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## Anatomical Characterization of Western Corn Rootworm Damage in Adventitious Roots of Maize

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Corn rootworms are one of the most economically damaging insect pests of maize, yet little is known about the feeding behavior of the larvae. This study was conducted to determine which tissues of the adventitious roots of maize are damaged by western corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval feeding. Root axes (10 cm long) were removed from the fifth node of greenhouse-grown maize plants. Root segments 2 cm long, excised 4 or 6 cm from the root tip, were infested with second or third stage larvae, respectively, (0, 1, 3, or 6 larvae per segment) for a period of 24 hours. Root segments were fixed, embedded and sectioned to a thickness of 16  $\mu\text{m}$  for light microscopy. Serial sections taken at 1 mm intervals were used to measure the amount of tissue removed during insect feeding. Light micrographs revealed that larval feeding damage was restricted to the root cortex, and no damage was visible in the pith. Up to 80% of the cortex was removed during feeding within a 24-h period. The suberized and lignified secondary walls of the endodermis and exodermis appear to act as barriers that either prevent or discourage larval feeding within the pith. These results are discussed in terms of possible explanations of the characteristics of corn rootworm damage under field conditions.

INDEX DESCRIPTORS: *Zea mays*, *Diabrotica virgifera virgifera*, corn

Northern and western corn rootworms are major insect pests of maize. Treatment costs and yield loss attributed to these pests have been estimated to be near \$1 billion per year (Metcalf, 1986). Little is known about the feeding behavior of the larval form of these insects (Strnad and Bergman, 1987).

It has been suggested that rootworm damage in adventitious roots is localized in the cortex, the primary tissue region between the vascular system and the epidermis (Apple and Patel, 1963). However, other workers have indicated that rootworm damage also occurs in the stele, the central cylinder of the root comprised of the vascular system and associated pith tissue (Howe and Britton, 1970). Because neither of these authors presented quantitative or pictorial data to support their observations, we decided to further investigate the feeding behavior of the western corn rootworm. Our objective was to characterize and quantify the root damage caused by different densities of second- and third-stage rootworm larvae.

### MATERIAL AND METHODS

Kernels of hybrid maize (A632  $\times$  A619) were planted in 2:1:1 (v:v:v) soil:peat:perlite contained in 25-cm diameter cellulose-fiber pots. Pots contained in 30-cm plastic saucers were placed in the greenhouse under natural lighting. Greenhouse temperature was maintained at approximately 30/20°C day/night, and relative humidity was 50  $\pm$  10%. Plants were watered as needed by placing water in the saucer. Fertilization was accomplished by placing 10 g of general purpose 20-20-20 water-soluble fertilizer in each saucer before watering. Plants were fertilized every other week.

After 40 days of growth under these conditions, the soil was carefully washed away from the root systems. Root axes from the 5th node, which were approximately 10-cm long, were removed from the stem and cut into 2-cm segments. The third and fourth segments (the first segment contained the root apex) were infested with second- and third-stage rootworm larvae, respectively, in the following manner.

Each cut end of the root segment was dipped into molten paraffin and sealed. Individual root segments were transferred to moist filter paper contained in petri dishes. Second- or third-stage larvae (determined by head capsule size) were placed on each root segment at a rate of 0, 1, 3, or 6 per segment. Petri dishes were then covered and placed into a dark incubator for 24 h at 25°C.

After incubation, the paraffin-covered root segment tips were trimmed and discarded, and the remaining 1-cm segments were fixed in FAA (formalin-acetic acid-alcohol), embedded in a paraffin, sectioned to a thickness of 16  $\mu\text{m}$  and stained with safranin-fast green

(Jensen, 1962). Sections were viewed and photographed using a light microscope.

The percentage of cortex removed by the feeding larvae was derived from embedded root segments by sectioning each root segment at 1-mm intervals (10 sections per segment). After removing the paraffin in xylene and staining with fast green, the sections were photographed. The resulting photographs were used to measure the percentage of the cortex removed by larvae feeding. The image of the root cortex, excluding the thick-walled cells of the exodermis, was cut from each photograph. The area of this portion of the photograph was measured with an area meter. Next, the region of the cortex that had been damaged by larval feeding was cut from the photograph, and the area again determined. The percentage of cortex removed was then determined by the equation:

$$\text{Cortex Removed (\%)} = \left(1 - \frac{D}{C}\right) \times 100$$

where D = area of cortex after damage was cut away and C = total area of cortex. Each section was evaluated in this manner, and the average for the 10 sections was used to compute the percent cortex removed from each segment. Means  $\pm$  standard error were calculated for 4 segments per larval treatment rate.

### RESULTS

Typical damage to roots caused by 3 second-stage larvae per segment is illustrated in Figs. 1 and 2. The damage consists of tunnels where root tissue has been removed by the feeding insect. A cross section of a larva as well as feces are present within these tunnels. Feeding damage was restricted to the root cortex (Fig. 1). Tissues of the pith and vascular system were not damaged. Only the thin-walled parenchyma cells of the cortex are removed by the feeding insect (Fig. 2). The thick-walled cells of the outer cortex (the exodermis) as well as the thick-walled, inner-most layer of cells of the cortex, the endodermis, are left intact.

Typical damage to roots as caused by 6 third stage larvae per root segment is illustrated in Figs. 3 and 4. Large areas of the cortex have been removed by the feeding insect (Fig. 3). An oblique section of a larva as well as feces are evident within the damaged cortex. No feeding damage to the pith or vascular tissue is evident. Larval damage to the thin-walled cells of the cortex as revealed by a higher magnification view is particularly striking (Fig. 4). The thick-walled cells of the outer cortex (the exodermis) and the thick-walled cells of the endodermis are left intact by the third stage larvae.

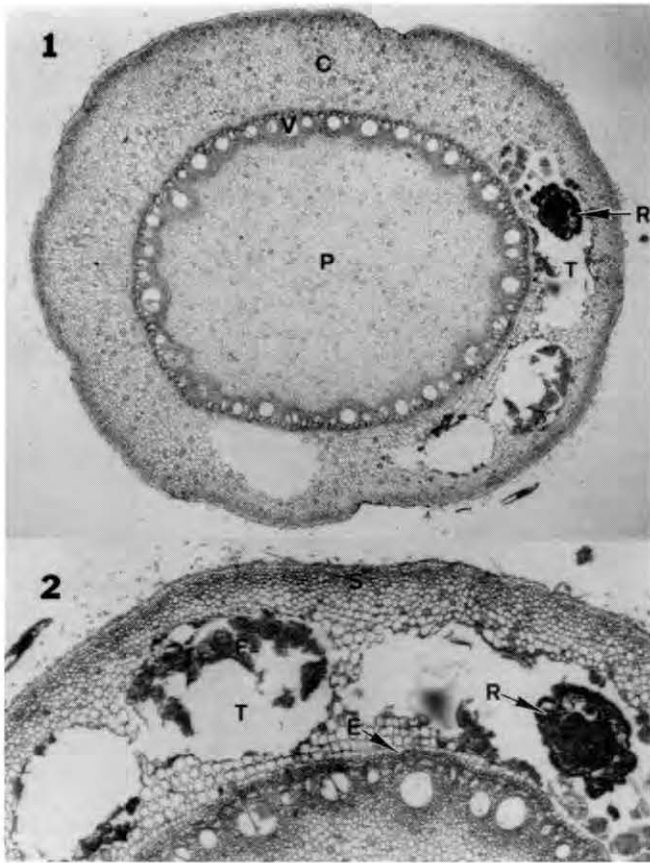


Fig. 1. Representative cross-sectional view of root segment infested with 3, second-stage larvae. Rootworm (R) is visible in tunnel (T) in the cortex (C) of the root. Pith (P) and vascular system (V) are undamaged. (X 15).

Fig. 2. Higher magnification view of Fig. 1. Rootworm (R) and feces (F) are visible in tunnel (T). Endodermis (E) and exodermis (S) are undamaged. (X 30).

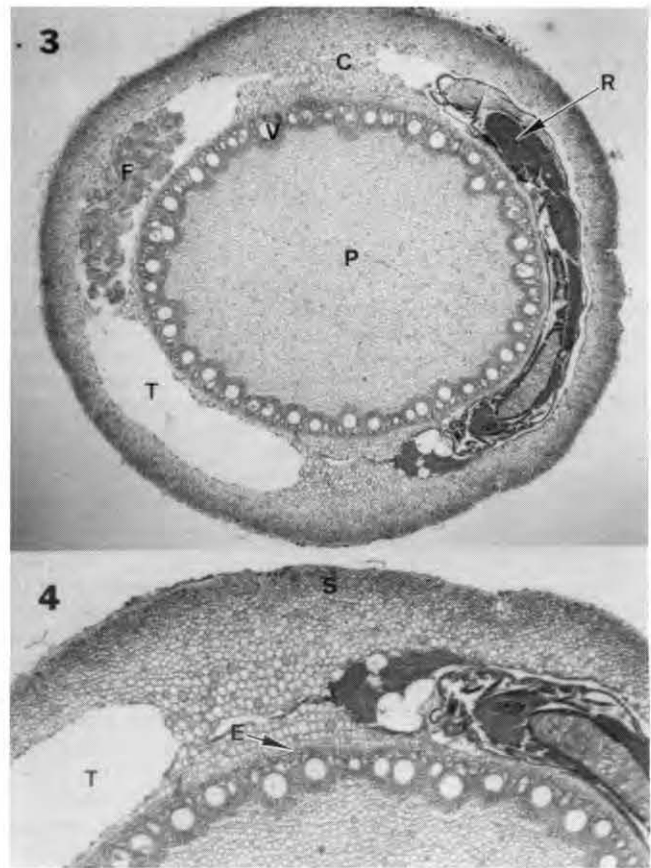


Fig. 3. Representative cross-sectional view of root segment infested with 6, third-stage larvae. Rootworm (R) and feces (F) are visible in tunnel (T) of cortex (C). Pith (P) and vascular system (V) are undamaged. (X 15).

Fig. 4. Higher magnification view of Fig. 3. Rootworm (R) is visible in tunnel (T). Endodermis (E) and exodermis (S) are undamaged. (X 30).

The percentage of cortex removed from root segments by second- or third-stage larvae over a 24-h period is shown in Fig. 5. The percentage of cortex removed increased with the number of larvae applied to the root segments. Second-stage larvae removed less cortex than equivalent numbers of third-stage larvae.

**DISCUSSION**

The results of this study indicate that second- and third-stage rootworm larvae damage the thin-walled cells of the cortex but do not damage the cells of the vascular system or the pith. This observation is in agreement with that of Apple and Patel (1963). It should be noted that when the present experiment was performed without capping the cut ends of the root segments with paraffin, rootworm larvae damaged the cells of the pith as well as the cortex. The report by Howe and Britton (1970), which was also based on laboratory observations, did not include a description of the methods used to evaluate the location of rootworm feeding. Because of this, it is impossible to know if these workers used intact roots or root segments. The use of root segments would have provided rootworms with easy access to the pith.

Maize root cells from both the cortex and the pith contain equal concentrations of solutes (Warmbrodt, 1987), suggesting that the

cells of either tissue would be of equal nutritional value to the insect. This observation, coupled with the previous observation that larvae will enter the pith through the cut ends of roots and feed on cells of the pith, supports the contention that the endodermis forms a barrier that prevents or discourages rootworms from crossing across the endodermis and vascular system into the pith during feeding. Cells of the endodermis of maize roots have lignified or suberized secondary walls (Peterson et al., 1982). We suggest that it is these secondary walls which act as a barrier to the rootworms. The effectiveness with which these cells act as a barrier is illustrated by the fact that even when almost 80% of the cortex was removed from the root, the third-stage rootworm larvae never damaged the endodermis and vascular tissue.

The cells of the exodermis in the outermost layer of cortex also appear to act as a selective barrier. If the larvae are outside the root, the exodermis does not prevent the insects from entering the cortex. Once inside the cortex, however, the larvae do not damage the exodermis. These observations are inexplicable given the present knowledge of larval behavior.

In general, two types of rootworm damage are considered when roots are evaluated for damage ratings: feeding scars and root pruning (Mayo, 1986). Feeding scars are the obvious result of larvae tunneling

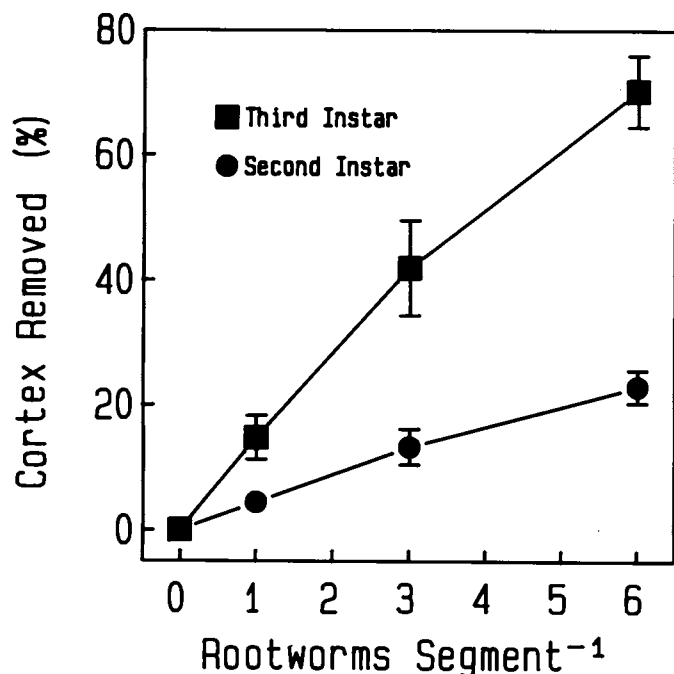


Fig. 5. Percentage of cortex removed from root segments by different numbers of larvae in a 24-h period. Values represent mean  $\pm$  standard error for four root segments per larval treatment rate. In one case (second instar with one rootworm per segment), the symbol used to denote the mean was larger than the standard error.

in the cortex. Root pruning, however, is harder to understand. In order to prune roots, defined as root axes destroyed to within 3.8 cm of the base of the plant (Branson, 1986), larvae would have to completely sever the endodermis and vascular cylinder at that point. Because the tissues of the endodermis and vascular cylinder are continuous in older regions of the root, the results of our study suggest that corn rootworms could not prune established roots. This suggestion, however, falls short of explaining the processes that cause the phenomenon of root pruning in the presence of corn rootworm under field conditions.

To explain the phenomenon of root pruning, one could speculate that mechanical breaks in the root could allow larvae to penetrate the stele in older regions of established roots. One could also speculate that root-rot pathogens associated with rootworms invade the endodermis and vascular cylinder at the point of damage to the cortex, causing deterioration of the root at that point (Palmer and Kommedahl, 1969). If this is the case, the customary practice of digging root ratings (Hills and Peters, 1971) at beetle emergence may exacerbate the appearance of root pruning. In a field situation, 10 to 14 days may pass between maximum root damage and the first adult emergence (Bergman and Turpin, 1986). If roots are evaluated at beetle emergence, then ample time exists for root-rot pathogens to destroy roots damaged by rootworm larvae, giving the impression that the insect pruned the root.

Another possible explanation of the phenomenon of root pruning is related to the feeding behavior of the rootworm larvae. Larvae show a progressive movement toward the succulent new growth of nodal root axes (Chiang, 1973; Strnad and Bergman, 1987). The apex of the nodal root axis contains regions of meristematic and undifferentiated cells (Esau, 1977). Because the tissues of the endodermis and vascular cylinder have not differentiated, the rootworm larvae would be able to penetrate the root in the region of the apex. Subsequent damage to the meristematic cells by the feeding insect would result in cessation of elongation of the root axis at that point, giving the impression that the insect pruned the root.

While the data presented in the present report do not demonstrate the root pruning by corn rootworms does not occur, it does generate questions as to the mechanisms which cause root pruning in the presence of corn rootworms under field situations. Further research is needed to answer these questions.

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