Histological Description of Tarnished Plant Bug (Heteroptera: Miridae) Feeding on Small Cotton Floral Buds[1]

Livy Williams, III[2] and N. Philip Tugwell

University of Arkansas, Department of Entomology, Agricultural Science Building 320, Fayetteville, AR 72701 USA

J. Entomol. Sci. 35(2): 187-195 (April 2000)

Abstract Microscopic features of small cotton floral buds abscised due to *Lygus lineolaris* (Palisot de Beauvois) feeding and non-insect factors were identified and contrasted with healthy buds. Feeding damage appeared to be most common on staminal columns, developing anthers, and corollas. These tissues exhibited gross enlargement and varying degrees of cellular degradation. Fragmented cell walls were thinner and stained lighter than those that were intact. Desiccation of buds abscised due to *Lygus* feeding was irregular. Tissues of buds abscised due to non-insect factors stained uniformly and all cells were intact. Uniform basipetal desiccation throughout the bud occurred in non-insect damaged buds, especially in anthers, staminal columns, and carpels. Tissues and cells of healthy buds stained uniformly and consistently and were without structural abnormalities. The biochemical composition of male reproductive tissue of cotton floral buds appears to play an important role in the nutritional physiology of *L. lineolaris.*

Key Words Cotton, feeding damage, floral bud abscission, extra-oral digestion

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton, *Gossypium hirsutum* L., in the southeastern USA (Hanny et al. 1977). Feeding by *L. lineolaris* is usually concentrated on actively-growing tissue, especially small (<3 mm diam) floral buds, and can result in abscission of these structures, thus reducing yield (Pack and Tugwell 1976). Cotton is especially vulnerable to economic loss of floral buds in the early season when plants initiate reproductive growth. At this time, cotton is growing rapidly and the young tissue is attractive to *L. lineolaris* dispersing from senescing spring weed hosts (Tugwell et al. 1976, Snodgrass et al. 1984). The resulting infestations often lead to annual losses exceeding $40 million for the southeastern USA (Williams 1999).

Histological studies of insect feeding provide insight into the mechanical and chemical components of the feeding process, and the nutritional requirements of the insect. Several such studies have been conducted with mirids. Pack and Tugwell (1976) reported on the feeding damage by *L. lineolaris* and *Neurocolpus nubilus* (Say) to cotton floral buds (>3 mm diam) and fruit. King and Cook (1932) provided histological descriptions of the damage to cotton stems and leaf petioles by several mirids, including *L. pratensis* (probl. *L. lineolaris*), *Psallus seriatus* Reut.) (=*Pseudatomoscelis seriatus* Reut.), and *Adelphocoris rapidus* (Say). Histological studies of feeding

[1]Received 29 January 1999; accepted for publication 16 August 1999.
[2]USDA-ARS Southern Insect Management Research Unit, P.O. Box 346, Stoneville, MS 38776-0346 USA.

damage to crops other than cotton have been made for *L. lineolaris* (Flemion et al. 1954) and *L. disponsi* Linnavuori (Hori 1971, Hori et al. 1987).

The present study was undertaken to investigate the nature of *L. lineolaris* feeding damage to young cotton tissue. Our objectives were to: (1) identify and describe histological characteristics of cotton floral buds abscised due to *L. lineolaris* feeding, and (2) contrast these characteristics with those of healthy buds and those buds abscised due to non-insect factors.

Materials and Methods

Cotton (cv. Stoneville 213) floral buds were subjected to feeding by adult *L. lineolaris* in a greenhouse study. Insects were caged individually on small (≤3 mm diam) floral buds for 24, 48, or 72 h. Sleeve cages constructed of polyethylene foam and nylon mesh were affixed to the first or second fruiting points of fruiting branches in the upper six nodes of plants. Identically caged buds were induced to abscise by mechanically injuring the pedicel beneath the bud. Healthy buds caged without *Lygus* served as controls. Ambient light was supplemented by artificial light on a 16:8 h L:D cycle. After the appropriate time interval, buds were removed from plants and prepared for histological examination. Voucher specimens of *L. lineolaris* are deposited in the senior author's personal collection.

Details of specimen preparation are given in Jensen (1962). Briefly, buds were prepared for staining in one of two ways. Buds to be utilized for paraffin section light microscopy were fixed in FAA (formalin-glacial acetic acid) under gentle vacuum infiltration, then dehydrated in a series of ethanol-tertiary butyl alcohol solutions. Specimens were then passed through a series of Paraplast® (Sherwood Medical Industries, Inc., Norfolk, NE) - paraffin oil solutions and embedded in Paraplast. A rotary microtome was used to section specimens longitudinally at 10 and 15 µm. Serial sections were then differentially stained with Toluidine Blue or Safranin-Fast Green and permanently mounted.

Buds to be examined by thin section light microscopy were fixed in Karnovsky's fixative under gentle vacuum infiltration, postfixed in osmium tetroxide, stained in uranyl acetate, then dehydrated in an ethanol-propylene oxide series, and embedded in Spurr's epoxy resin. Specimen blocks were sectioned at 1 µm with glass knives in a JB-4 DuPont microtome. Specimens were then stained with a Toluidine Blue-sodium tetraborate solution, after which slides were permanently mounted. Descriptions of treatment effects were made with the aid of a binocular microscope. Phase-contrast photomicrographs were made with an Olympus Vanox light microscope coupled with a PM-10 camera unit.

Results and Discussion

Tissue and cell characteristics of healthy cotton floral buds were described and contrasted with those of buds that abscised due to: (1) *L. lineolaris* feeding and (2) non-insect factors. Floral buds from the three treatment categories exhibited distinguishable characteristics.

General characteristics of differentially-stained tissues and cells are as follows. In specimens stained with Safranin-Fast Green, the primary parietal and sporogenous layers, staminal column, carpels, trichomes, and vascular bundles stained green. Starch grains, cell walls, nuclei, epidermal cells, and tannin-containing cells appeared

pinkish or red. In specimens stained with Toluidine Blue, epidermal cells, and vascular bundles appeared bright blue or purple, and tannin-containing cells were orange. Other structures were deeper blue or green. Structures stained darkly red (Safranin-Fast Green) or blue (Toluidine Blue) appear black in the figures.

Healthy buds were characterized by uniform, consistent staining of tissues and cells, and were without structural abnormalities (Fig. 1). Floral buds abscised due to non-insect factors also stained uniformly, but were distinguishable from healthy buds and buds abscised due to *Lygus* feeding (Fig. 2). Cells in buds abscised due to non-insect factors were intact, and there was uniform desiccation throughout the bud. In particular, the staminal column was shrunken basipetally, giving rise to a disproportionately large void between the apex of the staminal column and the bud apex. Also, tannin-containing cells stained more intensely than in buds of the other treatments.

Feeding damage by *L. lineolaris* was generally restricted to the male reproductive structures. Damaged staminal columns were characterized by gross enlargement, and various degrees of cellular degradation which appeared as amorphous red (Safranin-Fast Green) or blue (Toluidine Blue) deposits (Fig. 3). Damage appeared to progress with time so that buds subjected to feeding for 72 h exhibited nearly complete cellular dissolution. Damage symptoms appeared to proliferate from areas where feeding occurred. In some cases, entire staminal columns were destroyed (Fig. 4). Fragmented cell walls were thinner and stained lighter than those that were intact. Cells adjacent to damaged tissue were usually in various stages of disorganization and stained red or blue, depending upon the staining technique. Damaged anthers and corollas were shrunken and stained deeply red (Fig. 5). Damage to corollas was restricted to areas adjacent to damaged anthers or staminal columns, and thus may have been a result of stylet passage through these structures. The presence of cell wall fragments and amorphous material staining either red or purple suggest the activity of pectinase on cell walls (Laurema and Nuorteva 1961), as well as mechanical damage to cells by stylets during feeding (Flemion et al. 1954). Breakdown of cell walls might aid *L. lineolaris* in 'pooling' the cell sap and semi-solid cell components into a relatively large accumulation, thereby facilitating ingestion. The origin of the amorphous masses may also result from compounds released from damaged tissue as a wound response by the plants and from cell contents not ingested by the bug.

The damage symptoms of small cotton floral buds described in the present study are consistent with those reported by other investigators. Pack (1973) reported that plant bug damage to large floral buds was concentrated on anthers and pollen grains therein. Damaged anthers and pollen grains were irregularly shrunken and darkly stained. Dissolution of the staminal column was also observed on buds that were heavily fed upon. King and Cook (1932) found that external swelling and split lesions were characteristic of *L. lineolaris* feeding on cotton stems and petioles. These symptoms were a result of enlargement and subsequent rupture of cortex and collenchyma cells. The fragments of ruptured cells and their contents formed an amorphous mass that was readily stained. Flemion et al. (1954) reported that *L. lineolaris* feeding on vegetables resulted in ruptured cell walls that were more lightly stained than in undamaged areas. Feeding by *L. disponsi* on sugarbeet and rape caused cell lysis and the formation of a darkly stained amorphous substance (Hori 1971).

The present study showed that feeding by *L. lineolaris* on small cotton floral buds is concentrated on male reproductive tissue (i.e., staminal columns and developing anthers). Feeding damage to larger buds occurs predominantly on anthers and pollen

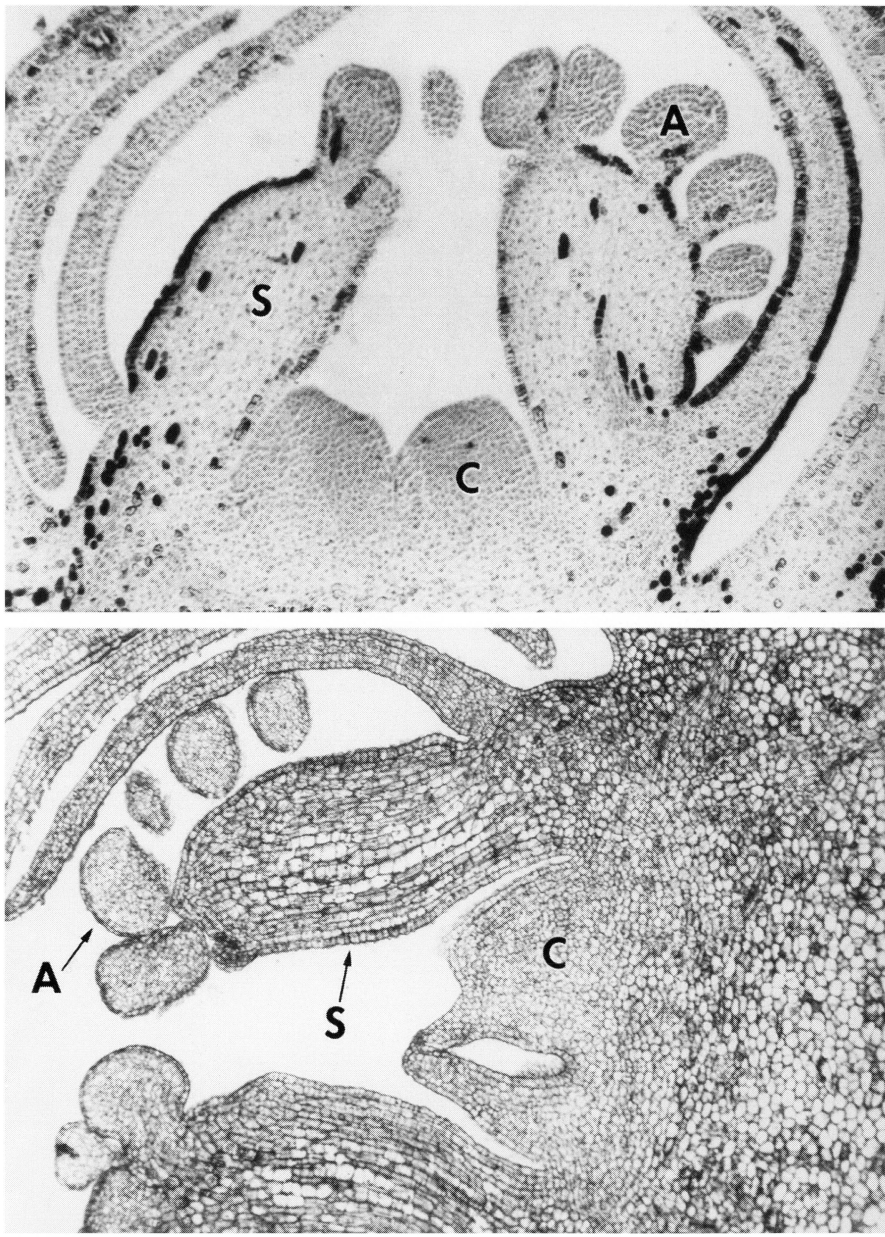

Fig. 1. Median longitudinal sections (10 μm) of healthy cotton floral buds. Cells of anthers (A), staminal column (S), and carpels (C) are evenly stained and intact. 25X.

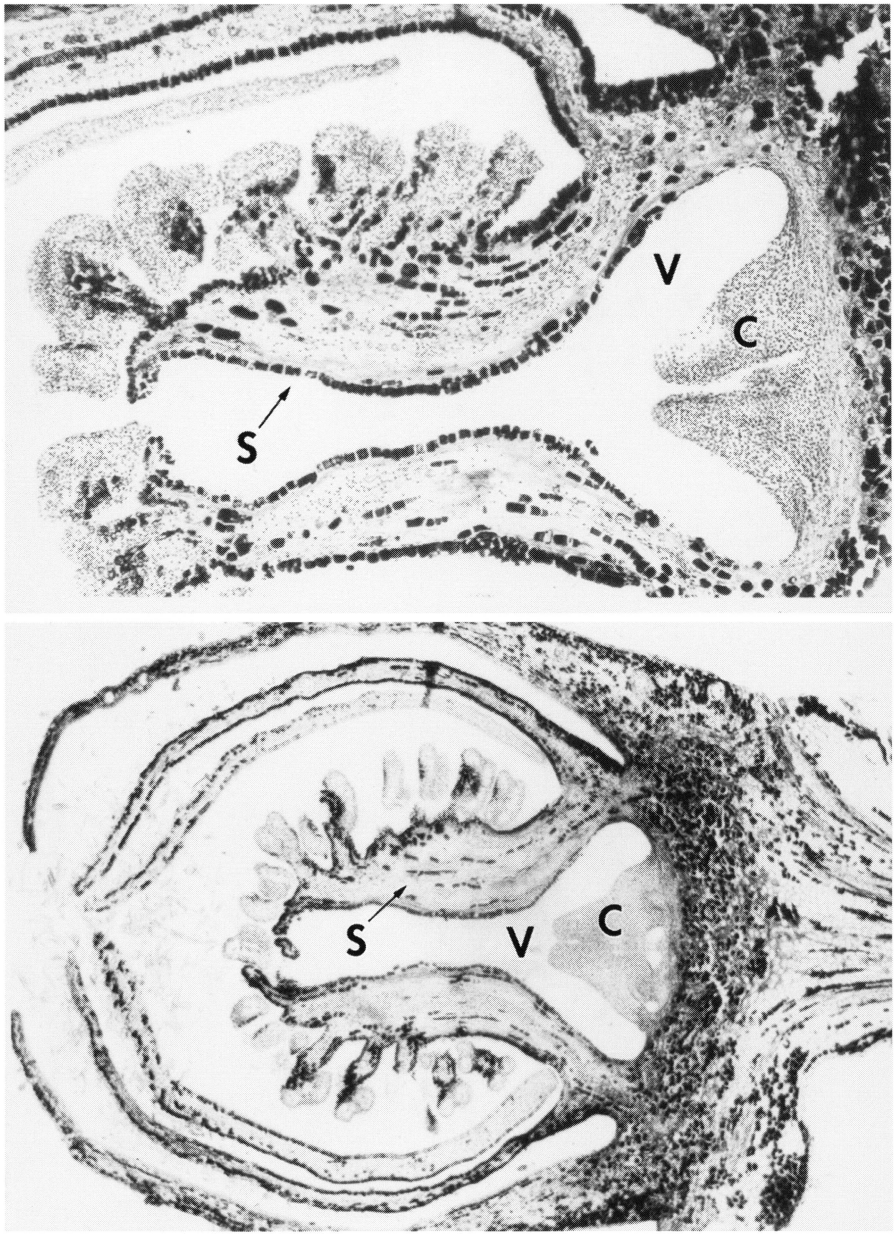

Fig. 2. Median longitudinal sections (10 μm) of cotton floral buds abscised due to non-insect factors. Cells of staminal column (S), and carpels (C) are intact but shrunken basipetally creating voids (V). Top 25X, bottom 10X.

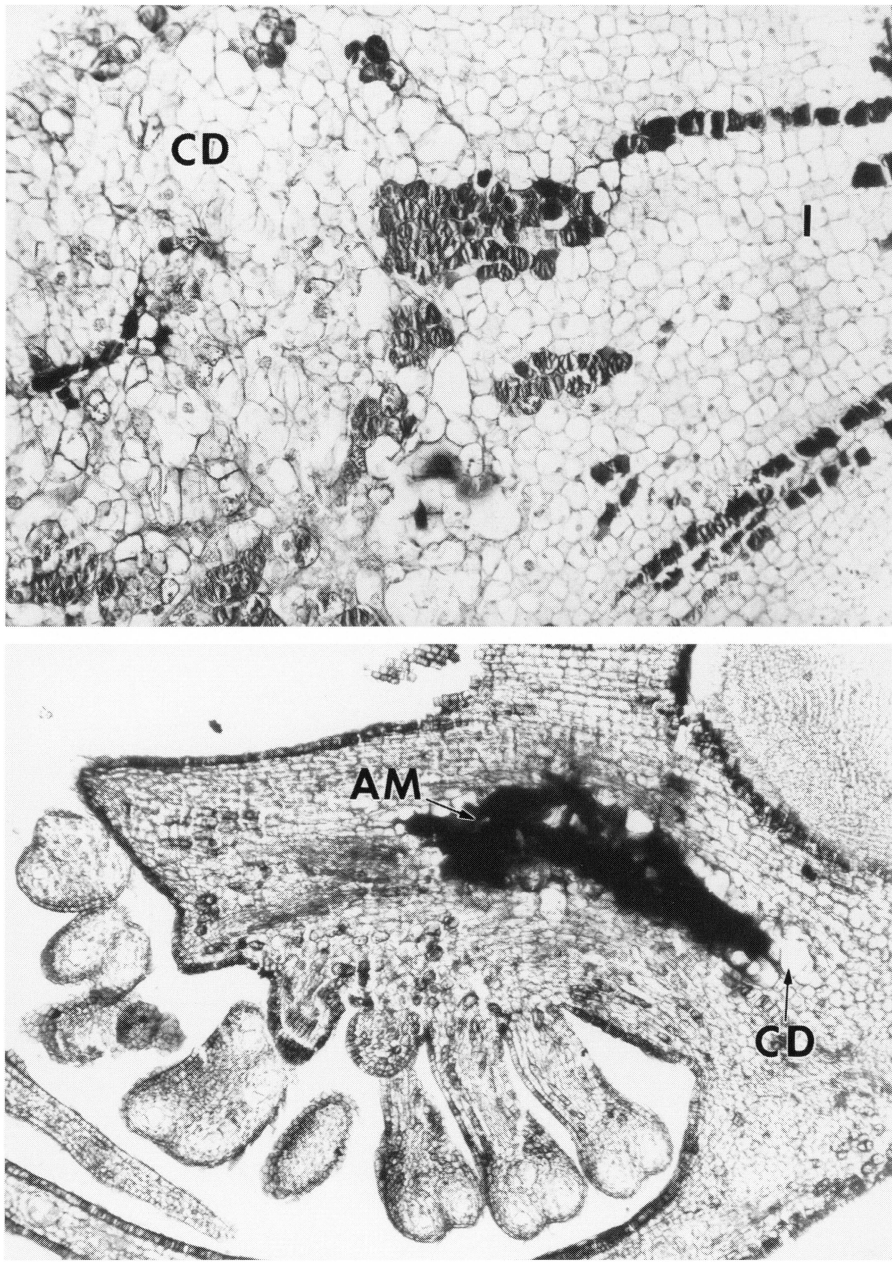

Fig. 3. (Top) Cellular degradation (CD) and intact cells (I) in staminal column due to *Lygus lineolaris* feeding. 33X. (Bottom) Median longitudinal section (10 μm) of cotton floral bud abscised due to *Lygus lineolaris*. Note cellular degradation (CD) in staminal column and the associated darkly stained amorphous material (AM). 10X.

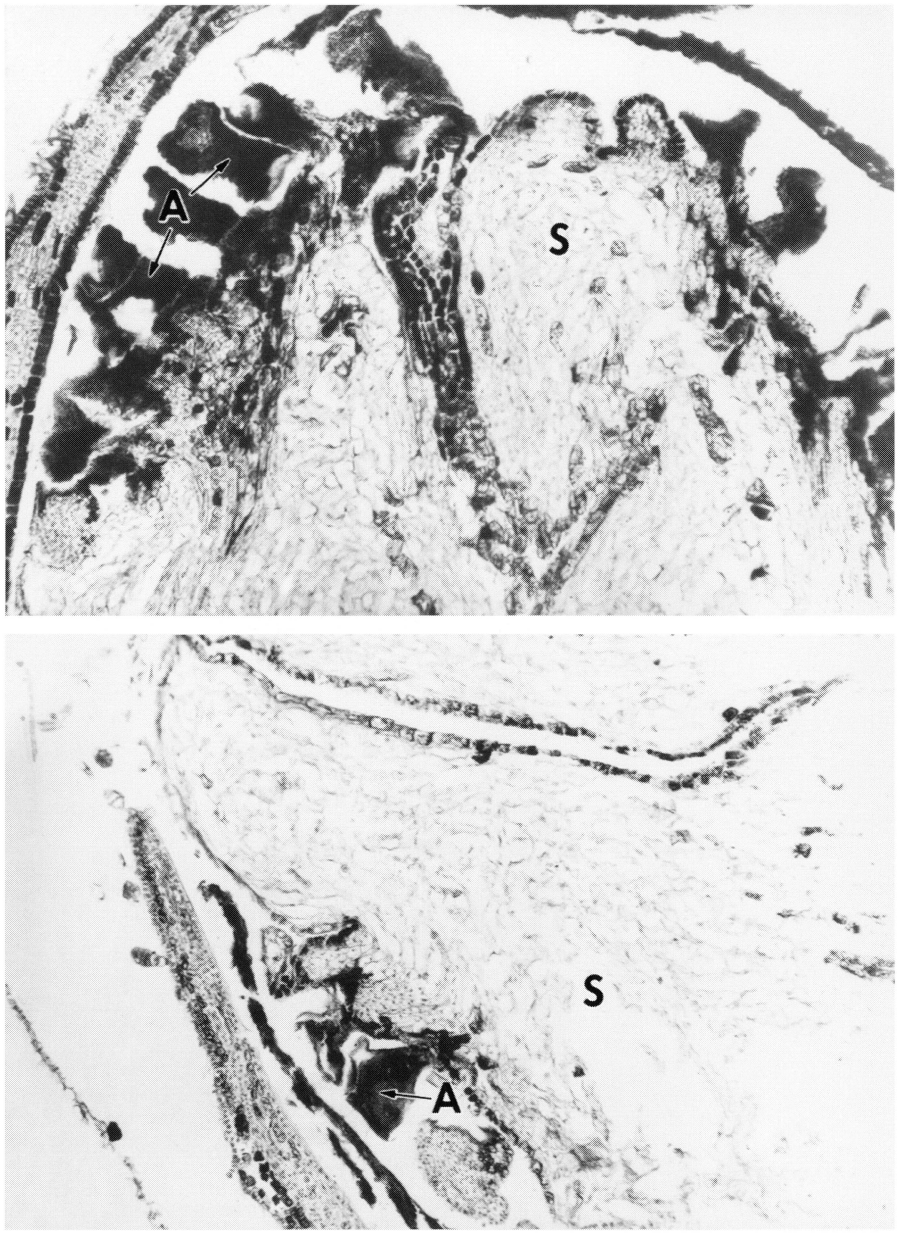

Fig. 4. Median longitudinal sections (10 μm) of cotton floral buds abscised due to *Lygus lineolaris* feeding. Anthers (A) are shrunken and darkly stained, while cells of the staminal columns (S) are lysed. 25X.

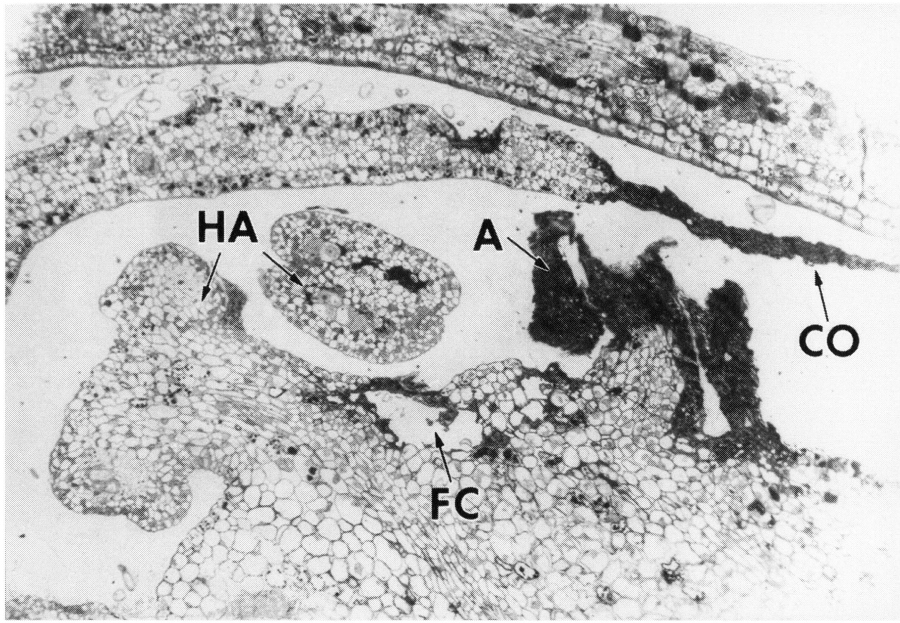

Fig. 5. Longitudinal section (1 μm) of bud abscised due to *Lygus lineolaris* feeding. Damaged anthers (A) and corolla (CO) are shrunken and darkly stained. A feeding cavity (FC) in the staminal column is visible, as are healthy anthers (HA). 33X.

grains (Pack 1973). Thus, *L. lineolaris* apparently prefers feeding on male reproductive tissue throughout development of the bud. A partial explanation for feeding site specificity in small buds may be that the staminal columns and anthers comprise a considerable proportion of the total bud tissue available to *L. lineolaris.* However, in larger buds the proportional area of staminal columns and anthers decreases due to growth of female reproductive tissue. Thus, it appears that the relative size of potential feeding sites within cotton buds does not completely explain feeding site specificity.

Differential levels of nutrients and allelochemicals in tissues within buds are probably also important in determination of feeding sites. While there is little information available on this topic for cotton buds, some general statements can be made. Pollen is an abundant source of free amino acids, proteins, carbohydrates, and lipids (Stanley and Linskens 1974). Vascular bundles in staminal columns and anthers are sources of nutrients for these structures as well as pollen. Therefore, it appears that male reproductive tissue of cotton floral buds is a rich food source for *L. lineolaris.* Research conducted on the allelochemical constituents of mature buds (1 cm diam) indicates that although anthers contained less condensed tannin than other structures in buds (Chan et al. 1978), gossypol content in anthers (Chan et al. 1978) and pollen (Hanny 1980) was relatively high. Hanny (1980) concluded that gossypol content of anthers was responsible for growth suppression of *Heliothis virescens* (F.) larvae. It

is not known if the allelochemical composition of large buds reflects that of the smaller buds studied in the present investigation. Detailed investigations on the biochemical composition of male reproductive tissue within cotton floral buds throughout the period of development might lead to a better understanding of the nutritional physiology of *L. lineolaris*.

Acknowledgments

We are grateful to J. R. Phillips for guidance throughout the study. B. Martin and W. M. Harris assisted with histology and microscopy, and K. C. Vaughn and R. N. Paul helped produce the figures. Helpful comments on the manuscript were given by F. A. Harris, G. L. Snodgrass, and two anonymous reviewers.

References Cited

Chan, B. G., A. C. Waiss, Jr., R. G. Binder and C. A. Elliger. 1978. Inhibition of lepidopterous larval growth by cotton constituents. Ent. Expl. Appl. 24: 294-300.

Flemion, F., M. C. Ledbetter and E. S. Kelley. 1954. Penetration and damage of plant tissues during feeding by the tarnished plant bug *(Lygus lineolaris)*. Contrib. Boyce Thompson Inst. 17: 347-357.

Hanny, B. W. 1980. Gossypol, flavonoid, and condensed tannin content of cream and yellow anthers of five cotton (*Gossypium hirsutum* L.) cultivars. J. Agric. Food Chem. 28: 504-506.

Hanny, B. W., T. C. Cleveland and W. R. Meredith, Jr. 1977. Effects of tarnished plant bug, (*Lygus lineolaris*), infestation on presquaring cotton, (*Gossypium hirsutum*). Environ. Entomol. 6: 460-462.

Hori, K. 1971. Studies on the feeding habits of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) and the injury to its host plants. I. Histological observations of the injury. Appl. Ent. Zool. 6: 84-90.

Hori, K., H. Torikura and M. Kumagai. 1987. Histological and biochemical changes in the tissue of pumpkin fruit injured by *Lygus disponsi* Linnavuori (Hemiptera: Miridae). Appl. Ent. Zool. 22: 259-265.

Jensen, W. A. 1962. Botanical Histochemistry: Principles and Practice. W. H. Freeman Co., San Francisco. 408 pp.

King, W. V. and W. S. Cook. 1932. Feeding punctures of mirids and other plant-sucking insects and their effect on cotton. USDA Tech. Bull. 296. 12 pp.

Laurema, S. and P. Nuorteva. 1961. On the occurrence of pectin polygalacturonase in the salivary glands of Heteroptera and Homoptera Auchenorryhncha. Ann. Entomol. Fenn. 27: 89-97.

Pack, T. M. 1973. A description of symptoms caused by the feeding of two common plant bugs, *Lygus lineolaris* (Palisot de Beauvois) and *Neurocolpus nubilus* (Say) on cotton squares. M. S. Thesis, Univ. of Arkansas. 44 pp.

Pack, T. M. and P. Tugwell. 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage on fruit parts. Arkansas Agric. Exp. Stn. Report Series 226. 17 pp.

Snodgrass, G. L., W. P. Scott and J. W. Smith. 1984. Host plants and seasonal distribution of the tarnished plant bug (Hemiptera: Miridae) in the delta of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 13: 110-116.

Stanley, R. G. and H. F. Linskens. 1974. Pollen: Biology, Biochemistry, Management. Springer-Verlag, New York Heidelberg Berlin. 307 pp.

Tugwell, N. P., S. C. Young, Jr., B. A. Dumas and J. R. Phillips. 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury and significance of wild hosts near cotton. Ark. Agric. Exp. Stn. Rep. 227. 24 pp.

Williams, M. R. 1999. Cotton insect losses 1998. Proc. Beltwide Cotton Conf. Pp. 785-809.