Bulletin of Entomological Research (2006) **96**, 439–444 DOI: 10.1079/BER2006440

SHORT COMMUNICATION

Histological studies of damage by pod-sucking bugs (Heteroptera: Coreoidea) associated with cowpea *Vigna unguiculata* ssp. *unguiculata* in Nigeria

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Abstract

Histological studies were conducted on cowpea pods fed upon by the coreoid pod-sucking bugs, *Anoplocnemis curvipes* (Fabricius), *Clavigralla tomentosicollis* Stål, *C. shadabi* Dolling, *Riptortus dentipes* (Fabricius) and *Mirperus jaculus* (Thunberg). Various degrees of tissue and cellular disruption were apparent, especially in the brachysclereids. They were manifested in terms of plasmolysis, cell enlargement and cell wall disintegration depending on the coreoid species involved. The feeding site and its periphery had all the cells of the brachysclereids plasmolysed, with the parenchyma having broken cell walls in the case of *A. curvipes*. *Riptortus dentipes* and *M. jaculus* showed similar patterns of feeding activity but less extensive levels of damage. The *Clavigralla* spp., however, caused cell enlargement in the brachysclereids, and broken cell walls in both the brachysclereids and the parenchyma. Damage symptoms were observed in cells far away from the feeding sites of the bugs, suggesting the possibility of sucrase activity which has been reported to cause osmotic pump feeding in the Coreidae.

Keywords: cowpea, damage symptoms, Coreidae, Alydidae, histopathology, *Anoplocnemis curvipes*, *Riptortus dentipes*, *Mirperus jaculus*, *Clavigralla tomentosicollis*, *Clavigralla shadabi*

Introduction

Cowpea, *Vigna unguiculata* ssp. *unguiculata* (L.) Walp., is mostly grown for grain and therefore, arthropods damaging the developing seeds are of economic importance (Dreyer & Baumgartner, 1995). Several important pests attack the crop at all stages of growth and after harvest, causing economic damage (Jackai & Daoust, 1986). The pod-sucking bug complex (members of the families Coreidae and Alydidae) accounts for most of this loss in West Africa (Dreyer *et al*., 1994). They include *Anoplocnemis curvipes* (Fabricius), *Clavigralla tomentosicollis* Stål, *C. shadabi* Dolling, *Riptortus dentipes* (Fabricius) and *Mirperus jaculus* (Thunberg); and cause premature drying and shrivelling of pods or the production of half-filled pods.

Phytophagous hemipterans employ a macerate (or lacerate) and flush feeding strategy involving the use of their stylets and watery saliva from their salivary gland complex (Miles, 1972; Hori, 2000). The adults and nymphs pierce and cut plant tissues with their stylets, and inject saliva containing digestive enzymes through the salivary canal to liquefy the food into a nutrient-rich slurry. The food slurry is ingested through the food canal and passed into the

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Table 1. Relative degree of cell damage observed in transverse sections of cowpea pods fed upon by various species of pod-sucking bugs.

Pod-sucking bug species	Instar	% Plasmolysed brachysclereids	% Parenchymatous cells with broken walls
Anoplocnemis curvipes	Nymph	100.0	100.0
	Adult	100.0	100.0
Riptortus dentipes	Nymph	100.0	100.0
	Adult	94.3	75.0
Mirperus jaculus	Nymph	99.1	100.0
	Adult	100.0	87.1
Clavigralla spp.		All the brachysclereids became enlarged and some of them (as indicated below) had broken cell walls.	
C. tomentosicollis	Nymph	81.6	99.1
	Adult	75.9	99.0
C. shadabi	Nymph	73.4	66.9
	Adult	62.8	57.2

alimentary canal where it is further digested and absorbed (Cohen, 2000).

Stylet penetration through plant tissues has been studied in aphids (Pollard, 1973) and other homopterans (Blanco, 1994; Ecale & Backus, 1995). Similar investigations have been conducted on some species of heteropterans (Strong, 1970; Miles, 1987; Hori & Miles, 1993; Miles & Taylor, 1994; Steinbauer *et al*., 1997). Pod penetration and consequent cellular damage by *Lygus* spp. in lima beans (*Phaseolus lunatus* L.), and green beans (*P. vulgaris* L.) have been reported by Elmore (1954) and Flemion *et al*. (1954). However, no such reports exist in respect of feeding by coreoid pod-sucking bugs on cowpea. The present study was therefore conducted to find out some of the cellular changes that accompany the feeding action of these bugs, and the consequent injury to cowpea pods.

Materials and methods

Feeding of the bugs

This study was conducted in a screenhouse using 90 cm × 62 cm × 62 cm wire-net screen cages inside which Ife Brown cowpea plants were maintained. Each plant with a single 8-day-old pod was utilized in feeding a single fourth instar nymph or adult of each of the coreoid bugs, *Anoplocnemis curvipes*, *Clavigralla tomentosicollis*, *C. shadabi*, *Riptortus dentipes* and *Mirperus jaculus*. The insects were obtained from cultures maintained specifically for that purpose in the screenhouse, and each was allowed to complete a single feeding session on a pod. Each treatment, comprising an instar of each of the pod-sucking bug species per 8-day-old pod, was replicated twice. Pods fed upon were left for 12 h and thereafter, removed and prepared for histological studies.

Histological studies

The sucked portions of the pods obtained from above were cut into small slices and placed in Bouins fluid (fixing agent). Using a rotary microtome, thin transverse sections (6 μm) were cut from the slices after being embedded in rubberized paraffin. Staining was done with haematoxylin and eosin while DEPEX was used as mountant for permanent slides. The slides were examined under a phase contrast microscope (Leica Gallen III) and photomicrographs (×175) of the damaged cells were taken. Slides of thin sections from healthy pods (i.e. not exposed to pod-sucking bugs) were similarly prepared for use in comparison. To allow for a quantitative assessment of the damaged cells, visual counts were made of all cells in the field of the microscope, separating them into plasmolysed cells, enlarged cells, cells with broken walls, and normal cells. This was repeated for three transverse sections for each bug species. Data obtained were used to calculate percentage cells in each damage category as indicated in table 1.

Results

Transverse section through a healthy cowpea pod (fig. 1) revealed that it is made up largely of special types of sclerenchyma (sclereids or sclerotic cells) in addition to parenchyma and other plant tissues. The sclereids comprise four main forms as follows:

1. Macrosclereids. These are columnar cells forming a solid palisade-like epidermal layer in the pod wall.
2. Osteosclereids. These are also columnar in nature and found at the base of macrosclereids in the pod wall. Most are dilated at one or both ends, somewhat like bones and shorter than the macrosclereids.
3. Astrosclereids. These are irregular in shape with radiating arms of varying lengths and are found in the grain.
4. Brachysclereids. These are roughly spherical in shape and make up a larger proportion of the grain (fig. 2).

The general patterns of damage resulting from the feeding action of the pod-sucking bugs included plasmolysis (fig. 3), broken cell walls (fig. 4) and cell enlargement (fig. 5). These patterns are readily discernible by comparing with figs 1 and 2 which show transverse sections through a normal healthy pod. A quantitative assessment of the patterns of damage by the various species of pod-sucking bugs is presented in table 1. As evident in the table, 100% plasmolysed brachysclereids and broken parenchymatous cells were observed at the feeding sites and their periphery in the case of both the nymphs and adults of *A. curvipes*. Similarly, the alydids caused very high to total cell damage with only the adults showing somewhat less reduced levels

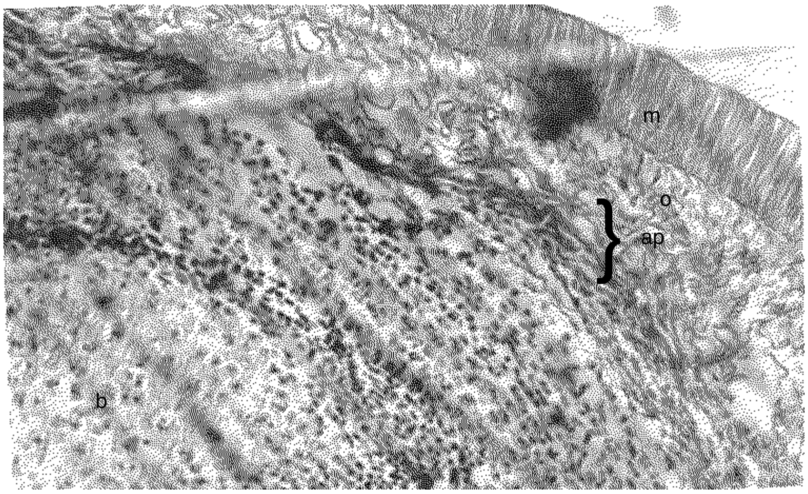

Fig. 1. Photomicrograph of a transverse section of healthy cowpea pod showing the macrosclereids (m), osteosclereids (o), brachysclereids (b) and the region occupied by astrosclereids and parenchyma (ap) (×70).

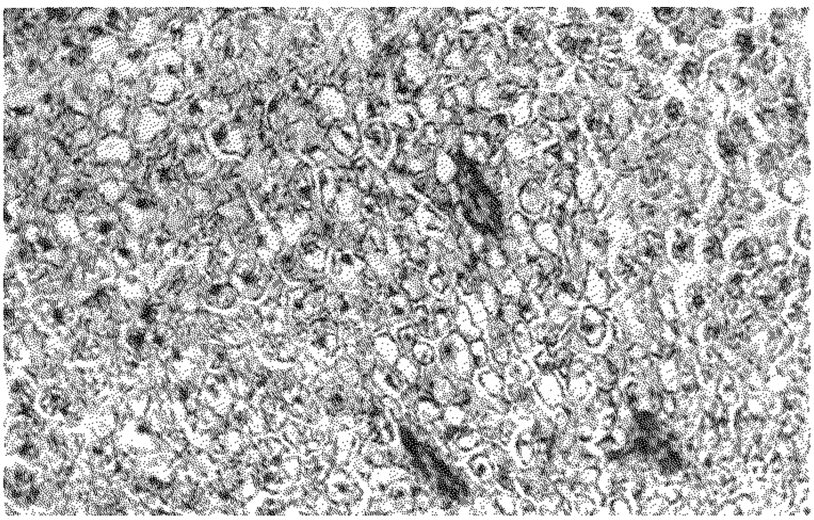

Fig. 2. Photomicrograph of a transverse section of healthy cowpea pod showing the brachysclereids (×175).

of broken parenchymatous cells (75.0% in *R. dentipes* and 87.1% in *M. jaculus*). The feeding sites and their periphery in the case of the *Clavigralla* spp. had all the brachysclereids enlarged and in addition, both the brachysclereids and parenchymatous cells showed varying degrees of broken cell walls which generally was much higher for *C. tomentosicollis* than *C. shadabi*. Among the species, wherever there was any difference in the level of cell damage between the fourth instar nymph and the adult, it was the nymphs that exerted the higher level of damage.

Discussion

The piercing and sucking action of the investigated pod-sucking bugs caused water-soaked lesions at the feeding sites on their food plants. These were especially evident in the feeding of *A. curvipes* where the effect was immediate and most conspicuous. Such insects are known to feed typically on the parenchyma in and surrounding vascular tissues (Miles & Taylor, 1994). Histologically, they cause partial to complete loss of cellular integrity.

The ability of the heteropterans to cause remarkable damage appears to reside in the chemical nature of their salivary products. A number of studies have linked cellular damage in plant tissues to salivary constituents of phytophagous insects. Miles (1987) observed that some of the localized, necrotic lesions caused by tropical coreid species on their food plants are superficially similar to those caused by mirids. Strong & Kruitwagen (1968) had earlier convincingly ascribed such symptoms induced by mirids to the secretion of a salivary pectinase which coreids are not known to secrete. Miles & Taylor (1994) later attributed the

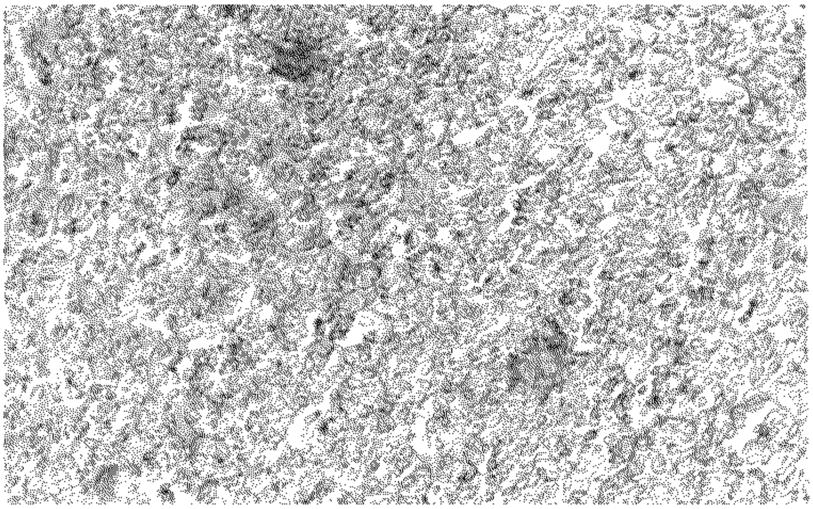

Fig. 3. Photomicrograph of a transverse section of cowpea pod fed upon by *Anoplocnemis curvipes* showing plasmolysed brachysclereids (×175).

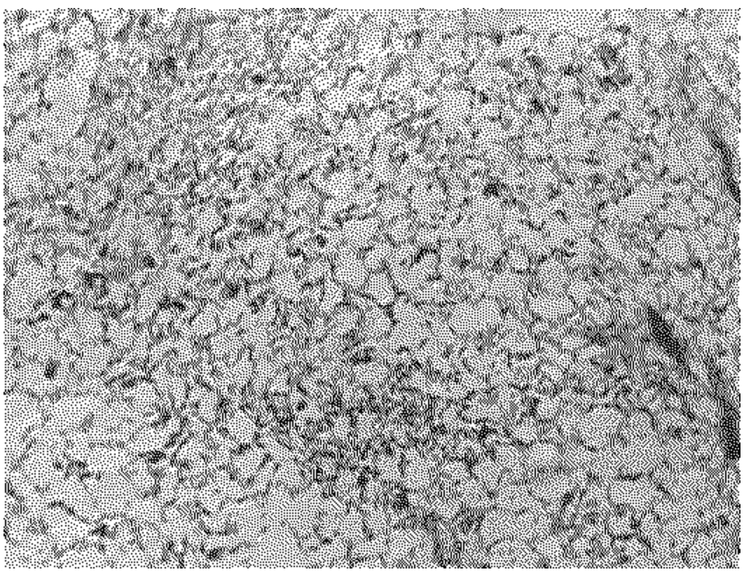

Fig. 4. Photomicrograph of a transverse section of cowpea pod fed upon by *Mirperus jaculus* showing broken walls of brachysclereids (×175).

water-soaked lesions resulting from the feeding action of the coreid, *Mictis profana* (Fabricius) to a salivary sucrase. An investigation on the digestive enzymes in the salivary gland extracts of the preceding coreoid cowpea pod-sucking bugs (Soyelu, 2005) revealed the presence of proteases, α and β-amylases, and amyloglucosidase. The proteases were preponderant (especially in *A. curvipes*) and were believed to be largely responsible for the feeding damage caused by the bugs. The plasmolysis of the brachysclereids and the broken cell walls of the parenchymatous cells seem logical, given the mechanical puncturing by the stylets of the bugs, the digestive action of the proteases, and the eventual sucking of solutes from the cells. However, the cell enlargement due to the feeding action of *Clavigralla* spp. was unexpected and more difficult to explain. Presumably, the salivary gland secretion contains some other substances capable of stimulating cell enlargement. This would be a subject for further investigation. Enlarged cells have been reported in the vascular cambium and phloem, as well as other tissues in the stem of alfalfa, *Medicago sativa* L. fed upon by the potato leafhopper, *Empoasca fabae* (Harris) (Kabrick & Backus, 1990). The enlarged cambial cells appeared to crush the phloem and were thus presumed to cause the hopperburn symptom characteristic of the leafhopper damage. Presumably, a similar mechanism may have been involved in the case of the feeding damage of

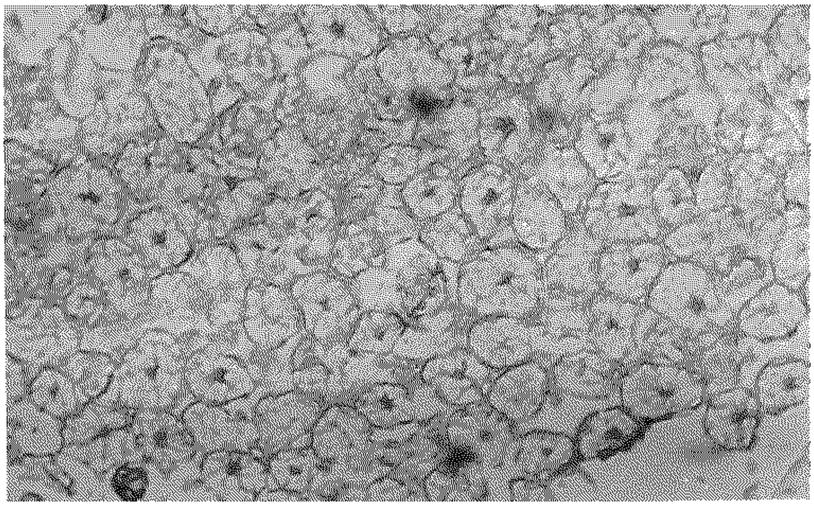

Fig. 5. Photomicrograph of a transverse section of cowpea pod fed upon by *Clavigralla tomentosicollis* showing enlarged brachysclereids (×175).

Clavigralla spp. on cowpea pods. The enlarged brachysclereids seem to crush the adjoining layers of astrosclereids and parenchyma, compressing them with the osteosclereids and macrosclereids.

The extensive damage inflicted on cells of cowpea pods by both adults and nymphs of *A. curvipes* and the fourth instar nymphs of *R. dentipes* indicated that cells not touched by the inserted stylets also suffered substantial damage. This shows that the feeding mechanism of the pod-sucking bug species does not consist of mechanical puncturing of tissue and sucking of cell sap in the immediate vicinity of the inserted stylets alone. Rather, it also involves what Miles & Taylor (1994) termed osmotic pump feeding. Coreid bugs are known to secrete the salivary sucrase, α-D-glucohydrolase, which they use in this process to convert endogenous sucrose to glucose and fructose, thereby causing unloading of solutes into the apoplast from where the bugs suck the leaked contents of affected cells. Hence, they can suck the contents of many cells from a single locus.

A critical assessment of the damage done by coreoid pod-sucking bugs has revealed that *A. curvipes*, *M. jaculus* and *R. dentipes* can cause more significant damage to cowpea at lower densities than the *Clavigralla* spp. (Soyelu, 2005). The present findings on the patterns of cellular damage also corroborate the observation. The plasmolysis of pod cells due to feeding by *A. curvipes, M. jaculus* and *R. dentipes* is a more severe instantaneous reaction. The enlargement of the brachysclereids due to feeding by *Clavigralla* spp. and its mechanical effects in compressing the adjoining layers of astrosclereids and parenchyma with the osteosclereids and macrosclereids, is much less severe. Hence, it would require a higher frequency of feeding by *Clavigralla* spp. to elicit similar level of damage from a single feeding action by these other pod-sucking bugs.

Acknowledgements

The authors thank Drs J.O. Faluyi and H.C. Illoh of the Department of Botany and Mr J. Ibeh of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife for their assistance in the histological preparations and the photomicrographs.

This paper forms part of the senior author's MPhil. thesis submitted to the Postgraduate College, Obafemi Awolowo University, Ile-Ife, Nigeria.

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(Accepted 25 April 2006)