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HISTOLOGICAL STUDIES ON THE STYLET PATHWAY, FEEDING SITES AND NATURE OF FEEDING DAMAGE BY *PLANOCOCCUS CITRI* (RISSO) (HOMOPTERA: PSEUDOCOCCIDAE) IN SWEET ORANGE.

ABSTRACT

HISTOLOGICAL STUDIES ON THE STYLET PATHWAY, FEEDING SITES AND NATURE OF FEEDING DAMAGE BY *PLANOCOCCUS CITRI* (RISSO) (HOMOPTERA: PSEUDOCOCCIDAE) IN SWEET ORANGE.

Histological studies on the stylet pathway, feeding sites and cell damage caused by the citrus mealybug, *Planococcus citri* (Risso), on sweet orange (*Citrus sinensis* (L.) Osbeck) fruit and leaves are described. The frequency of stylet pathways that were exclusively intercellular did not differ significantly from those that were both inter- and intracellular. Stylet track terminations were significantly more frequent in the phloem and its proximity than elsewhere, indicating that the phloem was the preferred feeding site in both fruits and leaves. None of the observed stylet tracks had multiple branches. The majority of plant cells surrounding the stylet tracks showed no detectable damage; only in the fruit did some cells appear slightly enlarged and had a different pigmentation, suggesting that the damage was caused by diffusion of saliva from the stylet. The pierced cells appeared otherwise healthy.

Key words: feeding behaviour

INTRODUCTION

The citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae), is a world-wide polyphagous insect, considered to be a key pest of citrus in many countries. Feeding injuries caused by *P. citri* on citrus include chlorotic spots on fruit and leaves, deformation of the peel and fruit splitting (Silva, 1994, 1998; Silva & Mexia, 1997). Despite the importance of the damage caused by *P. citri* on citrus, there are no studies of its feeding behaviour on citrus and the physiological damage caused by this species is poorly understood.

The present study was developed to study the feeding behaviour (i.e., stylet pathway, stylet track terminations and proximity to phloem) of *P. citri* and the nature of any cell damage caused by it to sweet orange fruit and leaves. This study adds to the present understanding of the histology and damage already outlined in previous studies (Silva, 1994, 1998; Silva & Mexia, 1997).

MATERIALS AND METHODS

Selection of experimental units. Fruit and leaves were collected from 10-year old, sweet orange trees (cultivar "Navelina") budded on to Citrange Troyer rootstock, in a grove with an area of 3600m^2, in Setubal (38° 33' N, 8° 53' W; 28m altitude), Portugal.

Before the fruit and leaves were used in the experimental study, they were submitted to a phase of isolation and infestation in cages, as described previously (Silva, 1994; Silva & Mexia, 1997). All fruits and leaves were harvested on the 12 November 1996, during the normal harvesting period. A total of 10 fruits with feeding injury (i.e. chlorotic spots and peel deformation) and 10 healthy fruits (i.e. without any injury and with characteristic orange colouration) and a total of 10 leaves with and without feeding injury caused by *P. citri* (i.e. with chlorotic spots) were harvested from the grove.

Fixation and sectioning. Small pieces (approximately 1cm^2) of citrus peel and leaf tissue were removed, with those from the infected samples being taken from selected chlorotic areas within a *P. citri* colony while the uninfected leaves were from healthy, green regions.

The method of Graça & Pereira (1990) was slightly modified for fixation and sectioning. The samples were fixed in FAA (10 parts 95% ethanol, 1 part glacial acetic acid, 2 parts formaldehyde [37-40%], 7 parts distilled water), according to the formula presented by Washington & Walker (1990) for 2 days at laboratory temperature. After fixation, the samples were washed in distilled water several times for 24 hours and then were gradually embedded in polietilenoglicol (1,500 g/mol) (PEG), first with 20% PEG for 24 h at 60°C and then each sample was placed in an appropriate mould and a solution of 100% PEG was added. The pieces were sectioned (0.8µm) at right-angles to the surface of fruits and leaves, with an appropriate adhesive ribbon, using a hand microtome. The sections were stained with toluidine blue (0.05%). Prior to mounting on microscope slides, each section was separated from the adhesive ribbon with gelatinous glycerine and xylol. All the sections were carefully examined under the light microscope (1000x) for (i) stylets or stylets sheaths (i.e. the stylet pathway and termination of stylets at feeding sites) and (ii) the condition of the chlorotic cells (e.g., cell appearance, cell size, alterations of chloroplast number and aspect) and whether these cells surrounded the stylet tracks or were some distance away, when compared with healthy cells.

Statistical analysis. Chi-square analyses ($P<0.01$) (Zar, 1984) were used to compare frequencies of stylet pathways, stylet tracks terminations and their proximity to phloem in the fruits and leaves.

RESULTS AND DISCUSSION

Stylet track pathways.

There was no significant difference in the frequency of stylet pathways that were exclusively intercellular and those that were both inter- and intracellular in either fruits (53% as compared with 45.2%; χ^2=0.62, df=1, P>0.01) or leaves (42.4% as compared with 57.5%; χ^2=1.22, df=1, P>0.01)). Stylet pathways that were exclusively intracellular occurred rarely in the fruit (1.8%) and never in the leaves.

These observations suggest that *P. citri* stylets tend to follow a mixed inter- and intracellular pathway or an exclusively intercellular pathway. No stylet track had multiple branches, suggesting considerable ability by *P. citri* to locate the feeding sites.

Stylet track terminations and proximity to phloem.

Stylet-track termination was as follows: (a) in the proximity of vascular bundles: 49.6% in fruits and 72.7% in leaves, (b) in the phloem: 10.4% in the fruits and 6.1% in the leaves, and (c) in the mesophyll, distant from the vascular bundles, 40.0% in fruits and 21.2% in the leaves. The percentage of the stylet tracks that reached the phloem or were located near the vascular bundles was 60.0% in the fruit, 78.8% in the leaves, significantly more frequent than other stylet terminations distant from vascular bundle (fruits: χ^2=4.0, df=1, P<0.05; leaves: χ^2=33.29, df=1, P<0.01). Thus, the phloem is apparently the preferred feeding site in both the fruits and leaves of sweet orange, as suggested by Silva (in press).

Nature of cell damage caused by stylets.

The majority of plant cells surrounding the stylet tracks showed no detectable damage, suggesting that the cellular contents were not removed or that there was little or no saliva injection into these cells. This is similar to observations with other scale species, e.g., *Aonidiella aurantii* (Homoptera: Diaspididae) (Washington & Walker, 1990), *Unaspis citri* (Comstock) (Homoptera: Diaspididae) (Albrigo & Brooks, 1977) and *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae) (Calatayud *et al.*, 1994). However, some cells distant from stylet tracks appeared slightly enlarged within the fruits (32.2%) and had different pigmentation (5.2%). Because there was no detectable damage to cells pierced by *P. citri* stylets during penetration, it is suggested that the damage (i.e. chlorotic spots on the fruit and leaves and the citrus peel deformation) may be caused by diffusion of saliva away from the stylet. In addition, the toxicity of saliva may be enhanced by the high population densities of *P. citri* on sweet orange. However, it was not possible to detect any changes in the number and appearance of chloroplasts within the chlorotic cells (Silva, in press).

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