

# Temperature-dependent and Diurnal Root Conductivities in Two Citrus Rootstocks<sup>1</sup>

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Additional index words. *Citrus sinensis*, water relations, cold hardiness

**Abstract.** Root conductivity was measured in decapitated 18-month-old citrus rootstocks using a modified pressure chamber apparatus. Carrizo citrange [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* L. (Raf.)], a frost-hardy species, exhibited a log-linear decrease in conductivity over a temperature range from 40 to 10°C. Rough lemon (*C. jambhiri* Lush.), a frost-susceptible species, also exhibited decreasing conductivity from 40 to 20°, but conductivity was similar at 10 and 20°. No endogenous diurnal cycling of root conductivity was observed in Carrizo citrange rootstocks.

Citrus is a cold-sensitive evergreen which under appropriate environmental conditions exhibits a limited degree of acclimation to low temperatures and resistance to freezing injury (20). One of the most important factors in this acclimation process, and consequent tolerance of freezing in citrus, is the use of a cold-hardy rootstock (4, 19). Scions on rough lemon, a widely used rootstock, are very susceptible to freeze damage (4, 5, 9, 16). Those on Carrizo citrange, a rootstock used in many new Florida plantings, are generally more cold tolerant (15, 16).

The difference in hardiness between scions on rough lemon and Carrizo citrange rootstocks has been attributed to differences in growth patterns and water relations in the 2 species (20). Tissue maturity, water content, and growth status are 3 factors associated with hardiness in citrus (20). Winter temperatures of 8.8 to 15.5°C will induce quiescence or non-apparent growth in scions on citrange rootstocks. Such growth cessation is essential to the low-temperature acclimation process (20) and is accompanied by decreased water absorption and lowering of water potential and water content in the scion (17, 18, 19). Scions on rough lemon, however, exhibit growth over a wider range of temperatures than other citrus types. Active growth occurs in lemons at temperatures as low as 4.4°, producing well-hydrated and thus freeze-susceptible growth flushes at times when freezing air temperatures are most likely to occur (20).

The main resistance to water flux through a plant, especially at low soil

temperatures, is in the root (7). Crocker et al. (2) reported that under field conditions, 'Orlando' tangelo on rough lemon had an average leaf water potential of -7.87 bars, while 'Orlando' on Carrizo averaged -11.41 bars. Differences observed in leaf water potential were attributed to rootstock effects since soil type, water supply, and evaporative demand were assumed uniform in the experiment. Water conductivity through roots is a temperature-dependent process (7, 11, 12, 13). Thus, control of plant water relations at the root would effect growth patterns and acclimation to cold. Inherent differences in temperature-dependent root conductivity could provide such control.

Both temperature-dependent and diurnal conductivity patterns in Carrizo citrange and rough lemon were examined in this study. Temperature-dependent conductivity was examined to see if there is an inherent difference in temperature response in root conductivity which could explain the difference in water relations and hardiness of a common scion on these 2 rootstocks. Diurnal conductivity patterns were examined in Carrizo roots to determine whether endogenous rhythms have an effect on conductivity.

Measurements of diurnal and temperature-dependent conductivity were made on root systems of 18-month-old Carrizo citrange and rough lemon seedlings which were greenhouse-grown in a 1 sand:1 peat:1 pine bark (v/v) soil mixture. The greenhouse temperature fluctuated with the ambient air temperature from 10 to 30°C. Plants were routinely sprayed with insecticides, fertilized, and watered as necessary. Plants were removed from the greenhouse several hours before measurements were begun to standardize experimental conditions and tops were severed just above the soil line, after which roots were carefully rinsed in 25° water and placed in a modified pressure

chamber filled with water (11, 14). The apparatus was suspended in a temperature-controlled water bath, and conductivity through the severed stem was measured by collecting the exudate. A constant pressure of  $0.50 \pm 0.25$  MPa was maintained in the chamber. Four root medium temperatures, 10, 20, 30 and 40° were used. Measurements were performed on 9 trees per temperature for each rootstock. Exudate was collected for 30 min at each temperature following a 15 min equilibration period after each temperature change. Ramos and Kaufmann (11) observed uptake rates in rough lemon differed when measurements were made from 35 to 5° rather than from 5 to 35°. Consequently, roots were exposed to the 4 temperatures in random order. Addition of ions to the water did not alter conductivity. Conductivities were standardized on a root volume basis to eliminate variation due to different size root systems. Diurnal conductivity was measured in 3 Carrizo citrange seedlings at a constant temperature of 25° and pressure of  $0.50 \pm 0.25$  MPa over a 24 hr period. Root exudation was measured hourly.

Conductivity of Carrizo citrange roots decreased log-linearly as temperature decreased from 40 to 10°C (Fig. 1). Similar temperature-dependent decreases have been observed in soybean (8), cotton (13), and citrus (11). Such temperature responses have been associated with decreased membrane permeability and increased viscosity of water at low temperatures (6, 11). Conductivity in rough lemon also decreased in a log-linear fashion from 40 to 20°, however, there was no further decrease from 20 to 10° (Fig. 1, Table 1). Although rough lemon and Carrizo exhibit similar conductivity at 20 and 30°, there are significant differences between the 2 cultivars at 10 and 40°. Nearly all of the variability in conductivity associated with temperature can be accounted for in Carrizo over the entire temperature range ( $r^2 = 0.98$ ) (Fig. 1). The  $r^2$  for rough lemon over the entire range was 0.88; but increased to 0.98 with the exclusion of the conductivity value at 10°. Values deviating from linearity, such as those representing rough lemon conductivity at 10°, (starred data point) are considered anomalies.

Greater conductivity (Table 1) in rough lemon as compared to Carrizo at 10 and 40°C would allow greater water uptake at these temperatures. Growth continues over a broader temperature range in lemons than in citranges (20). Root efficiency and leaf water potentials have also been observed to be greater in rough lemon than in Carrizo under field conditions (1, 2). The observed differences in plant water relations and growth between these 2 cultivars may be a result of differences in temperature-dependent root conductivity. The greater root conductivity over a broader temperature

<sup>1</sup>Received for publication September 29, 1980. University of Florida Journal Series No. 2643.

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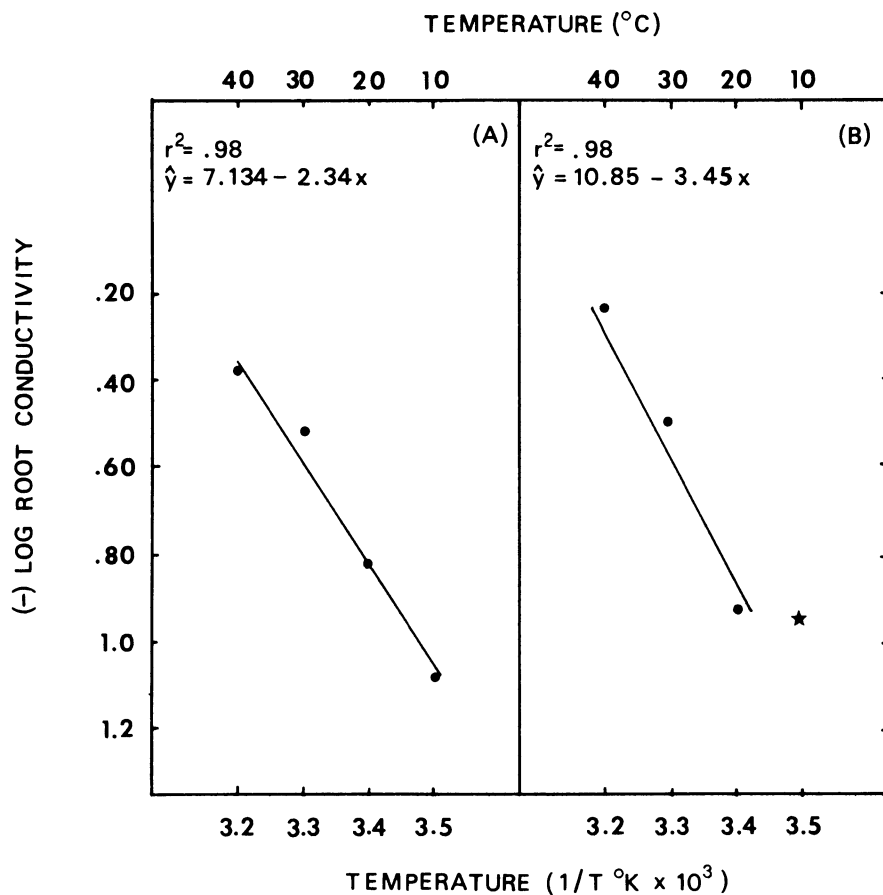


Fig. 1. Temperature dependent root conductivity (mls exudate/cc root volume-hr-MPa) in (A) 18-month-old Carrizo citrange seedlings (B) 18-month-old rough lemon seedlings. (Each point is the mean of 9 individual plant measurements).

range exhibited by rough lemon may result in maintenance of plant water status favorable for growth during most of the year. As a result of high root conductivity at low root temperatures, active freeze-susceptible growth may be induced in scions on rough lemon rootstocks during the winter months when quiescent mature tissue is most desirable. Thus, differences in growth patterns, water relations, and cold hardiness between rough lemon and Carrizo citrange may be a consequence of inherent differences in root conductivity characteristics.

Diurnal conductivity of Carrizo citrange roots varied little over a 24-hr period (Fig. 2). Some random, but not statistically significant ( $p < 5\%$ ), variation occurred in the late afternoon and pre-dawn hours; however, this variability probably resulted from occasional fluctuations in pressure within the chamber. It is significant in view of similar studies on conductivity in other species (8, 13) that conductivity in Carrizo citrange was identical at 1200 and 2400 hr, since these were the times of maximum and minimum conductivity, respectively, in cotton (10). Lower root pressures were utilized in conductivity experiments with herbaceous species (8, 10); however, 0.50

Table 1. Temperature-dependent root conductivity in rough lemon and Carrizo citrange. Values are means of 9 plants  $\pm$  SE.

Rootstock	Root conductivity (ml exudate/cc root vol-hr-MPa)			
	10°C	20°C	30°C	40°C
Rough lemon	0.11 $\pm$ .01	0.12 $\pm$ .02	0.32 $\pm$ .04	0.59 $\pm$ .06
Carrizo citrange	0.08 $\pm$ .01	0.15 $\pm$ .02	0.31 $\pm$ .04	0.41 $\pm$ .04

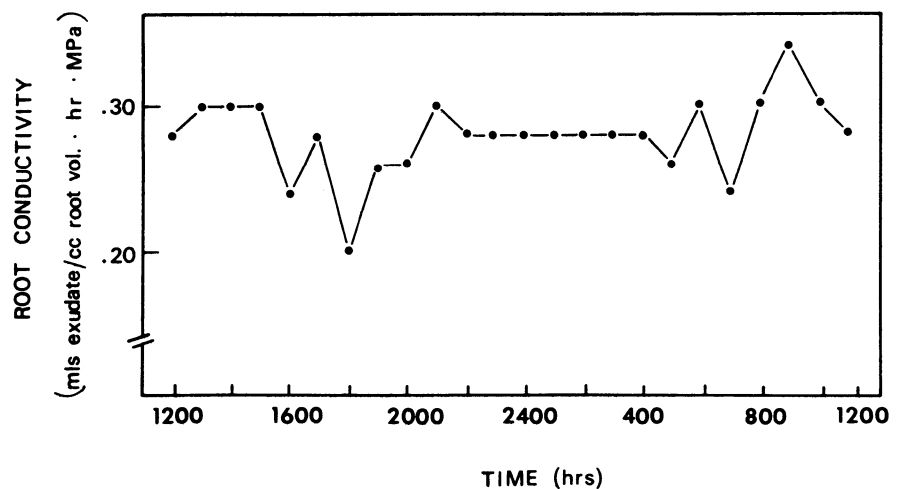


Fig. 2. Diurnal root conductivity in 18-month-old Carrizo citrange seedlings. (Data are from measurements made over a 24-hour period on a representative plant).

MPa pressure used in this study is well within normal water potential ranges found in citrus (2, 3). The absence of a cyclical pattern of conductivity in Carrizo citrange indicates diurnal fluctuations in stomatal resistance (2, 3) and water potential (3) are dependent upon environmental factors and not endogenous rhythms in root conductivity.

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HortScience 16(3):305-306. 1981.

## In Vitro Propagation of Carrizo Citrange<sup>1</sup>

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Additional index words. tissue culture, micropropagation, *Citrus* spp.

**Abstract.** In vitro proliferation of shoot tips was attempted with nucellar seedlings of sour orange (*Citrus aurantium* L.), Carrizo citrange ('Washington' navel × *Poncirus trifoliata* (L.) Raf.), and Cleopatra mandarin (*C. reshni* Hort. ex Tanaka). Shoot multiplication was achieved with Carrizo citrange shoot tips cultured on Knop's medium (with organics) supplemented with Murashige and Skoog microelements, 5 mg/liter 6-benzylamino purine (BA), 3 — 4% sucrose and at a light intensity of 2.2 klx. No effect on shoot proliferation was obtained with 6-furfurylamino purine (kinetin), 6 γ-γ-dimethylamino purine (2iP), agar concentration, addition of orange juice, or nitrogen source. Proliferated shoot tips of Carrizo citrange rooted when cultured on Murashige and Tucker's medium containing 1 mg/liter naphthaleneacetic acid and gelled with 0.5% agar. Plants were successfully established in soil.

Several fruit tree species, including apple (1, 6, 7), plum (3, 8, 11) and cherry (5, 8) have been clonally propagated by *in vitro* techniques. Although clonal propagation by nucellar seedlings is possible with some citrus rootstocks, micropropagation from non-nucellar vegetative tissue would be desirable as several years are required before sufficient seed can be produced from a newly introduced rootstock. Some rootstock clones, although desirable in other respects, may not produce nucellar seedlings, may be seedless, or produce few seed; and scion cultivars may fruit more precociously on rootstocks derived from vegetatively propagated shoots than vegetative seeds. The following research was undertaken to determine the feasibility of tissue culture-propagation of citrus by shoot tip culture.

**Media.** On the basis of a preliminary study, the basal medium for shoot proliferation consisted of Knop's macroelements (13) plus the microelements of the Murashige and Skoog (MS) high salt medium (9). The following organics were added to the basal medium, in mg/liter: pyridoxine-HCl, 0.5; thiamine-HCl, 0.4; nicotinic acid, 0.5; myo-inositol, 100; adenine sulfate, 80; sucrose, 3000; and agar, 1000. Rooting of proliferated shoots was evaluated using Murashige and Tucker medium (10) with the following addenda, in mg/liter: pyridoxine-HCl, 10; thiamine-HCl, 10; nicotinic acid, 5; myo-inositol, 100; adenine sulfate, 80; sucrose, 5000; and agar, 1000. The pH of the media was adjusted to 5.7 ± 0.1; 25 ml of medium were placed in 25 × 150 mm culture tubes capped with polypropylene closures and autoclaved for 15 minutes at 121°C.

**Rootstocks.** Seeds of sour orange, Carrizo citrange, and Cleopatra mandarin, currently the most commonly propagated rootstocks in Florida, were sown on January 23, 1979 in flats containing a mixture of 1 perlite:1 vermiculite:1 peat (v/v/v) and grown in a growth chamber illuminated with Westinghouse Cool White

bulbs (0.9 klx at plant height) 16 hr a day. The temperature averaged 26° (day) and 21°C (night).

**The explant.** Shoot tips 2-cm long were excised when seedlings were 12 weeks old and every 6 weeks thereafter. Larger leaves were removed and shoots were trimmed to 1 cm. Groups of 10 prepared shoot tips were placed in small cheesecloth bags and disinfested as follows: 70% ethanol for 30 sec, 1 rinse with sterile water, 0.5% sodium hypochlorite (10% commercial laundry bleach) plus 0.1% Tween 20 for 5 min followed by 3 rinses with sterile water. The tips were then trimmed to 5 mm, treated again with 0.5% sodium hypochlorite for 1 min, and rinsed once with sterile water before explanting. To obtain shoots for rooting experiments, 10 tips obtained from *in vitro*-proliferated shoots were further multiplied in 125 ml Erlenmeyer flasks each containing 30 ml of medium. Illumination was provided by Sylvania Lifeline bulbs for 16 hr per day with an average light intensity of 2.2 klx. Culture room temperature averaged 29°C. Experiments were terminated after 6 weeks.

**Proliferation.** The effect of 3 cytokinins (BA, kinetin, and 2iP) at 0, 0.2, 1.0, and 5.0 mg/liter on shoot tip proliferation of each of the 3 rootstocks was tested (Table 1). BA induced meaningful prolifer-

Table 1. Influence of cytokinins on shoot proliferation of 3 citrus rootstocks (10 shoots per treatment).

Cytokinin	Concn (mg/liter)	No. shoots per explant		
		Carrizo citrange	Sour orange	Cleopatra mandarin
Control	0.0	1.0a <sup>2</sup>	1.0a	1.0a
BA	0.2	1.2a	1.0a	1.0a
	1.0	1.8b	1.1a	1.2a
	5.0	3.1c	0.9a	1.3a
Kinetin	0.2	1.0a	1.0a	1.0a
	1.0	1.0a	1.0a	1.0a
	5.0	1.0a	1.0a	1.0a
2iP	0.2	1.0a	1.1a	1.0a
	1.0	1.0a	1.1a	1.0a
	5.0	1.5a	1.2a	1.0a

<sup>2</sup>Mean separation in columns by Duncan's multiple range test, 5% level.

<sup>1</sup>Received for publication November 17, 1980. Florida Agricultural Experiment Stations Journal Series No. 2600.

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