

fatto che deve sempre essere tenuto presente nella valutazione dei singoli reperti citochimici che ad essi si riferiscono e che può facilitare la spiegazione di taluni loro comportamenti apparentemente anormali. I molti elementi già raccolti in proposito, saranno discussi in altra sede¹.

Per ora mi preme sottolineare il fatto che l'esistenza nei granuli neutrofili di una componente lipidica risulta inequivocabilmente confermata, e che le divergenze esistenti fra il comportamento di tali granuli e quello delle più comuni localizzazioni lipidiche di altri tessuti, non infirmano il significato tradizionale fin'ora attribuito alla sudanofilia dei granuli neutrofili.

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Summary

On the grounds of cytochemical findings and of critical arguments, the author reaffirms that the sudanophilia of neutrophile leucocytes depends on their lipidic content and not on some chemical combination of the dyes with cytoplasmic constituents, as yet unexplained, as suggested by LILLIE and BURTNER, and by GOMORI.

¹ S. PERUGINI e D. BOTTINO (in stampa). – S. PERUGINI e M. SOLDATI, Biol. Latina (in stampa); Riv. Istoch. norm. pat. (in stampa).

² Borsista del Consiglio Nazionale delle Ricerche presso l'Istituto di Anatomia Comparata dell'Università di Pavia.

Feeding Habits and Physiology of Digestion of Certain Leafhoppers Homoptera: Jassidae

Investigations on the feeding habits of certain leafhoppers have revealed that they show a certain degree of specificity to a particular plant tissue for drawing their food-sap. On this basis they can be grouped into two categories. One of these includes forms which feed mainly upon the mesophyll tissue. The species recorded under this category are: *Empoasca bifurcata* DeL., *E. erigeron* DeL., *E. filamenta* DeL., *E. abrupta* DeL., and *E. maligna* (Walsh) (SMITH and POOS¹), and *Typhlocyba pomaria* McATEE (PUTMAN²). The other category includes forms which suck the sap primarily from the vascular bundles, particularly from the phloem tissue. The species recorded under this group are: *Empoasca fabae* (HARRIS) (SMITH and POOS¹), *Homalodisca triquetra* (Fab.) (KING and COOK³), *Graphocephala versuta* (Say) (KING and COOK³), *Eutettix tenellus* Bak. (BENNET⁴), *Cicadulina mobila* (Naude) (STOREY⁵), *Macropsis trimaculata* (Fitch) (PUTMAN²), and *M. insignis* (Van Duzee) (PUTMAN²).

The significance of this specificity to a particular plant tissue shown by jassids is not clear. In the case of *Eutettix tenellus*, which feeds upon the phloem tissue of sugar-beet petioles, FIFE and FRAMPTON⁶ have shown that the stylets of this insect are guided to their ob-

jective by a pH gradient which exists between the epidermis and the phloem. Their experiments, however, do not throw light on the physiological value of this tissue-specificity. Experiments of BENNET¹ on the same leafhopper reveal that the individuals fed on the parenchyma of sugar-beet petioles show a much higher rate of mortality than do those fed on the phloem tissue. This indicates that the parenchyma does not serve as a favorable source of food for these insects.

Further elucidation of the physiological relationship between the leafhoppers and the plant tissue attacked requires a knowledge of their physiology of digestion. At present our knowledge of this aspect is confined to HERFORD's² record of the occurrence of two enzymes, diastase and invertase, in the salivary secretion of *Empoasca solana* DeL. The study of this aspect was, therefore, taken up in four species of jassids, namely, *Empoasca kerri* Pruthi, *E. devastans* Dist., *Tettigella spectra* (Sign.) and *Parabolocratrus porrectus* Walk.

The methods employed for the study of stylets' track through the plant tissue were those adopted by SMITH³ and PUTMAN⁴. For the determination of digestive enzymes the usual practice of preparing the extracts of digestive organs by grinding was avoided in order to exclude the endoenzymes. The latter are not liberated in the lumen of the gut and, hence, cannot take part in the digestion of food prior to its absorption. Only the contents of the lumen were drawn out and tested for the presence of various enzymes. The technique for their detection is described elsewhere.

Of the four species included in the present study, *Tettigella spectra* and *Parabolocratrus porrectus* confine their feeding to plants of the family Graminae. They show a great preference for doobgrass (*Cynodon dactylon*), maize (*Zea mays*), and, sometimes also for paddy (*Oryza sativa*). On the other hand, *Empoasca kerri* and *E. devastans* do not feed on gramineous plants. *E. kerri* feeds mostly on plants of pigeon pea (*Cajanus indicus*), potato (*Solanum tuberosum*) and castor (*Ricinus communis*) while *E. devastans* feeds on malvaceous plants like cotton (*Gossypium* spp.), *Hibiscus* spp., and also on potato, and brinjal (*Solanum melongena*). The first two species suck the sap from the phloem tissue while the latter two feed mostly on the mesophyll tissue.

The distribution of enzymes in different parts of the digestive tract is shown in the accompanying table. It is apparent from this table that the enzymes present in the mesophyll-feeding *E. kerri* and *E. devastans* are: amylase, maltase, invertase, alkaline proteinase, polypeptidases, chlorophyllase and, in *E. kerri* only, lipase. These enzymes act upon starch, maltose, sucrose, proteins, polypeptides, chlorophyll and fat globules respectively to yield diffusible substances. On the other hand, in the phloem-feeding jassids, *T. spectra* and *P. porrectus*, the enzymes amylase, proteinase, lipase and chlorophyllase are absent. Hence, these insects cannot utilise nondiffusible substances like starch, native proteins and lipids, which are abundant in the mesophyll cells. The sap in the phloem is, however, rich in soluble substances which are either already in a diffusible state or can be rendered diffusible by the enzymes present in these insects. When they are artificially fed on solutions of starch, casein, gluten or oil emulsion they die much more quickly than do the individuals fed on solutions of maltose, cane sugar, glucose and tryptophane. In the former case the

¹ F. F. SMITH and F. W. POOS, J. Agri. Res. 43, 267 (1931).

² W. L. PUTMAN, Canad. Ent. 73, 39 (1941).

³ W. V. KING and W. S. COOK, U. S. Dep. Agri. Tech. Bull. No. 296 (1932).

⁴ C. W. BENNET, J. Agri. Res. 48, 665 (1932).

⁵ H. H. STOREY, Proc. Roy. Soc. (London) [B] 125, 455 (1938).

⁶ J. M. FIFE and V. L. FRAMPTON, J. Agri. Res. 53, 581 (1936).

¹ C. W. BENNET, J. Agri. Res. 48, 665 (1932).

² G. V. B. HERFORD, Ann. Appl. Biol. 22, 301, 306 (1935).

³ K. M. SMITH, Ann. Appl. Biol. 13, 109 (1926).

⁴ W. L. PUTMAN, Canad. Ent. 73, 39 (1941).

nutrients have been observed to get clogged in the midgut and thus cause a distention of the 1st ventriculus. Sometimes the latter may even rupture.

Table showing the distribution of digestive enzymes of the leafhoppers

Name of insect	Organ	Amylase	Maltase	Invertase	Alkaline Proteinase	Polypeptidase	Lipase	Chlorophyllase
<i>E. kerri</i> . . .	Salivary glands	×	—	×	—	—	—	—
	Midgut	×	×	×	×	×	×	×
<i>E. devastans</i> .	Salivary glands	×	—	×	—	—	—	—
	Midgut	×	×	×	×	×	—	×
<i>T. spectra</i> . .	Salivary glands	—	—	×	—	—	—	—
	Midgut	—	×	×	—	×	—	—
<i>P. porrectus</i> .	Salivary glands	—	—	×	—	—	—	—
	Midgut	—	×	×	—	×	—	—

Note: (i) '×' denotes the presence and '—' the absence of enzymes.
(ii) The hind gut did not show the presence of these enzymes.

These observations suggest that the specificity of jassids to a particular plant-tissue is related to the distribution of enzymes in their digestive tract and, hence, to their capacity to utilize various nutrients.

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Department of Zoology, University of Delhi, February 15, 1954.

Zusammenfassung

Die Zikaden *Empoasca kerri* und *E. devastans* saugen in erster Linie den Saft des Mesophyllgewebes, während *Tettigella spectra* und *Parabolocratrus porrectus* sich hauptsächlich vom Phloemgewebe ernähren. Bei den mesophyllfressenden Formen sind folgende Fermente vorhanden: Amylase, Maltase, Invertase, alkalische Proteinase, Polypeptidasen, Chlorophyllase und allein bei *E. kerri* Lipase. Die Phloemfresser besitzen nur Maltase, Invertase und Polypeptidase.

On the Ovary Development in Queenless Worker Bees (*Apis mellifica* L.)

Introduction. If the queen of a colony of honeybees gets lost, a new one is reared from a fertilized egg or a young female larva. If, however, in a queenless colony, no eggs or young larvae are available, a development occurs in the ovaries of a great number of worker bees resulting in the production of haploid eggs from which only drones emerge. As long as a queen is present in a colony, the ovaries of the worker bees remain small and undeveloped with no differentiation in the ovarioles. Thus the presence of the queen prevents the development of the worker bee ovary. The nature of the factors responsible for the inhibition of the worker bee ovary in a queen-right colony is still unknown, and so far experi-

mental data concerning this problem are scanty and contradictory¹.

In an attempt to obtain more information on this problem, experiments were performed with small groups of bees kept under laboratory conditions.

Material and methods. Very young worker bees, emerging from a comb with sealed brood in an incubator, were used as experimental material. Within 24 h after emergence, groups of about 50 bees were transferred into LIEBEFELDER experimental cages. The caged bees were kept at 30°C throughout the experiment and supplied with food and water *ad libitum*. The food consisted of sugar candy containing bee gathered pollen in a concentration of 15 % of the dry sugar. (For further details of this method of keeping bees see².) After about 3 weeks, the bees were killed with chloroform and dissected under 70 % alcohol. The development of the ovaries was studied under a binocular microscope using the classification of developmental stages as given by HESS³. According to this method, the degree of development is qualified by the figures 1–5, representing a development of respectively none, first signs, distinct, high and highest. Based on the situation in a normal bee colony, the stages 1 and 2 were considered as undeveloped and only the stages 3, 4, and 5 as developed.

Results. In preliminary experiments, it was observed that the degree of development of the ovaries of young worker bees caged without a queen is highly dependent on the amount of pollen consumed. In order to eliminate as far as possible the variable factor of pollen intake, which might influence the results, the bees were forced to consume the pollen by incorporation in sugar candy. A pollen concentration of 15 % was sufficient to obtain a development of more than 50 % of the ovaries within a period of 21 days. At higher pollen concentrations many bees died, apparently as a result of accumulation of indigestible material in the gut. In subsequent experiments a pollen concentration of 15 % was therefore used.

Under the conditions of our experiments, the presence of a queen resulted in a complete inhibition of ovary development of the worker bees during the first three weeks, whereas, in the controls without a queen, the number of developed ovaries after three weeks varied between 50 and 100 %. A virgin queen prevented the ovary development as well as a mated one. More surprising was the observation that the inhibiting influence is not only exerted by a living queen but also by the presence of a dead one. In general the effect of dead queens decreased with increasing time after the queen had been killed, but in some experiments even after 12 months a distinct inhibition was obtained.

In further experiments it turned out that especially certain parts of a dead queen attract the worker bees and exert a distinct influence. If only the head of a queen was present an almost complete suppression of ovary development was obtained. The influence of an

¹ G. HESS, Über den Einfluss der Weislosigkeit und des Fruchtbarkeitsvitamins E auf die Ovarien der Bienenarbeiterin, Beih. Schweiz. Bienenz. 1, 33 (1942). — A. MÜSSBICHLER, Die Bedeutung äusserer Einflüsse und der Corpora allata bei der Afterweiselenstehung von *Apis mellifica*, Z. vgl. Physiol. 34, 207 (1952). — J. PAIN, L'alimentation et le développement des ovaires chez l'ouvrière d'abeille, Arch. int. Physiol. 59, 203 (1951); La «substance de fécondité» dans le développement des ovaires des ouvrières d'abeilles (*Apis mellifica* L.). Critique des travaux de MÜSSBICHLER, Insectes Sociaux 1, 59 (1954).

² A. P. DE GROOT, Protein and Amino Acid Requirements of the Honeybee (*Apis mellifica* L.), Physiol. Comp. Oecol. 3, 197 (1953).

³ G. HESS, Über den Einfluss der Weislosigkeit und des Fruchtbarkeitsvitamins E auf die Ovarien der Bienenarbeiterin, Beih. Schweiz. Bienenz. 1, 33 (1942).