METHOD OF ENTRY AND SPEED OF ACTION OF LIQUID PARATHION IN RELATION TO THE CUTICULAR COMPOSITION AND EXOSKELETAL FEATURES OF EURYGASTER INTEGRICEPS, PUT.

by

Abdul Monim S. Talhouk

A thesis

submitted in partial fulfillment for the requirements

of the degree of Master of Science

in the Biology Department of the School of Arts and Sciences of the

American University of Beirut

Beirut, Lebanon.

1955

Parathion Entry in Eur. integriceps

TABLE OF CONTENTS

ABSTRACT.

Method of Entry and Speed of action of Liquid Parathion in Relation to the cuticular Composition and exoskeletal Features of Eurygaster integriceps, Put.

Eurygaster integriceps Put. is an important wheat pest in Syria, Turkey, Iraq, Iran, Pakistan, and parts of Russia. Biological and chemical methods of control against this insect have met with little success. Among the more successful chemicals reported was calcium arsenite dust in irrigated fields (Starostin, 1944; Peredelskii, 1947). Later, parathion dusts were tried in Russia (Paikin, 1950) and in Syria (Talhouk, 1951, 1954), and were found very effective against all post-embryonic stages of this pest.

However, there are rather conflicting points of view in the literature regarding the method and place of entry of contact insecticides. For this reason the present study was undertaken. It attempts to correlate the cuticular composition and the exoskeletal features of Eurygaster integriceps with their role in the entry and speed of action of parathion.

In beginning this work, the first few weeks were spent in the detailed, microscopical examination of the external anatomy and sense receptors.

The second half of the work consisted of the experimental application of parathion at eleven different points on the insect's body. There were five replicates of ten insects each for the different treatment groups. Parathion droplets were obtained by means of a syringe-

micrometer attachment.

The experimental lots differed significantly in their LT_{50}'s as well as the mean length of time required for complete kill.

The analysis of variance of LT_{50} (in hours) indicates a very high significance that can be attributed to treatment.

The significance of the difference between means was calculated for "t" values by Student's Method.

The speed of penetration of parathion at the eleven different points of application was termed their "effectiveness". There were seven categories of effectiveness, viz.,

a. The effectiveness of the tip of the proboscis was highest in permitting the penetration of liquid parathion as proved by the onset of its physiological effect. It constituted the only locus in the first category.

b. The effectiveness of the coxopleurite was much greater than any other locus in groups III - VII.

c. The third category included the metathoracic tarsi, the female external genitalia, the first antennal segment, and the sixth abdominal spiracle.

d. Next, the sixth abdominal sternite, the sixth abdominal tergite, and the fifth-sixth intersegmental membrane were included in the fourth category.

e. Group V included the back of the head, and

f. Group VI comprised the scutellum which showed the least effectiveness of all the treated areas.

g. The Control was placed in Group VII because its "t" value with the scutellum (4.987) was greater than the "t" value between any two experimental groups.

However, certain "t" values show insignificant differences so that Groups III, IV, and V to some extent merge into one another. For this reason the seven categories can be divided into five more comprehensive divisions namely: Highly effective, Group I; effective, Group II; moderately effective, Groups III, IV, and V; slightly effective, Group VI; and finally, the control, Group VII.

Three loci were chosen for a study of the possible relation between cuticular thickness and "effectiveness". The sixth abdominal sternite, the back of the head and the scutellum showed possible correlation between cuticular thickness and the average length of life after treatment.

The experimental work is discussed and certain explanations and conclusions are given.

I. PREFACE.

Eurygaster integriceps, Put., commonly called the "Wheat Shield Bug", is a very serious pest of wheat and barley in Syria, Turkey, Iran, Iraq, Pakistan, and parts of Russia around the Black and Caspian Seas (Zwolfer, 1930; Blachowsky et Mesnil, 1935; Distribution Maps of Insect Pests, 1954).

The financial loss it causes to Syrian wheat is estimated to be about L.S. 2,500,000 per year. In the region around Teheran, agriculture suffers an annual loss of about 100 million rials because of this pest (Alexandrov, 1948). Adle (1932) states that 40-50%, and even 90% loss of the wheat crop occurs in Persia from this pest. Turkey and Russia suffer great losses too, but the extent of their losses is not known to the writer.

The wheat (or barley) crop is attacked twice during the growing season. The amount and degree of damage is dependent on the abundance of the pest and the stage of growth of the crop at the times of attack. A short account of the life-history and habits of this insect will therefore help visualize its economic importance and the reason for which the present study was undertaken.

II. LIFE-HISTORY AND HABITS.

The life history and habits of Eurygaster integriceps have been investigated by Vasiliev (1913), and more thoroughly by Zwolfer (1930), and Peredelskii (1947).

Their work indicates that the insects overwinter as adults in the

hills. In late March or early April, they fly down to the wheat and barley fields to feed for two to three weeks before the attainment of sexual maturity. It is during this part of their life-cycle that they cause great damage to the plants that are fed upon (i.e., stung) since the latter wilt, become yellow and produce more or less aborted inflorescence or almost empty seeds.(1) In most regions, copulation begins during the first half of April. Oviposition follows in three to five days, and continues for ten to twenty days. The total number of eggs that a female carries in her ovaries averages between 150 and 180 eggs (Zwolfer, 1930). The eggs, as they mature, are laid in batches of 12 to 14. The incubation period lasts about eight to ten days. The nymphs pass through one pre-feeding and four post-feeding moults. They reach the adult stage in four to five weeks after egg-hatching. By this time the wheat would be in the "milk stage", or, very nearly ripe and dry.(2) It is during this stage that most of the damage is done, since there are records when 15-25 of these bugs can be seen feeding on one ear (Hibrawi, 1930), and, in some exceptional cases, 50-60 bugs were counted feeding on one ear in Varamine, Iran (Alexandrov, 1948). Right after harvesting and carrying of the wheat, the adults scatter to the

---

(1) Dr. A. Davachi, in an oral communication, says that in the district of Varamine in Iran, 30% of the loss of wheat crop is due to the feeding of adults before and during copulation and egg-laying.

(2) The physical damage caused to the grain by the feeding bugs is a reduction in weight from 15 to 60%.

Apart from that, the straw from attacked plants is refused by domestic animals. The flour milled from attacked grain suffers a reduction in its total nitrogen content (Kretowitch & Tokareva, 1942). This is accompanied by an increase of proteolytic enzymes, and a high amylase activity in the flour (Kretowitch et al. 1943), resulting in deterioration in the baking quality of the flour (Marushev et al. 1938).

hills where they seek shelter under the fallen leaves of trees and shrubs and remain practically without food until the following spring.

III. REVIEW OF LITERATURE ON CONTACT INSECTICIDES

Since the beginning of the 20th. century, Russian entomologists have devoted a great deal of study to this pest. They have published many important papers on its biology, and especially on biological control through its egg parasites (Vasiliev, 1913, and amongst the recent ones, Meir, 1940; Alekseev, 1940; Fedotov, 1944; Rubtzov, 1947). Biological control has always proved inadequate, and this has led to the trials of chemicals for its control. Amongst the more successful chemicals reported was calcium arsenite dust in irrigated fields (Starostin, 1944, Peredelski, 1947).

During, and after World War II, the insecticides DDT and BHC (benzene hexa-chloride) were tried with varying success. (Tzitovich, 1950; Kuznetzov, 1950; Paikin, 1950). Later, parathion dusts were tried in Russia (Paikin, 1950), and in Syria (Talhouk, 1951, 1954) and were found very effective against all post-embryonic stages of this pest.

In addition to the work on E. integriceps, a number of different investigators have studied the effect of contact insecticides. Among earlier papers are Shafer (1915) on penetration of lipoid insoluble dusts, Richardson et al. (1934), on the penetration of insecticidal vapours through the cuticle, Tischler (1935) on the penetration of derris through the body wall of Bombyx, and O'Kane et al. (1940) on the penetration of

certain liquids through the pronotum of Periplaneta. Following the discovery of DDT, greater interest was taken in the development of contact insecticides. Many papers appeared on the mode of entry, and place of action of these insecticides. Potts et al. (1945) conducted experiments on Tse-tse flies whose feet only came in touch with DDT for 2 seconds. The flies were killed with this very short exposure. They concluded that the size of the pulvilli is directly proportional to the speed of action of DDT, and that the poison enters through it. Hicken (1945) experimented with two species of lice that have very poorly developed pulvilli. He made the lice touch the insecticidal surfaces only by means of their tarsi. When made to walk on n-carbitol thiocyanate, the lice were dead in 6 minutes, whereas when on DDT, it took them 120 minutes to die. Hicken concludes that the relative size of the pulvilli is of no special significance in the entry of insecticides, and different insecticides show a wide variation in their speed of action on the same species. Richards et al. (1949), working on isolated larval cuticles taken from presumably identical individuals of the same culture of Phormia, found that there were considerable differences in permeability of these cuticles to inorganic and organic salts. Serial sections of these cuticles showed no parallel variation in total cuticular thickness. They conclude that "the variation must be conditioned by variation in the thickness of an exceedingly thin component layer (presumably the lipid epicuticle) or by variation in the sub-microscopic porosity of one or more of the component layers". They warn against correlating variation in cuticular permeability between different individuals with variation in biological responses such as sensory reactions and

mortality from contact insecticides.

Savit et al. (1946) have compared body-surface application versus intra-abdominal injection of y-BHC in Periplaneta americana, and found no statistically significant differences between the LD_{50}'s. Similar results were obtained by them for DDT. They concluded that their findings emphasize the importance of the absorptive capacity of the cuticle. Dresden and Krijsman (1948) found that DDT, BHC, and rotenone display about the same activity when applied to the skin of an insect or injected into it. They conclude that the cuticle does not act as a barrier, but permits quick and complete penetration. This, they conclude, explains the apparent specific toxicities of these chemicals to insects.

To test the permeability of the cuticle to electrolytes and non-electrolytes, Skvortzov (1946) filled cultures of living paramecia into (emptied) larval skins of Musca domestica, which he tied at both ends, and suspended in aqueous solutions of these salts. His experiments proved to him that electrolytes penetrated the cuticle more slowly than non-electrolytes that are highly soluble in lipoids. His opinion is that non-electrolytes penetrated the lipoid phase of theepicuticle by dissolving it, while electrolytes pass through the pores of the protein phase; his conclusion is that in this case penetration is affected by the size of the molecules, as well as by adsorption. Wiesmann (1946, 1949) has shown that the penetration of DDT-in-oil into the bodies of Calliphoria vomitaria and Phyllodromia germanica is dependent on the structure and chemical composition of the cuticle. He found that in the parts that were

not permeable, resistance was due to the high sclerotin content of the exo- and endo-cuticle, while the sense organ pores showed permeability because of their lipoid content and their thin, unpigmented, flexible exo- and endo-cuticle containing little or no sclerotin. Permeability was possible also through the non-sclerotized intersegmental membranes. Wigglesworth (1948 b), writing on the mode of action of DDT, says that the shape of a molecule is very important in determining the ease and the speed of its passage through the cuticle. Substances soluble in oils are known to penetrate the cuticle much more quickly than those that are lipophobic. Their "partition coefficient", or as Richards (1951) prefers to call it, the "chemical potential", helps to decide on the ease of penetration. Wigglesworth (1948 a) states that if a toxic substance can pass through the epicuticle and reach the pore canals, it will have only a very short way to travel before it reaches the soft tissues of the insect. Pfaff (1952) found that parathion enters the body of Periplaneta americana only "at points determined by nature for the reception of chemical and physical stimuli, the sense organs, as well as through the spiracles and hair roots, and in this way reaches the place in which it takes effect", but that it cannot penetrate through the "homogenous cuticle", meaning the undifferentiated cuticle. Fisher (1952) studied the effectiveness of DDT-benzene droplets applied on different loci of the body of adult Musca domestica. His results showed that the effectiveness increased as the loci of application approached the body or the head; was greatest when the labella were treated, and was increased as the area of intact integument surrounding the locus was increased. In his discussion, he mentions

the importance of the cuticle as a tissue that may change DDT after its passage through it, into a toxic compound. He bases this hypothesis on the fact that when he placed crystalline DDT in large doses directly into the tissues of the mesonotum, it did not have any toxic effect. But when DDT droplets were placed on the cuticles, it showed its characteristic toxic effects. Ball and Beck (1951) working with Periplaneta americana found that parathion is transported by both the ventral nerve cord as well as the blood, but that the former was more effective. Armstrong et al. (1951) found that the amounts of the alpha, beta, gamma and delta isomers of BHC that penetrated the epi- and exo-cuticles of Calandra granaria were in the approximate ratio of their sulubilities in hydro-carbon solvents, and that the gamma isomer penetrates through the outer layers of the insect integument much more rapidly than the other three isomers tested. They conclude, therefore, that the first stage of pick-up of the insecticide by the insect is simple solution in the outer waxy layers of the epicuticle and that structural effects play an important role in the penetration through the insect cuticle as well as in toxic effects at the site of action. Fernando et al. (1952), working with radioactive organic phosphates, including parathion, found that following topical application of the insecticides to the cervical membrane of Periplaneta americana, the insecticides were chiefly transmitted by the blood, and that very little parathion was found in the central nervous system, but quite enough to inhibit cholinesterase, however.

Thus it is evident that there are rather conflicting points of

view on the method and place of entry of contact insecticides in various insects. For this reason the present study was undertaken. It attempts to correlate the cuticular composition and the exoskeletal features of Eurygaster integriceps with their role in the entry and speed of action of parathion.

If penetration is limited to specific areas only, it is necessary to determine whether these regions were characterized by an abundance of sensory hairs or other sense-perceiving structures as contrasted with the undifferentiated cuticle. In beginning this work, therefore, the first few weeks were spent in the detailed, microscopical examination of the external anatomy and sense receptors. This work is described in the next section.

IV. MORPHOLOGY

Techniques.

Adult E. integriceps were collected from wheat fields in June 1953, killed and fixed in 10% formaldehyde for several months. The formalin-fixed material was then embedded in celloidin by the method of Wall (1932), or double embedded by Peterfi's method (McClung, 1950). The celloidin embedded material was sectioned from 16-18 u, while that embedded by Peterfi's method was cut in ribbons 7.5 u thick. The sections were stained in either Delafield's haematoxylin-eosin or Heidenhain's iron haematoxylin-eosin, or Mallory's triple stain, or Liang's method of nerve staining (Liang, 1949). The different methods gave variable results.

Delafield's haematoxylin-eosin gave more uniform results and was used almost exclusively, except when similar sections were stained by other methods as a control. The best sections obtained were from material embedded by the hot celloidin method of Wall. The major difficulty in working with such material is the difficulty of getting serial sections from it.

External and Peripheral sensory Anatomy

The morphology of the systems of this insect has been studied extensively (Trukhanov, 1947). The exoskeleton of E. integriceps shows a great deal of variation in the amount and shape of various structures covering it. Some regions are more or less smooth and naked, while others may be pitted, hairy, or warty in different degrees. The thickness, degree of sclerotization, and pigmentation of the cuticular layers vary greatly in the different regions.

In surface view, pore canals are clearly seen by phase-contrast illumination. Their number is very large in an area of a few square micra (Fig. 1).

Below, each of the general anatomical regions is described in turn.

a. The head (Figs. 1, 2).

The head is characterized by the dark pits scattered widely over its surface (Figs. 1, 2). These pits are circular to slightly ovate, and are surrounded by a slight ridge 2-3 micra in height. Arising from a domed prominence at the posterior portion of each pit is a small seta (28-30 u in length) which is apically bent and points posteriorly. Cross

sections thru these pits (Fig. 2) show that the seta is joined directly to a thin nerve. The exocuticle is thickest at the base of the pit, and is tanned dark brown in this area. The exocuticle in this region varies between 12 and 20 u being thickest at the posterior end of the depression, while the endocuticle varies between 30 and 55 u, being thinnest at the posterior section of the pit.

Other topographical features in the head, other than the pits, are the dermal glands. Their canals start from enlarged cells in the hypodermis. They traverse the cuticle straight or in zig-zag line, gradually get thinner when they enter the exocuticle (see Figs. 1, 2). In surface view the opening looks like two concentric rectangles at right angles to one another (Fig. 1).

Apart from the pits and dermal glands, there are canals of unknown function and the pore canals (Fig. 2).

b. The antennae. (Figs. 3, 4, 5)

Except for the last antennal segment which has dark brown cuticle, all the segments are transluscent.

All nymphal instars have four antennal segments, while the adult has five. The shape and length of the segments differ (Fig. 3).

The wall of the antennary segments is of medium thinness, lined by hypodermal cells with large nuclei. Except for segment four in the immature forms, and segments four and five in the adults, the walls are

very sparsely clothed with hairs. The third segment bears highly enervated setae at its tip and few minute ones on its walls. Segments four in the nymphs, and four and five in the adults, are thickly clothed with setae that usually curve distally upwards. All along the length of the antennae a thick tracheal tube accompanies the antennary nerve. This nerve splits at the proximal end of the second segment, as it enters the third, so that two branches of it are found in the third, fourth, and (in the adult) fifth segments. A number of groups of sensory cells (4-6 cells per group) send their proximal processes to the antennary nerve, while their terminal ones are drawn into a fascicle that passes through the cuticle and enters the setae that cover the walls of the fourth and fifth segments. The cells are ovate. They possess large dark staining nuclei (Fig. 5).

c. <u>The proboscis</u> (Fig. 6).

When at rest the proboscis extends to the tip of the metathoracic coxae. Its tip is covered with long, thin setae of the chemoreceptor type and are considered by Weber (1930) to be "Taste Borste".

These hairs cover a very small part on the proximal end of the proboscis which does not exceed 1/50th of its entire length. A section thru the proboscis at this level shows that it is well supplied by trachea, nerves and muscles. The type of columnar epithelial cells that surround the mandible in the labium, as well as other anatomical components of the proboscis are shown in figure 6.

d. The regions around the meso-, and metathoracic legs of adults (Fig. 7)

Between the prothoracic legs of the adult there are no signs of any differentiation of the cuticle. The surface here is typically that of the sternites as a whole, with the pitting, dermal glands, and occasional very short and fine setae.

Between the mesothoracic legs the region is well differentiated. It has a high double ridge running across the middle of the metasternite. This double ridge is the "median sternal groove" (Snodgrass, 1935). It is thickly clad with sensory setae that are traversed by thin protoplasmic strands which in turn connect with columnar cells that stain deeply. These cells are very densely massed (Fig. 7). Lateral to the double ridged structure, the cuticular surface is ornamented with the same pattern of conical projections found on the abdominal tergites (Figs. 9, 10). The same description holds true for the metasternite, except that the cuticular ornamentation typical of the abdominal tergites does not exist here.

e. Thoracic and abdominal spiracles (Figs. 8, 8a).

There are two pairs of thoracic spiracles, and seven pairs of abdominal ones that can be seen in females of this insect.

The thoracic ones are not exposed; their openings are found on the intersegmental membrane between the pro- and meso-thorax, and the meso- and meta-thorax, and belong to the meso- and meta-thorax respectively (Trukhanov, 1947). The shape of the opening is ovate, slit-like and is appreciably large. They have the normal "lip-type" closing

mechanism.

All abdominal spiracles have circular, hard, atrial chambers surrounded by a peritreme. The first abdominal spiracle is usually hidden by the metathoracic sternite in dead specimens. Except for the first and last abdominal spiracles, the five other pairs are associated each with a pair of trichobothria, having a comparatively long hair, either posterior or lateral to the spiracular openings (Fig. 8a). On no other parts of the body do these trichobothria occur(3). Their function remains unknown. However, their position leads one to suspect that they have a relation with the opening and closing of the trachea by the occlusor muscle.

f. The scutellum

The scutellum develops gradually with each molt. This is a very important practical significance since it acts as a shield in protecting the more delicate abdominal tergites, especially in the adult stage. In the nymphal stages, the abdominal tergites seem to be topographically composed of the same exo-skeletal features as the pronotum and the abdominal sternites.

In nymphs, up to the third instar, the scutellum is comparatively very small; it does not cover any abdominal tergite. In general, it is in the form of an open W (ω), covering on either side the growing wing pads. In the fourth instar, the scutellar shield is slightly longer,

---

(3) There seem to be exceptions since one whole mount of an abdomen shows only one trichobothrium posterior to the right spiracular opening on the VI abdominal segment, and two posterior to the left spiracular opening on the same (VI) abdominal sternite.

and partially covers the lateral parts of the first and second abdominal terga.

In the adult stage, the scutellum covers both pairs of wings, and the soft, thin abdominal tergites, that have lost their hard structure with the pre-imaginal molt.

In cross-section the upper side of the scutellum shows the same pitting that characterizes the head and abdominal sternites. The underside, which overlies the abdominal tergites, has the same tooth-like cuticular projections that cover these tergites described below (Figs. 9, 10.) Sections through the scutellum show that they are lamellate and are traversed longitudinally with trachea (slide No. 2 AWM), and perpendicularly by many glandular canals.

g. The abdominal tergites (Figs. 9, 10.)

In the nymphal stages, these tergites are of the same topographic composition as the abdominal sternites descrived above. They also possess three lip-like openings with thickened edges on the 4th, 5th, and 6th segments; these are the openings of the stink glands. With the last nymphal moult the lips seem to fuse together and the structures become vestigial in the adult, and the whole topographic features are changed from the pitted type in Figs. 1 and 2, to that in Figs. 9 and 10. As a result of this moult, the abdominal tergites of the adult become thinner than those of the 4th instar nymph.

In general, there are two different types of surfaces on the adult abdominal tergites. One is covered with the rough conical pro-

jections seen in Figs. 9 and 10, and composes all of the tergites I, II, III, IV and the lateral parts of V, VI and VII. The other type is the "smooth" type; it is found in the mid-dorsal part of tergites V, VI, VII, and all of the VIIIth and IXth tergites. The posterior part of the IXth tergite is covered with longer setae than those found on the other tergites. This is quite expected as this part is very near to both the rectal and genital openings, and the hairs serve a tactile function.

h. The abdominal sternites

The abdominal sternites have numerous pits of the type described on the head. However, the setae of these pits point posteriorly, in contrast to the anteriad ones of the head.

In addition to the pits, the 2nd, 3rd, 4th, 5th and 6th abdominal sternites each bears 4 trichobothria, 2 on either side posterior to the spiracular openings. No sections were obtained through these, and their function is unknown; however, their long setae arouse the suspicion of a sensory function.

The ninth abdominal sternite in the adult female bears on its posterior as well as its inner lateral ends, several medium-sized setae of the sensory type found on the tibia. The rest is of the typical sternite ornamentation.

The thickness of the cuticle varies greatly in the same sternite as well as between different sternites.

i. The paratergites

The paratergites are in their surface features, exactly like the abdominal sternites, except that the diameter of the pits on their surface is about twice as great as that of the ones on the head, or the abdominal sternites. There are several pits per segment, and the degree of sclerotization is different in each segment.

j. The anal ring

The anal ring is covered with comparatively long slender setae that are tactile in function. They are arranged along both margins of the posterior half of the ring.

k. The female external genitalia

The female genital opening is slit-like, and extends the length of the 8th abdominal sternite. Along its extreme posterior margins are medium-sized, probably sensory, setae.

l. The legs

The coxae move easily in the sternal sockets (coxo-pleurites). Their movement is free in the plane that runs parallel to the body in an antero-posterior direction. Surface features on the coxae afford no interest from the toxicological point of view. However, the coxal articulation with the pleuron affords a very quick means of entry of chemicals, in the form of liquids and fine dusts between the pleuron and the coxa. This results in an intimate contact between these chemicals and the coxal corium.

The trochanter, too, does not show any interesting surface features such as spines, setae or sensory hairs, but the articular membrane joining the trochanter and coxa is very well exposed on the outer lateral side, while guarded by a sort of evagination of the distal part of the coxa with which the trochanter articulates at two points. This evagination is not very tight around the base of the trochanter, so that it allows liquids and fine dusts to collect inside this funnel-like articulation. The dusts, especially, cause the abrasion of the articular membrane at the joints, thereby making possible the entry of poisons, as well as the evaporation of water from the insect's body. (Wigglesworth, 1947).

The femur does not display interesting outside features, and is almost devoid of any cuticular outgrowth.

The tibia of the fore leg is armed at its distal ventrad end with three strong short peg-like setae, and a row of very fine bristles forming a comb-like structure. Opposite these are four prominences, two of which are empty sockets, alternating with two others that bear no sharp outgrowths (Fig. 14). Between these two sets of outgrowths there are rows of fine setae very much similar to those found on the ventral surface of the first and second tarsal segments. On the whole length of the femur are large numbers of pointed pegs and setae set in longitudinal rows. The setae are of varying length and thickness.

Histological examination of the pegs indicates a probable sensory function.

On the anterior tibia, and on its ventrad surface, there is an inclined triple U-shaped prong or fork which, according to Weber (1930) is termed "Fuhler Borste", or "antennary bristle".

On the tibia and the femur (especially of the meso-, and meta-thoracic legs) are groups of dark spots, which upon some magnification appear as dark, tanned areas of the cuticle in which are a number of more or less circular figures. Sections through these areas show that they are points of muscle attachment on the exoskeleton of the leg called tonofibrillae. These tonofibrillae can be seen extending to the dark cuticular markings. The number of circles in each dark spot corresponds to the number of fascicles in the individual muscle attached to it (Fig. 13). In all of the nymphal stages of this hemipteron, these dark spots are not apparent. In some whole mounts of legs that have been cleared in sodium benzoate or in benzene, the muscles of the femur (especially), and tibia can be seen by transparency. A very large trachea runs spirally inside the femur and the tibia, and gives off a number of branches especially to the femoral muscles. The muscles occupy almost all the cavity in the femur and the tibia. The tarsi are three-segmented in the adults, and have well developed pretarsi. On their ventral surfaces, the first and second segments bear longitudinal rows of thin, densely-packed setae of medium length.

The thinness of their walls and the fact that their sensory cells are very closely packed in a tight space indicate a probable chemoreceptor function (Figs. 14, 15).

On the dorsal and lateral sides of the first and second segments of the adult tarsi, however, very few of these setae are found. This also holds true in the case of the third tarsal segment which is very sparsely covered on its ventral side by the type of setae just described, but has on the dorsal and lateral sides much longer ones that are slightly

curved at their tips (Fig. 14). These are the so-called "sensory hairs" (Weber, 1930).

The pretarsus is composed of chitinized, undulated, sclerotized pulvilli, developed auxilia, and a strong pair of claws (Fig. 14).

V. EXPERIMENTAL RESULTS

Materials and Methods

The second half of the work consisted of the experimental application of parathion at eleven different points on the insect's body. A total of 550 experimental animals were tested in this work. There were five replicates of ten insects each for the eleven different points of application. In addition 550 controls were run against the experimentals.

In order to have a sufficiently large number of individuals of E. integriceps on which to perform the insecticidal experiments, it was decided to collect the eggs of this insect from the field and keep them in cold storage. Then, as they were needed, batches of 150 - 200 eggs were taken from the refrigerator, placed in an incubator, where in a few days hatching started and the newly born nymphs were fed on wheat planted in flats for this purpose. Attempts to breed the insect from collected eggs were not successful, however. This project was therefore abandoned and the material on which the tests were carried out was collected in the Rouj Valley (Syria) on 9/6/54 from a small wheat field. About 6000 to 6500 adults were collected and placed in three insect-rearing cages together with some wheat ears and straw.

The entire apparatus was brought to the laboratory. Here the insects were freshly supplied with food and water. The food consisted of (a) fresh ears of wheat which were placed in water, and (b) a 0.5% glucose solution in distilled water which was placed in a petri dish in which some absorbent cotton was soaked to provide a feeding-place for the bugs. Another petri dish contained absorbent cotton soaked with tap water. The insects refused to feed on the wheat or drink from the solution or the tap water.(4)

Paration droplets were obtained by using a syring-micrometer attachment. This consisted of a 1 cc. tuberculin syringe graduated to .01 ml. The piston was pushed by a fine micrometer screw. The syringe was fitted with a 27 gauge needle, on which was cemented a bent capillary glass point, drawn very fine to further reduce the gauge. Very fine uniform droplets were obtained by this apparatus. The syringe-micrometer attachment was held by a clamp that could be rotated horizontally or vertically so that the tip of the glass point could be made to touch any locus on the insect's body. This was accomplished with the help of a binocular dissecting microscope (Fig. 18).

---

(4) Only on one occasion was it noticed that one insect sucked some tap water. Most of the insects climbed on the wire screen or on the wheat ears, without paying any attention to the food or water.

At any rate, in nature, these insects stop feeding as soon as the wheat grain passes from the "milk stage" to the mature stage. They remain without taking food all summer, autumn, winter, and part of early spring. When they come down to the wheat fields from their higher hibernating quarters in the spring these adults would have depleted their fat body reserves (Strogaya, 1950). They feed on the young wheat, barley or oats for ten to fifteen days after which time they become sexually mature.

The average weight of one droplet obtained by this apparatus was 8 ugm. (one hundred drops weighed), and the parathion was 98% pure. One droplet was used per treated insect in all the eleven different treatments given in Table I.

For the different treatments the insects were stuck on paper cards by means of rubber cement. Batches of ten adults were subjected to one treatment. Each treatment was attempted five times giving a total of fifty individuals per treatment. The different loci treated together with the LT_{50}'s are found in Table I. This table not only indicates the median lethal time in hours for each of the five replicates but gives the percentage death in the control at the time when 50% of the experimentals had died.

The next table (Table II) indicates the analysis of variance of LT_{50} (in hours) for the treatment groups to determine if the differences between the treated groups were significant or due to chance. This method attempts to partition the variance as to cause. The column headed by MS is comparable to variance (V or s^2) and is obtained by dividing the column SS by Df. The symbol SS literally means sum of squares, or sum of squares of deviations of individual values from their mean according to the following formula:

$$SS = Ex^2 = EX^2 - \frac{(EX)^2}{n}$$

The symbol Df refers to degrees of freedom or number of independent comparisons. It is usually one less than the number of items. Thus in this example the total number of items was 55 and the number of treatment

groups was eleven. Of the 54 degrees of freedom for total, 10 were between treatments and 44 were within treatment groups.

The test for significance in this analysis is known as the "f" test. This is the ratio of the error term (variation within the treatment groups) divided into the variance due to treatment. Theoretically if treatment did not affect the groups we should expect a ratio of 1:1. The probability of obtaining a ratio larger than this can be read from tables found in textbooks of statistics such as Snedecor (1946). The "f" value, in this case, was 322.519/18.032 = 17.88, which indicates a very high significance that can be attributed to treatments.

Table III gives the statistical constants of the experimental lots. This table indicates the locus of treatment, numbers of insects included, average length of life ($\bar{m}$), standard deviation (σ), and the standard error of the mean ($\sigma_{\bar{m}}$). The mean length of life was calculated by the short method,

$$\bar{m} = a + i\left(\frac{\Sigma fd'}{n}\right)$$

The standard deviation was calculated from the formula

$$\sigma = i\sqrt{\frac{\Sigma fd'^2}{n} - \left(\frac{\Sigma fd'}{n}\right)^2}$$

and the standard error of the mean was calculated from the formula

$$\sigma_{\bar{m}} = \frac{\sigma}{\sqrt{n}} .$$

Table I. Median lethal times for the treatment of different loci on adults of E. integriceps Put

Locus of treatment		LT_{50} in hrs.	D.C.*
1. Tip of proboscis	a.	0.53	0%
	b.	0.41	0%
	c.	1.0	0%
	d.	1.13	0%
	e.	1.41	0%
	av.	0.896	0%
2. Coxopleurite	a.	1.5	0%
	b.	1	0%
	c.	3.33	0%
	d.	1.25	0%
	e.	4.75	0%
	av.	2.36	0%
3. Metathoracic tarsus	a.	5.1	0%
	b.	5	0%
	c.	7.8	0%
	d.	6	0%
	e.	7.66	0%
	av.	6.31	0%
4. 1st antennal segment	a.	4.33	0%
	b.	8.16	0%
	c.	6.75	0%
	d.	10.66	0%
	e.	5.83	0%
	av.	7.14	0%
5. External genitalia of the female	a.	6	0%
	b.	8.1	0%
	c.	7	0%
	d.	8.2	10%
	e.	7	0%
	av.	7.26	2%
6. 6th abdominal spiracle	a.	7.5	10%
	b.	7.6	10%
	c.	8.75	0%
	d.	9.00	0%
	e.	20.00	30%
	av.	10.57	10%
7. 6th abdominal	a.	10.66	10%
	b.	10.00	10%
	c.	9.50	0%
	d.	21.00	0%
	e.	12.66	0%
	av.	12.76	4%
8. 6th abdominal tergite	a.	12.75	0%
	b.	6.00	40%
	c.	15.66	0%
	d.	27.00	30%
	e.	18.66	0%
	av.	16.01	14%
9. 5th-6th intersegmental membrane of sternum	a.	9	0%
	b.	26	10%
	c.	25.5	10%
	d.	18.8	20%
	e.	14.0	0%
	av.	18.66	8%
10. Vertex (of head)	a.	27.66	0%
	b.	16.4	0%
	c.	25.66	0%
	d.	20	0%
	e.	20.5	0%
	av.	22.04	0%
11. Scutellum	a.	28.66	10%
	b.	23.00	10%
	c.	22.66	0%
	d.	25.66	0%
	e.	27.33	0%
	av.	25.46	4%

*D.C. = The perdentage death in the controls when the LT_{50} was recorded for each separate replicate.

Table II. Analysis of variance of LT_{50} (in hrs.) for the treatment groups.

Source	Df.	SS.	MS.
Total	54	4018.62	
Treatments	10	3225.19	322.519**
Within treatments	44	793.43	18.032

Table III. Statistical Constants of the experimental Lots.

Lot		n^*	$\bar{m}$	σ	$\sigma_{\bar{m}}$
1	Tip of proboscis	49	1.71	1.2	0.17
2	Coxopleurite	49	3.61	4.23	0.604
3	Metathoracic tarsus	50	7.44	5.34	0.752
4	Female external genitalia	50	9.6	7.23	1.02
5	First antennal segment	50	10.54	9.60	1.35
6	Sixth abdominal spiracle	47	12.06	7.2	1.05
7	Sixth abdominal sternite	46	15.1	7.78	1.15
8	Sixth abdominal tergite	48	17.6	8.74	1.26
9	Fifth-sixth intersegmental membrane of the sternum	47	19.12	12.97	1.89
10	Vertex (of head)	49	22.89	8.22	1.17
11	Scutellum	48	27.91	7.83	1.13
12	Control	48	64.12	56.24	8.12

* A few insects were able to free themselves from the cards on which they were stuck; others were not affected by the treatment and had to be neglected.

VI. DISCUSSION AND CONCLUSION

The present study attempts to correlate the cuticular composition and the exoskeletal features of Eurygaster integriceps with their role in the entry and speed of action of parathion. The experimental work has been divided into two categories, viz., (1) histological examination of tissues, particularly sensory hairs and other peripheral sense organs, and (2) experimental application of parathion droplets at eleven different points on the insect's body.

The entry of parathion into the body of this insect causes clear-cut external symptoms of muscular non-coordination, excitation, convulsions, and paralysis. These definite physiological reactions take place in the sequence enumerated, terminating in the death of the insect. This latter event was taken as the criterion in judging the speed of entry of the insecticide into the different exoskeletal areas treated. The speed of penetration of parathion at the different points of application is hereafter termed their "effectiveness".

As has been indicated earlier, Table II shows the highly significant difference between the treated groups as compared to the variation within the groups (Probability <0.01). This is evidence that the treatments significantly lowered the $LT_{50's}$ of the treated lots. Further reference to Table I shows the order of "effectiveness" of the eleven different loci treated. These loci are given again below in order of decreasing effectiveness for the $LT_{50's}$:

1. tip of proboscis
2. coxopleurite
3. metathoracic tarsi
4. first antennal segment
5. female external genitalia
6. sixth abdominal spiracle
7. sixth abdominal sternite
8. sixth abdominal tergite
9. fifth-sixth intersegmental membrane of the sternum
10. vertex of head
11. scutellum

This is almost exactly the "effectiveness" indicated in Table IV which is based on the significance of the difference between means calculated for "t" values by Student's method.(5) The only difference is in the third category (Group III). The "effectiveness" of the female external genitalia and first antennal segment is reversed in the latter table.

Table IV, however, indicates the following:

1. The "effectiveness" of the tip of the proboscis was highest in permitting the penetration of liquid parathion as proved by the onset of its physiological effect. It constituted the only locus in Group I (Table IV).

2. This was followed by the area of the coxopleurite, which constituted Group II.

3. Group III included the metathoracic tarsi, the female external genitalia, the first antennal segment, and the sixth abdominal spiracle.

---

(5) The arbitrary limit of statistical significance has been taken at $P = 0.05$. This indicates a "t" value of approximately 2.0.

4. Next, the sixth abdominal sternite, the sixth abdominal tergite, and the fifth-sixth intersegmental membrane were included in Group IV.

5. Group V included the back of the head, and

6. Group VI comprised the scutellum, which showed the least "effectiveness" of all of the treated areas.

7. The Control was placed in Group VII because its "t" value with the scutellum (4.987) was greater than the "t" value between any two experimental groups.

However, the "t" values between (1) the sixth abdominal spiracle and the sixth abdominal sternite, and (2) the fifth-sixth intersegmental membrane of the abdomen and the back of the head show no significant differences, so that Groups III, IV, and V to some extent merge into one another. For this reason it is possible to divide the seven categories into five more comprehensive divisions namely: highly effective, Group I; effective, Group II; moderately effective, Groups III, IV and V; slightly effective, Group VI; and finally, the Control, Group VII.

The tip of the proboscis has a lining of columnar epithelium and is covered distally by a dense mass of fine chemoreceptors, or "taste hairs", that are highly innervated. Taste perception requires that the substance to be tasted should be in solution (Heilbrunn, 1952), and since the parathion was applied as fat-soluble droplets, it could quickly enter these hairs. If the substance is distasteful, an avoidance reaction will follow. Otherwise, it will be sucked in. During the experiments it was not possible to treat

the tip of the proboscis without touching the hairs. In fact, the contact of liquid parathion with these hairs was constantly observed to have stimulated the functioning of the buccal apparatus of the treated individuals. Due to the low surface tension of liquid parathion, the insecticide spread over the area of application, reached the food channel, and was sucked in. This fact is responsible for the very quick action of parathion on this locus.

The coxopleural area was found to be much more "effective" than any other locus in Groups III - VII (Table IV). In all cases when parathion droplets were applied at the base of the coxa, the insects moved their legs in an excited manner, and helped the quick conveyance of the insecticide to the coxopleural articular membranes, i.e., the coxal corium. This resulted in quick contact with an extremely large, and highly important nerve center, leading to paralysis and death. Therefore, the position of part of the fused thoraco-abdominal ganglion, directly underneath the coxopleural legs, and the quick penetration of parathion into the coxal cavity accounts for the effectiveness of this locus (Ball and Beck, 1951; Fisher, 1952).

The metathoracic tarsi were third in "effectiveness." The effectiveness of this locus can probably be explained by the presence of many hairs having thin walls that permit the quick entry and diffusion of parathion to the nerve branch to which their sense cells are connected (Figs. 14, 15).

The penetration of the insecticide at the female external genitalia was undoubtedly facilitated by the presence of sensory hairs at its posterior margin as well as by direct contact with the inner cuticular layers of the

vagina and their connection with the tenth nerve that innervates the genital organs (Trukhanov, 1947). Next in effectiveness were the first antennal segment and the 6th abdominal spiracle. However, the "t" values for the four loci in Group III do not show any statistically significant differences. A comparison of the 6th abdominal spiracle with its sternite gave an insignificant "t" value. This showed that the chemical did not act like a fumigant under the conditions of the experiment, or that the insect was capable of occluding the tracheal trunk leading from the 6th abdominal spiracle (Weber, 1930).

The three loci of application in Group IV failed to show any statistically significant values for "t".

However, the mean length of time required to kill the treated insects in the fifth category was 13.4 times greater than the tip of the proboscis.

The scutellum was the least effective of all the treated areas.

There are references in the literature on the possible importance of the thickness of the cuticle and entry or resistance to insecticides.

Klinger (1936) found that the steep increase in resistance of caterpillars of Bombyx mori, Vanessa io, and Laspeyrisia pomonella, as they mature to the last larval stage, may be correlated with a considerable increase in cuticular thickness. Wiesmann (1947) states that the resistant houseflies from Sweden had thicker cuticles than a susceptible strain from Switzerland. Wigglesworth (1948a) says that the thickness of the endocu-

ticle is important in slowing down the rate of entry of pyrethrum into the cuticle of Rhodnius.

On the other hand there are opposite views in the literature, amongst which are: Richards and Fan (1949) (see Review of Literature p. 4.) who found considerable variation in permeability of isolated cuticle but not variation in thickness. They warned against correlating variation in cuticular permeability with biological response. Ricks and Hoskins (1948) found that the rate of penetration of trivalent arsenic through the cuticle is proportional to the concentration of non-ionized arsenious acid except in the presence of fairly strong alkali that cause an unknown change in the protein constituents of the cuticle. Such a change alters the properties of the lipid layer of the integument as well as its permeability.

Certain loci which were not complicated by the presence of chemoreceptors were chosen for a study of possible relation between cuticular thickness and "effectiveness."

Individuals treated on the sixth abdominal sternite with an average cuticular thickness of 35.15 u, had an average length of life of 15.10 hours; those treated on the back of the head with an average thickness of 59.61 u, had an average of 22.89 hours; and those treated on the scutellum with an average thickness of 78.36 u, had an average of 27.91 hours.

These three loci showed a possible correlation between total cuticular thickness and the mean length of life after parathion application. It is clear that there are individual variations in susceptibility and other

factors that may affect such measurements.

This work indicates that the mean length of life of treated individuals of E. integriceps depends on:

1. the position of the locus in relation to the fused thoraco-abdominal ganglion,
2. a possible relation with columnar epithelium beneath the treated locus,
3. the concentration of chemoreceptor hairs, sensillae, and other setae, and
4. the possibility of a relation with total cuticular thickness.

Table IV. Groups of "Effectiveness", and "t" Values between Groups

Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
		6. 6th abdominal spiracle				
		"t" = 0.88				
		5. 1st antennal segment	9. 5th-6th intersegm abdom. membrane			
		"t" = 0.55	"t" = 1.90 "t" = 0.67			
		4. ♀ external genitalia	8. 6th abdominal tergite	"t" = 1.69		
		"t" = 0.05	"t" = 1.46			
1. tip of proboscis	2. Coxopleurite	3. Metathoracic tarsus	7. 6th abdominal sternite	10 Back of head	11. Scutellum	12. Control
	"t" = 3.06	"t" = 3.91	"t" = 3.62	"t" = 4.86	"t" = 3.08	"t" = 4.987

VII. SUMMARY

1. This work attempts to correlate the cuticular composition and the exoskeletal features of Eurygaster integriceps with their role in the method of entry and speed of action of parathion.

2. In beginning this work, the first few weeks were spent in the detailed, microscopical examination of tissues, particularly sensory hairs and other peripheral sense organs.

3. The second half of the work consisted of the experimental application of parathion at eleven different points on the insect's body.

4. Parathion droplets were obtained by using a syringe-micrometer attachment. The average weight of one droplet obtained by this apparatus was 8 ugm. (one hundred drops weighed), and the parathion was 98% pure.

5. Batches of ten adults were subjected to one treatment. Each treatment was attempted five times giving a total of fifty individuals per treatment.

6. The analysis of variance of LT_{50} indicates a very high significance that can be attributed to treatments.

7. The speed of penetration of parathion at the eleven different loci was termed their "effectiveness".

8. There were seven categories of effectiveness, viz.,

a. The effectiveness of the tip of the proboscis was highest in permitting the penetration of liquid parathion as proved by the onset of its physiological effect. It constituted the only locus in the first category.

b. The effectiveness of the coxopleurite was much greater than any other locus in groups III-VII.

c. The third category included the metathoracic tarsi, the female external genitalia, the first antennal segment, and the sixth abdominal spiracle.

d. Next, the sixth abdominal sternite, the sixth abdominal tergite, and the fifth-sixth intersegmental membrane were included in the fourth category.

e. Group V included the back of the head, and

f. Group VI comprised the scutellum, which showed the least effectiveness of all the treated areas.

g. The control was placed in group VII because its "t" value with the scutellum (4.987) was greater than the "t" value between any two experimental groups.

9. The significance of the difference between means was calculated for "t" values by Student's Method.

VIII. ACKNOWLEDGEMENT

The experimental work was done under the direction of Dr. Kenneth Christiansen to whom I wish to express my sincere thanks. I am greatly indebted to Dr. Harry M. Smith, Chairman of my Committee, under whose direction the thesis was written.

Thanks are extended to my colleagues in the School of Agriculture, visiting Professor Hassan A. El-Tobgy and Professor Maurice Shelton, for their help in the statistical analysis; to Mr. H. Artenian who photographed the drawings for this work; to Mlle Raymonde Romailler who supplied me with most of the original Swiss and German articles; and to Dr. John Mirhij, Chairman of the Biology Department, for helpful advice and criticism.

I am especially grateful to Dean S. W. Edgecombe who kindly arranged a grant in aid for me to defray the expenses of my tuition during the two years' work.

Finally I wish to thank the clerical staff of the School of Agriculture for typing this thesis.

IX. BIBLIOGRAPHY

1. Adle, A. H. - Persia Insect Pests. International Review of Agriculture, XXIII (1) Rome, (1932).

2. Alekseev, Ya. A. - The biological Control of the noxious Corn Bug E. integriceps Put. by means of Egg Parasites. Bull. Plt. Protection (4) 81-88. Leningrad (1940). Abstract in English R.A.E. (A) 30 : 299 - 300, (1942).

3. Alexandrov, N. - Eurygaster integriceps Put. à varamine et ses parasites. Entom. et phytopath. appliquées. (5,6,7,8) Tehran, (1948).

4. Armstrong, G., Bradbury, F. R., and Standen, H. - The Penetration of the Insect Cuticle by Isomers of Benzene Hexachloride Ann. appl. Biol. 38 (3): 555 - 566 London (1951). R.A.E. (A) 40: 37, (1952).

5. Balachowsky, A. et Mesnil, L. - Les Insectes nuisibles aux Plantes cultivees p. 1093-6. L. Mery, ed. Paris, 1935.

6. Ball, H. J. and S. D. Beck - The Role of the Circulatory and Nervous Systems in the toxic Action of Parathion; Journ. Econ. Ent. 44 (4): 558 - 564, (1951).

7. Distribution Maps of Insect Pests. Eurygaster integriceps, Put. (40) Commonwealth Institute of Entomology. London, (1954).

8. Dresden, D. and B. J. Krijsman. - Physiological Action of Contact Insecticides. Bull. ent. Res. 38: 575 - 578, (1948).

9. Fedotov. D. M. - Relations between E. integriceps and Phasiid Flies parasitic on the Bug, and Phasiids as Means for controlling it. C. R. Acad. Sci. URSS (N.S.) 43 (3): 134 - 136. Moscow, (1944). Abstracted R.A.E. (A) 33: 301 - 2, (1945).

10. Fernando H. E., C. C. Roan, and C. W. Kearns. - The Penetration and Metabolism of organic Phosphates in the American Roach, Periplaneta americana (Linn.) Ann. ent. Soc. Amer. 44 (1951) (4) pp. 551 - 565, Columbus, Ohio, 1952. R.A.E. (A) 40 pp. 386-7, (1952).

11. Fisher, R. W. - The Importance of the Locus of Application on the Effectiveness of DDT for the House Fly, Musca domestica L. (Diptera : Muscidae) Canadian Journal of Zoology, 30 : 254-266. August, (1952).

12. Goulden, C. H. - Methods of Statistical Analysis. 2nd ed. John Wiley & Sons, Inc., New York, 1952.

13. Heilbrunn, L. V. - An Outline of General Physiology. pp. 671-3. W. B. Saunders Co., 3rd ed. Philadelphia, 1952.

14. Hibrawi, M. - Contribution a l'etude biologique et systematique de Eurygaster integriceps Put. en Syrie. Rev. Path. veg. Ent. Agr. 17: 97-160, (1930).

15. Hickin, N. E. - Mode of Entry of Contact Insecticides. Nature 156: 753-4 (1945).

16. Klinger, H. - Die insektizid Wirkung von Pyrethrum und Derrisgiften und ihre Abhangigkeit von Insektenkorper. Arb. physiol. angew. Entom. Berl. 3 (1-3): 49-69, 115-151, (1936). R.A.E. "A" 24: 454-6, (1936).

17. Kretowich, W. L., Bundel, A.A., & Pshenova, K.V. - Mechanism of Wheat Injury by E. integriceps C. R. Acad. Sci. URSS (N.S.) 39 (1): 31-33, Moscow (1943). R.A.E. "A" 32: 160-161, (1944).

18. _____, and Tokarewa, R. R. - Character and Identification of Injury to Grain by Pentatomids. Mullerei u. Elevatorwirtsch. (1940). R.A.E. "A" 31: 38, (1943).

19. Kuznetzov, K. P. - The Effectiveness of Applications of certain Chemical Preparations in the Control of the Little Tortoise Bug. (1950) R.A.E. "A" 41: 9-10, (1953).

20. Liang, H. M. - A new Method for staining Nerves and their endings using the Schiff reaction. Anat. Rec. 99: 511, (1947).

21. McClung, R. J. - Microscopical Technique. p. 37 3rd ed. Paul B. Hoeber, Inc., New York, 1950.

22. Marushev, A. I. and Grivanov, K. P. - The Effect of Injuries caused by Eurygaster integriceps Put. on the baking properties of Wheat. Social Grain Fmg. 4: 187-191 Saratov (1938) R.A.E. "A" 27: 554-5, (1939).

23. Meir, N. F. - Parasites hatched in USSR in 1938-1939 out of the Eggs of the Corn Bug E. integriceps Osch. Bull. Plant Prot. 1940 3: 79-82, Leningrad (1940). R.A.E. "A" 30: 240, (1942).

24. _____.- Results of the Work of the Laboratory of biological Control at the Institute of Plant Protection. Lenin Acad. Sci.: 121-124. Leningrad (1940). R.A.E. "A" 29: 582, (1941).

25. O'Kane, W. C., L. C. Glover, R. L. Blickle, and B. M. Parker - Penetration of Certain Liquids through the Pronotum of the American Roach. Studies of Contact Insecticides XIV. New Hampshire Ag. Exp. Sta. Tech. Bull. (74), (1940).

26. Paikin, D. M. - The Results of the Testing of Thiophos for the Control of the Noxious Little Tortoise. (1950) R.A.E. "A" 41: 52, (1953).

27. Peredelskii, A. A. - Biological Foundations of the Theory and Practice of the Control of E. integriceps. Moscow, Akad. Nauk USSR 2: 89-270, (1947) R.A.E. "A" 40: 309-311, (1952).

28. Pfaff, W. - Investigation of the Construction of the Cuticle of Insects and of the Mechanism of Penetration by the Contact Insecticide E 605. Hofchen-Briefe 5 , (3): 93-160, (1952).

29. Potts, W. H. and F. L. Vanderplank. - Mode of Entry of Contact Insecticides. Nature 156: 112. (1945) Biol. Abst. 20, Item 4002, (1946).

30. Richards, A. G. - The Integument of Arthropods. p. 315-317. University of Minnesota Press, Minneapolis, 1951.

31. _____, and H. Y. Fan. Studies on Arthropod Cuticle V. The Variation in Permeability of Larval Cuticles of the Blowfly Phormia regina. Journ. Cell and Comp. Physiol. 33 (2): 117-198. 1949. Biol. Abst. 24, Item 5291. (1950).

32. Richardson, C. H., L. H. Glover, and L. O. Ellisor. - Penetration of Gaseous Pyridine, Piperidine and Nicotine into the Bodies of certain Insects. Science, 80:76-77 (1934).

33. Ricks, M. and W. M. Hoskins. - Toxicity and Permeability. 2. The entrance of arsenic into larvae of the flesh fly, Sarcophaga securifera. Physiol. Zool., 21: 258-272, (1948).

34. Rubtzov, I. A. - On two Species of the Family Phasiidae (Diptera) parasite on Eurygaster integriceps Put. (Hemiptera). Rev. Ent. USSR 28 (3-4) pp. 85-199 Leningrad, (1947) R.A.E. "A" 36: 241. (1948).

35. Savit, J., J. J. Kollros, and J. M. Tobias. - Measured Dose of y-BHC required to kill Flies and Cockroaches, and a Comparison with DDT. Proc. Soc. Exptl. Biol. and Med. 62 (1): 44-48. 1946. Biol. Abst. 20. Item 18888, (1946).

36. Shafer, G. D. - How Contact Insecticides kill. Part 3. Michigan Agri. Coll. Expt. Sta. Tech. Bull. 21, 1915. R.A.E. "A" 3: 691-2, (1915).

37. Skvortozov, A. A. - On the Permeability of Insect Integuments for Contact Insecticides. Advances Mod. Biol. 21 (2) pp. 249-256, Moscow (1946). R.A.E. "A" 37, 43-4 (1948).

38. Snedecor, G. W. - Statistical Methods. Collegiate Press, Ames, Iowa, 1946.

39. Snodgrass, R. E. - Principles of Insect Morphology. p. 69 McGraw-Hill Book Co., Inc., New York, 1935.

40. Starostin, S. - Control of Eurygaster integriceps Put. by Aircraft. Proc. Lenin Acad. Agric. Science, USSR 9, pp. 25-27, (1944). R.A.E. "A" 33: 303-304, (1945).

41. Talhouk, A. S. - The Effect of Parathion Dusts on Eurygaster integriceps Put. (Hem.). Bull. Ent. Res. 42 (2): 375-377 London (1951). R.A.E. "A" 40: 2, (1951).

42. _____. - Further Trials with Parathion Dusts against Eurygaster integriceps Put. (Hemipt., Pentatomidoe). Bull. Ent. Res. 45 (3): 495-500, (1954).

43. Tischler, N. - Studies on how Derris kills Insects. J. Econ. Entom., 28: 215-220, (1935) Biol. Abst. Item 6515, (1936).

44. Trukhanov, I. F. - Contribution to the Morphology of E. integriceps. Akad. Nauk SSSR, 2 (1): 10-34, Moscow (1947).

45. Tzitovich, I. K. and Snitko, Yu. S. - The Susceptibility of Phases of one Generation of E. integriceps to DDT. (1950. R.A.E. "A" 40: 94-5, (1951).

46. Vasiliev, J. V. - La Tortuettle nuisible Eurygaster integriceps (Osch.) Put. et les nouvelles Methodes de Lutte contre elle avec l'aide de ses Parasite du Monde des Insectes. Travaux Entomologiques du Comite Savant. Ministere d'Agriculture. St. Petersbourg, (1913).

47. Wall, G. L. - Hot Celloidin Technique for Animal Tissues. Stain Technology. 7 (4): pp. 135-145. (1932).

48. Weber, H. - Biologie der Hemiptern. Verlag von Julius Springer. Berlin, (1930).

49. Wiesmann R. - Untersuchungen uber das physiologische Verhalten von Musca domestica L. verschiedener provenienzen. Schweiz. Entomol. Gesell. Mitt. 20 (5): 484-504, (1947).

50. _____. - Untersuchungen uber die Eintrittspforten des Dichlordiphenyltrichloraethan (DDT) in den Insektenkorper. Verh. schweiz. naturf. Ges. 126: 166-167 (1946). Aaran, 1946. R.A.E. "A" 37, p. 115. (1949).

51. _____. - Die Eintrittspforten des p, p-Dichlordiphenyl trichlorathans am Insektenkorper. Mitt. schweiz. ent. Ges. 32, (3): 257-291. Lausanne (1949).

52. Wigglesworth, V. B. - The Site of Action of Inert Dusts on certain Beetles infesting Stored Products. Proc. Roy. Ent. Soc. (London) 22: 65-69. (1947).

53. _____. - The Insect Cuticle. Biol. Reviews, 23: pp. 408-451. (1948a).

54. _____. - Mode of Action of new Insecticides. Rpt. fifth Commonwealth entomological Conference. London (1948b).

55. Zwolfer, W. - Beitrage zur Kenntnis der Schadlingsfauna Kleiasiens I. Zeitschr. ang. Ent. 17: 227-252. Berlin, (1930).

X. APPENDIX

List of Figures

Explanation of Abreviations

Abbreviation	Explanation
an	antennary nerve
cc	columnar cells

cec	columnar epithelial cells
cm	comb
cp	conical projection
d	terminal strand
dg	dermal gland
end	endocuticle
epd	epidermis
exct	exocuticle
l	auxilia
m	muscle
n	nerve
odg	opening of dermal gland
p	pit
pg	peg
ps	protoplasmic strand
pv	pulvillus
s	seta
sc	sense cell
sh	sensory hair
ss	sensory seta
tar	tarsi
tb	tibia
tf	tonofibrillae

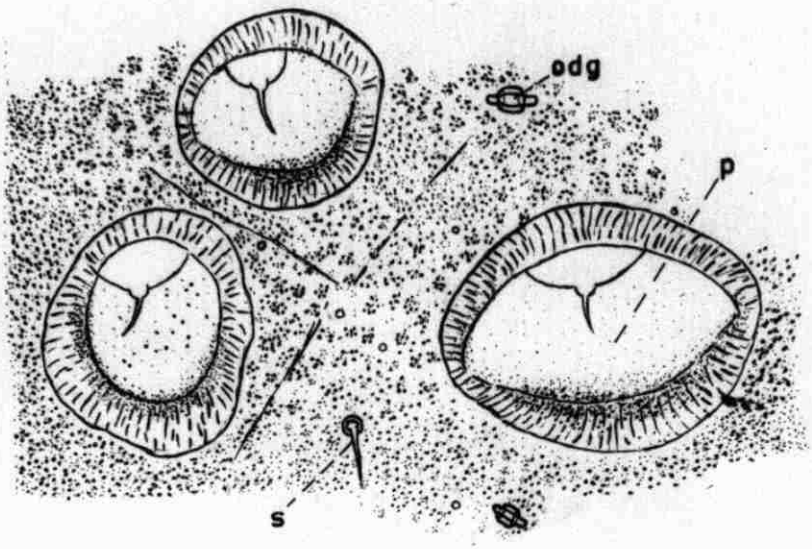


Fig. 1

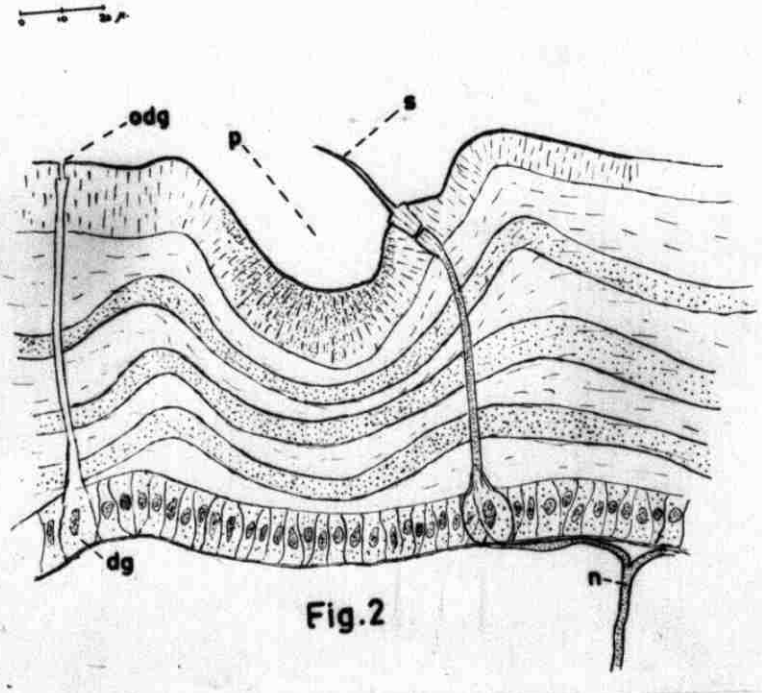


Fig. 2

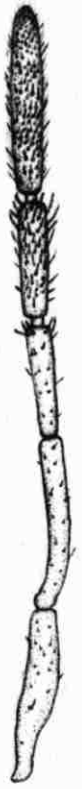


Fig. 3

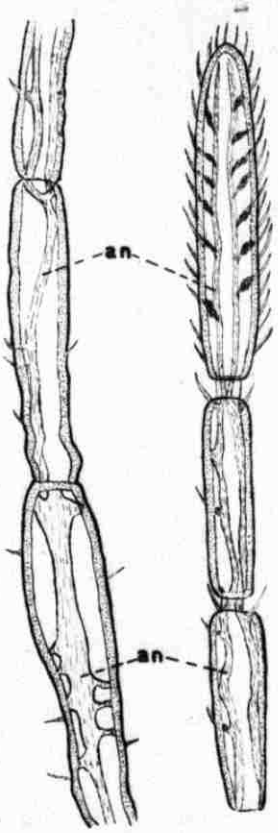


Fig. 4

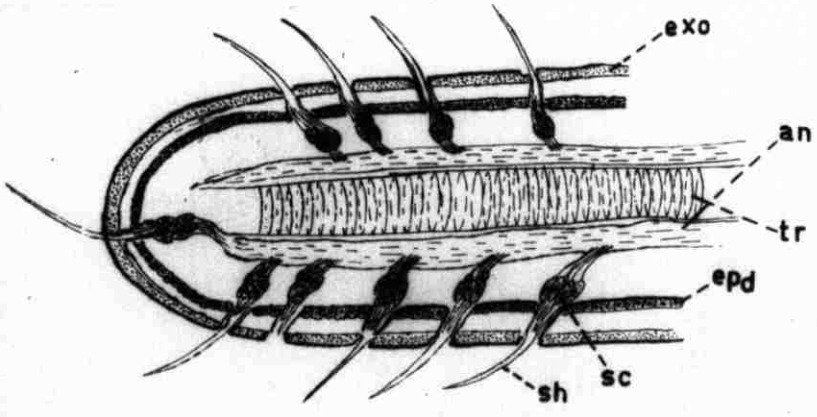


Fig. 5.

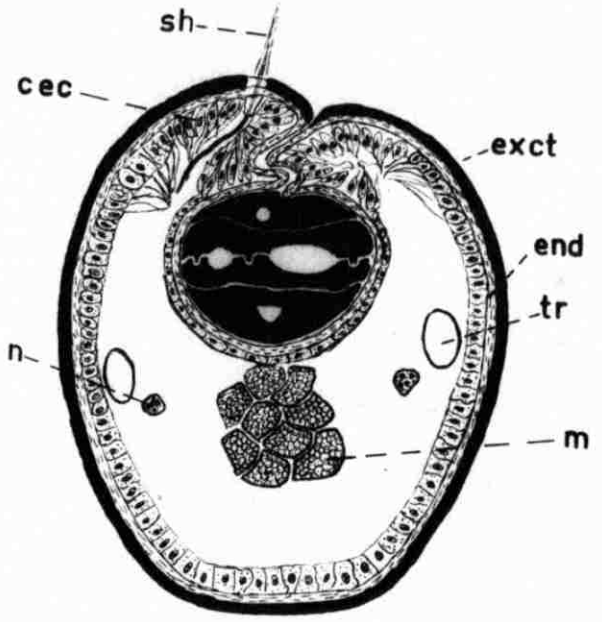


Fig. 6

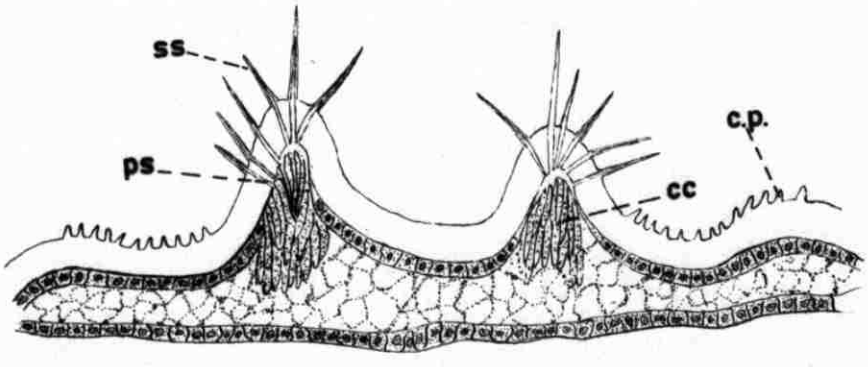


Fig. 7

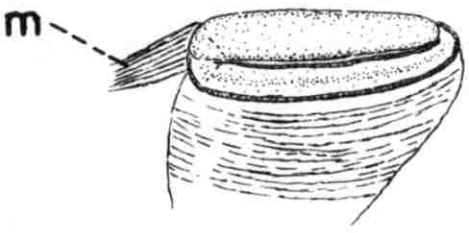


Fig. 8.

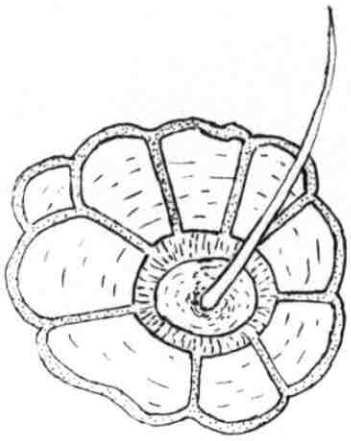
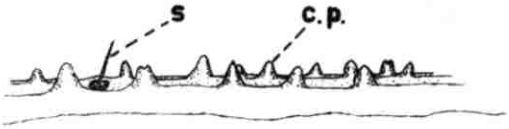
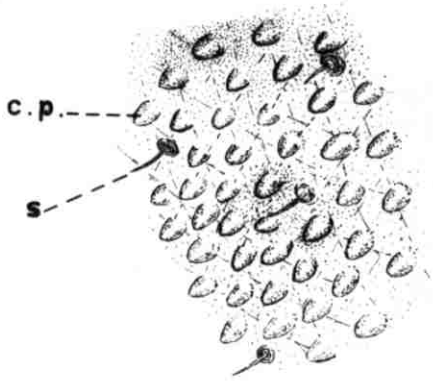


Fig. 8a





Figs. 9 & 10

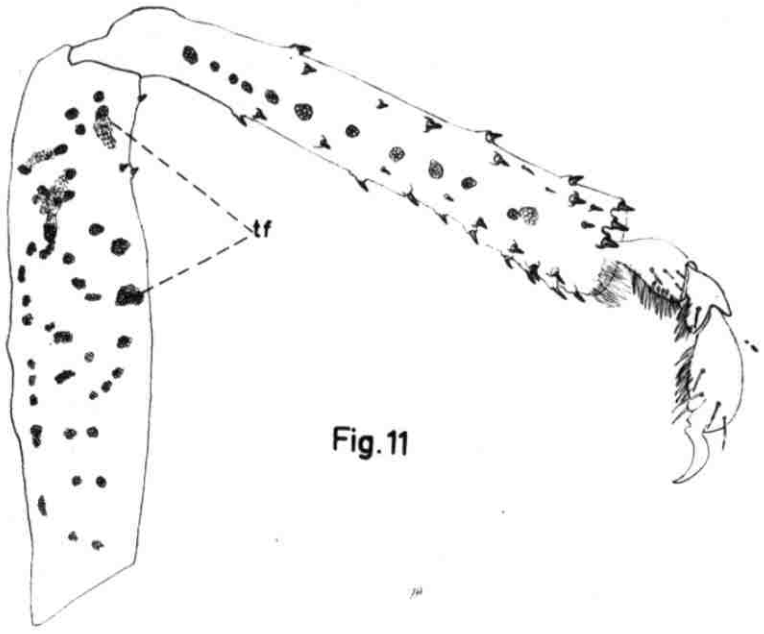


Fig. 11

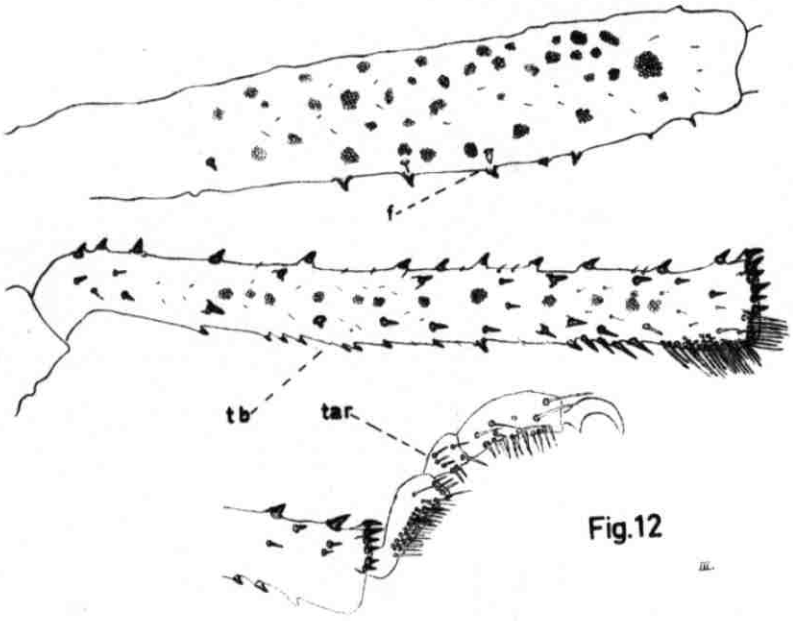


Fig. 12

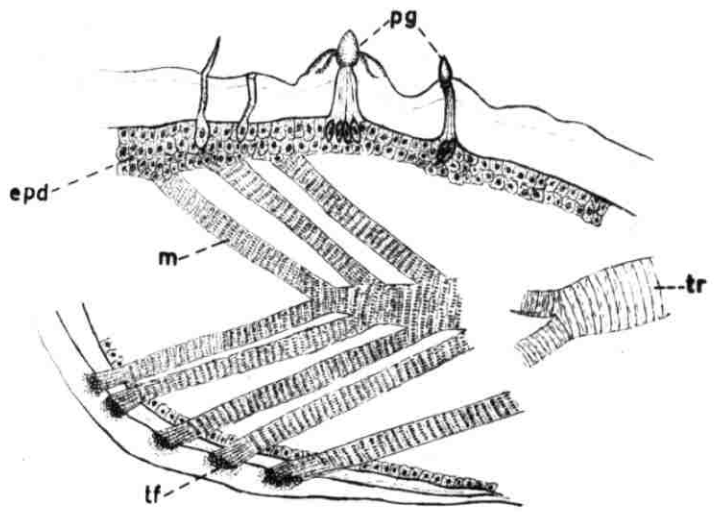


Fig.13.

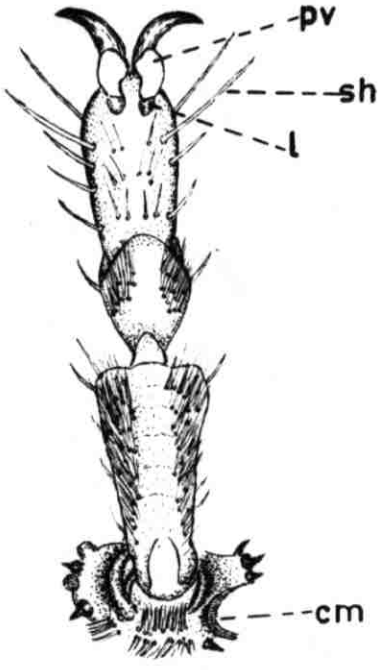


Fig. 14

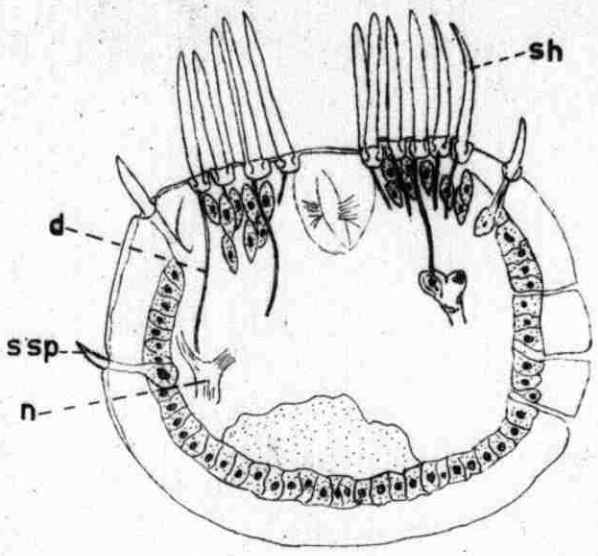
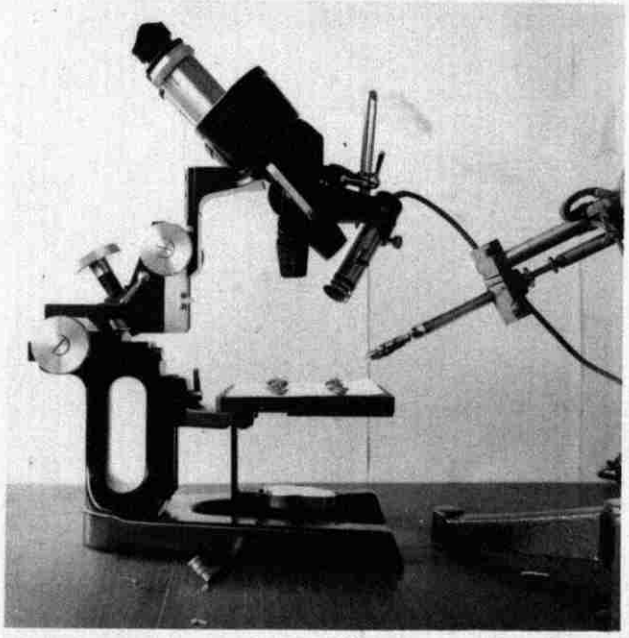


Fig. 15.



Fig, 16.