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Use of Arginine and Total Amino Acids to Determine Site of Stress in Citrus Trees with Young-tree Decline

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Young-tree decline (YTD) is a new and increasingly serious disease threat to citrus trees in Florida. YTD first came to attention about seven years ago in trees six to eight years old (hence the name YTD) located on flatwoods soils near the east coast. Since then, even much older trees—40 years or more—growing on better drained, sandy soils of the central ridge area of the state are found with the same or similar symptoms. The name "sand hill decline" is currently used for the disease in that area.

Symptoms of YTD develop progressively, usually beginning in single, isolated trees, with development of dull green foliage and upright growth of younger leaves. Leaves are smaller and fewer in number in subsequent flushes, with twig dieback and leaf wilt on one or more branches. These symptoms are accompanied by a reduction in size and number of fruit. Other citrus tree diseases produce the same or similar symptoms so that additional criteria are currently used for diagnosis of YTD. These include: (1) determining random distribution of gum pockets in stock bark at the bud union; (2) determining the presence of several small pegs in stock bark with corresponding pits in wood near the bud union; and (3) establishing that trees are growing on Rough lemon rootstock. The disease seems to be confined to trees on Rough lemon rootstock although it has been reported on trees on other, unidentified rootstocks.

Trees with YTD are usually replaced two to three years after onset of symptoms, by which time production is no longer economical. The rates of tree decline and spread of YTD are such that loss of trees and of production has ranged up to 50 per cent in some groves within six years (*3*). The number of groves with affected trees has increased considerably during the past six years, and losses are currently estimated to be about 50,000 acres.

The etiology is at present unknown. Neither mineral nutrition (*1*) nor nematodes (*11*) appears to be responsible for this decline. Patterns of distribution of affected trees in groves in the early stages of YTD and the subsequent spread to other trees in affected groves indicate that YTD may be an insect-vectored disease (*3*). Results of attempts to transmit a causal agent of YTD by bud grafting are inconclusive..

This investigation was undertaken to measure changes in amounts of free amino acids in YTD-affected trees and to determine the site of stress, since we do not know whether YTD is a stock or a scion disease. It was also desirable to determine if these data would support the concept that areas of stress in citrus can be detected by increases in total free amino acids, arginine, and proline. Increases of arginine, proline, and total amino acids in specific sites of stress in citrus have been reported for trees affected by the burrowing nematode (*Radopholus similis* (Cobb) Thorne) (*5, 6, 7*), for seedlings inoculated with exocortis virus (*8*), for young girdled trees (*9*) and in iron- and zinc-deficient chlorotic leaves (*10*).

MATERIALS AND METHODS

Four groves, all on Rough lemon rootstock, with 10 to 15 per cent of the trees exhibiting YTD symptoms, were selected for this investigation. Two groves are on flatwoods soils located near the east coast, one of 25-year-old grapefruit trees and the other, 10-year-old Valencia trees. The other two groves are also Valencia, located in the central ridge district, and are approximately 25 and 48 years old, respectively.

Bark samples were collected from immediately above and below the bud union of five apparently healthy trees and five trees exhibiting slight to moderate YTD symptoms. Samples, collected in September, 1970, and January, April, and July, 1971, were frozen when collected, and stored at –20°C.

To determine the effect of complete girdling on proline content in bark of mature citrus trees, two healthy, 12-year-old Valencia trees in a grove with no symptoms of YTD were included in this investigation to serve as a type of "proline control." One tree was completely girdled mechanically at the bud union, and the other tree served as a nongirdled control. Bark samples from immediately above and below the bud union on both trees were collected six times during the growing season (April through October, 1971).

Procedures used for processing bark samples and for quantification of amino acids by two-way paper chromatography are described elsewhere (*6*). Total amino acids were calculated as the sums of the amounts of individual amino acids in a sample after elution from the chromatagrams. Although our primary concern was to determine total free amino acids, arginine, and proline, the amounts of 11 other free amino acids were measured to determine if changes in amounts of any of these amino acids could possibly serve as a presumptive diagnosis for YTD.

RESULTS

Total amino acids. The 13 amino acids and amides isolated from extracts of citrus bark were considerably fewer than the usual 17 to 21 amino acids generally found in citrus leaves and feeder roots (*4, 5, 6*). In addition to proline and arginine, the following amino acids and amides were also found in the bark samples: aspartic, glutamic, alanine, γ-aminobutyric, lysine, threonine, glycine, serine, ethanolamine, aspargine, and glutamine. Those amino acids not detected in bark samples but usually found in leaves or roots were either absent or were present in amounts less than 0.5 μg/gm.

Total amino acids for each stock and scion bark sample are given in table 1. Totals ranged from 644 to 3,555 μg/gm of bark (stock bark, YTD-affected trees, January, Groves D and A, respectively).

Relative increases in total amino acids in stock bark from diseased trees were, for the most part, greater than corresponding increases in total amino acids in scion bark from the same trees (see ratios in table 1). With few exceptions, stock and scion bark from diseased trees contained more total amino acid than did stock and scion bark from corresponding healthy trees (table 1).

Combined amounts of total amino acids in stock and scion bark from diseased trees were always greater at each sampling period than were combined amounts found in stock and scion bark from corresponding healthy trees.

Arginine. Arginine content of stock and scion bark varied considerably from grove to grove and from season to season. The range was from 15 μg/gm of bark (stock, healthy trees, Grove D, January) to 650 μg/gm of bark (scion, diseased trees, Grove B, September, table 2). Combined amounts of arginine in stock and scion bark from diseased trees were always greater than corresponding amounts from healthy trees in each collection, except for Grove C in January (table 2). With but two exceptions (Grove C, January, table 2), scion bark

TABLE 1

TOTAL AMINO ACIDS IN STOCK AND SCION BARK FROM HEALTHY (H) AND FROM YTD-AFFECTED (D) CITRUS TREES, AND RELATIVE INCREASES (D/H) IN TOTAL AMINO ACIDS IN STOCK AND SCION BARK FROM DISEASED TREES

Grove	Cultivar and bark	Tree age (yrs)	Amino acids (μg/gm) at collection dates in:											
			Sept.			Jan.			April			July		
			H	D	(D/H)	H	D	(D/H)	H	D	(D/H)	H	D	(D/H)
A	Valencia:	48												
	Stock.....		1,469	2,487	1.69	2,887	3,555	1.23	2,079	2,704	1.30	2,464	2,955	1.20
	Scion.....		1,221	1,331	1.09	2,965	3,273	1.10	2,147	2,474	1.15	2,083	3,065	1.47
B	Valencia:	25												
	Stock.....		1,271	2,387	1.88	1,546	2,411	1.56	2,134	2,794	1.31	1,763	2,592	1.47
	Scion.....		2,457	2,775	1.13	2,345	2,691	1.15	2,681	2,853	1.06	2,095	2,991	1.43
C	Valencia:	10												
	Stock.....		1,097	1,369	1.25	1,932	2,507	1.30	1,692	2,231	1.32	1,984	2,378	1.20
	Scion.....		1,057	1,236	1.17	1,040	924	0.89	2,348	2,397	1.02	1,548	1,979	1.28
D	Grapefruit:	25												
	Stock.....		1,207	2,647	2.19	707	644	0.91	1,173	2,423	2.07	1,390	2,822	2.03
	Scion.....		1,206	2,227	1.85	889	1,344	1.51	1,713	2,326	1.36	1,658	2,351	1.42

TABLE 2

ARGININE IN STOCK AND SCION BARK FROM HEALTHY (H) AND FROM YTD-AFFECTED (D) CITRUS TREES, AND RELATIVE INCREASES (D/H) IN ARGININE IN STOCK AND SCION BARK FROM DISEASED TREES

Grove	Cultivar and bark	Tree age (yrs)	Arginine (μg/gm) at collection dates in:											
			Sept.			Jan.			April			July		
			H	D	(D/H)	H	D	(D/H)	H	D	(D/H)	H	D	(D/H)
A	Valencia:	48												
	Stock.....		64	157	2.5	78	338	4.3	91	312	3.4	65	143	2.2
	Scion.....		104	208	2.0	364	560	1.5	429	618	1.4	312	403	1.3
B	Valencia:	25												
	Stock.....		39	286	7.3	39	111	2.9	91	338	3.7	52	221	4.3
	Scion.....		338	650	1.9	198	270	1.4	455	618	1.4	208	468	2.3
C	Valencia:	10												
	Stock.....		12	12	1.0	92	52	0.6	104	169	1.6	52	46	0.9
	Scion.....		111	169	1.5	52	46	0.9	260	280	1.1	65	176	2.7
D	Grapefruit:	25												
	Stock.....		18	247	13.7	15	117	7.8	26	176	6.8	39	221	5.7
	Scion.....		72	390	5.4	91	377	4.1	78	319	4.1	65	312	4.8

from healthy or YTD-affected trees contained more arginine than did stock bark collected from the same trees.

The arginine content in both the stock and scion bark from diseased trees was generally greater than in similar bark from the corresponding healthy trees, and scion bark almost always contained more arginine than did the corresponding stock bark. It was therefore necessary to determine and evaluate the extent of the increases of arginine in both the stock and scion bark, respectively, from YTD-affected trees. The magnitude of increases in arginine in stock and scion bark from diseased trees is best indicated by comparing individual ratios (D/H) for both stock and scion bark (table 2). Arginine ratios in stock bark (diseased tree to healthy tree) were, with few exceptions, greater than those in scion bark (diseased tree to healthy tree) collected at the same time in the same grove. The exceptions were in Grove C in September, January, and July (table 2). Thus the magnitude of the arginine increase in the stock bark from diseased trees was greater than that in the scion bark from the same trees.

Proline. Proline was most abundant of all the amino acids, and ranged from 364 to 1,764 μg/gm, accounting for 31.2 to 65.3 per cent of the total amino acids found in bark samples.

Although proline content in both stock and scion bark was determined, only data for scion bark are reported here (table 3) since girdling of young citrus trees results in an accumulation of proline above the girdle (*9*).

Changes in proline content in scion bark from diseased trees were most evident when percentage of proline in scion bark from diseased trees was compared with corresponding data from healthy trees. Ratios which indicated increases in proline were found only for samples from Grove A in April, Groves B and C in September and July, and Grove D in January (table 3).

These increases in proline at times noted above did not show any correlation with tree condition. Absence of persistently greater amounts of proline in diseased trees, however, indicates that girdling is not a continuing condition in YTD-affected trees.

A healthy tree, 12 years old, completely girdled mechanically at the bud union, showed a sustained increase in proline in scion bark beginning 30 days after girdling (table 3).

TABLE 3

PROLINE AS A RATIO OF THE PERCENTAGE FOUND IN SCION BARK FROM DISEASED CITRUS TREES TO THAT FOUND IN SCION BARK FROM HEALTHY TREES, AND AS A SIMILAR RATIO FOR SCION BARK FROM A MECHANICALLY GIRDLED AND A NONGIRDLED, HEALTHY TREE

Grove	Cultivar	Tree age (yrs)	Dates of collection			
			Sept.	Jan.	April	July
A	Valencia	48	0.65	0.94	1.23	0.87
B	Valencia	25	1.14	0.95	0.98	1.12
C	Valencia	10	1.12	1.03	1.04	1.08
D	Grapefruit	25	0.95	1.08	0.93	0.92

Days after girdling	Effects of mechanical girdling					
12-year-old healthy Valencia	0	30	60	90	150	190
Girdled/nongirdled	1.05	1.36	1.35	1.09	1.38	1.25

DISCUSSION

Increases in amounts of total amino acids and disproportionately greater increases of both arginine and proline appear to be characteristic of citrus trees under stress (*5, 6, 7, 8, 9, 10*). Increase in total amino acid content, especially in stock bark from YTD-affected trees, is similar to increases in amino acid content reported for many other species of virus-infected plants (*2*). In YTD-affected trees, greater increase of amino acid content in stock bark indicates that the rootstock is the site of stress or at least is under greater stress than the scion.

The extent of the increases of arginine and proline may also be indicative of the nature of the stress as well as its site. Although arginine increased in leaves of trees affected with spreading decline disease, it increased more in feeder roots, the primary area affected by the burrowing nematode (*5, 6, 7*). In contrast, arginine as well as proline increased far more in leaves than in roots as a consequence of girdling very young trees (*9*). Arginine also increased in chlorotic citrus leaves deficient in zinc and iron (*10*). There was a "striking accumulation of arginine and proline" in leaves of Etrog citron inoculated with either moderate or severe strains of citrus exocortis virus (*8*). In the last instance, this could be attributed to disruption of phloem tissue in young bark and in midribs of leaves.

In the above-cited responses of citrus tissue to four unrelated causes of stress, each area of stress was also the area in which the greatest accumulation of total amino acids and arginine or proline, or all three, occurred. Therefore, the accumulation of both arginine and total amino acids in greater amounts in stock bark than in scion bark of trees affected with YTD indicates that the rootstock of YTD-affected trees is the primary site of stress. Further support of this evaluation of YTD as a rootstock disease is found in verified reports that affected trees are confined to Rough lemon stock. Recovery of YTD-affected trees as a consequence of scion rooting (nine months' duration) tends to eliminate YTD as a scion disease (*4*).

In Grove C (10-year-old Valencia), unlike the three older groves, ratios obtained for arginine were greater in stock than in scion bark only in the April collection of samples. However, total amino acids and their ratios, as well as proline data for Grove C, were comparable with the three older groves. Difference in extent of accumulation of arginine in stock bark from Grove C as compared with stock bark from the other three groves may possibly be attributed to differences in ages of the trees (10 years vs 25 to 48 years), even though the extent and duration of stress in these trees are unknown.

Mild gumming of inner bark near the bud union and subsequent wilting in later stages of the disease syndrome indicate that some type of restricted translocation occurred. Fluctuation of amounts of proline from season to season in scion bark from YTD-affected trees suggests either a transitory type of girdling or periodic restrictions of translocation. Nonpermanent wilting of leaves on either a single branch or a few isolated branches in later stages of the disease is also indicative of restricted translocation. Temporary recovery of some trees in early stages of the disease has been observed near the middle of the growing season (May through September). This occurs probably because of rapid development of both new xylem and phloem in trees which are still fairly vigorous. However, recurring restrictions eventually reduce vigor of growth to a level from which YTD-affected trees do not recover. Rapid decline is often evident during dormancy in trees bearing a moderate crop.

It is very doubtful that these changes in amino acids are responsible, either directly or indirectly, for any of the field symptoms characteristic of YTD. The changes are more likely a consequence of other, more serious modifications of normal biochemical processes. Extent and location of the changes in the amounts of arginine, proline, and total amino acids indicate Rough lemon rootstock to be the primary stressed area (although not necessarily the primary site of the etiological agent), and provide some indication of the type of internal tissue disorder.

CONCLUSIONS

Young-tree decline (YTD) is a new and increasingly serious disease threatening citrus trees growing on Rough lemon rootstocks in Florida. The etiology of YTD has not yet been determined, but the rate and pattern of spread of the malady are typical of insect-vectored virus diseases. Loss of production in affected groves reached 50 per cent or more within six years of onset of YTD, and at present more than 50,000 acres of citrus are affected.

Disproportionate increases in both arginine and total amino acids of stock bark indicate the stock or root system to be the primary stressed area in YTD-affected trees although the site of the etiological agent is undetermined. Location and nature of amino acid changes, particularly those of arginine, proline, and total amino acids, along with internal gumming of stock bark near the bud union, characterize a developing condition best described as a transitory restriction of translocation which ultimately leads to a complete and permanent loss of vigor.

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