PHYSIOLOGICAL ADAPTATIONS OF DUSKY COTTON BUG, *OXYCARENUS HYALINIPENNIS* (COSTA) (HETEROPTERA; LYGAEIDAE) TO ITS HOST PLANT, COTTON

Pt. I. Digestive Enzymes in Relation to Tissue Preferences

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Abstract

Present study was undertaken to ascertain correlation between relative preference of *Oxycarenus hyalinipennis* (Costa) for different parts of cotton plant and its ability to utilise the nutrients available in these parts.

The insect shows maximum preference to cotton leaf as the source of food and least to cotton-seed. While feeding on leaf they draw food-sap mostly from mesophyll and phloem tissues, rarely from xylem vessels and never from the oil-glands scattered all over the leaf and other parts of the plant.

The insects feeding on cottonseed draw food material from outer or inner integument of the seed coat. They never penetrate their stylets deeper than the seed coat and, consequently, never feed on the kernel or embryo of the seed.

Nutrients available to the insects feeding on cotton leaf are proteins, some free amino acids, starch, sugars (sucrose, glucose and fructose), and fats. Those available in the cottonseed coat are pentosans, sugars (raffinose, sucrose, glucose and fructose), free amino acids, slight quantities of proteins and fats.

Study of the distribution of digestive enzymes indicates that cellulase, hemicellulases, inulase, β-glucosidases, β-galactosidases, polypeptidase, and lipase are absent. Amylase, maltase, invertase, α-galactosidase, proteinase and esterase occur in the salivary secretion and in the midgut. Their occurrence in the hindgut is quite irregular. Trehalase has been detected only in the midgut. Maximum activity of almost all these enzymes occurs at *p*H 5.4. The invertase, present in the digestive tract of *Oxycarenus*, is a glucosaccharase.

Correlation between the nutrients available in cotton leaf or in seed coat and ability of the insect to utilise the nutrients has been discussed. Cotton leaf appears to be a much better source of food for *Oxycarenus* than cottonseed.

Introduction

Genus *Oxycarenus* Fieber includes a number of species which are known to be pests of cotton plant in different parts of the world. Their biology has been studied by various workers and there has been some disagreement regarding the site of feeding, and nature of injury caused by these insects. Most workers consider that *Oxycarenus* feeds mainly on the seeds of cotton and causes severe damage to their tissues (Peacock, 1913; Balls, 1915; Misra, 1921; Kirkpatrick, 1923). Kirkpatrick (1923) further believes that this insect visits green leaves or epicalyces etc. only to imbibe moisture from the glands present on those parts of the plant. According to some other workers *Oxycarenus* feeds on the blossoms (Sickenberger, 1890), squares, buds and young bolls of cotton (Willcocks, 1906; Adair, 1918; Misra, 1921) in addition to the seeds. Investigations of Saxena and Krishna (1958) on the orientation and site of feeding of *O. hyalinipennis* on cotton plant indicate that this insect, when offered a choice from amongst cotton leaves, epicalyces, green bolls, opened bolls, seeds, lint, flowers and flower buds, prefers to feed mostly on green leaves or epicalyces. Next in order of preference come

other green parts of the plant, and seeds come the last. In fact seeds are fed upon much less frequently than the leaves.

Little is yet known whether *Oxycarenus* is better adapted to utilise nutrients available in the cottonseed than those in the leaf or vice versa. Present work has been undertaken in order to investigate this aspect.

Material and Methods

Adults of *Oxycarenus hyalinipennis* (Costa)* were used for the present work. For all the experiments reported in this paper freshly collected insects were allowed to feed on distilled water for 48–72 hours in order to clear their alimentary canal by flushing.

In order to determine the plant tissue from which the food-sap is drawn, the insects were given access to different parts of cotton plant and allowed to feed on them. Since cottonseed has been reported by Kirkpatrick (1923) to be the chief site of feeding and leaf to be the main source of food by Saxena and Krishna (1958), only these two parts were selected for the present study. Insects to be fed on leaves were provided with short twigs of cotton plants bases of which were kept immersed in water to prevent wilting. The seeds supplied to the insects were freshly collected from fresh or old open cotton bolls and were delinted as completely as possible. Some fuzzy hairs were, however, left behind on the seed-surface. The seeds from freshly opened bolls had a fine film of moisture on their surfaces due to moist lint surrounding them. The seeds from the old opened bolls had, on the other hand, dry surface. It was difficult to make the insects feed on cottonseed. However, they could be made to do so with a little less difficulty by applying a fine film of moisture to the surface of the seed. This was done by giving the delinted seed a dip in water and then wiping its surface with filter paper, drying the fuzzy hairs as completely as possible. Whatever trace of moisture was left on the surface of the seed was enough to induce the insects to feed on it. It may be noted that no water could diffuse into the interior of the seed by this treatment nor was there sufficient moisture for the insects to drink it from the surface and prevent them from penetrating their stylets deeper into the seed tissues.

Whenever an insect penetrated its stylets into the leaf or seed tissue it was allowed to feed for 1–2 minutes and then its beak was clipped off by means of a pair of very fine scissors. In the case of insects feeding on cotton leaf the stylets *in situ* with the leaf were fixed in Formol—acetic acid—50 per cent alcohol mixture (5 : 5 : 90 v/v) for about 12 hours. Freehand sections of the fixed material were cut, stained in Safranin, counterstained in Fast Green and finally mounted in canada balsam. In case of insects feeding on cottonseed, the stylets *in situ* with the seed were treated with 10% KOH solution for 4–7 days in order to soften the seedcoat. Fairly thick sections of these seeds were then cut, mounted in glycerine and examined in reflected light under a microscope. Various parts of the seed, including the two distinct outer and inner integuments of the seedcoat, could be differentiated without difficulty.

In order to ascertain the nutrients that may be available to the insects feeding on cottonseed and leaf, a knowledge of chemical composition of these parts is essential. Some information on chemical composition of the entire cotton leaf and different parts of cottonseed is already available and may be made use of in this paper. However, additional information on the free amino acids and sugars present in the cotton leaf and seedcoat (hull) was desirable and was obtained in the present work.

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* The insects were kindly identified by Dr. Reece I. Sailer, U. S. Dep. Agric. Entomological Research Bureau, Washington, D.C., for our colleague Dr. M. K. Dutt.

Cottonseed hulls from 100 freshly collected seeds and 50 fresh leaves were separately homogenised in 80 per cent ethanol. The leaves used for extraction were collected both at night as well as during the day and were transferred to 80 per cent ethanol immediately after plucking. The homogenate was centrifuged and the supernatant was concentrated to a volume of 5 ml. by evaporation in a stream of air. 0.05 ml. samples of the concentrated extract were applied on 18″ by 18″ sheets of filter paper (Whatman No. 1) and subjected to paper chromatography by the descending technique. For sugars single dimensional chromatograms were run using the solvent system *n*-butanol—acetic acid—water (4 : 1 : 5 v/v). For amino acids two dimensional chromatograms were run employing the above solvent for the first run and phenol-water (8 : 2 w/w), containing 0.04 per cent 8-hydroxyquinoline, as the second solvent. Sugars on the chromatograms were revealed as brown or yellowish brown spots after spraying with benzidene-trichloracetic-acid reagent (Bacon and Edelman, 1951) and heating at 105°C for about 10 minutes. Amino acids were revealed by spraying with 0.25 per cent solution of ninhydrin in acetone and heating at 60°C for about 10 minutes.

To determine the distribution of enzymes in different parts of the digestive tract of *Oxycarenus*, the insects fed on water for 48–72 hours were starved for 3–4 hours under 0% R.H. They were then made to drink water. An hour later they were dissected one by one and aqueous extracts of their salivary glands, first, second, and third ventriculi, and the hindgut were prepared according to the method described by Saxena (1954). For each enzymatic determination extracts from 10–12 individuals were pooled together, centrifuged and the volume of the supernatant was made up to 1 ml. with distilled water. The clear extract was divided into five equal lots of 0.2 ml. each, arranged in two series A and B. The former contained three lots while series B contained two lots. The extracts of series B were heated in boiling water bath for about an hour and served as controls. To each of the extracts of both the series were added 0.1 ml. of a suitable substrate solution or suspension and 0.2 ml. of a suitable citrate-phosphate buffer. In the absence of any information on the *p*H optima of the digestive enzymes of *Oxycarenus*, the three extracts of series A were buffered to three different values of *p*H 3.0, 5.4, 7.2, selected arbitrarily. The reaction of the two extracts of series B was adjusted to *p*H 3.0 and 5.4 respectively. The extract-substrate-buffer mixtures were incubated at 37°C for about 12–24 hours and the presence or absence of the substrate or products of its hydrolysis was chemically determined as described below.

Names of enzymes investigated and the substrates employed for their detection are given in Table 1. For the detection of amylase, 0.03 ml. samples of all the five incubated mixtures were placed 1″ apart on a strip of filter paper. After drying, the strip was given a dip in 1.0 per cent ethanolic solution of iodine. Incubated mixtures containing undigested starch would show up as blue spots on the paper strip, the intensities of the spots depending on the amount of starch present. In case of complete digestion of starch no blue colour would develop. Visual comparison of the intensities of blue colour gave an approximate idea about the relative activity of amylase in extracts of different regions of the gut under different conditions of hydrogen ion concentration.

For detection of the rest of the carbohydrases, presence or absence of hydrolytic products of the substrates in incubated mixtures was determined by paper partitian chromatography. 0.03 ml. samples of incubated mixtures were placed 1″ apart along one of the longer edges of 22″ by 18″ sheet of filter paper (Whatman No. 1). Suitable reference sugars were also applied on the same paper-sheet. The solvent *n*-butanol-acetic acid-water (4 : 1 : 5 v/v) was allowed to run by descending technique for 48 hours. The chromatogram, after drying, was developed with benzidene-trichloracetic acid reagent as described before. Sugars in the incubated mixtures, which showed up as brown or yellowish brown spots on the chromatogram,

were identified by comparing their positions on the chromatogram with those of the reference sugars. Appearance of sugars other than the substrate in any incubated mixture would indicate hydrolysis of the substrate due to the activity of the corresponding enzyme. Visual comparison of the intensities of spots of undigested substrates would give a rough idea about the degree of enzymatic activity at three different *p*H values under consideration.

For proteinases a different procedure was adopted. As stated before, 1 ml., of the original extract of each region of the digestive tract was divided into five lots of 0.2 ml. each, arranged in two series A and B, the latter serving as control. All of these were buffered as in the previous cases but no substrates were added to the extracts. On the other hand, 0.1 ml. samples of each of the five lots of buffered extracts were placed, side by side, on the gelatinised surface of a 5″ by 1.25″ strip of a photographic plate. The extracts on the strip were incubated in a moist chamber for about 12–14 hours and then the strip was washed in cold distilled water. After drying in air it was immersed in a bath of 0.1 per cent ethanolic solution of bromophenol blue for about 5 minutes. After drying it again, the strip was washed in a bath of 1 per cent acetic acid. The gelatin of the photographic plate, being a protein, would take up a deep blue stain which would persist even after acidifying with acetic acid. Hydrolytic products of proteins such as

TABLE I

List of the enzymes tested for and the substrates employed

Name of Enzyme	Name of substrate employed
CARBOHYDRASES :	
Amylase	Soluble starch, 0.3% aqueous solution containing a little sodium chloride.
Inulase	Inulin, 1% suspension in water.
Cellulase	Cellulose powder, 1% suspension in water.
Hemicellulases	*a*. Pentosans extracted from cottonseed hull according to the method of Jermyn (1955), 1% suspension in water.
	b. Gum arabic, 1% aqueous solution
	c. Agar agar, 1% aqueous solution.
Maltase	Maltose, 5% solution.
β-glucosidases	*a*. Cellobiose, 5% solution.
	b. Salicin, 5% solution.
α-galactosidases	*a*. Raffinose, 5% solution.
	b. Melibiose, 5% solution.
β-galactosidase	Lactose, 5% solution.
Invertases :	
B-h-fructofuranosidase	*a*. Sucrose, 5% solution.
	b. Raffinose, 5% solution.
Glucosaccharase	*a*. Sucrose, 5% solution
	b. Melezitose, 5% solution
Trehalase	Trehalose, 5% solution
PROTEINASES :	Gelatin, on photographic plate.
POLYPEPTIDASES :	Peptone, 1% solution.
ESTERASES :	
Lipase	Olive oil emulsion, prepared as described by Baldwin and Bell (1955)
Esterase	Ethyl butyrate.

polypeptides and amino acids do not give this test since their blue stain changes to yellow on acidifying (vide Fiegl, 1954, for explanation). Presence of proteolytic

enzymes in any of the incubated mixtures would bring about digestion of gelatin at the spot where the incubated mixture was placed on the photographic plate. As a result of this the blue colour at that particular spot would fade out. The degree of fading of blue colour would depend on the enzymatic activity and in the case of complete digestion of gelatin the spot might become transparent. Visual comparison of the intensities of blue colour developed by different incubated mixtures would give a rough idea about the degree of proteolytic activity in different parts of the gut and under different conditions of hydrogen ion concentration.

For the detection of lipase the method described by Baldwin and Bell (1955) was employed. Esterases acting on lower esters were also tested in the same way, with emulsion of ethyl butyrate as the substrate.

Tissue Preferences

As mentioned before, *Oxycarenus hyalinipennis* has been reported to feed mainly on cottonseeds by some workers, particularly by Kirkpatrick (1923). On the other hand, Saxena and Krishna (1958) observed that this insect prefers to feed on green parts of the cotton plant, particularly on leaves. Since each of these parts is made up of a number of different tissues which differ in their structure and chemical composition, the nutrients available to *Oxycarenus* in any one part of the plant will depend upon the tissue from which the sap is drawn. It was, therefore, considered necessary to determine the exact tissue of cottonseed or of leaf from which the insect draws its food. Leaf was taken to represent the green parts of the cotton plant since it is shown maximum preference by the pest.

Preference for Leaf-Tissues. Both the adults as well as the nymphs feed mostly on the lamina of cotton leaf, and occasionally they also feed on leaf-petiole. The insects feeding on the lamina may attack it from any of the two surfaces, preferably the one in shade. The point at which the stylets are introduced into the leaf-tissue is selected quite carefully. The insect always avoids the so-called 'oil-glands' which are profusely distributed all over the cotton plant (Brown, 1938). The stylets are pierced through the surface of the leaf in-between the oil-glands. In most cases the point of entry lies in the angles of veins or veinlets; but occasionally it may lie in the area in-between the veins or directly on the vein or veinlet. As the stylets penetrate into the tissues intracellularly the cells are torn off.

The tissue up to which the stylets extend and from which the sap is drawn out may vary with different individuals and with the same individual. Stylets of insects feeding on the lamina extend mostly into the mesophyll tissue ; both the palisade layer of cells as well as the spongy parenchymatous tissues are equally preferred. In the case of insects feeding directly on the vein or veinlet the stylets usually extend up to the phloem tissue also. Both the phloem parenchyma and sieve tubes may be tapped. The insects which feed on leaf-petiole introduce their stylets mostly into the parenchymatous tissue but often they are found to tap the phloem tissue as well. Rarely the stylets have been noticed to extend up to xylem vessels. There is no indication that the introduction of the stylets into one or the other tissue is limited by the length of the stylets or of the labium. On the same leaf some individuals may extend the stylets up to the phloem tissue and others may stop short at the parenchymatous tissue.

These observations make it clear that so far as leaf as the source of food is concerned, *Oxycarenus* draws food sap from almost all parts of the cotton leaf, except from the oil-glands. However, the insect shows a little greater preference to mesophyll tissue and phloem parenchyma than to phloem vessels.

Preference for Seed-Tissues. The insects have been observed feeding on cottonseed much less readily than on leaf. Presence of a fine film of moisture on the surface of the seed is fairly helpful in making the insects feed on the seed.

Still, hardly 8–10 insects out of a batch of 50 feed on the cottonseed in about 6–8 hours. Most of them probe their beaks here and there on the surface of the seed and then move away.

Insects feeding on cottonseed do not seem to show any choice for any particular spot on the surface of the seed for introducing their stylets. Sections of the seeds *in situ* with the stylets of the feeding insects show that, in many cases, the stylets extend into superficial layers of the seedcoat i.e. into the outer integument, and they easily get detached from the seed as soon as the beak of the feeding insect is clipped off. In a number of other cases stylets extend deeper into the seedcoat, reaching the parenchymatous layer of the inner integument. These stylets remain intact with the seed after they are clipped off. Sometimes, however, the stylets may be introduced into or in-between the bases of the fuzzy hairs present on the surface of the delinted seed. *Oxycarenus* has never been found introducing its stylets into the seed beyond the seedcoat into the kernel or embryo.

Chief Nutrients available in Host Tissues

The nutrients required for the growth and maintenance of insects, like those for other animals, can be grouped under the following categories: Proteins and amino acids, carbohydrates, fats, minerals, and accessory growth factors like vitamins, sterols etc. Requirements for these substances vary with different species of insects. Minerals and accessory growth factors are mostly in such a form that they can be readily absorbed and utilised by the insects. The nutrients of the first three categories, however, may or may not be in a diffusible form. In case they are nondiffusible they must be brought into diffusible form by the action of digestive enzymes before they can be absorbed and utilised. In case an insect does not possess suitable enzymes it cannot utilise corresponding substrates. Since main object of the present study is to examine the correlation between the nutrients available to *Oxycarenus* in its preferred host-tissues and its ability to utilise those nutrients, it is necessary to have information on various nutrients occurring in the preferred host-tissues. Only the nutrients belonging to the first three of the above mentioned categories will be considered because they may or may not need enzymatic action prior to absorption. Minerals and vitamins etc. can be readily absorbed and are, therefore, not being taken into consideration here.

Chemical composition of the different parts of the cotton plant has been determined by a number of workers and their results have been included in reviews by Brown (1938), Leahy (1948), Tharpe (1948), Boatner (1948), Dollear and Markley (1948) and Dunning (1948). Table II, partially taken from Brown (1938), summarises the data on chemical composition of different parts of the mature cotton plant. It is apparent from the table that relative concentration of various nutrients in different parts of the plant is in the following order:

Carbohydrates: leaf > stem, root > seed boll > lint.

Proteins: seed>leaf>boll>stem, root>lint.

Fats: seed>boll>leaf>root>stem>lint.

Such data, however, do not give any information whether the proteins reported upon are all in the form of *native* proteins or free amino acids or both. Similarly it does not give any idea as to what poly-, oligo-, or monosaccharides are included in the total carbohydrates determined. In order to gain all this information free amino acids and sugars in the cottonseed hull and leaf were determined and the results will be presented in due course.

It may be further noted that *Oxycarenus* prefers to feed on one or the other tissue of cottonseed or of leaf. Therefore, chemical composition of entire seed or leaf, given in Table II, will not give much information on the nutrients available to the insect. For this purpose a knowledge of the constituents of different tissues of cottonseed and leaf is important.

So far as the leaf is concerned, it may be recalled that insects draw food-sap from almost all the tissues of the leaf except from the oil-glands. Therefore, constituents like gossypol, ethereal oils, tannins, resins, pigments etc., present in these glands (Brown, 1938), will not be available to the insects. Xylem vessels, on which the insects rarely feed, are known to contain mostly water and inorganic solutes. Most of the proteins, amino acids, starch, sugars and fats, present in the leaf, are distributed amongst the rest of the tissues, particularly phloem and mesophyll tissues, on which the insects mainly feed.

TABLE II

Chemical Composition of Mature Cotton Plant (Brown, 1938)

Part of the Plant	Ash	Protein	Fat	Carbohydrates
Roots	3.72	3.00	2.78	49.88
Stem	3.09	4.00	1.11	46.49
Leaves	12.55	14.06	8.49	56.19
Seed	3.65	22.13	23.05	39.26
Lint	1.25	1.12	0.61	10.00
Bolls	4.74	11.44	9.81	29.07

As regards the identity of sugars present in the leaf, Mason and Maskell (1928) reported that concentration of sucrose and reducing sugars of the leaf-sap undergoes diurnal variation—their quantities increasing during the day and decreasing during the night. Since *Oxycarenus* feeds on cotton leaf during the day as well as during night fluctuations in sugar content of the leaf will not affect their ingestion by the insect. However, Mason and Maskell (1928) did not examine whether sugars of cotton leaf included oilgosaccharides other than sucrose; also, they did not establish the identity of reducing sugars present in the leaf. Results of the present study indicate that cotton leaf contains only three different sugars, namely, sucrose> glucose>fructose. Besides sugars, the following free amino acids are also present in cotton leaf : aspartic acid, histidine, serine, threonine and tyrosine.

Studies on the chemical composition of different parts of cottonseed by various workers have revealed that the chemical constituents undergo a great change in their distribution and concentration in different tissues of the seed during its development. Most of these changes are complete by the time the cotton boll opens. After its opening there may be a slight change in the concentration of certain constituents, particularly in the kernel, but no change in the distribution of various constituents amongst different tissues (Tharpe, 1948). Since, in the normal course, the cottonseed becomes available to *Oxycarenus* only when it is ripe and the boll has opened, a knowledge of the nutrients available to the insect can be gained only from the chemical composition of mature cottonseed.

Although kernel of the cottonseed is vary rich in proteins, oils and sugars like raffinose, the seedcoat or hull, from which the insect draws the food-sap, is quite poor in these constituents. Information on the chemical constituents of the hull has been incorporated in reviews by Dunning (1948), Leahy (1948), Tharpe (1948), Dollear and Markley (1948). Dunning (1948) has compiled data on the composition of cottonseed hull from several sources, which is given in Table III.

TABLE III

Chemical Composition of Cottonseed Hull (*Dunning*, 1948)

Constituent	Average % weight. Oven-dry basis
α-Cellulose	43.9
Cross and Bevan Cellulose	46.9
Pentosans	29.5
Lignin	21.95
Ash	1.79
Protein	3.30
Crude fibre	49.19

Pentosans and sugars have been reported to occur in the parenchymatous cells of the hull (Leahy, 1948). Galactose and traces of sucrose are the only sugars reported to occur in the seedcoat (Dollear and Markley, (1948). The fat content of the hull, like that of proteins (3.3 per cent), is very low (0.9 per cent) (Guthrie *et al.*, 1944). In addition to these, the hull also contains certain free amino acids and sugars presence of which was qualitatively determined in the present work. The free amino acids present are alanine, asparagine, aspartic acid, glycine, methionine, serine, tryptophane and two unidentified amino acids. The sugars present in the seed hull are : raffinose>glucose>sucrose>fructose. No galactose was found in the hull.

DIGESTIVE ENZYMES

Digestive organs of *Oxycarenus hyalinipennis*, like those of *Dysdercus koenigii* (Saxena, 1955) and some other Heteropterous insects, include a pair each of principal and accessory salivary glands, foregut, midgut and hindgut. The midgut is the longest division of the alimentary canal and is differentiated into three distinct regions : a wide, sac-like, *first ventriculus*, a narrow, tubular, *second ventriculus*, and a short, bulbous, *third ventriculus*. The hindgut is very short and not further divisible into intestine and rectum. Gastric caeca are not present in this insect.

Distribution of various enzymes in different parts of the digestive tract is given in Table IV. Of the enzymes tested for, cellulase, hemicellulases, inulase, β-glucosidase, β-galactosidase, polypeptidase and lipase were found to be completely absent. The remaining enzymes were detected in one or the other region of the digestive tract and are described below.

Amylase. Strong amylolytic activity was always detected in the salivary glands, all the regions of the midgut, and the hindgut. Although maximum activity occurred at *p*H 7.2, appreciable activity was also evident at *p*H 5.4. At *p*H 3.0, however, no amylolytic activity was detected at all.

Maltase. This enzyme was detected in the salivary glands and in all the three regions of the midgut but not in the hindgut. Its activity could be detected only at *p*H 5.4. Even at this *p*H value the enzymatic activity was quite weak since only a little quantity of glucose was liberated after an incubation of 36 hours ; most of the substrate remained undigested.

α-galactosidase. Both raffinose and melibiose were separately used as substrates to detect the presence of this enzyme. Paper chromatograms revealed the presence of galactose in the incubated mixtures containing extracts of salivary glands and of the three regions of the midgut. This clearly indicates the presence

of an α-galactosidase in each of the region mentioned above. Activity of the enzyme was detected at *p*H 5.4 but not at *p*H 3.0 or 7.2. It may be noted that in the incubated mixtures containing raffinose as the substrate no fructose or glucose was liberated as a result of hydrolysis of the trisaccharide. Instead, sucrose was detected in addition to galactose.

Invertase. In order to detect and characterise the enzyme invertase in the digestive tract of *Oxycarenus*, sucrose, melezitose and raffinose were separately used as substrates. With sucrose as the substrate, hydrolysis of the sugar, yielding glucose and fructose, was obtained with extracts of salivary glands and first and second ventriculi. This indicated presence of invertase in these regions. In the third ventriculus presence of this enzyme was detected only occasionally. In the hindgut also its occurrence was found to be quite irregular. In all experiments, maximum activity of the enzyme occurred at *p*H 5.4 ; very little activity occurred at *p*H 7.2 and none at *p*H 3.0. In comparison to other carbohydrases detected in the digestive tract of *Oxycarenus* the concentration of invertase was noticed to be much greater.

TABLE IV

Distribution of enzymes in salivary glands and alimentary canal of Oxycarenus hyalinipennis (*Costa*)

Enzymes	Salivary glands	1st Ventriculus	2nd Ventriculus	3rd Ventriculus	Hindgut
Amylase	+++	+++	+++	+++	+
Maltase	+	++	++	++	—
α-galactosidase :					
Acting on raffinose	+	+++	++	++	—
Acting on melibiose	++	+++	+	—	—
Invertase :					
Acting on Sucrose	++	+++	++	—	±
Acting on melezitose	+	+++	+	+	—
Trehalase	—	+++	++	—	—
Proteinase	++	+	+	—	—
Esterase	++	++	++	++	+

Note.—(*i*) *Plus* sign indicates the presence and *minus* the absence of the enzyme; ± indicates presence of traces of the enzyme.

(*ii*) The number of plus signs gives an approximate estimate of the concentration of the enzyme in different parts of the gut.

(*iii*) The enzymes cellulase, hemicellulase, inulase, β-galactosidase, β-glucosidase, polypeptidase, and lipase were totally absent.

When the gut extracts were incubated with melezitose, instead of sucrose, hydrolysis of the substrate occurred in the extracts of the same regions which showed hydrolysis of sucrose. The hydrolysis of melezitose also occurred at *p*H 5.4 only, yielding glucose and turanose. This again must be due to the activity of invertase. With raffinose as the substrate only galactose and sucrose were found to be the hydrolytic products, as reported above. No fructose was liberated, which shows that the enzyme concerned with the digestion of raffinose is an α-galactosidase only.

Trehalase. Activity of this enzyme was detected only in the first and second ventriculi at *p*H 5.4. At *p*H 7.2 slight activity of the enzyme was noticeable in the first ventriculus but not in the second. No activity was detected at *p*H 3.0.

Proteinase. Fairly strong proteolytic activity was noted in the extracts of salivary glands, first and second ventriculi but none in the third ventriculus and hindgut. In the extract of salivary glands the activity of the enzyme was greater at *p*H 7.2 than at *p*H 5.4. Even then fairly good activity was noticeable at *p*H 5.4. In the extracts of the first two ventriculi no difference in the activity of the enzyme was perceptible between *p*H 5.4 and 7.2 However, at *p*H 3.0 only slight proteolytic activity was detected.

Esterase. Enzyme capable of splitting up lower esters of fatty acids was detected in all the regions of the digestive tract.

Discussion

As mentioned before, there has been some diversity of opinion regarding the feeding behaviour of *Oxycarenus* on the cotton plant. According to some workers (Sickenberger, 1890 ; Willcocks, 1906 etc.) the insect feeds on seeds as well as on buds, flowers and young bolls of cotton. Peacock (1913) reports that *Oxycarenus*, when infesting cotton plant, feeds almost exclusively on seeds ; but, on some other host plants, such as *Hibiscus* species, it feeds on almost all parts of the plant. Kirkpatrick (1923) has given an exhaustive discussion on the site of feeding of *Oxycarenus*. According to him all the stages of this insect "feed solely on the cottonseed", piercing "the testa of ripe seeds with their setae and extracting the juices of embryo". He also reports that adults and nymphs "may be seen sucking at the gland on the under surface of the midrib of the leaf, near the base ; but in the writer's (Kirkpatrick's) opinion this is merely a means of obtaining moisture in the absence of dew". He also reports that the bugs may draw moisture from the glands of epicalyces as well. He does not clarify whether the glands he refers to are the 'oil-glands' or the nectaries present on the undersurface of large veins of the leaf.

It may be remarked that Kirkpatrick (1923) makes no mention of the method he adopted to determine the relative preference of *Oxycarenus* for different parts of cotton plant and for the determination of tissues of the seed or leaf from which, he claims, the insect draws its food-sap. Furthermore, he does not give any data nor any experimental evidence in support of his conclusions regarding the site of feeding of the pest. The only evidence he cites in support of his views is the loss in weight of cottonseeds from plants infested with *Oxycarenus*. This sort of evidence is very much indirect and cannot be relied upon since a number of other factors may be responsible for the loss in weight of cottonseeds in the fields.

Experimental investigations of Saxena and Krishna (1958) have indicated that *Oxycarenus* prefers to feed on green parts, particularly the leaf, of cotton plant much more than on mature seed. Results of the present study go a step farther to contradict the conclusion of Kirkpatrick (1923). Sections of cotton leaf *in situ* with the stylets of insects feeding on it clearly show that the stylets penetrate deeper than the epidermis, extending mostly up to mesophyll or phloem tissues and rarely to xylem vessels. They have never been observed to extend into the nectaries or into the oil-glands scattered over the leaf. This observation is in conflict with the view of Kirkpatrick (1923) that the insect goes to leaf only for getting moisture from the glands. Similarly, sections of cottonseeds *in situ* with the stylets of insects feeding on it show that the insect never penetrates its stylets into the seed deeper than the seedcoat or the hull and, therefore, its stlyets can never reach kernel or embryo of the seed as contended by Kirkpatrick (1923). It appears that very hard texture of the seedcoat also plays an important rôle

in inhibiting its complete penetration by the stylets which can extend up to the parenchymatous tissues of the inner integument of the seedcoat.

The facts presented in this paper make it possible to consider the correlation between the nutrients available in cottonseed or leaf and the ability of *Oxycarenus* to utilise those nutrients. It has been observed that the insects feeding on cotton leaf draw food-sap from both mesophyll and phloem tissue at one time or the other. They rarely suck juice from xylem vessels and never from the oil-glands. In view of this, substances like gossypol, ethereal oils, resins, tannins, flavones, etc. (Brown, 1938), which occur in oil-glands and which are specific to cotton and certain other Malvaceous plants, will not be available to the insects. According to our present knowledge of plant physiology xylem vessels contain mainly water and minerals which, therefore, will be available to the insects only occasionally. Most of the proteins, free amino acids, starch, sugars and fats of the leaf are distributed amongst the mesophyll and phloem tissues and will, therefore, be mostly available to the insects feeding on cotton leaf.

As to the nutrients available to insects feeding on cottonseed, it may be noted that the seed is rich in proteins, fats and sugars (Table II) but these are mostly concentrated in the kernel. The seedcoat, from which the insects draw food-sap, is very poor so far as proteins (3.3 per cent; Dunning, 1948) and fats (0.9 per cent; Guthrie *et al.*, 1944) are concerned. The carbohydrates, in addition to cellulose, present in the seedcoat are: pentosans (29.5 per cent; Dunning, 1948), raffinose, sucrose, glucose and fructose (vide p. 252). Small amounts of free amino acids also occur in the seedcoat (p. 252).

Of the constituents of cotton leaf and seedcoat, reported above, free amino acids and sugars like glucose and fructose are in a diffusible form and can be readily utilised by the insect. Substances like proteins, starch, pentosans, sucrose, raffinose and fats are non-diffusible and have to be digested before they can be absorbed. Digestive enzymes present in the digestive tract of *Oxycarenus* are: proteinase, amylase, maltase, invertase, α-galactosidase and an esterase. Their presence bestows upon the insect ability to digest proteins starch and sucrose available in the leaf and also, to digest raffinose, sucrose and slight amount of proteins available in the seedcoat. Fats present in the leaf and seedcoat cannot be utilised by *Oxycarenus* due to the absence of lipase. Similarly, pentosans present in the seedcoat cannot be utilised owing to the lack of any hemicellulase. Absence of cellulase in the insect rules out the possibility of any digestion of cellulose.

It may be noted that cotton leaf contains a much greater proportion of proteins, carbohydrates and fats (Table II) than the seedcoat (Table III). Free amino acids and sugars are the only constituents of the seedcoat which may be utilised by *Oxycarenus* for its nourishment. However, it is noteworthy that the insects feeding on seedcoat have access to raffinose which is not available in the leaf. It is difficult to say whether ingestion of raffinose is in any way important in the nutrition of *Oxycarenus*.

These facts appear to show that, so far as proteins, carbohydrates, and fats are concerned, leaf is much better a source of food for *Oxycarenus* than cottonseed hull.

On the basis of some of the results presented in this paper, nature of invertase present in the gut of *Oxycarenus* may be considered. Two types of invertases are known to occur in different organisms: β-*h*-fructofuranosidase and glucosaccharase (Kühn, 1923 *a*, *b*). Both of these enzymes can act on sucrose yielding glucose and fructose as the initial products; but the mechanisms of their action is different. The first enzyme acts on sucrose by attacking fructose moiety of the molecule. The same enzyme is capable of acting on raffinose (6-α-*d*-galactopyranosyl-α-*d*-glucopyranosyl-β-*d*-fructofuranoside), liberating fructose and melibiose, since in this trisaccharide fructose part of the molecule is again free and accessible to the enzyme. However, the enzyme cannot act on melezitose

(3-α-*d*-glucopyranosyl-β-*d*-fructopyranosyl-α-*d*-glucopyranoside) in which the fructose moiety of the molecule is blocked by glucose.

The second type of enzyme i.e. glucosaccharase, also termed α-*n*-glucosidoinvertase (Neuberg and Mandl, 1950) is an α-glucosidase (Gottschalk, 1950) which attacks sucrose from the free glucose end of the molecule. It can also act on melezitose where the glucose part of the molecule is free. On the other hand, raffinose, where glucose moiety is blocked by galactose, cannot be acted upon by glucosaccharase. The fact that invertase present in *Oxycarenus* acts upon sucrose and melezitose and not on raffinose indicates that the enzyme concerned is glucosaccharase and not β-*h*-fructofuranosidase.

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