

Tree Water Status and Exogenous Abscisic Acid Affect Freeze Tolerance in 'Valencia' Trees

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Abscisic acid (ABA) plays an important role in cold temperature responses of plants and has been reported to increase during cold acclimation. The application of exogenous ABA may alter ABA metabolism and induce cold tolerance. Drought stress also interacts with ABA metabolism and can increase cold acclimation. In this study, exogenous 1 mM ABA was sprayed during late winter onto 14-year old 'Valencia' sweet orange trees that were under three different irrigation regimes: 1. drought stress (no irrigation with a rain shield covering the soil during winter) 2. rain only (no irrigation) and 3. normal irrigation plus rain throughout the winter. Freezing tolerance estimated from electrolyte leakage from leaves at freezing temperature was evaluated one and three weeks after ABA spray applications. Overall, trees under the greatest drought stress had greater freezing tolerance than well irrigated trees. Exogenous ABA did not improve freeze tolerance one week after application but ABA sprays increased freeze tolerance in irrigated trees during the third week, although was not as effective in drought stressed trees. Thus, drought stress and exogenous ABA can both increase freezing tolerance in 'Valencia' orange trees although we observed no additive effect in this experiment.

Freezing temperatures are the largest environmental limitation to worldwide citrus production. Florida's subtropical winters are characterized by intermittent cold and warm periods of unpredictable length (Downton and Miller, 1993). Freezes in Florida (Figs. 1–2) negate citrus production in the northern part of the state and freezing temperatures can reduce citrus production during any year (Attaway, 1997). Florida's winter records show six major freezes from 1981 to 1989 killed or damaged thousands of acres of citrus trees (Ferguson, 2002) and yearly probability of temperatures as low as -5 °C (24 °F) has been estimated between 60% and 80% (Sauls and Jackson, 2000).

Wintertime drought stress can delay flowering and extend mechanical harvesting in late season cultivars (Melgar and

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Syvertsen, 2008). Abscisic acid (ABA) accumulation has been correlated with increased drought tolerance (Wang et al., 2004) as roots in drying soils have been reported to synthesize ABA that is transported to the shoot acting as a signal that promotes stomatal closure (Gowing et al., 1990; Zhang and Davies, 1989, 1990) and activates the expression of drought stress-related genes (Matsui et al., 2008). ABA also plays an important role in cold temperature responses as its concentration increases in herbaceous and woody plants exposed to low temperatures (Lee and Chen, 1993; Li et al., 1997; Yelenosky, 1985) and some authors have reported a relationship between drought stress and possible ABA-meditated increases in cold hardening and freeze tolerance in citrus (Yelenosky, 1979). Exogenous ABA applications may alter ABA metabolism and improve freezing tolerance (Kalberer et al., 2006; Thomashow, 1999; Zhang et al., 2008) by promoting increased concentrations of endogenous ABA as has been shown



Figs. 1-2. 'Valencia' trees after freeze temperatures of 26 °F on 22 Jan. 2009 at the Citrus Research and Education Center in Lake Alfred, FL.

in herbaceous plants. However, the effect of exogenous ABA applications on physiological properties under water deficits has been studied little in woody plants as only low concentrations of exogenous ABA has been applied to seedlings (Gibson et al., 1991; Yin et al., 2003).

We determined effects of winter time drought stress and exogenous ABA applications on freeze tolerance in 'Valencia' trees in the field. We tested the hypothesis that winter time drought stress and exogenous ABA applications can increase freezing tolerance.

Materials and Methods

PLANT MATERIAL AND GROWING CONDITIONS. In mid-February, a 1 mM concentration of ABA was sprayed onto leaves on tagged branches of 14-year-old 'Valencia' sweet orange trees that were under three different irrigation regimes: 1) drought stress (2-month no irrigation treatment with a rain shield covered soil during winter beginning in mid December); 2) rain only (uncovered, 2-month no irrigation treatment); and 3) normal irrigation and no cover throughout the winter. There were three single tree replicates in each irrigation treatment.

STEM WATER POTENTIAL. In order to assess tree water status, stem water potential (SWP) was measured at solar noon (13:00–14:00 HR) in three adult leaves in the west side of each tree. Leaves to be sampled were enclosed for at least 1 h in aluminum foil covered plastic bags to allow leaf water potential to equilibrate to stem water potential (McCutchan and Shackel, 1992). Covered leaf water potential (=SWP) was then determined using a Scholander-type pressure chamber (PMS instrument, Corvallis, OR; Scholander et al., 1965).

DETERMINATION OF FREEZING TOLERANCE. Leaf freezing tolerance was estimated from lethal freezing temperatures from electrolyte leakage from leaf tissue (EC; Lindén and Palonen, 2000). Lethal freezing temperatures were evaluated one and three weeks after ABA applications. Three sets of six leaves were taken from the ABA sprayed branches of each of three trees from the three irrigation regimes. Another three sets of six leaves were sampled from control (non-sprayed) branches. Sampled leaves were gently washed in deionized water. Leaf discs, 6 mm (0.24 inches) in diameter, were punched from each set of leaves (six discs per leaf) and placed into six test tubes (six discs per tube). Three tubes per tree were used for each temperature tested, considering three tubes as one replicate. Tubes were immersed in a glycol-refrigerated bath and exposed for 1 h to a range of six different temperatures between -3.33 °C (26 °F) and -8.88 °C (16 °F), at intervals of 2 °F per hour. A small chip of ice of deionized water was dropped into each test tube to nucleate the leaf tissue. Replicates for the first temperature were removed after 1 h and the refrigerating bath was reset to the next lower temperature. After removing the tubes from the bath, 10 mL deionized water were added to each tube. Tubes were covered with parafilm and incubated overnight at room temperature (25 °C). The initial electrolytic conductivity of each solution (EC₁, in μ S·cm⁻¹) was measured using an Orion 3 Star conductivimeter (Thermo Scientific, Beverly, MA). Test tubes were autoclaved (20 min, 121.0 °C) to completely disrupt the tissues and when the samples had cooled to room temperature, electrical conductivity was measured again (EC₂). Electrolytes that leaked into the solution at each freezing temperature were expressed as a percentage of total electrolytes leached after autoclaving. Percentage of electrolyte leakage (EL) was calculated as $EL = (EC_1/EC_2) \times 100$.

STATISTICAL ANALYSIS. The relationship between EL and temperature was described by a sigmoid response curve, which was fitted using the following logistical function:

$$EL(\%) = \{a/[1 + e^{b(T-c)}]\} + d$$

where T was temperature, a was the range of EL from minimum to maximum temperatures tested, b was the slope coefficient at the temperature of the inflection point, c was the temperature at the inflection point, and d the minimum value of EL. Three replicates per temperature were used for calculating the sigmoid curve. The inflection point of the sigmoid curve (parameter c) corresponds to the subzero temperature at which 50% ion leakage occurs, a point reported to correspond to irreversible lethal damage, and has been used in fruit trees (Anderson et al., 1983; Barranco et al., 2005; McKellar et al., 1992; Palliotti and Bongi, 1996) and in herbaceous crops (Cardona et al., 1997; Li, 1984; Sukumaran and Weiser, 1972; Zhang et al., 2008) for predicting the lethal freezing temperature (LT_{50}). Parameters were estimated using the curve fitting on non-linear regression methods of the statistical software package Sigmaplot for Windows, version 10.0 (Systat Software Inc., San Jose, CA). Lethal temperature values (parameter c) were expressed as mean \pm standard error. Stem water potential data were analyzed using analysis of variance on the three irrigation treatments with three replicate trees in each treatment and three leaves per tree. When treatments were significant, means were separated using Duncan's multiple range test at P < 0.05.

Results and Discussion

Tree water status measurements showed that stem water potential in drought stressed trees was significantly lower than in moderate stressed trees under the rain-only treatment, which were under a moderate stress (Table 1). Trees under drought stress showed greater freezing tolerance (lower LT_{50} values, Table 2) than well irrigated trees. This supports the idea that cold tolerance may be increased by increased levels of ABA synthesized as a consequence of drought stress (Yelenosky, 1979). There is little published information, however, about changes in leaf ABA content or other metabolites in citrus during winter (Gomes et al., 2004), when non-irrigated trees in Florida may experience drought stress in some years when rains are infrequent. Exogenous ABA did not improve freeze tolerance one week after application but ABA increased cold tolerance in irrigated trees by the third week after the spray treatment. Changes in leaf ABA content after the exogenous application need to be determined, as ABA has been previously reported to increase substantially during cold acclimation (Lee and Chen, 1993; Zhang et al., 2008). ABA applications in drought stressed trees were not as effective at increasing freeze tolerance as in well-irrigated trees (Table 2). Leaves on irrigated trees may have been more physiologically active and foliar absorption may have been greater in irrigated trees than in trees

Table 1. Stem water potential (MPa) in trees under three different irrigation regimes: drought stress, rain, or rain + irrigation. Measurements were performed 1 and 3 weeks after exogenous ABA applications, at midday on clear days.

	5	5		
		Drought	Rain only	Rain + irrigation
Week 1		-2.93 a ^z	–2.08 b	−1.80 b
Week 3		–3.07 a	−1.88 b	-1.29 c

²Values within a row followed by unlike letters are significantly different at P < 0.05 as tested by Duncan's multiple range test.

Table 2. Lethal temperature (LT_{50}) values (F) in control or ABA applied leaves of 'Valencia' sweet orange under three different irrigation regimes (drought stress, rain or rain + irrigation). Measurements were done 1 and 3 weeks after exogenous ABA applications.

	Drot	Drought		Rain only		Rain + Irrigation	
LT ₅₀	Control	ABA	Control	ABA	Control	ABA	
Week1	19.81 ± 0.85	21.66 ± 2.87	20.72 ± 0.40	22.35 ± 0.88	21.60 ± 0.77	21.61 ± 0.31	
Week 3	19.11 ± 4.45	18.34 ± 1.80	21.07 ± 0.74	20.82 ± 0.36	22.30 ± 0.75	20.98 ± 0.30	

that were drought stressed for two months, although it could have also been an effect of leaf ABA contents already raised in drought stressed trees. Changes in levels of endogenous ABA would be probably occur if different exogenous ABA concentrations were sprayed. In summary, wintertime drought stress and exogenous ABA can both increase freezing tolerance in 'Valencia' orange trees, although we observed no additive effect of both ABA and drought stress in this experiment.

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