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Preliminary Observations on Abnormal Depositions and Forms of Siliceous Compounds in a Blight-Affected Sweet Orange Tree from a Brazilian Tropical Humid Area

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ABSTRACT. Qualitative thermal tests and light and scanning electron microscopy of leaves, roots and trunks for opal-A (amorphous silica (Si)) as well as total leaf analysis for Si were performed on 11- to 12-yr old Hamlin sweet orange trees on Rangpur lime with and without blight symptoms and grown on soils classified as Oxisol. High amounts of silicified opaline plugs were found in the outermost active xylem in trunks of blight-affected trees, but very few in healthy-looking trees. In the case of the leaves, much smaller amounts of opal plugs were found in a blighted tree in comparison with healthy looking trees. However, total Si contents of the Hamlin leaves did not show differences. Further microscopic observations indicated that leaves of a blight-affected sweet orange tree have more silica in the form of randomly distributed colloid bodies (silica-gel), whereas leaves from healthy trees had silica in the opal phytoliths form, which is known to be beneficial when it is located in the epidermis and between the cell walls.

Key words: Silicon, phytoliths, citrus decline.

Decline or blight of citrus is a disorder of undetermined etiology (7). It occurs mainly in tropical and subtropical humid areas, causing serious plant damage and sometimes death of the plant (3). The incidence is estimated to be around 6% in the State of São Paulo, Brazil (2), where soils classed as Oxisol and Ultisol (1) dominate. The various symptoms of the disease have already been extensively described. Most seem to be related to restrictions of xylem water flux (6). Nutrient concentrations in blight-affected trees often differ from healthy trees, and differalso found between ences are asymptomatic and symptomatic leaves, the latter having lower concentrations of Mg, Mn, B and Si (9).

In Uberlândia, southwestern Minas Gerais State, Brazil, white siliceous cylinders, identified *in situ* as partially opalized branched vegetative axes from *Cecropia* sp., a common native tree, were found in a medium textured oxic soil horizon. The bark and internal tissues were completely decomposed without silicification. Silicon was dominant, but Zn and Cu were also detected. Since some of the *Cecropia* sp. trees show symptoms similar to citrus blight, which also occurs in the area, material from a blighted citrus tree was sampled and examined to determine its silica content. The observations are presented in this paper.

The area of this study is located at the border of the "cerrado" (edaphic savanna) ecosystem, about 19°26"S and 48°35'W. Climate is classed as "Aw" according to Köppen (megathermic rainy tropical, with a long dry winter). Rainfall is about 1,700 mm yearly with a 6 mo dry season. Soils are medium textured Oxisols (Rhodic Haplustox) (7). Topsoil in the orchard had an initial pH of 4.8 to 5.0 and was limed to pH 6.0 to 6.5. High fertilizer amounts (soil and leaf) were applied each year since planting. A 12-yr-old Hamlin sweet orange grafted on a Rangpur lime rootstock showing clear blight symptoms and a nearby healthylooking tree were chosen. Blight occurrence was tested using the syringe method (5), and the bulk density of lateral fresh roots (8). Samples were taken from: a) lateral roots (near trunk); b) trunk active

xylem and bark, about 10 cm above and below bud union and c) base and apex leaves around middle crown. Roots and leaves from a nearby old Rangpur lime tree were also sampled. Whole fresh leaves were first boiled for 30 min. in 2 M HCl (to remove basic cations such as Ca, Mg and K), washed with deionized H_aO, set between two glass slides and heated for 8 to 10 h at 550°C. The resulting whitish residue is mainly biogenic opal "silicaphytolits". According to Jones and Handreck (3), opal phytoliths are a result of the process by which certain plants deposit solid silica in intracellular or extracellular locations as unwanted material or reinforcement of cell structures after absorbing silica in a soluble state (monosilicic acid) from the soil. Whole leaves were also dried (60°C). crushed on a Willey-mill to 60 mesh and analyzed for total silica (Si).

Paraffin impregnated and colored thin sections of tissues were observed and photographed on light and scanning electron microscopes. Leaves were also treated with hydrogen peroxide $(20\% \text{ at } 45^{\circ}\text{C})$ and sodium perborate solution (for tissue thinning) and observed by petrographic microscopy, to identify and locate the phytoliths. Thermal and thin section observations exposed considerable amounts of opal in the active xylem of the trunk of the blighted tree but none, or very few, in the healthy tree. Thermal tests indicate that blight-affected leaves have much smaller opal amounts as compared to healthy leaves (Fig. 1). Total Si content among all the basal sweet orange leaves did not show much difference (Table 1). Rangpur lime basal leaves had higher amounts of total Si, suggesting that their roots may absorb more soluble Si (probably in the form of silicic acid) than the Hamlin sweet orange needs. 1) lead to the hypothesis that in blightaffected leaves Si compounds are mostly in a form different to the opal



Fig 1. Thermal tests on Hamlin sweet orange leaves. Left: from plant without blight symptoms; Right: with blight symptoms. The lesser amounts of the whitish residue indicate much smaller amounts of opal in the leaf from blight affected plant as compared to the healthy leaf.

phytoliths. The latter are known to be beneficial especially when located within the epidermis and between the cell walls, where they are supposed to inhibit water stress, protect against pathogens, and make a healthier leaf that enhances photosynthesis (4). It was further observed that in blight-affected leaves, most of the Si seems to be in the form of silica gel-like colloid bodies. These most probably act as a desiccant which should be detrimental. Heavy mineral ligaments (such as Cu and Zn), transformation of silica gel into opal as well as organic compounds, and microbial action are some of the possible interfering agents that need to be investigated in further studies. It should also be taken into consideration that processes involved in the formation of the opal phytoliths deserve further studies since it appears to be related to the citrus blight syndrome. These kinds of phytoliths (e.g., silicophytoliths) are characteristic of several plants especially ones from more humid environments. These ideas are reinforced

(g/Kg)

4.33.7

3.62.0

5.7

2.8

AFFECT OF BLIGHT ON TOTAL SI LEVELS OF HAMLIN SV		
Cultivars and description	Leaf type (age)	Si
Hamlin/Rangpur lime		

	TABLE 1	
AFFECT OF BLIGHT	ON TOTAL SI LEVELS OF	F HAMLIN SWEET ORANGE

base (mature)

apex (mature)

base (mature)

base (mature) apex (young)

apex (some young)

^zSi content based on the dry weight of leaves.

when one considers that the citrus blight does not occur where the plants are grown under a semiarid environment such as California and the Mediterranean fringe. In these conditions, plants usually have phytoliths mostly in the form of calcium oxalate crystals.

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Blight

Healthy

Rangpur lime Healthy