MARINA SEMTŠENKO

Plant root behaviour: responses to neighbours and physical obstructions

Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu

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Supervisors: Prof. Kristjan Zobel, University of Tartu
Prof. Michael J. Hutchings, University of Sussex, UK

Opponent: Prof. Hans de Kroon, Radboud University Nijmegen, The Netherlands

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers that are referred to in the text by Roman numerals:

I. **Semchenko M, Hutchings MJ, John EA. 2007.** Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume. *Journal of Ecology* **95**: 252–260.

II. **Semchenko M, Zobel K, Heinemeyer A, Hutchings MJ. 2008.** Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytologist* **179**: 1162–1170.

III. **Semchenko M, John EA, Hutchings MJ. 2007.** Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. *New Phytologist* **176**: 644–654.

IV. **Semchenko M, Zobel K, Hutchings MJ.** Competition between species with contrasting root responses to neighbours: is avoidance of competition inferior to a strategy of contesting resources? Manuscript.

The participation of the author in preparing each of the listed publications is as follows: collecting and analysing data (90%), preparing the text (80%).

INTRODUCTION

Current evidence suggests that plants can actively forage for belowground resources and that they have evolved a variety of root growth strategies that are comparable to sophisticated behaviours observed in animals. Changes in root growth in response to the presence of competitors have generally been interpreted as reactions to resource depletion caused by neighbours' roots. However, much evidence has accumulated showing that neighbouring roots can also interact directly using allelochemicals and non-toxic signals (Mahall & Callaway, 1992, 1996; Krannitz & Caldwell, 1995; Ridenour & Callaway, 2001; reviewed in Schenk, 2006). Physiological coordination between roots belonging to the same plant, or to connected ramets belonging to the same clone, and the genetic relatedness of different plants, have also been shown to affect the growth and spatial distribution of roots (Mahall & Callaway, 1996; Falik *et al*., 2003; Holzapfel & Alpert, 2003; de Kroon *et al*., 2003; Gruntman & Novoplansky, 2004). Moreover, a recent study suggested that roots can also detect solid objects in the soil and reduce growth towards them (Falik *et al.,* 2005), indicating that plants may be able to forage not only for nutrients and water, but also for rooting space. These elaborate root interactions suggest that evolutionary theories that have been successfully used to explain many behavioural phenomena in animals could also give a new level of understanding in plant studies. However, experimental studies attempting to demonstrate the applicability of certain evolutionary theories to plants are faced with the challenge of discriminating between active communication between roots and root responses to the availability and distribution of nutrients and space.

Game-theoretic models of nutrient foraging predict that, compared with plants growing in the absence of belowground competition, plants competing for a common pool of soil-based resources should overproduce roots at the expense of reproduction (Zhang *et al*., 2001; Gersani *et al*., 2001; Maina *et al*., 2002). Simultaneous exploration of the same soil volume by numerous roots results in extensive overlap between root depletion zones and therefore causes a reduction in the efficiency of resource acquisition (the amount of resource acquired per unit of root length and time). As within-individual competition is highly wasteful, avoidance of overlap between the depletion zones created by different roots from the same plant should be particularly advantageous (Falik *et al.*, 2003; Gruntman & Novoplansky, 2004). However, when there is competition between several plants, the production of additional roots in the space shared with competitors can potentially improve the relative fitness of an individual by allowing it to capture new resources that would otherwise be acquired by its competitors (Zhang *et al.*, 1999; Gersani *et al.*, 2001; Maina *et al*., 2002; O'Brien *et al*., 2007). A plant may therefore benefit from the ability to discriminate between 'self' and 'non-self' roots, and the restriction of root growth in soil volumes already occupied by its own roots but proliferation of roots in substrate occupied by the roots of competitors. If all competitors adopt

this strategy, however, each plant manages only to "steal" as many resources from competitors as it loses to them. The benefit of "stealing" resources from neighbours is therefore lost, but all plants still have to bear the costs of producing additional roots and of having fewer resources to invest in reproduction (Zhang *et al*., 1999; Gersani *et al*., 2001). This phenomenon has been described as a "tragedy of the commons" (after Hardin, 1968). Unless plants cooperate and actively prevent invasion by non-cooperators, the over-production of roots in the presence of competitors for soil-based resources appears to be the only evolutionarily stable strategy. Thus, the tragedy of the commons may well be a widespread phenomenon.

Several studies have attempted to test the prediction that there will be over-production of roots in the presence of competitors, but the experimental designs used have often failed to account for the possibility that roots may not only detect and interact with each other, but that they may also respond to inert objects in the substrate, such as pot walls and partitions used to prevent belowground competition in control treatments. Experiments on the responses of plants to the presence of competitors for soil-based resources have often involved treatments that provide a constant volume of substrate, and amount of nutrients, per plant (Gersani *et al*., 2001; Maina *et al*., 2002; Falik *et al*., 2003; O'Brien *et al*., 2005; Falik *et al*., 2006). As a result, the substrate volume potentially accessible by each plant was twice as large for plants sharing rooting space with a competitor as for plants growing in the treatment where rooting space was partitioned to prevent competition. Thus, the effects of the presence of neighbours may have been confounded with an effect of changing the rooting volume potentially available to each competitor (Schenk, 2006). If the availability of rooting space determines root production, the increase in root production exhibited in the presence of a competitor may be due to the larger rooting volume potentially available for each plant in this treatment compared with the treatment without competition. Reanalysis of the data from previous studies on the tragedy of the commons and self/non-self root discrimination confirmed that some of the results of previous studies can be explained by differences in the substrate volume provided in different treatments (Hess & de Kroon, 2007).

Moreover, the distribution of resources in the substrate will be affected by the presence and activity of a competitor's roots in a way that may make root growth in the presence of competitors less efficient. If root systems of competitors overlap, each plant will need to explore a greater volume of substrate, and may need to produce a greater root length, to acquire the same amount of nutrients as in a treatment without root competition. The overlap between two root systems may lead to lower nutrient uptake rates per unit of root length compared with plants grown alone because there will be fewer resources in substrate already occupied by a competitor's roots (Schenk *et al*., 1999). Therefore, increased root production and poorer performance of plants in the presence of competitors, compared with plants grown without competitors for

soil-based resources, may be a consequence of their inability to avoid overlap with the root systems of competitors, rather than active neighbour detection.

Independent evidence clearly demonstrates that limited rooting space can have profoundly detrimental effects on plant growth, and cause significant physiological changes, even when plants are sufficiently supplied with nutrients, water and oxygen (Carmi & Heuer, 1981; Tschaplinsky & Blake, 1985; Hameed *et al*., 1987; McConnaughay & Bazzaz, 1991; Kharkina *et al*., 1999). Under natural conditions, the availability of usable belowground space is modified by the presence of physical obstructions such as compacted soil, neighbouring roots, organic debris, stones and other objects with a variety of sizes and densities. Such obstructions can interfere with root growth by reducing space for root deployment, fragmenting usable parts of the substrate and hindering roots from locating and exploiting resources. This interference is likely to reduce resource acquisition efficiency (McConnaughay & Bazzaz, 1992; Nielsen *et al*., 1994; Rubio *et al*., 2001). Plants could therefore benefit from being able to forage for usable space by avoiding substrate with a high density of obstructions in favour of substrate in which resources can be easily accessed and exploited. However, there have been no studies to assess the ability of roots to avoid obstructions, to determine how widespread any such ability might be, or to explore the conditions that might favour the evolution of this ability. For example, habitat productivity is likely to affect the cost of inefficient root placement (McConnaughay & Bazzaz, 1992), and therefore influence the adaptive value of the ability to avoid obstructions.

Our knowledge of the mechanisms that plants could use to assess the density of obstructions in the substrate, and to adjust root behaviour appropriately, is very limited. Studies on chemical signalling suggest that plant responses to limited availability of space are not related to changes in the nutritional status of plants, but to changes in hormone production in roots and to sensitivity of roots to the accumulation of their own allelopathic exudates in the vicinity of obstructions (Goss & Russell, 1980; Carmi & Heuer, 1981; Moss *et al*., 1988; Sarquis *et al*., 1991; Falik *et al*., 2005). Although demonstrated under highly artificial conditions, these mechanisms may ensure efficient root placement in natural soil where obstructions are unevenly distributed.

Theoretical models that have been used to estimate the adaptive value of different degrees of root proliferation as a function of the presence or absence of competitors, have based their predictions entirely on cost-benefit analysis (Gersani *et al*., 2001), ignoring variation in many fundamental species traits that are known to be important determinants of species success in a community. This simplification has lead to the conclusion that the only evolutionarily stable strategy for plants that lack the capacity for territory defence *via* toxin production is to overproliferate roots in the presence of neighbours (Gersani *et al*., 2001; Maina *et al*., 2002; O'Brien *et al*., 2007). However, the actual diversity of responses to neighbouring roots that has been documented is striking. For example, roots of some species have been shown to avoid growth only towards

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roots belonging to the same individual or a connected ramet from the same clone (Falik *et al*., 2003; Holzapfel & Alpert, 2003). Other species are unaffected by contact with, or close proximity to, roots of the same individual but reduce overlap with the root systems of neighbours belonging to the same genotype or population, or all neighbours, independent of their identity (Mahall & Callaway, 1992, 1996; Schenk *et al*., 1999; de Kroon *et al*., 2003). However, detailed comparative studies involving several co-occurring species have rarely been undertaken (e.g. Mahall & Callaway, 1992), and the factors promoting the evolution and coexistence of different rooting behaviours remain largely unexplored. Importantly, the adaptive value of different patterns of root behaviour has not been considered in the context of different species' aboveground characteristics and life-history traits.

The objectives of the investigations reported in this thesis were:
- to investigate aspects of the experimental design used in previous studies on the tragedy of the commons in root competition that may have affected plant growth in a way that could be interpreted as due to self/non-self discrimination (**I**).
- to determine the ability of roots to avoid obstructions and forage for usable space, and to examine variation in this ability between species characteristic of habitats with contrasting productivity (**II**).
- to examine mechanisms that could be involved in root communication, and in the responses of plants to the presence of obstructions in the substrate (**I, II**).
- to investigate how physical connection between ramets, and the genetic identity of a neighbouring ramet, affect the spatial distribution of entire root systems and elongation rates of individual roots in two clonal species with markedly different stolon internode lengths (**III**).
- to make a first attempt to investigate the association between species rooting behaviour and other fundamental species traits, and to demonstrate how such associations can modify the relative performance of species in intraclonal and interspecific competition as predicted from root behaviour alone (**IV**).

MATERIALS AND METHODS

Paper I –
Confounding effects of neighbour presence and substrate volume

An experiment was conducted using spring oats (*Avena sativa* L.) as a model species and included treatments similar to those used in previous studies on the tragedy of the commons in root competition: (i) two plants sharing the whole substrate, and (ii) two plants with root systems separated by a solid partition and each having access to only half of the substrate. In addition, treatments with (i) mesh partitions, which permitted the movement of resources and root exudates between root systems, but prevented direct contact between roots of different plants, and (ii) the addition of activated carbon to the substrate, were established. Activated carbon adsorbs organic root exudates from the substrate, reducing their possible effects on root growth (Mahall & Callaway, 1992; Ridenour & Callaway, 2001). This resulted in a 2×3 factorial design, with addition of carbon as one factor and pot partitioning as the other. There were 20 replicate pots per treatment. Plants were harvested after 38 days of growth, and shoots and roots of each plant were dried separately at 75°C for 48 hours and weighed.

General linear mixed models were used to test for the effects of pot partitioning, the addition of activated carbon to the substrate, and size inequalities between individuals within a pot, on the root, shoot and total dry mass of plants. Pot was included in the models as a random factor. A one-way ANCOVA was used to examine the effects of different treatments on biomass allocation between shoots and roots, with shoot mass as a dependent variable, treatment as a fixed factor and root mass as a covariate.

Paper II –
Avoidance of physical obstructions by plant roots

Pots were filled with two different substrates using a vertical partition inserted across the pot centre, namely substrate containing only sand (particle diameter < 1 mm) and substrate containing a 4:1 volumetric mixture of sand and gravel (nominal particle diameter 4 mm). The larger size of gravel particles presented greater potential for obstruction of roots than that of sand particles. Seedlings were transplanted individually into the centre of pots, at the boundary between the two substrate types. Four treatments were established that combined two factors: the addition or absence of activated carbon, and low or high nutrient concentration.

Eight species from four genera were used in the experiment: *Agrostis stolonifera* L.*, Agrostis vinealis* Schreber*, Festuca pratensis* Hudson*, Festuca ovina* L.*, Phleum pratense* L.*, Phleum phleoides* (L.) Karsten*, Poa trivialis* L. and *Poa bulbosa* L. Within each genus, the species listed first is characteristic of nutrient-rich habitats and the second of nutrient-poor habitats (Hill *et al.*, 1999). There were 12 replicate pots per treatment×species combination, except for *P. phleoides* and *P. bulbosa*. These species could only be replicated seven and six times per treatment, respectively. Species characteristic of nutrient-rich habitats were harvested earlier (55–60 days after planting) than species characteristic of nutrient-poor habitats (65–80 days after planting) to reduce size differences between species. At harvest, roots in each half of the pot were harvested separately and a representative primary root axis with all its associated laterals was selected from each pot half for morphological analysis. The remaining parts of each plant were dried separately at 70°C for 48 hours and weighed.

General linear mixed models were used to estimate the effects of species habitat type, addition of activated carbon and nutrient levels (fixed effects factors), and substrate type within a pot (repeated measures factor), on root mass and morphological root traits. Genus was included in the models as a random effect.

Paper III –
Effects of neighbour identity
on root-placement patterns

Fragaria vesca L. and *Glechoma hederacea* L. are winter-green, clonal perennial herbs that produce numerous ramets connected by stolons. The habitat range of both species includes woodland margins, scrub and hedgerows (Grime *et al.*, 2007). These species were selected because they are easy to propagate and manipulate, and have markedly different stolon internode lengths, which may result in different frequencies of intraclonal competition. The ranges of stolon internode lengths in *G. hederacea* and *F. vesca* are 2–11 cm and 19–49 cm, respectively (unpublished data, M. Semchenko).

In the first experiment, four treatments were established for each focal species in which the following combinations of ramets were planted: (T1) two connected ramets (i.e. the stolon internode between the ramets was intact); (T2) two disconnected ramets that grew from the same mother ramet (the stolon was severed immediately before the start of the experiment); (T3) two ramets of different genotypes of the same species; and (T4) interspecific ramet pair. Each treatment was replicated 12–15 times. New ramets produced during the experiment were prevented from rooting. After eight weeks of growth, roots were harvested from five separate fractions of the substrate in each tray (Fig. 1

in **III**). Aboveground parts associated with each ramet, and roots from each fraction of the substrate, were dried separately at 75°C for 48 hours and weighed.

Predictions tested using data obtained from the first experiment are presented in Table 1 in **III**. Repeated-measures ANOVA was carried out separately for each focal species, with root mass away from, or towards, the neighbouring ramet as a repeated measure within a tray, and treatment as a fixed factor. The interspecific treatment was split into two levels in the treatment with *Fragaria* as a focal species: root mass of *Fragaria* and root mass of *Glechoma*.

In the second experiment, three treatments were established: (T1) two disconnected ramets of *Glechoma*; (T2) two disconnected ramets of *Fragaria*; and (T3) a ramet of *Fragaria* and a ramet of *Glechoma*. Each treatment was replicated five times. Chambers with the following dimensions were made from transparent acrylic: 2 cm wide, 21 cm long and 22.5 cm deep. Chambers were oriented at an angle of 45° from the vertical so that roots would grow geotropically along the lower wall. Between the tenth and 28th day from root initiation, high-resolution images of the lower walls of the chambers were taken at two day intervals, and the elongation rates of individual roots were calculated. The date and type of root contact (intra- or interplant) were recorded for each root.

In the treatment with pairs of *G. hederacea* ramets, very few inter-ramet root contacts were observed. Therefore, for this treatment, roots were assigned to one of two categories: roots growing towards the neighbouring ramet or roots growing away from it. A general linear mixed model was used to examine the effects of time (repeated measures factor), ramet identity within a chamber and the direction of root growth (fixed factors) on root elongation rates. In the treatments with pairs of *F. vesca* ramets and interspecific ramet pairs, roots were assigned to one of three categories: (i) non-contact roots, (ii) only intra-ramet contact roots, and (iii) inter-ramet contact roots. Two separate general linear mixed models were used to examine the effects of time, ramet identity within a chamber and the category of root contact on root elongation rates in each treatment. Chamber was included in the models as a random factor.

Paper IV –
Association between species rooting behaviour and other fundamental traits

Sixteen treatments were established that combined three factors: identity of the focal species (a ramet of either *Fragaria* or *Glechoma* established in the centre of each of the trays), mode of competition (root *and* shoot competition or only root competition) and planting pattern (focal ramet without neighbours; two neighbours without a central focal ramet; intraclonal monoculture; and inters-

pecific mixture; Fig. 1 in **IV**). To prevent aboveground competition in the "only root competition" treatment, white opaque plastic partitions were placed vertically and perpendicularly across the trays between the focal ramet and its neighbours. Each treatment was replicated 12 times. New ramets produced during the experiment were prevented from rooting. Treatments with all combinations of the presence and absence of partitions, and the presence and absence of focal plants and neighbours, allowed testing and controlling for the effects of partitions themselves on the growth of focal and neighbouring ramets (see McPhee & Aarssen, 2001). After eight weeks of growth, the roots and above-ground parts of each mother ramet were harvested separately, dried at 75°C for 48 hours and weighed.

Growth responses to competition were analysed using a general linear model that included four factors: three treatment factors as fixed effects (treatments with three ramets per tray only were included in the analysis) and ramet position within a tray as a repeated measures factor with three levels (focal ramet and each neighbouring ramet). The intensity of competition was estimated using a standardised variable: ln(mean dry mass of the focal ramet in a competition treatment/mean dry mass of the focal ramet in an appropriate control). Treatments with single ramets and no partitions were used as the control for full competition treatments, and treatments with single ramets and partitions present were used as the control for only root competition treatments (McPhee & Aarssen, 2001).

RESULTS

Paper I –
Confounding effects of neighbour presence and substrate volume

The addition of activated carbon did not affect the growth of plants sharing substrate with a competitor, but it led to increases in total plant mass and allocation to shoots in treatments where root systems were separated by pot partitions (Figs 2–3 in **I**). When activated carbon was added to the substrate, plants that were separated by a solid pot partition performed significantly better than plants sharing the whole volume of a pot (Fig. 2 in **I**). Plant mass was not significantly affected by the presence of root exudates, root contact or resource movement between the root systems within a pot (Fig. 2 in **I**).

Paper II –
Avoidance of physical obstructions by plant roots

Only species characteristic of nutrient-poor habitats were able to significantly restrict root mass placement in substrate containing obstructions (Fig. 1 in **II**). This response was not observed when activated carbon was added to the substrate. No selective root placement was observed in species characteristic of nutrient-rich habitats. Branching density was significantly reduced in substrate containing gravel compared with substrate without gravel, but this effect was not observed in pots containing activated carbon (Fig. 2 in **II**). The presence of gravel in the substrate did not affect the elongation rates of single roots (Fig. 3 in **II**). Roots growing in the half of the pot with gravel had lower specific root length (root length per unit of root mass) and produced fewer laterals than roots growing in the half of the pot without gravel (Fig. 3 in **II**).

Paper III –
Effects of neighbour identity on root-placement patterns

Irrespective of the identity of neighbours, *Glechoma* ramets placed significantly less root mass towards the neighbouring ramet than away from it (Figs 2–3 in **III**). By contrast, *Fragaria* ramets exhibited similar root growth towards and away from all types of neighbours (Fig. 3 in **III**). The contrast between the two root-placement patterns was greatest in the interspecific treatment (Fig. 3 in **III**). In root observation chambers, roots of *Glechoma* avoided contact with

roots of neighbouring ramets and exhibited significantly slower elongation rates towards the neighbouring ramet than away from it (Figs 4 and 6a in **III**). Elongation of individual roots of *Fragaria* was significantly stimulated by contact with roots of *Glechoma* (Fig. 6b in **III**).

Paper IV –
Association between species rooting behaviour and other fundamental traits

Focal ramets of *Glechoma* grew significantly larger in the interspecific competition treatment than in the intraclonal competition treatment. The increase in growth was mainly due to an increase in the number and total mass of daughter ramets produced by mother ramets; no changes in the root mass, height and shoot mass of mother ramets were observed (Fig. 3 in **IV**). The reduction in the intensity of competition in the interspecific treatment compared with intraclonal treatment was greater when aboveground competition between ramets was prevented (Fig. 4 in **IV**). By contrast, the intensity of competition experienced by focal *Fragaria* ramets was not significantly different between intraclonal and interspecific treatments, either with full competition or root competition alone (Figs 3–4 in **IV**).

DISCUSSION

The results of the present study showed that differences in plant growth in the presence *vs*. the absence of root competition can be caused by factors other than neighbour recognition (**I**). In the control treatment without root competition, plant growth was limited by the availability of rooting space. This limitation was mediated by the accumulation of inhibitory root exudates in the vicinity of the pot partitions that were used to prevent belowground competition: the addition of activated carbon to the substrate in partitioned pots resulted in improved plant growth, despite there being no changes in space or nutrient availability (Fig. 2 in **I**). When activated carbon reduced the effect of self-inhibition, plants whose root systems were separated by a partition to prevent belowground competition performed better than plants that had free access to the competitor's rooting space. However, root exudates, physical contact and resource movement between root systems did not significantly affect plant growth, and were not apparently involved in communication between plants. If plants do not possess a mechanism to detect neighbours and to avoid overlap between their root systems, each plant may need to explore a considerably larger volume of substrate in the presence of a competitor to acquire the same amount of resources as when competition is prevented by partitioning of rooting space. This may lead to a reduction in resource acquisition efficiency and reduced performance (Brisson & Reynolds, 1997; Schenk *et al*., 1999). Therefore, self-inhibition of root growth in a limited volume of substrate in a control treatment without root competition, and inefficient root placement in larger substrate volumes that are shared with the roots of a competitor, may be important determinants of plant performance in studies on root competition. These factors create a challenge for experimental studies to discriminate between changes in plant growth that are attributable to communication between plants and responses of roots to changes in soil spatial characteristics.

Investigation of the ability of plants to forage for usable space in substrate with a heterogeneous distribution of obstructions demonstrated that self-inhibition of root growth due to the accumulation of root exudates in the vicinity of obstructions (as described in Falik *et al*., 2005 and Paper **I**) may facilitate efficient root placement (**II**). When presented with the choice of growing into substrate containing only sand or into substrate with a high density of larger obstructions, grass species characteristic of nutrient-poor habitats significantly limited root mass placement in the substrate with a high density of obstructions. The involvement of root exudates in the avoidance of obstructions was indicated by the fact that the addition of activated carbon to the substrate rendered plants of these species incapable of placing roots preferentially in substrate with few obstructions (Fig. 1 in **II**). Responses mediated by root exudates may reduce root production in substrate with many obstructions before resource uptake becomes directly affected by root crowding in limited space. This phenomenon is similar to other examples of stress avoidance where plants

use cues or signals to modify their behaviour *before* adverse conditions impact upon them (Ballaré *et al*., 1987, 1990; Shulaev *et al*., 1997; Baldwin *et al*., 2006; Heil & Silva Bueno, 2007). Although physical space is not a resource in the same sense as nutrients or water, it may indicate the accessibility of nutrients within the substrate. It is also worth noting that obstruction avoidance may play an important role in root competition because neighbouring roots, in addition to depleting resources and releasing chemicals, may physically obstruct each other's growth.

The ability to limit root mass placement in substrate with many obstructions was only observed in species characteristic of nutrient-poor habitats. Several factors could impose a stronger selective pressure on species from nutrient-poor habitats to evolve the ability to avoid obstructions in the soil. Firstly, such species are characterised by high root construction and maintenance costs, making efficient root placement particularly critical for them (Poorter *et al*., 1990; Wahl & Ryser, 2000). Secondly, the restriction of root systems to obstructed substrate caused greater reductions in plant growth at low nutrient concentrations (McConnaughay & Bazzaz, 1992), suggesting that growth in densely obstructed substrate may be more costly when nutrients are scarce.

It has been suggested that species from nutrient-rich habitats should exhibit more pronounced morphological plasticity in response to heterogeneous distribution of nutrients in the soil than species from nutrient-poor habitats (Grime *et al.,* 1986). In infertile soils, nutrients become available in short, unpredictable pulses that can be exploited most efficiently by large long-lived root systems with little morphological plasticity. By contrast, species from nutrient-rich habitats can afford to exhibit fast morphological responses to nutrient patches by producing inexpensive short-lived roots. Experimental tests of this prediction have produced conflicting results (Crick & Grime, 1987; Campbell & Grime, 1989; Grime *et al*., 1991; Fransen *et al*., 1998, 1999; reviewed in Hutchings & John, 2003). Root morphological responses to obstructions differed from those documented in response to low nutrient availability, suggesting that the presence of obstructions in the soil, and concurrent changes in space availability, represent different environmental challenges from changes in nutrient levels (**II**). Due to the ephemeral nature of nutrient pulses in infertile soil, high nutrient concentration may be an unreliable cue for the initiation of morphological responses in roots. By contrast, physical obstructions in the soil have a long-term effect on the availability of rooting space. Therefore, excessive nutrient-led proliferation of roots in obstructed substrate should be detrimental to long-term nutrient uptake efficiency. Nutrient-poor soils often contain higher proportions of larger particles than fertile soils (Borchers & Perry, 1992; Hassink, 1992), which may also render the ability to forage for usable space more critical for species that are characteristic of infertile soil. Given the limited number and variety of species used in this study, further research on a wider range of species is needed to fully analyse the effects of habitat fertility, frequency of obstructions in the substrate and other

factors (e.g. factors affecting root exudation) on the prevalence of obstruction avoidance amongst species found in different plant communities.

Experimental studies that test recent predictions of game-theoretic models of root competition often involve a single species and examine only intraspecific competition (Gersani *et al.*, 2001; Maina *et al.*, 2002; Falik *et al.*, 2003; O'Brien *et al.*, 2005; Murphy & Dudley, 2007). In a study in which interspecific interactions were also considered (**III**), contrasting responses to neighbouring roots were observed in two herbaceous clonal species: *Fragaria vesca* (wild strawberry) and *Glechoma hederacea* (ground ivy). Ramets of *Fragaria* expanded their root systems in all directions irrespective of the presence and identity of neighbours. By contrast, ramets of *Glechoma* exhibited avoidance of belowground competition with their neighbours by limiting root growth towards neighbours and by selective proliferation of roots in substrate that was free of competitors. Game-theoretic models of root competition predict that nutrient-efficient rooting strategies (such as avoidance of overlap between root systems) will be competitively inferior to more aggressive rooting strategies that lead to a reduction in plant performance due to excessive production of roots in areas where root systems overlap (O'Brien *et al.*, 2005, 2007). Results from the competition experiment clearly demonstrated for *Glechoma* and *Fragaria* that the avoidance of competition with neighbours was not accompanied by competitive inferiority to a species that does not avoid competition (**IV**). *Glechoma* achieved greater growth in the mixture with *Fragaria* than in the intraclonal monoculture, whereas *Fragaria* ramets did not show significant growth reductions in mixture with *Glechoma* compared with monoculture. In addition to contrasting rooting behaviour, differences in the pattern of allocation of resources between the aboveground parts of the mother and daughter ramets could allow an increase in growth of *Glechoma* without a corresponding reduction in the growth of *Fragaria*. The additional resources presumably acquired by *Glechoma* ramets in the interspecific treatment, compared with the intraclonal treatment, were invested in the production of vegetative offspring, rather than in enhancement of the capacity of the mother ramet for localised competition against neighbouring *Fragaria* ramets. Thus, *Fragaria* ramets did not experience more intense competition from *Glechoma*, even though they may have ceded some nutrients to it. By contrast, *Fragaria* invested in local persistence by preferential allocation of resources to the mother ramet and by increasing its capacity to shade competing vegetation. Therefore, the outcome of competition, and the capacity for species with different root growth strategies to coexist, may be contingent upon a combination of different species characteristics.

The persistence of avoidance behaviour in *Glechoma* is intriguing because simple game-theoretic models predict that the avoidance genotype should be outcompeted within a species by a genotype that overproduces roots in the presence of competitors (Gersani *et al.*, 2001; Maina *et al.*, 2002). The adaptive value of an avoidance rooting strategy may depend on the long-term probability of competition between genetically identical or closely related individuals (**III**).

Within-genotype competition is regarded as highly wasteful and to be avoided wherever possible (Mahall & Callaway, 1996; Schenk *et al*., 1999; Falik *et al*., 2003; Gruntman & Novoplansky, 2004). Due to its relatively short stolon internodes, *Glechoma* is likely to experience frequent root contact between ramets of the same clone and could therefore benefit from being able to avoid competition between neighbouring ramets. In contrast, *Fragaria* clones produce significantly longer stolon internodes that are likely to result in frequent competition with unrelated individuals, favouring the evolution of a more aggressive rooting strategy.

The study of root responses to neighbours can be addressed using a range of approaches, though conclusions can differ dramatically depending on the chosen approach. Morphological responses to environmental variation have traditionally been studied in the framework of phenotypic plasticity (reviewed in Hutchings & John, 2003; Hodge, 2004; de Kroon *et al*., 2005). The null hypothesis in such studies is that plants lack plasticity, and if alterations in plant development in response to an environmental change are observed, they are often considered adaptive. A number of costs and limitations on the evolution of phenotypic plasticity have been suggested, including the unreliability of environmental signals, deficient sensory capabilities, a time lag between environmental and phenotypic change, the maintenance cost of the genetic and cellular machinery needed to produce a plastic response, and genetic correlations between traits (reviewed in Schmitt, 1997; DeWitt *et al*., 1998; Pigliucci, 2001; Diggle, 2002; van Kleunen & Fischer, 2005). A completely different approach was taken by a group of studies that examined root responses to neighbours (Gersani *et al*., 2001; Maina *et al*., 2002; O'Brien *et al*., 2005). In these, the adaptive value of different root responses to the presence of competitors was assessed using evolutionary game theory, in which the optimal root production of one individual depends on the rooting strategies of other plants. It was predicted that plants should respond to the presence of neighbours with increased root production at the expense of reproductive yield. In contrast to plasticity studies, no constraints on the expression of such behaviour were considered.
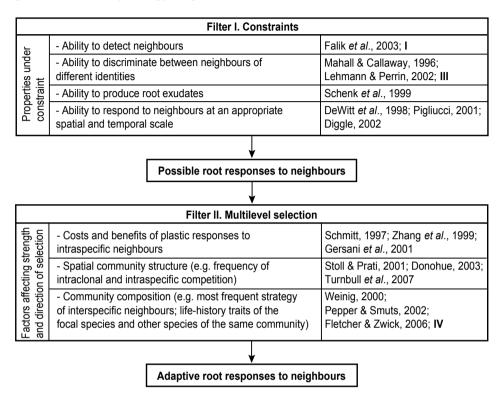
Root responses to neighbours can fall along a gradient from complete avoidance of competition with neighbouring roots, through no response to the presence of a competitor's roots, to intensified root growth towards the roots of a competitor (**III**). In the framework of phenotypic plasticity, avoidance and aggressive rooting patterns would be regarded as expressions of phenotypic plasticity that are likely to be adaptive, whereas no response to neighbours would signify the existence of constraints on the evolution of plasticity (Sultan, 2000; Alpert & Simms, 2002). Most of the studies on root communication report limited overlap between neighbouring root systems and interpret it as either an adaptation to reduce intraclonal competition, territory defence *via* toxin production or a result of efficient foraging for nutrients (Krannitz & Caldwell, 1995; Holzapfel & Alpert, 2003; reviewed in Schenk *et al*., 1999). In

the framework of game theory, any deviance from complete root segregation towards higher root density in substrate shared with competitors would be regarded as evidence that plants are involved in a tragedy of the commons (O'Brien *et al*., 2005, 2007). This prediction cannot be readily tested experimentally, however, because of the confounding effects of factors other than neighbour recognition (**I–III**).

Future studies could greatly benefit from combining different approaches to root research (Table 1). Root behaviour is likely to be determined by many interacting factors that can be represented as two filters. Firstly, genetic constraints on the evolution of mechanisms to detect and respond to neighbours at an appropriate spatial and temporal scale determine the range of root responses that can conceivably be produced. These possible root responses to neighbours provide material for the second filter: multiple selection pressures that act at an ecological scale eliminate root behaviours that are maladaptive in a given environmental setting (Table 1). While the costs and benefits of phenotypic plasticity in response to intraspecific neighbours have received considerable attention in plant science, the importance of biotic community-level processes for the evolution of different behavioural patterns has been appreciated in very few studies (references in Table 1). For example, community spatial structure and composition determine the similarity between interacting individuals (i.e. genetic and phenotypic similarity) and the intensity of interactions between neighbouring individuals (e.g. density of individuals and degree of competition avoidance; Donohue, 2003, 2004; **IV**) that may in turn affect the adaptive value of different behavioural patterns. The importance of these factors for the evolution of root behaviour is virtually unknown.

A major challenge for future theoretical and experimental studies will be to account for complex interactions between roots and their environment, including the types of interactions demonstrated in the present study (**I–IV**). Consideration of community-level processes and of possible limits to plasticity in response to the presence of neighbours may promote the development of more realistic predictions and explanations regarding variation in root behaviour between populations and species. In conclusion, it is clear that interactions between plant roots represent a challenging but promising model system for testing major hypotheses in evolutionary biology.

Table 1. A representation of the ways in which root responses to neighbours could be determined by constraints on the ability to detect neighbours and respond to them at an appropriate temporal and spatial scale, and by the adaptive value of those responses within a community setting. Examples of relevant studies from plant ecology and general evolutionary biology are presented.

Filter I. Constraints		
Properties under constraint	- Ability to detect neighbours	Falik *et al*., 2003; **I**
	- Ability to discriminate between neighbours of different identities	Mahall & Callaway, 1996; Lehmann & Perrin, 2002; **III**
	- Ability to produce root exudates	Schenk *et al*., 1999
	- Ability to respond to neighbours at an appropriate spatial and temporal scale	DeWitt *et al*., 1998; Pigliucci, 2001; Diggle, 2002

Possible root responses to neighbours

Filter II. Multilevel selection		
Factors affecting strength and direction of selection	- Costs and benefits of plastic responses to intraspecific neighbours	Schmitt, 1997; Zhang *et al*., 1999; Gersani *et al*., 2001
	- Spatial community structure (e.g. frequency of intraclonal and intraspecific competition)	Stoll & Prati, 2001; Donohue, 2003; Turnbull *et al*., 2007
	- Community composition (e.g. most frequent strategy of interspecific neighbours; life-history traits of the focal species and other species of the same community)	Weinig, 2000; Pepper & Smuts, 2002; Fletcher & Zwick, 2006; **IV**

Adaptive root responses to neighbours

CONCLUSIONS

Research on root interactions has moved beyond the assumption that resource depletion is the main mechanism underlying interactions between roots and the soil environment. The findings of this study reveal complexity and interspecific variation in root interactions, setting challenges for experimental design and opening new avenues for future research.

The studies reported in this thesis demonstrate that, in addition to responses to the presence of neighbours, two confounding processes could have determined plant growth in the experimental design used in previous studies on the tragedy of the commons in root competition: (i) greater root self-inhibition in the limited substrate volume of the control "no competition" treatment, and (ii) inefficient nutrient uptake in the competition treatment in which larger substrate volume is shared with roots of a competitor. These findings demonstrate the need for new experimental designs that take into account these confounding factors.

Investigation into the ability of roots to avoid physical obstructions in the soil and forage for usable space revealed that self-inhibition of root growth due to the accumulation of root exudates in the vicinity of obstructions may facilitate more efficient root placement in substrate with a heterogeneous distribution of obstructions. Responses mediated by root exudates may ensure a reduction in root production in densely obstructed substrate patches before nutrient uptake becomes directly limited by root aggregation in limited space. This mechanism is similar to other examples of signal-mediated stress avoidance.

Examination of root-placement patterns in two herbaceous clonal species demonstrated that co-occurring species can exhibit a variety of responses to the roots of neighbours, including avoidance of root growth towards neighbours, no response, and even intensified root growth towards a neighbour's roots. The underlying mechanisms and causes of the evolution and coexistence of different rooting behaviours remain largely unexplored.

Ignoring functional variation in fundamental plant traits that are known to determine the success of a species in a community prevents theoretical models from explaining the variation in root behaviour that has been observed in plants. The results of this study show that, in contrast to the predictions of simplified game-theoretical models, the avoidance of competition with neighbours is not synonymous with competitive inferiority to a strategy that does not avoid competition. The outcome of competition, and the potential for species with different rooting behaviours to co-exist, is clearly contingent upon other species characteristics. A goal of future research should be to identify the constraints and species- and community-level traits that shape the evolution of root behaviour.

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Taimejuurte käitumine: reaktsioonid naabertaimedele ja füüsilistele takistustele

Tänaseks on selge, et taimed on võimelised tajuma naabertaimede juuri ja reageerima nende olemasolule mitmel moel. Lisaks otsesele toitainete omastamisele võivad juured mõjutada naabrite kasvu nii läbi allelopaatiliste eritiste kui ka läbi mittetoksiliste signaalide. Kasvu füsioloogiline koordineerimine ühe ja sama taimevõsu või samasse klooni kuuluvate rametite juurte vahel ning konkurentide geneetiline sugulus võivad samuti mõjutada juurte kasvu ja ruumilist paigutust. Keerukate taimedevaheliste interaktsioonide eksisteerimine avab võimaluse kasutada taimeuuringutes samalaadseid evolutsioonilisi käsitlusi, mida on edukalt rakendatud loomade käitumismustrite seletamisel. Empiirilised tööd, mis seavad eesmärgiks testida erinevate evolutsiooniliste teooriate rakendatavust taimeriigis, seisavad aga silmitsi raskusega eristada aktiivset juurtevahelist kommunikatsiooni reaktsioonidest toitainete kättesaadavusele ja ruumilisele jaotusele.

Evolutsiooniline mänguteooria ennustab, et taimed peaksid konkurentide mõjuväljas üleprodutseerima juuri, makstes selle eest väiksema seemnetoodanguga. Kui taimejuurte aktiivse ressursiomastamise tsoonid kattuvad, tekib konkurents toitainete pärast ja toitainete omastamise efektiivsus langeb, sest sama toitumistsoon on kasutuses mitme juure poolt üheaegselt. Kuna otsene konkurents ühe ja sama indiviidi juurte vahel oleks äärmiselt raiskav, peaks võime vältida juurkonkurentsi ühe isendi piires andma evolutsioonis eelise. Kui konkurendiks on aga teine taim, võiks juurte paigutamine konkurendi poolt asustatud alasse vastupidi parandada taime kohasust sest see võimaldab konkurentidelt lisatoitainete "varastamist". Kuna konkurendid omastavad oodatavalt ajapikku sama strateegia, peaks iga taim lõpuks suutma „varastada" sama palju toitaineid nagu teised "varastavad" temalt. Seega kaob algne lisajuure moodustamise eelis ja kõik taimed peavad maksma lisajuurte moodustamise hinda seemnetoodangu arvelt. Sellist nähtust on kirjeldatud kui ühisomanditragöödiat. Kui taimed ei suuda koopereeruda ja vältida isekate genotüüpide invasiooni, peaks ühisomanditragöödia olema ainukeseks evolutsiooniliselt stabiilseks strateegiaks.

Ülaltoodud mõttekäigu paikapidavust on mitmetes uurimustes püütud empiiriliselt testida. Paraku ei ole seniste eksperimentide disain võtnud arvesse asjaolu, et taimejuured on võimelised tajuma ja reageerima mitte ainult teiste juurte lähedusele vaid ka füüsilise ruumi kättesaadavusele ja takistuse olemasolule mullas. Töödes, kus on otsitud kinnitust ühisomanditragöödiale juurkonkurentsis, on toitainete hulka ja mulla ruumala ühe taime kohta hoitud konstantsena kõigis töötlustes. Selle tulemusena on mulla koguruumala, mida ühe taime juured võivad asustada, olnud kaks korda suurem töötluses, kus kaks

taime konkureerivad maa-aluste ressursside pärast, võrreldes töötlusega, kus juurkonkurentsi tõkestatakse kasutades maa-alust vaheseina kahe taime vahel. Seega juurekonkurentsi mõju võib nimetatud uurimustes olla segunenud ühe taime juurtele maksimaalselt kättesaadava ruumi mõjuga.

Selle doktoritöö üheks eesmärgiks oli välja selgitada ülaltoodud segavate faktorite mõju taimede kasvule ja uurida, kuidas see on võinud mõjutada eelmainitud eksperimentaalset disaini kasutanud tööde järelduste õigsust. Selleks kombineerisin seniste uurimuste disaini lisatöötlustega, mis võimaldasid uurida taimede reaktsioone füüsilise ruumi kättesaadavusele ja taimede võimet tuvastada konkurentide kohalolekut. Tulemusena leidsin, et taimede kasv kontrolltöötluses oli piiratud ruumi vähese kättesaadavuse tõttu. Taimed reageerisid ruumi kättesaadavusele toksiliste juureeritiste akumulatsiooni tõttu piiratud ruumis. Kui sellist kasvu eneseinhibitsiooni pidurdati aktiveeritud söe lisamisega, mis adsorbeerib juureeritisi ja vähendab nende mõju taimekasvule, kasvasid taimed väiksemas mullaruumalas suuremaks kui need taimed, mis pidid jagama ruumi naabriga. Samas, lisatöötlused näitasid, et taimed ei reageerinud ei naabri juureeritistele, juurtevahelisele kontaktile ega toitainete liikumisele kahe juuresüsteemi vahel. Seega ei mänginud need faktorid rolli naabrite äratundmises. Kui taimed ei olnud võimelised naabreid tuvastama ja neile reageerima, siis eri taimede juuresüsteemid põimusid. Ressursside omastamine kahe taime läbipõimunud juuresüsteemi poolt muutub aga ebaefektiivseks: iga taim peab kasvama läbi oluliselt suurema ruumi ja võib selleks vajada rohkem juurepikkust, et omastada sama palju ressursse nagu taimed, mis kasvavad üksi poole väiksemas ruumis. See omakorda kahandab taime üldist kasvu.

Sõltumatud uuringud näitavad selgelt, et maa-aluse kasvuruumi vähene kättesaadavus võib taimedel põhjustada olulist kasvulangust, isegi kui toitainete, vee ja hapniku kättesaadavus on piisav. Looduslikes tingimustes sõltub kasvuruumi kättesaadavus füüsiliste takistuste suurusest ja tihedusest mullas. Takistuseks võib olla kokkupressitud muld, kiviosakesed, juured, orgaaniline kõdu ja muud sarnased objektid. Need vähendavad juurte paigutamiseks vajalikku ruumi, killustavad kasutuskõlblikku mulda ja raskendavad ressursilaikude leidmist ning hõlvamist. Seega võiks taimede kohasust parandada võime otsida kasvuruumi, sealjuures vältides juurte kasvu mullalaikudes, kus on palju takistusi, ja eelistada mulda, milles ressursid on kergemini kättesaadavad. Samas puuduvad uuringud, mis hindaks taimede võimet vältida maa-aluseid kasvutakistusi, ja mis näitaks kui levinud selline võime taimeriigis on, või millised faktorid sellist võimet soodustavad.

Selle doktoritöö teiseks eesmärgiks oli uurida taimede võimet vältida maaaluseid takistusi ja selle taga peituvat mehhanismi ning uurida selle võime sõltuvust liigiomase kasvukoha produktiivsusest. Eksperimendi tulemused näitasid, et ainult toitainetevaeste kasvukohtade liigid olid võimelised paigutama juuri eelistatavalt sellisesse substraati, kus takistusi oli vähe, ja vältima kasvu takistuste poole. Nagu eelmises uuringus, nii ilmnes ka nüüd, et võime vältida takistusi põhines juurte tundlikkusel omaenda eritiste akumulatsiooni suhtes:

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aktiveeritud söe lisamisel kaotasid taimed võime tajuda kasvutakistusi. Võib välja tuua mitu asjaolu, mis võiksid mullas olevate takistuste vältimise teha eriti oluliseks toitainetevaeste kasvukohtade liikidele. Esiteks on juurte moodustamise ja ülalpidamise kulud oluliselt suuremad liikidel, mis asustavad väheviljakaid kasvukohti, võrreldes toitaineterikaste kasvukohtade liikidega. Samuti sisaldavad toitainetevaesed mullad tihtipeale rohkem suuremamõõtmelisi takistusi kui viljakad mullad.

Need teoreetilised mudelid, millega on püütud ennustada juurte erinevate käitumismustrite adaptiivset väärtust, on seni käsitlenud taimi identsetena kõigi tunnuste osas, välja arvatud juurte produktsioon konkurentide puudumisel või olemasolul. Täiesti on igoreeritud varieeruvust paljudes funktsionaalsetes tunnustes, mis on tuntud kui olulised liigi edukuse määrajad kooslustes. Selline lihtsustus on viinud järelduseni, et ainukeseks evolutsiooniliselt stabiilseks strateegiaks on juurte produktsiooni suurendamine konkurentide olemasolul. Samas näitavad empiirilised uuringud üllatavat mitmekesisust taimejuurte käitumises.

Selle doktoritöö raames uurisin, kuidas kaks oluliselt erineva kasvumustriga klonaalselt paljunevat liiki reageerivad kokkupuutele enda juurtega ja naabrite juurtega. Täpsemalt selgitasin kuidas füüsiline ühendus rametite (ehk klooni eri moodulite) vahel ja naabertaimede geneetiline identsus mõjutavad juurte ruumilist paigutust ja üksikute juurte kasvukiirusi. Uuritavateks liikideks valisin hariliku maajala (*Glechoma hederacea*) ja metsmaasika (*Fragaria vesca*) kui liigid, millele on iseloomulikud vastavalt lühikesed ja pikad keskmised vahemaad samasse klooni kuuluvate naaberrametite vahel. Tulemused näitasid, et *Glechoma* rametid vältisid järjekindlalt maa-alust konkurentsi, paigutades juuri naabertaimest eemale sõltumata naabri identsusest. Seevastu *Fragaria* taimed demonstreerisid pigem sümmeetrilist juurekasvu naabertaime suunas ja temast eemale sõltumata naabertaime identsusest. *Glechoma* rametid konkureerivad looduses suure tõenäosusega enda lähisugulastega ja konkurentsi vältimine peaks selle liigi jaoks osutuma kasulikuks strateegiaks. *Fragaria* kloonides konkureerivad rametid enamasti mittesugulastega, mis võib soosida agressiivsemat kasvustrateegiat.

Järgmise sammuna kasutasin samu liike uurimaks juurte käitumismustri seost teiste liigiomadustega ja selgitamaks, kuidas selle seose arvestamine võiks muuta ainuüksi juurte käitumisel põhinevat ennustust liikidevahelise konkurentsi tulemuse kohta. Kui teised liigiomadused ei mõjuta juurte käitumismustri adaptiivset väärtust, peaks liik, mis väldib konkurentsi teiste taimedega (*Glechoma*), jääma konkurentsis alla liigile, mis ei väldi konkurentsi naabritega (*Fragaria*). Konkurentsikatse tulemused näitasid aga, et *Glechoma* saavutas suurema kasvu segus *Fragaria* taimedega kui sama klooni monokultuuris. Samas ei vähenenud *Fragaria* kasv segus *Glechoma* taimedega, võrreldes enda monokultuuriga. Lisaks erinevale juurkäitumisele erines *Glechoma* konkurendist suurema kasvukiiruse poolest ja suunas enamuse lisaressursse vegetatiivsesse paljunemisse ning uute ressursilaikude asustamisse, selle asemel, et tugevdada emataime konkurentsivõimet. Seevastu *Fragaria* investeeris klooni

püsimisse juba asustatud laikudes, paigutades suure osa ressurssidest emataime maapealse konkurentsivõime parandamisse ja investeerides taime pikaea-lisusesse, mis kompenseeris ebasoodsat aeglast kasvu. Seega ei vii maa-aluse konkurentsi vältimise strateegia tingimata konkurentse väljatõrjumiseni agres-siivsema juurkäitumise strateegiaga liigi poolt. Konkurentsi tulemus ja eri juurte käitumismustrite potentsiaal kooseksisteerimiseks võivad sõltuda sellistest liigi-omadustest nagu suhteline kasvukiirus, kudede ja rametite eluiga, ressursside paigutamine emataime ja tütarvõsude vahel ning samasse klooni kuuluvate rametite ruumiline paigutus.

Kokkuvõtteks toovad selle doktoritöö tulemused esile vajaduse parema eks-perimentide disaini väljatöötamiseks, mis võimaldaks eristada juurte kommu-nikatsioonil põhinevat käitumist mulla toitainete ning ruumi jaotusest tingitud reaktsioonidest. Lisaks naabertaime äratundmise võimele omavad mõned liigid ka võimet tuvastada ja vältida juurte kasvu füüsiliste takistuste poole mullas, enne kui toitainete omastamine muutub ebaefektiivseks juurte kuhjumise tõttu piiratud ruumis. Selline võime on sarnane teiste nähtustega, kus taimed kasu-tavad signaale stressi vältimiseks enne kui stressifaktor hakkab otseselt taimi mõjutama. Juurte käitumismustri adaptiivse väärtuse hindamisel on oluline arvestada põhiliste liigiomadustega aga ka koosluse ruumilise struktuuri ja koosseisuga.

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CURRICULUM VITAE

I. General

Name: Marina Semchenko
Date and place of birth: 08.05.82 Tallinn
Citizenship: Estonian
Language skills: English, Estonian, Russian
Address: Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai St., 51005 Tartu, Estonia
Phone: +372 737 6188
e-mail: marina.semchenko@ut.ee

Current position: University of Tartu, Institute of Ecology and Earth Sciences, researcher

Education and professional employment:
1988–1997	Tallinn Secondary School No. 11
1997–2000	Laagna Secondary School, Tallinn
2000–2004	University of Tartu, Estonia (B.Sc. in botany and ecology, *cum laude*)
2004–2005	University of Tartu, Estonia (M.Sc. in plant ecology and ecophysiology)
2005–2008	University of Tartu, Estonia (Ph.D. student in plant ecology and ecophysiology)
2005–2007	University of Sussex, UK (visiting Ph.D. student)
2007–present	University of Tartu, Institute of Ecology and Earth Sciences, researcher

II. Scientific and research activity

Research interests:
phenotypic plasticity in plants; plant root intearactions and self/non-self discrimination

Publications:
Semchenko M, Hutchings MJ, John EA. 2007. Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume. *Journal of Ecology* 95: 252–260.
Semchenko M, John EA, Hutchings MJ. 2007. Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. *New Phytologist* 176: 644–654.

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Semchenko M, Zobel K. 2007. The role of leaf lobation in elongation responses to shade in the rosette-forming forb *Serratula tinctoria* (Asteraceae). *Annals of Botany* 100: 83–90.

Semchenko M, Zobel K, Heinemeyer A, Hutchings MJ. 2008. Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytologist* 179: 1162–1170.

Conference presentations:

Semchenko M. Phenotypic plasticity in response to different types of biotic and abiotic environment (Discussion of PhD projects). 8th Clonal Plant Workshop: Generality, specificity and diversity of clonal growth. 27–30 June 2006, Estonia.

Semchenko M, John EA, Hutchings MJ. Root placement patterns in two clonal species. British Ecological Society's Annual Meeting. 10–12 September 2007, University of Glasgow, UK

Semchenko M, Zobel K, Hutchings MJ. Foraging for space by plant roots: a comparative study of grasses from contrasting habitats. 51st Annual Symposium of the International Association for Vegetation Science. 7–12 September 2008, University of Stellenbosch, South Africa

Awards and scholarships:

2004   Yearly Award, Biosciences and Environment – Estonian National Contest for Young Scientists at university level, I Prize

2005, 2006 Kristjan Jaak's scholarships

2007   Doctoral School of Ecology and Environmental Sciences, graduate student scholarship

Other scientific activities:

Reviewer in Evolutionary Ecology, Journal of Ecology, Journal of Vegetation Science, New Phytologist, Plant Ecology

ELULOOKIRJELDUS

I. Üldandmed

Nimi: Marina Semtšenko
Sünniaeg ja koht: 08.05.82 Tallinn
Kodakondsus: Eesti
Keelteoskus: eesti, inglise, vene
Aadress: Tartu Ülikool, Ökoloogia ja Maateaduste Instituut, Botaanika osakond, Lai 40, 51005 Tartu, Eesti
Telefon: +372 737 6188
e-mail: marina.semchenko@ut.ee

Praegune amet: Tartu Ülikool, Ökoloogia ja Maateaduste Instituut, erakorraline teadur

Haridus ja erialane teenistuskäik:
1988–1997   Tallinna 11. Keskkool
1997–2000   Laagna Gümnaasium, Tallinn
2000–2004   Tartu Ülikool, Eesti (B.Sc. botaanika ja ökoloogia erialal, *cum laude*)
2004–2005   Tartu Ülikool, Eesti (M.Sc. taimeökoloogia ja ökofüsioloogia erialal)
2005–2008   Tartu Ülikool, Eesti (doktorant taimeökoloogia ja ökofüsioloogia erialal)
2005–2007   Sussexi Ülikool, Suurbritannia (külalisdoktorant)
2007–   Tartu Ülikool, Ökoloogia ja Maateaduste Instituut, erakorraline teadur

II. Teaduslik ja arendustegevus

Peamised uurimisvaldkonnad:
Fenotüübiline plastilisus taimedel; taimejuurte käitumismustrid ja naaberisendite läheduse tuvastamise mehhanismid

Publikatsioonide loetelu:
Semchenko M, Hutchings MJ, John EA. 2007. Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume. *Journal of Ecology* 95: 252–260.
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Semchenko M. Phenotypic plasticity in response to different types of biotic and abiotic environment (Discussion of PhD projects). 8th Clonal Plant Workshop: Generality, specificity and diversity of clonal growth. 27–30 June 2006, Estonia.

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Saadud uurimistoetused ja stipendiumid:

2004 I preemia bio-geoteaduste valdkonnas Eesti üliõpilaste teadustööde riiklikul konkursil

2005 Kristjan Jaagu tasemeõppe stipendium

2006 Kristjan Jaagu osalise õppe stipendium

2007 Ökoloogia ja Keskkonnateaduste doktorikooli toetus teadustööks ja teaduskonverentsil osalemiseks

Muu erialane tegevus:

Retsensent ajakirjades Evolutionary Ecology, Journal of Ecology, Journal of Vegetation Science, New Phytologist ja Plant Ecology

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