also positively phototactic under different lighting conditions, temperatures, humidities, and physiological states. Only when exposed to host egg masses before tests were run was a definite neutral photoresponse observed, and at no time was negative phototaxis exhibited. The results are discussed as they relate to the microhabitat distribution of the parasitoid in nature.

The behavioral responses of insects to abiotic factors of their environment are varied. Not only do different species respond diversely, but responses of the same individual may vary under different conditions (Green 1954a, 1954b; Henson 1964; Jack and Williams 1937; Perttunen and Ahonen 1956; Roth and Willis 1951).

Responses of entomophagous insect parasitoids to abiotic environmental factors may be related to the habitats within which the parasitoids search for hosts. Information on habitat selection will help in evaluating the searching capacity of these species and might lead to ways to augment their effectiveness in suppressing host populations.

Little experimental work on habitat selection has been carried out with parasitoids. As one example, Simmonds (1955) found that individuals of *Spalangia drosophilae* Ashmead responded positively to high

[1] Hymenoptera: Encyrtidae.

[2] Received for publication Jan. 26, 1971.