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# Urease Activity in Ortho Red-Yellow Podzolic Soil Planted With Pera Orange Trees Affected With Declínio

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**ABSTRACT.** Urease activity of soil under declínio-affected citrus trees was studied to determine a possible relationship of declínio with soil and rhizosphere. Forty trees of each of four stages of declínio: healthy trees, initial, intermediate and severe stages of the disease were examined. The soil samples at 0-20 cm were collected from four different locations at the dripline of each tree, in September and November 1988 and in January and April 1989. Higher urease activity was found in all soil samples under declínio-affected trees. Differences were statistically significant only for the samples collected in September 1988 and January 1989. These data suggest that there may be a higher biological activity in nitrogen metabolism under declínio-affected citrus trees.

Characteristics of declínio of citrus trees in Brazil, which is similar to blight in Florida, suggest that the disease may be, in some way, related to soil and rhizosphere (13). Wutscher has observed the internal redistribution of nutritional elements in blight-affected trees, and soil sample analysis may suggest nutritional disorders (17). In Brazil, concentrated mineral fertilizers are widely used in citrus orchards because of the need to add relatively high amounts of nutrients (3, 12). One hundred fifty g of N per tree is recommended for each 40 kg-box of orange, divided in three applications in August, November and February (11). Urea is considered important as a N fertilizer because of its low cost. During 1980-1981, urea corresponded to 12% of  $12.7 \times 10^6$  metric tons of fertilizers used in the U.S. and to 70% of N fertilizers used in Asia (5).

When applied to the soil, urea is hydrolyzed by urease, resulting in higher soil pH and concentration of ammonium which evolves in ammonia (5). Urease seems to be liberated in the soil by the death and rupture of ureolytic soil microorganisms and by plant organs where it is adsorbed to the soil colloids or remaining in soil solution (6). Recently, research has been carried out on the possible relations of  $N-NH_4^+$  (2) and on the high values of soil pH (16) with blight.

The objective of this work was to determine urease activity in soil under healthy and declínio-affected trees.

## MATERIALS AND METHODS

The trial was carried out in an orchard at Monte Alto city, São Paulo State, Brazil. The soil is classified as Ortho Red-Yellow Podzolic soil. The topography is relatively flat; the soil is well drained. The trees were 12-yr-old Pera orange on Rangpur lime.

The experimental design was in randomized blocks, in each block five trees each of the following disease rating were selected: healthy trees, initial, intermediate and severe stages of declínio. Samples were collected from eight blocks. In all, soil samples were collected from 160 trees. The parameters used for declínio evaluation were visual symptoms (4), water uptake by the syringe injection method (7) and zinc accumulation in the trunk above the bud union (19).

Soil samples were collected at the dripline of trees, at 0-20 cm depth. Four samples were collected for each tree and combined as one sample. The samples for each disease rating were combined for the five trees in each of the eight blocks. Soil collections were made in September 1988, November 1988, January and April 1989.

Urease activity was determined in their air dried samples by May and Douglas method (8). Three g of soil plus 0.5 ml of toluene were put in a 125 ml erlenmeyer flask and left for 10 min. Twelve ml of (0.067 M) phosphate buffer pH 8.8 was added, and after 10 min 3 ml of urea solution (1000  $\mu$ g N-urea/

ml) was added and incubated at 37 °C for 4 hr. After incubation, 15 ml of (2.0 M) KCl - phenyl mercury acetate (dissolve 150 g KCl in 800 ml water and 5 mg phenyl mercury acetate in 100 ml hot water, combined these solutions and make the volume to 1 liter) was added, and shaken for 5 sec. Ammonium content was determined in 10 ml of the filtered solution by the vapor distillation method proposed by Bremner and Keeney (1). The control was urea solution added after the KCl - phenyl mercury acetate solution.

## RESULTS AND DISCUSSION

The results obtained for urease activity are summarized in Table 1. Urease activity was higher under decline-affected trees in the samples collected in the four dates. Significant differences between treatments were found in the samples collected in September 1988 and January 1989. Higher activity were observed for the trees in the three stages of decline, which did not differ one from the other, but statistically differed from the healthy trees.

Urease activity values varied from 15.7 to 42.4  $\mu\text{g N-NH}_4^+$  per g soil per 4 hr (or 3.92 to 10.60  $\mu\text{g N-NH}_4^+$  per g soil per hr). These results, when compared with the ones obtained are similar to values obtained by May and

Douglas in 1976 (8) for Derrimut, a soil similar to the Ortho Red-Yellow Podzolic soil.

The effect of pH on soil urease activity has been observed by several authors. Pettit *et al.* (9) stated that the optimum soil pH for urease activity was 6.5 - 7.0. Tabatabai and Bremner in 1972 (14) and May and Douglas in 1976 (8) found the optimum pH to be 8.8 - 9.0. Rachhpal-Sing and Nye (10) found optimum pH values of 6.0 - 6.8. As the soil pH decreases, urease activity decreases, suggesting that in tropical, generally acid soils, urease activity must be low. This was observed in this experiment.

The soil pH in this study (in  $\text{CaCl}_2$ ) varied from 5.0 to 6.0 which may be responsible for the variations between samples (Table 1). Wutscher, using a detailed method for collecting soil samples (16) at each 5 cm depth to 45 cm in five Florida regions, found that the mean pH was 0.5 - 1.2 pH units in water and 0.5 - 1.6 pH units in 0.01 M  $\text{CaCl}_2$  higher under blight-affected trees than under healthy trees. These pH differences may result in higher urease activity under blight-affected trees. Moreover, the alkaline products of urea hydrolysis (ammonium bicarbonate and ammonium hydroxide) may raise the pH in soil environment resulting in loss of ammonium from the soil

TABLE 1  
UREASE ACTIVITY IN RED YELLOW ORTHO-PODZOLIC SOIL PLANTED WITH TREES OF PERA SWEET ORANGE ON RANGPUR LIME ROOTSTOCK SHOWING DECLINIO SYMPTOMS (MEANS OF 8 REPLICATIONS)

Treatment	Samples			
	Sep88	Nov88	Jan89	Apr89
	----- $\mu\text{g N-NH}_4^+/\text{g soil}/4\text{ hr}$ -----			
Healthy trees	15.7a <sup>z</sup>	19.5	19.4 a	38.7
Initial stage of decline	17.8ab	17.5	25.8ab	39.5
Intermediate stage of decline	20.9b	22.6	25.4ab	42.4
Final stage of decline	21.2b	23.0	28.0 b	41.4
F <sub>(treat)</sub>	7.8**y	3.0 <sup>ns</sup>	4.0*	0.6 <sup>ns</sup>
F <sub>(B)</sub>	1.3 <sup>ns</sup>	2.0 <sup>ns</sup>	1.9 <sup>ns</sup>	1.8 <sup>ns</sup>
CV <sub>(%)</sub>	14.1	20.8	21.1	15.3
DMS <sub>(5%)</sub>	3.7	6.0	7.3	8.6

<sup>z</sup>Means followed by the same letters do not differ significantly by Tukey's Test,  $P \leq 0,05$ .

<sup>y</sup>Significant at 5% level (\*) or 1% \*level \*or not significant (ns), by the F Test.

by volatilization (15). The loss of urea nitrogen by the action of urease, giving high ion ammonium concentrations, is favored by a high soil pH, low buffer capacity and high temperature (5).

Ammoniacal nitrogen has been thought to be related to blight and to other citrus abnormalities in Florida and some South American countries (18). Some observations suggest that N, mainly in ammoniacal form, may cause damage to citrus by causing root stress when applied in high dosages, producing injuries for entry for microbial infection or increasing fungal activity and pathogenicity. The need of N under ammoniacal form for toxin pro-

duction by *Fusarium solani* has been reported (2). Burnett *et al.* (2) have reported the development of blight-like symptoms in healthy citrus trees after application of some amounts of  $\text{NH}_4\text{OH}$ .

The higher urease activity under declínio-affected citrus trees when urea is present, mainly after the application of the N-fertilizer, can result in high levels of N-ammonium and in higher pH in soil microenvironments close to the roots, which may cause injuries to the plants, a factor enhancing declínio symptoms as a stress factor.

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