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STUDIES ON THE CHEMICAL BASIS OF HOSTPLANT SELECTION IN THE GENUS *EPILACHNA* (COLEOPTERA, COCCINELLIDAE)—I. A VOLATILE PHAGOSTIMULANT IN *SOLANUM CAMPYLACANTHUM* FOR *EPILACHNA FULVOSIGNATA*

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Abstract—At least two phagostimulants for the larvae of *Epilachna fulvosignata* occur in the leaves of *Solanum campylacanthum*. One of these is steam volatile, the other not. An oil containing rather more than 95 per cent of a single component has been prepared from the fresh leaves. Larvae responded to concentrations of 2 p.p.m. of this oil incorporated in suitable agar mixtures.

INTRODUCTION

ALTHOUGH the significance of chemical factors in hostplant selection has been studied in several insects (see reviews by FRAENKEL, 1959; THORSTEINSON, 1960) little is known concerning its evolution. One reasonable approach to this problem is to examine the role of chemical phagostimulants and inhibitors in a number of closely related insects having different hostplant preferences. The lines of evolution of hostplant preferences suggested by such work might then be corroborated, or otherwise, by taxonomic considerations. The coccinellid genus *Epilachna* appears to be particularly suitable for such a study. The present paper is concerned with part of an investigation into the chemical factors acting as phagostimulants on the larvae of *Epilachna fulvosignata* Rche. var. *suahelorum* (Weise), a species feeding on certain Solanaceae. To date the presence of two phagostimulants has been demonstrated, one being steam volatile and the other not. Only the demonstration of the steam volatile factor is described here.

FEEDING BEHAVIOUR AND HOSTPLANT SELECTION IN *EPILACHNA*

Both larvae and adults of *Epilachna* are phytophagous and probably tend to have similar hostplant preferences (STRIDE and WARWICK, 1960). The method of feeding is apparently the same in both stages and has been described in detail for the larvae of the Mexican bean beetle, *E. varivestis*, by HOWARD (1941). The mandibles are opened widely on the flat surface of a leaf, embedded in the leaf tissues, and then closed together, thus scraping together the leaf tissues into a

pulpy mass. From this the insect extracts the tissue juices on which it feeds, leaving the solid residue behind on the leaf membrane. In this way feeding insects produce semi-transparent patches on leaves, the areas of which are a convenient measure of the amount of feeding.

Although the genus *Epilachna* is associated with a wide range of plant families, a majority of species feeds on either the Solanaceae or the Cucurbitaceae. Host-plant records suggest that most species are oligophagous, feeding predominantly on plants of a single family. Records of *Epilachna* species feeding on a wide range of plant families should be treated with reserve, since until DIEKE (1947) showed the taxonomic value of the male genitalia, the identification of some species was uncertain. Species feeding on the same family of plants may show very different preferences within that family. In East Africa *E. gibbosa* Crotch and an unidentified species are both found on *Momordica foetida* but not on *Melothria maderaspatana*, *Cucumis sativus*, or *Cucurbita pepo*. *E. chrysomelina* feeds on the latter group of cucurbits but not on *M. foetida*.

The existence in Australia of the two species *E. sparsa* and *E.28-punctata*, morphologically very similar, but feeding respectively on the Solanaceae and Cucurbitaceae only, suggests that one of these feeding habits may have been derived directly from the other. If this is so, a knowledge of the chemical bases of hostplant selection in *Epilachna* species feeding on these two plant families would provide a good foundation for an experimental investigation into the mutability and evolution of hostplant relationships.

CONDITIONING EFFECT OF LARVAL FOODPLANT ON ADULT PREFERENCES

Before commencing the chemical investigations a general examination was made of the food preferences and feeding behaviour of *E. fulvosignata*.

Adult beetles, which had been reared on *Solanum campylacanthum*, were confined in Petri dishes with leaf squares taken from mature leaves of various solanaceous plants. The following order of preference, based on the areas of leaf eaten, was established for these beetles:

1. *Solanum campylacanthum* eaten very readily
2. *Solanum tuberosum* } eaten readily
3. *Solanum nigrum* }
4. *Lycopersicum esculentum* } eaten less readily
   Physalia peruviana }
5. *Datura stramonium* }
   Datura arborea }
   Nicotiana tabaccum } not eaten
   Capsicum annuum }

These results indicated that *E. fulvosignata* differed in its selection of solanaceous plants from the Australian *E. sparsa* since both *D. arborea* and *D. stramonium* were acceptable to the latter species (STRIDE and WARWICK, 1960). It may be noted that the plants in the above list that were rejected by *E. fulvosignata* were all

eaten by the tobacco hornworm, *Protoparce sexta* (Johan.), which probably accepts a much wider range of solanaceous plants (YAMOMOTO and FRAENKEL, 1960).

Four groups of thirty-six larvae were reared on *S. campylacanthum*, *S. tuberosum*, *L. esculentum*, and *P. peruviana* respectively. The numbers of larvae that survived to pupation were twenty-five (*S. tuberosum*), eighteen (*S. campylacanthum*), fourteen (*L. esculentum*), and seven (*P. peruviana*). The newly emerged adults from these larvae were given the choice of the foodplants, and the amount of feeding that occurred on each was recorded. Statistical analysis suggested that insects reared on *S. tuberosum* greatly preferred this plant and *S. campylacanthum* to the other two plants, and showed an overall preference for *S. tuberosum*. Adults reared on *L. esculentum* seemed to feed equally readily on this plant, *S. tuberosum*, and *S. campylacanthum*, and greatly preferred these plants to *P. peruviana*. Adults reared on *S. campylacanthum* showed a general preference similar to those reared on *S. tuberosum*, except that they preferred *S. campylacanthum* to *S. tuberosum*. These simple tests indicated that adult preferences could be modified by the larval foodplant.

Those newly hatched larvae placed on *L. esculentum* became very restless and wandered around the container for several hours before settling down to feed. No such wandering occurred on the other three plants. Thus innate hostplant preferences of the newly hatched larvae were not completely correlated with the ability of a plant to promote larval development, since the latter was more rapid on *L. esculentum* (pupation on twentieth to twenty-second day after hatching) than on *P. peruviana* (pupation on thirty-second day and after).

MATERIAL AND METHODS

The insect species used in this investigation was identified by Mr. R. D. Pope at the Commonwealth Institute of Entomology as *Epilachna fulvosignata* Rche. var. *suahelorum* (Weise). Adults laid readily when confined in cages with sprays of *Solanum campylacanthum*, and batches of eggs were removed daily and kept in plastic pill boxes until hatching. The newly hatched larvae were transferred to sleeve cages on *Solanum campylacanthum* in the field. There were four larval instars, and in Uganda the life cycle was completed within a few weeks. The larvae attacked artificial foods much more readily than did the adults; they were used throughout the work described in this paper unless otherwise stated.

In the feeding preference tests on leaves described above, adult insects were confined in Petri dishes containing a damp filter paper on which rested square pieces of the leaves being offered. The Petri dishes were kept in an incubator at 26·7°C for a day, after which the areas of the leaves eaten were measured.

Several methods of presenting extracts and chemical fractions of *S. campylacanthum* to the larvae were tried.

Methods using filter papers, and techniques essentially similar to those used by LIPPOLD (1957) and NAYAR and FRAENKEL (1963), were tried but were discarded as being too insensitive. The surface of the filter paper was marked only when the insects chewed vigorously, and weaker responses were unrecorded. Furthermore,

there was the undesirable possibility that volatile substances might evaporate from the filter paper before the tests were complete.

Cells were made by fixing glass rings, 0·5 cm deep and 1·5 cm dia., to glass cover slips, and were filled with various substances as a base for carrying the extracts. Of these cellulose powder was the most successful, and the larvae fed readily from the surface of cellulose powder wetted with attractive solutions. As with filter paper, however, only chewing responses were recorded. Certain cellulose derivatives (Cellophas B) proved unsuitable as it was impossible to obtain a sufficiently smooth surface to show up the attack of the insects.

The most satisfactory method involved the use of agar disks. 100 ml of 2% agar solution, containing the extracts under investigation, was equally divided among three 9 cm Petri dishes. When cold the agar plate was removed carefully from the Petri dish and inverted so that the very smooth surface that had gelled in contact with the glass lay uppermost. Disks 15 mm in diameter were cut out of the plate with a cork borer and used in the insect tests. Prepared in this way the disks were about 5 mm thick, which was sufficiently thick to prevent drying out but sufficiently thin to ensure that the insects attacked the surface of the disks and not the sides. The heat necessary to prepare agar solutions might have been disadvantageous where very volatile substances were concerned. To minimize this trouble a plant extract known to contain volatile material was added only when the agar solution had cooled somewhat and was nearly ready for pouring into the Petri dishes. The Petri dishes were closed immediately after pouring.

Generally larvae were only given the choice of two types of agar in any one test, and if comparisons were required of several agars they were tested in pairs. A test consisted of ten Petri dishes (9·5 cm dia.) each lined with damp filter paper and containing four agar disks (two of each type under test) and the *Epilachna* larvae. Disks of the same agar lay at opposite points of the compass, and all were placed sufficiently far from the wall of the dish so that they were not automatically encountered by larvae walking around the sides of the dish. Five or six early third or fourth instar larvae were placed in each of the dishes, which were kept in a dark-room during the test period. They were examined at a convenient period during the next 24 hr. Placing the insects in an incubator at 26·7° speeded up the test so that the result might be apparent after only 4 hr. When volatile substances were present, however, the use of an incubator was felt to be unsuitable since the air therein soon became tainted with their smell. The larvae were allowed to feed until they were required for the tests, since it seemed likely that replete larvae would show a greater differential response to slight differences in the phagostimulatory properties of the agars. Hungry larvae would be more likely to chew persistently at the first suitable agar disk that they encountered even though it might not be the more attractive of the two agars present.

In some of the earlier experiments (e.g. those recorded in Figs. 2, 3, and 4) less than ten dishes were used in each test.

Epilachna larvae may mark agar disks in three ways, referred to here as clawing, biting, and chewing. It is often important to differentiate between these.

Clawing. Feeding larvae grasp the leaf surface very firmly with their claws, and agar disks frequently carried clawing marks. They were recognizable as long scratches, deepening and ending in a small pit where the claws had sunk into the agar. It was uncertain whether such marks were associated with a very weak feeding response or with subtle differences in the consistency of different agars.

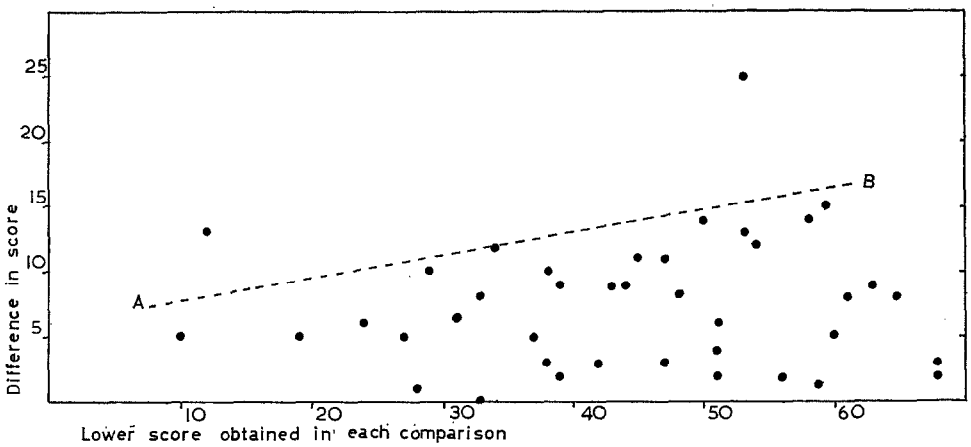

FIG. 1. Differences in the score obtained by the two members of pairs of similar agars when offered to larvae under the standard conditions of the test for phagostimulation. The line AB has been inserted somewhat arbitrarily to mark the limit of the expected experimental error. It is likely to be exceeded only occasionally.

Biting. Biting marks resulted from the open jaws being thrust into the agar and then being withdrawn without closure. They appeared as pairs of deep triangular pits scattered over the surface of the agar.

Chewing. Chewing marks were seen as deep scours on the surface of the agar, similar to the marks made on leaves.

When the agar disks were examined at the end of a test they were scored in the following grades:

Grade 0, less than ten biting marks, scored 0
Grade 1, less than quarter of disk surface destroyed, scored 1
Grade 2, less than half of disk surface destroyed, scored 2
Grade 3, less than three-quarters of disk surface destroyed, scored 3
Grade 4, less than entire surface destroyed, scored 4
Grade 5, entire surface destroyed, scored 5

Clawing marks were ignored. Successive quarter-disk increments on this scale were not of equal value since there was no evidence that the larvae attacked only the undamaged agar surface. As destruction of the unbroken agar surface proceeded presumably a greater proportion of new bites fell on previously damaged areas and were thus unrecorded.

It was necessary to determine at what level differences in the scores obtained by two agars were likely to represent true differences in acceptability to the larvae.

Forty pairs of similar agars were compared using the standard number of ten Petri dishes for each comparison. Some of the pairs of agars were made less attractive than others in order to obtain information at different levels of acceptability, and observations were made at various times between 4 and 18 hr after setting up the experiments. In Fig. 1 the difference between the two scores obtained in each comparison has been plotted against the lower of the two scores. It will be seen that, with two exceptions, the results fell within a clearly defined zone of experimental error. For convenience the apparent limit of this zone was defined, somewhat arbitrarily, by the broken line inserted in Fig. 1. During the subsequent investigation it was considered that if two agars were compared in two separate tests and if the two results thus obtained both lay above the broken line then the difference was due, at least in part, to a difference in phagostimulatory properties.

CHEMICAL INVESTIGATIONS

The sensitivity of the method used to test for the presence of phagostimulants is conveniently demonstrated by the experiment summarized in Fig. 2. This was one of duplicate experiments done to investigate the effect of adding glucose to the test agars. Certain soluble carbohydrates are known to act as general phagostimulants to certain insects (YAMOMOTO and FRAENKEL, 1960), and it seemed possible that the differing responses of the larvae to different concentrations to carbohydrates introduced in the plant extracts might obscure the responses to more specific phagostimulants. There is also evidence (DETHIER, 1953) that some insects do not distinguish between a number of different soluble carbohydrates or their derivatives. By adding a large amount of glucose (5%) to the test agars it was hoped to swamp any variations in larval response due to differences in carbohydrate content arising from the plant extracts used.

From Fig. 2 it is evident that adding glucose to the agars greatly increased the sensitivity of the tests. The leaf extract used for this experiment was prepared by macerating 100 g freshly gathered *S. campylacanthum* leaves in 300 ml water and straining the suspension obtained through coarse cloth. It was approximately a dilution of 1 part original leaf juice in 4·7 parts final solution. In the absence of glucose a strong feeding response was obtained only to the 100% extract agar, but the presence of 5% glucose promoted strong responses to all the agars containing juice. Thus in the absence of glucose the difference in the response to the 1% extract agar and the water agar (test 4), though discernible, was slight. It was greatly increased by the addition of glucose to both agars (test 5). Essentially similar results were obtained in the duplicate experiment, except that in test 1 the difference between the two agars lay within the range of experimental error. Addition of glucose was particularly valuable for enhancing the response to weakly phagostimulating material—in this experiment the presence of a 1 in 470 (approximately) dilution of leaf juice was readily detected. A 5% glucose agar to which no leaf extract had been added was attacked only slightly more than a water agar.

Phagostimulants were isolated in several ways including the organic extraction of dry leaves, and the organic extraction or distillation of water extracts from

fresh leaves. The first and second methods indicated the presence of a powerful non-volatile phagostimulant with solubility properties rather similar to those of the gustatory stimulant for *Protoparce sexta* (YAMOMOTO and FRAENKEL, 1960). This will form the subject of a separate report. The last method demonstrated the

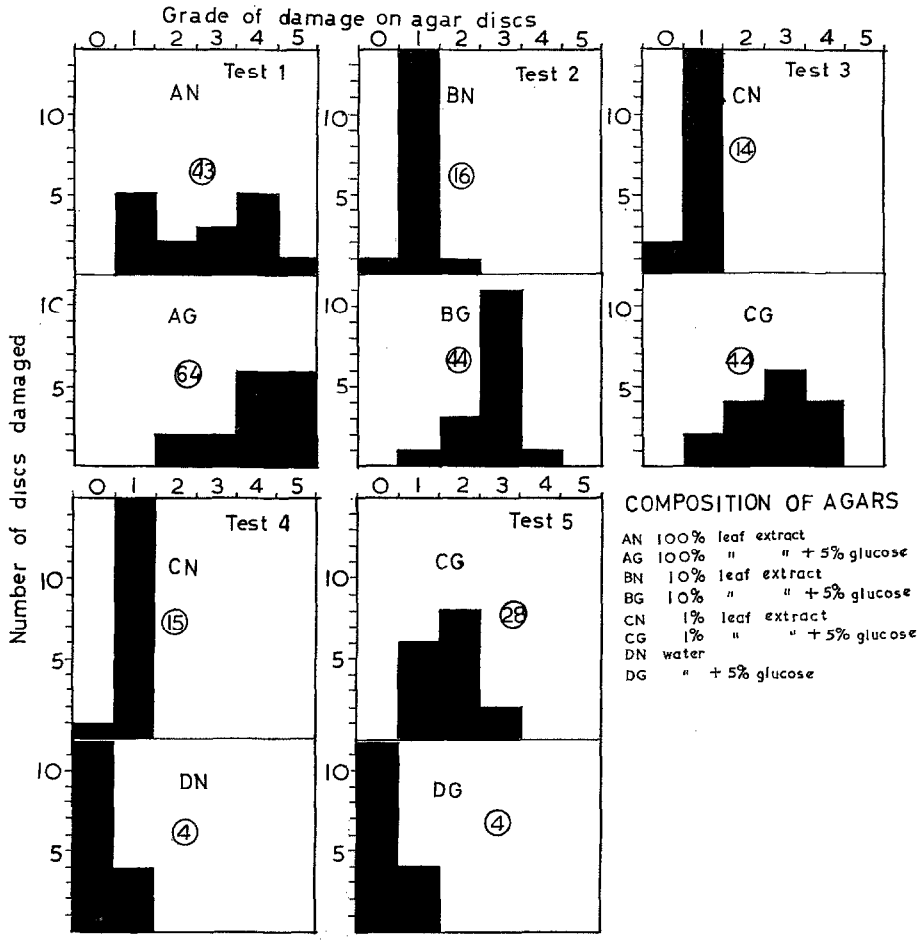

FIG. 2. Comparison of larval damage to agar disks containing various concentrations of leaf extract, some with glucose and some without. The figures in circles indicate the score obtained by the agars.

presence of the steam volatile phagostimulant with which this paper is mainly concerned.

The steam distillation of the water extract from macerated fresh leaves

200 g fresh mature leaves were finely macerated with 600 ml water, and expressed through a fine nylon gauze to give 600 ml of a dark green liquid. By steam distillation this liquid could be separated into three components:

(a) the steam distillate
(b) a dark green coagulum } in distillation residue.
(c) a brown liquor

The coagulum was separated from the brown liquor by filtration, and the phagostimulatory properties of the three components compared separately and in combination, using agars with and without added glucose. The coagulum was heavily contaminated with the brown liquor. Certain results from an experiment in which no glucose was added are shown in Fig. 3. All components were

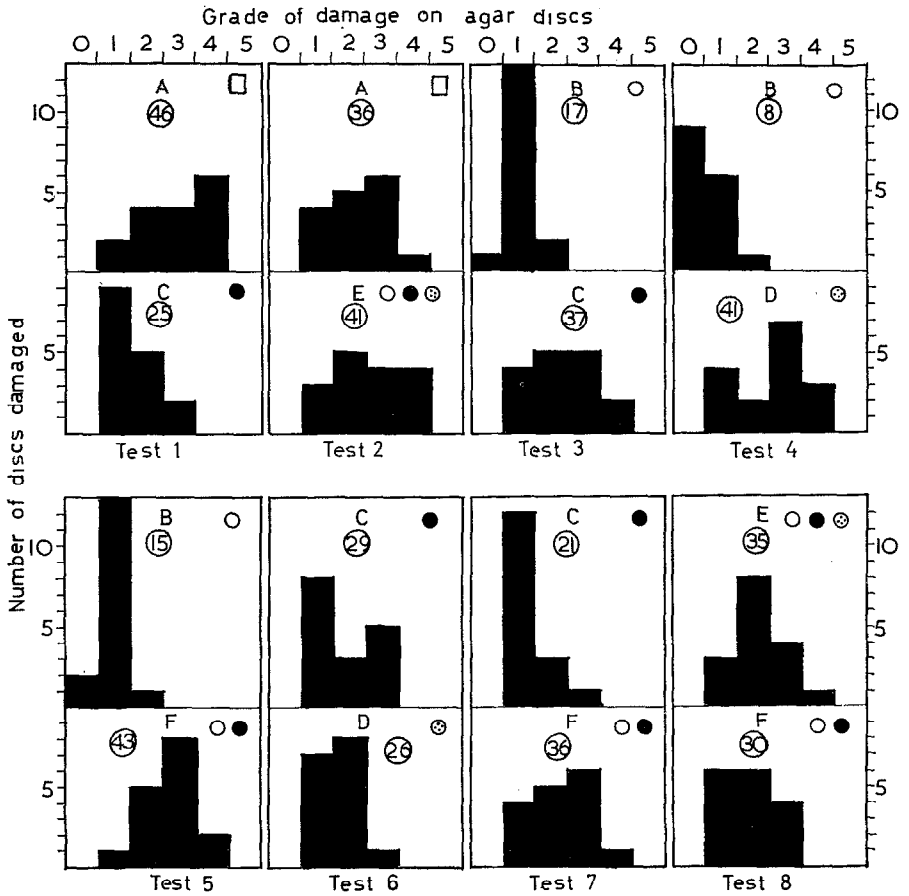

FIG. 3. Comparison of larval damage to 2% agar disks containing water extract of fresh leaves or various components of this extract after steam distillation. The contents of the agars are indicated as follows: White square, leaf extract before distillation; White circle, steam distillate; Black circle, filtered brown mother liquor from distillation residue; Dotted circle, green coagulum from distillation residue.

Composition of agars: Agar A, whole leaf extract; Agar B, steam distillate; Agar C, mother liquor; Agar D, coagulum; Agar E, steam distillate + mother liquor + coagulum (i.e. reconstituted extract); Agar F, mother liquor + coagulum.

incorporated in the agars in amounts equivalent to 50 ml of the original extract per 100 ml final agar solution.

Steam distillation did not affect the phagostimulatory properties of the extract since the agar containing the three components recombined after steam distillation

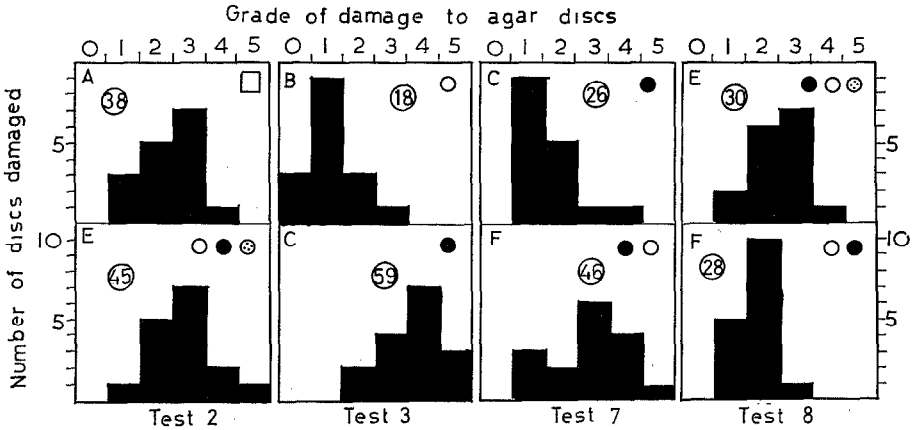

FIG. 4. Damage to 2% agar disks containing 5% glucose and the water extract of fresh leaves or the components of this extract after steam distillation. The contents of the agars are indicated as in Fig. 3.

was as heavily attacked by the larvae as that containing the original extract (test 2). A similar result was obtained when glucose was incorporated in the agars (Fig. 4).

Considering the three components separately, the brown liquid and coagulum were attacked most heavily and the steam distillate least (Fig. 3, tests 3, 4, and 6). The addition of glucose did not change this preference, but exaggerated the differences (Fig. 4). The heavy contamination of the coagulum by brown liquor undoubtedly contributed much to its acceptability by the larvae and it is probable that the same chemical phagostimulant was present in both fractions. The coagulum presumably arose from colloidal proteinaceous materials liberated by the vigorous maceration of the leaves. In later work a more gentle method of water extraction was adopted in which the leaves were deep frozen, roughly crushed, and then soaked in water. Water extracts prepared in this way did not produce the coagulum when heated.

The presence of a steam volatile phagostimulant is shown in test 7 (Fig. 3) where a combination of steam distillate and brown liquor was more heavily attacked than the brown liquor alone. This was confirmed in further tests using glucose agars (Fig. 4).

Isolation of the steam volatile phagostimulant

In order to test for the presence of the steam volatile phagostimulant (hereafter referred to as VPS) suspected fractions were incorporated in agar consisting of 2% agar, 5% glucose, and 40% brown liquor obtained from the residue of a distillation. The presence of VPS is readily detected when it is added to this

type of agar, which also had one further advantage; although all reasonable precautions were taken there was a possibility of distillates being contaminated by small amounts of brown mother liquor carried over as a fine spray. Use of an agar already containing a large proportion of brown liquor eliminated the risk of mistaking a response to unsuspected brown liquor contamination for a genuine response to VPS. Brown liquor to be used in the preparation of agars was steam distilled for at least 30 min before use to remove VPS.

The pH of the extract from which VPS was distilled was approximately 6·0. VPS could be redistilled from a steam distillate under either alkaline or acidic conditions, and presumably lacked acidic or basic properties that could be utilized for its extraction. Extraction of the steam distillate with organic solvents was unsuitable on grounds of expense and availability of the solvents.

The following method was eventually devised and adopted for the routine separation of VPS. Each day a batch of 8 kg fresh leaves was collected and placed in a deep freeze. After 24 hr it was removed, roughly broken, and pounded while still frozen and brittle. 16 l. of water (i.e. twice the weight of the leaves) was then added and the leaves pounded again. The leaves were allowed to soak in the water with occasional pounding for a further 24 hr. The water was then expressed from the leaves and steam distilled in a metal boiler. The first 500 ml distillate was collected and stored in a refrigerator. When four such distillates had been collected (from 32 kg leaves) they were bulked and fractionated through a 40 cm Vigreux column. The amount of organic material in the steam distillate was small and this fractionation required care. The temperature of the column head was raised slowly to about 80°C and then maintained between 80°C and 90°C for about 30 min. After this time it was again allowed to rise slowly until steam came over into the collecting flask. When sufficient steam had passed over to sweep the organic material into the flask the distillation was stopped. A small Claisen flask surrounded by freezing mixture made a convenient receiver. During the earlier stages of this distillation a small quantity of a sweetly smelling oily mixture came over; it was miscible with the water that came over in the final stages.

The Claisen flask was removed and placed in a water bath. The temperature of the bath was raised until that at the top of the distillation column reached 80°C. The column temperature was maintained at 80°C for as long as possible but not allowed to rise higher. When that part of the liquid which would distil over at this temperature had done so the column temperature fell. When it had fallen to 50°C the distillation was ended. During this distillation the bulk of the organic material passed over into the receiver, and a small amount of a pale yellow oil separated out on the surface of the residual water in the flask. This oil contained VPS. It was removed from the water by extraction with ether, and the ether extract was dried with anhydrous magnesium sulphate, before being filtered through a sintered glass filter, also covered with a layer of the drying agent. Finally the ether extract was evaporated in a small pear-shaped flask on a water bath. VPS remained in the residue in the flask. The yield was small, about 0·3 g oil per 100 kg fresh *Solanum* leaves.

It seemed essential that the fractionation through the Vigreux column should be carried out very slowly, and that the top of the column should be kept at a high temperature for a long period. Apparently the VPS collected just above the steam front in the column. Examination by gas chromatography showed that probably eight volatile substances were present in the distillate from the Vigreux column. One of these was apparently unstable and interfered with the examination. An unpleasant smelling grease could be extracted with ether from the residue of the distillation. This was not a phagostimulant.

In several experiments the distillate from the Vigreux column was split into two fractions, the fractionating temperature being controlled so that it did not rise above 75–80°C. In each experiment the residue had much greater phagostimulatory powers than did the distillate. In some instances the distillate was phagostimulatory, in others not so.

THE ABILITY OF THE LARVAE TO DETECT THE PHAGOSTIMULANT AT LOW CONCENTRATIONS

Tests were done in which standard agars (40 ml brown liquor, 60 ml water, 5 g glucose, 2 g agar) containing low concentrations of the phagostimulant were compared with similar agars containing no phagostimulant. In order to measure

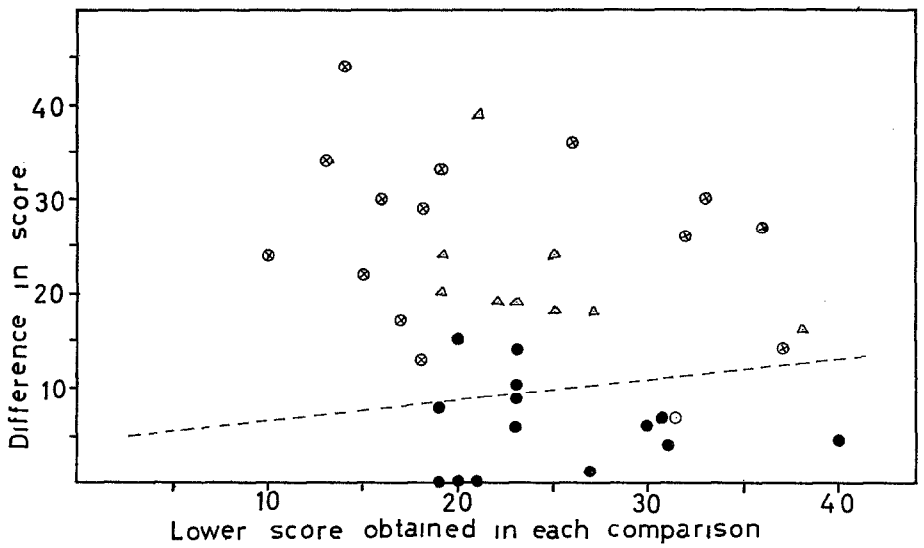

FIG. 5. Differences in score obtained by agars containing 10, 4 and 2 p.p.m. of the volatile phagostimulant when compared with control agars containing no phagostimulant. All agars were prepared with the standard recipe of 2 pt agar, 5 pt glucose, 40 pt brown mother liquor, and 60 pt water. ⊗, 10 pt phagostimulant. △, 4 pt phagostimulant. ●, 2 pt phagostimulant. ○, 2 pt phagostimulant.

In all comparisons except the one shown as an open circle, the attack on the agars containing phagostimulant was equal to or in excess of that on the controls.

The broken line marks the limit of expected experimental error—see Fig. 2.

the small quantities of phagostimulant required it was necessary to dilute it with acetone to concentrations of $\frac{1}{10}$ or $\frac{1}{25}$, the phagostimulant then being added to the agar in acetone solution. An equivalent amount of acetone was added to the control agar. The amount of acetone added to any agar never exceeded 0·05 ml per 100 ml agar solution, other tests having shown that at this concentration acetone had no phagostimulatory effect on the larvae.

The results obtained with the lowest concentrations of the oil containing VPS are shown in Fig. 5. They indicated that at concentrations of 10 and 4 p.p.m. this oil acted as a phagostimulant. Differences obtained with agars containing only 2 p.p.m. tended to fall within the range of expected experimental error. That eleven tests favoured the VPS agar and only one in the control showed, however, that even at this concentration the VPS was acting as a weak phagostimulant.

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Mr. E. V. PEARSON, Commonwealth Institute of Entomology, kindly read the original manuscript.

The chemical nature of VPS is now being investigated by Dr. R. D. M. MURRAY, Department of Chemistry, University of Glasgow.

REFERENCES

DETHIER V. G. (1953) *Insect Physiology* (Ed. by ROEDER K.), pp. 544–576. John Wiley, New York.

DIEKE G. H. (1947) Ladybeetles of the genus *Epilachna* (sens. lat.) in Asia, Europe, and Australia. *Smithson misc. Coll.* **106**, 1–183.

FRAENKEL S. G. (1959) The raison d'être of secondary plant substances. *Science* **129**, 1466–1468.

HOWARD F. N. (1941) Feeding of the Mexican bean beetle larva. *Ann. ent. Soc. Amer.* **34**, 766–769.

LIPPOLD P. C. (1957) The history and physiological basis of host specificity of the Mexican bean beetle, *Epilachna varivestis* (Muls.), Coleoptera, Coccinellidae. Ph.D. Thesis, University of Illinois, Urbana.

NAYAR J. K. and FRAENKEL G. (1963) The chemical basis of host selection in the Mexican bean beetle, *Epilachna varivestis* (Coleoptera, Coccinellidae). *Ann. ent. Soc. Amer.* **56**, 174–178.

STRIDE G. O. and WARWICK E. P. (1960) On two species of *Epilachna* (Coleoptera, Coccinellidae) from Australia. *Proc. Linn. Soc. N.S.W.* **85**, 208–214.

THORSTEINSON A. J. (1960) Host selection in phytophagous insects. *Annu. Rev. Ent.* **5**, 193–218.

YAMOMOTO R. T. and FRAENKEL G. (1960) Assay of the principal gustatory stimulant for the tobacco hornworm, *Protoparce sexta*, from solanaceous plants. *Ann. ent. Soc. Amer.* **53**, 499–503.