Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination

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Abstract

1 An increasing number of studies have shown that plants produce more root mass when sharing rooting space with an intraspecific neighbour as compared with plants growing alone. This so-called self/non-self discrimination has been suggested as a mechanism by which plants may prevent wasteful competition with their own roots and enhance their competitive ability for nutrients with roots of neighbouring plants. The overproduction of root biomass is said to result in a ‘tragedy of the commons’, because it appears to occur at the expense of reproductive biomass.

2 Studies on self/non-self root discrimination have commonly used a split-root design to distinguish self from non-self competition, while keeping the total amount of nutrients available per plant the same. This design has recently been criticized because the rooting volume differs between treatments.

3 Here, we use three general hypotheses to explain the published results without invoking the mechanism of self/non-self discrimination. The hypotheses propose that differences in root mass are due to differences in rooting volume, and differences in nutrient availability determine whole plant growth. More root mass without more growth results in less reproductive biomass.

4 A reanalysis of the results of root self/non-self discrimination confirms these hypotheses. Root overproduction in the presence of another plant, as found in nearly all studies, is consistent with effects of a larger soil volume available to these plants as compared with plants growing alone. Under the same total nutrient availability, total plant weight was the same or higher when more roots were produced. Inevitably, a larger root production with the same total biomass implies that less reproductive biomass is produced.

5 Although our analysis can explain most of the results of the split-root experiments, we cannot rule out the possibility that self/non-self root discrimination did take place. We discuss a limited set of experiments for which volume effects cannot explain the results, suggesting, in fact, that direct self/non-self root interactions have operated. We suggest experimental designs that can demonstrate their ecological significance in the future.

6 We conclude that there is ample evidence that plants can sense the volume of available rooting space, and a limited number of studies on individual roots show that plant roots may sense the identity of neighbouring roots and respond accordingly. The significance of these responses for whole plant growth and reproduction in relation to well-known resource competition effects is yet largely unknown and in urgent need of further study.

Key-words: roots, root growth, rooting volume, nutrients, self/non-self discrimination, root recognition, allocation, split-root design, competition

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Introduction

There is an increasing awareness that the roots of plants do not interact solely through the depletion of soil resources but may also interact directly and that this may have profound consequences for plant growth and competition. Over the last 15 years, a number of studies have shown that root growth is differently affected when roots encounter roots of another plant than when roots encounter roots of the same physiological individual (e.g. Mahall & Callaway 1991; see Callaway 2002; de Kroon et al. 2003). This phenomenon is now referred to as self/non-self (S/NS) root discrimination. Recently, several studies have been published on the implications of S/NS root discrimination for whole plant performance in competitive settings (Gersani et al. 2001; Maina et al. 2002; Falik et al. 2003; O’Brien et al. 2005; Falik et al. 2006). These studies have claimed that plants overproduce roots upon encounters with non-self roots as compared with encounters with self roots, at the expense of reproductive biomass. In this paper we take a critical look at these results and determine whether ‘overproduction’ of roots as a result of S/NS recognition takes place, or whether the observed allocation patterns can be explained by well-known ecological hypotheses.

These studies consistently showed that plants produced more roots and yielded less seed when they compete with another plant than when the plants were grown alone. Thus, the results suggest that roots can discriminate between their own roots and those of other plants, and subsequently overproduce roots in non-self encounters. This response has been interpreted as an ability of plants to minimize competition with its own roots and to maximize root competition with a neighbour (Gersani et al. 2001; Falik et al. 2003). The ‘overproduction’ of roots appears to occur at the expense of reproductive biomass. It has further been inferred that the plants are thus caught in a ‘tragedy of the commons’ (Hardin 1968) because root competition inevitably invokes root overproduction of all competitors in order not to lose out in competition, but in doing so less of the ‘common good’ (i.e. soil nutrients) is available for reproduction for all plants involved (Gersani et al. 2001).

Most of these studies rely on a common split-root design in which the roots of a plant intermingle in two different pots and the growth responses are compared with a plant growing in a single pot in the absence of roots of another plant (Gersani et al. 2001; Maina et al. 2002; Falik et al. 2003, 2006; O’Brien et al. 2005; see Fig. 1a,b). Schenk (2006) recently argued that such a design fails to account for the fact that the absolute size and shape of the soil volume can strongly affect plant growth and development (Hanson et al. 1987; McConnaughay & Bazzaz 1991; van Iersel 1997; NeSmith & Duval 1998). In fact, Schenk (2006) showed that if soil volume is taken into account, the data from Maina et al. (2002) and Gersani et al. (2001) suggest that root mass of legume plants increases in response to an increase in absolute soil volume, but is not affected by the presence of a competitor. This calls into question the conclusion reached by Gersani et al. (2001), Maina et al. (2002) and O’Brien et al. (2005) that the increase in root mass was a response to detecting a neighbouring plant with which resources were shared.

We take Schenk’s (2006) analysis a step further by not only explaining the root biomass with rooting volume per plant but also by explaining the total biomass and allocation, by taking into account the nutrient availability per plant. Moreover, we analyse related severing studies on S/NS recognition in which responses of severed plants to previously attached plants (ramets) are explored. We will approach these studies in the same way as we approach the S/NS split-root studies to investigate whether there is any basis for the existence of S/NS recognition and subsequent ‘overproduction’ of root biomass.

Three straightforward hypotheses will be used, which together can explain not only most of the root biomass results but also the biomass allocation results:

1. Root mass is a function of available rooting volume independent of nutrients, i.e. a plant will fill up the available volume with its roots.

We assume that when two plants have access to a pot, both have access to the full pot volume. The actual volume taken up by the roots of a plant is thus assumed to be
negligible compared with the pot volume, and the plants are not aware of the presence of each other.

**II** The growth of a plant is limited by the total amount of nutrients available to the plant.

A neighbouring plant does not influence available soil volume, but does affect the availability of nutrients. Hence, when two plants of comparable biomass have access to a pot, only half of the nutrients in the pot are available to each plant. When plants have the same amount of nutrients available, they produce the same total plant biomass.

**III** It follows that if a plant produces more root biomass but similar total biomass, it will produce less reproductive and/or shoot biomass.

We use these three hypotheses for a systematic reanalysis of studies on S/NS root discrimination. First, to underpin Hypothesis I, we briefly review results from the literature on the effects of pot volume on root mass, with special attention to studies that were able to separate the effects of rooting volume and nutrient availability. Second, we reanalyse the split-root studies on S/NS discrimination, and test the results against our alternative hypotheses. Next, severing studies and particularly studies with clonal plants, which include other treatments than the split-root design, are reviewed and similarly tested. Based on these results, we re-evaluate the current evidence for root S/NS discrimination, give an overview of results that cannot be explained by the volume and nutrient hypotheses, and make suggestions for future research.

**Available volume affects root growth: independent evidence**

Many studies have emphasized the fact that the size and shape of the soil volume can strongly affect plant growth and development (see, for example, Crist & Stout 1929; Dibbern 1947; Biran & Eliassaf 1980; McConnaughay & Bazzaz 1991; NeSmith & Duval 1998; Matthes-Sears & Larson 1999). Although it is extremely difficult to separate the effects of rooting volume and nutrients on plant growth, there is ample evidence for Hypothesis I. Here, we discuss a number of studies in which the separation of the confounding factors (volume and nutrients) has been accomplished.

In most of the studies, the effects of the confounding variables were separated by simultaneously varying pot volume and nutrient concentration (Gurevitch et al. 1990; McConnaughay & Bazzaz 1991; Xu et al. 2001). Gurevitch et al. (1990) simultaneously varied container volume and nutrient concentration to investigate competition between three old-field perennials. The 5.6-fold increase in pot volume resulted in a 3.6-fold increase in root mass at the high nutrient level, and a 2.3-fold increase at the low nutrient level. This larger root biomass in larger pots corresponds with Hypothesis I.

McConnaughay & Bazzaz (1991) grew several annual species over a wide range of pot volume and nutrient supply and distinguished statistically the effects of rooting volume, nutrient concentration and total nutrients per pot. All species grew larger roots and shoots in larger pot volumes, independent of the amount of nutrients, but the species differed quantitatively in their response to physical space in both vegetative and reproductive parameters. Some species nearly doubled their root and shoot mass with doubling of the rooting volume, whereas for other species this increment was less than 50%. *Setaria faberii* Herrm. responded to smaller volumes with earlier flowering and greater reproductive output. With more reproduction and smaller roots, the allocation pattern of this species is in accordance with Hypothesis III. *Abutilon theophrasti* Medik., however, decreased its allocation to reproductive tissues relative to vegetative tissues in smaller rooting volumes (McConnaughay & Bazzaz 1991).

Hypothesis I is further supported by hydroponic studies. Bar-Tal et al. (1995), for instance, grew tomato plants aero-hydroponically at two different nutrient concentrations, and root restriction was achieved by confining the roots in 0.4- or 1.0-L bags that were permeable to the nutrient solution but not to roots. In this way root volume was manipulated independent of the nutrient status of the volume. Plants grown in the smaller volume had a root mass of 37 and 38 g (low- and high-nutrient solution concentration, respectively), whereas the plants grown in the larger volume at the high concentration had a root mass of 69 g. Root mass was thus affected by volume, but not by the nutrient concentration of the solution. Several other hydroponic studies have produced results that are consistent with those of Bar-Tal et al. (1995), in that the decrease of the available rooting volume has led to a decrease in root dry weight (Hameed et al. 1987; Ternesi et al. 1994; Kharkina et al. 1999).

A very different approach was taken by Loh et al. (2003) who grew young *Ficus benjamina* L. trees in a stone-soil blended skeletal soil material compared with plants grown in loam soil only. The stone fragments in the skeletal soil treatment created relatively large voids which were filled with soil. By combining these two different substrates with different container volumes and slightly different soil compaction, Loh et al. (2003) were able to create treatments that differed three-fold in void volume (i.e. rooting volume) but with the same soil weight (i.e. nutrient availability). This three-fold increase in soil volume resulted in a 1.5-fold increase in root dry weight, while shoot dry weight was unaffected (Table 1). The results of Loh et al. (2003) thus confirm Hypothesis I. In other treatments, shoot weight was positively correlated with the soil weight per container. This result suggests that Hypothesis II can also be applied to the above-ground vegetative mass, i.e. that not only total plant biomass but also the stem and leaf mass is determined by the nutrients available to the plant.

We conclude that there is ample independent evidence supporting Hypothesis I – that root mass is a function of available rooting volume, independent of the available nutrients, although it is difficult to disentangle the effects...
of volume and nutrients, and it has only been achieved in a few studies. In an experiment applying different root partitions and activated carbon, Semchenko et al. (2007) recently showed that the effect of volume on root growth is likely due to root self-inhibition in smaller pots, partly mediated by growth limiting effects of root exudates.

**Re-analysis of root S/NS discrimination studies**

**SPLIT-ROOT STUDIES**

In the majority of studies on S/NS recognition, an experimental design is used as depicted in Fig. 1: either a split-root plant had its own pot (this treatment is called ‘1 pot per plant no root competition’ and is also referred to as ‘Owners’ in the original studies), or its roots were spread over two pots together with another plant (this treatment will be called ‘2 pots per plant with root competition’ and is referred to as ‘Sharers’ treatment in the original studies). The rationale behind these treatments is that plants in both treatments have the same amount of nutrients available and the only factor that differs between them is the fact that sharing plants share the rooting space with another plant and plants that have the pot to themselves do not. However, a ‘1 ppp no rc’ plant and a ‘2 ppp with rc’ plant also differ in available rooting volume per plant, and here we explore its consequences following our Hypotheses I–III.

Table 1 Growth response of *Ficus benjamina* to limited soil volume and soil nutrient availability, using two different substrates (from Loh et al. 2003). Container volume and soil medium were varied in such a way that nutrient availability (given by soil weight) and soil volume were manipulated independently. See text for further explanation. Different letters indicate a significant difference ($P < 0.05$) in mean root dry weight or shoot dry weight between soil treatments.

<table>
<thead>
<tr>
<th>Medium type</th>
<th>Container volume (dm$^3$)</th>
<th>Soil weight (kg)</th>
<th>Soil volume (dm$^3$)</th>
<th>Soil compaction (Mg m$^{-3}$)</th>
<th>Root dry weight (g)</th>
<th>Shoot dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Loam</td>
<td>11</td>
<td>15</td>
<td>5</td>
<td>1.37</td>
<td>89$^a$</td>
<td>89$^a$</td>
</tr>
<tr>
<td>2: Skeletal soil medium</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>1.94</td>
<td>44$^b$</td>
<td>25$^c$</td>
</tr>
<tr>
<td>3: Loam</td>
<td>54</td>
<td>74</td>
<td>26</td>
<td>1.37</td>
<td>275$^a$</td>
<td>327$^a$</td>
</tr>
<tr>
<td>4: Skeletal soil medium</td>
<td>54</td>
<td>15</td>
<td>15</td>
<td>1.94</td>
<td>130$^b$</td>
<td>85$^b$</td>
</tr>
</tbody>
</table>

Table 2 The effect of planting treatment on root, shoot and pod masses of 60-day-old *Phaseolus vulgaris* plants (from Maina et al. 2002). The weights are given on a per plant basis. The capital letters correspond to planting set-ups: A = ‘1 pot per plant no root competition’, B = ‘2 pots per plant with root competition’, C = ‘2 pots per plant no root competition’ and D = ‘1 pot per plant with root competition’ (see Fig. 1). Different superscripts indicate significant differences between treatments ($P < 0.05$). The analysis shows that root mass corresponds with the volume available per plant (v) following Hypothesis I, and that total mass (as well as shoot mass) corresponds with available nutrients per plant (n), following Hypothesis II. Hypothesis III states that increased allocation to root biomass because of more available rooting space, but equal biomass production because of nutrient limitation, will inevitably result in decreased allocation to reproductive biomass.

<table>
<thead>
<tr>
<th>Hypothesis tested</th>
<th>Corresponding volume (v) or nutrients (n) per plant</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>H I Root mass (g):</td>
<td>B (2v) = C (2v) &gt; A (1v) = D (1v) = B (3.93)$^a$ = C (3.64)$^a$ &gt; A (1.44)$^b$ = D (1.28)$^c$</td>
<td></td>
</tr>
<tr>
<td>H II Shoot mass (g):</td>
<td>C (2n) &gt; B (1n) &gt; A (1n) &gt; D (1/n) = B (4.93)$^a$ &gt; C (4.02)$^b$ &gt; A (1.99)$^c$</td>
<td></td>
</tr>
<tr>
<td>H III Pod mass (g):</td>
<td>A (1n &amp; 1v) &gt; B (1n &amp; 2v) = C (7.03)$^a$ &gt; B (4.82)$^b$ &gt; A (2.29)$^c$</td>
<td></td>
</tr>
<tr>
<td>H II Total mass (g):</td>
<td>C (2n) &gt; B (1n) = A (1n) &gt; D (1/n) = C (15.60)$^a$ &gt; B (8.30)$^b$ &gt; A (8.25)$^c$ &gt; D (3.84)$^c$</td>
<td></td>
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Our analysis starts with the results of Maina et al. (2002) with *Phaseolus vulgaris* L., who used two other treatments in addition to the ‘1 ppp no rc’ and ‘2 ppp with rc’ treatments, as illustrated in Fig. 1. The available rooting volume and the available nutrients per plant in each of these four treatments are as follows:

A ‘1 pot per plant no root competition’ (1 ppp no rc; ‘Owners’): each plant has one unit of rooting volume and one quantity of nutrients (1v, 1n);
B ‘2 pots per plant with root competition’ (2 ppp with rc; ‘Sharers’): each plant has access to two units of rooting volume and one quantity of nutrients (2v, 1n);
C ‘2 pots per plant no root competition’ (2 ppp no rc): the split root system of one plant is divided over two pots – each plant has two units of rooting volume and two quantities of nutrients (2v, 2n); and
D ‘1 pot per plant with root competition’ (1 ppp with rc): each plant has one unit of rooting volume and half a quantity of nutrients (1v, 1/n).

The results of Maina et al. (2002) are consistent with our hypotheses I–III (Table 2). Plants that had access to two units of soil volume (two pots) produced a root mass per plant that was more than twice as large as the plants that had access to one unit of soil volume. Root mass can thus largely be explained by the available rooting volume per plant (Hypothesis I): the more soil volume is available, the more root mass a plant produced. This effect of volume on root biomass is independent of nutrient availability, as ‘2 ppp with rc’ and ‘1 ppp no rc’
plants have a different rooting volume but an equal amount of nutrients available. Shoot mass and total biomass were related to the available nutrients, and not to rooting volume or root mass (Table 2); the more nutrients available the more shoot and total mass was produced, in accordance with Hypothesis II: a neighbouring plant does not influence available soil volume, but does affect the availability of nutrients. Obviously, the results of 

Maïna et al. (2002) are also in accordance with Hypothesis III, as ‘2 ppp with rc’ plants produce more roots than ‘1 ppp no rc’ plants without gaining more nutrients and allocate less biomass to reproduction.

The results of other studies using the same design as in Maïna et al. (2002) also agree with our hypotheses I–III (Fig. 2). Doubling the rooting volume from ‘1 ppp no rc’ to ‘2 ppp with rc’ (i.e. from Owner to Sharer) consistently increased the root biomass 1.4–3-fold in seven out of eight cases. Only in the high-nutrient treatment of O’Brien et al. (2005) did doubling the rooting volume resulted in a marginal (but significant) increase in root mass. By contrast, and consistent with Hypothesis II, total biomass was similar in ‘1 ppp no rc’ and ‘2 ppp with rc’ plants in five out of eight cases. In the three exceptions, plants were larger in the ‘2 ppp with rc’ treatment than in the ‘1 ppp no rc’ treatment, and moreover in two of these cases the plants were harvested before seed-set (Falik et al. 2003; H.J. Schenk, unpublished data). Finally, in all six cases in which the plants flowered, ‘2 ppp with rc’ plants consistently produced a lower seed mass than ‘1 ppp no rc’ plants (cf. Hypothesis III).

Finally, we will discuss the study by O’Brien et al. (2005) in which not only was the effect of the presence of roots of another plant studied, but volume and nutrient availability per plant were also manipulated in an attempt to disentangle the effects of presence of non-self roots, the effects of rooting volume and the effects of nutrients on the root biomass of a plant. In a two-pot experiment, the roots of single Pismum sativum L. plants were distributed over two pots, and the volume or the nutrient availability of one of the two pots was manipulated. In the treatment with a half-sized pot, half the amount of root mass was produced in the half-sized pot compared with the pot of standard size (treatment M; Table 3a), following Hypothesis I. If the nutrient concentration was halved in one pot while the volume remained unaltered (treatment L), the plants selectively placed their roots in the control pot containing more nutrients (cf. Hodge 2004).

O’Brien et al. (2005) further conducted a three-pot experiment in which two plants share one pot and each plant has access to an exclusive pot. In one treatment a barrier was placed in the shared pot, effectively creating a ‘1.5 ppp no rc’ treatment with access to 1.5 pot volume per plant: treatment O. Again, the rooting patterns followed Hypothesis I: the plants produced similar root biomass in the exclusive and the shared pots of similar volume (treatment N; Table 3b), while the plants produced only half of the root mass in the half-sized pot created by the barrier (treatment O).

Combining these two experiments of O’Brien et al. (2005), we can make an interesting comparison between the two-pot treatment in which one of the pots had half of the nutrient concentration of the other (treatment L; ‘2 ppp no rc’) and the three-pot treatment in which a plant was subjected to a competitor plant in the shared pot (treatment N; ‘2 ppp with rc’). If we assume that the competitor plant consumes half of the soil nutrients, we have exactly the same nutrient and volume availability for the roots of the plants in both of these treatments (Table 3) except for the cause of the lower nutrient availability in the second pot, i.e. a lower nutrient concentration in treatment L, and a competitor plant in treatment N. Interestingly, the root responses in these two treatments were quite different. In the two-pot situation of treatment L, the plants showed selective root placement, resulting in a root distribution over the pots that mimicked the nutrient distribution. By contrast, in treatment N, the root mass was almost the same in both pots, suggesting root overproduction in the presence of the roots of another plant.

SEVERING STUDIES

Here we discuss studies that investigate SNS recognition in clonal plants in which independent physiological individuals are created through severing clonal connections, and studies in which ramets or seedlings are split into two halves. The experimental set-ups can be compared with the ‘2 ppp with rc’ and ‘1 ppp no rc’ design (i.e. Sharers and Owners design), which was discussed in the previous section. In treatment E (Fig. 3), two connected ramets (or one single ramet) were used, similar to the ‘1 ppp no rc’ treatment. In the ‘1 ppp with rc’ treatment F (Fig. 3), the connection between the two ramets is severed (or a ramet is split into two halves), resulting in two physiologically separate plants each exploring the soil volume. The two separate plants in treatment F, initially half the size of the original plant, share a single pot. In some studies, there were two different treatments with severed plants, all referred to as ‘1 ppp with rc’: in treatment F, the two plant ‘halves’ originated from the same plant (Fig. 3), while in treatment H (Fig. 3), the two halves originated from different plants, either of the same or a different genotype. Gruntman & Novoplansky (2004) and Falik et al. (2006) combined these treatments with the split-root design (G in Fig. 3) in which two two-ramet plants were growing in two pots, comparable with the ‘2 ppp with rc’ treatment B in Fig. 1B.

When analysing the results of these experiments in the same way as those of the non-separated split-root experiments from the previous section, we should consider the number of physiological individuals and the rooting volume and nutrients available to them. Analogous to Fig. 1, we can characterize the four treatments in Fig. 3 as:
E ‘1 ppp no rc’ (Intact = Owner): each plant has access to one unit of rooting volume and one quantity of nutrients (1v, 1n);

F ‘1 ppp with rc’ (Severed Self): each plant has one unit of rooting volume and half a quantity of nutrients (1v, 1/2n); the two plants originate from the same plant;

G ‘2 ppp with rc’ (Intact Non-self = Sharer): each plant has two units of rooting volume and one quantity of nutrients (2v, 1n);

Fig. 2 The effect of planting arrangement (‘1 pot per plant no root competition’ and ‘2 pots per plant with root competition’, i.e. Treatments A and B, respectively, in Fig. 1) and nutrient level on root, seed and total masses of plants in five different studies. Maina et al. (2002) grew *Phaseolus vulgaris* plants in either 0.1 or 0.5 Hoagland solution (a, b). Gersani et al. (2001) used two different cultivation treatments with *Glycine max*, either split-root plants in pots (c), or non-split root plants in boxes (d). To create two root systems for the split-root plants Gersani et al. (2001) cut the radicle of the seedlings; this was not done in the box treatments. O’Brien et al. (2005) grew *Pisum sativum* plants in either 0.1 or 0.6 Hoagland solution (e, f). Falik et al. (2003) grew seedlings of *Pisum sativum* in 0.1 Hoagland solution (g). Shoot biomass values were recalculated from the root : shoot ratios given in Falik et al. (2003). Schenk (2006, and unpublished data) grew *Glycine max* plants as in Maina et al. (2002) (h). In studies (g) and (h) the plants were harvested before seed set. Means and SE values were read from the graphs in the papers and re-plotted. Stacked bars indicate, from bottom to top, the mass of roots (dark shading), shoots (speckled) and reproductive structures (light shading). Statistical results were obtained from the original papers; a difference in letter indicates a significant difference in root, shoot or reproductive biomass between the treatments.
H ‘1 ppp with rc’ (Severed Non-self): each plant has one unit of rooting volume and half a quantity of nutrients (1v, 1/2n). The two plants in each pot originate from different plants.

One important difference with the split-root studies of Fig. 1 is that the starting weights of the physiological individuals to which rooting volume and available nutrients were assigned differed two-fold between the severed and intact treatments. However, if the plants grow considerably beyond their initial weight, and if nutrients were limiting final biomass production, these differences in initial weight should play a minor role in comparisons of final biomasses and allocation.

We can now test the results of S/NS studies on clonal plants and severed ramets against the volume and nutrient hypotheses I–III we set out at the beginning.

We first analyse the results of Falik et al. (2006), who conducted an experiment with Trifolium repens L. plants in which all four treatments of Fig. 2 were represented. A ‘2 ppp with rc’ plant (treatment G) with access to two pot volumes produced 51% more root biomass than a ‘1 ppp no rc’ plant (treatment E) that had access to only one pot volume (Table 4a). This is in accordance with Hypothesis I, which predicts a larger root biomass as a result of a larger rooting volume. Severed plants in the ‘1 ppp with rc’ treatments F and H produced somewhat smaller root mass than the (Intact) plants of the ‘1 ppp no rc’ treatment E, although they had access to the same unit of rooting volume (Table 4a), perhaps because they started growing as a single rooted ramet rather than two-rooted ramets. Plants in the ‘1 ppp no rc’ treatment E had access to the same quantity of nutrients as plants in the ‘2 ppp with rc’ treatment G and their shoot masses did not differ significantly (Table 4a). The severed plants in treatments F and H, growing with only half the nutrient availability per plant, also produced half the shoot biomass of the intact plants in treatments E and G, indicating that the shoot mass of a plant closely follows the total nutrients available per plant, as in other studies described above.

Table 3 Volume and nutrient availability effects on root mass in Pisum sativum (from O’Brien et al. 2005). (a) Two-pot experiment with single split-root plants (cf. Treatment C in Fig. 1), where in one of the pots either the nutrient concentration was halved (treatment L) or the pot volume was halved (treatment M), in addition to the control treatment K in which the split roots had access to two similar pots. Statistically significant differences (MANOVA as tested by O’Brien et al. 2005) are indicated by differences in superscript letters. (b) Three-pot experiment in which two split-root plants shared a single pot and each plant also had access to a control pot that they did not share with other plants. In treatment N, the roots in the common pot grew intermingled; in treatment O a divider was present in the shared pot so that the roots did not interact and the rooting volume was effectively halved. The root mass of a plant in treatments N and O in the pot with root competition (rc) was calculated by including half of the total root biomass in the pot that was shared by two plants, as given in O’Brien et al. (2005). Statistics on these plant values are not available. For both sets of treatments, the total nutrient availability per pot (n) is based on the assumption that when two plants have access to a pot, half of the nutrients were available to each plant. Means and SEs were read from the graphs in the original paper. Rc is an abbreviation for root competition.
Gruntman & Novoplansky (2004) observed root growth responses in *Buchloe dactyloides* Engelm. after the plants were grown in the presence of neighbours of variable physiological and genetic identities. In addition to treatment E (‘1 ppp no rc’), in treatment F1 ramets were split longitudinally and the two halves were grown together (‘severed-self’). In two further treatments, the plant-halves were alienated from each other by letting them grow separately from each other (‘alienated’) for a period of 7 or 60 days before planting them together again (treatments F2 and F3, respectively). In the final treatment, treatment H, the second half originated from a plant of a different genotype (‘alien’). All the treatments depicted in Fig. 3 were thus conducted by Gruntman & Novoplansky (2004) except treatment G. Hence, all plants in this study had the same rooting volume and we do not expect a difference in root biomass between the treatments if root mass is solely determined by available volume per plant (Hypothesis I). The individual plants of each of the (Severed) treatments F2, F3 and H did indeed have a root biomass (17–21 mg) comparable with the root mass of plants of (Intact) treatment E (20 mg; Table 4b). Treatment F3 did produce a significantly greater root mass than treatment F2, suggesting that the period of alienation prior to the start of the experiment had affected root biomass. Remarkably, the un-alienated severed plants of the F1 treatment had only half the root biomass of the

**Table 4** Volume and nutrient availability effects in two severing studies with clonal plants. (a) Falik et al. (2006) arranged intact or severed two-ramet *Trifolium repens* plants over one or two pots resulting in treatments E–H as depicted in Fig. 3. The two original plants in the non-self treatments (G and H) belonged to the same genotype. The plants were harvested before seed set. Different superscript letters indicate statistically significant differences from a one-way ANOVA and Tukey HSD post-hoc test (from Falik et al. 2006). Because we halved the original values for treatments F and H as given in the original paper to obtain masses per physiological individual, statistical information for these groups is not given. (b) Gruntman & Novoplansky (2004) used symmetrical two-branched ramets of the stoloniferous *Buchloe dactyloides* that were either longitudinally severed into two genetically identical but physiologically separate halves (treatment F) or left intact (treatment E). Severed ramet halves of different origins were placed together: the two halves originating from the same ramet were physiologically separated immediately before the onset of the experiment (treatment F1). In the Severed-7 days (F2) and Severed-60 days (F3) treatments, the halves originating from plants of the same genotype were grown in separate pots for 7 and 60 days, respectively, before severing. Finally, in treatment H, the two ramet halves originated from two different clones (genotypes). ‘Plant’ refers to a single physiologically separate individual, i.e. either an intact ramet (treatment E) or a severed ramet half (treatments F and H). Mean values were read from the graphs in the original paper and re-plotted; we recalculated values per physiological individual by halving the masses per pot. Shoot masses were computed from the root mass and the root mass ratio, and therefore there is no statistical information on shoot mass. Different superscript letters indicate statistically significant differences from a one-way ANOVA, followed by a Fisher’s least significant difference comparison (from Gruntman & Novoplansky 2004). We only show statistical results for the severed F and H treatments, because we halved the original numbers to obtain root mass per plant, and could not compare them statistically with treatment E (not halved).
other four treatments (11 g), despite the same rooting volume. Shoot mass again closely followed the available nutrients per plant in all treatments (Table 4b).

The study of Falik et al. (2003), who split Pisum sativum plants longitudinally creating three treatments, i.e. E, F and H of Fig. 3 ('1 ppm no rc', '1 ppm with rc' and '1 ppm with rc', respectively), bears some resemblance with that of Gruntman & Novoplansky (2004). With the same rooting volume per plant, plants in treatment E and H had a similar root biomass (6.0 and 5.5 g, respectively). Interestingly, the plants in treatment F ('1 ppm with rc' or Severed Self), where two halves of the same severed plant remained growing together, had a much smaller root biomass (3.5 g), reminiscent of the lower root mass in treatment F1 of Gruntman & Novoplansky (2004).

Discussion

Our analysis of S/NS studies shows that increases in root mass, which have widely been attributed to the presence of another plant in the pot, can largely be explained by the larger soil volume that these plants have at their disposal. It appears that the difference in absolute rooting volume per plant between treatments is an important factor that was not taken into account in the analysis of split-root experiments, as has already been pointed out by Schenk (2006). Specific S/NS discrimination mechanisms need not to be inferred to explain the rooting patterns found in most of these studies. Although there is an extensive literature on the importance of rooting volume on plant growth, very few authors have attempted to separate the effects of volume and nutrients on root growth and even fewer have looked specifically at root biomass. From studies in which the confounded factors were separated and root biomass was measured, in particular McConnaughay & Bazzaz (1991) and Loh et al. (2003), it is clear that there is an effect of volume on root mass that is independent of nutrients (see also Semchenko et al. 2007).

Not only can root biomass be explained by volume, differences in total and reproductive biomass can also be explained by differences in nutrients and volume available to a plant. This applies to studies on non-clonal plants as well as on severed (clonal) plants. As these are well-known and well-accepted responses, we cannot accept the explanation given by a number of authors (Gersani et al. 2001; Maina et al. 2002; Falik et al. 2003; O’Brien et al. 2005) that the reduced flowering and fruiting are due to S/NS interactions and the result of an evolved response to avoid wasteful self-competition below ground.

EXCEPTIONS TO THE VOLUME–NUTRIENT HYPOTHESES

Although the majority of the growth and allocation patterns in the S/NS studies are consistent with our three hypotheses, our reanalyses revealed some results that could not be immediately explained by our hypotheses on volume and nutrients. We highlight three specific results, some of which indeed suggest that root growth stimulation may occur in response to the presence of another plant in the pot.

First, the results of two different experimental treatments of O’Brien et al. (2005) (Table 3; treatments L vs. N) suggest that plants produce more roots when nutrients are depleted as a result of S/NS interactions. These two treatments comprise one of the very few comparisons for which the effects of volume can be safely ignored, and thus generate important evidence for the occurrence of S/NS interactions. However, the degree of nutrient depletion of the competitor plant was not measured and it could have been incomparable with the treatment in which the nutrient concentration was lowered. Moreover, it is very difficult to translate this result to the many other treatments in this study and decipher the quantitative importance of the volume effects vs. the S/NS effects in the responses obtained.

Second, S/NS discrimination or other effects may have influenced the root responses on top of effects of rooting volume. Schenk (2006, fig. 6) has already shown that the degree of root increment as a function of volume may differ widely among species (see also
Fig. 2). In particular, Maina et al.'s (2002) study stands out with an up to three-fold increase in root mass with doubling the rooting volume. Such an increase is larger than would be expected on the basis of increasing rooting volume alone. In the independent rooting volume studies doubling of the rooting volume did not result in double root mass, but usually a much smaller increase. Such disproportional increase in root mass may be due to non-self interactions increasing the root production in this particular experiment, but other effects cannot be ruled out. These results call for a quantitative assessment of root responses to rooting volume and nutrients, in conjunction with quantifying the effects of resource competition and direct root interactions.

Finally, for the split-ramet Buchloe dactyloides plants of Gruntman & Novoplansky (2004) the rooting volume was the same in all treatments, leading to comparable root masses per plant except for the treatment in which split-ramets from the same node remained together immediately after severing, where only half of the roots per plant were produced (Table 4b). Looking at the results in this way, it is not surprising that the 'alienated' ramets 'overproduce' roots when grown together as these effects can be attributed to volume effects (a split-ramet individual having the same rooting volume as an intact ramet individual – see Table 4b). Rather, the remarkable result is that the plants in this particular severing treatment underproduce roots when not yet alienated, despite their similar shoot mass as alienated plants. It is unclear how this may be explained by S/NS interactions or otherwise, and repetitions of this experiment are needed to test the generality of this particular response. It is noteworthy that in Trifolium repens immediate severing, albeit of stolons rather than ramets split into two halves, resulted in root development and growth in accordance with the volume and nutrient hypotheses set out at the beginning of our paper (Falik et al. 2006).

Conclusions

There can be little doubt that individual plant roots of at least some species can sense the roots of neighbouring plants and respond to them according to their identity, i.e. whether they are from the same plant, from a different physiological individual, a different genotype or a different species. Since the classical studies of Mahall & Callaway (1991, 1996) with Ambrosia dumosa and Larrea tridenta, evidence for root–root recognition has been obtained in studies of individual roots in Pisum sativum (Falik et al. 2003) and the grass Miscanthus sinensis Anderss. (Nishiwaki, unpublished data, in De Kroon et al. 2003). We conclude from our reanalysis of S/NS root discrimination studies that it is yet unknown how important such direct discrimination is for plant interactions in relation to well-known effects of resource competition.

The very few comparisons between treatments that can be made without confounding factors involved do suggest that root overproduction may take place in the presence of neighbouring roots. More research on these responses is thus highly pertinent. Experimental designs are needed in which the effects of volume and nutrients, and the effects of identity of neighbouring roots are properly disentangled. There are studies that have successfully separated the effects of volume and nutrients, for example McConnaughay & Bazzaz (1991) and Loh et al. (2003). In addition, clever split-root designs in which plants are subjected to similar nutrient limitation but by different causes (lower nutrient concentration or a neighbouring root system; see O'Brien et al. 2005) call for repetition, especially if also plant nutrient concentrations are measured to assess resource limitation. Semchenko et al. (2007) used different types of root partitioning in combination with activated carbon showing how absorption of organic root exudates partly reversed root growth inhibition due to pot partitioning but did not alter neighbour interactions. Finally, it is important to include more non-legume plant species in these experiments in order to exclude possible effects of root symbionts on the responses.

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