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The effect of pot size on growth and transpiration of maize and soybean during water deficit stress

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Abstract

Many experiments are conducted in greenhouses or growth chambers in which plants are grown in pots. Considerable research has shown that pots can have a limiting effect on overall plant growth. This research was undertaken to examine the effects of pot size specifically on transpiration response of maize (*Zea mays* L.) and soybean (*Glycine max* L.) plants undergoing water-deficit stress. Maize and soybean experiments were conducted similarly, but as separate experiments. Maize plants were grown in 2.3, 4.1, 9.1, and 16.2 l pots sealed to prevent water loss except by transpiration. For each pot size, plants were divided into two watering regimes, a well-watered control and a water-deficit regime. Water deficits were imposed by simply not rewatering the pots. Soybean was examined in a similar manner, but only the three larger pot sizes were used in the experiment. For both maize and soybean, and in both watering regimes, there was a significant reduction of shoot dry weight and total transpiration with decreasing pot size. However, there were no significant differences among pot sizes in the fraction of transpirable soil water (*FTSW*) point at which transpiration began to decline (*FTSW* $\approx$0.31 for maize and $\approx$0.35 for soybean) or in the overall relationship of transpiration rate to soil water content in response to water deficits. These results indicated that, regardless of pot size or plant size, the overriding factor determining transpirational response to drought stress was soil water content.

Key words: Drought, maize, pot size, transpiration.

Introduction

Many plant physiology experiments are conducted in controlled environments where the plants are grown in pots with limited soil volume. A well-recognized problem with growing plants under these conditions is the possibility that the plants may become 'root bound'. Numerous studies have shown a general reduction in growth associated with smaller pot sizes (Peterson *et al.*, 1984; Robbins and Pharr, 1988; Townend and Dickinson, 1995; Whitfield *et al.*, 1996). This reduction in growth has been shown to occur in both solid soil media as well as in hydroponic nutrient solution (Carmi *et al.*, 1983).

The mechanisms by which overall plant growth is inhibited by restricted root growth are unknown (Carmi, 1993). Pot size, however, has been shown to affect a number of physiological processes including nutrient efficiency (Huang *et al.*, 1996) and photosynthesis rates (Robbins and Pharr, 1988; Carmi *et al.*, 1983; Krizek *et al.*, 1985; Arp, 1991). It should be noted that the relationship between photosynthesis rates and pot size has not been consistent. Photosynthetic rate has been shown to increase (bean, *Phaseolus vulgaris* L., Carmi *et al.*, 1983), decrease (tobacco, *Nicotiana tabacum* L., Herold and McNeil, 1979), or not change (soybean, Krizek *et al.*, 1985) with decreasing pot size.

Considering the number of pot studies done on plant response to soil water deficits, it is surprising that no information exists on the possibility that pot size might influence the sensitivity of plants to soil drying. This is especially true because of the fact that root growth and the mechanisms of water uptake might be influenced by the soil volume. This study was undertaken to examine explicitly the sensitivity of transpiration rate to soil drying in pots of differing volume.

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To characterize the degree of soil drying among pots, transpiration was examined as a function of available soil water. Ritchie (1980) proposed that there was a consistency in plant response to the fraction of available soil water across a wide range of conditions. Transpiration, for example, was unaffected by soil drying until about only one-third of the available soil water remained in the soil. Below the one-third level of available soil water, transpiration rate decreased essentially linearly until all the available soil water was exhausted. This approach is now used widely to characterize plant response to soil water deficits (Sadras and Milroy, 1996).

In this paper, dehydration experiments to examine explicitly the possibility whether pot size might influence the response of transpiration to drying soil are reported. Maize and soybean plants were grown in a range of pot volumes that had a clear influence on plant growth. However, these experiments provided important documentation that the response to drying soil was not influenced by pot size.

Materials and methods

Four sizes of pots were selected for use in the maize experiment and three in the soybean experiment. The smallest pot size was a tapered cylindrical pot (16 cm height × 16 cm top diameter × 11 cm bottom diameter) commonly used in greenhouse experiments. This pot was approximately 2.3 l in volume. The other three pot sizes were made from polyvinyl chloride (PVC) pipe of 10.2, 15.2 and 20.3 cm diameters cut to 50 cm lengths. One end of the pipes was sealed with PVC caps with small holes drilled in the bottom for drainage. This produced pots with approximate volumes of 4.1, 9.1 and 16.2 l. In the soybean experiment, only the PVC pipe pots were used (the 4.1, 9.1 and 16.2 l pots).

In both experiments, pots were filled to within approximately 2.5 cm of the top with a commercial sandy loam potting soil (Grace Serria, Milpatis, CA). In the maize experiment, three seed (Pioneer International Hybrid 3165) per pot were sown in on 3 June 1995, and in the soybean experiment, three seed ('Biloxi') per pot were sown on 27 January 1997. In each case, plants were thinned to one plant per pot 2 weeks after sowing. The plants were grown in a greenhouse subjected to natural solar radiation with the air temperature regulated between approximately 20 and 28 °C (night/day). Daylength was extended to 16 h by incandescent lamps over the soybeans to keep the plants in vegetative development throughout the experiment. For both experiments, vegetative growth stage (V-stage) was defined as the number of fully expanded leaves.

On the evening before the water deficit was initiated (13 d after sowing for maize and 34 d after sowing for soybean), all pots were watered to soaking and then allowed to drain overnight. On the following morning, pots were sealed to prevent water loss except by transpiration. All pots were weighed and this weight was defined as the initial pot weight. The 2.3 l pots were sealed by placing the entire pot in a plastic bag, bunching the bag opening around the plant stem and attaching it securely with a twist tie. In the larger pots, the bottom of the bags were cut out and the bag slipped over the pot. The top of the bag was sealed around the plant stem as was done for the 2.3 l pots, and the bottom of the bag was sealed by taping the bag to the pot. For all pots, a 5 ml pipette tip was then inserted between the plant and the bag in order to facilitate watering.

The experiments were designed with pot size as the treatment, and within each pot size there were two watering regimes; a well-watered control (three pots for each pot size) and a water-deficit regime (six pots per pot size, except for the 4.1 l pot size in the maize experiment for which there were seven pots). To maintain well-watered conditions but prevent anaerobic conditions in the control pots, water was added, when necessary, to maintain a pot weight 200, 600, 1200, and 2000 g below the initial saturated pot weight for the 2.3 l, 4.1 l, 9.1 l, and 16.2 l pots, respectively.

Transpiration was measured by weighing the pots every afternoon at approximately 15.30 h. Daily transpiration was calculated as the difference in pot weight from that on the previous day. For the smaller 2.3 l pots in the deficit watering regime of the maize experiment, any water loss over 70 g d^{-1} was added back to the pot. This ensured that the dry down did not proceed too rapidly for accurate measurements of the point at which transpiration began to decline. On average, a total of 424 g of water was added to each 2.3 l pot, primarily during the first few days of the experiment. No water was added to any of the other pots in the stress treatment at any time during the dry down.

The transpiration data were analysed by the procedure previously described by Sinclair and Ludlow (1986) and Ray and Sinclair (1997). To minimize the influence of large variations in daily transpiration across days, the daily transpiration rates of the water-deficit pots were normalized against the transpiration rates measured for the well-watered plants on each day. That is, the daily normalization was achieved by dividing daily transpiration of each individual plant in the water-deficit regime by the daily mean transpiration of the well-watered control plants for each pot size,

$$TR = \frac{\text{transpiration of stressed plant}}{\text{average transpiration of control plants}} \tag{1}$$

The values of *TR* varied among individual plants because of differences among plants within a pot size. To facilitate comparisons among plants, a second normalization was done so that the normalized transpiration rate of each plant was centred on a value of 1.0 when the soil water content in each pot was high. Therefore, a mean *TR* was calculated for each plant for the first 2–5 d of the experiment. The daily *TR* for each pot was divided by the mean *TR* during the well-watered stage to give a daily normalized transpiration ratio (*NTR*).

Pots were allowed to dry through transpirational water loss until *NTR* dropped below 0.10, which was defined as the endpoint for the water-deficit treatment (Sinclair and Ludlow, 1986). From the data on initial pot weight and the endpoint, the transpirable soil water available to the plant in each pot could be calculated as the difference between these two measures. The use of transpirable soil water as the basis of comparing plant response to soil drying under a range of conditions has been effectively used in a number of studies (Weisz *et al.*, 1994; Ray and Sinclair, 1997).

The comparisons among pot sizes was further facilitated by expressing the available soil water as the fraction of transpirable soil water (*FTSW*) for each pot in the water deficit treatment on each day. Daily values of *FTSW* for each pot was calculated by dividing the daily pot weight minus the final pot weight by the transpirable soil water of that pot,

$$\textit{daily FTSW} = \frac{\text{daily pot weight} - \text{final pot weight}}{\text{initial pot weight} - \text{final pot weight}} \tag{2}$$

The relationship of *FTSW* and transpiration for each pot size was evaluated using non-linear regression procedures of Prizm (GraphPad Software, San Diego, CA) to fit the equation for maize described by Muchow and Sinclair (1991),

$$NTR = 1/[1 + A \times \exp(B \times FTSW)] \qquad (3)$$

Comparisons of the curve generated by Equation (3) for each pot size and between maize and soybean were based on 95% confidence intervals of coefficients A and B. To determine the specific *FTSW* value (threshold) at which transpiration began to decline, the plateau regression procedures of SAS were employed (SAS Institute, Inc., 1988). Because of the double normalization used to calculate *NTR*, the plateau was constrained to equal one. Within each experiment (maize and soybean), analysis of variance and Tukey's honestly significant difference test were used to examine the effects of pot size based on the *FTSW* thresholds determined for each individual plant. Comparison of the *FTSW* thresholds between the maize and soybean experiments were made based on the 95% confidence intervals of the composite threshold values generated for all maize and soybean plants, regardless of pot size. Analysis of variance and Tukey's honestly significant difference were also used to evaluate the effect of pot size on shoot dry weight, total water transpired, and days to endpoint.

Results and discussion

The results of these experiments were consistent with previous reports (Peterson *et al.*, 1984; Robbins and Pharr, 1988; Townend and Dickinson, 1995; Whitfield *et al.*, 1996) in that large differences existed among pot sizes in shoot dry weight of well-watered plants at the end of the experiment (Table 1). In the maize experiment, the shoot weight of the well-watered plants grown in the 2.3 l pots was only 44% of the plants grown in the 16.2 l pots. The difference in plant size was also reflected in the difference in cumulative transpiration amounts among well-watered plants grown in pots of differing sizes (Fig. 1). This difference in plant size appeared to develop during the 24 d of the experiment because at the beginning of the drying cycle the maize plants in all the pots were nearly the same size and growth stage (V5, all plants). The transpiration rate of the well-watered plants was also equivalent among the various pot sizes on the initial days of the experiment (Fig. 1).

Table 1. *Mean total water transpired and the final shoot dry weight for plants in the well-watered treatment for both maize and soybean over various pot sizes*

Pot size	Total water transpired (g)	Shoot dry weight (g)
Maize		
2.3 l	4941 a[a]	20.7 a
4.1 l	5926 a	29.9 b
9.1 l	7795 b	43.9 c
16.2 l	8609 b	46.7 c
Soybean		
4.1 l	4593 a	16.5 a
9.1 l	6555 b	23.1 b
16.2 l	9920 c	36.9 c

[a]Means within columns with the same letter are not significantly different at the 0.05 level of probability using Tukey's honestly significant difference test.

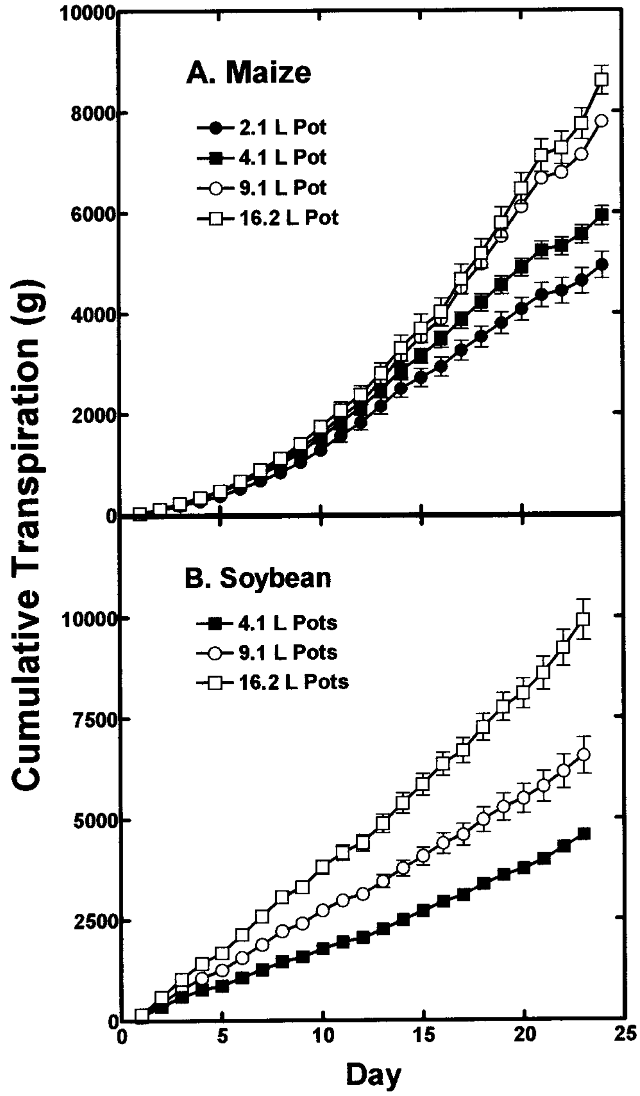

Fig. 1. Cumulative transpiration of control plants (continuously well-watered) in the maize and soybean experiments separated by pot size. Points are means ±SE ($n=3$).

Similar to maize, the final shoot weight of well-watered soybean plants grown in the 4.1 l pots were only 45% of those grown in 16.2 l pots (Table 1). This difference in shoot weight was consistent with the differences among pot size in cumulative transpiration (Fig. 1). In contrast to maize, however, the influence of pot size was already being expressed by the time the drying cycle was initiated at 34 d after sowing. This was apparent in the transpiration data recorded on the first days of the experiment when all plants were essentially still well-watered. Over the first 4 d of the experiment, plants in the 4.1 l pots averaged only 56% of the transpiration rate of the 16.2 l pots and those in the 9.1 l pots 79% of those in 16.2 l pots (Fig. 1). Vegetative growth stage data indicated that plants in the 4.1 l pots (average V-stage, 7.6) were slightly, but significantly ($P<0.05$) different than those in the 16.2 l pots (average V-stage, 8.5).

Not surprisingly, the size of the pots had a large influence on the amount of water available for transpiration. The total transpirable soil water for each pot size corresponded closely with the volume of the pot (Table 2). Because of this large difference in transpirable soil water, there was a large difference in the rate at which the *FTSW* decreased in each pot size (Fig. 2). The smaller pots dried much more quickly and these plants were subjected to a fairly rapid imposition of water-deficit stress (Fig. 2).

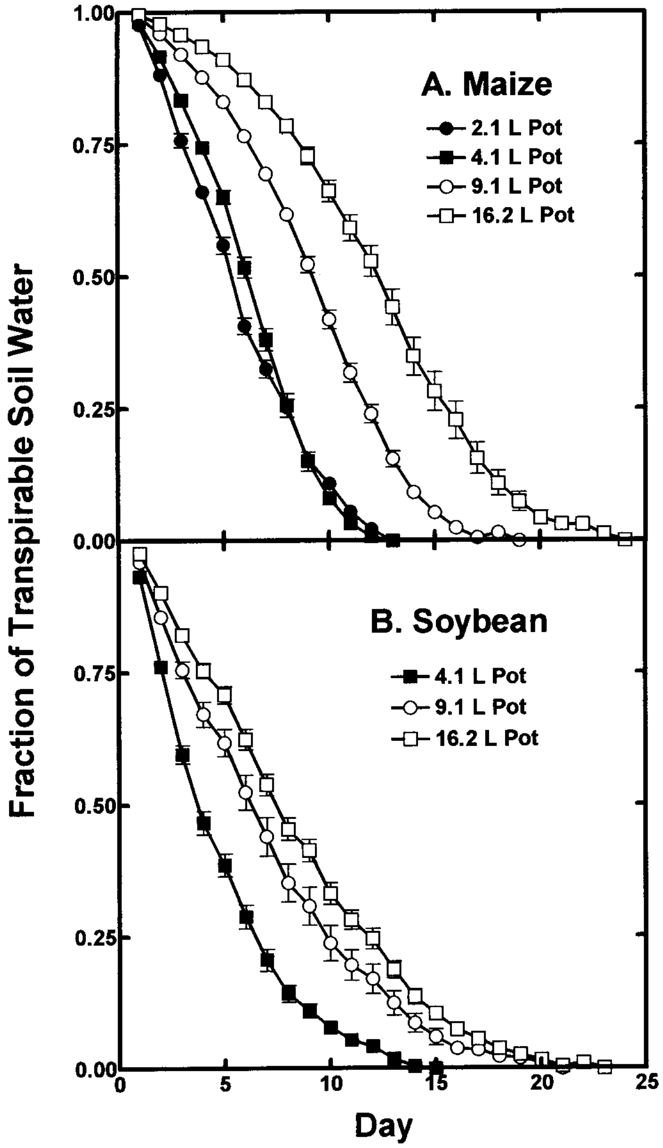

Fig. 2. *FTSW* plotted against time for maize and soybean plants in the water-deficit regime separated by pot size. Points are means ±SE ($n=6$, except $n=7$ for the maize 4.1 l pot size).

For the very smallest pot volume of 2.3 l in the maize experiment, the addition of water was essential to avoid extremely rapid soil dehydration. By additions of water to the 2.3 l pots, the rate of soil drying was slowed so that rate of *FTSW* decrease was similar to that in the 4.1 l pots (Fig. 2A). By the end of the experiment, the total water transpired from the 2.3 l and 4.1 l pots were equivalent (1217 g for the 2.3 l pot and 1239 g for the 4.1 l pot).

The primary objective of this research was to determine if pot size had an effect on the relationship between transpiration and soil water content. *NTR* was the basis for expressing relative transpiration rate, and *FTSW* expressed the relative soil water content. The plots of *NTR* against *FTSW* for both maize and soybean showed a similar pattern that was independent of pot size (Fig. 3). This pattern was consistent with the commonly reported

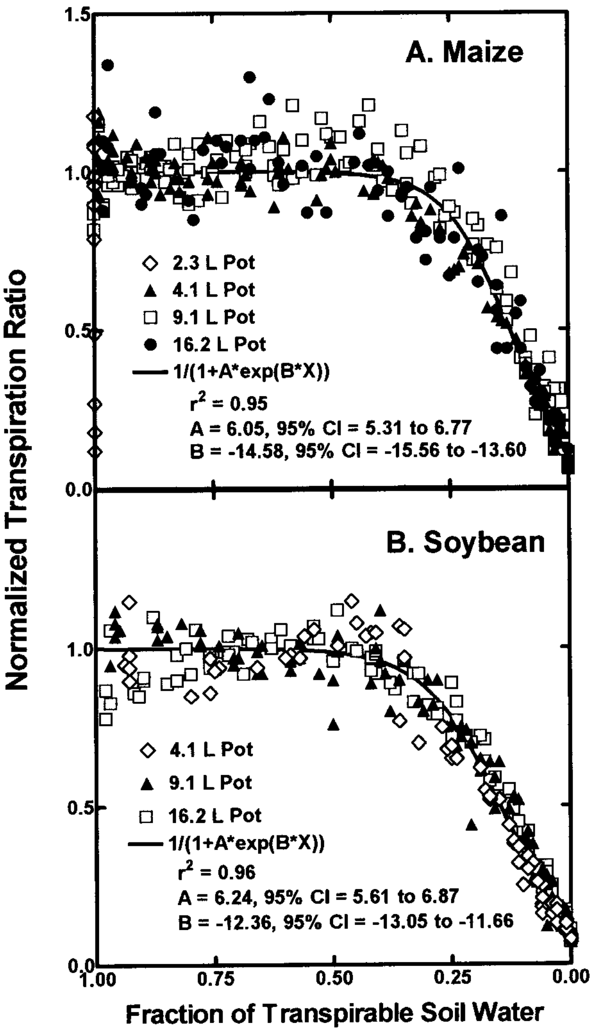

Fig. 3. *NTR–FTSW* response curve of maize and soybean plants in the water deficit regime. Symbols represent daily values for each individual pot, data from all pot sizes are presented. The solid line represents the composite fit of all data to the equation of Muchow and Sinclair (1991).

pattern of transpiration response to decreases in soil water content (Weisz *et al.*, 1994). In these experiments, there was no difference across pot size in the fact that *NTR* was constant until *FTSW* decreased to about 0.30 to 0.35, and then there was a uniform decrease in *NTR* until *FTSW* reached zero.

Calculation of the *FTSW* threshold for decline in transpiration by the plateau regression technique showed no significant difference across pot size within either plant species (Table 2). The calculated thresholds among the four pot sizes tested with maize ranged from 0.27 to 0.35 and among the three pots sizes tested with soybean ranged from 0.33 to 0.37. The important result of these experiments is the demonstration that pot size did not influence the response of transpiration to soil water content. The stability in response independent of pot size helps to explain the apparent universality observed in transpiration response to relative available soil water.

The data on the *FTSW* threshold at which transpiration began to decline (Table 2) allow a comparison between maize and soybean. The mean of the *FTSW* threshold for maize (0.31) was significantly different from that of soybean (0.35) based on non-overlapping 95% confidence intervals. Since these differences are actually quite small and it has been demonstrated that there are significant differences in the threshold among maize genotypes (Ray and Sinclair, 1997), it would be premature to conclude that important differences actually exist between these two species.

Equation (3) proposed by Muchow and Sinclair (1991) to describe the response of *NTR* to *FTSW* for maize also fits the data obtained across pot sizes in these experiments ($r^2=0.95$ for maize and $r^2=0.96$ for soybean). Individual curves for each pot size showed no differences based on overlapping 95% confidence intervals of the coefficients. In comparing species using Equation (3), the 95% confidence intervals for coefficient A overlapped which indicates that there was no significant difference. On the other hand, the 95% confidence interval for coefficient B in Equation (3) did not overlap, indicating a significant difference between maize and soybean. Again, the relatively small difference in this coefficient indicates that caution is needed before concluding that species differences exist.

In summary, the results from these soil drying experiments demonstrated that despite the large effect of pot size on plant growth, there was no significant effect of pot size on the overall relationship between *NTR* and *FTSW* within a species. Specifically, no significant effect of pot size on the *FTSW* threshold at which transpiration begins to decline was detected for either maize or soybean. These data are consistent with a general description of plant transpiration responses to soil drying across a wide range of conditions based on the fraction of extractable soil water.

Table 2. *Mean transpirable soil water* (TSW), *number of days to the endpoint* (NTR ≤ 0.10) *of the drying cycle, final shoot dry weight, and* FTSW *point at which transpiration begins to decline for both maize and soybean over various pot sizes*

Means are for six plants of each pot size (seven plants for the 4.1 l pot size of maize).

Pot size	*TSW*[a] (g)	Days to endpoint (d)	Shoot dry weight (g)	*FTSW* point of transpiration decline
Maize				
2.3 l	793 a[b]	13 a	7.2 a	0.32 a
4.1 l	1239 b	12 a	8.2 a	0.30 a
9.1 l	2724 c	17 b	15.9 b	0.35 a
16.2 l	5021 d	23 c	26.4 c	0.27 a
Soybean				
4.1 l	1432 a	14 a	7.5 a	0.37 a
9.1 l	3455 b	20 b	15.2 b	0.35 a
16.2 l	5725 c	21 b	22.7 c	0.33 a

[a]Except for the 2.3 l pot, *TSW* reflects the total water transpired. During the dry down water was added to the 2.3 l pots bringing the total water transpired to 1217 g.

[b]Means within columns with the same letter are not significantly different at the 0.05 level of probability using Tukey's honestly significant difference test.

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