

Considerations on the Possible Nature of Citrus Decline

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ABSTRACT. Citrus decline, locally known as "declinio" was observed in citrus groves of the State of São Paulo, Brazil starting in 1970 and since then has spread, causing extensive losses. Symptoms of the disease are very similar to those of blight or young tree decline in Florida. The etiology of this decline has not yet been determined. Valencia, Natal, Pera and Hamlin orange trees budded on Rangpur lime are the scion-rootstock combinations more frequently affected. Field observations suggest an infectious cause for the problem, however transmission tests here were negative. Nematode studies revealed the presence of *Trichodorus*, *Xiphinema*, *Helicotylenchus*, *Criconemoides* and *Hemicriconemoides* spp. on the roots of affected and neighbouring healthy trees. Tomato plants grown in pots with soil taken from areas under diseased trees or with soil contaminated with nematodes from diseased roots developed yellow leaf spotting. Field surveys also revealed a coincidence of the appearance of citrus decline and the invasion of citrus areas by sugarcane plantings. Ratoon stunting, a disease associated with the presence of bacteria (*Erwinia herbicola*, *Pseudomonas* spp. and *Xanthomonas albilineans*) in the xylem, is frequently found in these sugarcane plantings. Incidence of declining trees is greater in citrus groves in the warmer areas, while the disease is nearly absent in cool areas.

Index words. citrus blight, "declinia," xylem bacterium.

Thousands of citrus trees are declining in Brazil, mainly in the State of São Paulo, with symptoms resembling those of blight or young tree decline. The disease was first observed in the early 1970's, in trees of a citrus farm, where buds from Argentina and Florida had been brought in a few years before. Nursery trees produced in this and neighbouring farms were sold to growers all over the country. No phytosanitary restrictions were imposed and nurseries are still operating on properties with plantings severely affected by "declinio". Affected trees are now occurring also, in groves in the northern states of Bahia and Sergipe. Some growers call the disease "tristeza of Rangpur lime rootstock", but the name "declinio" is more generally used throughout the country.

The abnormality was first described by Rodriguez *et al.* (9) in 1979. However, its similarity with Florida's young tree decline had been earlier pointed out by Dr. E. P. DuCharme, during a visit to that farm with the first focus of the disease. This possibly is the ex-

planation for the name "declinio" (citrus decline).

All efforts to transmit citrus tree decline (CTD) yielded negative results (7, 8, 9, 10). Here we report field observations and the results of studies aimed to increase the understanding of this serious problem that is threatening the entire citrus industry of Brazil.

FIELD OBSERVATIONS

Incidence of citrus decline.

Citrus decline is presently the most serious problem affecting the citrus groves in the State of São Paulo, according to field inspections and growers opinion. Rough estimates are that over one million trees were already uprooted or are declining. Symptoms of the disorder have been carefully described by Rossetti *et al.* (10) and are nearly identical to those of citrus blight or young tree decline in Florida (17).

Field observations revealed that CTD has accelerated its spread since 1977 and diseased trees are found in most citrus groves. The problem is more serious in planta-

tions with better care; those receiving higher amounts of fertilizer. The number of affected trees varies considerably from a few trees in some groves up to 50% of the trees in a few plantations.

The incidence of CTD was highest in groves of the Bebedouro-Barretos area, a northern warmer region of the State of São Paulo, less prevalent in the cooler Limeira-Araras region and practically absent in the cool south region of Sorocaba-Tatui-Botucatu.

In a recent survey, Muller and Prates (8) encountered respectively 3.0%, 1.5% and 0.0% in the official agricultural regions (DIRAs of Ribeirão Preto, Campinas and Sorocaba) corresponding to the above-mentioned citrus areas. In a new northern citrus area (DIRA of S. José do Rio Preto) they found 0.5% of the trees declining.

Curiously, the area most affected by CTD coincides with the area more or less recently planted to sugarcane. The area of Bebedouro-Barretos was previously a cattle raising area and especially around Barretos this is still an important enterprise. Ratoon stunting of sugarcane, (RSD) a disease associated with bacteria like *Erwinia herbicola*, *Pseudomonas* spp, and *Xanthomonas albilineans* in the xylem is frequently encountered in the plantings (15). Colonial grass, *Panicum maximum* Jacq. is abundant in one farm in Barretos area, where the citrus orchard having the highest percentage of declining trees was found. The RSD agent has been transmitted to or found in a wide variety of graminaceous plants (5, 14) and some relationship was suspected.

Stionic combinations and age of affected trees. Field inspections revealed a range of susceptibility of commercial stionic combinations to CTD. The late ripening varieties, Valencia, Natal and Pera oranges

budded on Rangpur lime rootstock are most affected. These orange varieties represent over 60% of all citrus grown in São Paulo and Rangpur lime is practically the only rootstock (over 90%) used there. Symptoms of decline were found on trees of Hamlin, Baianinha Navel and Piralima oranges. Only rarely trees of other scion varieties were declining. Based on these observations scion varieties were tentatively rated accordingly: a.) Very susceptible—Valencia, Natal and Pera oranges; b.) Susceptible—Hamlin, Baianinha Navel and Piralima oranges and Tahiti lime; c.) Moderately susceptible—Murcott tangor, Cravo and Ponkan tangerines and Mexican lime; and d.) Tolerant or resistant—Eureka lemon and grapefruit.

Rangpur lime, Volkamer lemon and trifoliolate orange are considered intolerant rootstocks and Cai-pira sweet orange, Cleopatra mandarin and possibly Orlando tangelo are apparently tolerant or resistant. These are, of course, ratings based on field inspections and need experimental confirmation.

Some minor scion and rootstock varieties found free from CTD may actually be intolerant to the problem, and existing trees have just escaped the causal agent. In one large carefully inspected, citrus farm, some groves were found free from CTD, while others showed a high percentage of diseased trees. Twenty-year-old Valencia orange trees on Rangpur lime rootstocks had 10% CTD and neighbouring sister trees on Cai-pira sweet orange were all healthy. Baianinha navel trees on Rangpur lime rootstock of the same age were also healthy.

The incidence of CTD was not related to whether the scions were of old-line or nucellar origin. The extensive use of healthy nucellar clones in the citrus orchards in the last two decades, practically elimi-

nated virus diseases like psorosis and viroids like exocortis and xyloporosis from the citrus plantings in São Paulo. Tristeza is, however, endemic and new strains of the virus probably arise periodically and are spread by *Toxoptera citricida* Kirk.

CTD affects bearing trees independent of age. Inspections in commercial orchards revealed declining trees from four to 20 years old or more. Apparently, the disease does not occur before trees flower and produce a heavy crop. Higher percentage of affected trees were found between five to ten years of age. Incidence of CTD was somewhat proportional to precocity of the varieties grown.

EXPERIMENTAL TRIALS AND RESULTS

Indexing for tristeza. Young Mexican lime seedlings were infected with blind buds and root pieces from diseased and healthy trees. Donors were five trees each of Valencia, Pera and Baianinha Navel oranges with CTD and same number from neighbouring apparently healthy trees. Inoculations were made in October 1982 and seedlings were decapitated 30 days later. New sprouts were periodically inspected for leaf symptoms of tristeza. In April 1983 all new sprouts were peeled and rated for stem pitting. All test plants were positive for tristeza stem pitting ranging from a rating of two to four on a scale from zero to five. There was no significant difference between CTD and healthy trees. Thus, apparently tristeza expressed as stem pitting is not involved in the citrus decline problem. The tristeza virus may however play a role in the CTD problem by weakening trees and predisposing them to stress.

Perpetuation and transmission trials. Young branches and roots were collected from 20-year-old de-

clining nucellar Valencia orange trees on Rangpur lime and trifoliolate orange rootstocks from a badly affected orchard (the first orchard showing CTD). Buds and root pieces were used to inoculate, by lateral grafting, young potted test plants of Valencia orange on Rangpur lime rootstock. Inoculations were made in October 1982, with one tree plots and five replications. Noninoculated plants were left as healthy controls. Periodic inspections, up to eight months following inoculations revealed no differences between infected plants and the healthy controls.

In another series of tests, propagations were made by grafting young sprouts from the same trees, 10 cm long, on Rangpur lime and Mexican lime seedlings. New growth was apparently normal in all plants, except for irregular margins in the first leaves of most test plants. Leaves with irregular margins were more numerous in the Valencia plants grafted on Mexican lime.

Root pieces from CTD affected plants were also induced to sprout by planting them in small plastic bags with fertile soil and favorable moisture. Only Rangpur lime roots sprouted and no sprouts were obtained from trifoliolate orange root pieces. Sprouting was poor and in three of 20 plants showed conspicuous chlorotic leaves.

Nematode studies. Root pieces and soil taken under declining trees growing in heavy and sandy soils were examined for nematodes. These studies indicated the presence in practically all samples of the following nematodes: *Trichodorus*, *Helicotylenchus*, *Cricone-moides*, *Hemicricone-moides* and *Xiphinema* spp. Curi *et al.* (2) found the same nematodes in the roots of declining trees but were unable to establish any relationship between their presence and CTD. In the present work, the highest

populations observed were of *Xiphinema americanum* Cobb, which was found on trees of Valencia orange budded on Rangpur lime (1 larva/ml), on Caipira sweet orange (1 larva/ml) and on trifoliolate orange (5 larvae/ml). Since nematodes are involved in the transmission of tomato ringspot virus to apple trees (1) a series of trials were conducted to verify the possibility of its action as a vector of CTD to tomato plants.

Young tomato seedlings were transferred to pots filled with soil infested with *Xiphinema americanum* and other nematodes. Treatments were: 1) pots filled with soil taken under declining citrus trees; 2) pots filled with clean soil that was infested with *X. americanum* from diseased roots; 3) pots filled with clean soil, but a small Valencia orange seedling collected from under declining trees was planted in each pot. Yellow leaf spotting suggestive of initial Mg deficiency appeared in tomato plants of all treatments. Symptoms occurred in the basal leaves only and reappeared in the new sprouts after plants were cut back. New experiments in progress may answer a possible relationship of these symptoms to CTD.

TABLE 1
ATTEMPTS TO TRANSMIT CITRUS
TREE DECLINE (CTD) TO TOMATO
PLANTS BY THE USE OF
NEMATODES

Treatments	No. of tomato plants	
	Total	With leaf yellow spots
CTD soil	60	17
Clean soil plus nematodes	20	12
Clean soil plus orange seedlings	6	4
Clean soil	20	0

DISCUSSION

Several possible causes have been raised to explain the abnormality named "declinio" or CTD and among them are a nutritional imbalance, phytotoxicity, and an infectious disease. Other causes have been disregarded in light of presently available information (7, 11, 12).

All transmission tests up to now have yielded negative results. However, the spread of the problem in mature groves and the difference in the reaction of various stionic combinations favour the thesis of an infectious agent that moves by a vector.

Citrus decline was found to affect trees of any age, provided the tree has bloomed and yielded heavily, suggesting that a stress may be necessary for the onset of symptoms or that pollen may be involved in the transmission of the causal agent. Similarly, symptoms of greening or leaf mottling disease normally develop only after production stress and some strains of cherry leafroll virus are seed-borne and pollen transmitted and natural spread does not occur until trees bear flowers (6).

Rangpur lime rootstock seems to be hypersensitive to the citrus decline agent while sweet orange and Cleopatra tangerine are apparently tolerant or resistant. This varietal reaction is similar to that of the wilt disease induced by Pierce's disease bacterium (4) and also to that observed for Florida blight (18). Citrus decline has external and anatomical symptoms nearly identical to those described for blight or young tree decline in Florida, USA (17), for "Marchitamiento repentino" in Uruguay (13) and for "declinamiento" or "fruta bolita" of Argentina (16, 17). Exchange of plant materials among these countries may have occurred just before the appearance

of these diseases. Thus, the spread of an infectious agent could be easily explained. To attribute these diseases to noninfectious cause(s) would require a more complicated mechanism, like a toxic effect of a chemical product distributed by a multinational company. A possible carbamate toxicity enhanced by the presence of organic solvents was suggested (11) but found little support in field observations.

Evidence was found that heavy applications of fertilizers and dolomite induce a higher incidence of "declinio". Nutrition certainly plays an important role in the incidence of the abnormality, possibly as a factor inducing high yields and consequently stress. However, it would be difficult to explain the appearance of decline symptoms in trees growing under extremely different soil and climatic conditions, of different ages and stionic combinations and to attribute it to nutrition.

The higher incidence of citrus decline in the areas invaded by sugarcane plantations and the certain similarities of xylem symptoms of declining citrus trees and

sugarcane with ratoon stunting disease may be merely a coincidence. It should however be investigated, since all aspects leading to the possible cause of the decline are important. Moreover, this hypothesis would be in agreement with the work of Feldman and Hanks (3).

All transmission trials conducted by the authors resulted in negative results. However, the presence of high populations of nematodes, especially *X. americanum* was noted. Yellow spots were induced in the leaves of tomato plants grown in soil taken from under diseased plants or soil infested with those nematodes. Further studies to establish a possible relationship of the tomato leaf spotting and citrus decline as well as the role of nematodes as vectors of the problem are in progress.

All available information points toward an infectious agent as the cause of citrus tree decline (CTD). Research is needed to determine the nature of this pathogen and the relative resistance of cultivars and rootstocks, as well as the form of spread of the disease.

LITERATURE CITED

1. CUMMINS, J. N., D. GONSALVES, and D. ROSENBERGER
1982. Interactions of apple rootstocks and fruiting cultivars with Tomato ring-spot virus. 21st Intern. Hort. Congress, Hamburg. 1: 1399. (Abstract).
2. CURI, S. M., S. G. P. SILVEIRA, and H. S. PRATES
1981. Nematodes and the abnormality "declinio" of citrus trees in the State of São Paulo. V Reunião Brasileira de Nematologia, Londrina, p. 9. (Abstract).
3. FELDMAN, A. W. and R. W. HANKS
1980. Recovery of rickettsialike bacteria from xylem of healthy and young tree decline-affected citrus trees, p. 264-269. *In Proc. 8th Conf. IOCV. IOCV, Riverside.*
4. HOPKINS, D. L.
1982. Relation of Pierce's disease bacterium to a wilt-type disease in citrus in the greenhouse. *Phytopathology* 72: 1090-1092.
5. LIAO, C. H. and T. A. CHEN
1981. Isolation, culture and pathogenicity to Sudan grass of a *Corynebacterium* associated with ratoon stunting of sugarcane and with Bermuda grass. *Phytopathology* 71: 1303-1306.
6. MIRCETICH, S. M., R. R. SANBORN, and D. E. RAMOS
1980. Natural spread, graft-transmission, and possible etiology of walnut blackline disease. *Phytopathology* 70: 962-968.
7. MULLER, G. W. and H. S. PRATES
1981. Observations about the decline of citrus trees. *Laranja* 2: 1-12.

8. MULLER, G. W. and H. S. PRATES
1982. Investigações sobre o declínio dos citros no Estado de São Paulo. *Laranja* 3: 143-154.
9. RODRIGUEZ, O., V. ROSSETTI, G. W. MULLER, C. S. MOREIRA, H. S. PRATES, J. D. DENEGRI, and A. GREVE
1979. Decline of citrus trees in the State of São Paulo, Brazil, p. 927-932. *Anais do V Congresso da Soc. Bras. Fruticultura, Pelotas*.
10. ROSSETTI, V., H. K. WUTSCHER, J. F. CHILDS, O. RODRIGUEZ, C. S. MOREIRA, G. W. MULLER, H. S. PRATES, J. D. DENEGRI, and A. GREVE
1980. Decline of citrus trees in the state of São Paulo, Brazil, p. 251-259. *In Proc. 8th Conf. IOCV. IOCV, Riverside*.
11. SALIBE, A. A.
1982. Decline of citrus trees and related problems. p. 195-209. *Planta Cítrica, Cordeirópolis*.
12. SALIBE, A. A.
1983. Considerações sobre a natureza da doença "declínio" dos citris. VI Congresso Paulista de Fitopatologia, Araras. *Book of Abstracts*, p. 20.
13. SALIBE, A. A., J. C. TUCCI, P. B. GIRARDIN, and H. G. CAMPIGLIA
1976. "Marchitamiento repentino," an infectious disease of citrus trees, p. 152-156. *In Proc. 7th Conf. IOCV. IOCV, Riverside*.
14. STEINDL, D. R. L.
1961. Ratoon stunting disease, p. 433-459. *In J. P. Martin, E. V. Abbott, and C. G. Hughes (eds.) Sugarcane diseases of the world. Vol. 1. Elsevier Publ. Co., Amsterdam, London and New York*.
15. TOKESHI, H.
1982. Diseases of sugarcane. *Bull. Inst. do Açúcar e do Alcool Planalsucar, Piracicaba*. 70p.
16. WUTSCHER, H. K., H. G. CAMPIGLIA, C. HARDESTY, and A. A. SALIBE
1977. Similarities between marchitamiento repentino disease in Uruguay and Argentina and blight of citrus in Florida. *Proc. Fla. State Hort. Soc.* 90: 81-84.
17. WUTSCHER, H. K., R. E. SCHWARZ, H. G. CAMPIGLIA, C. S. MOREIRA, and V. ROSSETTI
1980. Blightlike citrus tree declines in South America and South Africa. *HortScience* 15: 588-590.
18. YOUNG, R. H., L. G. ALBRIGO, D. P. H. TUCKER, and G. WILLIAMS
1980. Incidence of citrus blight on Carrizo citrange and some other rootstocks. *Proc. Florida State Hort. Soc.* 93: 14-17.