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Interactions between cassava mealybug and cassava: cytochemical aspects of plant cell wall modifications

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Introduction

The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), a serious pest of cassava in Africa, is an oligophagous insect mainly living on cassava (*Manihot esculenta* Crantz) (Matile-Ferrero, 1976). Following the demonstration of an aphid-type phloem-feeding behaviour of cassava mealybug with an aphid-like predominance of extracellular pathways of stylets (Calatayud *et al.*, 1994), host-plant resistance of cassava to *P. manihoti* has been investigated. The hypothesis was that *P. manihoti* secretes enzymes to facilitate intercellular stylet penetration by digestion of plant middle lamellae and primary cell walls. We suggested that pectinesterase isolated from mealybugs could be involved in plant pectin degradation; however, no polygalacturonase was detected (Calatayud, 1993).

In this paper, we present cytochemical evidence that mealybug pectinesterase found in salivary secretions is associated with the degradation of middle lamellae. Also, plant reactions resulting from the rupture of cell walls were cytochemically investigated. We showed that callose deposits were associated with damage caused by the stylet penetration within the phloem cells.

Materials and methods

Plant material. A cassava cultivar, *Manihot esculenta* Crantz var. Fetonegbodgi, was used in this study.

Insects. A culture of *Phenacoccus manihoti* was maintained on cassava (*M. esculenta*, var. Fetonegbodgi) under laboratory conditions at 22–25 °C and L12:D12 photoperiod.

Detection of pectinesterase and polygalacturonase from salivary secretions of living mealybugs. Gels (pectin 0.1%-agarose 1% gel plates) were prepared by incorporating commercial pectin (Sigma P-9135) into agarose (Biorad 162–0126). The pH of gel plates prepared in sterile petri dishes was adjusted to values between 3.8 and 8.0. About 50–80 mealybugs of L3 and L4 were caged for feeding during 12–24 h on pectin-agarose gel plates. After feeding, the mealybugs were removed from the gel plates with a small brush, and the plates were incubated at 37 °C for 1–3 h. Gels were then stained with 0.02% ruthenium red (Sigma R-2751) for 30–60 min. After a few minutes of destaining in water, the gels were examined under a microscope. To assess the effect of pH on enzyme detection, gels were prepared with 9 different pH levels, ranging from 3.8 to 8.0 (4 replicates each). As controls, the same procedure was used either without mealybugs, or with crushed mealybugs and two enzymes (pectinesterase E.C. 3-1-1-11, Sigma 9025-98-3 and polygalacturonase E.C. 3-1-1-15, Sigma 9032-75-1) (Ma *et al.*, 1990).

Cytochemical study. Leaf portions used were either free of mealybugs or infested by the insects for 48 h. Immediately before fixation, the mealybugs were removed from the leaf with a small brush after the stylets have been quickly cut with a scalpel. Only the leaf portions showing the stylet stump were selected. After fixation and embedding, all leaf portions were sectioned with a diamond knife on a Reichert Ultracut E microtome. Sections were collected on nickel grids and processed

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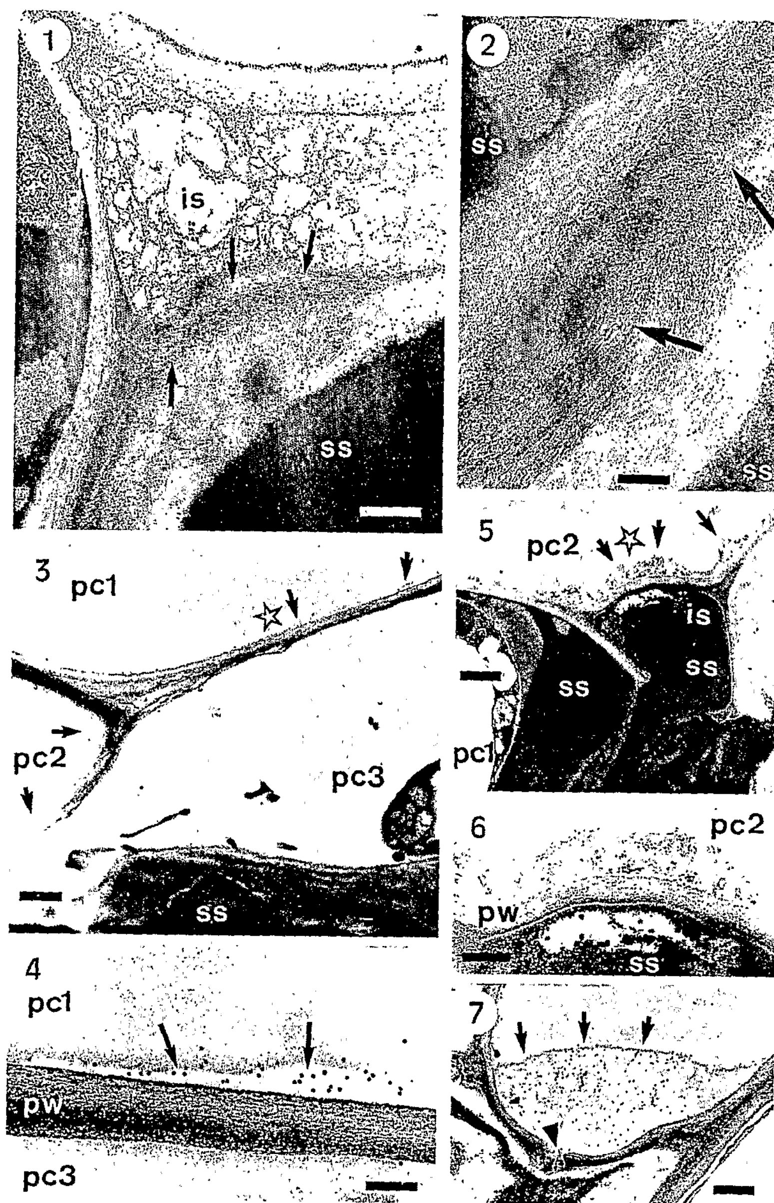

Figures 1–7. (1) Micrograph of infested cassava leaf phloem tissues fixed in glutaraldehyde/osmium tetroxyde. Sections were treated with JIM5 anti-pectin antibodies. Portions of the middle lamella and primary wall (arrows) of a phloem cell adjacent to the insect salivary sheath (ss) show an uneven labelling; no gold particles are seen over the sheath. The fibrillar network present in the intercellular space (is) is labelled (bar = 0.7 μm). (2) Same material as in Figure 1. A portion of the phloem cell wall close to the salivary sheath (ss) shows degradation pattern of the middle lamella (arrows) (bar = 0.25 μm). (3) Micrograph of infested cassava leaf tissues fixed in glutaraldehyde/paraformaldehyde. Sections were incubated in β(1,3)-D-glucopyranose polyclonal antibodies. An even gold labelling (arrows) is visible over paramural material deposited close to the primary walls of phloem cells (pc1, pc2) adjacent to the punctured cell (pc3) containing the salivary sheath (ss) (star: portion enlarged in Figure 4) (bar = 0.6 μm). (4) Enlarged portion of Figure 3 showing gold labelling of callose (arrows) in the electron-lucent material deposited in the paramural area of a phloem cell (pc1). No gold particles are seen over the primary wall (pw) (bar = 0.2 μm). (5) Micrograph of infested cassava leaf vein fixed in glutaraldehyde/osmium tetroxyde. Sections were treated with anti β(1,3)-D-glucopyranose polyclonal antibodies. The salivary sheath (ss) is in intracellular position in the phloem cell (pc1) and fills the intercellular space (is). Material deposited on the phloem cell wall (pc2), near the salivary sheath is weakly labelled (star: portion enlarged in Figure 6) (bar = 0.5 μm). (6) Enlarged portion of Figure 5 showing material deposited on the phloem primary cell wall (pw: primary wall, pc2: phloem cell). This material reacts positively to anti-β(1,3)-D-glucopyranose antibodies (bar = 0.2 μm). (7) Micrograph of infested cassava leaf tissues fixed in glutaraldehyde and osmium tetroxyde. A papilla (arrows) occludes a plasmodesmatal area (arrowhead) of a phloem cell in the insect feeding zone. The papilla appears evenly labelled after treatment with anti-β(1,3)-D-glucopyranose polyclonal antibodies (bar = 0.4 μm).

for gold labelling. For the detection of β-(1,4)-glucans, sections from samples embedded in LR White were treated with an exoglucanase conjugated to colloidal gold (Boher *et al.*, 1995a). Immunocytolocalization of β-(1,3)-glucans and pectin was performed on samples embedded in Epon 812 according to Gollotte *et al.* (1993) and Boher *et al.* (1995a), respectively. The grids were examined using a Jeol 100 electron microscope (LPRC-CIRAD) operating at 80 kV.

Results

Detection of pectinesterase and polygalacturonase from salivary secretions of living mealybugs. Mealybugs probing into pectin-agarose gels left branched stylet sheaths. After staining the gels with 0.02% ruthenium red, the activity of pectinesterase secreted by mealybugs was detected on the red background by formation of dark-red halos around the site of each stylet sheath. The activity of pectinesterase isolated from mealybugs (crushed material) was rendered also visible. No polygalacturonase activity was detected. Variations in pH did not qualitatively modify pectinesterase activity.

Cytochemical studies. Gold probes were used to gain a better insight into molecular and cellular mechanisms involved in leaf cell wall degradation resulting from leaf penetration by the insect stylets (stylets were present but are not visible in the figures). The use of anti-pectin monoclonal antibodies revealed that the plant middle lamellae and the primary cell walls close to the stylet pathway sheath were altered (Figures 1 and 2). These degradation patterns were observed close to the salivary sheath material (Figures 1 and 2). The use of a β-(1,4)-exoglucanase-gold complex for the cytolocalization of β-(1,4)-glucans did not show any alteration of plant cell walls. After application of anti-β-(1,3)-D-glucopyranose polyclonal antibodies, labelled papillae for callose detection were seen in phloem cells associated with stylet penetration (Figures 3, 4, 5, 6 and 7).

In controls, no primary cell wall and middle lamellae degradations were found after the use of gold probes. Callose deposits were only seen in degenerated sieve tubes at the phloem periphery. A weak labelling also occurred over plasmodesmatal areas in phloem cells, as already stated by Boher *et al.* (1995a, b).

Discussion

Pectinolytic enzymes are important to sap-sucking insects because of their role in the degradation of pectin, a major component of middle lamellae and primary cell walls of plants (Dreyer & Campbell, 1986, 1987). With EPG (i.e. electrical penetration graph technique – DC system) studies, we demonstrated that the path, resulting from the penetration of *P. manihoti* stylets, is predominantly intercellular (Calatayud *et al.*, 1994). Our present results showed that during probing, mealybugs secrete pectinolytic enzymes that may be involved in the degradation of middle lamellae and primary cell walls to facilitate ingress of stylets within host tissues. Nevertheless, no change in cellulose was detected. Although cellulase activity was not determined in our study, it is well documented that salivary cellulases are absent from a number of aphid species (Miles, 1988). In field conditions, cassava damage caused by mealybugs is characterized by apex and leaf deformation. It is likely that stylet sheaths left in plants by feeding mealybugs may not only cause mechanical damage to host tissues, but may also be residually toxic due to the presence of pectic enzymes remaining.

Callose deposits in phloem cells associated with stylet penetration are interpreted as a plant response to the breakdown of host cell walls. According to the contribution of callose deposits in cassava resistance to *Xanthomonas campestris* pv. *manihotis* (Boher *et al.*, 1995b) and in plant resistance to fungal infection (Skou *et al.*, 1984; Kovats *et al.*, 1990), it is reasonable to assume that callose formation could be involved in resistance mechanisms of cassava to *P. manihoti* infestation.

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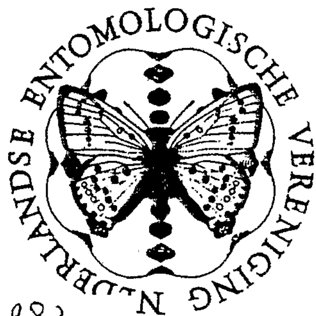

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