THE IDENTIFICATION OF DIGESTIVE ENZYMES IN SEVERAL SPECIES OF DESTRUCTIVE GRASSHOPPERS; A QUALITATIVE ANALYSIS OF DIFFERENT ENZYMES PRODUCED IN VARIOUS STAGES OF DEVELOPMENT

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INTRODUCTION

The study of the feeding of insects is of immense practical importance. Damage resulting from the feeding activities of phytophagous insects directly affects agriculture, our basic industry. The economic security of prairie regions adapted to the growing of cereals is thus partly dependent upon the balance between insect populations and adequate control methods.

Grasshopper populations often have reached concentrations sufficient to cause widespread economic losses. Present control methods include cultural practices, the growth of resistant varieties, and the application of insecticides, but all methods have been applied without thorough knowledge of the physiological processes involved.

It is recognized that cultural control methods require a knowledge of the life history of the grasshopper. The development of resistant varieties of grain is dependent upon the physiology of the plant in relation to the physiology of the grasshopper. There are indications that the toxicity of insecticides also involves physiological processes.

One of the main physiological processes is the utilization of food. Nutrients must be made available to the grasshopper tissue for the maintainence of growth, reproduction and energy. The ability to make nutrients available lies in the digestive system. Nutritive organic

materials are hydrolyzed with the aid of active agents in the alimentary canal. These hydrolytic substances make up the enzyme complement upon which digestion depends.

As an outgrowth of a nutritional investigation the study of digestive enzymes in Melanoplus mexicanus mexicanus (Sauss.), Melanoplus bivittatus (Say), and Camnula pellucida Scudd. was undertaken.

LITERATURE REVIEW

Enzymes are organic catalysts produced as a result of cellular activity but which function independently of these cells (Harrow 1946[11]).

There are two main classes:

(1) Hydrolases which breakdown or build up protoplasm through the addition or removal of water, and

(2) Desmolases which break the C-C bonds and produce energy.

The hydrolases are protein in nature and show the properties of colloidal systems (Harrow 1946[11]). The digestive enzymes of an animal are hydrolases which catalyze the breakdown of food so that it can be assimilated by the tissues.

In the majority of insects so far investigated, the digestive enzymes are strongly similar to, if not identical with, the corresponding ones of vertebrates (Hoskins 1940[16]). Certain minor differences in pH optima have been noted as with proteinases of fleshfly larvae (Hobson 1931[14]) and of tse-tse flies (Wigglesworth 1929[38]). Insect enzymes capable of digesting the same substrates as vertebrate enzymes, have thus been given the same names.

Although similar in properties and reactions to vertebrate activating agents, insect enzymes have not yet been isolated.

An exhaustive summary of the literature dealing with the occurrence of hydrolytic enzymes in insects is given by Uvarov (1928[36]). It indicates that insects as a class possess an extremely wide range of digestive enzymes.

Omnivorous insects (Blattidae, Gryllidae) have a varied enzyme complement (Lafon 1951[17]). Schlottke (1937[28] b) showed that in Blattidae there is a powerful amylase produced by the labial glands, a maltase, a proteinase active in an alkaline medium, a dipeptidase and a lipase.

Predaceous insects produce a strong proteinase as in the tsetse fly (Wigglesworth 1929[38]). In Dytiscus marginalis, tryptase is abundant (Duspiva 1939[17])[v]. In contrast to their widespread occurrence in vertebrates, proteolytic enzymes in an acid medium have not been detected in insects (Wigglesworth 1947[39]).

Phytophagous insects show a tendency towards more frequent specialization of food habits. Strict monophagy is not rare, especially among caterpillars, but the digestive abilities vary little from one species to another in spite of the diversity of food. The potato beetle, which feeds on Solanaceous plants, produces a maltase, saccharase, esterase and a proteinase (Busnel 1939[17])[v]. The same enzymes are found

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[v] Lafon, M., Quelque documents sur l'appetit et la consommation alimentaire chez les insectes. Annales de la nutrition et de l'alimentation. V: 492-495, 1951.

plus an amylase in the silkworm which feeds exclusively on mulberry leaves (Shinoda 1931[29]), (Yamafugi 1934[17])[v].

Phytophagous insects do not usually possess a cellulase. The cellulose of leaves is excreted as such, the nourishment being drawn from the cellular contents of the parenchyma (Lafon 1951[17]).

Cellulose is digested by certain highly specialized wood-eating insects (Yonge 1937[41]). Larvae of Cerambycidae and Anobiidae have a cellulase capable of hydrolyzing filter-paper in vitro (Mansour and Mansour-Bek 1934[19]).

Other xyllophagous insects harbor micro-organisms which enable them to utilize cellulose. The alimentary canal of the termite contains a ciliate, Trichomonas termopsis, which secretes a cellulase capable of hydrolyzing cellulose to glucose. A temperature of 36°C kills this micro-organism without affecting the insect. The termite is then incapable of utilizing its habitual food (Trager 1932[17])[v].

Other so-called wood-eating insects live only on the sapwood and feed on the simpler carbohydrates in this region (Parkin 1936[23]). Some live on the impurities present in the wood (Lafon 1951[17]).

Insects that live on nectar (some adult Lepidoptera) produce invertase in abundance (Lafon 1951[17]). Fraenkel (1940)[9] found the larvae of Lucilia sericata (Meig.) to be especially adapted to the digestion of sugars. They possessed an alpha glucosidase and an alpha galactosidase.

Lipase appears to be widely distributed in insects without any apparent regard to the feeding habits. Uvarov (1928[38]) feels that some

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[v]Lafon, M., Quelque documents sur l'appetit et la consommation alimentaire chez les insectes. Annales de la nutrition et de l'Alimentation. V: 492-495, 1951.

of the records probably referred to lipase charged with a metabolic rather than a digestive function. Busnel (1939[39])[vv] claims that lipase is specific to insects of a carnivorous diet and that one need not incorporate fat into a synthetic medium offered to phytophagous insects.

Other enzymes reported in Uvarov's summary (1928[36]) are: wax-digesting enzyme, chlorophyllase, xyllanase, inulase, glycogenase, dextrinase, raffinase, melezitase, trehalase, glucosidase, formizyme, an enzyme acting upon chitin, coagulating enzymes, haemolysins, rennet, fibroin protease, asparaginase and an enzyme acting on keratin.

It is seen that insects having unusual diets usually possess special enzymes adapted to the decomposition of these foodstuffs (Hoskins 1940[16]). Wigglesworth 1947[39]) states: "Insects which live on a food rich in some particular substance generally produce the appropriate enzyme in greater abundance. The enzymes are correspondingly limited in insects which live on a highly restricted diet."

Quantitative differences in enzyme activity are sometimes very evident in the developmental stages of an insect (Wigglesworth 1947[39]). Qualitative differences are also found in various stages, e.g. inulase present in Lepidoptera larvae is absent from the pupae but appears in the adults (Straus 1909[36])[v].

[vv]Wigglesworth, V.B., The principles of insect physiology. Methuen & Co., Ltd., London, 274-276, 1947.

[v]Uvarov, B.P., Insect nutrition and metabolism Trans. Ent. Soc. London, II, 1928.

Marked differences between species in their abilities to produce certain enzymes was observed by Schlottke(1937[28] b). Differences were apparent be tween certain species of Blattella and Periplaneta in their abilities to produce amylase. These observations were confirmed by Day and Powning (1950)[5]. Invertase was found in the salivary glands of Blattella germanica (L.) but not in Periplaneta americana (L.) or Periplaneta orientalis (Timon-David 1941[35]).

Most of the active enzymes appear to be secreted by the midgut and its caeca (Fink 1932[7], Simmons 1939[30]) although amylase and invertase are often secreted by the labial glands (Wigglesworth 1947[39]). Fink(1932[7]) found anylase, lactase, invertase, lipase and proteolytic enzymes to be secreted only in the midgut and regurgitated liquid. These observations were made on normal Leptinotarsa decemlineata (Say).

Abbott(1926[1]) established that in Periplaneta australasiae (Fab.) lipase secretion takes place only in the midgut and that the presence of lipase in the crop is due to regurgitation.

Because enzyme activity is limited to a definite range of H-ion concentration, it is helpful to know the range of pH in the gut contents during the process of digestion. Skrjabina(1936[37] b)[v] reported a cyclical variation in pH within the midgut of Euxoa segetum larvae, Pieris brassicae larvae and the nymphs of Locusta migratoria. The acidity of the midgut contents increases as soon as acid food reaches the midgut, but later

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[v]Uvarov, B.P. Recent advances in Acridology. Trans. Roy. Ent. Soc., London 99. 1: 12-14, 1947.

the alkalinity increases, probably owing to the secretion of the digestive juice (Uvarov 1928[36]).

Hastings and Pepper (1943[12]) found the pH of regurgitated digestive juices of six species of grasshoppers to range from 5.2 to 5.8. Melanoplus bivittatus (Say) was reported to exhibit the following range of pH in the gut: Crop 5.60; caecum 5.98; midgut 6.40; hindgut 6.58 (Swingle 1931[34]). The pH of the gut of Melanoplus species in general was summarized: Crop 5.5-5.9; caecum 6.0; midgut 6.5; and regurgitated liquid 5.2-5.8 (Prosser et al 1940[25]).

The pH of the digestive fluid of the larvae of Leptinotarsa decemlineata (Say) was compared with that of the adults (Busnel 1939[37])[v]. The only variation appeared in the posterior intestine which was slightly more acidic in the adult than in the larva.

A satisfactory correlation was not established between the acidity of the gut and the type of food ingested by the insect (Swingle 1930[34]). The mixture within the digestive tract of Popillia japonica Newm. had a fairly constant pH regardless of the pH of the soil in which the larvae were feeding.

A knowledge of digestive processes is necessary for the intelligent formulation of certain types of control measures. It was thought that the toxicity of some insecticides involved the action of digestive juices on the poisons taken into the digestive tract. Fink (1932[7]) attempted to relate the toxic effect of arsenic to its action on digestive processes,

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[v]Uvarov, B.P., Recent advances in Acridology. Trans. Roy. Ent. Soc., London 99. 1: 12-14, 1947.

especially enzyme complexes. He found that poisoned foliage eaten by the potato beetle caused little retardation of the activity of amylolytic and tryptic enzymes and none at all of enzymes active in the digestion of di-saccharides and fats. However, the injection of arsenical suspensions directly into the insect mouth resulted in complete inhibition of the activity of proteolytic enzymes.

Further work on the effect of poisons on digestive processes was done by Day and Powning (1950[5]). Changes produced in the midgut by substances other than arsenicals are not sufficient to account for the death of the insect. There was evidence of cell breakdown in the midgut when sodium arsenite was used.

In a study of the enzymes occurring in the crop of the grasshopper Stenobothrus sp. and Tettigonia cantans. Schlottke (1937[27] a) found the action of proteinase to be completely inhibited by hydrogen cyanide and to be strongly depressed by hydrogen sulfide (Hoskins 1940[16]). Lipase from the midgut was also reduced in activity.

Two techniques for studying digestive enzymes are quoted by Uvarov (1928[36]). In one method, the enzymes are extracted from the tissues, their activity being measured on various substrates.

A more exact method involves the feeding of chemically pure substances. The length of life is compared between insects fed on a pure diet and those fed water only. This method cannot readily be extended to all insects.

Franklin and Quastel (1949[10]) report preliminary results of an investigation of the paper chromatography of proteins and enzymes. By this method it is possible to bring about the separation of the components of enzyme mixtures.

The detection of enzymes by the chromatographic brush method is described by Zechmeister and Rohdewald (1950[42]). The invisible zones of some enzymes absorbed on alumina can be located by brushing the chromatographic column along its main axis, first with a suitable substrate, and later after a brief incubation period with a color reagent. The color of the streak where it crosses the enzyme zone will then be different from that observed in the enzyme free section of the column.

MATERIALS AND METHODS

LIVING MATERIAL

Nymphs and adults of Cammula pellucida Scudd., Melanoplus mexicanus mexicanus (Sauss.) and Melanoplus bivittatus (Say) were used for identification tests of digestive enzymes. All the grasshoppers were hatched in a rearing room under controlled conditions. A constant temperature of 30°C ± ½ and an RH of 40-50% was maintained.

Nymphs were placed within a few hours after hatching in 2-quart glass jars with screen tops. They remained in the rearing room under these controlled conditions throughout their development.

The food consisted exclusively of Renown wheat seedlings supplied fresh every day. The wheat was grown in the greenhouse by subirrigation methods on a complete nutrient solution. Individuals from each instar of each species were allowed to feed several days after moulting before dissection.

The entire digestive tracts of nymphs of the first two instars were used. The contents were removed from each individual by sharp forceps. The alimentary canals of third, fourth and fifth instar nymphs and adults were divided into fore, mid and hindgut regions and caeca. A suspension was made of the regurgitated fluid.

The entire alimentary canals of freshly hatched first instar grasshoppers which had not been allowed to feed, were similarly prepared for analysis.

The labial glands were dissected from adult M. mexicanus, M. bivittatus, and C. pellucida for enzyme determinations.

METHODS USED FOR ENZYME DETERMINATIONS

The digestive tracts of freshly killed grasshoppers were freed from adhering tissues and scraped clear of food particles. These tissues were accumulated in small vials in a few drops of 0.2% NaCl. Toluene was added as a preservative. This material was stored at -25°C until needed.

The frozen tissue was allowed to thaw at room temperature and then was homogenized with a glass rod. Half the material was allowed to extract at room temperature in distilled water for carbohydrase tests. The other half was extracted in 50% glycerol for proteinase and lipase tests. This division is necessary because glycerol in certain concentrations is known to inhibit the activity of certain carbohydrate enzymes (Hinman 1933[13]). Toluene was used throughout to prevent bacterial action.

The tissues were allowed to extract for an hour, then half of each extract was boiled as a control. Substrates were placed in small test tubes (10x 75mm), a few drops of extract added, and a layer of toluene. The test tubes were loosely corked and suspended in a water bath at 37°C for the incubation period.

Preliminary tests were run before the addition of substrate. The stomach contents of some insects may give a positive reaction prior to the addition of any substrate.

Proteolytic Enzymes

The determination of proteolytic activity included tests for pepsin, trypsin and dipeptidase.

Pepsin

Flakes of carmine-stained fibrin, well washed in distilled water, were placed in 0.01 N. HCl with several drops of tissue suspension. The

mixture was adjusted to a pH of 2.2 approximately with either dilute Na_2CO_3 or dilute HCl. Release of the stain upon incubation indicated peptic activity (Cole 1926[4]).

Trypsin

Flakes of fibrin stained with congo red were placed in a few drops of 0.5% Na_2CO_3 with the tissue suspension. The mixture was incubated for about an hour. Release of the stain indicated tryptic digestion. The fibrin shows signs of dissolution when the concentration of trypsin is strong (Cole 1926[4]).

The clotting of calcified milk was used as further proof of tryptic activity. A few drops of 5.5% $CaCl_2$ were added to fresh milk which was incubated with the tissue suspension. The milk had been previously boiled and cooled. Clotting occurs usually within a few hours to indicate tryptic activity (Cole 1926[4]).

Dipeptidase

A solution of 4% peptone was the substrate for dipeptidase tests. A few drops of brom-thymol blue indicator were added with the tissue suspension. Dilute Na_2CO_3 was added until the solution turned blue. Incubation of this mixture required at least 3 days and it is important to add sufficient preservative. The presence of dipeptidase, releasing amino acids from the peptone is indicated by a color change from blue to green or yellow (Cole 1926[4]).

Carbohydrate Enzymes

The carbohydrases tested for included amylase, maltase, lactase, invertase, and cellulase.

Amylase

A solution of 2% soluble starch formed the substrate for amylase tests. A drop of 5% NaCl was added with the tissue suspension to several cc's of starch solution. This mixture was incubated for at least 24 hours.

The iodine test for hydrolysis of starch was used primarily but the osazone test was later applied when it was found to be difficult to detect partial hydrolysis with iodine. Equal quantities of fresh phenylhydrazine solution and the incubated mixture were placed in test tubes and heated in boiling water for 30 minutes. Glucosazone precipitates while the solution is still hot; maltosazone precipitates only after cooling. The crystals formed were indentified under a microscops by their characteristic shapes (Morrow and Sandstrom 1935[18]).

Maltase

A solution of 2% maltose in the presence of phosphate buffer pH 6.8 was the substrate for maltase tests. The tissue suspension was added and this mixture incubated for 24 hours. The osazone test was applied, glucosazone crystals being present when hydrolysis has occurred.

Lactase

A solution of 2% lactose buffered with phosphate buffer pH 6.8 was the substrate for lactase tests. Tissue suspension was added to the mixture which was incubated for 24 hours. The osazone test was applied to detect hydrolysis. Lactose hydrolyzes to galactose and glucose and the characteristic osazones formed are easily identified under the microscope.

Invertase

The substrate for invertase tests was a 2% solution of sucrose with phosphate buffer pH 6.8. The tissue suspension was added and the

mixture incubated for 12 hours. Barfoed's test for the presence of monosaccharides was then applied. Equal quantities of the incubated mixture and Barfoed's reagent were heated in boiling water for not more than half a minute. Sucrose is easily hydrolyzed by heat and the boiling time was watched very carefully. A red precipitate of cuprous oxide appears almost immediately when invertase is present.

Cellulase

Filter paper fragments were shredded and added to the tissue suspension. This was incubated for about three days. Barfoed's reduction test for the presence of monosaccharides was then used. Cellulose hydrolyzes to glucose in the presence of cellulase which gives a red precipitate with Barfoed's solution when heated.

Lipeolytic Enzymes

The tissue suspension plus two drops of brom-thymol blue indicator were added to fresh milk which had been boiled and cooled. A solution of 1% KOH was added until the mixture turned light blue. If lipeolytic activity is present, the production of fatty acids will turn the blue milk yellow (Swingle 1925[32]).

To ascertain whether the enzymes could be obtained from the food itself, cuttings from Renown wheat seedlings were ground and subjected to the same tests used for enzymes in insect tissue extracts. This material was adjusted to the pH at which the grasshopper enzymes appeared to be active by the addition of dilute Na_2CO_3 or HCl.

pH DETERMINATIONS ON THE REGIONS OF ADULT DIGESTIVE TRACTS

The one-drop electrode attached to the Beckman pH meter was employed to determine the H-ion concentration of the different regions of the gut. The section of the gut to be tested was dissected from the freshly killed hopper, freed of food material and placed quickly in the electrode cup where it was gently macerated. The readings were taken directly on the moist tissue. The pH of the regurgitated fluid was measured by forcing the living hoppers to regurgitate over the electrode cup.

RESULTS

Wherever tests on the entire alimentary canal made after the insects had fed failed to show the presence of an enzyme, subsequent tests were not made on the various regions of the gut.

Tests with the entire alimentary canals of adult *M. mexicanus* and *M. bivittatus* did not establish the presence of dipeptidase, pepsin, lactase or cellulase. The entire digestive tracts of fourth instar *M. mexicanus* and first instar *M. mexicanus*, *M. bivittatus* and *C. pellucida* gave similar results. The other instars were not tested for these enzymes because of shortage of living material.

RESULTS OF QUALITATIVE ENZYME TESTS ON ADULT DIGESTIVE TISSUE

The extract prepared from complete digestive tracts of *M. mexicanus*, *M. bivittatus* and *C. pellucida* gave positive results for trypsin, lipase, amylase, maltase and invertase. Significant variations amongst species were not detected in the adult stage. Tests on the different divisions of the gut failed to show significant variations either.

TABLE I

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF ADULT MELANOPLUS MEXICANUS MEXICANUS (SAUSS.)

Region of gut	No. of ind.	Tryp-sin	Lip-ase	Amy-lase	Mal-tase	Invert-ase
Regurg-itated liquid	4	+	+	+	+	+
Caeca	9	slight	+	+	+	+
Midgut	9	slight	+	+	-	+
Foregut	4	slight	doubtful	-	slight	+
Hindgut	4	slight	slight	-	-	+

TABLE II

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF ADULT MELANOPLUS BIVITTATUS (SAY)

Region of gut	No. of ind.	Tryp-sin	Lip-ase	Amy-lase	Mal-tase	Invert-ase
Regurg-itated liquid	4	+	+	+	+	+
Caeca	4	+	+	+	+	+
Midgut	4	+	+	+	+	+
Foregut	4	+	-	slight	-	+
Hindgut	4	-	-	slight	-	+

TABLE III

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF ADULT CAMNULA PELLUCIDA SCUDD.

Region of gut	No. of ind.	Tryp-sin	Lip-ase	Amyl-ase	Mal-tase	Invert-ase
Regurg-itated liquid	3	+	+	-	+	+
Caeca	5	+	+	+	+	+
Midgut	5	+	slight	+	+	+
Foregut	5	+	slight	+	+	+
Hindgut	5	+	-	+	-	+

In Table III, the negative test for amylase in the regurgitated liquid from adult C. pellucida is attributed to a very low concentration of material. Amylase was detected in the other regions of the gut.

RESULTS OF TESTS ON IMMATURE STAGES

First instar

Tests made on freshly hatched hoppers of M. mexicanus, M. bivittatus and C. pellucida indicate that enzyme activity is very weak before food is taken into the gut.

TABLE IV

DIGESTIVE ENZYMES IN THE GUT OF FRESHLY HATCHED GRASSHOPPERS BEFORE AND AFTER FEEDING.

Species	No. of ind.	Trypsin un-fed	Trypsin fed	Lipase un-fed	Lipase fed	Amylase un-fed	Amylase fed	Maltase un-fed	Maltase fed	Invertase un-fed	Invertase fed
M. mexicanus	20	-	+	-	+	-	-	-	-	+	+
M. bivittatus	20	-	+	doubt-ful	-	-	-	-	+	+	+
C. pellucida	20	-	+	+	slight	-	-	-	-	+	+

In the newly hatched nymphs only invertase was present in appreciable quantities. After feeding, tryptic and lipeolytic activity was detected. The test for maltase was positive in first instar M. bivittatus but amylase activity was not indicated in the first instar of these three species.

Second instar

Trypsin, invertase and lipase were found in both first and second instar of M. mexicanus and M. bivittatus. Tests for amylase and maltase were negative in both species.

TABLE V

COMPARISON OF DIGESTIVE ENZYMES IN TWO SPECIES OF SECOND INSTAR GRASSHOPPERS

Species	No. of indiv.	Trypsin	Lipase	Amylase	Maltase	Invertase
M. mexicanus	20	+	+	-	-	+
M. bivittatus	20	+	slight	-	-	+

Second instar C. pellucida were not available for comparison.

Third instar

More variation in enzyme activity between species was present in third instar nymphs than in the previous instars. Trypsin, lipase and invertase were detected in all regions of the gut of M. mexicanus. Amylase was not detected in the hindgut and only slight hydrolysis of starch occurred with the extract from the other regions. The results of the maltase tests were weak, first tests being negative. When the incubation period was doubled, several positive tests were obtained with extracts of midgut, caeca and regurgitated fluid. Maltase was not detected in the hindgut.

TABLE VI

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF THIRD INSTAR MELANOPLUS MEXICANUS MEXICANUS (SAUSS.)*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	+	+	+	+
Midgut	+	+	+	+	+
Foregut	+	+	+	+	+
Hindgut	+	+	-	-	+

* Based on 10 individuals dissected.

The tests on M. bivittatus were positive for both trypsin and invertase as on M. mexicanus in all regions of the gut except the hindgut where trypsin was not detected. Lipeolytic activity appeared much weaker

than in M. mexicanus, only one positive test being obtained from the extract of caeca. A positive test for maltase was obtained with the regurgitated fluid only.

TABLE VII

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF THIRD INSTAR MELANOPLUS BIVITTATUS (SAY) *

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	-	+	+	+
Caeca	+	slight	+	-	+
Midgut	+	-	-	-	+
Foregut	+	-	-	-	+
Hindgut	-	-	-	-	+

* Based on 5 individuals dissected.

Fourth instar

The enzyme activity in fourth instar nymphs showed little variation amongst species. Trypsin and invertase were found in all regions of the gut of the three species. Lipeolytic activity appeared stronger in C. pellucida than in the other two species.

TABLE VIII

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FOURTH INSTAR MELANOPLUS MEXICANUS MEXICANUS (SAUSS.)*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	slight	+	+	+
Midgut	slight	slight	-	-	+
Foregut	+	+	+	-	+
Hindgut	+	-	-	-	+

* Based on 5 individuals dissected.

TABLE IX

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FOURTH INSTAR MELANOPLUS BIVITTATUS (SAY)*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	slight	+	+	+
Midgut	+	-	+	-	+
Foregut	+	-	+	-	+
Hindgut	+	-	-	-	+

* Based on 5 individuals dissected.

TABLE X

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FOURTH INSTAR CAMNULA PELLUCIDA SCUDD.*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	+	+	+	+
Midgut	+	+	+	-	+
Foregut	+	+	+	+	+
Hindgut	+	+	-	doubtful	+

* Based on 8 individuals dissected.

Fifth instar

Tests for maltase were particularly weak in fifth instar nymphs of M. bivittatus. A positive test was obtained with a suspension of the regurgitated liquid. Tests on the other regions of the gut were negative. There was no significant variation in the occurrence of the other enzymes in the three species.

TABLE XI

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FIFTH INSTAR MELANOPLUS MEXICANUS MEXICANUS (SAUSS.)*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	+	+	+	+
Midgut	+	+	+	+	+
Foregut	+	+	+	+	+
Hindgut	+	+	-	-	+

* Based on 9 individuals dissected.

TABLE XII

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FIFTH INSTAR MELANOPLUS BIVITTATUS (SAY)*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	+	+	-	+
Midgut	+	slight	+	-	+
Foregut	+	slight	+	-	+
Hindgut	+	slight	-	-	+

* Based on 10 individuals dissected.

TABLE XIII

DISTRIBUTION OF DIGESTIVE ENZYMES IN REGIONS OF THE GUT OF FIFTH INSTAR CAMNULA PELLUCIDA SCUDD.*

Region of gut	Trypsin	Lipase	Amylase	Maltase	Invertase
Regurgitated liquid	+	+	+	+	+
Caeca	+	+	+	+	+
Midgut	+	+	+	+	+
Foregut	+	+	+	+	+
Hindgut	+	+	slight	+	+

* Based on 6 individuals dissected.

Labial glands

Lipeolytic activity was not detected in the labial glands of the three species of adults tested. Invertase and trypsin tests were positive but amylase and maltase tests indicated slight activity only after incubation times had been increased from 24 to 36 hours.

TABLE XIV

DIGESTIVE ENZYMES IN LABIAL GLANDS OF THREE SPECIES OF ADULT GRASSHOPPERS

Species	No. of Indiv.	Trypsin	Lipase	Amylase	Maltase	Invertase
M. mexicanus	4	doubtful	-	+	-	+
M. bivittatus	2	+	-	-	slight	+
C. pellucida	5	+	-	-	-	slight

RESULTS OF ENZYME TESTS ON RENOWN WHEAT SEEDLINGS

These tests were made in case a positive test for enzyme activity might be obtained from food material adhering to the gut.

Slight lipase activity was the only evidence of probable enzyme activity which could be attributed to the seedlings. The optimum pH for enzymes in growing grain is less alkaline than that maintained in the grasshopper gut.

RESULTS OF pH DETERMINATIONS ON ADULT GRASSHOPPERS

The ranges of H-ion concentration were very similar in the three species.

TABLE XV

RANGE AND AVERAGE OF H-ION CONCENTRATION IN THE DIFFERENT REGIONS OF ADULT GRASSHOPPERS*

Species	Regurgitated Liquid		Foregut		Midgut		Hindgut	
	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.
M. mexicanus	5.1 - 5.6	5.4	5.9 - 6.4	6.0	6.0 - 6.2	6.1	6.4 - 6.7	6.5
M. bivittatus	5.4 - 5.6	5.5	6.0 - 6.4	6.2	6.4 - 6.6	6.5	6.4 - 6.5	6.3
C. pellucida	5.2 - 5.5	5.3	6.1 - 6.4	6.2	5.9 - 6.4	6.2	6.1 - 6.4	6.3

* Based on 5 individuals in each species.

DISCUSSION

The H-ion concentrations in regions of the digestive tracts agree closely with published data. The pH range in regions of the gut of M. bivittatus observed here shows little variation from that reported by Swingle (1931[34]). Hastings and Pepper (1943[12]) found the pH of regurgitated digestive juices of six species of grasshoppers to range from 5.2 to 5.8. In this study of M. mexicanus, M. bivittatus and C. pellucida the range of 5.3 to 5.5 was obtained for the regurgitated liquid.

The elaboration of enzymes in insects is attributed to the midgut tissue with its caeca and to the labial glands (Swingle 1930[34], Fink 1932[7], and Simmons 1939[30], Day and Powning 1950[5]). The presence of activity in other regions of the gut, according to Fink (1932[7]) is probably a result of regurgitation of digestive juices or of the passage of the enzyme-food mixture along the digestive tract. Observations made during this investigation indicate the strongest activity in the caecal extract and in the suspension of regurgitated liquid. Enzyme activity is perceptible in other regions but is definitely weaker. This suggests that the midgut with its caecal diverticula is responsible for the production of the majority of enzymes in the three species of grasshoppers studied. The labial glands contribute some trypsin and invertase, but the greatest concentration appears to be in the midgut.

Tests for tryptic activity were made primarily with congo-red fibrin. When the stained fibrin was allowed to digest for periods longer than an hour, there was a color release in the controls in several

cases. If the concentration of the enzyme was not strong enough to release the dye within the first hour, an additional test was made with calcified milk.

Peptic activity is present only in an acid medium of a pH of approximately 2.2. Determinations of pH of the gut showed the range to be from 5.1 to 6.7. Negative results were obtained from preliminary trials on the entire alimentary canals, hence pepsin tests were discontinued.

Difficulties in the technique of detecting lipase activity were reported by other workers (Evenius 1927[6], Hinnman 1933[13]). Tests made with regurgitated liquid or with caeca usually showed lipase activity within a few hours. When the concentration was less as in the extract of regions of the gut, very slight color changes in both extract and controls were difficult to interpret. Slight lipeolytic activity was noted with an extract of wheat seedlings and there is the possibility that some of the food material may have adhered to the gut during the process of dissection. This may have contributed to the difficulty experienced with lipase test.

Iodine tests for the detection of unhydrolyzed starch failed to show slight amylolytic activity. Osazone tests were found to be more reliable and the degree of hydrolysis was more easily determined by the identification of the osazones formed. In some of the tests for amylase, the hydrolysis had been carried to the glucose stage indicating that maltase was also active. In other tests where the enzymes may not have been as concentrated an osazone appeared,

together with glucosazone and maltosazone, which was not identified. When it appeared alone, the tests were considered positive because some activity was obviously present.

Uvarov (1928[36]) states that invertase is common in phytophagous insects in general. Invertase tests on regions of the adult gut and on the digestive tracts of all immature stages gave positive results for the three species under investigation here.

The ability to digest protein is also common. The only negative results of trypsin tests were obtained from the extracts of hindgut tissue.

Lipase activity is well distributed, although weaker in the immature stages of M. bivittatus than in corresponding stages of the other two species. This wide distribution of lipase irrespective of feeding habits is also mentioned by Uvarov (1928[36]).

Except for one positive test for maltase, the results of tests for amylase and maltase were negative in the first two instars of each species tested here. Because one positive test was obtained, there is the possibility that the use of a greater number of individuals and an increase in incubation time might indicate the presence of these enzymes. However, if present, the concentration of these enzymes in each individual must be very small.

Brown (1937 a, seen in Uvarov 1947[37]) investigated the utlization of different foods by M. bivittatus. He reported that this species is unable to utilize starch grains (potato and arrowroot) and that the starch grains appeared unchanged in the excreta. Tests for

amylase in M. bivittatus were conducted in this investigation and results were negative using a starch substrate. When soluble starch was used, amylase activity was detected.

The results obtained in this investigation using M. mexicanus, M. bivittatus and C. pellucida indicate the presence of trypsin, lipase, amylase, maltase and invertase. These observations agree with the literature concerning the occurrence of digestive enzymes in grasshoppers. No correlation between the type of food consumed and the enzyme complement was established.

With the possible exception of the absence of amylase and maltase in the first two instars, no striking changes in enzyme activity in different instars is exhibited.

SUMMARY

(1) Tests on the digestive tracts of adult M. mexicanus, M. bivittatus, and C. pellucida revealed the presence of invertase, amylase, maltase, trypsin and lipase. There was no significant variation amongst species.

(2) The presence of cellulase, lactase, pepsin or dipeptidase was not established.

(3) The majority of tests for amylase and maltase were negative in second instar M. mexicanus and M. bivittatus.

(4) Invertase and trypsin appeared in all regions of each instar in the three species. Invertase activity was noted in the gut of freshly hatched nymphs even before feeding. Trypsin was not detected until there had been food in the gut.

(5) Indications of lipeolytic activity were present before feeding in M. bivittatus and C. pellucida but not in M. mexicanus.

(6) Lipeolytic activity was weak in third instar M. bivittatus as compared to that in third instar M. mexicanus; its activity in fourth instar C. pellucida was strong as compared to the other two species.

(7) Third, fourth and fifth instar nymphs showed little variation amongst species in their enzyme complement other than in lipase activity.

(8) Invertase was found in the labial glands of the three species tested. Lipase was not detected and amylase and maltase results were doubtful.

(9) The suspension of regurgitated liquid and extract of gastric caeca showed the strongest enzyme activity. This suggests that most of the enzymes are produced by the midgut and its caecal diverticula.

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