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Carbohydrate Status of Mycorrhizal and Nonmycorrhizal Citrus Rootstocks[1]

S. Nemec[2]
Agricultural Research Service, U.S. Department of Agriculture, Horticultural Research Laboratory, Orlando, FL 32803
G. Guy[2]
Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108

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Abstract. **Carbohydrates were analyzed in mycorrhizal and nonmycorrhizal citrus rootstock seedlings in greenhouse studies. Inoculated seedlings grew taller or weighed more and their leaves contained greater amounts of total soluble sugar, sucrose, reducing sugars, starch, and total nonstructural carbohydrate per gram of tissue than noninoculated controls, in a low P soil (9–12 ppm). Only the reducing sugars, fructose and glucose, increased slightly in roots of inoculated seedlings over those levels found in uninoculated control seedlings. Levels of reducing sugars were higher in leaves than in roots. Uninoculated seedlings grown in high P soil (210 ppm) were about the same height and their leaves contained levels of total soluble and reducing sugars similar to those in inoculated rootstocks grown in low P soil. It does not appear that these mycorrhizal fungi mobilize sugars as a sink for photosynthate in roots.**

Studies have shown that, both in the ectomycorrhizal and vesicular-arbuscular mycorrhizal fungal (VAM) symbiosis, carbon derived from host-photosynthate is transferred from the host to the fungus (2, 4, 9). Assimilate was incorporated in both the chlamydospores (9) and hyphae (2, 4) of the VAM fungi studied. However, no sugar distinctive for VAM fungi has been found (2, 7), and this distinguishes it from the carbon transfer mechanisms of ectomycorrhizas in which carbohydrate of the host is converted to specific fungal carbohydrates, trehalose and mannitol, and ultimately the storage polysaccaride glycogen (15). Neither trehalose nor mannitol has been detected in VAM (8). Bevege (2) reported sucrose was the major component followed by glucose in VAM fungi and uninfected hoop pine (*Araucaria cunninghamii* Act.) roots but that extracts of infected and noninfected hoop pine and clover (*Trifolium subterraneum* L. var Bacchus March) roots contained similar levels of the same sugars. Hayman (7) and Hepper and Mosse (8) also indicated that soluble carbohydrates did not differ in roots of VAM fungus-infected and noninfected plants.

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[2]The authors thank Kathleen Munroe for her assistance in analyzing sucrose, glucose, and fructose.

However, other researchers (1, 14, 20) reported soluble sugars and starch increase in roots of plants infected by VAM fungi over levels found in noninfected plants.

This study was designed to examine the carbohydrate status in roots and leaves of endomycorrhizal citrus, and discuss the implications of carbohydrate metabolism in endomycorrhizal citrus.

Materials and Methods

Chlamydospores of *Glomus etunicatus* Becker & Gerd., *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and *G. macrocarpus* Tul. & Tul. were used as sources of inoculum in this study. Spores were produced on either citrus rootstock seedlings or sudangrass (*Sorghum bicolor* var. *sudanense* [Piper] Stapf.) grown in steamed-pasteurized Astatula fine sand subsoil low in phosphorus (<9–12 ppm P). Steaming was done at 83°C for 1-1/2 hr in a Lindig Aerator. Soil containing each *Glomus* species was used as inoculum at spore populations ranging from 320 to 704 per 15-cm diameter clay pot, depending on the test. Populations that large were used to obtain consistent establishment of infected plants. Single citrus rootstock seedlings were grown from seed or from transplants in each pot in all experiments reported in Tables 1, 2 and 3. Four seedlings were grown per pot in the experiments reported in Table 4. All experiments were maintained in a greenhouse for 6 to 7 months.

Because VAM fungi effect the uptake of P in citrus (5, 17), and ultimately the growth of plants, fertilizer programs regulating soil P were varied as follows. The sweet orange seedlings (*Citrus sinensis* (L.) Osb.) reported in Table 2 were fertilized monthly with a liquid 12N-6P-6K, and the rough lemon (*C. limon*. (L.) Burm. F.) and sour orange (*C. aurantium* L.) were fertilized with a liquid 12-0-6. This test contained 7 single-plant replicates per treatment. Soil in half the experiment reported in Table 3 was amended with 210 ppm P, the other half was left unamended, all plants were fertilized with 12-0-6. Ten single-plant replicates per treatment were used in this test. The same fertilizer, a 12-0-6 solution containing nitrogen as urea, was used monthly in the remaining tests. Three single-plant pot replicates were used in the experiment reported in Table 1, and 4- and 7-pot replicates were used in the tests reported in Table 4. Minor elements were supplied to plants in all experiments in a supplemental solution developed by Hoagland and Arnon (10).

Leaves, roots or both were collected from each plant for carbohydrate analysis at the termination of each test. Roots were washed to remove soil and organic matter, and all roots and leaves sampled was dried at 90°C for 90 min and then at 70° for 22 hr. Dried tissue was weighed then ground to a fine powder in a Wiley mill. Leaves and roots were analyzed for total soluble sugars by the Anthrone method (11) and for reducing sugars by the Somogyi-Nelson method (11). In addition, starch was determined by hydrolyzing it to glucose with amyloglucosidase (12). Only reducing sugars were determined in roots of plants reported in Table 4. Fructose, glucose, and sucrose were analyzed in leaves of plants reported in Table 2 by extraction of tissue in Soxhlet tubes followed by filtration and injection of the sugars in a Waters Associates 201/401 Liquid Chromatograph. The solvent used was acetonitrile/water (80/20 v/v) with a flow rate of 1 ml/min in a Waters Associates micro carbohydrate column.

Results

Both Cleopatra mandarin (*C. reticulata* Blanco) and sweet orange inoculated with *G. macrocarpus* were significantly taller than the noninoculated controls (Table 1). Chlamydospores of *G. etunicatus* were also found along with those of *G. macrocarpus* in soils of inoculated pots indicating that the original inoculum used did not contain a single species. Total soluble sugars, sucrose, reducing sugar, starch, and total nonstructural carbohydrate were all significantly greater in leaves of inoculated Cleopatra mandarin than in the control plants. Similar increases in sugars were present in leaves of inoculated sweet orange, but not in roots of either rootstock. There appeared, however, to be less starch and a slight increase in reducing sugars in roots of inoculated plants.

Fresh top weight of rootstocks inoculated with *Glomus* species, whether fertilized with 12N-6P-6K or 12-0-6, was greater than their respective controls (Table 2). In general, higher levels of fructose, glucose, and sucrose were present in leaves of inoculated rootstocks grown in a low P sand and fertilized with a fertilizer deficient in P, than in leaves of plants grown in the same sand fertilized with a complete fertilizer. Sucrose levels were higher than those of the reducing sugars in leaves of plants treated with both fertilizers but no consistent differences between levels of glucose and fructose were noted.

Both Cleopatra mandarin and rough lemon, inoculated with *G. etunicatus* and grown in low P soil, were taller than their respective controls. Only inoculated rough lemon seedlings grown in soil containing 210 ppm P were taller than their controls (Table 3). Levels of total sugars and reducing sugars in leaves were higher in inoculated Cleopatra mandarin and rough lemon than in the controls in low P soil, but these differences did not occur in plants grown in soil containing 210 ppm P. Total sugars in roots did not differ between inoculated and control plants, regardless of

Table 1. Carbohydrates in leaves and roots of 2 citrus rootstocks inoculated with *Glomus macrocarpus*.

Citrus rootstocks and mycorrhizal treatment	Plant height (cm)	Carbohydrates (mg/g)									
		Total soluble sugars		Sucrose		Reducing sugar		Starch		Total nonstructural carbohydrate[z]	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Cleopatra mandarin *G. macrocarpus*	51.8**	54.9**	39.7^{NS}	37.9**	31.8^{NS}	17.0**	7.9^{NS}	32.6*	50.3^{NS}	87.4**	90.0^{NS}
Cleopatra mandarin Control	20.3	31.3	34.7	24.3	29.4	6.9	5.3	5.6	55.7	36.9	90.5
Sweet orange *G. macrocarpus*	43.4**	66.0*	64.5^{NS}	47.3^{NS}	49.6^{NS}	18.7*	16.8^{NS}	54.6^{NS}	39.2*	120.6^{NS}	103.7^{NS}
Sweet orange Control	10.8	33.7	70.1	28.9	63.4	4.8	6.7	11.3	56.0	45.0	126.1

^{NS}, *,**Nonsignificant (NS) or significant at 5% (*) or 1% (**) level from the control. Three-pot replicates, 1 seedling per pot used in test.
[z]Total nonstructural carbohydrate = total soluble sugars + starch.

Table 2. Sugar content in leaves of mycorrhizal and non-mycorrhizal citrus rootstocks fertilized with a complete fertilizer and one deficient in phosphorus.

Fertilizer mycorrhizal and rootstock treatments	Top weight (g)	Sugars (mg/g) Fructose	Glucose	Sucrose
$12^{N}6^{P}6^{K}$				
Sweet orange *Glomus mosseae*	5.8**	2.77**	<0.01^{NS}	12.52^{NS}
Sweet orange *G. macrocarpus*	4.9**	1.16^{NS}	2.27^{NS}	18.39^{NS}
Sweet orange Control	1.6	<0.01	1.39	16.63
$12^{N}0^{P}6^{K}$				
Rough lemon *G. macrocarpus*	3.57**	4.32**	4.77**	20.44**
Rough lemon Control	1.49	0.43	<0.01	14.37
Sour orange *G macrocarpus*	3.05*	1.63^{NS}	3.57**	29.65^{NS}
Sour orange Control	0.93	<0.01	<0.01	22.81

^{NS},*,** Nonsignificant (NS) or significant at 5% (*) or 1% (**) level from the respective control. Seven-pot replicates, 1 seedling per pot used.

soil P content. Levels of reducing sugars were significantly higher in roots of inoculated plants in the high soil P treatment.

Reducing sugars were significantly higher in inoculated plant roots than in the controls in 1 of 2 tests in which sour orange was inoculated with *G. mosseae* (Table 4).

Discussion

Citrus seedlings in soil deficient in P or devoid of mycorrhizas are typically stunted, the leaves are smaller than normal and are dull green in color (17). Results of this study show these symptoms are corrected by inoculation with *Glomus* species or by mixing P into soil. By mediating P uptake, *Glomus*-inoculated citrus mimics the normal growth of plants present in fertile soils. Total sugars, sucrose, reducing sugar, starch and total nonstructural carbohydrates all increased in leaves of plants inoculated with *Glomus* species compared to uninoculated controls, and

Table 3. Total and reducing sugars in 2 citrus rootstocks, Cleopatra mandarin (Cleo) and rough lemon (RL) inoculated with *Glomus etunicatus* and grown in soil low (9-12 ppm) and high in phosphorus (210 ppm).

Treatments	Height (cm) Cleo	RL	Sugars (mg/g) Leaves Cleo	RL	Roots RL
Total sugars					
Low P, *G. etunicatus*	68.5**	47.1*	75.2**	82.6*	15.6^{NS}
Low P, Control	28.9	35.0	53.8	74.8	13.9
High P, *G. etunicatus*	84.3^{NS}	46.5**	64.0^{NS}	65.9^{NS}	15.6^{NS}
High P, Control	68.2	40.1	67.2	74.6	15.3
Reducing sugars					
Low P, *G. etunicatus*			16.8**	28.1^{NS}	2.1^{NS}
Low P, Control			2.7	26.4	1.6
High P, *G. etunicatus*			10.9^{NS}	30.4^{NS}	2.5*
High P, Control			12.7	27.9	1.8

^{NS},*,** Nonsignificant (NS) or significant at 5% (*) or 1% (**) from respective controls. Ten-pot replicates, 1 seedling per pot used in test.

Table 4. Reducing sugars in roots of sour orange citrus rootstock inoculated with *Glomus mosseae* and grown in soil low in phosphorus.

Treatments	Height (cm)	Reducing sugars (mg/g)
Test A		
G. mosseae	52.8**	5.0**
Control	26.2	0.7
Test B		
G. mosseage	54.4**	5.4^{NS}
Control	29.1	3.6

^{NS}, ** Nonsignificant (NS) or significantly different from respective control at 1% level. Three-pot replicates for test A and 7-pot replicates for test B, 4 plants per pot each test.

these sugar levels were similar to those in plants grown in soil containing adequate P.

The carbohydrate balance in roots of *Glomus*-inoculated citrus differs little from the content in control roots. In inoculated plant roots there was a trend towards an increase in reducing sugars and a decrease in starch content. Safir (19) also found an increase in reducing sugars in onion roots infected with *Endogone mosseae* Nicol. & Gerd. It is possible reducing sugars also accounted for the increase in total soluble sugars measured in infected roots in 3 other studies (1, 14, 20). The identify of the reducing sugars in infected roots is yet to be determined. If these reducing sugars were composed of principally glucose, their increase may be due, in part, to the hydrolysis of starch which is depleted in cells surrounding and occupied by the fungus (5, 13, 18). Whether the fungus obtains its carbohydrate though starch hydrolysis or some other mechanism is not clear.

Carbon transfer mechanisms of vesicular-arbuscular mycorrhizal fungi are distinctly different from those of ectomycorrhizas. Host photosynthate is assimilated by VAM fungi, but the only apparent change in the carbohydrate status of the infected root is in the reducing fraction. Host photosynthate is apparently synthesized rapidly into lipid, which is present in large quantities in VAM fungus structures (4, 18). Significantly more lipid has been extracted from VAM fungus-infected roots than from noninfected roots (3, 16). Synthesis of lipid by the fungal endophyte is suggested as an alternate storage sink for the plants' photosynthates (4, 6), and probably serves as a form of stored energy for the fungi (18).

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Maturity Index for the Color Grade of Canned Dark Sweet Cherries[1]

S. R. Drake, E. L. Proebsting, Jr., and S. E. Spayd[2]
Irrigated Agriculture Research and Extension Center, Prosser, WA 99350

Additional index words. Prunus avium, reflectance color, anthocyanins, soluble solids, titratable acidity

Abstract. **Highly significant correlations were obtained for reflectance color (Agtron) with anthocyanin content in both fresh and canned dark sweet cherries (*Prunus avium* L.) and also for both reflectance color and anthocyanin content with subjective USDA color. Soluble solids, titratable acidity, pH, and a soluble solids/titratable acidity ratio were not good indicators of color development in sweet cherries. Reflectance color could be used to predict USDA color from fresh or canned dark sweet cherries.**

To a large degree the USDA grade and consumer acceptance of canned dark red sweet cherries depends on the color of the fruit and syrup, and subjective evaluation of skin color of the fresh fruit is only as good as the observer. A more accurate measurement of skin color would be useful in determining the maturity that is needed to produce a highly colored canned product.

Proper maturity for the fresh market has been the goal of most maturity indices. Soluble solids, acidity, soluble solids/acidity ratios, skin color, and firmness have all been used as indices of maturity (1, 2, 5). Very few studies have been concerned with the proper maturity for canned fruit. Chemical analysis of anthocyanins (6), or raw fruit absorbance at 520 nm, has been proposed as a maturity index for canned fruit. This chemical procedure is quick, simple, and requires only limited analysis, but it was developed before widespread use of reflectance color instrumentation. The use of a reflectance color instrument has been proposed to measure color development in cranberries, strawberries, and grapes (4, 7, 9). Reflectance color of cranberries was not correlated with pigment content and could not be used to predict the color of a processed product, but could be used to measure the degree of coloring at harvest.

Reflectance instruments for color determination are now used by most processing plants for quality control. The use of reflectance color as a reliable maturity index for dark sweet cherries would be most useful. This study was conducted to determine the feasibility of using reflectance color as a maturity index for the harvesting of dark sweet cherries for canning, and as an objective measurement of color for grading canned cherries.

Materials and Methods

Fruit from 'Bing', 'Chinook', 'Lambert', and 'Van' cherry trees growing at or near the Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA, were used. During the 1977 to 1980 growing seasons fruit was harvested at 7-day intervals starting when the fruit had reached maturity for fresh shipment. Plots consisted of 8 trees each of 'Bing' and 'Chinook', and 3 trees each of 'Lambert' and 'Van', for each growing season. Due to the known effects (3) of butanedioic acid mono-(2,2-dimethylhydrazide) (daminozide) on

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[2]Research Food Technologist, USDA/ARS: Horticulturist, Dept. of Horticulture; Assistant Food Technologist, Dept. of Food Science and Technology, respectively.

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