ally numerous in the colonies of these ants (Silvestri 1932).

Six males and six females of *T. borgmeieri* were found in an emigration column of *Nomamyrmex esenbecki crassicornis* (F. Smith) (colony E-164). All were running in the column. Ten additional specimens were seen in the column but not taken. Because no specimen of *borgmeieri* was taken with *Labidus praedator* on Barro Colorado Island, it must be rare or absent there with *praedator*.

Grassiella rettenmeyeri Wygodzinsky

Grassiella includes at least 12 species which have been found either associated with ants and termites or apparently free-living in soil. The most recent revision includes seven Neotropical species in addition to the description of *rettenmeyeri* Wygodzinsky (1958) (fig. 1). Ten specimens of this species were found on Barro Colorado Island with *Nomamyrmex esenbecki, Eciton burchelli, E. vagans, E. dulcius crassinode, E. mexicanum* Roger and *Neivamyrmex gibbatus* Borgmeier. In addition, three specimens were found in leaf litter on the forest floor or running on the ground. The latter records and the wide range of hosts are typical for species not closely associated with ants. Furthermore, *Grassiella* shows no morphological adaptations for myrmecophily except possibly the limuloid form which is similar to that of many free-living Thysanura (Wygodzinsky 1958: 99). It is amazing that such a soft-bodied, fragile insect can live within the colonies of army ants. All specimens taken died a few hours after they were collected whether or not they were kept with ants. All specimens were taken with an aspirator, and at least half of them appeared to be stunned or permanently injured when captured. *G. rettenmeyeri* was found both during the day and night. The single specimen found with *burchelli* was running in the area of anastomosing columns just behind the advancing swarm front at about 11:45 a.m. The thysanuran may have just joined the column as the swarm front was advancing across the forest floor.

Two additional specimens of *Grassiella* sp. were taken with *L. praedator,* including one found by T. C. Schneirla in Mexico. These could not be identified but may belong to *G. rettenmeyeri.* No previous records of *Grassiella* found with *Labidus* are known to me.

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The Chemical Basis of the Host Selection in the Mexican Bean Beetle, *Epilachna varivestis* (Coleoptera, Coccinellidae)[1]

J. K. NAYAR AND G. FRAENKEL

Department of Entomology, University of Illinois, Urbana

ABSTRACT

Fractions containing phaseolunatin and/or related cyanogenetic glycosides were isolated from leaves of bush and lima beans, and were shown to elicit strong biting response from the Mexican bean beetle, when present at low concentrations in combination with glucose. After acid hydrolysis these fractions elicit a negligible response. At higher concentrations they act as inhibitors. It has been suggested that phaseolunatin in high concentrations is responsible for the resistance of certain varieties of bush beans and lima beans to the Mexican bean beetle.

Lippold (1957) demonstrated the oligophagous habits of the Mexican bean beetle, *Epilachna varivestis* Muls., showing that this insect feeds almost exclusively on the plants of the genus *Phaseolus.* Olfaction, he showed, is of minor importance in host selection by this insect, but gustatorily stimulating ethanol- and water-soluble substances, possibly glycosides, stimulate feeding. He isolated a fraction containing a triterpenoid saponin from the leaves and seeds of the bush bean, *Phaseolus vulgaris,* and showed that it elicits a strong biting response from

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the larvae. He also demonstrated that the Mexican bean beetle feeds and grows relatively well on leaves of the lima bean, *P. lunatus,* which contain the cyanogenetic glycoside, phaseolunatin (linamarin). Higher concentrations of phaseolunatin are known to be both poisonous and repellent to higher animals (Viehoever 1940). Therefore it was considered desirable to investigate whether this glycoside plays any part in the food selection of this insect. This report deals with the effects of phaseolunatin or similar cyanogenetic glycosides on the food selection of the Mexican bean beetle.

MATERIALS AND METHODS OF BIOASSAY

Insects used in the bioassays were reared in the laboratory. Bean plants used for the isolation of the gustatory stimulants were grown on the horticulture farm of the University of Illinois. Leaves from month-old plants were collected, dried at room temperature, and ground to a fine powder in a domestic hand grinder.

The filter paper technique used by Lippold (1957) was modified as follows: Whatman No. 1, 9 cm.-diameter filter paper was marked with equally spaced three 2cm.-diameter semicircles on its circumference. The semicircles were ringed with molten wax, to prevent the fractions in the semicircle from being diluted or diffusing out of the marked area. All fractions to be tested were concentrated and tested on the basis of milligrams per milliliter of water, and 0.2 ml. of the solution was applied on the semicircle. One of these semicircles contained only glucose in a suboptimal concentration; in the second semicircle, the fraction to be tested was applied alone; and in the third, the fraction and glucose were applied in combination. Before the fractions were applied to the semicircles, the rest of the filter paper was moistened with distilled water to provide enough moisture to prevent the area in the semicircles from drying during the test period. Two freshly molted, fifth-instar larvae that had been starved for 4 to 6 hours were used for each test. These tests were run for 12 hours. The larvae were then removed and relative amounts of attempted feeding, i. e., biting into the filter paper, were estimated. Bitten places were recognized by the yellow spots formed as a result of reflux spitting and by tufts of filter paper loosened by the bite. Three or more positive tests out of five tests were taken to mean that there was enough stimulant present to warrant further isolation. These tests gave only qualitative estimates of the biting responses.

Isolated fractions were tested also on lettuce discs. Lettuce serves as an almost neutral plant and the larvae feed on it only when hungry. When stimulating fractions were coated onto 12-mm. lettuce discs punched out from the smooth area of the leaf with a cork borer, the larvae reacted to the treated discs almost immediately. These tests were run for 6 to 12 hours and the amount of biting was judged qualitatively at the end of the test period.

PROCEDURES OF ISOLATION, AND RESULTS

Lippold (1957) showed that the water solution of the concentrated alcoholic extract of bush bean leaves produced a strong feeding response by the larvae and adults of the Mexican bean beetle. He attributed this reaction to a triterpenoid saponin. His methods, however, did not remove sugars and cyanogenetic glycosides which could have been responsible for the feeding response. Acid hydrolysis of the gustatorily active fraction made such extracts completely inactive for the insect. This suggested that the gustatory stimulant might be a glycoside, as suggested by Lippold (1957). Dunstan and Henry (1903) and Dunstan and Auld (1906) isolated a cyanogenetic glucoside phaseolunatin from the leaves of *Phaseolus lunatus* and *Linum usitatissimum.* It was assumed that a similar glucoside is present in the leaves of bush beans, and the method used by Dunstan and Auld (1906) was adopted for its isolation.

The details of the method are as follows: Air-dried bush bean leaf-powder was extracted with 95% ethanol in the Soxhlet apparatus for about 36 hours. The alcoholic extract was concentrated under reduced pressure and the concentrate dissolved in water. The solution of water (fraction 1a), which produces a

Table 1.—Biting responses of fifth-instar larvae of the Mexican bean beetle to various fractions of the ethanolic extract of the leaves of bush beans. The degree of response is expressed by a graded number of plus and minus signs: (+++++) biting on ethanolic solution, and (−) no biting at all. The tests were run for a period of 12 hours.

	Fractions	Mean biting responses of five replicates
1.	Glucose 0.15 M	++
2.	Fraction 1a	+++++
3.	Fraction 1a in 1 ml. of 0.15 M glucose solution	+++++
4.	Fraction 2a	+++
5.	Fraction 2a in 1 ml. of 0.15 M glucose solution	+++++
6.	Fraction 3a	++
7.	Fraction 3a in 1 ml. of 0.15 M glucose solution	+++++
8.	Fraction 3a hydrolyzed with dil. H_2SO_4	−
9.	Fraction 3a hydrolyzed with dil. H_2SO_4 in 1 ml. of 0.15 M glucose solution	+
10.	Linamarin & Lotaustrin mixture 2 mg./ml.	+
11.	Linamarin & Lotaustrin mixture 2 mg. in 1 ml. of 0.15 M glucose solution	++++

Notes: a) Fractions 1a to 3a correspond to those described in the text.
b) Fractions in tests No. 2, 4, and 6 were tested at 5-mg./ml. concentrations. Fractions in tests No. 3, 5, and 7 were tested by dissolving 5 mg. of the corresponding fractions in 1 ml. of 0.15 M glucose solution. All the fractions when tested were applied in 0.2 ml. at each of the semicircles.

strong biting response (table 1), was filtered and decolorized by the addition of a slight excess of neutral lead acetate.

The filtrate was treated with H_2S to remove the excess lead and was concentrated under reduced pressure. This fraction (fraction 2a) elicited a strong biting response from the beetle larvae (table 1). Crystallization of any material from this fraction was unsuccessful because of the presence of an excess of syrupy material. This fraction was redissolved in 95% ethanol and re-extracted thrice with ether. Ether and ethanol were evaporated from the combined ether layers, the dry concentrate was dissolved in ethanol, and water was added until the solution was slightly cloudy. It (fraction 3a) was then set aside in the refrigerator and after some days needle-like crystals settled out (fraction 4). In the original procedure by Dunstan and Auld (1906), which we followed closely, fraction 3a contained the phaseolunatin. The syrupy residue from the ether-ethanol mixture (fraction 3b) also elicited a biting response, which was probably due to the presence of large amounts of sugars. However, the concentrate of the ethyl ether and ethanol mixture (fraction 3a) did not elicit appreciable biting responses by itself; but when 5 mg. of the almost dry fraction were dissolved in 1 ml. of 0.15 M glucose solution, and 0.2 ml. of this mixture was applied on the semicircle, it elicited a strong biting response (table 1). The crystalline fraction 4 was also active when tested in combination with sugar. It was also observed that once the larvae were stimulated to bite, they reacted strongly even to suboptimal amounts of sugar alone. Tests were repeated at least five times. After acid hydrolysis fraction 3a did not stimulate biting responses either alone or in the presence of glucose (table 1).

A preparation of glucosides, isolated from white clover and stated to contain linamarin and the closely related lotaustrin in about equal amounts was obtained from Dr. G. W. Butler of D. S. I. R., Palmerston, New Zealand. This mixture of glucosides did not elicit biting responses by itself but did so in combination with glucose (table 1). This biting response was similar to that obtained with fraction 3a from bush beans, when tested with 0.15 M glucose solution (table 1).

A fraction containing phaseolunatin was similarly prepared from the leaves of lima beans. When fraction 3a from lima beans was tested at various concentrations, with and without glucose, the following results in table 2 were obtained. These results indicated that the phaseolunatin fraction, by itself, did not elicit any extensive biting response. Solutions of 0.5 or 1.0 mg. of fraction 3a in 1 ml. of 0.15 M glucose solution did elicit extensive biting responses. Concentrations of 2.0 mg. or more of fraction 3a in 1 ml. of 0.15 M glucose solution did not elicit appreciable biting, though the larvae visited these semicircles repeatedly. On the contrary, higher concentrations of fraction 3a inhibit biting. These results suggested that phaseolunatin serves as a biting stimulant at fairly low concentrations but at higher concentrations acts as a repellent.

Table 2.—Biting responses of fifth-instar larvae of the Mexican bean beetle to various concentrations of the fractions containing phaseolunatin obtained from the leaves of lima beans. The degree of response is expressed by a graded number of plus and minus signs: (+++++) biting on ethanolic solution of bush bean leaves, and (—) no feeding at all. The tests were run for a period of 12 hours.

	Fraction	Mean biting responses of five replicates
1.	Glucose 0.15 M	++
2.	5 mg. of ethanolic solution of bush bean in 1 ml. of 0.15 M glucose solution	+++++
3.	Fraction 3a. 0.5 mg./ml. of the lima bean extract	+
4.	Fraction 3a. 0.5 mg. in 1 ml. of 0.15 M glucose solution	+++++
5.	Fraction 3a. 1.0 mg./ml.	+
6.	Fraction 3a. 1.0 mg. in 1 ml. of 0.15 M glucose solution	+++++
7.	Fraction 3a. 2.0 mg./ml.	+
8.	Fraction 3a. 2.0 mg. in 1 ml. of 0.15 M glucose solution	+++
9.	Fraction 3a. 5.0 mg./ml.	—
10.	Fraction 3a. 5.0 mg. in 1 ml. of 0.15 M glucose solution	+
11.	Fraction 3a. 10.0 mg./ml.	—
12.	Fraction 3a. 10.0 mg. in 1 ml. of 0.15 M glucose solution	—

Notes: a) Fractions 3a correspond to that described in the text.
b) 0.2 ml. of test fraction was applied at each of the semicircles.

CHARACTERIZATION OF THE PHAGOSTIMULANT

Systematic characterization of the crystalline substance (fraction 4) could not be conducted as sufficient material was not available. So tests were made with a crude fraction (fraction 3a) of bush beans and lima beans, to demonstrate the presence of a cyanogenetic glycoside.

The active fraction obtained from bush bean leaves (fraction 3a) was yellow, syrupy, and possessed a cool, bitter taste. It did not reduce Benedict's reagent unless it had been acid-hydrolyzed. This indicated that something in the active fraction yielded a reducing substance on hydrolysis. Paper chromatography showed that the hydrolyzed extract contained a substance identical with glucose.

The aglycone portion of a cyanogenetic glycoside should contain hydrocyanic gas. This was demonstrated by the following tests:

a) Alkaline picric acid paper test according to McElroy (1951) was positive.

b) Iodine-starch paper test according to Guatelli (1954) was positive.

c) Benzidine-copper acetate test according to Cheronis and Entrikin (1957) was also positive.

Paper chromatography of fraction 3a from bush

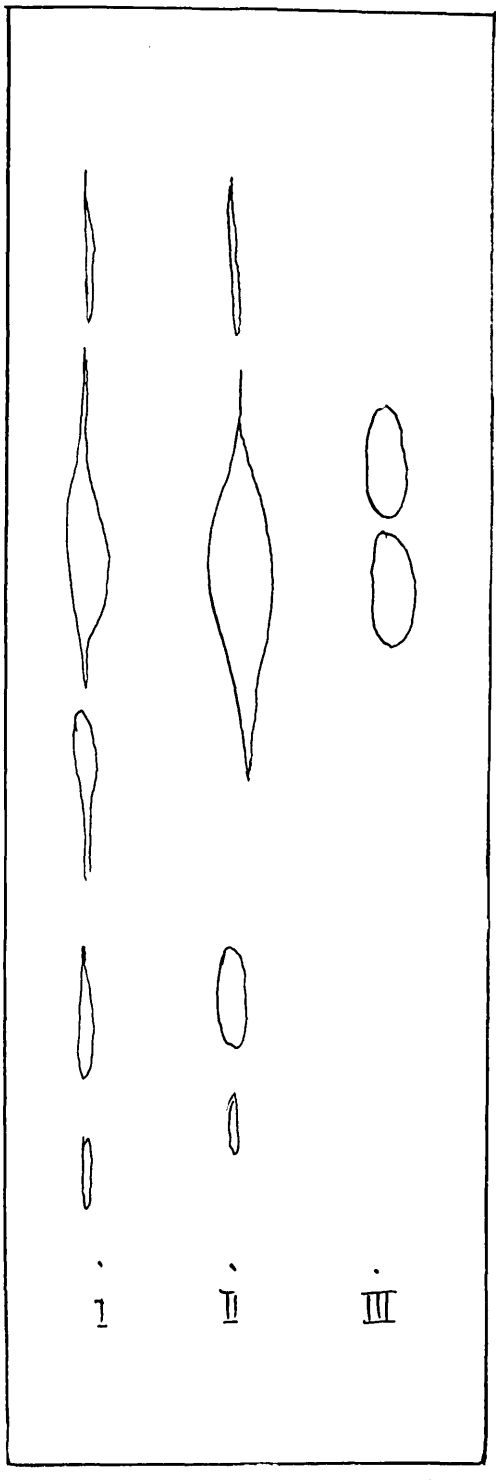

FIG. 1.—Paper chromatogram of fraction 3a from the leaves of *Phaseolus vulgaris* (streak I) and *Phaseolus lunatus* (streak II) and of a linamarin and lotaustrin mixture (streak III).

beans, from lima beans, and of the mixture of linamarin and lotaustrin in a solvent mixture of *n*-butanol:acetone:acetic acid:water (4:1:1:3) showed that all these three mixtures contained compounds with similar Rf values. The chromatogram was sprayed with a 5% aqueous $AgNO_3$ solution and then with a 5% aqueous NaOH solution, and dried at room temperature. After about one-half hour, dark brown spots could be seen on a slightly brownish background. The phaseolunatin-lotaustrin mixture gave two closely spaced spots, and the other two preparations gave a single spot in the same region and additional spots representing impurities (figure 1).

These studies showed that the leaves of bush beans and lima beans contain a cyanogenetic glycoside, probably phaseolunatin, which acts as a stimulant at low concentrations for the Mexican bean beetle.

Comparison of the amounts of the cyanogenetic glycoside in the leaves of bush beans and lima beans, using the method described in Official Methods of Analysis of the A. O. A. C. (1955) showed that the air-dried bush bean leaves contain about 0.016% of HCN and room-dried lima beans about 0.045% of HCN.

DISCUSSION

These studies indicated that a similar substance was extracted from both bush beans and lima beans. By itself this substance elicits no biting responses from the larvae of the Mexican bean beetle, but it does do so in the presence of 0.15 M glucose. This response could be observed clearly from the bite marks produced by the larvae on filter paper.

As shown above, this glucoside is present in three times higher concentrations in lima beans than in bush beans, and this factor alone might explain the lesser feeding of the Mexican bean beetle larvae on lima beans. This glucoside varies in concentration in different varieties of lima beans (Viehoever 1940), which renders some of them with higher concentrations inedible or poisonous for human consumption. Similar findings have been reported in the case of different varieties of lima beans which are not consumed by higher animals (Dunstan and Henry 1903; Razafirmahery 1954). Painter (1951) stated that sorghum contains a similar type of cyanogenetic glucoside. "Under certain environmental conditions the sorghum develops a high content of HCN, which differs in different varieties. The presence of this poison has suggested the possibility that it may be related to the cause of resistance to certain insects in sorghum. No clear evidence supporting this idea had been reported." It has been shown that a fraction of cyanogenetic glucosides, containing in all probability phaseolunatin acts as a phagostimulant for the Mexican bean beetle larvae at lower concentrations, but at higher concentrations it acts as a deterrent or inhibitor. It is, thus, possible that phaseolunatin, when present at higher concentrations, may cause resistance to the Mexican bean beetle in different varieties of either bush beans or lima beans.

The present work does not directly contradict Lippold's (1957) conclusions as to the attractant proper-

ties of a triterpenoid saponin, contained in bean leaves, for the Mexican bean beetle. No such compound was, however, detected in our active fractions, as demonstrated by the absence of foaming.

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A Successful Technique for Mass-Rearing Cabbage Loopers on a Semisynthetic Diet[1]

CARLO M. IGNOFFO

Entomology Research Division, Agric. Res. Serv., U. S. Department of Agriculture
Brownsville, Texas

ABSTRACT

The medium, apparatus, and procedures used in rearing cabbage loopers (*Trichoplusia ni* (Hübner)) are described. Studies on the biology of diet-reared loopers include information on number of larval instars and duration, measurements of head-capsule width and body length, rates of prepupation, pupation, and adult emergence, and average longevity of the different stages.

Presently, basic studies on the cabbage looper, *Trichoplusia ni* (Hübner), are curtailed because of difficulties encountered in rearing. The present paper describes a technique for effectively rearing loopers in the laboratory with ordinary equipment and handling procedures. The development of this technique should serve as a stimulus to studies of diseases, irradiation, nutrition, sex attractant, and insecticide screening of the cabbage looper, all of which depend upon the use of large numbers of insects. More importantly, it should also stimulate the commercial production of highly virulent looper-polyhedra for wide-scale field application. The rearing technique has been successfully employed for six generations with the polyhedrosis limited to less than 5% of the numbers reared. Modifications should further increase the efficiency of the collecting procedures and reduce the loss of loopers due to polyhedrosis.

DESCRIPTION OF REARING APPARATUS AND PROCEDURE

Oviposition Cage.—The oviposition cage (fig. 1*A*) is essentially a cylinder enclosed on both ends by pie pans. The bottom of the cage (*a*) is a porcelain-coated pie pan (outside diameter 10 ¼ inches, inside diameter 8 inches, depth 1⅜ inches) filled with 1 inch of sand (*b*) and wet with 250 milliliters of water. The lower part of a ½-pint milk carton (*c*) with a 1-inch hole punched in the bottom supports an 8-dram feeding vial (*d*) containing 50% honey in water. A dental roll (*e*), recessed in a bored cork (*f*) to provide a feeding trough, is used as a wick. The adults are contained in a 4-mesh, galvanized, screen-wire cage (*g*), cylindrical in shape (8×11 inches) and enclosed at the top. A 2-inch square opening (*h*) is cut in the top of the cage to insert newly emerged adults. Eggs are laid on white paper toweling (*i*) tightly wrapped around the outside of the cage, secured with masking tape (*k*), and thoroughly wet with sprayed water. The wetting insures a high humidity and also shrinks the paper, allowing it to fit tighter around the cage. Another pie pan (*l*) is inverted over paper toweling (*j*) placed on top of the cage. Figure 1*B* shows the oviposition cage ready to receive adults.

Excellent results in egg recovery are also obtained with paper toweling that lines the insides of pliable, cardboard and plastic cylinders. However, the use of screen cages with the toweling wrapped around the outside facilitates egg collecting and conserves time.

Emergence Cages.—The emergence cage (fig. 1*C*) is a lantern globe with a petri dish bottom and a screen-lid top. The 4×0.9-inch petri dish (*a*) con-

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