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# Below Ground Pot-in-Pot Effects on Growth of Two Southwest Landscape Trees was Related to Root Membrane Thermostability<sup>1</sup>

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## Abstract

Two southwestern desert landscape trees, *Acacia smallii* L. (sweet acacia) and *Cercidium floridum* Benth. ex A. Gray (blue palo verde), were grown outdoors in full sun during Summer 1997 in 19-liter (#5) containers placed either pot-in-pot (PIP) below ground or unshielded in above-ground containers (AGC). Soil moisture sensors wired to electronic solenoid valves regulated occurrence of six cyclic micro-irrigation pulses per day (0600, 0900, 1200, 1500, 1800, and 2100 HR) such that container substrate moisture tensions were continuously maintained between  $-0.005$  to  $-0.01$  MPa (90% of water holding capacity) in both PIP and AGC. Mean maximum recorded root-zone temperatures in PIP containers were  $19\text{C}$  ( $34\text{F}$ ) lower than for AGC. Micro-irrigation volumes were 40% less for trees grown PIP compared with those in AGC. Growth of sweet acacia was enhanced by PIP placement while in containers and one year after transplanting trees into field plots in 1998. Only caliper growth of blue palo verde was increased by PIP placement while in containers, but had no effect on blue palo verde growth one year after transplanting into field plots. The critical killing temperature ( $T_M$ ) for root tissues of sweet acacia and blue palo verde were  $45.3 \pm 1.8\text{C}$  ( $113.5 \pm 3.2\text{F}$ ) and  $49.4 \pm 0.8\text{C}$  ( $120.9 \pm 1.4\text{F}$ ), respectively, indicating differences in root membrane thermostability. Based on our data, we suggest that sweet acacia trees benefitted from PIP placement more than blue palo verde trees because root-zone temperatures in PIP containers were lower than for AGC in central Arizona, and sweet acacia roots were more susceptible to injury by supraoptimal root zone temperatures.

**Index words:** container production, pot-in-pot, cyclic micro-irrigation, heat stress, membrane thermostability.

**Species used in this study:** sweet acacia (*Acacia smallii* L.), blue palo verde (*Cercidium floridum* Benth. ex A. Gray).

## Significance to the Nursery Industry

Two southwestern desert landscape trees responded differently to below ground PIP placement compared with those in AGC in Tempe, AZ. Compared with growth in AGC, growth of *Acacia smallii* (sweet acacia) was enhanced by PIP placement, whereas growth of *Cercidium floridum* (blue palo verde) generally was not. Enhancement of sweet acacia growth by PIP placement was still evident one year after transplanting trees into field plots. Roots of blue palo verde were more heat tolerant than sweet acacia roots as quantified by electrolyte leakage techniques. Maximum root-zone temperatures of PIP containers were  $19\text{C}$  ( $34\text{F}$ ) lower than for AGC. This study suggests that sweet acacia roots have a lower tolerance of supraoptimal root-zone temperatures than blue palo verde and will benefit more from PIP placement because the rooting substrate is insulated from supraoptimal temperature extremes. In contrast, nursery production of blue palo verde trees in a below ground PIP system appears less beneficial.

## Introduction

Pot-in-pot (PIP) production is increasingly used by nursery operators to grow trees in large containers (4, 5, 10). In a below ground PIP system, a holder or socket pot is permanently positioned in the ground and a container plant is then placed inside the socket pot. Compared with traditional above-

ground container (AGC) production methods, PIP methods can be less costly to nursery operators due to less intensive, labor-saving cultural practices and the ability to grow larger trees quickly (1).

Recent studies have shown that PIP methods can increase tree growth (11, 12, 14), insulate roots from high and low temperature extremes (15, 16), increase fertilizer longevity (12), and decrease daily container evapotranspiration (3), though one study reported a gross increase in evapotranspiration water loss due to increased tree size (14). In each of these studies, irrigation volumes applied to PIP and AGC were similar, even though evapotranspiration water loss by PIP and AGC might vary. In order to elucidate PIP effects on growth independent of effects on container substrate moisture availability, it is important to maintain similar substrate water contents for trees grown in either production method.

Nursery operators in the arid, southwestern United States contend with supraoptimal air and root-zone temperatures, high surface evaporation rates, and limited water resources. In this region, daily maximum air temperatures above  $38\text{C}$  ( $100\text{F}$ ) occur from May to October. Temperatures as high as  $60\text{C}$  ( $140\text{F}$ ) have been recorded in AGC substrates in central Arizona during the summer months (15). Because of extreme summer heat, many southwestern nursery operators cyclically irrigate [daily water allocation in more than one application (2)] container grown nursery trees during the mid-day and afternoon hours to cool container substrates, maintain adequate substrate moisture levels, and conserve water resources (5). Under southwest condition, below ground PIP placement of containers might insulate tree roots from supraoptimal root-zone temperatures common in AGC, and conserve water resources via less evapotranspiration.

Objectives of this research were to 1) determine growth effects of two southwest landscape trees in response to placement below-ground PIP compared to AGC, and 2) to ascer-

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tain if there is a relationship between PIP-related growth enhancement, insulation of roots from supraoptimal root-zone temperature extremes which normally occur when containers are positioned above ground, and tree root membrane thermostability. In this paper, we report on our use of soil moisture sensors to regulate pre-programmed pulses of cyclic micro-irrigations by overriding a scheduled irrigation event if substrate moisture levels were above a preset condition. Using this technology, we were able to maintain similar substrate moisture levels in PIP and AGC despite the differential evapotranspiration rates associated with these two production methods. Using electrolyte leakage techniques, we quantified tree root membrane thermostability expecting that trees most benefitting from PIP placement would have lower root membrane thermostability.

## Materials and Methods

**Tree species.** Sweet acacia (*Acacia smallii*) and blue palo verde (*Cercidium floridum*) were used in this research. Both species are in the Family *Fabaceae* and are common urban landscape accent trees in lower Sonoran Desert cities. Sweet acacia is native to upland seasonally-moist regions of central Mexico and in the urban landscape grows into an evergreen, small, multiple-trunk tree [7.6 m (25 ft) mature height] having a highly fragrant, orange winter blossom. Blue palo verde is native to riparian washes in the lower Sonoran Desert and in the urban landscape grows into a medium-sized, multiple-trunk tree [10.6 m (35 ft) mature height]. It has bluish-green photosynthetically active stems, drought deciduous leaves, and lemon yellow flowers in April.

**PIP production.** Research was conducted outdoors under full sun in quarantined field plots at Desierto Verde Wholesale Nursery in Tempe (33.5N 112W), AZ, during April 1997 to October 1998. On April 21, 1997, six-month-old, uniform seedlings [20 cm (8 in) in height] of sweet acacia and blue palo verde were transplanted into #5 (19-liter) black polyethylene containers filled with a regionally common substrate mixture of forest mulch, silt and sand (6:1:1 by vol) amended with 3.1 kg/m<sup>3</sup> (9 lb/yd<sup>3</sup>) 20N–3.0P–8.3K (custom blend 20–7–10, N derived from urea-formaldehyde) controlled-release fertilizer plus micronutrients formulated for a 8 to 9 month release. Physical characteristics of the container substrate mixture at the start of the experiment were as follows; 661 ± 31 kg/m<sup>3</sup> (1908 ± 89 lb/yd<sup>3</sup>) dry bulk density, 56.0 ± 0.6% total porosity, 43.1 ± 0.4% water holding capacity (WHC), and 12.8 ± 0.3% aeration porosity, n = 5.

After transplanting, all containers were placed 1.1 m (4 ft) on center either PIP or above-ground, unshielded on a gravel-covered surface and grown for 5 months. The #10 (38-liter) PIP holder or socket pots were placed in the ground. A spray stake (Terracotta Spot Spitter, Roberts Irrigation Products, San Marcos, CA) micro-irrigation delivery system was electronically controlled to deliver 3-min cyclic pulses of water each day at 0600, 0900 1200, 1500, 1800, and 2100 HR at the rate of 210 ml (0.05 gal)/min/container. Soil moisture sensors (Intellisense<sub>™</sub> 100 Soil Moisture Sensor, Rainbird Irrigation, Tucson, AZ) were inserted vertically into the container substrate 20 cm (8 in) below the substrate surface and 10 cm (4 in) inside the container wall. Moisture sensors were directly interconnected to the electronic solenoid valves by electrical wire, and calibrated to allow or prevent pulses of water to each plant at the programmed time so that container

substrate water tensions at 20 cm (8 in) below the substrate surface and 10 cm (4 in) inside the container wall were maintained between –0.005 to –0.01 MPa (about 90% of WHC or consistently moist) for both above ground and below-ground PIP container substrates. Container substrate moisture tensions were monitored throughout the duration of the study by soil tensiometers. The position of the soil moisture sensors and tensiometer ceramic tips within the container profile were identical. Both tree species were arranged in a completely randomized block design of two container placement (PIP and AGC) treatments and 10 single tree replications which equaled 40 total trees.

Cyclic micro-irrigation application volumes and container substrate temperatures were recorded on September 3 to 10, 1997; all were cloudless days with a mean daily air temperature range of 40C (104F) max/28C (83F) min, and mean daily dew point temperature range of 21C (69F) max/16C (60F) min. Container substrate temperatures were recorded with a 21× micrologger and AM32 multiplexer (Campbell Scientific, Inc., Logan, UT) using copper constantan thermocouples positioned one-half way down the container profile at the east and west cardinal coordinates, 2.5 cm (1 in) from the rooting substrate/container wall interface, and at the center location. Temperatures were logged every 5 min and averaged for each 30-min interval.

Monthly measurements of tree height and trunk caliper at 5 cm (2 in) above the substrate surface were made. A visual evaluation of root presence on the root ball surface was made by three independent observers on September 17, 1997 using a pre-established visual rooting scale. The rooting scale was as follows: 0 = no surface roots present; 1 = surface roots on bottom surface only; 2 = surface roots present on bottom and one wall quadrant; 3 = surface roots present on bottom and two wall quadrants; 4 = surface roots present on bottom and three wall quadrants; and 5 = surface roots present on bottom and all wall quadrants. On October 1, 1997, half of the plants were harvested and dry weights determined.

**Post production.** The remaining unharvested trees were transplanted into field plots at 3 m (10 ft) on center to assess the post-transplant growth response of trees for one year to container placement treatments. Transplant field soil (clay loam, pH = 8.0, EC = 2.78 dS/m, available N = 129 lbs/A, organic matter = 0.6%) was unamended and plants were not fertilized. Each tree was drip irrigated once every week for eight hours with three 3.8-liter (1 gal)/hr drip emitters per tree. Final measurements of height and trunk caliper were made on November 5, 1998. All growth data were analyzed using the GLM procedure of SAS (SAS version 6.03, Cary, NC) to test for significant treatment main effects and interactions. Statistical comparison of temporal changes in height and caliper data as affected by treatments combinations were made by repeated measures analysis (8).

**Root membrane thermostability.** Electrolyte leakage procedures, as described in detail by Ingram and Buchanan (6) and Martin et al. (9), were used to quantify root membrane thermostability of sweet acacia and palo verde trees grown outdoors in #5 (19-liter) black polyethylene containers during summer 1998. Seven 1-gram samples of non-suberized root tissue from each tree species were harvested at random from the inner portion of an outdoor nursery production block, enclosed in test tubes with de-ionized water, and exposed to

one of nine temperature treatments [25.0C (77F), 40.5C (105F), 45.6C (114F), 47.5C (118F), 50.5C (123F), 53.0C (127F), 57.0C (135F), 62.0C (144F), and 70C (158F)] for 35 min in a temperature-controlled circulating water bath. Electrical conductivity, representing the solutes lost or membrane leakage caused by the water bath treatments, was measured for each sample with a conductivity bridge. Root samples were then autoclaved for 15 min at 100C (212F) and 15 psi. Electrical conductivities were re-measured and percent electrolyte leakage ( $L_E$ ) was computed as the conductivity measurement after water bath temperature treatments divided by the conductivity measurement after autoclaving multiplied by 100. Any additional solute loss from sample root tissue after the autoclaving was attributed to total membrane dysfunction. The  $L_E$  from excised roots graphed against bathing temperature (T) resulted in a sigmoidal response curve. A sigmoidal equation:

$$L_E = [(x - z) / (1 + \exp[-k(T - T_M)])] + z$$

where  $L_E$  was percent leakage,  $z$  = baseline level of electrolyte leakage,  $x$  = maximum proportion of electrolyte leakage or asymptote,  $T_M$  = critical killing temperature,  $k$  = slope of the predicted response curve at  $T_M$ , and  $T$  = water bathing

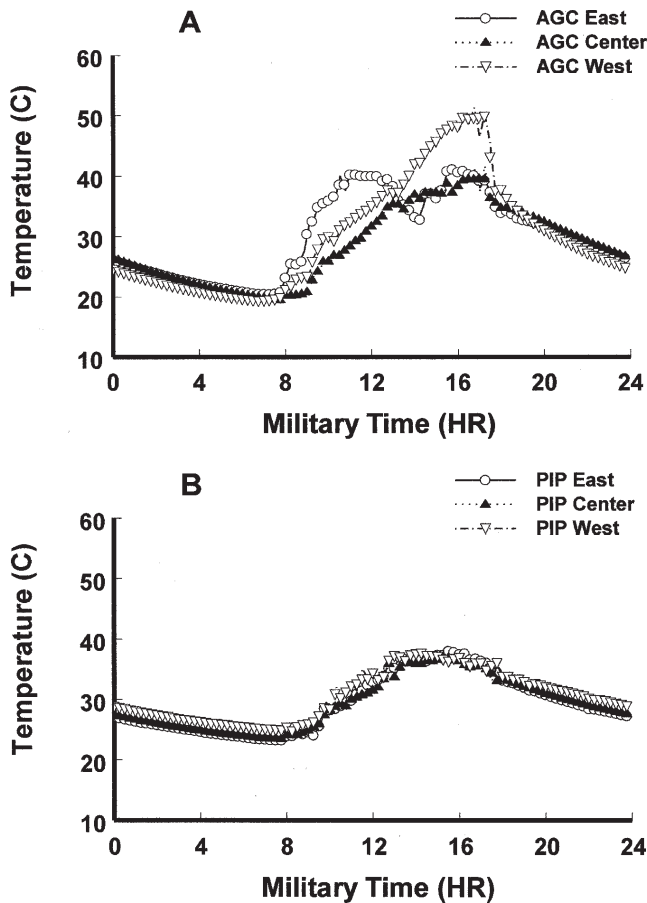


Fig. 1. Mean daily container substrate temperature patterns in the east, center and west quadrants of #5 black polyethylene containers (A) unshielded, above ground, or (B) placed PIP below ground during September 3 to 10, 1997. Data are mean values,  $n = 6$ .

Table 1. Repeated measures analysis of variance of significant polynomial time trends and treatments contrasts for increases in height and caliper of sweet acacia and blue palo verde in response to container placement treatments<sup>a</sup>.

Source of variation	MS	F	P value <sup>b</sup>
<i>Sweet acacia</i>			
Height increase			
Time	6745.3	531.6	0.0001L
Time $\times$ Placement, PIP vs AGC	47.2	5.1	0.0425C
Caliper increase			
Time	2258.8	834.9	0.0001L
Time $\times$ Placement, PIP vs AGC	10.9	30.1	0.0001C
<i>Blue palo verde</i>			
Height increase			
Time	6080.1	271.2	0.0001L
Time $\times$ Placement	11.3	0.5	0.4911
Caliper increase			
Time	5.0	10.9	0.0058Q
Time $\times$ Placement, PIP vs AGC	2.4	5.4	0.0364C

<sup>a</sup>Container placement treatments were PIP = below ground pot-in-pot or AGC = unshielded above ground.

<sup>b</sup>Linear (L) or cubic (C) fitted line responses across time.

temperature, was used to fit the data. The NLIN procedure of SAS (SAS version 6.03, Cary, NC) was used to estimate the equation parameters. There were 63 sample observations per tree species.

## Results and Discussion

**PIP production.** Mean maximum recorded root-zone temperatures in containers placed PIP averaged 19C (34F) lower than for those in AGC (Fig. 1A&B) and failed to exceed 40F (104F) (7). Earlier studies reported that root-zone temperatures were 13C (23F) lower if containers were placed PIP compared with those above ground (15, 16). 3.2 liters (0.8 gal)/pot/week or 5.3 liters (1.4 gal)/pot/week if placed PIP or in AGC, respectively, were required to keep moisture tensions for all rooting substrates between  $-0.005$  and  $-0.01$  MPa using cyclic micro-irrigation. For trees in AGC, soil moisture sensors usually overrode scheduled cyclic micro-irrigation during the mornings and permitted cyclic micro-irrigations during the afternoon or early evening hours. For trees placed PIP, there were no apparent relationship between when an irrigation event occurred and time of day. Rainfall during this container phase of the study totaled 8.0 cm (3.2 in), of which 6.9 cm (2.8 in) occurred during three days in August. In contrast with an earlier report (11), no incidences of rooting-out were observed with trees placed PIP below ground.

Height and caliper growth of all sweet acacia increased linearly over time (Table 1). Moreover, height and caliper growth of sweet acacia placed PIP increased cubically and linearly, respectively, compared with those in AGC. The significant cubic contrast in height growth between trees placed PIP and those in AGC was evidenced as an increase in the rate of growth of trees placed PIP during the month of July (Fig. 2). Shoot and root dry weight of sweet acacia placed below-ground PIP were 2.2 and 1.7 times greater, respectively, than for trees in AGC (Table 2). The visual root index value was higher for trees placed PIP below ground compared with those trees placed above ground which suggested that roots of sweet acacia placed PIP were redistributed to-



ward the rootball surface. Sweet acacia shoot to root ratio was not significantly affected by container placement.

Height and caliper growth of all blue palo verde increased linearly and quadratically over time, respectively (Table 1). Container placement did not affect blue palo verde height; however, caliper growth of trees placed PIP increased cubically over time compared with those in AGC (Table 1). The significant cubic contrast in caliper growth between trees placed PIP and those in AGC was evidenced as an increase in the rate of caliper increase in trees placed PIP during the month of July (Fig. 3). Shoot and root dry weights and shoot to root ratio were not affected by container placement, though shoot dry weight of trees placed PIP tended to be greater than for those in AGC (Table 2). Similar to sweet acacia, the visual root index values were higher for those trees placed PIP compared with those in AGC which suggested that roots of blue palo verde placed PIP were also distributed toward the rootball surface (13).

*Post production.* One year after transplanting into field plots, height and trunk caliper of sweet acacia were greater for trees previously grown PIP compared with those previ-

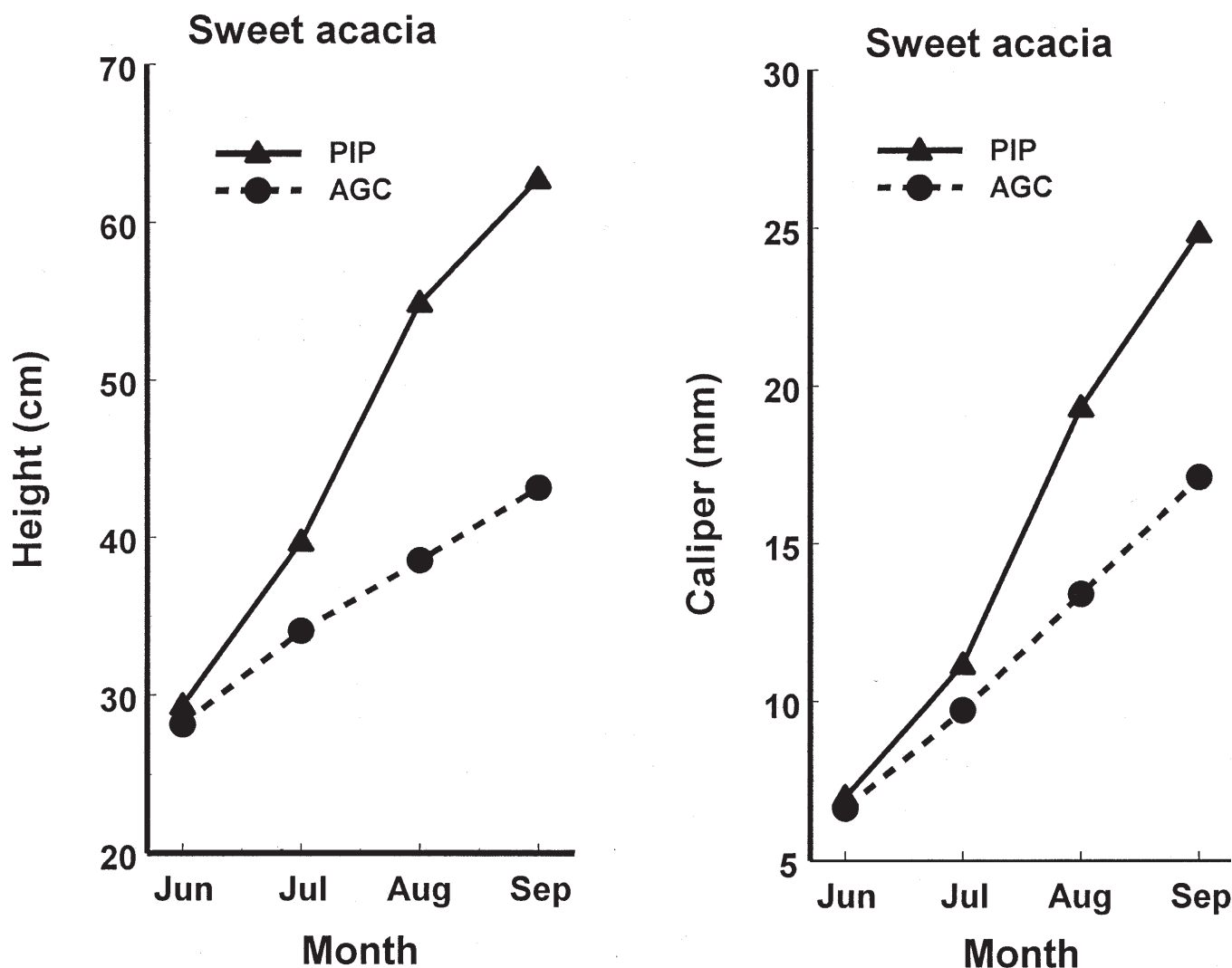
**Table 2.** Shoot and root dry weights, shoot to root ratio (S:R), and visual root index of sweet acacia and blue palo verde in response to container placement treatments.

Treatments <sup>a</sup>	Shoot dry weight (g)	Root dry weight (g)	S:R	Root index <sup>b</sup>
<i>Sweet acacia</i>				
PIP	346a <sup>c</sup>	167a	2.4a	4.5a
AGC	160b	97b	2.3a	3.1b
<i>Blue palo verde</i>				
PIP	166a	58a	2.9a	4.0a
AGC	112b	45a	2.5a	3.2b

<sup>a</sup>Container placement treatments were PIP = below ground pot-in-pot or AGC = unshielded above ground.

<sup>b</sup>Rooting index specifications were 0 to 5 where 0 = no surface roots present, 1 = surface roots on bottom surface only, 2 = surface roots present on bottom and one wall quadrant, 3 = surface roots present on bottom and two wall quadrants, 4 = surface roots present on bottom wall and three quadrants, and 5 = surface roots present on bottom and all wall quadrants.

<sup>c</sup>Treatment means, n = 5. Means separation in columns (within species) by Fisher's LSD test, P = 0.005.



**Fig. 2.** Height and caliper growth of sweet acacia as affected by container placement treatments. PIP = containers placed pot-in-pot below; AGC = unshielded containers above ground. Values are treatment means, n = 5.

**Table 3.** Height and trunk caliper of sweet acacia in response to precedent nursery container placement treatments<sup>a</sup> one year after transplanting into field plots.

Treatments	Height (cm)	Trunk caliper (mm)
<i>Sweet acacia</i>		
PIP	288a <sup>b</sup>	25a
AGC	184b	17b

<sup>a</sup>Nursery container placement treatments were PIP = below ground pot-in-pot or AGC = unshielded above ground.

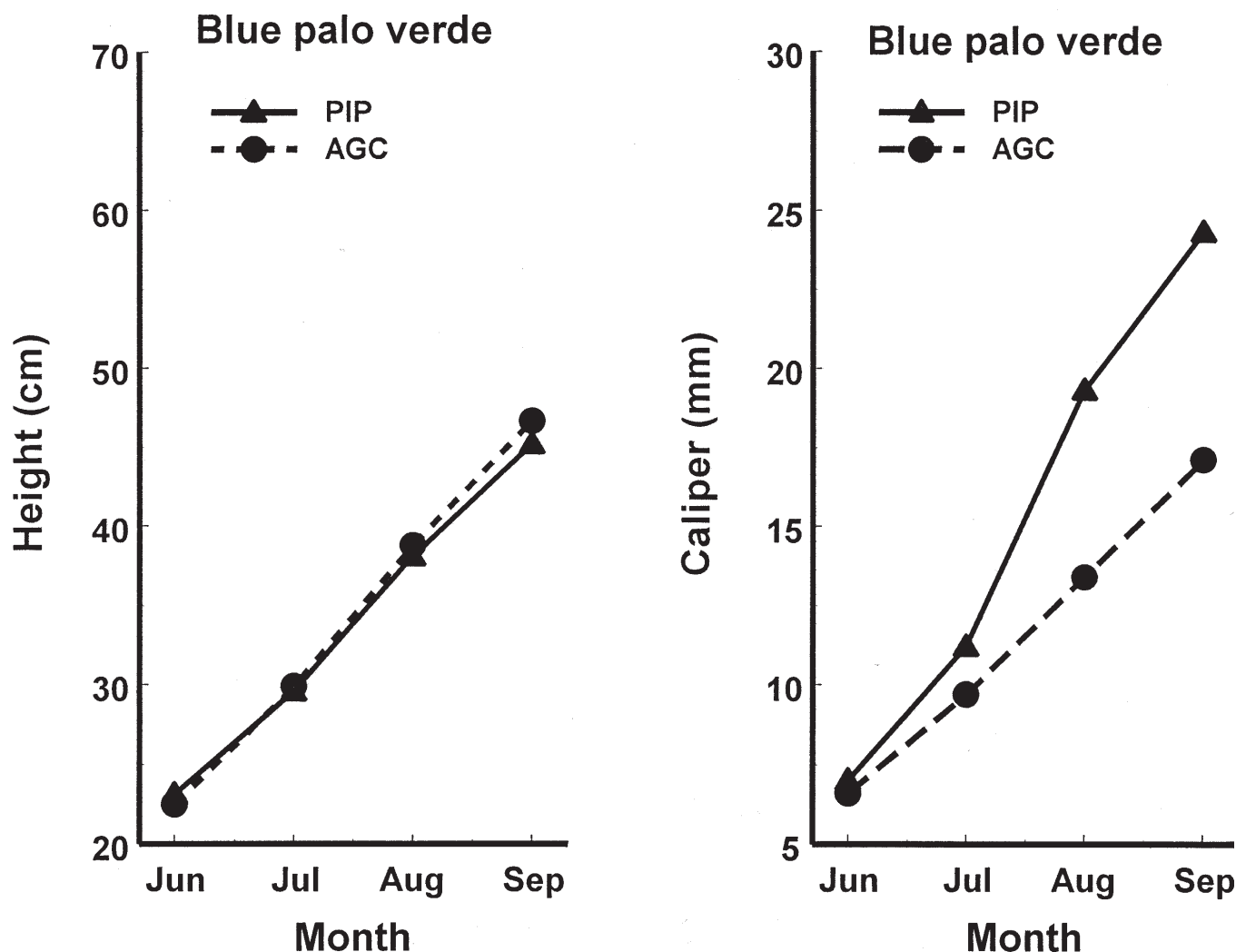
<sup>b</sup>Mean separation in columns (within species) by Fisher's LSD test,  $P = 0.005$ ,  $n = 5$ .

ously grown in AGC (Table 3). Conversely, height and trunk caliper of blue palo verde trees were not affected by previous container placement treatments ( $P = 0.357$ , data not shown).

**Root membrane thermostability.** The relationship between electrolyte loss and temperature was correctly depicted by

the sigmoidal equation ( $r^2 = 0.78$ ). The  $T_M$  for blue palo verde roots was  $49.4 \pm 0.8^\circ\text{C}$  ( $120.9 \pm 1.4^\circ\text{F}$ ). The  $T_M$  for sweet acacia roots was  $45.3 \pm 1.8^\circ\text{C}$  ( $113.5 \pm 3.2^\circ\text{F}$ ). In addition to the higher  $T_M$ , blue palo verde roots had a narrower temperature response range than sweet acacia roots. The narrower temperature response of blue palo verde roots was reflected in their  $k$  values,  $1.4 \pm 0.4$  compared with  $0.2 \pm 0.04$  for sweet acacia roots. Because  $T_M$  and  $k$  values were both higher for blue palo verde roots than for sweet acacia roots, higher temperatures would be required before induction of root membrane leakiness. Therefore, it was concluded that blue palo verde roots had a higher root membrane thermostability than sweet acacia roots.

In summary, root-zone temperatures in PIP containers were lower than for AGC. Micro-irrigation volumes measured during one week in September were 40% less for trees grown PIP compared with those in AGC. Shoot and root growth of sweet acacia was enhanced when grown in containers placed PIP below ground, while only caliper growth of blue palo verde was enhanced by PIP placement. In contrast to an earlier study (11), PIP growth enhancement of sweet acacia during the container-phase of the study was still evident af-



**Fig. 3.** Height and caliper growth of blue palo verde as affected by a factorial combination of container placement. PIP = containers placed pot-in-pot below; AGC = unshielded containers above ground. Values are treatment means,  $n = 5$ .

ter one year in the field. Blue palo verde roots were more heat tolerant than sweet acacia roots as evidenced by a higher critical killing temperature. Based on our data, we suggest that sweet acacia trees benefitted from PIP placement more than blue palo verde trees because root-zone temperatures in PIP containers were lower than for AGC, and sweet acacia roots were more susceptible to injury by supraoptimal root-zone temperatures. Nursery operators should consider growing sweet acacia in a below ground PIP system; however, the added expense of producing blue palo verde trees in a below-ground PIP system appears to less beneficial.

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