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ORIGINAL ARTICLE

Pot binding as a variable confounding plant phenotype: theoretical derivation and experimental observations

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Abstract

Main conclusion **Theoretical derivation predicted growth retardation due to pot water limitations, i.e., pot binding. Experimental observations were consistent with these limitations. Combined, these results indicate a need for caution in high-throughput screening and phenotyping.**

Pot experiments are a mainstay in many plant studies, including the current emphasis on developing high-throughput, phenotyping systems. Pot studies can be vulnerable to decreased physiological activity of the plants particularly when pot volume is small, i.e., "pot binding". It is necessary to understand the conditions under which pot binding may exist to avoid the confounding influence of pot binding in interpreting experimental results. In this paper, a derivation is offered that gives well-defined conditions for the occurrence of pot binding based on restricted water availability. These results showed that not only are pot volume and plant size important variables, but the potting media is critical. Artificial potting mixtures used in many studies, including many high-throughput phenotyping systems, are particularly susceptible to the confounding influences of pot binding. Experimental studies for several crop species are presented that clearly show the existence of thresholds of plant leaf area at which various pot sizes and potting media result in the induction of pot binding even though there may be no immediate, visual plant symptoms. The derivation and experimental results showed that pot binding can readily occur in plant experiments if care is not given to have sufficiently large pots, suitable potting media, and maintenance of pot water status. Clear guidelines are provided for avoiding the confounding effects of water-limited pot binding in studying plant phenotype.

Keywords Leaf area development · Pot volume · Soil medium · Soil water · Stomatal conductance · Water limitation

Introduction

Controlled environment studies of plants are almost always done in pots. What is often overlooked is that the growth and development of plants can be altered by what is commonly labeled as "pot binding" (Nesmith and Duval 1998; Poorter et al. 2012). For example, direct measures of physiological response to pot binding showed downregulation of photosynthesis rate of pot-bound plants (Ronchi et al. 2006; Kasai et al. 2012). Importantly, recent research has now shown that phenotypic comparisons among genotypes can be quite sensitive to pot size. Bourgault et al. (2017) compared the growth of four genotypes of wheat (*Triticum aestivum* L.) grown in 7.5-L columns (10 cm diameter, 100 cm tall) and in 1.4-L pots (13.7 diameter at the top, 14.0 cm tall). The potting media was a mixture of 50% peat and 50% sand. The pots were watered daily. Observations on development and growth

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showed inconsistencies in the genotypic comparisons between the two pot sizes. Dambreville et al. (2017) documented a large number of plant properties for three genotypes of oilseed rape (*Brassica napus* L.) grown in either 6-L pots (20 cm diameter, 24 cm tall) or 0.22-L pots (7 cm diameter, 10 cm tall). A mixture of 50% loamy soil and 50% organic compost was used in this experiment. Watering of the well-watered pots was done daily. Ranking among genotypes for several plant characteristics, including total plant dry mass, was different between the two pot sizes. These results, therefore, highlighted the potential for a major confounding of results for phenotypic comparison due to pot size. This may be a particular problem in high-throughput phenotyping approaches in controlled environments where there is pressure to maximize the number of plants being studied by minimizing pot size.

Unfortunately, no fundamental guidelines exist on requirements to avoid pot binding. Poorter et al. (2012) offered an empirical recommendation to avoid pot binding based on a meta-analysis of 65 studies. They suggested that the ratio of plant mass to pot volume should not generally exceed $1\ \mathrm{g\ L^{-1}}$. While this empirical guideline is useful, understanding a fundamental basis of pot binding and developing a quantitative description about the specific conditions that result in pot binding would offer direct guidance in designing experiments. However, such a fundamental description of pot binding does not exist although several possibilities have been advanced including those based on hormones (Ismail et al. 1994) and nutritional (Hess and De Kroon 2007) status of the plant. Poorter et al. (2012) suggested water availability might be a limitation but did not describe a quantitative basis to parameterize water balance on pot binding.

In this paper, the "water availability" hypothesis is explored and shown to be a strong quantitative candidate to describe pot binding. First, a quantitative description of pot binding based on pot water balance is derived. The two key features of pot water balance are (1) amount of water contained in the potting media that can be readily extracted by plants, and (2) amount of water extracted by plants dictated by plant size and atmospheric vapor density deficit. Second, experimental results are presented showing the leaf area threshold for pot binding on a daily basis based on decreasing plant vapor conductance for transpiration. Results from a series of experiments involving several crop species and a range of pot sizes are presented to document the circumstances when vapor conductance of plants is limited. These studies clearly showed threshold plant leaf areas depending on pot volume and potting media when vapor conductance is decreased, i.e., potting binding.

Pot-binding derivation

Pot transpirable water

The amount of extractable soil water from the potting medium is finite and clearly linked to the volume of the potting media. Often, the amount of extractable water available in a pot to support transpiration, CO_2 assimilation and plant development appears to be overestimated. One error in this overestimation is that it is assumed that much of the water in the media at water contents below "pot capacity" is freely available to plants. In fact, only a portion of the water in the potting media is available to support physiological activity. Ratliff et al. (1983) showed for many natural soils that the volumetric soil content of extractable water by plants is approximately $0.13\ \mathrm{cm^3\,cm^{-3}}$ over a wide range of agricultural soils. They found the extremes of extractable soil water were limited to the range from $0.08\ \mathrm{cm^3\ cm^{-3}}$ for sand and $0.15\ \mathrm{cm^3\ cm^{-3}}$ for silt. In all cases, the amount of soil water available to plants from the potting media can be determined for each potting mixture, and the hypothesis that limited water availability may impose a water limitation on plant activity is explored in the following derivation. All variables used in the derivation are defined in Table 1.

In this analysis, the amount of water in the soil that is available to support stomatal gas exchange is labeled as total transpirable soil water (TSW, $\mathrm{cm^3}$). The value of TSW is generally similar to extractable soil water, which is based on assumptions about the water potential endpoints for soil water availability. The TSW in a pot is defined simply as the soil volume explored by roots (SV, $\mathrm{cm^3}$), multiplied by the fraction of volumetric available soil water (VASW, ~0.13 as described above). Given that the focus of pot binding is on small pots in which roots can readily explore the full soil volume, SV usually represents pot volume although this assumption does not necessarily limit the derivation.

$$\mathrm{TSW} = \mathrm{SV} \times \mathrm{VASW} \tag{1}$$

However, it is often overlooked in pot studies that only a portion of the total extractable water from the soil medium is readily available to the plant. It is well established that only during the initial, irrigated stages of the potting media is water freely extractable by most plants. In their review of soil water extraction, Sadras and Milroy (1996) showed that under the wetter soil water conditions water was freely extracted by plants, but when the fraction of available volumetric water content decreased to the range of 0.25–0.40 there was a limitation of transpiration rate and leaf development rate (Sadras and Milroy 1996). The response of transpiration rate to soil drying is illustrated in Fig. 1a for a cowpea genotype

Table 1 List of acronyms of variables used in the derivation to describe pot binding based on water limitation

Acronym	Description	Unit
$AW_{threshold}$	Available water in soil above threshold to support uninhibited gas exchange	cm^3
FTSW	Fraction of transpirable soil water	–
g_p	Plant vapor conductance	cm day^{-1}
LA	Leaf area	cm^2
SV	Soil volume	cm^3
TA	Daily transpiration amount	cm^3 day^{-1}
TH	FTSW threshold for decline in physiological activity (e.g., transpiration, leaf area expansion)	–
TSW	Total transpirable soil water	cm^3
VASW	Volumetric soil water to available plants	cm^3 cm^{-3}
VDD	Vapor density deficit	cm^3 cm^{-3}

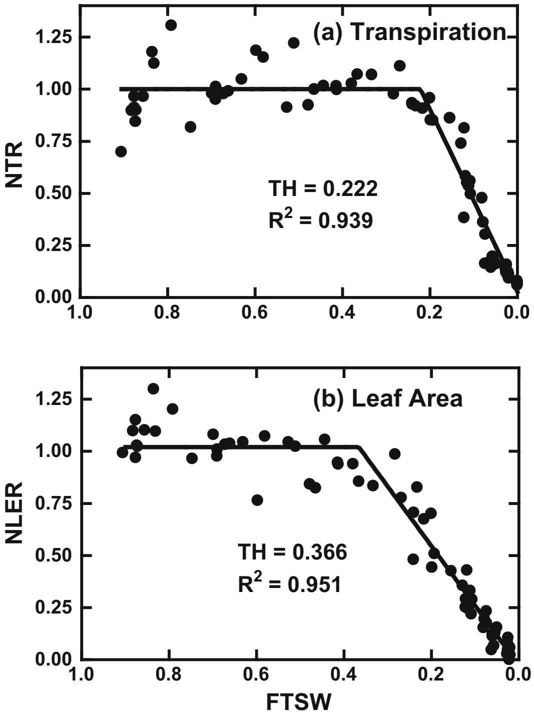

Fig. 1 Plots during a soil drying experiment in which daily observed **a** normalized transpiration rate (NTR) and **b** normalized leaf expansion rate (NLER) are plotted against fraction of transpirable soil water (FTSW) remaining in the soil. The results are for the cowpea cultivar IT89KD-288 (Manandhar et al. 2016)

by plotting as a function of fraction of transpirable soil water (FTSW), which is based directly on the volumetric water available in the soil to support plant transpiration. In this example in which the data were represented by two linear segments, the decrease in transpiration rate was initiated at FTSW equal to 0.22. It should be noted that at the initiation of the decline in transpiration rate the plants did not exhibit decreased water status, i.e., relative water content or leaf water potential, nor visible signs of wilting, but indeed a decrease in vapor exchange by the plants had been induced. Interestingly, Ray and Sinclair (1998) found over a range of pot sizes from 2.1 to 16.2 L with maize (*Zea mays* L.) and soybean [*Glycine max* (Merr.) L.] that while plant growth in the small pots was less than half of that in the large pots, the basic response of transpiration rate to decreasing FTSW as illustrated in Fig. 1a was unchanged.

In addition, at or before the threshold for transpiration decrease, there is an initiation of a decrease in leaf area development (Sadras and Milroy 1996). In this cowpea genotype, the initiation of decline in leaf area development occurred at FTSW equal to 0.37, which indicates another potentially important aspect of pot binding (Fig. 1b). Therefore, pot binding as reflected in early decrease in leaf area development may be the first indication of water-limited pot binding.

Only water in the pot above the thresholds for decline in transpiration and leaf area development as illustrated in Fig. 1 allows uninhibited plant growth. Therefore, the FTSW threshold (TH) at which there is a decrease in transpiration and leaf area development is crucial in defining the water available to support uninhibited gas exchange ($AW_{threshold}$, cm^3), and the associated cascade of limited physiological processes. The calculation of the amount of water to allow uninhibited transpiration (Fig. 1a) or leaf area expansion (Fig. 1b) can be calculated from Eq. (1) multiplied by (1-TH).

$$AW_{threshold} = SV \times VASW \times (1 - TH) \qquad (2)$$

In comparisons of genotypes, genetic variations are commonly observed in the threshold for the decline in transpiration rate and leaf area development (Gholipoor et al. 2013; Devi et al. 2013; Sinclair et al. 2015). As indicated in Eq. (2), such variation in TH clearly can result in genotypic differences in expression of pot binding.

In addition, the dependence of TH on the potting media is an important consideration. The TH values described previously were for plants growing on mineral soil. However, artificial potting media, which are very commonly used in pot studies, results in expression of very different TH values. Potting medium with substantial sand content causes VASW to be relatively low (Sinclair et al. 1998), and this low water-holding capacity makes an experiment more vulnerable to develop water deficiency. Of special concern are artificial potting medias that are mixtures of materials such as perlite, vermiculite, sphagnum peat moss, and tree bark. These media have special water-holding characteristics so that water is extracted by plants slowly over longer time periods, and hence, avoid visual wilting symptoms. One consequence is that TH values of these media are especially high. Wahbi and Sinclair (2007) found that a potting mixture containing vermiculite and sphagnum moss, which is quite common in many pot studies, exhibited a very high TH value of about 2/3. That is, plants grown on such artificial media can have decreased plant gas exchange very early in the potting media drying cycle, and consequently the induction of pot binding.

Transpiration

The second key feature of the pot water balance is the amount of cumulative plant transpiration. Pot binding will be induced whenever soil water content decreases to the threshold where transpiration rate and leaf development rate are inhibited, as illustrated in Fig. 1 for a soil drying experiment with cowpea (*Vigna unguiculata* L.) (Manandhar et al. 2016). For pots that are watered once a day, the daily transpiration loss relative to $\mathrm{AW}_{\mathrm{threshold}}$ is critical in assessing pot binding. Daily plant transpiration loss is dependent on the atmospheric humidity around the plant and the leaf area of the plant. The driving gradient for plant water loss is the difference in humidity between the leaf and the atmosphere. A large vapor gradient, i.e., dry air of low humidity, will result is more rapid exhaustion of water for pots due to high transpiration rates. For pot studies in controlled environments, low-humidity conditions may exist since temperature regulation is often achieved by flowing air over cooling coils, which may be sufficiently cold to result in vapor condensation.

The second component of plant water loss is plant leaf area. Plants with larger leaf area are likely to have greater water loss rates, and more likely suffer pot binding. Therefore, as plants develop greater leaf area in an experiment, the potential for pot binding increases.

Expression of daily transpiration amount (TA, cm^3 day^{-1}) for a potted plant can be approximated as a multiplication of the plant vapor conductance (g_p, cm day^{-1}) for the photoperiod, plant leaf area (LA, cm^2), and air humidity. The following equation is used based on expression of air humidity as vapor density deficit (VDD, $\mathrm{cm}^3\ \mathrm{cm}^{-3}$), which can be obtained directly from air vapor pressure deficit (VPD, kPa) and temperature (*T*, °K) such that VDD = 0.0022VPD/T.

$$\mathrm{TA} = g_\mathrm{p} \times \mathrm{LA} \times \mathrm{VDD} \tag{3}$$

Pot binding

Based solely on the potting media water balance, pot binding would occur whenever the amount of water required for daily unrestricted transpiration (TA) exceeds the amount of water that was freely available in the soil, that is, pot binding is hypothesized on a daily basis whenever $\mathrm{TA} > \mathrm{AW}_{\mathrm{threshold}}$. This criterion assumes a daily watering of pots, which may be overcome by more frequent watering of pots than once a day. Therefore, setting Eqs. (2, 3) equal allows calculation of the circumstances under which pot binding might be initiated.

$$\mathrm{SV} \times \mathrm{VASW} \times (1 - \mathrm{TH}) = g_\mathrm{p} \times \mathrm{LA} \times \mathrm{VDD} \tag{4}$$

Equation (4) can be rearranged to define the limit for vapor conductance.

$$g_\mathrm{p} = \mathrm{SV} \times \mathrm{VASW} \times (1 - \mathrm{TH})/(\mathrm{LA} \times \mathrm{VDD}) \tag{5}$$

Only under conditions when the right-hand side of Eq. (5) exceeds g_p is there no water limitation, i.e., pot binding. Sufficiently large pot size, i.e., large SV, low TH, small LA, and low VDD are required to avoid pot binding.

Another useful expression from rearranging Eq. (4) is to define a threshold leaf area where pot binding would be initiated ($\mathrm{LA}_{\mathrm{pb}}$, cm^2), that is,

$$\mathrm{LA}_{\mathrm{pb}} = \mathrm{SV} \times \mathrm{VASW} \times (1 - \mathrm{TH})/\left(g_\mathrm{p} \times \mathrm{VDD}\right) \tag{6}$$

Experimental materials and methods

A series of experiments were done with four crop species of different plant types: maize, soybean, wheat and cowpea to determine if pot binding as reflected in g_p can be observed. The basic experimental design was the same across experiments. Plants were grown in controlled environment chambers in which temperature was regulated at a constant value and VDD was regulated using humidifiers and dehumidifiers placed in the chambers. The photosynthetically active radiation in these chambers was 550–600 µmol $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. The plants were watered daily in all experiments.

After an initial growth of 2–3 weeks, measurements were initiated on daily transpiration and plant leaf area. Soil evaporation was prevented by either sealing the top of the pots, or placing foil or mulch on the soil surface. Daily transpiration amount was obtained by measurements of changes in pot weight between consecutive days. The daily transpiration amounts were used to determine the amount of pot watering to return the pots to a well-watered condition on each day. Nondestructive measures of leaf area were done by documenting daily changes in leaf dimensions. Allometric relationships were developed for each species and genotype to relate leaf dimensions to the area of individual leaves.

Vapor conductance was calculated daily for each plant essentially using Eq. (3), that is, daily conductance was equal to transpiration amount divided by plant leaf area and vapor density deficit of the atmosphere in the controlled environments during the photoperiod.

Maize

Maize was studied in two experiments performed in walk-in growth chambers. The photoperiod was 16 h and the temperature was 32 °C/27 °C for the light and dark periods, respectively. The VDD during the photoperiod was 19×10^{-6} cm^3 cm^{-3}. In the first experiment, 1.8-L pots were used filled with a sandy loam soil (69% sandy 18% silt, and 13% clay). Eight lines of maize were tested representing a diversity of genetic backgrounds. The plants were grown in a growth chamber photoperiod temperature at 32 °C and a VDD of 22×10^{-6} cm^3 cm^{-3}. In the second experiment, 9-L pots were used and the soil media was an artificial one containing vermiculite and sphagnum moss. Two maize lines were tested in this experiment. The data for all genotypes were combined in each experiment.

Soybean

Three experiments were done with soybean. In the first experiment, the plants were grown in a growth chamber in 2.7-L pots filled with the same sandy loam soil as used in the maize experiment, but the soil was amended with sand to alter the texture to 77% sand, 14% silt, and 9% clay. During the first experiment the photoperiod was 12 h and the 12-h dark period was interrupted for 3 h using only incandescent bulbs. The temperature was 29 °C/25 °C for the light and dark periods, respectively. The VDD during the photoperiod was approximately 16×10^{-6} cm^3 cm^{-3}. Six genotypes were included in the test and the data were combined in the regression analysis.

The second and third experiments were done using sand. For both experiments, temperature was 30/26 °C for the light/dark periods. The photoperiod in each experiment was 12 h and the dark period included a 3 h interruption. The VDD during the photoperiod in the second experiment was 25×10^{-6} cm^3 cm^{-3} and in third experiment 24×10^{-6} cm^3 cm^{-3}.The pot volume differed between these two experiments with the second experiment having a volume of 2.7 L and the third having a volume of 3.5 L. Two genotypes were tested in each experiment and these data were combined.

Wheat

Wheat plants were grown in a glasshouse in 3.1-L pots filled with a composted mixture derived from 100% organic matter. Fertilizer was added to the compost mixture. The temperature in the glasshouse was controlled to a 16-h photoperiod with temperature at 29.5 °C during the light period and 23.5 °C during the dark period. The VDD during the photoperiod was 18×10^{-6} cm^3 cm^{-3}. Twenty-three bread wheats representing 120 y of cultivar selection were tested. Data from all cultivars were combined in the analysis for pot binding.

Cowpea

Cowpea plants were gown in a growth chamber in 4-L pots filled with the same sandy loam soil used in the maize experiments. The plants were grown in a growth chamber with a photoperiod of 16 h and temperature of 30 °C during the light period and 24 °C during the dark period. The VDD during the photoperiod was 18×10^{-6} cm^3 cm^{-3}. Five genotypes were tested and the results from the genotypes were combined.

Results

Equation (6) allows calculation of the maximum plant leaf area allowed before the water limitation results in pot binding. The estimate of LA_{pb} based on Eq. (6) in Fig. 2 against soil volume. In this example, VASW was set equal to 0.13 as discussed previously, daily g_p equal to 2.5×10^4 cm day^{-1} as an estimate for fully open stomata (e.g., Figure 3), and VDD equal to 20×10^{-6} cm^3 cm^{-3}. Calculations were done both for mineral soil (TH = 0.33) and an artificial soil mixture (TH = 0.67). The analysis results in a linear relationship between pot volume and LA_{pb} where limited water availability causes pot binding and limited plant growth. In those experiments in which soil volume may be only 1 or 2 L, this derivation indicates that for plants grown on mineral soil the initiation of pot binding occurs when leaf area of the plant is only 173 or 346 cm^2, respectively. The situation is even more limiting when plants are grown on artificial potting media. The artificial potting media in a 1-L pot based on this derivation

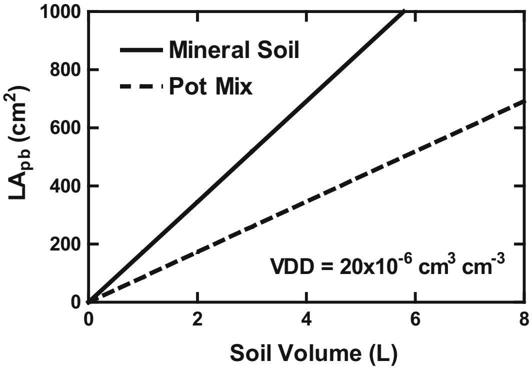

Fig. 2 Leaf area threshold for potting binding (LA_{pb}) calculated from Eq. (6) at which soil volume is limiting. Two potting media are shown: mineral soil and artificial potting mixture. These calculations were done assuming the vapor density deficit (VDD) of 20×10^{-6} cm^3 cm^{-3}

would result in pot binding for plants with a leaf area as small as 86 cm^2!

The results of all experiments showed the same general response in daily g_p to increasing plant leaf area. As shown in Fig. 3 for cowpea cultivar IT82E−18, g_p was essentially constant during the initial period of leaf area development. However, a LA_{pb} was eventually reached where water limitation resulted in a decline in g_p with increasing leaf area, as predicted by Eq. (5). In the example in Fig. 3, LA_{pb} was reached at 522 cm^2, which is approximately the predicted result as shown in Fig. 2.

The experimental results for all cases are presented in Table 2. The LA_{pb} across all experiments, including the maize experiment in 9-L pots with artificial potting mixture was 550 cm^2 or less. The value of LA_{pb} varied depending on soil volume, potting media, and the VDD during the experiment. In these experiments, pot volume and the potting media had especially large influences on observed LA_{pb}, as predicted.

Discussion

Both the hypothetical derivation and experimental results were consistent in showing that daily water limitation could readily be a possible explanation of pot binding. Both the derivation and experimental results showed also that the threshold plant response of transpiration or leaf area development to drying soil media can be critical in expression of pot binding. The water limitation was dependent on soil volume, i.e., pot size, potting media, and plant leaf area. Artificial potting mixtures with high thresholds for inducing decreases in vapor conductance (Wahbi and Sinclair 2007) can be especially problematic. The induction of pot binding as a result of artificial potting media and modest pot size clearly has the potential to readily confound phenotypic differences as observed in the ranking of genotypes in small-pot versus large-pot experiments (Bourgault et al. 2017; Dambreville et al. 2017). Even in the maize experiment with 9-L pots presented here, the LA_{pb} was still only 370 cm^2. These results both from the derivation and the experiments indicate considerable caution is needed to avoid the confounding effects of water limitation expressed as pot binding.

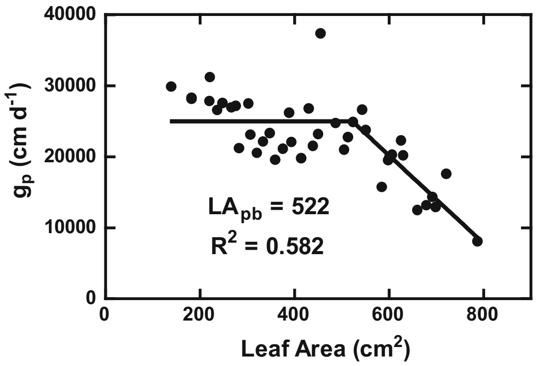

Fig. 3 Plot of daily calculated plant vapor conductance (g_p) as a function of plant leaf area during growth of well-watered cowpea genotype IT82E−18

One obvious solution indicated by the derivation to avoid pot binding is to water pots more frequently than once a day to ensure that soil water levels always remain greater than TH. The frequency of watering will depend both on pot volume and plant leaf area. In addition, these results indicate that environmental control that maintains a low vapor density deficit of the atmosphere will aid in avoiding pot binding. Higher VDD, as was the case in the soybean experiment with VDD of 25×10^{-6} cm^3 cm^{-3}, resulted in a lower LA_{pb}. On the other hand, a low VDD will allow larger LA_{pb}. Not shown in Table 2 is an experiment with cowpea in which the VDD was held at 10×10^{-6} cm^3 cm^{-3}. In this case, no LA_{pb} was observed up to the termination of the experiment with a plant leaf area of 700 cm^2.

Overall, this study offers a rather simple explanation for pot binding. Due to the limited amount of readily available water in pots, it is quite likely that with daily watering of pots there is the possibility of pot binding, especially as plants develop greater leaf area. In addition to pot volume, VDD and potting media, especially artificial potting mixtures, directly influence the susceptibility to pot binding. Variable plant traits including

Table 2 Experimentally determined mean maximum LA (LA_{pb}) across genotypes for decrease in vapor conductance for experiments involving four crop species with different pot volumes, potting media, and vapor density deficits (VDD)

Species	Pot vol. (L)	Potting media	VDD (cm^3 m^{-3})	LA_{pb} (cm^2)
Soybean	2.7	Sand	25	215
Maize	1.8	Sandy loam	22	255
Soybean	3.5	Sand	24	345
Soybean	2.7	Sandy loam	16	364
Maize	9.0	Artificial mix	19	370
Cowpea	4.0	Sandy loam	18	530
Wheat	3.1	Composted soil	18	535

Results are arranged in ascending LA_{pb}

stomatal conductance, TH, and plant leaf area are important in confounding pot results. In particular, the results presented here indicate that studies in pots designed for phenotypic comparisons can be confounded due to genotypic variability that will result from possible difference in the point of initiation of pot binding for transpiration and leaf area development. Each of the factors influencing pot binding as given in Eq. (6) must be considered carefully in setting up pot experiments to avoid the confounding consequences of limited plant growth and development due to pot binding.

Author contribution statement TS conceived the research and developed the theoretical derivation. AM, AS, P R-A, LB, RS, WS, and TR were responsible for the experiments that generated the data demonstrating pot binding.

References

Bourgault M, James AT, Dreccer MF (2017) Pot size matters revisited: does container size affect the response to elevated CO_2 and our ability to detect genotypic variability in this response in wheat? Func Plant Biol **(currently online, 8 Dec 2016)**

Dambreville A, Griolet M, Rolland G, Dauzat M, Bediee A, Balsera C, Muller B, Vile D, Granier C (2017) Phenotyping oilseed rape growth—related traits and their responses to water deficit: the disturbing pot size effect. Func Plant Biol **(currently online, 8 Dec 2016)**

Devi MJ, Sinclair TR, Beebe SE, Rao IM (2013) Comparison of common bean (*Phaseolus vulgaris* L.) genotypes for nitrogen fixation tolerance to soil drying. Plant Soil 364:29–37

Gholipoor M, Sinclair TR, Raza MAS, Loffler C, Cooper M, Messina CD (2013) Maize hybrid variability for transpiration decrease with progressive soil drying. J Agron Crop Sci 199:23–29

Hess L, De Kroon H (2007) Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination. J Ecol 95:241–251

Ismail AM, Hall AE, Bray EA (1994) Drought and pot size effects on transpiration efficiency and carbon isotope discrimination of cowpea accessions and hybrids. Aus J Plant Phyisol 21:23–35

Kasai M, Koide K, Ichidawa Y (2012) Effect of pot size on various characteristics related to photosynthetic matter production in soybean plant. Int J Agron 7, #751731

Manandhar A, Sinclair TR, Rufty TW, Ghanem ME (2016) Leaf expansion and transpiration response to soil drying and recovery among cowpea genotypes. Crop Sci **(in reviews)**

NeSmith DS, Duval JR (1998) The effect of container size. Hortech 8:495–498

Poorter H, Buhler J, van Dusschoten D, Climent J, Postma JA (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Func Plant Biol 39:839–850

Ratliff LF, Ritchie JT, Cassel DK (1983) Field-measured limits of soil water availability as related to laboratory-measured properties. Soil Sci Soc Am J 47:770–775

Ray JD, Sinclair TR (1998) The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. J Exp Bot 49:1381–1386

Ronchi CP, DaMatta FM, Batista KD, Moraes GABK, Loureiro ME, Ducatti C (2006) Growth and photosynthetic down-regulation in *Coffea arabica* in response to restricted root volume. Func Plant Biol 33:1013–1023

Sadras VO, Milroy SP (1996) Soil-water thresholds for the responses of leaf expansion and gas exchange: a review. Field Crops Res 47:253–266

Sinclair TR, Hammond LC, Harrison J (1998) Extractable soil water and transpiration rate of soybean on sandy soil. Agron J 90:363–368

Sinclair TR, Manandhar A, Belko N, Riar M, Vadez V, Roberts PA (2015) Variation among cowpea genotypes in sensitivity of transpiration rate and symbiotic nitrogen fixation to soil drying. Crop Sci 55:2270–2275

Wahbi A, Sinclair TR (2007) Transpiration response of abrabidopsis, maize, and soybean to drying of artificial and mineral soil. Env Exp Bot 59:188–192