A HISTOLOGICAL STUDY OF THE DAMAGE TO *GOSSYPIUM HIRSUTUM* (L.) RESULTING FROM ACTIVITIES OF *ANTHONOMUS GRANDIS* (BOH.)

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PREFACE

The cotton plant (Gossypium hirsutum) is an important crop plant of Oklahoma and other southern states. For over fifty years this crop has been severely injured by the boll weevil (Anthonomus grandis). Control measures against the insect and studies of gross damage to the plant have been abundant. No study of the damage to the individual plant at the microscopic level has ever been made. Such a study was attempted by the writer.

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CHAPTER I

INTRODUCTION

The boll weevil (Anthonomus grandis) has been a serious pest of cultivated cotton in the United States for over half a century. Attempts to determine the precise nature of the insect's damage to the host plant have been few in number. Studies have been of two types. One type dealt with the economics of its damage in terms of lost production while the other has included a macroscopic study of damage to the plant.

It was the intention of the writer to supplement our knowledge of the relationship between the host plant (Gossypium hirsutum) and the insect (Anthonomus grandis) by investigating the histological nature of the damage to the host plant. Such an investigation was considered to be a necessary and logical sequel to the investigations of gross damage which have preceded.

The study was begun in June of 1954 and was continued without interruption until May of 1957. The greater portion of the investigation was conducted in the Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

For all practical consideration, the insect is an obligate parasite on the domestic cotton plant (Coad, 1914). Certain wild cottons may serve in lieu of the domestic plant but their occurrence and frequency is so slight as to be negligible. The relationship between the host plant and the parasite is complete and it is probably the result of this that total damage is so large. An enumeration of the activities of the insect which result in visible damage to the cotton plant should illustrate this principle.

1. The adult feeds upon stems, leaves, vegetative buds, floral buds, and fruits of the cotton plant.
2. The adult female deposits eggs only in floral buds and fruits of the cotton plant.
3. The developing larva feeds only on a floral bud or a fruit of the cotton plant.
4. Several generations of weevils may be produced in one growing season of the plant.

CHAPTER II

REVIEW OF THE LITERATURE

The annual cotton crop has been severely damaged by the cotton boll weevil since the first appearance of the insect in the United States in 1892 (Brown, 1938; Cushman, 1911; Loftin, 1946). Annual losses vary with climatic conditions and control measures. Losses in the United States in terms of decreased yield are always large, commonly involving millions of bales (Brown, 1938; Cushman, 1911; Hunter and Hinds, 1905). It is significant that these loss estimates do not include the increased cost of production necessitated by control measures against the insect. An abundance of literature exists pertaining to methods of controlling the insect and several excellent studies of its general biology may be found (Brown, 1938; Cushman, 1911; Fenton and Dunham, 1929; Hunter and Hinds, 1905; Loftin, 1946; U.S.D.A., 1912).

The works of Fenton and Dunham (1929), Hunter and Hinds (1905), Coad (1914), Cushman (1911), and Loftin (1946) are especially valuable for their descriptions of the insect, its habits, and the gross damage to cotton plants.

Floral buds and fruits of the cultivated cotton plant are the most important sites of oviposition and larval

development of the boll weevil (Cushman, 1911; Fenton, 1952; Fenton and Dunham, 1929). It is the effect of these activities which results in the greatest economic loss to cotton growers (Hunter and Hinds, 1905; Loftin, 1946). The more mature fruits are less frequently used as food or as oviposition sites (Brown, 1938; Cushman, 1911; Fenton and Dunham, 1929; Hunter and Hinds, 1905; Isely, 1928).

Oviposition activities of the adult female are initially identical to those of individual feeding. According to Hunter and Hinds (1905) the site of the puncture is more carefully selected and if adequate numbers of organs are available only one puncture per organ is made for oviposition. After completing the excavation, the female reverses the position of the body, inserts her ovipositor, deposits the egg among the anthers and upon withdrawal of the ovipositor places a small drop of "mucilaginous material mixed with some solid excrement" into the opening (Hunter and Hinds, 1905). In approximately five days the site of the puncture is marked by a small rough protruberence usually termed a wart (Cushman, 1911; Hunter and Hinds, 1905). Hunter and Hinds (1905) noticed upon the internal wall of the carpel of the fruit, so marked externally, a mass of material they termed "gelatin". These workers attributed the external protruberance to pressure resulting from formation of the internal "gelatin". Following embryological development, the young larva begins to feed. The larva molts three to four times and completes pupation within the organ which -

received the egg (Fenton and Dunham, 1929). During this period, the floral bud is hollowed out and has abscissed. Young fruits may likewise be partially or completely hollowed out and abscise. Older fruits usually do not abscise although ovules and lint may be destroyed or badly stained (Cushman, 1911; Fenton and Dunham, 1929).

Infestation of floral buds and young fruits with developing larvae results in an opening and spreading of the involucral bracts prior to abscission of the organ. This response, commonly termed "flaring of the square" is a characteristic symptom of boll weevil infestation and/or other severe mechanical damage to these organs. Feeding upon the internal organs of the floral and fruiting structures occurs throughout the development of the larva but ceases during pupation (Fenton, 1952).

Abscission is a problem of special interest in a consideration of boll weevil damage. A conspicuous result of weevil infestation is the shedding of fruits and floral buds.

Although general physiological studies of abscission are available, as well as physiological studies of cotton abscission, there is a scarcity of information concerning the histology of cotton bud and fruit abscission.

Dutt (1928) published a report on cotton flower abscission. More recent physiological and anatomical studies of foliar abscission have been done by Leinweber (1956), Ramsdall (1954), and Walhood (1956). Eaton (1955), Addicott and Lynch (1955), and Walhood (1956) have recently investi-

gated the physiology of abscission in cotton, including flowers and fruits. Results obtained by these workers all demonstrate that a number of factors may influence the rate of bud and fruit shed. Dutt (1928) was particularly impressed with the effects of water stress. Walhood (1956), Addicott and Lynch (1955), and Eaton (1955) have investigated the effects of IAA, sodium 4-chlorophenoxyacetate, naphthalene acetic acid, auxin, and nutritional balance. Several theories have been postulated regarding the chemical initiation of abscission but at this time none is completely satisfactory. Eaton (1955) sums up the situation in these words: "In the light of available evidence it seems necessary to conclude that the cause, or causes of boll shedding are unknown."

Although numerous abscission studies on cotton have been made the majority of the interest has been directed along a quantitative physiological approach. Little histological work has been done. Leinweber (1956) examined the abscission region of the leaf petiole and Dutt (1928) describes histological studies of the zone in the pedicel. There are reasons to believe that Dutt (1928) misinterpreted some material. This will be referred to in the following discussion.

Leinweber (1956) found foliar abscission to occur chiefly by means of a lysigenous break across portions of a zone of undifferentiated cells. Separation involved a structure with four major vascular bundles which lacked an

interfasicular cambium.

Consideration of the basic phenomena of cell division and differentiation becomes important in the study of boll weevil damage. A characteristic of weevil oviposition is the production of a tumorous growth on the plant organ. Insect galls and other abnormal growths have received much attention because of the belief that their study could yield clues to the nature of basic processes.

A complete review of all pertinent literature is not possible here. Several recent reports of significance should be mentioned however.

Beck (1954) has investigated the nature of Solidago galls. LaRue (1941) and Bloch (1941; 1952; 1954) have done extensive work on wound healing and other abnormal growths. Sylwester and Countryman (1953) compared histologically the crown gall with a wound callus on apple. Outstanding reviews of the study of growth and differentiation have been done by Loeb (1924), Loomis (1953), Riker and Hildebrandt (1953), Sinnott (1953) and Swingle (1952). Went (1940) has been a prominent worker in the field of plant growth substances.

A large number of substances have been shown to induce tumorous response in higher plants. Among these are self-contained hormones (Loofbourow et al, 1941; Loofbourow, 1942), necrotic products (Riker and Hildebrandt, 1953) and many foreign substances artificially introduced (Bloch, 1954).

Further comment on the literature is deferred until discussion of related topics.

CHAPTER III

EXPERIMENTAL METHODS AND MATERIALS

The investigation was conducted by the traditional methods of the plant anatomist and histologist. Additional techniques, however, were employed whenever it was believed desirable. Of considerable value was the use of radioisotopes and autoradiographic techniques.

Materials for these studies were obtained from two sources. Specimens showing typical boll weevil feeding punctures were obtained from fields infested with the insect. In many instances, specimens were collected which bore an adult weevil which was feeding or ovipositing at the time. Other specimens were obtained from plants grown in the greenhouse under plastic covers and infested with one, or more, captive boll weevils. Specimens collected from these sources were compared with undamaged organs of the same size, taken where possible, from the same plant.

Determination of Feeding Damage

Histological Methods

Microscope studies were conducted upon stems, leaves, floral buds, fruits, and vegetative buds. These were examined with a low-power stereoscopic dissecting microscope

and the larger organs were dissected to determine the path of the feeding tunnel. Permanent microscope slides of other specimens were prepared using standard histological methods (Sass, 1951). Damaged and undamaged materials were killed and fixed in FAA, dehydrated with the normal butyl-ethyl alcohol process, embedded in paraffin, and sectioned at 15 microns. Several staining procedures were employed. For most routine observations the safranin O-fast green FCF proved most satisfactory. Some slides were stained with hemalum and safranin O for future photographic recording according to the procedure of Sass (1951).

Histochemical Methods

Earlier workers have noted that older fruits are not fed on by the weevil so long as young fruits and floral buds are available (Fenton and Dunham, 1929; Loftin, 1946). The writer had noted a larger number of incomplete feeding wounds in older fruits. The wounds in young fruits and buds were usually completed.

A study was therefore planned to determine whether the structure of the fruit wall was responsible for the apparent preference of the weevil in its feeding habits.

Microscope mounts were prepared as for other specimens. In addition, histochemical tests were performed in an effort to determine more accurately the chemical nature of the cell wall constituents. For this experiment, living materials were tested by standard histochemical methods. The tests

chosen were; the zinc-chloro-iodide test for cellulose and starch, the use of Meyer's modified hemalum as a selective stain for cellulose, a modification of McCready and Reeve's (1955) test for pectin, and the phloroglucin-hydrochloric acid test for lignin. Polarized light microscopy was also used. Tissues were cut by free hand and freezing microtome methods. Sections of 1-3 day old and 10 day old pericarps cut 15 to 50 microns in thickness were tested and examined.

Autoradiographic Methods

Studies of other plant-insect relationships have shown that foreign materials may be injected from the mouth parts of the insect into the host tissues (Chatters and Schlehuber, 1951; Diehl and Chatters, 1956; Flemion et al, 1951). Although these studies have been concerned with insects possessing piercing-sucking type mouth parts, rather than the biting-chewing type in the boll weevil, the hypothesis that this insect might inject materials was believed worthy of investigation.

Normal histological methods of staining and microscopic observation with white light and polarized light gave no indication of the presence of foreign materials in feeding wounds. Further studies using dark contrast and light contrast phase microscopy failed to indicate the presence of any such substances.

A series of experiments was planned to utilize an autoradiographic technique. It was believed that such a

technique could best demonstrate the transfer of a small amount of substance from the insect to the plant, if such a transfer occurred.

Previous experiences led the writer to select radiosulfur (S^{35}) for the tracer substance. Young potted cotton plants were selected as the "preferred" food of the adult weevil. The stems were severed at the base while submerged in water and transferred to a 100 ml. tap water solution containing 250 lambdas of S^{35} as $H_2S^{35}O_4$. Activity of this solution, as measured by a Berkley decimal scaler, was 19,500 counts per minute per 10 lambdas. The plants were placed under continuous fluorescent light illumination for twenty four hours. At the end of this period a captive adult boll weevil which had no measurable radioactivity was placed upon each of three plant specimens and then each specimen was covered with a ventilated plastic cage.

Feeding of the weevils upon the radioactive tagged cotton plants was allowed to continue for 96 hours at which time the weevils were removed and placed upon fresh non-radioactive cotton plants. This transfer was performed to allow feeding activities and contact with vegetative portions to remove radioactive debris from the weevil's exterior. It had also been observed that the adult weevil occasionally cleansed its own proboscis by rubbing the anterior legs along the length of its mouth parts. This cleansing period was continued for 72 hours at the end of which each weevil was placed in a vial of water and

agitated. It was believed that such a washing would insure that the external surface of the weevil would be free of any radioactive debris or fecal matter. Immediately following the washing, each weevil was placed upon a fresh non-radioactive cotton branch contained in a new plastic tube.

The weevils were kept under constant observation until feeding began. One weevil commenced feeding after 30 minutes. Upon the completion of two punctures in separate fruits the weevil was removed. The young fruits upon which it had fed were removed and preserved in a saturated solution of picric acid in 100% ethyl alcohol. As will be noted later, one of these punctures was used as an oviposition site.

One weevil refused to feed. Its behavior appeared to be abnormal and within 24 hours, it was dead. The third weevil did not complete two separate feeding punctures until after an interval of nine and one-half hours. Each of the punctures was in a separate small floral bud. These specimens were preserved in the same manner as described for the fruits.

Processing of the preserved materials was continued for the preparation of microscopic autoradiographs according to the techniques of Steffey (1953). One half of the specimens were processed using Ansco autoradiographic emulsion B and one half with Eastman Kodak NTB liquid emulsion. The length of exposure was 21 to 28 days.

The autoradiographs and the control histological sections were examined by ordinary light microscopy and by phase contrast microscopy.

A further experiment was conducted to test the hypothesis that transfer of substances occurred when the weevil engaged only in feeding activities. For this experiment it was not considered necessary to obtain micro-autoradiographs. The writer chose to repeat the previous experiments with two major differences. First, the involucral bracts of feeding material were removed to facilitate observing the exact nature of the weevil activities (feeding or oviposition). Secondly, upon securing a specimen the tissue was processed for gross autoradiographs only.

Two adult weevils were allowed to feed upon cotton plants containing S^{35} until they were moderately radioactive. A period of cleansing and feeding upon non-radioactive tissue followed. The weevils were bathed and placed upon undamaged, non-radioactive, young fruits. Continuous observation was maintained until feeding punctures were completed at which time the weevils were removed and the specimens collected. Counting of the tissues with a Berkley counter and a scaler showed that a small additional amount of radioactivity was present.

One fruit was immediately sectioned into three parts by free hand methods using a new unused razor blade. Each of these sections transected the principal puncture. One section also included a shallow puncture which the weevil had

begun but had not finished. These sections were placed upon a clean glass plate and covered with a sheet of thin polystyrene. A sketch of the tissues was made and the puncture sites indicated. A sheet of Ansco Isopan film was placed, emulsion side nearest the tissue, upon the polystyrene. Upon the back of the photographic film was placed a second glass plate. This entire unit was securely bound, wrapped in black paper, sealed in a box, and placed in a refrigerator for an exposure period of forty days.

Development of the film was according to the usual photographic procedures. At the time of development, a shadowgraph of the mounted sections was made and the site of the original puncture established in relation to the sketch made at the time the tissue was mounted.

Determination of Ovipositing Damage

Investigations performed by the writer into the nature of the damage resulting from oviposition activities were of six different approaches. Briefly enumerated, the techniques were: (1) Macroscopic observation of dissected structures to establish validity of descriptions previously made; (2) Histological study of damaged and ovum bearing organs in an attempt to determine precise cellular damage and response; (3) Preparation of microscopic autoradiographs to determine whether introduction of foreign substances occurred; (4) Chromatographic analysis of the animal excrement for the presence of the growth promoting substances of the

indol group; (5) Mechanical and chemical stimulation of floral and fruiting structures in an attempt to determine the nature of growth promotion at the oviposition site; (6) A study of the abscission of floral buds and fruits to determine if boll weevil-induced abscission differed from that resulting from other causes.

Macroscopic Methods

Structures showing evidence of weevil oviposition were examined with the unaided eye and by low-power magnigication. Some photographs were made of gross effects. Similar photographs may also be seen in the work of Hunter and Hinds (1905).

Histological Methods

Microscopic studies of the oviposition wound site were begun in an attempt to ascertain more precisely the nature of the plant response to the insect activities. Materials were prepared as described previously for feeding wounds. Several staining procedures were used. The safranin O and fast green FCF technique was found most satisfactory for routine studies.

Study of the oviposition tumors which arise on the locular side of the pericarp and which were termed "gelatin" by Hunter and Hinds (1905) involved the use of additional techniques. Additional microscopic studies were performed upon these growths. These consisted of teasing apart granular and "gelatinous" growths into aqueous mounts. Some of

these specimens were stained with neutral red dye for examination with an ordinary light microscope. Other specimens were not stained and were examined by phase contrast and polarization microscopy.

Autoradiographic Methods

Autoradiographic studies were made to determine if the insect injected materials into the host plant while depositing eggs. Because the weevil deposits the egg in a feeding cavity a separation of the two activities is difficult. The tracer experiments were performed simultaneously with the autoradiographic study of feeding and is described in that section. (See pages 11 and 12).

Allied Studies

Stimulation of Simulated Oviposition Tumors

Although deviating somewhat from the histological approach, the writer felt that the study would be incomplete without an attempt to determine the nature of agents capable of initiating cell division and enlargement in these tissues of the cotton plant. From the preceding discussion it is obvious that a number of complex materials could be possible agents. Among such substances would be the fecal matter used by the female in sealing the oviposition wound. Other materials would be soil from the insect's body or the plant surface, larval secretions, and adult reproductive secretions. It is known that indole acetic acid may function in

normal and abnormal plant growth (Riker and Hildebrandt, (1953; Skoog and Tsui, 1948). Auxin, which contains this compound, is known to occur in floral and fruiting tissues of cotton (Addicott and Lynch, 1955; Eaton, 1955). Since the diet of the insect normally is composed wholly of these tissues, a test was made to determine if this compound was present in the weevil feces.

The first sample of 1.3 mg of fecal material was ground into 0.1 ml of absolute ethyl alcohol and a chromatographic separation was attempted using the procedure of Bennet-Clark and Kefford (1953). In comparison with the indoleacetic acid standard the results were negative. A second trial was attempted using 100 mg of fecal material. No indoleacetic acid was detected by means of this test.

A series of experiments was conducted in which various agents were introduced in a manner which was thought to best duplicate the oviposition wound.

Three organs which serve as the oviposition sites and as principal food for the adult weevil were selected for experimental purposes. These were the floral buds of varying ages, but always four to six days short of anthesis, fruits of from one to seven days old, and fruits eight to fourteen days of age. Initial tests consisted of control punctures made with a sterile needle, punctures with inocula of soil, .03% indoleacetic acid in lanolin, boll weevil feces, an ether extract of ground weevil larvae, and colchicine. Each agent was selected either because of its

possible function as an inoculum of the boll weevil or because of its known growth promoting effect upon other plant tissues. Other workers (Beck, 1954) have reported that the lanolin often used as a vehicle for growth promoting substances was, in many instances, capable of stimulation. For this reason inoculation of lanolin alone was begun. Subsequently indolebutyric acid and petroleum jelly were added to the list of inocula.

All punctures were made by means of a No. 0 insect pin. A separate sterile needle was utilized for each puncture. This size pin produced a wound similar in diameter to that of the weevil's mouth parts. Penetration through the perianth and the carpel was effected by tactile perception. Upon penetration the pin was rotated 4 times to induce cellular damage and to distribute the inoculum about the perimeter of the wound. Each punctured organ was identified with a cardboard tag tied about the peduncle. Readings were made upon the organs after five days and results recorded. Some organs were lost by abscission. These were not recorded in the responses.

Abscission of Floral Buds and Fruits

The damage inflicted upon the cotton plant by the insect becomes most obvious upon abscission of floral buds and young fruits. Heavily infested fields may have the soil surface covered with the fallen structures (Hunter and Hinds, 1905; Loftin, 1946). Older fruits usually remain on the

plant even though containing several larvae. Floral buds and fruits younger than eight days invariably abscise soon after hatching of the weevil egg.

Studies of the physiology and anatomy of leaf abscission in deciduous species have long been of interest. Several anatomical types of abscission patterns have been described, and the causes of abscission have usually been related to auxin activities.

This writer wished to investigate the histology of abscission in healthy and weevil damaged floral and fruiting portions of cotton to determine if abscission of this type differed from abscission resulting from other causes. It was discovered that satisfactory descriptions of pedical abscission were not to be found and that foliar abscission although adequately described, was not comparable.

Most studies on abscission in cotton have been concerned with the physiological nature of foliar abscission. It has also been noted that the leaf and the fruit are more susceptible to abscission at certain times and highly resistant to shedding at other periods.

The writer undertook a study of the nature of abscission of floral buds and fruits resulting from boll weevil activity considering these two facts.

Two approaches were followed. Histological preparations were made of killed and fixed material. Histochemical tests were performed upon fresh material which was sectioned by free hand methods and with a carbon-dioxide freezing microtome.

The materials studied were abscission regions of; healthy young floral buds and fruits, healthy fruits older than 10 days, boll weevil damaged young buds and fruits, and boll weevil damaged fruits older than ten days.

Histochemical tests were; ruthenium red test for pectin substances (Glick, 1949), the hydroxylamine test for pectin (McCready and Reeve, 1955), phloroglucin and hydrochloric acid procedures for lignin, carbol thionin staining procedure for lignin, zinc-chloro-iodide for cellulose and starch, and procedures of Glick (1949) using oxalic and sulfuric acids for the presence of calcium.

CHAPTER IV

OBSERVATIONS AND RESULTS

All of the aerial organs of the cotton plant are subject to damage by the feeding of adult weevils. Structures most frequently serving as food are the floral buds and young fruits. It is claimed by some authors that the weevil has a preference for the pollen of the unopened floral bud (Fenton and Dunham, 1929; Hunter and Hinds, 1905). Observations made during the course of this investigation substantiate these reports. Whenever floral buds, fruits, and vegetative organs were available to captive weevils, the floral buds were usually completely hollowed before marked damage appeared in other organs. Young fruits of ages less than eight days were next to show evidence of feeding damage, followed by damage to older fruits. Vegetative structures are devoured only when other organs are not available.

The wound inflicted by the weevil consists of a round excavation of varying depth. The maximum depth is determined by the length of the long proboscis which, in a fully grown adult, is approximately 3 millimeters in length.

Excavation of plant tissues is accomplished by seizing, cutting, and tearing with the biting mouth parts which are

situated in the distal end of the proboscis. Although the resulting cavity is symmetrical, the description of some writers likening the feeding action to an augering, or boring, is incorrect. The weevil seldom changes the position of its feet while feeding or excavating for oviposition. There is some anterior-posterior motion as the cavity is deepened and some altering of the vertical angle of the head but the writer has never observed a twisting or pivoting action.

The excavated tissues are not deposited externally but are ingested by the weevil.

Macroscopically the wounds inflicted by the adult weevil appear similar in all organs of the host. A typical feeding cavity in a young stem is shown in Fig. 1, Plate I.

Internally however, the damage differed as a result of the nature and distribution of the tissues involved. For the purpose of clarity the organs shall be considered separately.

Feeding Damage

Vegetative Organs

Microscopic studies of young stems showed there was no tissue selection by the weevil when suffering starvation. Feeding in these organs progressed inwardly from the epidermis and proceded through the hypodermal collenchyma, phloem, cambium, xylem and into the pith.

The response of the plant to this kind of injury resulted in the proliferation of cells, especially pith and

phloem cells, which gave rise to a partial closure of the wound cavity. The production of cells was by an abnormal type of cell division. As may be seen in Fig. 2, Plate I, the proliferated tissue consisted of cells of irregular size and configuration. Walls were thin and demonstrated no secondary thickening, nuclei were quite small and remained adjacent to one another on opposite sides of the new transverse wall. In many instances nuclei were not observed in new cells. Cell contents were almost completely lacking or else consisted primarily of a large vacuole.

Feeding wounds in vegetative buds and leaves also included all tissues lying in the path of a straight tunnel. A puncture that was begun on one side of a vegetative bud or leaf was continued until the opposite surface was perforated. Extensive feeding results in shredding of the organ. Enclosed leaves, vascular tissue, and terminal meristems were consumed. Mature leaves were similarly injured. Large areas of upper epidermis, mesophyll and enclosed vascular bundles, and lower epidermis were consumed. This condition of gross damage has been observed by Hunter and Hinds (1905).

Floral Buds

When utilizing floral buds as food materials the method of penetration of the tissues is identical with that described for vegetative organs. Excellent illustrations of external damage to these organs may be seen in the work of Hunter and Hinds (1905). Microscopic studies show more

clearly the nature of the internal damage since the feeding site is marked externally only by a circular opening in the sepal, corolla, or pericarp.

Upon entrance of the insect's mouth parts into the floral cavity the ingestion of the staminal portions began. Ingestion of anthers and their pollen was continued. Movement of the feeding animal's body and head portions resulted in the change of the angle of the proboscis. Continued feeding at different angles was found to result in the formation of an approximately spherical cavity in the stamens. The tunnel leading to the cavity was seldom enlarged appreciably by the prolongation of feeding. New feeding punctures were made when additional materials could not be reached. When additional buds or fruits were not available, this manner of feeding was continued until only a shell composed of perforated calyx and corolla remained. In cases involving considerable feeding upon older buds, the staminal column, stigma, styles and enlarging carpels may be completely removed.

A comparison of internal damage to the floral bud may be made with the structure of an undamaged bud by reference to Figs. 3 and 4, Plate I.

Feeding tunnels in floral buds were found to begin either in the sepals or the corolla depending upon the age of the bud. In older buds the corolla extends beyond the sepals and feeding punctures are often begun in this extension. An excavation begun in a sepal differed only in the

increased number of tissue layers punctured by the proboscis prior to entrance into the anthers. These excavations were also formed by biting and ingesting portions of tissue. Torn cellular elements were noted along the boundary of the puncture. Regenerative activity was scant or lacking even in those areas which demonstrate this property in oviposition punctures. Tissues removed along the feeding path in order of their ingestion were: outer epidermis, mesophyll, inner epidermis of the sepal; outer epidermis, mesophyll, and inner epidermis of the petal. Incidental structures such as lysigenous cavities and glandular hairs were ingested if they lay in the feeding pathway.

Fruits

Feeding damage occurred primarily to younger fruits. Older fruits are considered herein as those beyond eight days following anthesis. Microscopic examination demonstrated a repetition of the feeding pattern found in the vegetative structures; that is, an unselective removal of the tissues of the pericarp beginning at the outer epidermis (exocarp) and terminating in the locule. The punctures were straight but varied in depth. Older fruits were found to contain greater numbers of punctures which did not completely penetrate the carpel.

Maturation of the cotton capsule was found to resemble that of capsules of other species. According to Esau (1953) such maturation is the result of an increase in the number

of cells as the organ increases in surface area. Accompanying the growth in size was an increase in the thickness of the walls of the individual cells. In stained sections, this thickening appeared to be the result of the deposition of additional cellulosic substances only, with no lignification. The vascular bundles are scattered within the pericarp. Incomplete punctures in the older pericarps were found which showed that lignified tissues were not serving to prevent feeding activities. Within the pericarp of the younger bolls there was a much greater abundance of starch than in the older tissues.

Autoradiographic Findings

Autoradiographs obtained by use of the larger grained Ansco emulsion, although indicating that radioactive substance was present in the host tissue, were not considered of sufficient resolution to be valid. Results obtained with the specimens processed using the finer grained Eastman emulsion gave positive evidence of the presence of radioactive substance in the damaged tissue. Control sections obtained from other levels of these organs and processed in an identical manner showed no radioactivity. Similarly, areas of sections which demonstrated the presence of radioactive substances in the wound did not indicate such substances in undamaged areas.

Within the autoradiograph of the floral bud specimen there was a small amount of sensitized emulsion in the tissue perfora-

tion and in the terminal cavity among the anthers. Fig. 1, Plate II, shows the presence of these particles which are not evident in the histological section shown in Fig. 2, Plate II.

Examination of the young fruit specimen revealed an unusual occurrence. What had been believed to be a feeding puncture was revealed to be an oviposition tunnel. As shown in Figs. 3 and 4, Plate II, the radioactive substance was more abundant. The emulsion granules indicated its presence throughout the tunnel and in greater abundance about the deposited ovum.

Examination of the gross autoradiograph which was prepared as a check revealed only three sensitized spots; the remainder of the emulsion was clear. When superimposed upon the shadowgraph the black spots were found to coincide exactly with the puncture sites. A duplicate of the autoradiograph super-imposed upon the shadowgraph is shown in Fig. 1, Plate III.

Ovipositing Damage

In these studies floral buds and fruits of varying ages which gave evidence of characteristic boll weevil oviposition, such as spread involucral bracts accompanied by the presence of a wart or rough tumor and/or an easily separated abscission area, were dissected and examined. Gross damage was found to be as described by Hunter and Hinds (1905) and Fenton and Dunham (1929). Although these effects

have been adequately described and illustrated by these authors, a brief review will be included in this writing for the purpose of clarity.

Floral Buds

A floral bud which contained an oviposition wound of four to five days of age bore an irregular lump approximately one to two millimeters in diameter. The crest of the lump or "wart" was rough and was either yellow or dark in color. Depending upon the age of the puncture, the involucral bracts may or may not have spread. A floral bud bearing a typical wart is shown in Fig. 2, Plate III.

Internally the damage to the floral bud varied with the stage of development of the new larval generation. An ovum was usually found lying among the anthers. See Fig. 3, Plate III. As the developing larva increased in size and age a progressively larger cavity was formed in the bud as a result of the ingestion of the tissues. Large larvae were found to have completely removed the androecium and gynoecium. The hollow shell in which pupation occurred consisted only of the base of the receptacle with the attached corolla and calyx. Lining the ovoid cavity was a dark brown paste-like substance of foul odor which was presumed to be larval excrement and decomposed floral tissue. A hollowed floral bud bearing a feeding larva may be seen in Fig. 4, Plate III. Structural details of the external protruberence were not visible internally with either the un-

aided eye or low-power magnifications.

Microscopic study of the oviposition wound showed that it was sealed with a material which contained pollen grains.

The tumor appearing at an oviposition site in a cotton floral bud was found to be the result of the proliferation of the mesophyll of the sepal. The nature of such a growth is shown in Fig. 1, Plate IV.

Fig. 2, Plate IV, shows an enlarged portion of the left base of the tumor pictured in Fig. 1, Plate IV. Examination of both figures discloses that the tumor mass has resulted from cell divisions and an increase in volume of the cells. Periclinal cell divisions occur principally along the periphery of the wound. As new cells are formed and enlarge, the proliferation region is appressed and bent. Bending of the dividing layer results in subsequent divisions being anticlinal to the original surface.

Initiation of cell division occurred almost entirely in the mesophyll along the periphery of the wound. The tissue produced consists of parenchyma-like cells which are vacuolate with very thin walls, small nuclei, and minute quantities of cytoplasm. Chloroplasts were not observed in the tumor tissue nor were vascular elements, lysigenous cavities, or other specialized structures. The single indication of organization is the hemispherical body of the mass. This structure apparently results from the divisions occurring in random planes and the mass of enlarging tissue being extended along the line of least resistance.

A number of the cells appeared to be normal sized parenchyma elements which had divided but had not deposited secondary wall material. The daughter nuclei of these cells may be observed adjacent to each other and separated only by the primary wall. Some of these cells are shown in Fig. 2, Plate IV. Each of these new cells was almost entirely filled by a single large vacuole. The nucleus was usually smaller than the normal parenchyma cell and was ovoid rather than spherical.

Two characteristics of these cellular elements indicated that the divisions producing them were amitotic. As may be seen at the right center of Fig. 2, Plate IV, two nuclei, not yet separated by a cell wall, appear in the interphase although there is no evidence of a mitotic spindle or cell plate. No mitotic figures were observed in the examination of several hundred sections of this material.

Fruits

Gross damage to cotton fruits parallels closely that of floral buds. Minor differences were seen which were attributed only to the differences in the nature of the tissues present.

Oviposition by the adult female weevil almost invariably resulted in the production of a tumorous growth which was visible upon the external surface of the carpel. Such an external growth is pictured in Fig. 3, Plate IV. These growths upon the fruit were less hemispherical than those

formed upon sepals. Instead, the wound consisted of the rough crest. Usually absent was the rounded distention apparent upon the sepal. It will be recalled that such a distention of the sepal was the result of proliferating cells within the sepal.

Microscopic study of oviposition wounds revealed that they, like similar wounds in the buds, were sealed with a material which contained cotton pollen grains.

Continued study of the specimens showed clearly the nature of the carpel growth response engendered by oviposition. Free cells of a parenchymatous nature were found to compose the inner growth. When teased apart the "gelatinous" and the "shining" growths separated into free cells which were very large, thin walled, vacuolate, and irregularly shaped. Some of these units bore cross walls which divided them into unequal portions. Some cells were nucleated, others were not. Cell walls were extremely thin and gave no evidence of secondary thickening. Several such free cells are shown in the phase photomicrograph in Fig. 4, Plate IV.

It may be noted in this photomicrograph that many small refractory bodies were present in the vacuole. Such structures were common in these growths. Staining with iodine and observation by polarization microscopy demonstrated a negative response for the presence of starch.

Permanently mounted and stained sections cut longitudinally through the oviposition site confirmed these observations and supplied additional details regarding the dif-

ferences in the tumors. A typical section of such a tumor may be viewed by reference to Fig. 1, Plate V.

Examination of such specimens disclosed that the "gelatinous" growths referred to by Hunter and Hinds (1905) are composed of a compacted mass of non-cohering parenchyma tissue identical with the granular growth. Frequently however, the endocarp, which is epidermal in nature, is proliferated and extended as a membranous sheath enclosing the free tumor cells. A portion of this structure is shown in Fig. 2, Plate V.

This study also revealed that the origin of the free tumor cells was in the parenchyma of the mesocarp. Random cell divisions initiated at the periphery of the wound refilled the excavated region and forced the cells into the locule. It is interesting to note that no re-differentiation of tumor tissue was observed. All cells produced were fundamentally of a parenchymatous type and arose from parenchymatous tissues.

Experimental Stimulation of Simulated Oviposition Tumors

Results of the experimental stimulation of tumors upon organs used as boll weevil ovipositing sites are shown in Table I.

Examination of the table discloses that sterile punctures were not important stimulators nor were inoculations of soil and feces. Other chemical agents show considerable uniformity in total responses although varying in the nature

of the response. The heterogeneous larval extract produced the largest percentage of responses in all organs.

TABLE I

RESULTS OF EXPERIMENTAL STIMULATION OF SIMULATED OVIPOSITION TUMORS

Column Number	Inocula
1.	Sterile
2.	Soil
3.	Feces
4.	Larval Extract
5.	IAA
6.	IBA
7.	Colchicine
8.	Lanolin
9.	Petroleum Jelly
10.	Totals

Column	1	2	3	4	5	6	7	8	9	10
Floral Bud										
Inoculations	10	10	14	15	10	10	10	10	10	99
External Response	0	1	3	10	9	9	10	8	2	52
Internal Response	0	2	3	10	9	9	10	7	1	51
1-7 Day Fruits										
Inoculations	8	9	15	12	9	7	5	10	10	85
External Response	0	0	2	6	1	4	0	2	0	15
Internal Response	1	5	3	8	7	7	1	10	2	44
8-14 Day Fruits										
Inoculations	12	13	10	10	9	5	10	10	11	90
External Response	0	4	1	3	4	0	4	0	2	18
Internal Response	3	7	2	5	4	2	6	4	9	42
Total Responses Each Agent	4	19	14	42	34	31	31	31	16	222

Abscission of Floral Buds and Fruits

Examination of the results of the comparative study of abscising organs of the cotton plant shows that four types of stelar structures are concerned, each differing in the

quantity, quality, or distribution of its tissues.

Foliar abscission concerns the separation of the petiole from a vegetative or a fruiting stem. The petiole contains four large, discrete, collateral bundles and four small, discrete, collateral bundles all of which are composed of primary tissues and which are not connected by an interfasicular cambium. Petiolar tissues are chiefly parenchymatous. Vascular bundles contain no secondary growth and the lack of patches of thick walled pericyclic fibers is especially noticeable.

The other three structures form an intergraded series of dissected siphonosteles. The pedicel is definitely more stem-like in its structure. Vascular bundles are numerous, closely appressed, and are connected by an interfasicular cambium. Some secondary xylem and phloem are present, especially in older organs. The amount of secondary tissues and their degree of thickening is proportional to the age of the structure. Thickened pericyclic fibers are also present in small patches in the pedicel. The quantity of these cells, too, increases as the structure ages.

Fruiting stems are intermediate in their structure to the pedicel and the vegetative stem. In each of these the radial alignment of secondary xylem, the obliteration of pith rays, and the increase in the amount of pericyclic fibers results in the formation of a continuous siphonostele of a woody nature.

Briefly summarized the petiole has a dictyostele, the

young pedicel a dissected siphonostele, the old pedicel a continuous siphonostele with little secondary thickening, the fruiting and vegetative stems possess continuous siphonosteles with amounts of secondary thickening proportional to their age.

Comparison of these structures may be made by reference to Figs. 3 and 4, Plate V, and Figs. 1 and 2, Plate VI.

Histochemical tests of these regions indicated also that the xylem and pericycle of the pedicel and stems contained greater quantities of lignin than was present in the petiole. Otherwise no significant chemical differences were noted either in abscising or healthy organs. Although Dutt (1928) reported a decrease in calcium and starch content, these conditions were not found. Starch concentration was found to be greatly variable and no significant decrease in calcium, as described by Dutt (1928), or pectic substances, as described by Facey (1950), prior to abscission could be seen.

Leinweber (1956) noted that the area of separation of the petiole was discernable externally as a "hyaline" ring and internally as an area in which the cell walls were thinner and had less affinity for stain. Abscission of the leaf was effected by hydrolysis of cell walls and cell division across the break.

Abscission of the floral organs resembles this in the presence of the hyaline ring. Separation is along this line. Internally, however, some differences exist. Along the line

of subsequent separation is a layer of flattened and compact parenchyma tissue usually 10-20 cells in depth. The long axis of these cells is at right angles to the axis of the pedicel. The walls of these cells are slightly thinner than those of other regions of pith. Separation occurs first in the cortex lateral to the vascular tissue and secondly in the vascular tissue and pith. The irregular edges of vascular tissues indicate that separation is by mechanical breakage. The line of separation in epidermis, collenchyma, and pith is marked by ruptured cells. There was no evidence of regular separation along intercellular junctions. Figs. 3 and 4, Plate VI illustrate the structures as seen in a young healthy pedicel and in a pedicel of a floral bud which showed external evidence of abscising from boll weevil damage. Fig. 1, Plate VII shows the external appearance of the abscission region, and the relative diameters of these structures in organs of four ages.

CHAPTER V

DISCUSSION

This work has been an attempt to determine histologically the nature of the damage inflicted upon the cotton plant as a result of activities of the boll weevil. Because the host plant serves as food for the adults and the larvae and also as a site for oviposition, the study has consisted of several separate investigations. Clarity may be achieved more easily if each of the several facets of the study is discussed individually.

Feeding Damage

All aerial portions of the host plant are subject to damage by the boll weevil. The direct damage is largely mechanical and results from ingestion of the tissues by the adult and the larva.

Vegetative organs may be injured but this occurs only when floral buds and fruits are not available. As has been noted, the weevil appears to have a very definite "preference" for certain organs of the plant. This study has shown that this "preference" does not extend to selection of tissues within any feeding site. Feeding punctures commonly were straight tunnels including all tissues in the

path. Results of the study of the structure of young and old carpels indicate that older carpels offer more mechanical resistance to the weevil's mouth parts. This resistance is likely due to thickening of the cell walls of the carpel.

Results of the autoradiographic studies of feeding have established that the adult weevil introduced a foreign substance into the host. The substance is minute in quantity and its nature was not determined.

In brief, the damage to the cotton plant consists of removal of tissues and injection of an oral secretion. Several effects may be suspected as resulting from these two factors. The removal of tissues by feeding may interrupt vascular continuity, result in additional metabolic stress, provide a portal of entry for pathogenic organisms or other foreign substances, and expose tissues to drying action of high temperatures and air. Severe wounds of many kinds, including weevil feeding, result in the abscission of buds and young fruits. Fragmentation of tissues results in debris within the wound which may be auto-toxic. Although admittedly speculative, the injection of the oral secretions may contribute to necrosis of tissue, toxicity to the plant, or perhaps to some other effect. At various times the cotton floral bud and young fruit are readily abscissed. It is considered possible that the introduction of a foreign substance might contribute to such abscission.

Additional investigation of a physiological and biochemical nature might contribute much toward determing the

effects which are not visible.

Ovipositing Damage

Conspicuous injury to the cotton plant as a result of egg laying activities are as described by earlier workers (Fenton and Dunham, 1929; Hunter and Hinds, 1905). The histological approach necessitated giving consideration to the oviposition tumors in the bud and fruit. These were found to be the result of the proliferation of mesophyll in all cases. Extrusion of newly formed cells may be in either direction in the tunnel. Internal masses of cells within the more flexible perianth of the bud often result in what would appear to be a large tumor. Actually, larger masses of cells are usually produced within the locule of the fruit but the more rigid carpel is not distended as is the perianth.

Hunter and Hinds (1905) noted in the fruit the internal production of the mass which they termed "gelatin". Indeed, macroscopically the growth appearing upon the inner surface of the carpel was often a white, shining, and irregularly shaped mass measuring up to four millimeters in diameter and offering very much the appearance of a mass of gelatinous substance. It was noted, however, that occasionally these inward proliferations appeared granular.

Results of the investigation of these two kinds of tumors have been described in a previous chapter. It appears to the writer that the growths are essentially the same; each consisting of a mass of separate, vacuolate, parenchyma like cells.

It is the conclusion of the writer that the fundamental difference in the two growths is the presence of a membrane covering free cells in the so called "gelatin". The membrane is a product of the endocarp and it is this membrane which conveys the shining gelatinous appearance to such structures. A portion of this structure is shown in Fig. 2, Plate V. If the cells of the endocarp were stimulated into dividing, the growing tumor was covered by the membrane. If, however, the endocarp was perforated or its rate of cell division did not maintain the membrane as the tumor grew, the free tumor cells were forced into the locule to form a "granular" structure. Of especial interest was the apparent complete individuality of each unit. The ease with which separation was effected strongly suggests the absence of a middle lamella. Some free cells from such a tumor are shown in Fig. 4, Plate IV.

Careful observation of the pericarp also led the writer to the belief that the numerous refractory bodies noted in the tumor cells were immature or abberant plastids. There are several reasons for this belief. The pericarp of the maturing cotton fruit is composed primarily of mesocarp of which the outer cell layers are largely chlorenchyma. In addition, deeper layers of the mesocarp contain numerous amyloplasts. Staining of fresh green sections of this organ with an IKI solution revealed a great abundance of discrete bodies which demonstrated a positive starch reaction. Measurements of the refractory bodies coincide closely with those of the plastids as given by Hayward (1938). These

measure from 4 to 6 microns. The refractory bodies and the chloroplasts are discoidal. It is generally accepted that plastids are self perpetuating and may, as a consequence, increase in number in newly formed cells (Esau, 1953; Hayward, 1938).

Comparison of the Structure and Stimulation of Oviposition Tumors in Cotton with Similar Growths in Other Plants

The production of specialized morphological units and of unspecialized growths from previously differentiated tissues has long been of biological interest. The basic biological phenomena of differentiation and cell division are apparent in such isolated growths. For this reason numerous studies of abnormal plant growths have been performed from the pure and applied approaches.

Two kinds of tumors have been described in the preceding pages. Both of these structures occur on the cotton plant as a result of activities of the cotton boll weevil. It will also be recalled that one of these growths was formed in the mesophyll of the sepal after oviposition and the other in the mesocarp of the fruit after oviposition. The growths do not usually appear in feeding wounds.

Growth is fundamentally the result of cell division, resulting in masses of new cells, and/or cell enlargement. A third factor, differentiation, may follow. According to one concept suggested by Esau (1953), "differentiation comprises the many interrelated processes of physiological and morpho-

logical nature which bring about the specialization of cells". Normal formation of an adult plant body, or replacement of removed portions, also involves organization of the differentiated tissues into morphological units.

Wound tissue may lack organization and thus the result is a mass of cells which may or may not be differentiated morphologically. It is often difficult to determine whether a cell is physiologically differentiated.

These abnormally produced growths involve certain changes not typical of normal growth. The first of these is a return of functioning cells to a meristematic condition. This is termed "dedifferentiation" by Bloch (1941). Daughter cells produced by these new meristematic regions are most commonly redifferentiated into tissue elements which may vary greatly in their specialization and organization.

A virus tumor in which considerable redifferentiation and organization occurred was studied by Kelly and Black (1949). It was found that the growth was initiated by tangential divisions in cells of the pericycle opposite the primary phloem. In this case the tumor resulted from abnormal cell multiplication rather than cell enlargement. Redifferentiation of phloem occurred at the base of the tumor and production of xylem followed at the periphery. Organization was not normal but did demonstrate special "tumor unit organization".

Investigations of crowngall and wound callus on apple have been numerous. This growth demonstrates a type in

which little organization and redifferentiation occurs. Wound callus on apple, as described by Sylwester and Countryman (1933), was produced by the primary cortex, the phloem, cambium, and regions corresponding to the endodermis and pericycle. Cells near an injured surface divided in three planes to produce a mass of loosely arranged, undifferentiated cells. Various portions of this spongy mass organized into irregular bands, whorls, cylinders, or spheres which were meristematic. Divisions of these cells occurred in one plane and their products were a cortical type parenchyma and a contorted form of xylem.

A third type of wound tissue is one in which only a mass of new cells is produced and which apparently lacks differentiation and organization. Such structures are termed kataplasmatic tumors by Bloch (1954). The "hemispherical wart", or tumor, which develops upon the fruit and floral bud of cotton, following oviposition by the cotton boll weevil, is of this type. Photomicrographs of a section through one of these growths are shown in Figs. 1 and 2, Plate V.

For over a decade the investigations of these problems have been largely of a physiological nature. They have for the most part been concerned with the effects of growth regulating substances. Swingle (1952) has stated that anatomical and morphological studies have not kept pace and a return to basic studies of protoplasm is needed.

Some of the more general investigations have been conducted by Bloch (1941; 1952). His work of 1952 stated that "the organizing principle controls, in some way, not only the production of many different patterns and specific individual forms but also their maintenance and regeneration under changing or adverse conditions". He also recognized that many environmental factors could vary the nature of regeneration of tissues in a given species. Along a more specific vein he stated "Cell division and growth are usually associated with decomposition processes in cells and with the formation of necrotic and autotoxic products, and may be related to them causally" (Bloch, 1941).

LaRue (1941) and Went (1940) have produced various growths by means of growth promoting substances as have Jablonski and Skoog (1954). Loeb (1924) found no such substance functioning in Bryophyllum but did attribute a "substance" in the descending sap with the ability to stimulate production. Loofbourow, et al (1941) described a proliferation-promoting hormone produced intercellularly as a direct response to injury. This "hormone" is in addition to other substances such as damaged cell products, adenine, adenosine triphosphate, and commonly known auxins.

In a study of the metabolism of insect galls, Newcomer (1951) found no evidence of growth substances or morphogenetic compounds being present. Examples were cited, however, of the many substances injected by adult insects or produced by larvae within the gall. Among these were diastase, invertase,

amylase and protease. He mentioned also the stimulation of gall production by injecting trypsin, amino acids, protein digests, and salivary gland extracts.

Skoog and Tsui (1948) believed that their work proved that organ formation was chemically controlled. They produced various growths, and inhibited others on tobacco callus and stem internodes by means of adenine, adenosine, alpha-naphthaleneacetic acid and increased phosphate and sucrose. Still other factors affecting differentiation are listed by Child (1940) and Riker and Hildebrandt (1953). Some of these are oxygen tension, hydrogen ion concentration, electric current, light, genetic constitution and vapor pressure. It is known that wound tissues sometimes contain cells with the chromosome number doubled or quadrupled (Sinnott, 1953). Sinnott (1953) implies cytoplasmic changes may be of primary importance.

The great diversity of findings in these studies indicates that cell division and differentiation are properties of living material which may be influenced by a multitude of conditions. No easy solution to their underlying principles can be expected nor should one expect a single chemical substance to stimulate and control these processes.

The situation has been summarized by Loomis (1953); "The causes of cell division remain among the unsolved riddles of biology". Sinnott (1953) believed differentiation begins early -- perhaps in the first cell division. He also stated that "histological differentiation is evident in all

tissues though the factors responsible for it are little understood".

In the writer's preliminary consideration of the wound tissue produced in the sepal of the cotton floral bud and the fruit carpel, a number of factors mentioned in the literature as possibly causal were observed. These factors are believed to be worthy of mention at this time.

A property of the oviposition puncture is that it initially is identical, in the manner in which it is made by the weevil, with a feeding puncture made by the same insect. It is of considerable interest that feeding punctures do not often produce the wound callus. This would appear to exclude as causal agents such substances as wound hormones, autotoxic products, and intercellular hormones or bacteria, viruses, and salivary secretions introduced by the mouth parts. The oviposition wound is subjected to three additional agents, however. These are; insertion of the weevil's ovipositor, presence of an ovum at the base of the excavation, and the sealing of the opening with an excretion. These differences provide opportunities for speculation regarding the nature of the stimulating agent or agents. Possible substances would include glandular secretions injected through the ovipositor, ovum secretions, digestive tract decomposition products, microorganisms, and insect enzymes.

This particular wound tissue is quite simple and shows no differentiation. The stimulating substance or substances have not been reported. Although this plant produces simple

wound tissue, it is noteworthy that it has considerable hereditary regenerative properties as demonstrated by its ability to produce secondary foliage after severe pruning. It therefore seemed clear that the act of oviposition or activities of the ovum in some manner contributed to the initiation of growth.

Comparison of Simulated Tumors with Natural Oviposition Tumors

The experimental stimulation of tumors described earlier supplied interesting, but varied, results. The results are given in Table I.

With the exception of the larger number of external responses in the floral bud recorded from the total of all agents, the structures appear to be very similar in their sensitivity. As was noted earlier, the floral bud tumor is essentially an internal growth and the outer protruberence is largely an expression of the degree of internal pressure on the thinner, more elastic perianth. For this reason, the 52 per cent external responses recorded for all substances in the bud cannot be considered significant.

Of especial interest are the total responses to each agent in the three ages of organs. It may be concluded that the tumor response is not the result of a wound hormone or autotoxic decomposition product of the damaged cells since sterile wounds only rarely resulted in a growth. Similarly, it is believed that general contamination as with soil and feces is not to be considered an important tumor inducing

substances. Lanolin, so often used as a solvent, was as effective in stimulating growth on buds and young fruits as were the commonly employed growth promoting substances. The larval extract resulted in the largest total number of responses and caused uniform stimulation in all three organs. Consideration must be taken, however, that this extract doubtlessly is a mixture of very diverse substances. Oviposition by an adult weevil nearly always results in a tumor, except perhaps in very old fruits. None of the plant growth promoting substances (i.e. IAA, IBA, and colchicine) approach this frequency. It would therefore appear that the substance, or substances, stimulating the natural oviposition tumor are extremely potent.

Additional investigations would be necessary to determine the chemical nature of the tumor stimulating substance. Such is not within the scope of this study. It is believed that since it has been shown that the weevil introduces foreign substances into the host tissues when ovipositing, due consideration should be given to the role of this material in growth stimulation.

Abscission

Although investigations of the chemical causes of abscission lie outside the realm of this study, two observations are believed to be related.

Recent reports on abscission in cotton have indicated that the mechanism involves either an auxin-auxin gradient

(Addicott and Lynch, 1955) or a growth stimulator-growth inhibitor balance (U.S.D.A., 1956).

Consideration of the activities of the boll weevil suggests four possibilities by which such mechanisms might be disturbed. Briefly these are:

1. The developing larva removes large amounts of tissue which could remove the source of the controlling substances.

2. The developing larva produces large quantities of damaged tissues and excrement. These could be toxic, or could interfere with or supplement the action of the controlling substances.

3. Secretions such as enzymes and hormones of the developing larva could interfere with or supplement the action of abscission regulating substances.

4. Foreign substances injected by the adult weevil, although minute in quantity, contain a growth promoting substance. It is considered possible that this substance could disturb the chemical balance which determines whether an organ remains attached or abscises.

Suggestions for Future Study

The study of this host-parasite relationship has brought to the writer several thoughts regarding additional investigations which could be of benefit to man through science.

The fundamental problems of the causes of cell division and differentiation well deserve increased attention of researchers. Likewise, satisfactory explanations of the causes of abscission are lacking.

Specific problems of the cotton plant and boll weevil relationship also are considered worthy of mention. Entomologists have made excellent progress in the control of many

insect pests. Systemic insecticides have been investigated. The complete dependency of the boll weevil upon the cotton plant would seem to hold unusual promise for the development of such a substance.

Within the plant sciences there is the possibility of developing varieties of cotton which would be resistant or unattractive to the insect. Determination of the nature of the substance injected into the plant by the weevil and of the effect of such substances would appear to be worthy of investigation.

In retrospect the writer feels that although man has learned much in his search for knowledge, the examination of one relationship such as this serves to show that the sum of his present knowledge is small in contrast to what remains to be learned.

Summary

This work has been an attempt to determine histologically the nature of the damage inflicted upon the cotton plant as a result of activities of the cotton boll weevil. Previous investigations of damage have been made by gross observation and measures of economic loss.

Damage is inflicted upon the host plant by all stages of the insect and all aerial portions of the plant are subject to damage.

Injury to vegetative organs results only under conditions of starvation for the insect. Feeding damage by the

adult and the larva on vegetative and floral organs is principally by physical removal of tissue. There is evidence that the adult weevil injects foreign substances into the host tissue when feeding and when ovipositing. The effect of these substances is not known but it is suggested that they may function in the production of abnormal growths or premature abscission.

Investigation of the abnormal tissue which marks the oviposition site showed it to be a plant response involving dedifferentiation and proliferation of parenchyma. The nature of the stimulating agent was not determined but studies showed that the host tissues were responsive to a large number of substances. It would appear that the stimulating substance is a secretion in minute quantities of the adult reproductive system.

Tumorous growths are undifferentiated and in some instances consist of free cellular elements. It is suspected that the free cells are produced by amitotic division.

The most important damage to the cotton grower is the premature abscission of floral buds and young fruits. A study of the abscission regions of the floral and fruiting structures shows them to be of different structure than that of the foliar abscission region. Resistance of older fruits is believed to be partly due to additional vascular and mechanical tissue. The study shows that abscission resulting from boll weevil activity does not differ from that initiated by other stimuli. The evidence indicates

that abscission in cotton is the result of a physiological complex which may be initiated by a number of stimuli.

Two responses of the cotton plant resulting from parasitism by the boll weevil are biological phenomena which have long been a challenge. Stimulation of tumorous growths such as are formed at oviposition sites pose questions regarding the nature of cellular differentiation and initiation of mitotic activity. The causes of abscission are of great interest and the relationship between the cotton plant and the insect demonstrates again the need for additional study and application of new techniques in its study.

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APPENDIX

Legend to Plate I

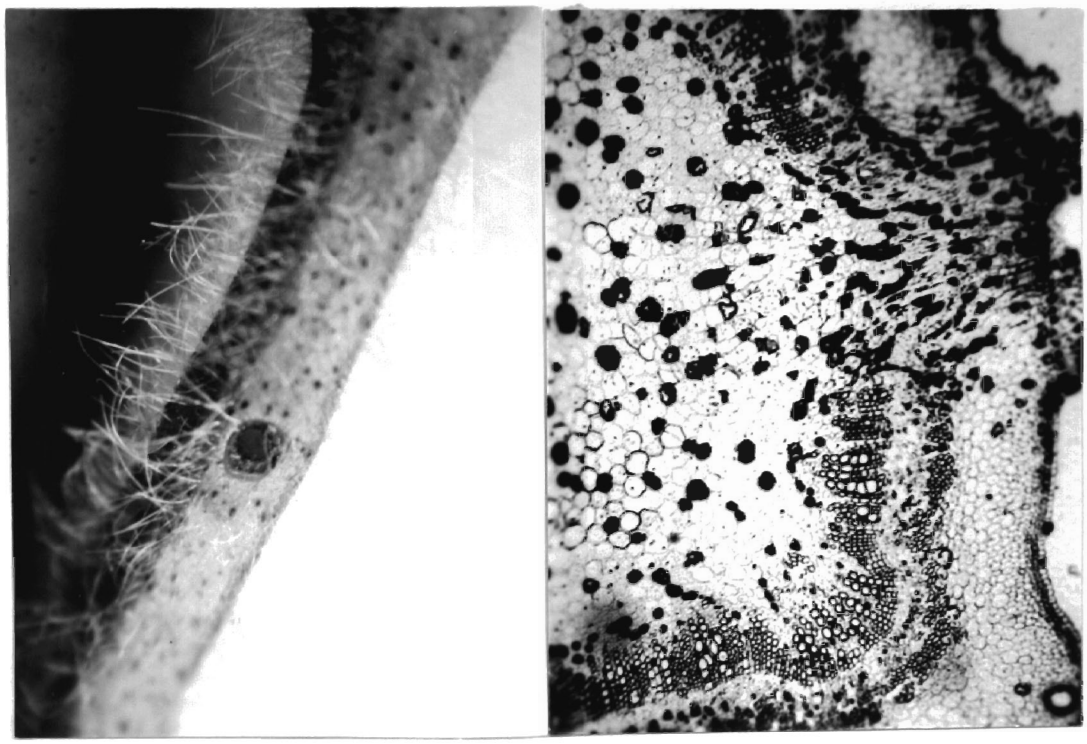
1. Photograph of a boll weevil feeding wound in a young cotton stem. (X6)

2. Proliferation of wound tissue in a young stem. (X60)

3. Photomicrograph of interior of young floral bud damaged by feeding of adult weevil. (X100)

4. Photomicrograph of a section of a normal bud. (X100)

PLATE I



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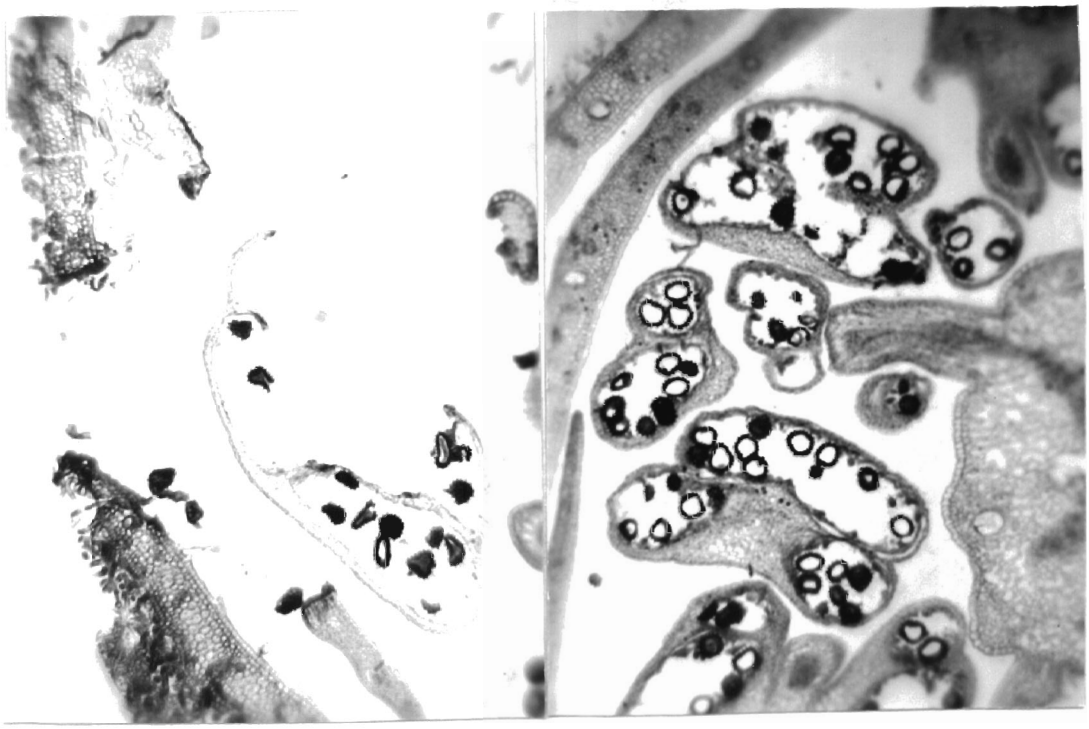


3 4

Legend to Plate II

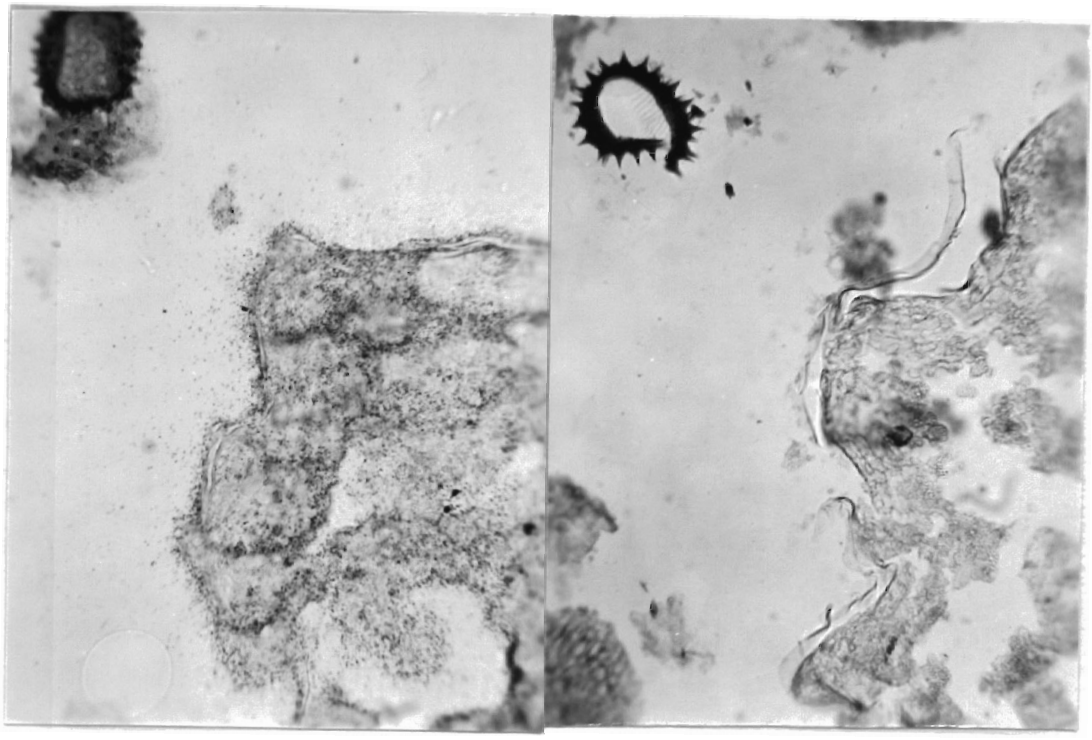
1. Autoradiograph of feeding area within floral bud made by radioactive boll weevil. (X440)

2. Photomicrograph of feeding area similar to above but made by non-radioactive weevil. (X440)

3. Autoradiograph of tissue fragment lying across oviposition tunnel made by radioactive weevil. (X300)

4. Autoradiograph of a portion of radioactive ovum deposited by weevil. (X300)

PLATE II



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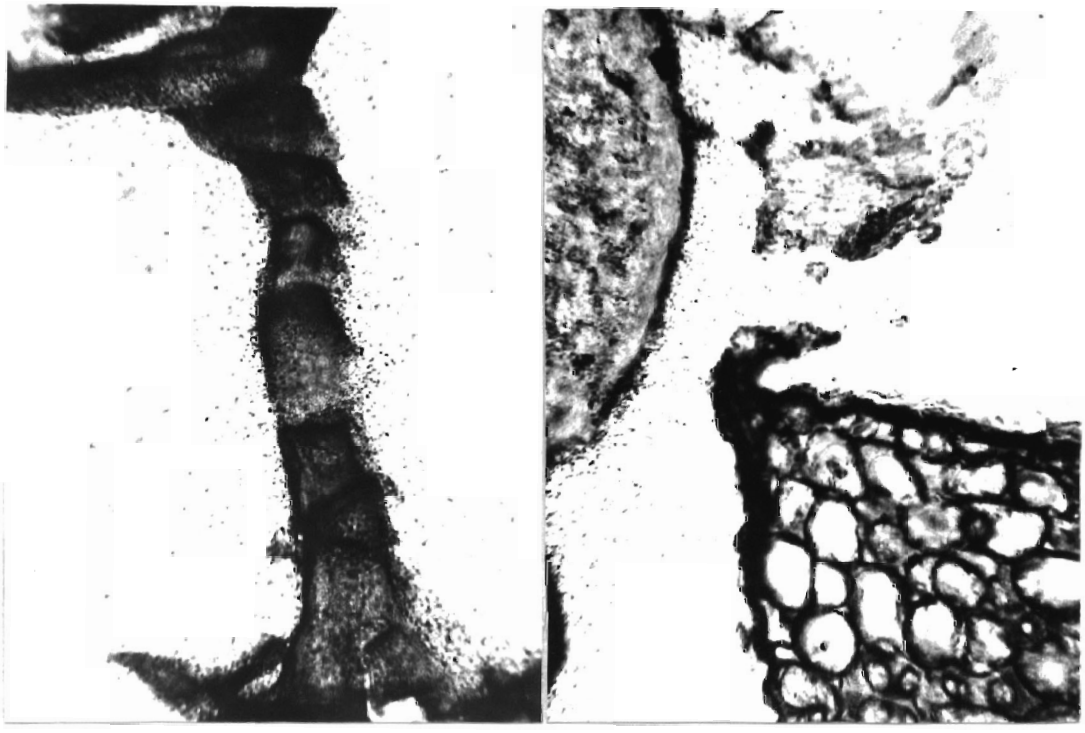


3 4

Legend to Plate III

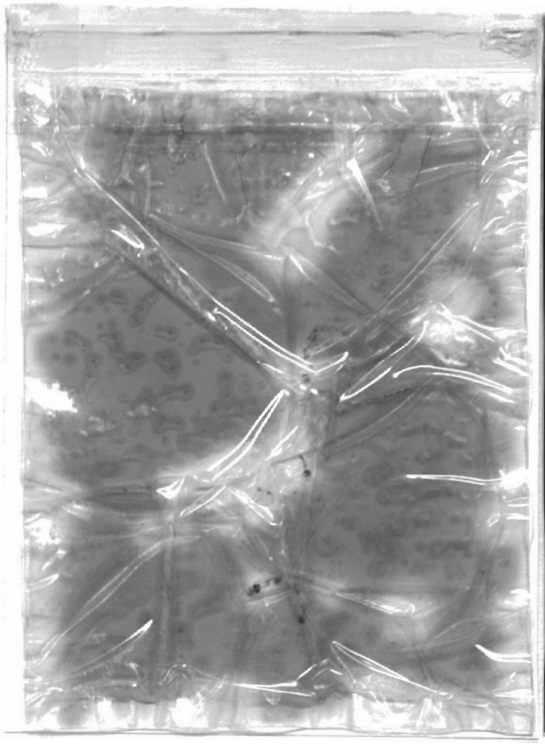
1. Shadowgram of portion of cotton fruit containing feeding puncture made by radioactive weevil. (X1) Copy of autoradiograph of same tissue is superimposed. (X1)

2. Photograph of cotton floral bud bearing a typical oviposition tumor at lower center. (X1)

3. Photograph of floral bud containing a boll weevil egg. (X8)

4. Photograph of floral bud hollowed by feeding of larva. Oviposition tumor also visible in left center. (X3)

PLATE III



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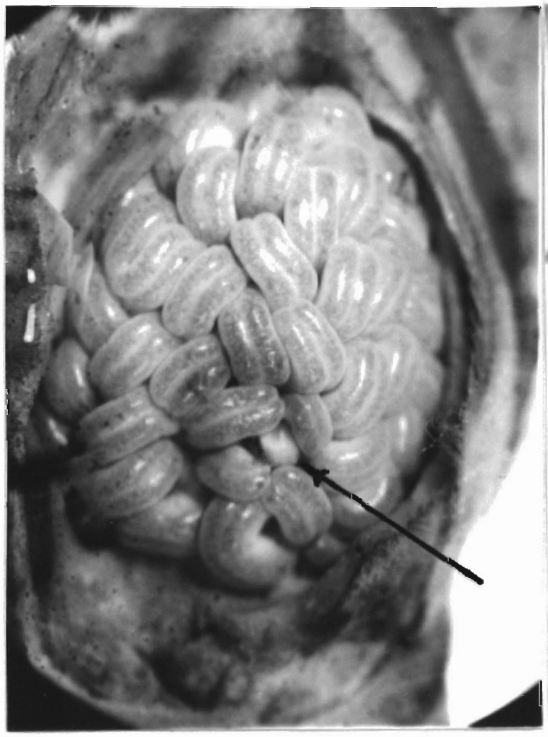

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4

Legend to Plate IV

1. Photomicrograph of oviposition tumor on floral bud sepal. (X60)

2. Photomicrograph of areas of cell division and enlargement shown in Figure 1. (Enlarged X400)

3. Photograph of oviposition tumor on ten day old fruit. (X4)

4. Photomicrograph of free cells from internal oviposition tumor on fruit carpel. (X400)

PLATE IV

1

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Legend to Plate V

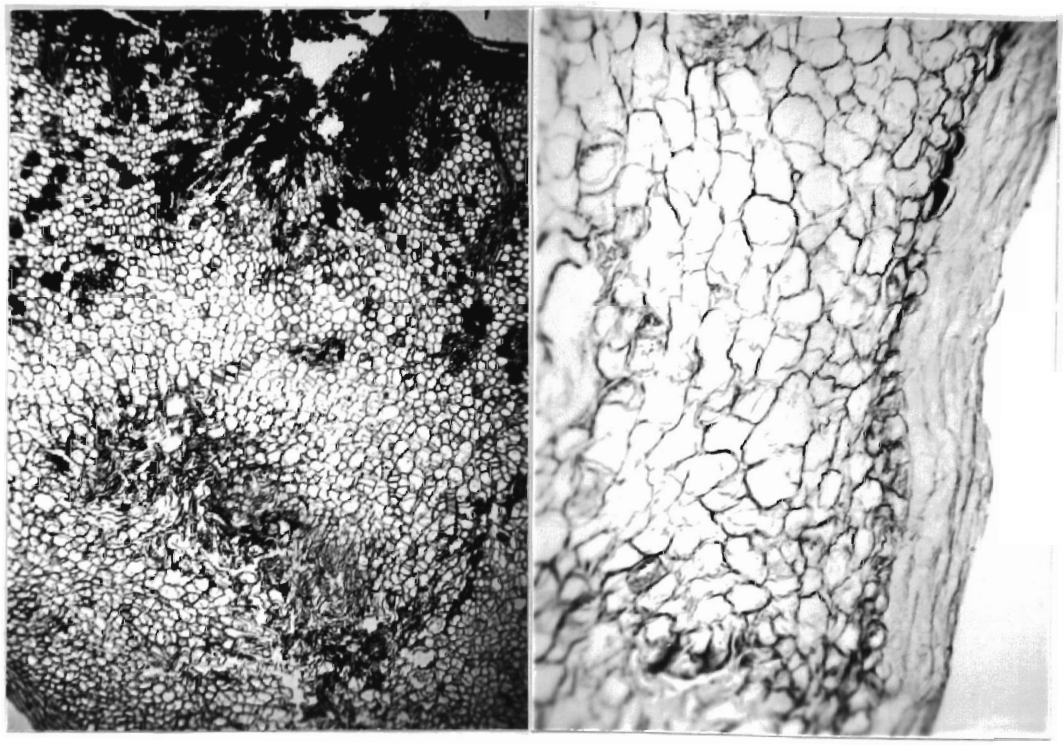
1. Photomicrograph of portion of oviposition tumor in cotton fruit carpel. (X30)

2. Photomicrograph of portion of oviposition tumor with covering membrane. (X100)

3. Photomicrograph of transverse section of mature petiole. (X80)

4. Photomicrograph of transverse section of young pedicel. (X80)

PLATE V



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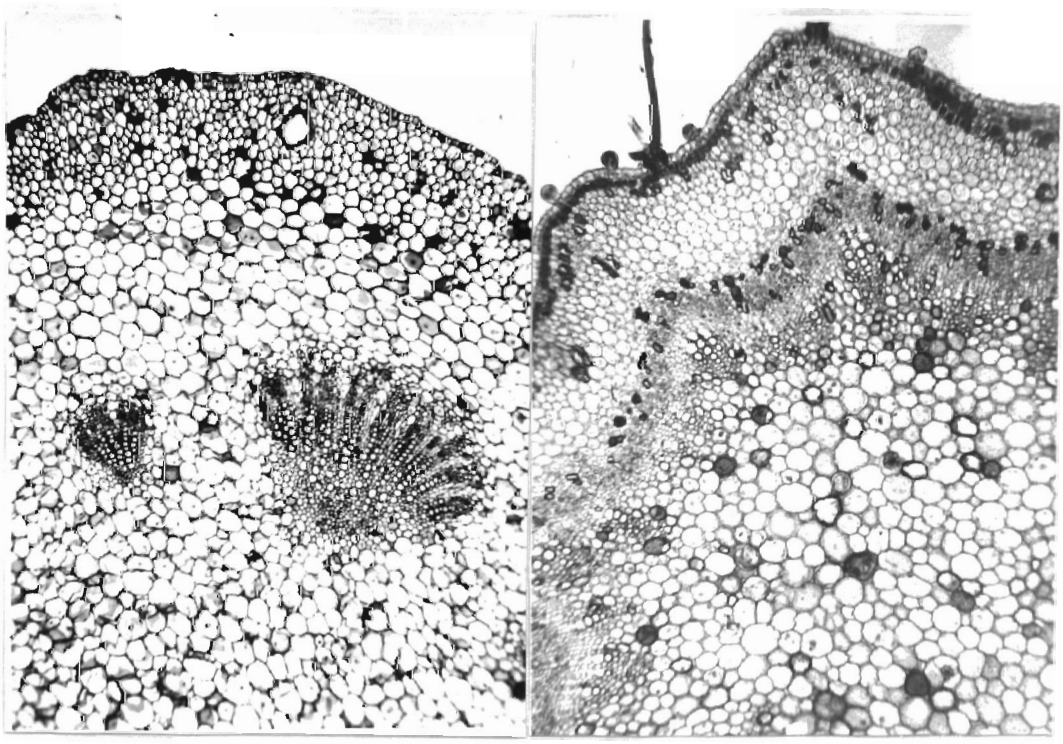


3 4

Legend to Plate VI

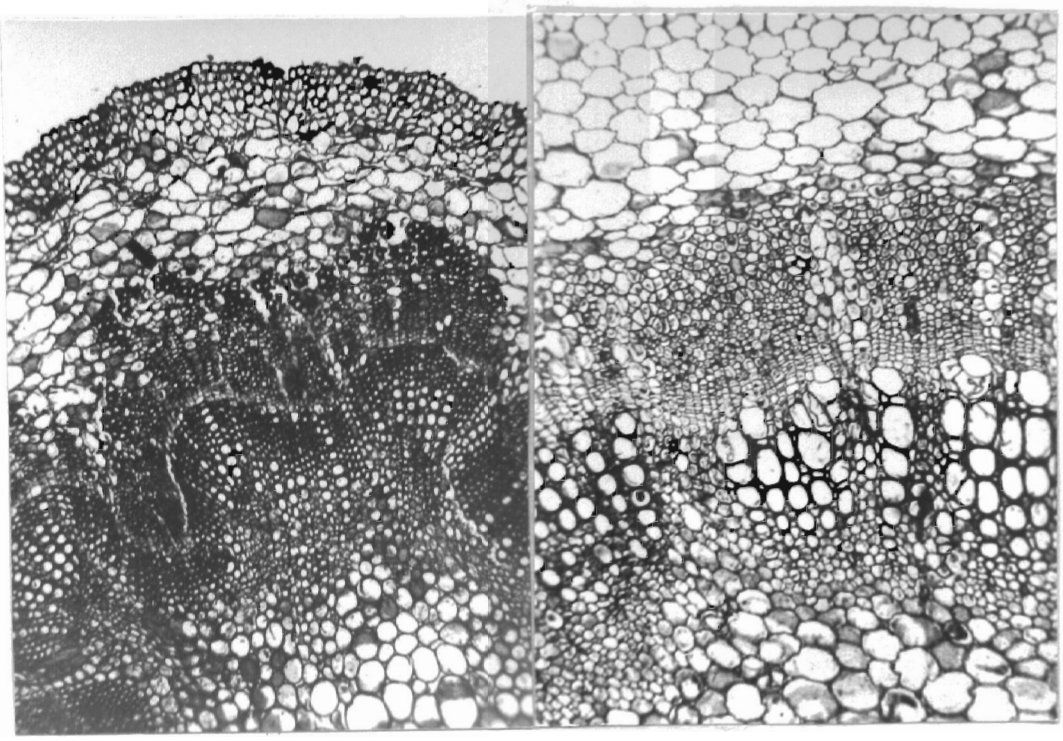
1. Photomicrograph of transverse section of pedicel of a ten day old fruit. (X80)

2. Photomicrograph of transverse section of vegetative stem. (X80)

3. Photomicrograph of tangential section of abscission region of normal young pedicel. (X80)

4. Photomicrograph of tangential section of abscission region of young pedicel in process of abscising. (X80)

PLATE VI



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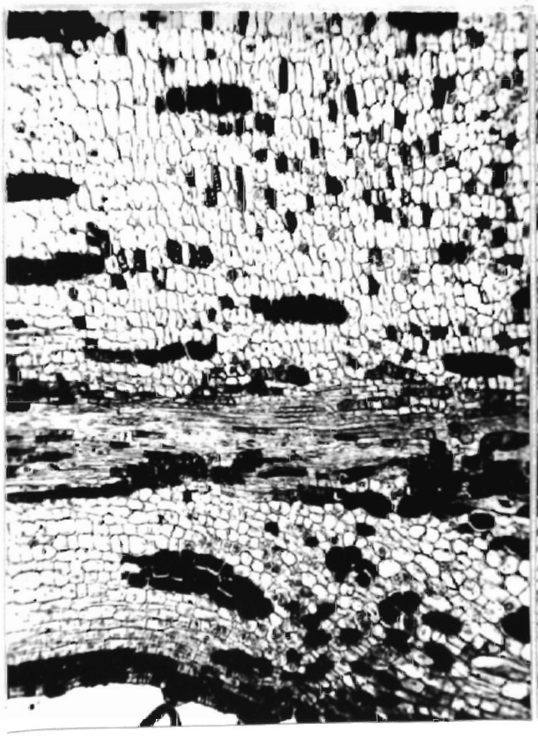

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Legend to Plate VII

1. External appearance of abscission areas of pedicels of different ages. ($X\frac{1}{2}$)

PLATE VII

1

VITA

Stanley Gregg Diehl

Candidate for the Degree of

Doctor of Philosophy

Thesis: A HISTOLOGICAL STUDY OF THE DAMAGE TO GOSSYPIUM HIRSUTUM (L.) RESULTING FROM ACTIVITIES OF ANTHONOMUS GRANDIS (BOH.)

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Personal data: Born in Kansas City, Missouri, September 22, 1919, the son of Howard B. and Gracie Lee Diehl.

Education: Attended elementary and secondary schools of Kansas City, Missouri, graduating from Paseo High School in 1936. Enrolled at Central Missouri State College, March 6, 1946 and received a Bachelor of Science in Education degree with a biological science major in May 1949. Received the Master of Science degree from Oklahoma Agricultural and Mechanical College in August, 1953, with a major in Botany. Completed requirements for the Doctor of Philosophy degree in July 1957.

Professional experience: Retail and wholesale business from 1936 to 1941. Enlisted service in the U.S. Army from October 1941 until May 1943. Active commissioned service from May 1943 until March 1946. Presently a Major in the Artillery United States Army Reserve. Instructor of biological sciences at Cameron State Agricultural College, Lawton, Oklahoma from January 1949 until June 1954. Assistant Professor of Botany and Biology at Southeast Missouri State College, Cape Girardeau, Missouri, from September 1956 to date.

Organizations: Kappa Delta Pi, Sigma Xi, Who's Who Among Students in American Universities and Colleges, 1947-48.