THE PECAN WEEVIL LARVA

CURCULIO CARYAE (HORN)

STUDIES ON ITS MORPHOLOGY AND BIOLOGY

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By

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BIOGRAPHY

Ferdinand Kern was born on the fourteenth of April, 1923, at Zürich, Switzerland. After eight years in grade school, he entered the cantonal Oberrealschule in Zürich, where he studied for three years. At that time, due to severe illness he was forced to leave school for a few months to recover, after which time he entered Professor Tschulok's Institute in Zürich. He passed the external federal examination, the "Matura," in September, 1943. This examination is an equivalent of the American college entrance examination.

As a prerequisite for entering the Agricultural Department of the Federal Institute of Technology, a practice period of at least six months on a farm is required. This requirement was fulfilled from September, 1943, to February, 1944. This was followed by a term of military training from February, 1944, to August, 1944. In October, 1944, he enrolled at the Agricultural Department of the Federal Institute of Technology in Zürich, from which he graduated as an Agricultural Engineer in May, 1948.

During the winter semester 1947-48 he began special studies in Entomology under Professor O. Schneider-Orelli. In the spring of 1948 he registered as a candidate for the Doctorate at this Institute, working principally on biological studies of the Brachkäfer, Amphimallua solstitialis (Scarabaeidae, Coleopt.)

In July, 1948, he was awarded an Exchange Scholarship at the Oklahoma Agricultural and Mechanical College in Stillwater to pursue postgraduate work.

The following thesis is the result of his special studies on the pecan weevil, Curculio caryae (Horn) during the fall semester of 1948 and the spring semester of 1949.

ACKNOWLEDGEMENTS

At this occasion I would like to give my thanks to the Committee for Exchange Scholarships and its Chairman, Dr. G. B. Hawkins, for the opportunity given to me to study at the Oklahoma Agricultural and Mechanical College.

The writer wishes to express his appreciation and thanks to Professor F. A. Fenton, Head of the Department of Entomology for the help and suggestions he gave to make my studies successful, to Dr. D. Ashdown, under whose directions these studies were made, for his generous help and assistance in conducting the research and to G. A. Bieberdorf for the leaving of data and his assistance in the photographic laboratory.

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INTRODUCTION

The pecan weevil, Curculio caryae (Horn) belongs to the order Coleoptera, family Curculionidae, which composes the typical snout beetles. According to Comstock (1940) this family is divided into thirteen subfamilies. Curculio caryae (Horn) belongs to the largest subfamily, the Curculioninae.

Studies of the pecan weevil larva were undertaken because of its economic importance as a destructive insect on pecan and hickory, and because very little is known about the morphology and biology of the larva.

The pecan weevil occurs all over the pecan growing area in Oklahoma and causes severe damage both by the feeding of the adult weevil as well as the larva on the kernels of the nuts.

Control measures against the adult weevil have been found, but no efficient control of the larval stage has been developed. Recommendations for the chemical control of the weevil are the application of a spray, using 6 pounds of 50 percent DDT wettable powder per 100 gallons of water. Applications are made when an average infestation exceeds 6 adult weevils collected per tree by the jarring method.

Life history of the pecan weevil, Curculio caryae (Horn).

The eggs of the pecan weevil are inserted into the nut during September and October. Upon hatching the larva feeds on the kernel and develops entirely on this source of nourishment. After reaching maturity in the fifth instar the larva eats a hole through the shell and husk, drops to the soil and burrows into the ground. There is no definite time for this movement from the nut into the soil. Larvae are found leaving the nuts as early as October, although larvae can be found in nuts as late as the following March.

After a diapause period of at least 14 months the first adult weevils

can be found in the soil. This appearance occurs in the winter time and the adults apparently remain in the soil until July, when they emerge to feed on young kernels. Other larvae remain in diapause for a longer period.

In general the pecan weevil has a two-year life cycle which means that a generation is completed in an average period of two years.

Characteristics of the pecan weevil larva.

The larva of the subfamily Curculioninae is principally characterized by the following characters:

1. a soft, white-yellowish, cylindrical body
2. legless (apodous)
3. a complete chitinized head capsule
4. absence of compound eyes, ocelli and antenal structures.

METHODS AND MATERIAL

1. SOURCES OF LARVAE

Larvae of the pecan weevil were collected several times in various stages of development. The first collection was made from infested nuts, collected at the Adams' orchard south of Stillwater on September 22. At this date samples of infested pecan nuts were taken from trees of the Stewart variety, which was the most infested one in this orchard. A second collection was made on October 10 and a third on November 6.

Other larvae were obtained by collecting the soil infesting or last larval instar found in the soil beneath infested trees. All these larvae were placed in moist soil in bottles, each of which contained 60 specimens.

2. PREPARATION METHODS

a. Preparation for Dissection.

Due to the unusually high fat content of the pecan weevil larva special techniques had to be devised for morphological and histological studies. No fat solvents were used in studies of the digestive system.

Larvae were killed in 95 percent alcohol and retained in this solution for 15 minutes to harden the body slightly. For the dissections the larva was then transferred into 30 percent alcohol. This technique was particularly suited to a study of the fat storing organs, and to obtain some information on the utilization of the fat. For permanent slides, the dissected parts were carried back into distilled water and preserved and mounted on the slides in poly-venyl alcohol. (PVA).

For other preparations, especially for histological studies, the fat was removed. The larva was killed by direct injection of absolute petroleum ether

into the larval body. Dissected parts to be mounted were run through the alcohol-xylene process. After dissection in 30 percent alcohol, such parts were treated as follows:

30 min. in 70 percent alcohol
15 min. in 85 percent alcohol (this step deleted in case of thin parts)
10 min. in 95 percent alcohol
immersion in xylene until the whole part was cleared.

The cleared tissues were then mounted on slides in xylene-Canada-balsam. (XCB).

b. Preparation for Sectioning.

The larva was killed in 70 percent alcohol. After complete dehydration of the body through the alcohol series, it was cleared in xylene. To insure better penetration of the paraffin in which the larva was embedded for sectioning, xylene was mixed with the same amount of technical acetone. The larva was then placed in pure acetone to which melted paraffin was slowly added. This mixture, containing the larva then was put into melted paraffin, to which a small amount of bees wax had been added to obtain better sectioning properties. The paraffin was kept at a temperature slightly above the melting point for a period of 30 minutes before being poured into paper blocks for the embedding. These blocks were hardened for 24 hours in running tap water.

c. Sectioning.

The block was sealed on a wooden carrier which was then fastened to the microtome. The microtome used was type No. 445 of the Spencer Lens Company of Buffalo, New York. The sections were made with a blue Gillette razor blade. The thickness of the sections was seven microns.

Special care had to be exercised in the temperatures of the paraffin block and the blade when sectioning was done. Best results were obtained with the following temperatures:

Paraffin 15 - 20°C.

Razor blade 30 - 35°C.

At these temperatures the different sections remained together, forming a ribbon without curling. Another very important factor in obtaining straight ribbons was to have the surfaces of the block even, especially the ones parallel to the blade.

d. Mounting and Staining of the Sections.

After the ribbons of tissue were cut, they were fixed to the slides with albumen-glycerine. A slight warming of the slides over the flame of a Bunsen burner flattened the sections to the surface of the slide. The paraffin was then removed from the sections with xylene. For staining of the tissue in borax-carmine (alcoholic) the sections had to be hydrated again. This was done by carrying them back through the alcohol series into 70 percent alcohol. After this hydration the stain was able to react with the tissue. Excess borax-carmine was washed out with distilled water and the sections brought through the alcohol series for dehydration. For the final preservation the slides were put into xylene and the cover glass mounted with xylene-Canada balsam.

Process of preparing, staining and mounting of the sections in figures:

Preparation:	Larva in	70% alcohol	15 minutes twice
		85 % alcohol	10 minutes
		95 % alcohol	10 minutes
		100 % alcohol	5 minutes
		Xylene	15 minutes
		Xylene:Acetone 1:1	10 minutes
		Acetone techn.	15 minutes Paraffin add.
		Paraffin liquid	30 minutes
		Paraffin block	24 hours

Sectioning:	Temperatures	Paraffin bloc	15 - 20°C
		Razor blade	30 - 35°C
Staining:	Sections in	Xylene	30 minutes
		95 % alcohol	2 minutes
		70 % alcohol	10 minutes
		Borax-carmine alc.	6 hours
		H_2O dist.	15 minutes
		70 % alcohol	15 minutes
		95 % alcohol	5 minutes
		100 % alcohol	1 minute
Mounting:		Xylene	5 minutes
		Xylene-canadian balsam	

e. Technique of Fat Extraction.

To obtain information as to fat deposition and its relationship to the food intake phase of the larvae, a series of fat extraction tests was conducted, using larvae collected on the following dates:

1. From nuts collected on the 10th of October.
2. From the soil, about 5 months after the larvae had emerged from the nuts.
3. From the soil, about 16 months after the larvae had emerged.

The groups of larvae collected at these times were dehydrated as described on page 4 in the alcohol series. The dry weight was then measured. The fat was extracted in a Soxlet extractor, using absolute petroleum ether. The extraction was continued for four hours at 55°C. Next the ether was evaporated, without vacuum, for one hour at 60°C. The residue, containing the extracted fat compounds (raw fat or ether soluble compounds), was weighed on a torsion balance, type 269 of the Torsion Balance Company, New York. Then the rest of the extracted larval tissue was also weighed. By weighing both, the larval body and its extracted fat residues, a more exact estimate of the body constituents was obtained.

A summary of the process is as follows:

Dehydration:	Larva in	70 percent alcohol	48 hours
		95 percent alcohol	48 hours
		100 percent alcohol	24 hours
Extraction:	in petroleum ether (absolute)		4 hours at 55°C
	evaporation		1 hour at 60°C

The following weights were measured:

a. bottle empty
b. dehydrated larvae (dry weight)
c. bottle plus ether soluble compounds
d. Larvae minus ether soluble compounds

Calculation of weights:

The content of ether soluble compounds (E.C.) was calculated as follows: the letters refer to the respective letters under the part: weights measured

1. $\frac{(b - d)\ (c - a)}{2} = E.\ C.$

2. $\frac{E.\ C.}{b} \cdot 100$ = percent raw fat content

This gives the content of ether soluble compounds (raw fat) in percentage of the dry weight of the larvae.

PART I

MORPHOLOGY OF THE LARVA

1. EXTERNAL MORPHOLOGY

a. The Head and its Appendages.

The eyeless head capsule of the pecan weevil larva is completely chitinized. Nevertheless, the coronal suture and the two frontal sutures are clearly visible. The frontal sutures are divided in their distal part again into two short sutures which circle the cranial articulation of the mandibles. (Fig.1).

Only a few bristles can be found on the head capsule. Five of them are located on each gena, two symmetrically on the frons.

There are no compound eyes; the larva lacks even the ocelli.

The mouthparts are of the biting-chewing type and are the only appendages on the head. No antennal structures are present.

The labrum is partially divided in its distal part into three lobes, the central of which is slightly longer than the outer ones. The external surface of the labrum is chitinized, while the internal surface is membranous and extends from the lobes into the preoral cavity, forming the epipharynx.

The very hard and strongly chitinized, double-toothed mandibles show the characteristic articulation of the typical mandibulate type of mouthparts. (Fig. 2).

In contrast to the mandibles, the maxillae appear rather weak. The cardo and the stipes are open, allowing space for the muscles entering the maxillae from the head. The short palpus consists of two segments. The ultimate segment rises slightly above the lacinia and bears on its distal end some short sensory palpi.

PLATE I

THE HEAD OF THE LARVA

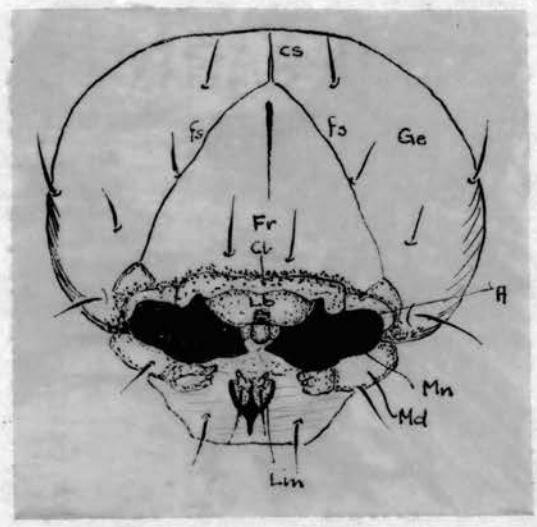

Fig. 1. - Head of the pecan weevil larva.

A articulation of mandibles
cs coronal suture
fs frontal sutures
Fr Frons
Ge Genae
Cl clypeus
Md mandibles
Mx maxillae
Lb labium
Lm labrum

PLATE II

MANDIBLE

Fig. 2. - Mandible of the larva.

A Articulation

The lacinia curves slightly toward the galea and bears on its dorsal surface a row of seven setae. There are two long bristles on the galea and one on the dorsal side of the stipes. (Fig. 3).

The labium is surrounded by a strip of chitinized tissue, which itself is divided by a second strip of chitin. The three branches of this chitinized part surround the two small two-segmented palpi. Two bristles are found between the palpi and the forklike chitinized part of the labium. (Fig. 4).

b. The Larval Body.

The larval body is, with the exception of the nine pairs of spiracles and the dorsal region of the first thoracic segment, not sclerotized. The more or less leathery body wall shows no appendages. Spiracles are found only on the first thoracic and on the first eight abdominal segments. The meso-and metathorax and the last three segments do not have respiratory openings.

On each segment are found eight bristles, six of which are on the dorsal part. These are reduced on the last two segments of the abdomen to two. Two bristles are found on the ventral side of each segment.

PLATE III

MAXILLA

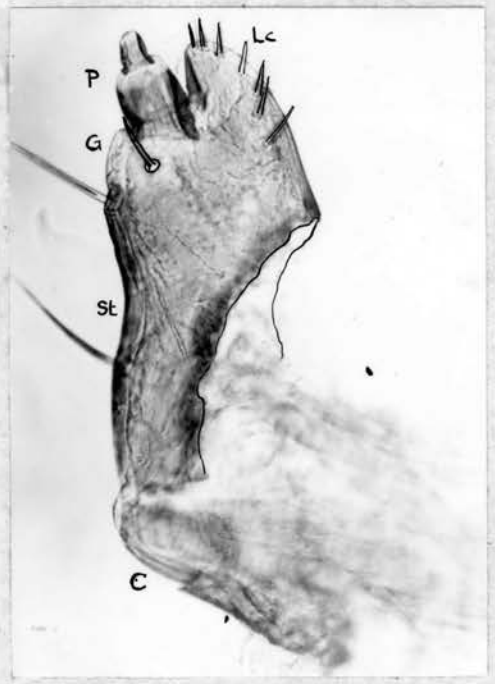

Fig. 3. - Maxilla of the larva.

C cardo
G galea
Lc lacinia
P palpus
St stipes

PLATE IV

LABIUM

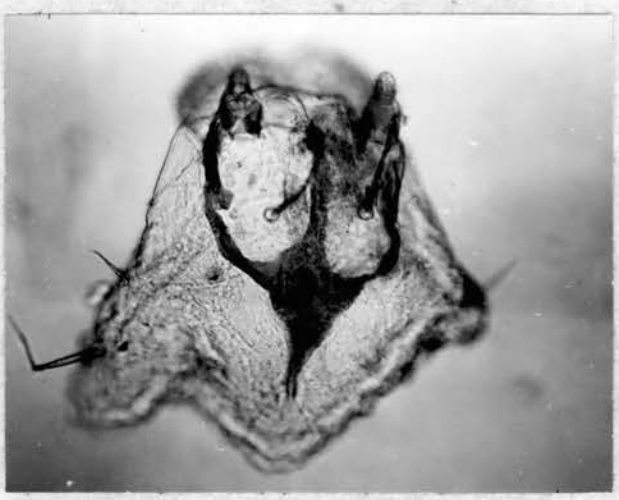

Fig. 4. - The labium of the larva.

PLATE V

THE BODY OF THE LARVA

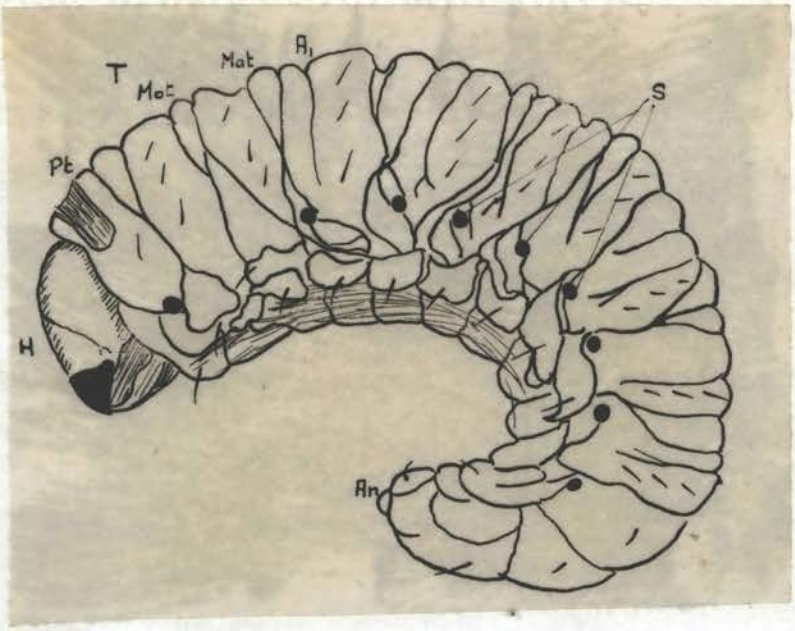

Fig. 5. - The larval body.

A_1	first abdominal segment
An	anus
H	head
S	spiracles
T	thorax
Mat	metathorax
Mot	mesothorax
Pt	prothorax

2. INTERNAL MORPHOLOGY

The internal morphology and anatomy of the pecan weevil larva shows some features of specialization, when compared with the morphology of the typical coleopterous larva. These specializations are principally involved in the digestive system and may be associated with the rather unusually nutritious food. The mass of the body cavity is filled with three sacs of a fat storing tissue, where the fat is stored by the larva during the actual feeding period. The digestive system, as well as the respiratory and nervous system are surrounded by and embedded in these fat bodies.

a. The Digestive System.

The first part of the alimentary canal conforms morphologically with that usually found in coleopterous larvae stomodaeum. From the buccal cavity the gut narrows down to the esophagus and in terms is enlarged into the crop. There is no distinct proventriculus noticed. The mesenteron or ventriculus, posterior to the crop is not enlarged, but is of approximatively the same size as the esophagus. Besides the size, there is no obvious differentiation between stomodaeum and mesenteron. The Malpighian tubules are six in number and originate at the junction of the mid and the hind gut. These tubules are in their proximal part thin and straight and pass through the wrinkled fat bodies. The second part of the Malpighian tubules then changes suddenly into another histological state. The straight tubes change from their membranous tissue into a glandular type of tubes. (Fig. 6, 7, 8, 9).

This distal portion of the Malpighian tubules is also closely attached to the fat body, from where the tubules pass to the posterior intestine (colon). Careful dissections have shown that the tubules enter the prerectal layer around the rectum. (Fig. 10). The Malpighian tubules lay therein a deepening

PLATE VI

THE HIND GUT

Fig. 6. The hind gut of the larva, showing the location and parts of the Malpighian tubules.

A. tubular type of the Malpighian tubules

B. glandular type of the tubules

C. junction between tubules and rectum

PLATE VII

THE MALPHIGHIAN TUBULES

I

Fig. 7. The two types of the Malpighian tubules.

A tubular type

B glandular type

PLATE VIII

THE MALPIGHIAN TUBULES

II

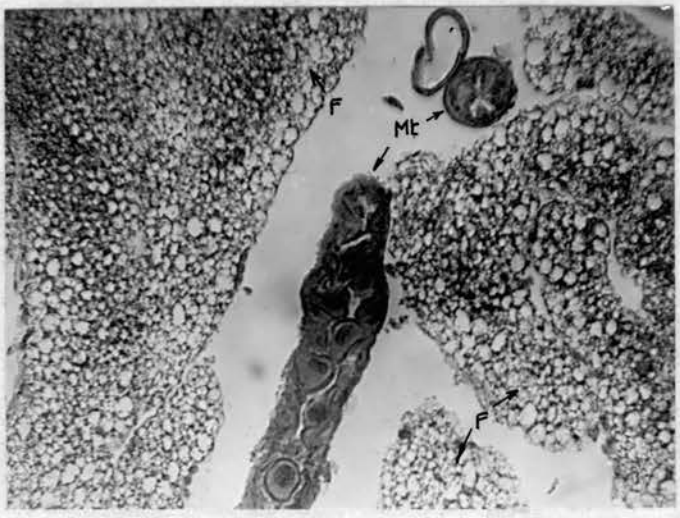

Fig. 8. Crossection through the glandular type of the Malpighian tubule

F Fat body

Mt Malpighian tubules

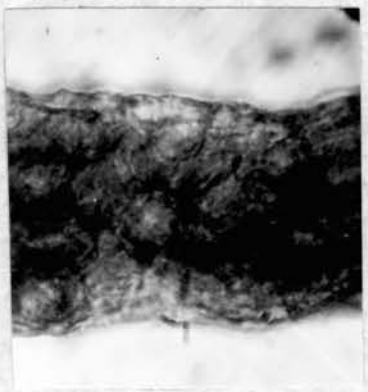

Fig. 9. The glandular type of the Malpighian tubule

PLATE IX

JUNCTION BETWEEN MALPIGHIAN TUBULES

AND RECTUM

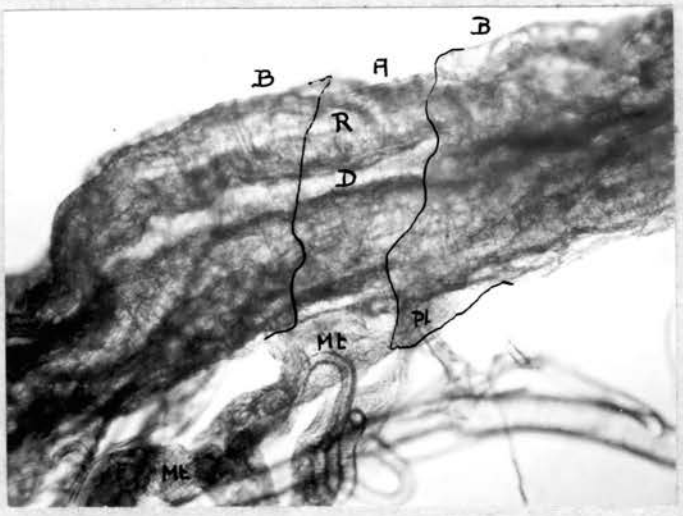

Fig. 10. Entrance of the Malpighian tubules into the prerectal chamber.

A rectum, prerectal layer removed

B rectum, prerectal layer not removed

D deepening, reduction of the rectal wall

Mt Malpighian tubule

Pl prerectal layer

of the inner wall of the rectum, apressed by the prerectal layer. This deepening of the inner rectal wall is due to a reduction of the wall into a porous membrane at this point. Direct openings are found between the prerectal chamber which contains the distal ends of the Malpighian tubules and the lumen of the rectum. (Fig. 11).

Similar observations are recorded by Wigglesworth (*). He states that this modification into the glandular type of the Malpighian tubules occurs also in two of the six Malpighian tubules in the Curculionid Apion. In the Chrysomelid Galerucella it was found, according to the same author, that the wall of the rectum is reduced to a thin layer of cuticle at the point where the Malpighian tubules are apressed by the prerectal layer to the rectum. Marcus (1930) states that only in a small proportion of the Coleoptera do the Malpighian tubules end freely in the body cavity. But he does not specify if this is true only in adults or larvae or in both. In his discussion of the Malpighian tubules Snodgrass (1935) says: "..in some cases...the distal part of the tube is distinctly differentiated into two sections by histological differences and by differences in the contents of the lumen." He does not mention in which groups of insects these specializations are found.

In the pecan weevil larva it was found that, in the case of the larva living in the soil (after they had ceased feeding) that the distal, glandular portion of the Malpighian tubules contained oil droplets, while the proximal, thin walled part of the tubules was empty. This was invariably true in all of the larvae examined. Physiological reasons for these observations and modifications are discussed later on in this paper.

b. The Nervous System.

Besides the brain and the subesophageal ganglia three pairs of thoracic

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* Publication date of book not given.

PLATE X

JUNCTION BETWEEN MALPIGHIAN TUBULES

AND RECTUM

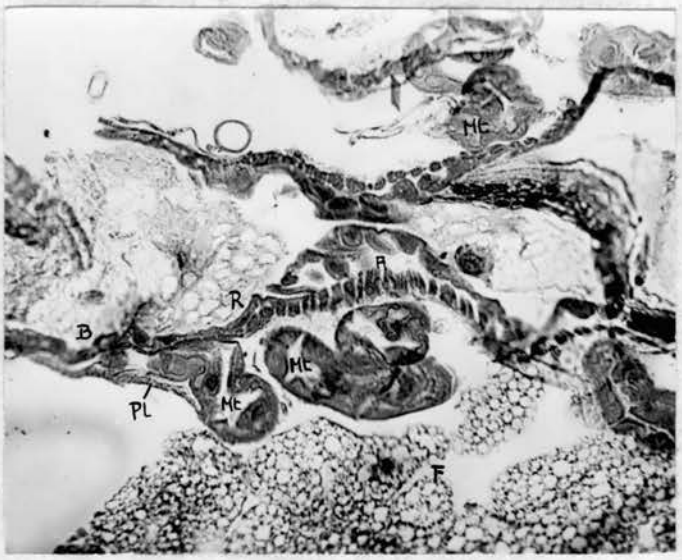

Fig. 11. Crossection through the junction between Malpighian tubules and the rectum.

A perforated rectal wall

B straight rectal wall

R rectum

Pl prerectal layer

Mt Malpighian tubules

F fat body

and eight pairs of abdominal ganglia are found in the larva. (Fig. 12). All these ganglia are located in the head and the first two thoracic segments. No parts of the central nervous system are found in the third thoracic and the abdominal segments. The reduction in the length of the ventral nerve cord is due to a reduction of the connectives between the pairs of ganglia which themselves are grown together. Therefore there is no distinct differentiation in the ventral nervous system into the two nerve cords. This reduction of the length of the central nervous system is also found in other coleopterous larvae, for example those of the June beetle, Amphimallus solstitialis.

The enervation of the third thoracic and of the abdominal segments is conducted by the peripheral nervous system. Nerves are leading from the ganglia to the respective segments of the larval body.

PLATE XI

THE NERVOUS SYSTEM

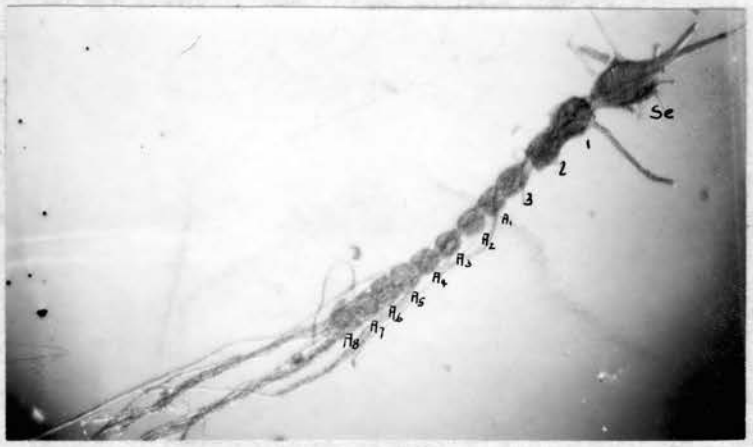

Fig. 12. The ventral nervous system of the larva.

Se Subesophageal ganglion

1 first thoracic ganglion

2 second thoracic ganglion

3 third thoracic ganglion

A_{1-8} abdominal ganglia

PART II

BIOLOGY

1. DEVELOPMENT OF THE LARVA AND DETERMINATION OF INSTARS

a. Development of the Larva in the Nut.

The eggs are deposited by the female through an egg puncture, leading through the husk and the wooden shell into the nut. Upon hatching the first instar larva burrows into the kernel tissue, where it completes its development through the fifth larval instar. Several methods were employed to establish the number of larval instars. No gross morphological differences were found which would divide the larvae into distinct instar groupings. The length or diameter of the larval body varied greatly, apparently depending on environmental and nutritional factors, and did not show constant enough features to identify larvae as to instars. The most consistent features to classify larvae into distinct groupings were the dimensions of the head capsule. The measurements were made with a micrometer eye-piece. The larva to be measured was laid on the glass stage of a binocular, the head laid flat on the plate, to insure a constant distance from the larva to the lenses. The width of the head capsule was determined by measuring the maximum distance between the two genae. The data obtained in these measurements fell into five distinct groups, indicating distinct instars. (Fig. 13, Table I).

Table I. - Width of the head capsule of the pecan weevil larva, showing the instar groupings.

Instar	Width of head capsule in mm	Number of larva measured
1	0.45 ± 0.01	4
2	0.99 ± 0.01	8
3	1.30 ± 0.02	21
4	1.52 ± 0.02	51
5	1.73 ± 0.02	81

PLATE XII

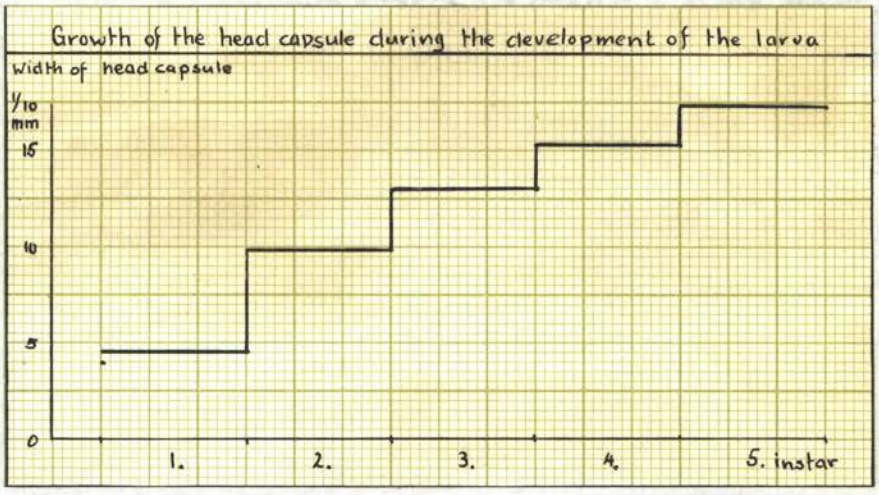

Fig. 13. The growth of the head capsule of the larva of the pecan weevil during the different moulting periods.

Samples of nuts were taken from the trees on the 22nd of September, on the 10th of October, on the 6th of November, 1948, and on the 15th of February, 1949.

Fig. 14 shows the seasonal appearance of the larval instars during a period of five months. The figure indicates that the hatching period was nearly over at the time of the first collection on September 22, 1948, as only a few first instar larvae were found at this date. On February 15, 1949, larvae still could be found in the nuts but in every case these were of the fifth instar.

b. Feeding Habits.

Upon hatching from the eggs, the larvae burrow into the kernel. The number of larvae present in a single nut varies considerably and from 1 to 8 were found in the same nut. Since larvae do not leave the nut in which they hatch until reaching maturity in the fifth instar and are not cannibalistic, the total available food supply for all larvae present is limited to the single nut. The whole kernel seems to be sufficient food for the development of 4 to 5 larvae. Where fewer are found, there is usually a portion of the nut left which is not consumed at the emergence of the larvae. Where more are found in the same nut, they are usually smaller in size when compared with those in the same instar from other nuts. Nevertheless even where extreme competitions for available food exist there seems to be no cannibalism among the larvae.

c. Emergence of the Larvae from the Nuts.

While in the fifth instar, one of the larvae chews a hole through the hard wooden shell and the surrounding husk. There could be found no evidence as to what feature of the development or impulse to emerge initiated the urge to leave the nut. In those cases where several larvae are present in a nut, it might

PLATE XIII

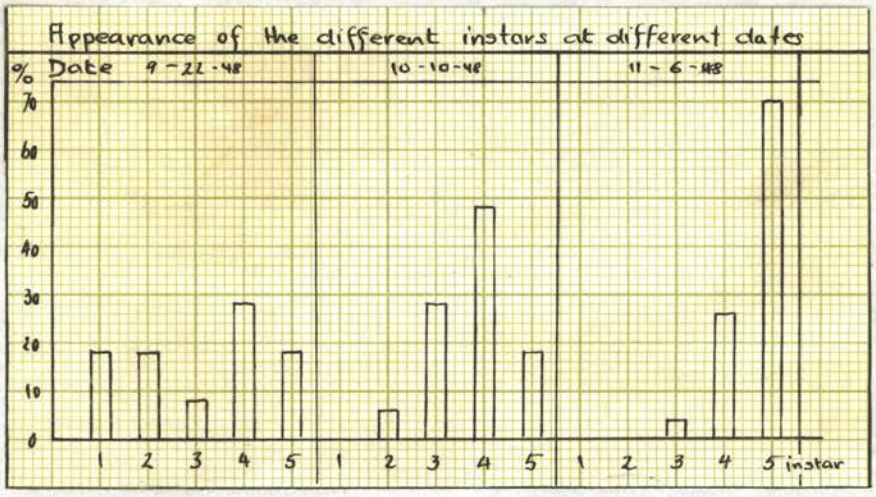

Fig. 14. The appearance of the different instars of the pecan weevil larvae over a period of 10 weeks. (The final investigation, made on February) (15th showed, that at this time all of the) (larvae were in the fifth instar.)

conceivably be a shortage of food--but in most cases observed larvae leave the nuts that still have considerable parts of unconsumed kernels in them. On the other hand, external ecological factors do not wholly account for emergence phenomena, for although most of the larvae leave the nuts between late October and early December, many larvae still remain within the nuts the following spring.

Neither did the influence of location of the nut determine emergence, for larvae can be found both in the nuts hanging on the trees and those which have fallen to the ground.

Even in cases where more than one larvae was found in a nut, there is only one hole chewed through the shell. In only one case out of the thousands examined was a second hole found. Rather frequently there are indications that another hole was begun, but never was finished. The reason could be found probably in light and air change effects through the first opening which may attract the remaining larvae and lead them out of the nuts.

d. Burrowing into the Soil.

Larvae leave the nuts that either still hang on the trees or that have fallen to the ground. After dropping the larva immediately begins burrowing into the soil. It does not have any appendages for this particular purpose. The burrowing is done by means of movements of the abdomen. This is pressed to the ground and by contraction and extension a tunnel is opened. This means that the larva borrows into the soil abdomen-first. It may be that the few bristles found on the abdomen help in keeping the larva from slipping. A definite geotropism was noted. Light seems to have little or no effect, as experiments showed that the same downward movement occured in complete darkness. This was demonstrated where larvae were laid on top of a bottle filled with moist soil and kept in complete darkness. Observations during the next

few days indicated that all the larvae had burrowed down to the bottom of the bottle.

The depth of the burrowing seems to depend to a major extent upon the physical properties of the soil and the subsoil. Observations in the orchard in October, when unusual drought conditions prevailed, showed that a harder plow sole limited the depth to which the larvae could burrow. This plow sole was at a depth of about 21 cm (7 inches). Most of the larvae were found on top of this hardened soil but a few of them had penetrated about two cm further down. Observations in another location showed most of the larvae at a depth of 15-20 cm (5-6 $\frac{1}{2}$ inches), the hardened layer there was found at a depth of about 13 cm (6 inches) which indicates again that the hard soil was a limiting factor in the depth of the penetration. Special experiments were made by Bieberdorf (1943) to obtain information about the depth to which the larvae burrow. Cages were placed into the ground to a depth of about 120 cm (40 inches). The soil was then replaced in the cages and the larvae laid on top of the soil. The distribution of these larvae was later compared with the distribution of other larvae in the soil under natural conditions.

Table II presents the findings in these experiments and indicates that the most limiting factor in the depth of the burrowing is the condition of the soil; a compact and hardened layer can not be penetrated.

* TABLE II - Distribution of the larvae in the soil under natural conditions and in cages. Depth of burrowing by 1 and 1½ years old larvae.

Depth in inches	larvae 1 year in soil		larvae 1½ years in soil	
	natural ** conditions	in cage	natural *** conditions	in cage
0 - 4	8	0	1	1
4 - 8	13	1	13	0
8 - 12	5	3	2	1
12 - 16	0	6	0	1
16 - 20	0	8	0	0
20 - 24	0	12	0	2
24 - 28	0	6	0	1
28 - 32	0	2	0	2
32 - 36	0	0	0	0
36 - 40	0	0	0	0

* Data obtained from experiments conducted by Mr. G. A. Bieberdorf, Assistant Professor of Entomology, Oklahoma Agricultural and Mechanical College.

** The plow sole was found here at a depth of 7 inches.

*** The hardened layer here was at a depth of 7½ inches. The soil used in the cages was of the same type as the one in the experiments under natural conditions, slightly packed.

2. STAGE IN THE SOIL

a. Diapause.

There seems to be a diapause during the time in which the larva remains in the soil. This means that the larva is in a resting stage which is apparently not influenced by obvious changes in the environmental factors. This concept agrees generally with that of Wigglesworth ( ), who also quotes a similar definition by Henneguy: ".. a spontaneous arrest of development which supervenes irrespective of the environmental conditions". The pecan weevil larva remains in the soil for at least 1½ years. Following this extended period of diapause a few of the larvae change into pupae and adults. There seems to be no definite time in which this change takes place. Adult weevil were found in the soil in October and December, as well as in March. Observations in tests conducted by Bieberdorf (1948) show that the first adults appear in the soil after about 16 months. The larger percentage of the larvae appear to remain in diapause for yet another season. No weevil appeared during the first year in the soil.

During this period of diapause the larva does not feed. In all the examined larvae taken from the soil, the fore and the mid-gut were completely voided, and even in the pharynx and esophagus there were was no indication that any food or soil had been ingested. The larva feeds exclusively on the relatively large amount of fat stored in its three fat bodies. Experiments were conducted to investigate the decrease of the fat during the period of diapause. Three groups of 20 larvae each were obtained, all in the fifth instar. The first group was taken directly from the nuts, the second after about 5 months in the soil and the third group after staying in the soil for about 16 months.

All these larvae were dehydrated and the fat extracted after Soxlet's method, with petroleum ether.

TABLE III - Comparison of the fat content in larvae at different times during the fifth instar.

	larvae out of nuts	about 5 months in the soil	about 16 months in the soil
Dry weight of one larva in grams. (Average of 20 larvae)	0.090	0.078	0.059
Fat content (ether soluble compounds) in percent. (Average of 20 larvae)	73.33%	64.69%	50.25%

These results show a distinct decrease in the total dry weight of a larva and in the total content of ether soluble compounds. An increase in size is evident in larvae that have been in the soil for 1½ years as compared with the average of the ones just leaving the nuts. This increase would therefore seem to be the result of a higher water content. In several of the examined larvae it was found that especially the oesophagus and crop were filled with a clear fluid, which might have been water.

b. The Fat Cycle. Hypothesis.

The pecan weevil larva exhibits an extraordinary nutritional cycle, characterized by a period in the nut of very rapid growth and food intake; this is followed by an extended period of diapause during which no organic food is ingested. This unusual nutrition is paralleled by a rather specialized digestive and excretory apparatus, especially in the Malpighian tubules. These tubules show features that are not described in available entomological literature. The author wishes to outline some of these facts and to propound

the following hypothesis showing possible relationship between food habits and the digestive system.

Observations of particular importance are as follows:

1. Fat is stored in the three large fat bodies during the actual feeding period.
2. During the period of diapause the gut, exclusive of the rectum shows no indications of any food contents.
3. The distal portion of the Malpighian tubules and the rectum are filled during diapause with fatty contents.
4. The connection between the Malpighian tubules and the rectum and especially the unusual histological arrangement of the rectal epithelium at the point of attachment, show that there is a possibility of a passage of products from the Malpighian tubules into the prerectal chamber and from there directly into the rectum.
5. It is generally agreed that digestion does not take place in the insect rectum, therefore the products found there have no further nutritional value, are therefore waste products.

The question naturally arises as to the site of the catabolism of the fat during diapause. It is apparently effected either by the fat body or by the Malpighian tubules. There is no evidence that the very loose and thin tissue of the fat body secretes enzymes or otherwise enters into the digestion of the fat. On the other hand, there must be a reason for the morphological and histological differentiation of the distal portion of the Malpighian tubules. The similarity between the histology of the salivary gland and of that of the distal part of the Malpighian tubules might conceivably lead to the assumption of a similar function--namely that there is a production of an enzyme by the Malpighian tubules, and that this enzyme functions as a fat

digesting compound or as a catalyst. Taking all these observations into consideration, the following hypothesis of the fat cycle in the larval body could be concerned:

While feeding on the kernel in the nut the larva assimilates the plant fat. The assimilated fat then is transferred through the blood and stored in the fat bodies. After the larva has ceased feeding and passes the period of diapause in the soil, fat is taken from the fat bodies into the distal portion of the Malpighian tubules. Here it is assimilated and the waste products are carried directly into the rectal lumen.

CONCLUSIONS

These studies on the larva of the pecan weevil, Curculio caryae (Horn), led to the following results and conclusions:

Upon hatching from the egg the larva completes its development in the nut. Five distinct groups of instars were obtained by comparative measurements of the dimensions of the head capsule. After reaching maturity in the fifth instar the larva chews a hole through the hard wooden shell and the husk and drops to the soil. No definite indications for the stimulus and the time of this emergence from the nut were found. The larva has positive geotrophism and burrows immediately into the soil. The depth of this burrowing seems to be limited in most cases by the conditions of the soil and the subsoil. No appendages for an active burrowing were found on the larva.

In the soil the larva passes through a period of diapause of at least 14 months, in which no actual feeding occurs. The period of diapause is often extended to at least 18 months. The pecan weevil therefore has at least a two-year life cycle. Active feeding occurs only during the period in which the larva lives in the nut.

Morphological and anatomical differentiations occur in the larva which seem to correlate with these particular feeding habits. There are three large fat bodies in the larva. These storage organs are filled during the actual feeding period. During the period of diapause the larva uses this stored fat. A significant decrease of the stored fat could be measured by the extraction of the remaining fat at different stages of the larval life.

A second differentiation occurs in the Malpighian tubules. These tubules show two different portions, first a proximal, straight and thin walled tubule and second a distal glandular type of tubule. This distal part is connected

with the colon. The tubules enter the thin prerectal layer and end in a prerectal chamber. At the point of attachment the rectal epithelium shows a distinct differentiation from the straight wall into a perforated membrane with direct openings from the prerectal chamber into the lumen of the rectum. The assumption was made that these differentiations are in connection with the unusual feeding habits of the larva and the following hypothesis of the cycle of the fat in the larva was made:

1. Fat is stored during actual feeding period into the fat bodies.
2. During the period of diapause the fat is utilized. The glanduler distal portion of the Malpighian tubules acts as a digestive organ. The fat is assimilated there and the waste products are carried through the tubules into the prerectal chamber and from there directly into the lumen of the rectum.

SUMMARY

Studies were conducted on the larva of the pecan weevil, Curculio caryae, (Horn), Curculionidae, Coleoptera.

Five different instars were found in the development of the larva.

After reaching maturity in the fifth instar the larva passes a period of diapause in the soil. This period lasts at least 14 months.

The only feeding period is during the development of the larva in the nut. No actual feeding is done during the period of diapause.

Morphological and anatomical adaptions of the digestive system, especially of the Malpighian tubules, seem to be related to the unusual feeding habits of the larva. A hypothesis was therefore proposed on the associations between the feeding habits, differentiations in the digestive system, and the cycle of the fat in the larval life.

LITERATURE CITED

Comstock, J. H.

An Introduction to Entomology. Ninth edition: 539. 1940.

Marcus, B. A.

Untersuchungen über die Malpighischen Gefässe bei Käfern. Zeitschr. Morph. Oekol. der Tiere, 19: 609-677. 1930.

Snodgrass, R. E.

Principles of Insect Morphology: 378-380. 1935.

Wigglesworth, V. B.

The Principles of Insect Physiology: 276, 305-319

Typist: Grace Peebles