Review

Pot size matters: a meta-analysis of the effects of rooting volume on plant growth

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Abstract.   The majority of experiments in plant biology use plants grown in some kind of container or pot. We conducted a meta-analysis on 65 studies that analysed the effect of pot size on growth and underlying variables. On average, a doubling of the pot size increased biomass production by 43%. Further analysis of pot size effects on the underlying components of growth suggests that reduced growth in smaller pots is caused mainly by a reduction in photosynthesis per unit leaf area, rather than by changes in leaf morphology or biomass allocation. The appropriate pot size will logically depend on the size of the plants growing in them. Based on various lines of evidence we suggest that an appropriate pot size is one in which the plant biomass does not exceed $1 \, g \, L^{-1}$. In current research practice ~65% of the experiments exceed that threshold. We suggest that researchers need to carefully consider the pot size in their experiments, as small pots may change experimental results and defy the purpose of the experiment.

Introduction

A large number of studies in plant biology focus on gene expression, physiology or biomass production of individually-grown plants. To this end, experiments are often conducted on plants grown in some kind of container, from here on referred to as 'pots'. Pot size has received special attention in forestry (Carlson and Endean 1976) and horticulture (Kharkina *et al*. 1999), where commercial companies can profit from choosing the smallest pot volume that still delivers a product with an appropriate quality. Occasionally, the issue of pot size has received attention in other fields of plant biology. Arp (1991), for example, emphasised that pot size might be an important issue in experiments that considered the effect of elevated CO_2 on plants; and recently, a discussion in the field of ecophysiology has emerged, where studies on the recognition of roots of neighbours are thought to be confounded by pot size (Hess and De Kroon 2007).

Apart from the fields mentioned above, pot size seems to have received little consideration in the scientific literature and is regulary not reported in the materials and methods section of publications. Nonetheless, it is an important issue. In most laboratories there is large demand for growth chamber facilities and the use of small pots generally implies more experiments or increased replication. Space is probably less of an issue in most glasshouses. However, the plant biology community currently makes a great effort to develop automated systems for plant phenotyping (Granier *et al*. 2006; Nagel *et al*. 2012). These high-throughput systems allow for the handling of many plants, which automatically implies that spatial demands on growth facilities will increase. The use of small pots has the additional advantage that it does not exceed the capabilities of the transport robots to move the weight of pot plus plant.

The use of small pots for research purposes may also have disadvantages, which are more related to biological constraints. A small container implies a small quantity of soil or other substrate and thereby, almost invariably, a reduction in the availability of water and nutrients to the plant. In addition to reduced resource availability, pots generally impede root growth. Many species easily produce roots of more than 1 m in length (Jackson *et al*. 1996), even at a relative young age (Drew 1975; Fusseder 1987), thereby exceeding the dimensions of most containers used. Large plants in small pots may have a large fraction of roots 'pot-bound', with all kind of secondary consequences (Herold and McNeil 1979). In this paper we explore the consequences of the choice of pot size for plants studied for experimental purposes. We first analysed in a quantitative way to what extent pot size affects plant growth in the studies that explicitly considered this factor. We did so by a meta-analysis of 65 studies reported in the literature. Second, we reported on some of the morphological and physiological components that might explain the observed growth patterns.

Third, we analysed whether there is a threshold in the plant size : pot size relationship above which growth is affected and compared the data for the current pot-size experiments with a more general database on plant research and to plants grown in fields. Based on these comparisons we suggest what might be an appropriate pot size for a given experiment.

1. Effect of pot size on plant biomass

To analyse the effect of rooting volume on plant growth, we screened the literature of the last 100 years and arrived at a total of 65 publications where this factor was studied in pots and ~10 where rooting volume was constrained in hydroponically-grown plants (see Appendix 1). For this analysis we will focus mainly on the results for the pot-grown plants. A description of the methodological approach is given in Appendix 2. The effect of pot size has been studied in a wide range of pot volumes, with values ranging from 5 mL for a herbaceous greenhouse crop (Bar-Tal and Pressman 1996), to 1700 L for trees growing over a period of several years (Hsu *et al.* 1996). The range of pot sizes used within the various experiments was also large, varying by a factor 2 at least and a factor 35 at most. A clear example in our compilation is the experiment by Endean and Carlson (1975), who followed the growth of *Pinus contorta* Douglas over time. In the very beginning of the experiment, plants grew presumably well in all pot sizes, but after 4 weeks of growth biomass was already reduced in the smallest pot volume (Fig. 1*a*), and by the last harvest, none of the pots seemed large enough to ensure unrestricted growth.

In the full compiled data set the picture is similar: in almost all of the experiments considered plants increase in weight with every larger pot that is used (Fig. 1*b*). Most experiments fall between the dotted lines, which indicate a fixed ratio of biomass to pot volume of 2 and 100 g L^{-1}, respectively, for the lower and higher line. Only a few experiments with trees in large containers exceed the 100 g L^{-1}. As all experiments start with small seedlings, plants move over time from the bottom part of the graph upwards (cf. Fig. 1*a*). Clearly, the experiments at the upper right end of Fig. 1*b* are also the ones that were planned to last for the longest time, 2 years in case of the experiment with the largest pots (Hsu *et al.* 1996), 5 years in case of the experiment with the highest ratio (Bar-Yosef *et al.* 1988).

Theoretically, dose–response curves should level off at higher pot sizes, as in Fig. 1*a*. However, this is not the case for many of the studies plotted in Fig. 1*b*, which mostly show linear responses. We discuss this further in section 4. To scale all data to an equal change in pot size, we calculated for each species in each of the experiments the average slope of the log-transformed mass versus pot size relationship and derived from those values an easy to understand expression that indicates the percentage increase in biomass for a 100% increase in pot size (Appendix 3). On average, plants increased 43% in mass for every doubling in pot size (Fig. 2, $P < 0.001$), with no significant differences in response between herbaceous and woody species. These response values are substantial and imply that plants are likely to be over 3 times larger in 2 L pots than in pots of 0.2 L. Given these differences, it is clear that pot size should be given careful consideration during the planning phase of an experiment.
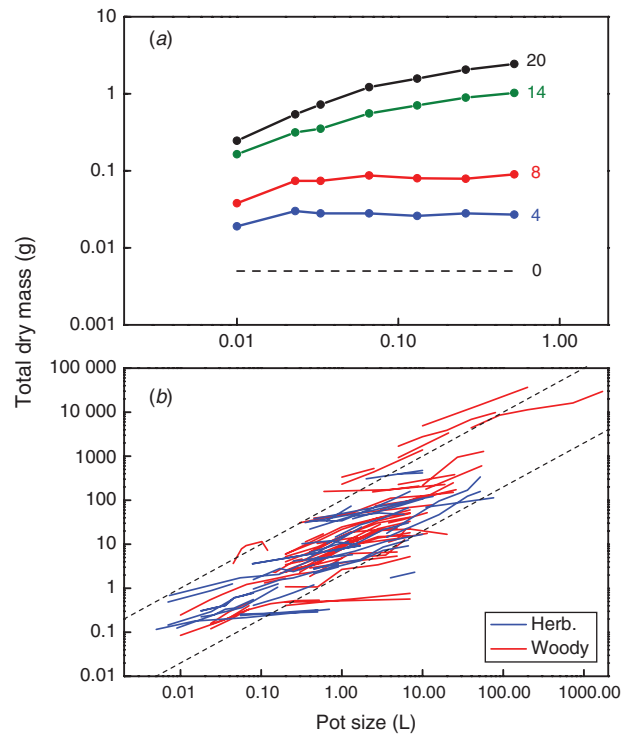

Fig. 1. (*a*) Dose–response curves of total plant dry mass of *Pinus contorta* as dependent on pot size. Plants were grown in pots with six different sizes and harvested at several times during a 20-week period. Numbers indicate the time of harvest after sowing, in weeks. Data from Endean and Carlson (1975), except for seed mass (week 0), which was taken from McGinley *et al.* (1990). (*b*) Summary of total biomass data of plants grown in pots of various sizes, as reported in the literature (65 experiments on 69 species, see Appendix 1). Each line connects the observations of one species or genotype in one experiment. Values in red indicate woody species, in blue herbaceous species. Dotted lines indicate a total plant biomass per unit pot volume of 2 (lower line) and 100 (upper one) g L^{-1}. Additional, unpublished data were obtained for Barrett and Gifford (1995) and Liu and Latimer (1995), as well as from L. Mommer and H. de Kroon, and R. Pierik.

2. Effect on components of the carbon-budget

What is the cause of the growth retardation in smaller pot sizes? As clearly shown by Endean and Carlson (1975), the effect of pot size on biomass gradually increases throughout (the later part of) the experimental period. The rate by which biomass of individual plants is accumulated is proportional to the size of the plant and conveniently described by the relative growth rate (RGR, the rate of increase in biomass per unit biomass present; Evans 1972). The differences in RGR of plants growing in different pot sizes are always smaller than the differences in biomass at the end of the experiment. This implies that the physiological and morphological factors that underlie the variation in biomass will also be affected to a smaller extent than biomass itself (Poorter *et al.* 2012*a*). We analysed growth in terms of the plant's carbon economy, using a top-down approach where the RGR is factorised into three components:

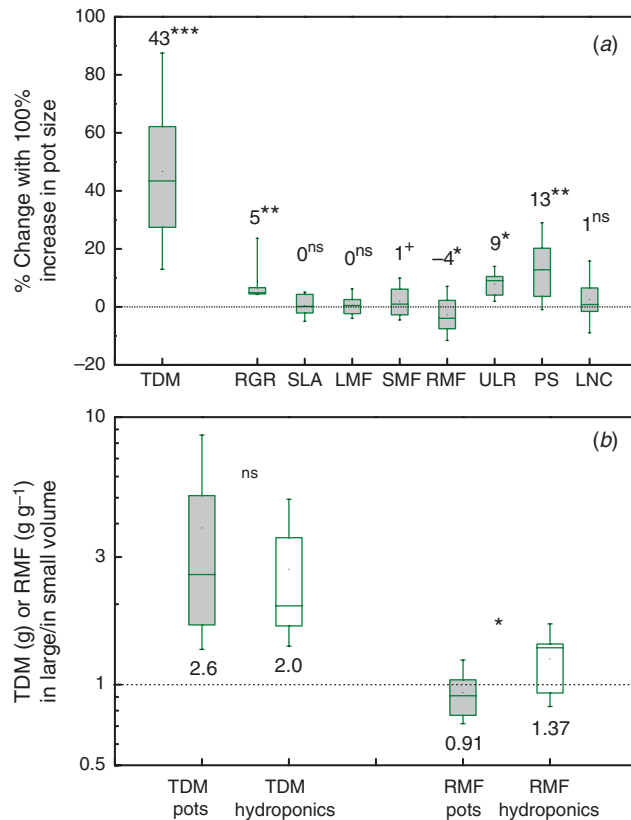$$RGR = SLA \times LMF \times ULR, \tag{1}$$

Fig. 2. (*a*) Distribution of the percentage increase in a range of growth-related traits when pot size doubles. The data are from a range of experiments described in literature (Appendix 1). The distribution is characterised by box and whisker plots, where the boxes show the 25th and 75th percentile and the whiskers the 10th and the 90th percentile. The median is represented by the line in the box and given as a number above the box plot. The total (rounded) number of species × experiment combinations on which the boxplots are based are TDM (total dry mass): 90; RGR (relative growth rate): 15; SLA (specific leaf area): 20; LMF (leaf mass fraction): 35; SMF (stem mass fraction): 30; RMF (root mass fraction): 80; ULR (unit leaf rate): 5; PSa (rate of photosynthesis per unit leaf area): 15; LNC (leaf nitrogen concentration): 15. The significance level of a test whether the observed distribution deviates significantly from 0 is given above the respective box plots: ns, not significant; $^+$, $0.05 < P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (*b*) Distribution of ratios in TDM and RMF, taken from pot size studies (values for the largest pot relative to those for the smallest pot within each experiment, $n = 65$) and studies where rooting volume was constrained for hydroponically-grown plants (ratio unconstrained/constrained, $n = 11$). The results of *t*-tests between the two groups are shown between the boxplots.

according to Evans (1972). Here, SLA denotes specific leaf area (leaf area per unit leaf dry mass; $m^2\,kg^{-1}$), LMF the relative allocation of biomass to leaves (leaf mass fraction, $g\,leaf\,g^{-1}$ plant) and ULR is a parameter that indicates the growth rate per unit leaf area (unit leaf rate, $g\,m^{-2}\,day^{-1}$). ULR is basically the net result of carbon gain through photosynthesis, corrected for the rate of respiration in the whole plant and the C-content of the newly added biomass. ULR and photosynthesis per unit leaf area are often strongly positively correlated (Poorter 2002).

A characteristic of RGR is that an *absolute* difference in RGR between treatments causes a *relative* difference in biomass over

time (Poorter and Navas 2003). However, in the case where we want to understand the reason for the difference in growth and only have fragmented information, it is more amenable to analyse the proportional differences in RGR relative to that of the three growth parameters that underlie RGR (Eqn 1). Only a few experiments report data on these underlying components, but the information we could gather points into the following direction (see Fig. 2 and Fig. S1, available as Supplementary Material to this paper): a doubling in pot size increases RGR by ~5%. Consequently, each of the growth parameters at the right-hand side of Eqn 1 may change by a very small proportion, or one of them by a somewhat larger proportion in the range of 5%. At the final harvest, SLA increased somewhat in some experiments and decreased in others. We could quantitatively compare the various experiments by calculating the percentage increase in SLA with a 100% increase in pot size and found that taken over all experiments, this variable did not deviate significantly from zero (Fig. 2). Most, but not all experiments with root restriction in hydroponics confirm this response (e.g. Carmi *et al*. 1983; Kharkina *et al*. 1999; but see Tschaplinski and Blake 1985).

Allocation patterns are more frequently reported, generally as the biomass ratio between shoot and root. We prefer a classification in at least three plant organs (Poorter *et al*. 2012*b*) and therefore use LMF, SMF and RMF, which are the fractions of total vegetative biomass invested to leaves, stems and roots respectively. The relatively scarce information indicates that LMF is not affected (Fig. 2*a*), whereas SMF increased slightly but non-significantly ($0.05 < P < 0.10$) with pot size. More information is present on RMF (or the root : shoot ratio). Reviews are not equivocal in their evaluation. NeSmith and Duval (1998) conclude that RMF often does not change with pot size. In contrast, Hess and De Kroon (2007) expect RMF to increase in larger pots. We found that over ~80 species × experiment combinations, RMF decreased on average by a small but significant extent (4% with a doubling in pot size, $P < 0.05$) We find no clear differences between woody and herbaceous plants in this respect (data not shown).

With two of the variables of Eqn 1 unaffected, we might expect that the observed differences in growth rates between plants grown in different pot sizes are caused largely by a change in net photosynthesis and hence we would expect a stimulation of ~4% in this variable. Some experiments report a more than 30% higher rate of photosynthesis with a 100% increase in pot size (Robbins and Pharr 1988; Ronchi *et al*. 2006; Fig. S1*g*). This is far more than the expected 4%. As pot size stress builds up gradually over time, results may depend on the actual timing of the measurements during the experiment. Moreover, obtained rates will also depend on the specific leaf measured and the time of the day that the measurement was taken. The growth parameter ULR is generally closely linked to the rate of photosynthesis and provides an estimate that is integrated over the whole plant and the whole growth period under consideration (Poorter 2002). A 2-fold difference is not reported in the literature (Fig. S1*f*), but the pot-size effects on ULR are significantly greater than zero and larger than for SLA and LMF (Fig. 2). The median increase in photosynthesis and ULR with a doubling in pot size is quite comparable to the median increase in RGR. Hence, we conclude that with the

limited and fragmentary evidence yet available, net photosynthesis is likely to be the process that is strongest affected by pot size and may explain best the observed pot size effect on biomass (Fig. 1*b*). Additional support comes from experiments where photosynthesis recovered quickly after plants were repotted in larger rooting volumes (Herold and McNeil 1979).

3. What mechanism could explain a reduced photosynthesis in smaller pots?

Several factors could explain the reduced rate of photosynthesis – and thereby growth – in smaller pots. A first possible explanation is that containers of smaller dimensions can be placed at a higher density, with less light available for each shoot and hence, a lower rate of photosynthesis. Although this could be the case in some of the compiled experiments, strong pot size effects on biomass and photosynthesis are generally also observed when density is specifically controlled for (e.g. Endean and Carlson 1975; Robbins and Pharr 1988; NeSmith *et al*. 1992; Climent *et al*. 2011).

In the type of experiments that we included in our meta-analysis, a smaller pot size will inadvertently decrease the total nutrient content in the pot. Low nitrogen and phosphorus availability are known to decrease photosynthesis (e.g., Sinclair and Horie 1989; Lynch *et al*. 1991) and growth and increase the root mass fraction (Poorter *et al*. 2012*b*). Thus, lower resource supply could form a plausible explanation. We calculated the response of leaf nitrogen concentrations to changes in pot size, expecting to see an increase if nutrient availability would explain the pot size effect. On average, there was a slight, but non-significant increase in leaf nitrogen concentrations with pot size (Fig. 2), suggesting that this factor cannot completely explain the observed differences in photosynthesis or growth. Similar results were found for phosphorus (Krizek *et al*. 1985). This conclusion is to a certain extent supported by observations on hydroponically-grown plants, which have decreased photosynthesis and growth (Fig. 2*b*) when the root volume was restricted, despite a continuous high supply of nutrients. However, unlike plants grown in pots, hydroponically-grown plants do not show an increased RMF when restricted (Fig. 2*b*). As increased RMF is a good indicator for nutrient stress, we presume that nutrient limitation in small pots is still a factor, although we cannot exclude possible allometric effects which could explain a larger RMF in smaller plants as well (Poorter *et al*. 2012*b*).

Water is the other commodity that may be in short supply. Small pots could negatively impact the water status of plants as they have a reduced total water holding capacity and will, therefore, dry out more quickly (Tschaplinski and Blake 1985) and at severe stress levels increase RMF (Poorter *et al*. 2012*b*). Ray and Sinclair (1998) demonstrated with their drought experiment that soil in small pots dries out faster and thereby caused more severe drought stress in plants. However, pot size does not necessarily affect stomatal conductance or leaf water potential (Ronchi *et al*. 2006) and – as for nutrients – with plants growing in hydroponics there is still a clear effect of root confinement, even though water availability is not restricted.

Besides resource availability, the temperature of the rooting volume could be affected by pot size (de Vries 1980). Pots can intercept a substantial amount of solar radiation especially in experimental gardens and glasshouses, which may increase the soil temperature at the edge and eventually in the middle of the pot if no precautions are made (Martini *et al*. 1991; Xu *et al*. 2001). Small pots have greater surface areas relative to their volume and thereby heat up more quickly. Townend and Dickinson (1995) measured 5°C higher day temperatures in 0.19 L pots compared with 1.9 L pots. Keever *et al*. (1986) suggest that the greater temperature fluctuations in small pots may explain the reduced growth of the plants. High temperatures in the pot may have several direct (respiration, root growth) and indirect (through increased microbial activity) effects on plant growth. Pot temperatures are rarely reported so it is difficult to evaluate how often temperature differences between pots contribute to reduced growth. However, given that growth reductions also have been observed in hydroponically-grown plants suggests that temperature differences alone cannot explain the observed pot effects either.

If neither nutrient or water availability nor higher temperatures can (fully) explain the pot size effects on photosynthesis and growth, it could be that root confinement *per se* may cause growth retardation, with reduced photosynthesis as a consequence. Root growth is known to respond directly to impedance. Impeded roots stay shorter whereas the initiation and growth of side branches increases (Bengough and Mullins 1991; Falik *et al*. 2005). Furthermore, Young *et al*. (1997) showed that within 10 min of increasing the impedance to root growth, leaf expansion rate is reduced. This suggests that some kind of signal may regulate shoot growth when a large proportion of the roots are impeded. The actual signal for such a response remains as yet unknown. Possibly a reduced sink strength of the root system could cause a direct negative feedback on photosynthesis (Paul and Pellny 2003). Alternatively, a specific root–shoot signal is involved (Jackson 1993).

A crucial point in the evaluation of the lastly discussed option is knowledge on the actual distribution of roots within the pots. Although the vertical root distribution is relatively easily measured (Price *et al*. 2002; Suriyagoda *et al*. 2010), analysis of the horizontal distributions is more complicated. Using non-destructive magnetic resonance imaging (MRI), we followed the root development of *Hordeum vulgare* L. and *Beta vulgaris* L. plants over time in three dimensional space. Representative nuclear magnetic resonance (NMR) images of root systems at the end of the experiment are shown in Fig. 3. We calculated the percentage of roots that was located in the inner half of the soil volume, furthest away from wall and bottom and the percentage of roots present in the outer 4 mm of the pot. Only 20–25% of the root biomass was in the inner part of the pot (Table 1), whereas ~50% was found in the outer 4 mm (20% of the total volume). The proportional distribution remained remarkably constant over time. Hence, if these observations have wider validity, we conclude that a relatively large fraction of roots is close to the edge of the pot, where unfavourable environmental conditions, for example, large temperature fluctuations and impedance of the pot wall may negatively impact growth.
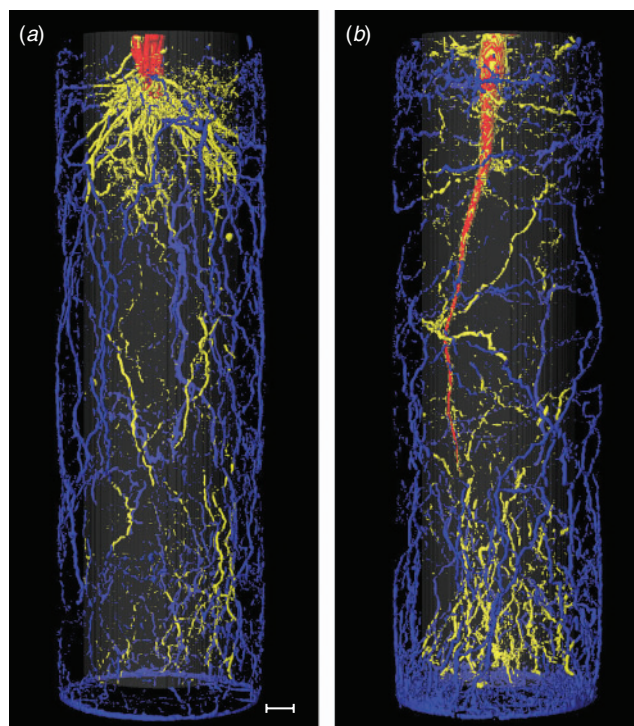
experiments last for sufficiently long time, even the largest pot size might not be large enough for unrestricted growth, i.e. the saturating part of the curve extends beyond the largest pots used. For the experimental data this implies that the relationship becomes close to linear again. In fact, many of the curves in Fig. 1b show a linear relationship. For experiments where only two pot sizes were used, it is impossible to deduce whether the response of the plants is indeed linear or not. But even in many of the other experiments in Fig. 1b no clear saturation is shown. What is the reason for that?

One objective way to relate plant and pot size across experiments is to calculate the plant biomass that is present at a given volume of rooting space. This variable, for which we use BVR as an acronym (total plant biomass : rooting volume ratio; $g L^{-1}$), has, to our knowledge, been used only by Kerstiens and Hawes (1994). BVR values vary widely and ranged in our database from as low as 0.01 in work by Climent *et al*. (2008) to over $300 g L^{-1}$ in work reported by Biran and Eliassaf (1980). The median value in the pot size experiments is around 9.5 for experiments both with herbaceous and woody species (Fig. 4b). We tested whether the BVR could explain the form of the dose–response curves in the 65 experiments shown in Fig. 1b, by calculating for each point what the slope of the dose–response curves was, as well as the BVR. In order to be consistent with Fig. 2, we derived the percentage increase in biomass with pot size doubling for these data as well. We found that very few experiments had BVR values lower than $2 g L^{-1}$ (Fig. 4b). Hence, for this part of the analysis we included not only data of the last harvest, but also data of earlier harvests where available. This may imply that not all data points in the analysis are formally independent, but it increases the power of our analysis in this crucial range. We binned all values in five BVR ranges and show the resulting distribution in Fig. 5a. Estimation of slopes is always more challenging than determining the absolute values per point and this may be one of the reasons that there is considerable variation within each category. In the category with a BVR between 1 and $2 g L^{-1}$ the effect of pot size is clearly noticeable. Pot size effects are saturating when BVR values exceed the $2 g L^{-1}$ ($P < 0.01$).

Another way to obtain a greater insight into the relationship between plant biomass and pot size is to express the biomass of

Fig. 3. (a) NMR image of a *Hordeum vulgare* plant grown in a pot with a volume of 1.3 L for 44 days. Roots in the inner 50% of the soil volume (furthest away from wall and bottom) are colour-coded yellow, roots in the outer 50% blue. The stem part that was masked from the analysis is shown in red. (b) Idem for a *Beta vulgaris* plant 48 days after sowing. The developing storage root is colour-coded red and was not included in this case.

4. When does pot size limitation starts?

In sections 1 and 2 we considered for each experiment the overall effect on plant growth, morphology and physiology when pot size was doubled. However, it is to be expected that a plant of a given size will be constrained more in a small than in a large pot. That is, young plants are initially not affected by pot size, but as plants grow older, the pot size effect becomes more pronounced, even in medium-sized pot volumes (Fig. 1a). When

Table 1. The proportion of the root mass that is present in the inner 50% of the pot volume (more than 12.5 mm from the wall or bottom) and in the space less than 4 mm from wall or bottom of the pot, for *Hordeum spontaneum* and *Beta vulgaris* plants growing in 1.3 L pots

Values are based on six plants followed over time. Time is days after sowing. Standard error of the mean was on average 1.6 and 2.9 percentage points for the inner half and outer 4 mm respectively

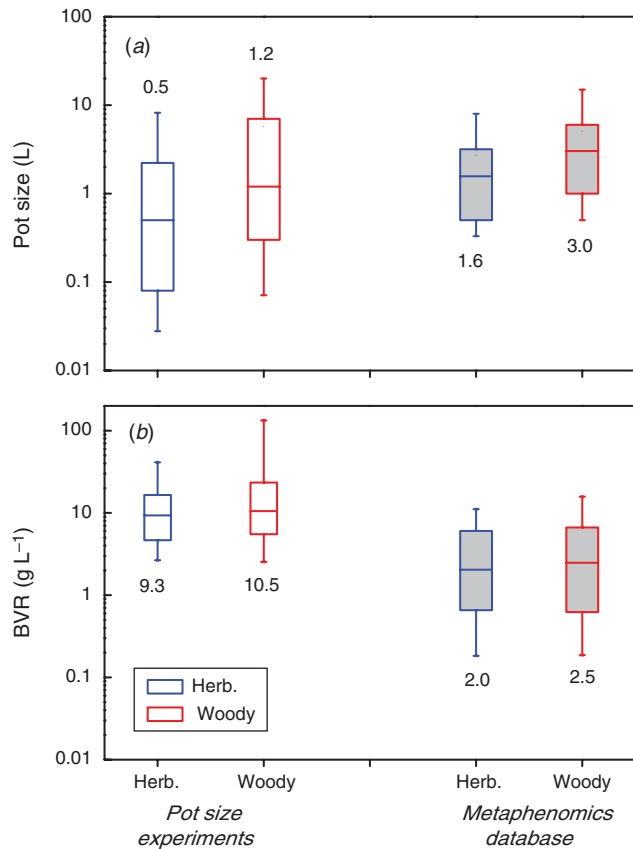| *Hordeum vulgare*			*Beta vulgaris*		
Time (days)	Root mass in inner half (%)	Root mass in outer 4 mm (%)	Time (days)	Root mass in inner half (%)	Root mass in outer 4 mm (%)
26	32.0	43.2	31	20.3	53.5
28	28.9	50.0	33	20.8	53.1
31	17.7	55.3	34	21.5	54.1
33	21.3	52.7	39	22.9	52.6
34	23.1	51.7	41	23.0	52.3
39	25.2	48.7	48	22.3	52.0
41	28.2	48.0	–	–	–
44	32.4	44.7	–	–	–

Fig. 4. (*a*) Distribution of pot volumes as represented in the current meta-analysis of pot size studies and in a compilation of ~800 studies on the effect of 12 environmental factors on growth and related ecophysiological traits (meta-phenomics database, Poorter *et al.* 2010). (*b*) Total plant biomass : pot volume ratios (BVR) in the current meta-analysis and the meta-phenomics database. The distribution is characterised by boxplots (see legend Fig. 2). Blue boxes indicate the values for herbaceous species, red ones for woody species. Numbers above/below each box show the median values.

Fig. 5. (*a*) Distribution of the percentage change in biomass with a doubling in pot size for a total of ~80 experiment × species combinations. Distributions are characterised by boxplots and given separately here for five classes of BVR (total plant biomass : pot volume ratio). The ANOVA was highly significant ($P < 0.001$), with an r^2 of 0.22. The (rounded) number of observations in the five classes are 30, 30, 90, 200 and 80 respectively. (*b*) The total dry mass of plants in various pot sizes, scaled to the biomass of the plants in the largest pot, plotted against BVR. Different lines indicate data from different experiments or different harvests within an experiment.

plants grown at various pot sizes relative to the biomass at the largest pot size and plot these values against the BVR (Fig. 5*b*). Experiments where pot sizes are limiting growth throughout the full range of pot sizes are characterised by lines that decline linearly with BVR. However, as long as biomass is not affected, the line will remain around 1 and only drop at greater BVR values when pot size starts to reduce growth. For the few experiments where this was the case, we could show that this inflection point occurred somewhere between 0.2 and $2\,g\,L^{-1}$ (Fig. 5*b*). Thus, from both the full sets of experiments compiled, as for the more detailed analyses over time, we derive that pot size effects are particularly strong when BVR values are greater than $2\,g\,L^{-1}$.

5. How do these BVR values relate to other experiments and the field?

As mentioned in section 1, the range of pot sizes used in this compilation is large. The median value is around 0.9 L (Fig. 4*a*). How does that compare to common practice in ecophysiological experiments? This will partly depend on the species studied.
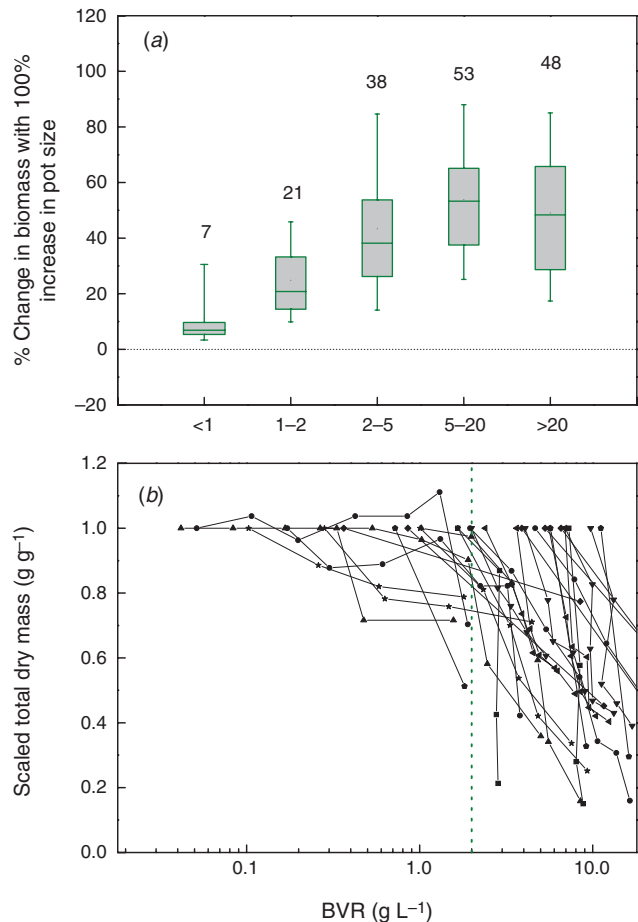
Arabidopsis, for example, is generally grown in much smaller pots (with an interquartile range of 0.08–0.21 L) than *Zea mays* L. (1.8–5.0 L). An overall impression of used pot sizes can be obtained from the metaphenomics database described by Poorter *et al.* (2010), where the response of ~900 different species to 12 different environmental factors is compiled for a total of ~800 experiments from the literature. The median pot size used in that compilation of experiments turns out to be ~2–3 times larger than those in the pot size studies (Fig. 4*a*), whereas the median BVR value is ~4-fold lower (Fig. 4*b*). Hence, we conclude that most studies on pot sizes have focussed on relatively small pots and have grown plants to larger sizes than is common in ecophysiological experiments. In contrast, experiments in plant biology generally use relatively larger pots and harvest plants at younger stages, when the BVR value is still below $8\,g\,L^{-1}$.
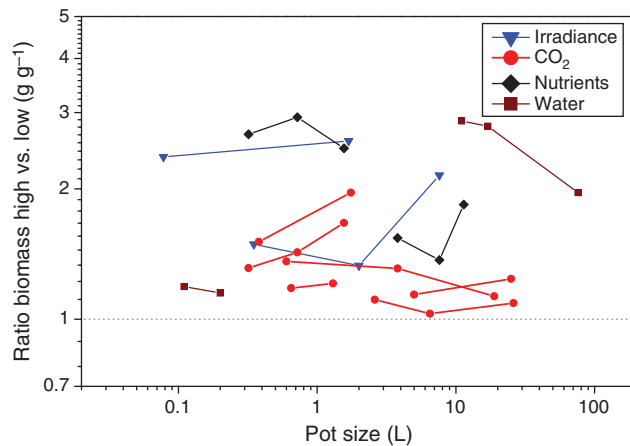
Fig. 6. Interaction of pot size with various abiotic factors. Results are given for total plant biomass and are expressed as the ratio between the biomass at a high level of that factor and the lower level. Data are from Bilderback (1985), Thomas and Strain (1991), McConnaughay *et al*. (1993), NeSmith (1993), Ismail *et al*. (1994), Nobel *et al*. (1994), Barrett and Gifford (1995), Will and Teskey (1997), Houle and Babeux (1998), Centritto (2000) and R. Pierik (unpublished data).

Most experiments with pots are conducted to eventually understand how (agro-)ecosystems function. It may therefore be relevant to consider what normal 'BVR' values are in the field. Maximum dry matter yield of major crops varies between 1000–3000 g m^{-2} (Unkovich *et al*. 2010; assuming 20% biomass in roots). If we assume a rooting depth of 1 m, this would correspond to BVR values in the range of 1–3 g L^{-1}. Given that at least half of the DM production takes place during grain filling, we can expect that during the vegetative state, the BVR value will not exceed 1.5 g L^{-1}. Similar calculations for natural ecosystems are more difficult as large variation exists in root depth and the standing biomass. Given a rooting depth of 0.35 m (Nagel *et al*. 2012) and a density of 500 plants per m^2, a field of *Arabidopsis thaliana* Heynh. plants of 0.1 g dry mass, would have a BVR of 0.15 g L^{-1}. Although we realise that plants in the field experience conditions that are very different from those where plants are grown singly in pots in controlled conditions, we conclude from these rough estimates that BVR values around 1 are of the same order of magnitude as those of vegetative plants in the field.

6. Does pot size affect experimental conclusions?

Up to now we have considered the effect of pot size *per se*. Most researchers are also interested in whether the outcome of their experiments is affected by the choice of the pot size. Arp (1991) was one of the first to draw attention to the fact that pot size might restrict the response to elevated CO_2. This would limit the possibilities to draw conclusions from experiments that have been conducted in this field.

The analysis by Arp (1991) was a compilation of different studies that worked with different pot sizes. Kerstiens and Hawes (1994), however, published a meta-analysis of the results of a range of studies with trees in which they show that the biomass responses to elevated CO_2 did not correlate with pot size, or even decreased with a BVR over 18 g L^{-1}. This suggests that small

pot sizes do not reduce responses to elevated CO_2 universally. However, in both meta-analyses the evidence could only be circumstantial, as pot size was not an experimental factor in the compilations. Here we analysed experiments where pot size was specifically included in the experimental design, not only for interaction with CO_2 but also for nutrients, water and irradiance (Fig. 6). Given that nutrient and water availability already increase when pot size is increased, we would expect less additional effect on plant growth if more nutrients or water were supplied. However, we would expect increasingly stronger growth responses with larger pot size when light or CO_2 would be increased, simply because of the higher demand for nutrients and water in larger plants. Although some of the experiments do indeed follow the expected trend, results are not equivocal. As results depend on the variability in at least four different harvests, the number of experiments is likely too small to draw any strong conclusion.

Besides possible interactions with abiotic factors, interactions with biotic factors have been shown. For example, vesicular arbuscular mycorhizae (VAM) infection rates, which would normally increase with reduced nutrient availability, are reduced in small pots (Bååth and Hayman 1984; Koide 1991). As a consequence, VAM colonisation is less beneficial for nutrient uptake and results in smaller growth increases when small pots are used (Kucey and Janzen 1987; Koide 1991). Similarly, Baldwin (1988) showed that pot-bound *Nicotiana* plants do not respond to leaf damage, whereas repotted plants do. Thus, although we cannot draw a firm conclusion here we suggest that the use of small pots with crowded roots carries a substantial risk of influencing the experimental results.

7. Other considerations

This analysis focussed on the effect of pot volume on plant growth. However, choosing a pot for an experiment not only includes choosing the right volume, but also the right shape. Although shape is less important than volume (McConnaughay *et al*. 1993), shallow and deep-rooting species may respond differently to the actual diameter and height of the pots, at equal pot volume (von Felten and Schmid 2008). Pot height is also an important factor in determining the free-draining water content of pots and thereby the water potential as well as the oxygen availability in the pots (Passioura 2006).

An alternative to standard plastic pots are containers that have ribbed inner sides and small air holes. Such containers promote self-pruning of roots close to the holes, which avoids root spiralling and promotes development of lateral roots (Rune 2003). Not only shape, but also the material (Bunt and Kulwiec 1970) and the colour of the pot (Markham *et al*. 2011) may affect plant growth, mainly through their effect on soil and root temperature. For a broader discussion on the use of pots for growing plants in the context of experimental setup see Poorter *et al*. (2012*a*).

Conclusions

A meta-analysis of the effects of pot size on growth shows that on average a doubling of the pot size results in 43% more biomass. In most cases reduced growth in small pots will be caused by a reduction in net photosynthesis. It is the plant

mass per unit rooting volume that is relevant rather than pot size *per se*. Large plant mass per pot volume not only reduces growth of plants but also carries the risk of influencing the relative differences between treatments. We conclude that it is important for researchers to minimise such effects by choosing pots that are large enough for their plants, even at later stages of growth. Our advice is to avoid plant biomass to pot volume ratio's larger than $2\,\mathrm{g\,L^{-1}}$ and preferably work with plant and pot sizes where this ratio is <1.

Acknowledgements

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Appendix 1.   List of references used for the meta-analysis

A. Pot studies

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B. Hydroponically grown plants

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Appendix 2

For this meta-analysis we screened the literature of the last 100 years. A total of 63 publications plus two additional unpublished experiments dealt with plants grown in pots of various sizes, 11 with plants grown in hydroponics with different levels of root confinement. The references are listed in Appendix 1. As we were interested in not only the physical aspect of container volume, but also in the resources that come with it, we compared pot size treatments based on size, including the possible additional benefits of increased nutrient and water availability. The experiments with hydroponically-grown plants are not included in the main analysis. Differences in the shape of the pots were not independently analysed either. In several publications only pot diameter was reported. For a sample of 30 different pots ranging in diameter (Ø) from 7 to 40 cm we derived an estimate for pot volume (V) based on the empirical equation $V = \pi(Ø/2)^2 \times (0.46 + 0.8397 \times Ø - 0.002307 \times Ø^2)$. If additional data were missing, we assumed pots to be filled with substrate up to the rim.

For each species or genotype in a given experiment, we determined the biomass at the last harvest and separated this variable in biomass of leaves, stems, roots and reproductive mass as far as data were provided. To capture the importance of pot size for growth we calculated for each experiment the proportional increase in total plant dry mass relative to the proportional increase in pot size, by calculating the slope of the lines that were fitted through the observed log-transformed plant masses and pot volumes, separately for each species in each experiment. Because experiments differed in the range of pot size used, we scaled these slopes in such a way that the number reflects the percent change in biomass (or another variable) given a doubling in pot size (see Appendix 3 for more details). For a more detailed analysis, log-transformed data from experiments which included three or more pot sizes were also fitted with a saturating equation, as described in Appendix 3 and calculated with the nls procedure in R (R Development Core Team 2011).

Appendix 3

Let V_1 and V_2 be pot volume 1 and 2 and B_1 and B_2 the total plant dry biomass that is observed at the respective pot volumes: because proportional differences are the focus of interest, we calculate the slope of the line that connects these points as:

$$S = \frac{\log(B_2) - \log(B_1)}{\log(V_2) - \log(V_1)}, \tag{1}$$

Suppose we want to know the fraction f by which plant mass increases when pot size doubles. Then

$$B_2 = f \cdot B_1, \tag{2}$$

and

$$V_2 = 2 \cdot V_1. \tag{3}$$

Assuming a common slope s over the whole trajectory of pot masses considered, this results then in

$$S = \frac{\log(fB_1) - \log(B_1)}{\log(2 \cdot V_1) - \log(V_1)} = \frac{\log(\frac{fB_1}{B_1})}{\log(\frac{2V_1}{V_1})} = \frac{\log(f)}{\log(2)}, \tag{4}$$

and, after rearrangement

$$f = 10^{s \cdot \log(2)} = (\log^{\log(2)})^S = 2^S. \tag{5}$$

The same procedure applies if slope s is determined by linear regression through more than two points.

To relate the slope of the line to the observed values of total plant mass per unit pot volume (BVR), we took the following approach: In cases where an experiment consisted of two pot sizes, a linear regression as above was calculated and the resulting slope attributed to both points. In case an experiment consisted of more than two pot sizes and a second order polynomial showed no significant saturating trend, we fitted a straight line through all points. Otherwise, a saturating curve was fitted through the points, of the form

$$B = \frac{a \cdot P + b}{P + c}, \tag{6}$$

with the constraint that a, b and c should be positive. The slope of the line at each pot size is then given by the derivative:

$$S = \frac{ac - b}{V + c}. \tag{7}$$

After the slope and f were calculated for each pot volume in each experiment, data were binned in five categories of BVR.

Appendix 4.

We measured the root distribution of *Hordeum vulgare* (barley) and *Beta vulgaris* (sugar beet), grown in a glasshouse in cylindrical containers with a volume of 1.3 L (length 26 cm, inner diameter 8 cm). Measurements were done by non-destructive imaging of roots using nuclear magnetic resonance imaging (MRI) as described extensively by Jahnke *et al.* (2009). This method is able to detect roots with a diameter down to 300 µM, which implies that fine roots go unnoticed. Image segmentation of the root system was done by thresholding the image and removing the stem and the sugar beet, with the RegionGrowingMacro module in MeVisLab (ver. 2.2.1; MeVisLab, Bremen, Germany). After segmentation, the pixel values were integrated for the inner and outer regions of the soil core and divided by the integral over the whole pot.