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rootstocks must be made under uniform growth and measurement

istics and hydraulic conductivity of intact root systems of 4 citrus

rootstocks commonly used in Florida. These data could then be used to determine whether potential differences in water transport

capability could be used to interpret observed differences in the

Materials and Methods

house-grown seedlings of RL, sour orange (SO), Carrizo citrange

(Car), and Cleopatra mandarin (Cleo). All seedlings were raised

in 4 x 21-cm plastic tubes filled with a commercial blend of peat,

perlite, and vermiculite (3:1:1, v/v) with added nutrients (5). The

seedlings received maximum irradiances of 700 μ E m⁻²s⁻¹ photo-

synthetically active radiation (400 to 700 nm) during natural pho-

toperiods during the 12-month duration of the study. Temperature

and relative humidity varied diurnally from 22 to 32°C and 40 to 100% respectively. The seedlings were kept well-watered and

After 6 months, a typical seedling had a stem diameter of 3 to 4

mm at the base and the root systemn had filled the small tubes.

Root conductivity was measured on 3 replicates of each root-

stock. In addition, 4 plants of each rootstock were transplanted in-

to 12 x 20-cm plastic pots containing the same growth medium.

After 6 additional months, these plants had a basal stem diameter

The plants used in this study were 6- and 12-month-old green-

The purpose of this study was to compare the growth character-

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Hydraulic Conductivity of Four Commercial Citrus Rootstocks^{1,2}

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Additional index words. Rough lemon, sour orange, Carrizo citrange, Cleopatra mandarin, temperature effects

Abstract. The hydraulic conductivities of intact root systems of 4 commercial citrus rootstocks were estimated using a pressure chamber technique. The rootstocks used were rough lemon (*Citrus jambhiri* Lush.), sour orange (*C. aurantium* L.), Carrizo citrange [*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osb.], and Cleopatra mandarin (*C. reshni* Hort. ex TAN). Carrizo and rough lemon seedlings had the highest root conductivity, whereas Cleopatra and sour orange had the lowest root conductivity. Although these rootstocks as seedlings produce root systems in pots that differ from those in the field, some of the growth, yield, and drought resistance chartacteristics that have been previously assoicated with these rootstocks may be at least partially explained by the hydraulic conductivity of their roots.

conditions.

water relations of citrus rootstocks.

fertilized as needed.

Citrus rootstocks can influence tree size (16, 18, 20), cold hardiness (21), relative wilting (10), leaf water potential (1, 6), transpiration rate (2, 16), fruit yield (11, 13), and juice quality (1, 9). Variations in citrus tree water relations that have been attributed to rootstocks are probably due to differences in root quantity, distrubtion (3) and/or apparent efficiencies in water uptake and transport (4).

Rough lemon (RL), a once-popular Florida citrus rootstock, is thought to be relatively drought-tolerant because of its extensive root system (3, 20). In addition, RL has higher stem conductivity (19) and transpiration rates (15), larger xylem vessels (14), and trees are larger on this rootstock than most other citrus rootstocks (3). Although Ramos and Kaufmann (17) described the effect of temperature on the hydraulic conductivity of RL roots, the hydraulic conductivity of RL roots has not been compared to that of other citrus rootstocks. Such comparisons should include the size and distribution of root systems as well as their water transport efficiency. Since many factors can influence root system development and hydraulic conductivity, any comparisons of different

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of 5 to 6 mm and were used to evaluate the effect of temperature on the hydraulic conductivity of intact root systems.

Methods of measuring the hydraulic conductivity were modified from Ramos and Kaufmann (17). Before each set of conductivity measurements, pots were saturated with water while shoots were maintained in ambient laboratory conditions of 25°C and 20 μ Em⁻²s⁻¹ for 30 to 60 min. This was done to close stomates and to minimize water tension in the xylem. The potting medium was then gently washed away from the root system and while under water, the stem was severed at its base. The intact root system was then submerged in a 19-liter pressure chamber containing 1.0 mM CaCl₂ (8) in deionized water. The chamber was large enough to allow the simultaneous measurement of 2 root systems. Each was sealed within the chamber with the cut stump protruding. The pressure was increased with compressed air at a rate of 0.05 MPa (0.5 bar)/min up to a final constant pressure of 0.3 MPa(3 bars). A controlled pressure relief valve allowed constant aeration of the root systems during measurements.

The volume of exudate was measured using a calibrated piece of plastic tubing attached to the stump. Preliminary experiments showed that exudation rates were constant over 8-hr periods and that the exudate volume increased linearly with an increase in pressure up to 1.0 MPa (10 bars). The temperature of the pressure chamber was regulated by circulating water from a controlledtemperature water bath through stainless steel tubing coiled inside the chamber. The measurement temperature of the 6-month-old seedlings was $23 \pm 0.5^{\circ}$ C; measurement temperatures for the 12month-old seedling were varied from 15 to 30°. These temperatures were selected as being typical of soil temperatures at rooting depths in Central Florida (7). All exudation rates were calculated from at least 4 exudate volumes taken at 5 to 10 min intervals after the exudation rate had equilibrated at a given temperature. Exudation volume vs. temperature for each root system yielded linear relationships with $R^2 = .83$ to .99.

After root conductivity measurements were completed, the root systems from all plants were divided into roots smaller than 1.5 mm (feeder roots) in diameter and all roots larger than 1.5 mm in diameter. The projected area of the smaller roots was measured using a Li-cor leaf area meter (12). Note that the root area measured is the projected longitudinal area, not the actual root surface area. Care was taken to minimize any root dehydration during measurement. All roots were then dried at 60°C for 24hr. The dry weight of the total root system (small and large roots) and that of the above ground shoot were used to calculate shoot:root ratios.

Total leaf area was also used as a measure of growth for analysis. The relationship between projected root area and root dry weight as well as the effect of temperature on root conductivity of the 12month-old seedlings were characterized using regression analyses. Root conductivities and growth data were compared using analysis of variance and Duncan's multiple range test.

Root conductivity data were expressed on a projected root area basis: cm^3 exudate cm^{-2} root area $Pa^{-1} sec^{-1} = cm Pa^{-1}s^{-1}$. The total length of the small roots of 3 seedlings was measured in mm to establish a relationship of cm of root length/mg dw to be used to express the conductivity data on a root length basis (calculated from feeder root dwt) for comparison to other similar published data (17).

Results and Discussion

Six-month-old RL seedlings had the largest leaf area while Car had the smallest; there was no difference among the root dry weights (Table 1). Rough lemon seedlings had higher shoot:root ratios than the other rootstocks. This may have been due to the small pots limiting root growth of the 6-month-old RL seedlings. Shoot:root ratios were similar among the other rootstocks and remained so in the 12-month-old plants. Sour orange seedlings had the greatest leaf area and root weight of the 12-month-old plants. Carrizo citrange seedlings remained the smallest, though their root mass was similar to that of RL and Cleo seedlings (Table 1).

The relationship between the projected root area and dry weight of roots less than 1.5 mm in diam from all 4 rootstocks of both ages was linear ($R^2 = 0.90$, Fig. 1). Although the root dry weight can be segregated into groups by rootstock variety, the good correlation between root area and dry weight indicates that the feeder roots probably have similar densities. In addition, this relationship indicates that root conductivity data can be compared on a feeder root dry weight or projected area basis without changing the results of such comparisons.

The hydraulic conductivity of roots of 6-month-old RL seedlings was significantly greater than that of SO and Cleo seedlings (Table 1). Condutivities of Car were intermediate and not significantly different from either RL or SO and Cleo. Carrizo had the highest conductivity of the 12-month-old seedlings, however (Fig. 2). Rough lemon seedlings had conductivities that were significantly less than those of Car, but greater than conductivities of SO and Cleo. Sour orange and Cleo had very similar root conductivities. It should be noted, however, that these data represent a

Table 1. Mean total leaf area, root dry weight, and shoot:root ratio of 4 citrus rootstocks of 2 different ages along with the hydraulic root conductivity of the 6-month-old seedlings.

Root- stock	Leaf area (cm ²)	Root dry wt (g)	Shoot-root ratio	Root conductivity (cm Pa ⁻¹ s ⁻¹ × 10 ⁻²)
Rough Lemon	246a ²	0.54a	3.2 ± 0.1^{y}	33.7a
Sourorange	196b	0.67a	2.2 ± 0.3	18.2b
Carrigo citrange	104d	0.67a	1.8 ± 0.3	23.5ab
Cleopatra mandarin	149c	0.58a	2.5 ± 0.5	17.9b
•		12-months-old		
Rough Lemon	553b	2.5b	2.1 ± 0.6	
Sour Orange	679a	3.6a	1.8 ± 0.3	
Carrigo citrange	216d	1.9b	1.7 ± 0.2	
Cleopatra mandarin	426c	1.7b	2.4 ± 0.6	

'Mean separation within columns by Duncan's multiple range test, P < 5%. ^y ± sD.



Fig. 1. The relationship between root dry weight and projected longitudinal root area of roots less than 1.5 mm in diameter from 6-month-old (inset) and 12month-old seedlings of 4 citrus rootstocks. Root area = $99.97 \times \text{root} \text{ dry}$ weight + 4.23, R² = 0.90. (RL = rough lemon; SO = sour orange; Car = Carrigo citrange; Cleo = Cleopatra mandarin.)

potential hydraulic conductivity of roots which were measured under standard conditions and are expressed on a per root basis. Even though Car tended to have the highest conductivity rates, it had a relatively small total root dry weight and low shoot:root ratio. Sour orange had the largest root system but the lowest conductivity. Apparently, hydraulic conductivity of roots does not, by itself, promote or inhibit shoot growth. Rough lemon had a relatively large root system and a high conductivity, whereas Cleo had a small root system and a low root conductivity. The low conductivity of SO and Cleo, along with the high conductivity of RL, reflect previous water relations studies in the field that compared these rootstocks (4, 6). Taken together, these data focus on the importance of root quantity and distribution as well as hydraulic conductivity in determining how these characteristics influence plant water relations.

The hydraulic conductivity of roots of all 4 rootstocks increased linearly with temperatures between 15 and 30°C (Fig. 2). The slope of root conductivity vs. temperature associated with the Cleo data is significantly less (P < 5%) than that of the other rootstocks. Visual inspection reveals that this difference probably does not represent a physiologically significant difference within the range of the data.

The total length of roots < 1.5 mm in diameter of a 12-monthold RL seedling was 2,348 cm. The mean length/dry weight ratio of this RL seedling along with that of a SO and a Car seedling was 2.37 ± 0.34 cm/mg. Using this ratio to express the data on a root length basis, the mean root conductivity of the 7 RL seedlings at 22° C was $1.58 \times 10^{6} + \text{cm}^{2}$ bar $^{-1}\text{s}^{-1}$ which equals a root resistance of 6.3×10^{6} bar s cm $^{-2}$. This value is approximately 10 times lower (higher conductivity) than the hydraulic resistance of RL roots reported by Ramos and Kaufmann (17). It is possible that differences in hydraulic conductivity of roots could result from differences in root extension or suberization as a result of differences in soil, water stress, or other environmental conditions.

Data obtained in this study on greenhouse seedlings grown in artificial potting medium support earlier field work which described trees budded on RL which were better adapted to sandy soils (5, 20). The relatively high root growth rate and hydraulic



Fig. 2. The effect of temperature on the hydraulic conductivity of intact 12month-old seedling root systems. Each point (\pm 1sD) is the mean of 4 replicate plants of each rootstock. Linear regression lines have been fitted to data from each rootstock ($R^2 = .42$ to .83). (RL = rough lemon; SO = sour orange; Car = carizzo citrange; Cleo = Cleopatra mandarin.)

conductivity of RL can be used to explain its greater efficiency of soil water extraction (5) and its ability to produce larger trees than many other citrus rootstocks (13, 18, 20). It is recognized that factors such as canopy development and total leaf area can also affect water transport characteristics of budded trees. It appears that the relatively low fruit production of trees on Cleo, the low drought tolerance of trees on SO, and the high drought tolerance attributed to trees on RL (20) can be at least partially explained by the hydraulic conductivity of these rootstocks. One might expect Car also to be drought tolerant, but its less extensive root system may negate any advantages imparted by its high root conductivity. The technique of measuring growth characteristics and the hydraulic conductivity of intact root systems of rootstocks as seedlings seems generally to support data from the field. Consequently, this technique, along with root system distribution and other rootscion relationships, may be of value in determining differences among potential water transport characterisics of rootstocks as seedlings rather than waiting for results from field trials.

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In Vitro Cotyledonary Development and Anthocyanin Synthesis in Zygotic and Asexual Embryos of *Theobroma cacao*¹

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Additional index words. cacao, tissue culture

Abstract. Cotyledonary development of asexual embryos of cacao (*Theobroma cacao* L.) in liquid medium on a rotary drum apparatus was similar to that of zygotic embryos *in vivo*. Immature zygotic and asexual embryos synthesized anthocyanins, characteristic of the developing zygotic embryo *in vivo*, when cultured *in vitro* in liquid or on semisolid media containing high concentrations of sucrose.

Secondary and storage products from embryos of cacao provide the flavor components and fats used in the production of cocoa and chocolate. Measurements of polyphenols and other secondary metabolites from callus or cell cultures initiated from cacao cotyledons have indicated low levels of these compounds relative to intact cotyledons (11, 12). However, chocolate aroma has been reported to emanate from roasted callus cells (23).

Results from this laboratory indicate that asexual cacao embryos proliferated and grown *in vitro* have the capacity to undergo normal maturation. Thus, asexual embryos may provide a model system for examining the regulation of the synthesis of metabolic products which are formed in cotyledons and could conceivably be used for the synthesis of secondary and storage products of cacao.

The purple color of mature Forastero or Trinatario cacao embryos is due to accumulation of the anthocyanins $3-\beta$ -D-galactosidly cyanidin and $3-\alpha$ -L-arabinosidyl cyanidin in the cotyledons (9). Synthesis of anthocyanins normally begins after rapid enlargement of the cotyledons has ceased, occurring first in localized zones, resulting in cotyledons having both pink and white areas. Cotyledons become entirely pink as they accumulate storage material and finally become purple at maturity. Results presented here indicate cotyledon enlargement can occur *in vitro* under certain conditions, and synthesis of anthocyanins in the developing cotyledons is regulated by the level of sucrose in the medium.

Materials and Methods

Culture of cacao embryos. Zygotic embryos of Amelonado cacao [obtained from Mayaguez Institute of Tropical Agriculture (USDA), Mayaguez, Puerto Rico] were excised from fruit and separated into 4 maturation stages as defined previously (17). Some of the immature white embryos and all of the immature pink/white, pink, and mature purple embryos were stored at -20° C until analyzed. The remainder of the immature white embryos were used as explants for culture. Asexual embryos were obtained from embryogenic tissues (17) maintained on semisolid basal medium. Those asexual embryos used in experiments had turgid, pale, translucent cotyledons and closely resembled zygotic embryos of the immature white stage.

Basal medium consisted of Murashige and Skoog salts (14) plus the following, in mg/liter: thiamine \cdot HCl, 0.1; pyridoxine \cdot HCl, 0.5; nicotinic acid, 0.5; glycine, 2.0; *i*-inositol, 100; and casein hydrolysate, 1,000. Unless otherwise stated, sucrose was included at 30,000 mg/liter and, when included, α -naphthalene-acetic acid (NAA) at 1.5 mg/liter (8 μ M) and deproteinized coconut water (CW), at 100 mg/liter.

Except where noted, embryos were cultured individually either on 15 ml of medium solidified with 1% agar in plastic Petri plates $(15 \times 60 \text{ mm})$ or in culture tubes $(25 \times 150 \text{ mm})$ covered with polypropylene closures containing 5 ml of liquid medium on a

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