Performance of two *Centaurea* species in response to different root-associated microbial communities and to alterations in nutrient availability

Mari Moora*, Maarja Öpik & Martin Zobel

*Institute of Botany and Ecology, Tartu University, Lai St. 40, Tartu, 51005, Estonia (*e-mail: mari.moora@ut.ee)*

The perennial forbs *Centaurea jacea* and *C. scabiosa* have relatively similar morphological traits, but different distributions and local abundance. Both occur in ruderal sites and in calcareous grasslands, but *C. jacea* may dominate in calcareous grasslands, while *C. scabiosa* is a subordinate species. We hypothesised that: (1) *C. jacea* is dependent on the presence of the root-associated microbial community of a calcareous grassland (CG inoculum), as opposed to the microbial community from a ruderal site (old field inoculum, OF), while *C. scabiosa* is not; and (2) if there is a positive response of *C. jacea* to CG inoculum, it will be more evident in oligotrophic soil conditions. In our experiment the two *Centaurea* species were grown with two different inocula (CG, OF) at three different nutrient levels. CG inoculation considerably increased the leaf number of *C. jacea* as compared with the OF inoculation. This response was interpreted as an investment into competitive ability in the future. CG inoculum resulted in a significantly higher root biomass in *C. scabiosa*, while the biomass of *C. jacea* did not vary among differentially inoculated plants. This was interpreted as an increased allocation to storage in *C. scabiosa,* to improve performance in stress conditions. Thus, compared with ruderal inoculum (OF), grassland (CG) inoculum enhanced 'competitive behaviour' in *C. jacea* and 'stress-tolerating behaviour' in *C. scabiosa*. Both responses were more evident at high nutrient levels. Fertilisation decreased the shoot to root ratio in both species, which may represent an 'element of stress-tolerating behaviour', much more common among rhizomatous perennial plants than is usually recognized.

Key words: allocation, arbuscular mycorrhiza, *Centaurea jacea*, *C. scabiosa*, growth response, plant reproduction, plant strategy, root-associated microbial community

Introduction

The ecological mechanisms responsible for the distribution and local abundance of any particular plant species in any particular site are different. Local populations may be seed-limited, which may be due either to dispersal limitation or low fecundity (Turnbull *et al.* 2000). Quite frequently, however, seed limitation is combined with some kinds of biotic and abiotic constraints within sites (Gustafsson *et al.* 2002). Abiotic constraints include local soil conditions, light availability, and microclimate (Eriksson 1998, Ehrlen & Eriksson 2000, Eriksson & Eriksson 2000, Zobel *et al.* 2000). Since both local light conditions and microclimate depend on standing crop, site productivity (or fertility) is a good surrogate characterising local abiotic conditions in general (Huston 1999, Grime 2001). Once established, seedlings may be suppressed by competition (Weiher & Keddy 1995) or herbivory (Hulme 1994, Gustafsson *et al.* 2002), while the outcome of particular biotic interactions is again dependent on site productivity.

Besides the impact of better known interactions such as competition and herbivory, there is increasing information that plant establishment, performance, and fecundity may to a great extent depend on relationships with microbes such as symbiotic fungi (van der Heijden & Cornelissen 2002, Öpik *et al.* 2003), N-fixing bacteria (Thrall *et al.* 2000), and soil-borne pathogenic organisms (Van der Stoel *et al.* 2002, van der Putten 2003). In recent years, interactions between plants and root-associated microbes have received more attention (Bever 2003, Sen 2003), and the number of works studying their impact on plant performance is increasing (Laakso & Setälä 1999, Klironomos 2002, Callaway *et al.* 2003).

There is, however, a shortage of information about the impact of microbial organisms on plant performance under particular site conditions, since most research has used highly artificial experimental systems. When studying the effect of arbuscular mycorrhizal fungi on plants, most of the work has been done with fungal strains that are easily cultured using trap plants, but rarely found in roots of native plants in field surveys (Clapp *et al.* 2002, Helgason *et al.* 2002). We mostly lack experimental evidence high-lighting differential plant species-related growth patterns in natural soils (Read 2002a, 2002b). The few exceptions include, for example, experiments by Johnson (1993), Moora and Zobel (1996) and Klironomos (2002).

In the present study we made an attempt to estimate the differential effects of root-associated microbial communities on plant growth and reproduction in different soil fertility conditions. We chose the perennial forbs *Centaurea jacea* and *C. scabiosa* as models for a comparative study. These species have rather similar morphological traits, but their distribution pattern in nature is clearly different. *Centaurea jacea* is a common inhabitant in dry and mesophytic grassland communities, where it can gain dominance (Kull & Zobel 1991), but it occurs in abundance also in ruderal sites such as road sides or old fields. *Centaurea scabiosa* occurs in calcareous grasslands as a constant subordinate species, which never dominates. It also grows in ruderal sites like road sides or old gravel pits, and sometimes achieves high abundance there, but is limited to calcareous or neutral soils. Thus, both species show a strong positive response to a surplus of resources in conditions where competition is not limiting growth. *Centaurea jacea*, however, is able to gain dominance in oligotrophic grassland communities, while *C. scabiosa* is not.

In particular, we aimed to understand the mechanisms behind the different distribution patterns and local abundances of *C. jacea* and *C. scabiosa*. We hypothesised that *C. jacea,* as an abundant inhabitant of intact grassland ecosystems, is dependent on the presence of root-associated microbial organisms originating from the sites where it predominates, while the scarce *C. scabiosa*, which may show higher abundance in some ruderal sites with evidently impoverished microbial communities, but not in grasslands, is less dependent on the presence or absence of particular root associated microbial organisms. We also hypothesised that if there is a positive effect of root-associated microbial organisms on *C. jacea*, it will become evident only at low soil fertility levels, i.e. in conditions corresponding to the situation in oligotrophic calcareous grasslands.

We established an experiment of factorial design where inoculation with root-associated

microbial communities of different origin and different soil fertility levels were used as treatments. In particular, we aimed to study: (1) whether there is any plant species-specific reaction to the presence of root associated microbial communities of different origin, and (2) whether the response of *Centaurea* species to different levels of soil fertility depends on the presence of particular microbial communities.

Material and methods

Experimental species

We used *C. jacea* and *C. scabiosa* (Asteraceae) as a phylogenetically independent contrast in order to avoid pseudoreplication due to the different phylogeny of species (Silvertown & Dodd 1996). These species are perennial hemicryptophyte forbs. *Centaurea jacea* is a common species in Estonian dry and mesophyte grasslands, where it sometimes predominates, although it also inhabits ruderal sites like old-fields and road verges. *Centaurea scabiosa* is a subordinate species in grasslands, but can sometimes be abundant in ruderal sites like road verges and gravel pits. According to ecological indicator values of (Ellenberg *et al*. 1991), they are both light-demanding species. *Centaurea jacea* occurs within a wide range along the moisture, soil pH and fertility gradients, while *C. scabiosa* is more confined to dry and neutral or basic soils with moderately low fertility. Grime *et al*. (1988) classified *C. scabiosa* as a stress-tolerator (S/C–S–R), but in an ordination scheme of Grime *et al*. (1997) it was positioned rather as a C–R/C–R–S strategist.

Seeds of the experimental plants were collected from Laelatu calcareous grassland in western Estonia before the start of the experiment. The seeds were kept in a refrigerator (3–4 °C) to break seed dormancy.

Sample communities

Living roots for inoculating experimental plants with rhizosphere communities were collected from two contrasting sites. The first site was

Laelatu, a mesic calcareous grassland in western Estonia on a soil of pH 7.0–7.5, where both *C. jacea* and *C. scabiosa* coexist (inoculum CG, calcareous grassland). *Centaurea jacea* predominates in mesophytic parts of the meadow, while *C. scabiosa* is a subordinate. According to historical notes, this grassland has never been cultivated (Kull & Zobel 1991). The fertilisation experiments in Laelatu grassland have shown that primary productivity is limited by the shortage of phosphorus and nitrogen (Sammul *et al*. 2003).

The second site was a ruderal one, a former arable field, located on slightly acid podzol (pH 5.5–6.0) in Kavastu, eastern Estonia, which was abandoned two years earlier (inoculum OF, old field) but cultivated prior to that since at least the beginning of the last century. *Centaurea jacea* was growing in abundance in an old-field community, but *C. scabiosa* was absent.

Experimental design

The experimental plant species were grown in a factorial pot experiment where two different root inocula and three levels of soil fertility were used as treatments. The growth substrate for the experimental plants was a mixture of steam-sterilized horticultural peat and sterilized fine sand. Inoculation of experimental plants was conducted with the help of fragments of living roots. This technique has previously been used to achieve mycorrhizal root colonization (e.g. Perumal & Maun 1999). Our primary intention was also to inoculate roots of experimental plants with different AM fungal communities. But, as other symbiotic, pathogenic and herbivorous microbial organisms may be transferred together with AM fungi, we call the treatment 'root inoculation'. The living roots of *C. jacea* were used to inoculate experimental plants. The presence of AM infection in roots was controlled in advance; the average colonization rate in the field was 40%–50%. Roots were collected from ten randomly selected individuals from the two contrasting sites immediately before the inoculation of experimental plants. The species composition of root-associated organisms was not recorded, but we assumed them to be different in the two contrasting ecosystems. The roots of

C. jacea plants were carefully washed under tap water, chopped into small pieces, and carefully mixed immediately before the inoculation of experimental plants. Approximately 4 g of fresh root pieces containing various microbial organisms was placed under the roots of experimental plants during the transplantation.

We used three soil fertility levels in the experiment, which we characterise as soil P (P_2O_5) concentration: 3 (low), 9 (medium), and 15 (high) mg/100 g. Initial pH was kept at 6.5 in every soil mixture. Different levels of soil fertility were achieved by mixing different quantities of horticultural peat (N 240 mg^{-1}, P 200 mg^{-1}, K 400 mg^{-1}, pH 5.5–6.5) and sand at the beginning of the experiment as follows: one part peat to two parts sand for low fertility level, one to one for medium level, and two to one for high fertility level. The different fertility levels were maintained by adding fertiliser solution in accordance with the initial nutrient proportions in the potting mixture every second week during the experiment. Although the mixture of horticultural peat and sand is quite an artificial environment as compared with field conditions it has been widely used in pot experiments and also with *C. jacea* (Zobel & Moora 1995). The main reason is that it is very difficult to create a fertility gradient and at the same time control soil microbial communities when working with natural soils.

For germination, on 9 May 1999 the seeds were placed on Petri dishes filled with filter paper and a 0.5-cm-thick layer of sterile sand. On 15 May, when most of the seedlings had two cotyledons, plant individuals were transplanted into experimental pots measuring $10 \times 10 \times 7.5$ (depth) cm. There were twelve different treatments in all (two plant species × three substrates with different fertility × two different root inoculations), each with 30 replicates. Pots were shuffled carefully during the whole experiment to avoid site effects.

The experiment was conducted outdoors in an experimental garden of Tartu University, from 15 May 1998 to 3 October 1998. Duration of the experiment followed the natural growth period in Estonia. Pots were watered during dry periods if needed. The number of leaves was counted every second week from 29 May. At the end of the experiment, plants were harvested and shoots and roots (including also rhizomes) were separated. Roots were carefully washed under tap water. Roots and shoots were dried at 85 °C for 24 hours and weighed.

Estimation of root AM colonisation

Root samples were taken from four randomly selected individuals of each treatment, for the determination of mycorrhizal root colonisation, at the end of the growing season. The percentage of AM fungal colonisation was estimated on the basis of 30 to 50, 1-cm-long root segments. To quantify AM infection, roots were stained (Koske & Gemma 1989) and the percentage of colonised root segments, containing arbuscules, hyphae, and/or vesicules, was determined (Rajapakse & Miller 1992). The presence of other microbial organisms was not recorded.

Statistical analysis

The number of leaves per plant was analysed by repeated measures analysis of variance (ANOVAr), where plant species (two levels), soil fertility (three levels), and origin of root inoculation (two levels) were considered as fixed factors, and time (10 measurements altogether) as a repeated measure factor. The biomass and mycorrhizal root colonisation data was analysed by three-way ANOVA, in which plant species, soil fertility, and origin of root inoculation were used as fixed factors. Biomass data was log-transformed and percentage data was calculated using an arcsine transformation prior to the statistical analysis. To estimate the degree of difference between different treatments, the Tukey HSD multiple comparison test was used. For all analyses, the Windows version of STATISTICA (StatSoft, Inc. 1998) was used.

Results

Leaf number

The leaf number of the two species was different: *C. jacea* had more leaves than *C. scabiosa*

(Table 1). Also, leaf number dynamics was different, since the leaf number of *C. jacea* individuals increased continuously during almost the whole growing season, while the leaf number of *C. scabiosa* reached a plateau approximately in the middle of the season (Fig. 1a).

Increasing soil fertility resulted in a higher leaf number. There was also a significant interaction between species and soil fertility; in *C. scabiosa* the increase of the number of leaves remained non- significant (Table 1 and Fig. 1b). Significant three-way interaction between species, fertility and time showed that, in low fertility soil, *C. jacea* stopped producing new leaves almost at the middle of the growing season, but in medium and high fertility soils the plants produced new leaves during the whole growing season.

The CG root inoculum increased the leaf number of *C. jacea* significantly more than the OF inoculum, but the leaf number of *C. scabiosa* was not dependent on different inocula (Table 1 and Fig. 1c). When *C. jacea* plants were CG inoculated, increasing soil fertility resulted in significantly more leaves at the highest soil fertility level, while at medium and low soil fertility the leaf number remained low. As compared with OF inoculated plants, CG inoculated *C. jacea* plants produced more leaves, especially during the second half of the season.

Table 1. Results of ANOVAr. The effect of plant species (Sp), soil fertility (So), root inoculum (In), as fixed factors, and time (Ti), as repeated measures factor, on leaf number of *Centaurea* plants.

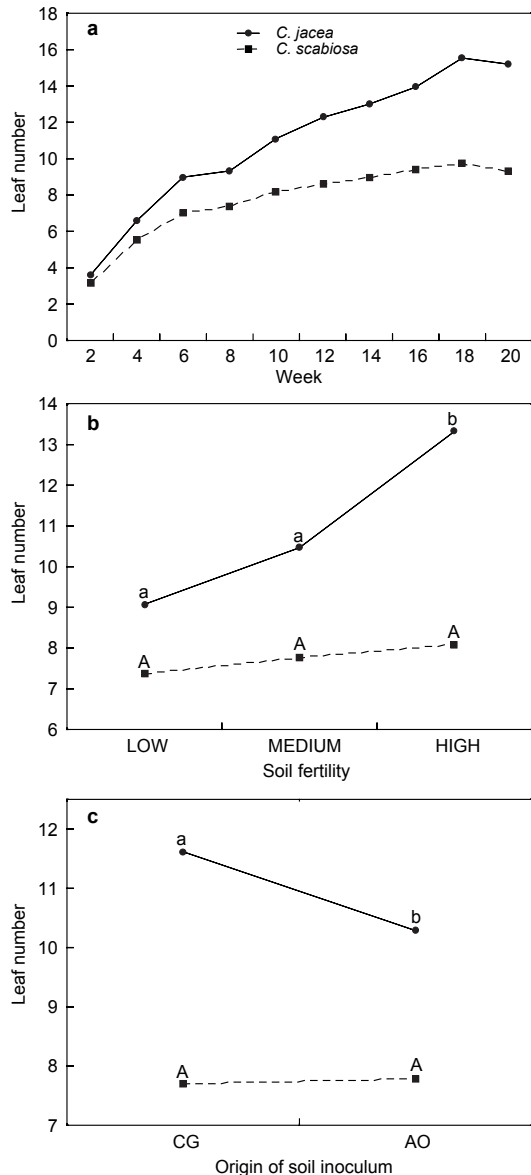| Source of variation	d.f.	F	P
Sp	1	93.9	0.00
So	2	19.3	0.00
In	1	3.6	0.06
Ti	9	431.4	0.00
Sp × So	2	9.95	0.00
Sp × In	1	4.6	0.03
So × In	2	3.8	0.02
Sp × Ti	9	44.0	0.00
So × Ti	18	10.1	0.00
In × Ti	9	2.4	0.01
Sp × So × In	2	3.8	0.02
Sp × So × Ti	18	10.0	0.00
Sp × In × Ti	9	4.8	0.00
So × In × Ti	18	5.8	0.00
Sp × So × In × Ti	18	4.0	0.00

Fig. 1. (**a**) Mean leaf number of *Centaurea jacea* (solid line) and *C. scabiosa* (dashed line) plants growing in (**b**) substrates with different fertilities, or (**c**) with different root inoculum from calcareous grassland (CG) and from old-field (AO). Points bearing the same letters are not significantly different (*P* < 0.05, Tukey test).

Biomass

The shoot and root biomass of *C. jacea* individuals was larger than that of *C. scabiosa*, but the shoot to root ratio was smaller in *C. jacea* (Table 2 and Fig. 2). With increasing soil fertility, shoot and root

biomass of both species increased, but shoot to root ratio decreased at the highest soil fertility (Tukey, $P < 0.05$). The two root inocula had no differential effect on the shoot biomass. In *C. scabiosa*, root biomass was higher in CG inoculated plants as compared with that in OF inoculated plants (Fig. 2). There was a significant interaction between soil fertility and the origin of inoculum in root biomass. When plants grew in low- and medium-fertility soils, there were no large differences between the inoculation treatments. When plants grew in high-fertility soil, CG inoculated plants had significantly larger roots than OF inoculated plants.

Mycorrhizal infection

The root AMF colonisation of experimental plants was relatively high for experimental conditions (average approximately 18%) but showed high variability (from 2% to 60%). The average colonisation rate was not dependent on plant species or soil fertility level. OF inoculated plants had significantly higher root colonisation rate than CG inoculated plants ($P < 0.05$). Production of arbuscules and vesicles tended to be plant-species specific: mycorrhiza in the roots of *C. jacea* had more arbuscules ($P < 0.05$), while mycorrhiza in the roots of *C. scabiosa* had more vesicles ($P = 0.055$).

Discussion

Centaurea jacea and *C. scabiosa* showed different patterns of growth and resource alloca-tion under conditions of different root inoculation and soil fertility. *Centaurea jacea* was distinguished by a more rapid increase in the number of leaves than *C. scabiosa*. The former increased the number of leaves continuously during the whole growing season, while *C. scabiosa* stopped producing new leaves already in the middle of the season. Though the biomass of *C. jacea* was higher than that of *C. scabiosa*, there were no differences between species in their biomass response to fertilisation. According to Grime (2001), *C. jacea* is characterised by an ability to rapidly capture resources, which makes it closer to being a competitive strategist, while *C. scabiosa* is more inclined towards a ruderal and stress-tolerating strategy (Grime *et al.* 1988, 1997). Thus, one might expect that different distributions and local abundances of these two species may be solely due to their differential capture of resources. Our experiment shows, however, that this is not the case, and that other factors such as biotic interactions with soil microbes may play an important role.

Different root-associated microbial communities had a differential effect on the traits of the two plant species studied. As compared with the root-associated microbial community of ruderal origin (OF), the grassland microbial community (CG) considerably increased the leaf number of *C. jacea*. Thus, our working hypothesis about the greater positive response of *C. jacea* to the root-associated microbial community from oligo-trophic grassland was confirmed. Increased allocation to growth meristems early in the plant's development could maximise size, which can be committed to reproduction later in develop-

Table 2. Result of ANOVA. The effect of plant species (Sp), soil fertility (So) and root inoculum (In) on the bio-masses and shoot to root ratio of *Centaurea* plants.

Source of variation	d.f.	shoot		root		shoot/root	
		F	*P*	*F*	*P*	*F*	*P*
Sp	1	49.4	0.00	73.1	0.00	17.5	0.00
So	2	59.3	0.00	85.2	0.00	15.6	0.00
In	1	2.2	0.14	8.0	0.01	3.1	0.08
Sp × So	2	0.3	0.71	1.1	0.33	2.2	0.12
Sp × In	1	0.2	0.65	0.8	0.36	0.6	0.46
So × In	2	0.8	0.43	3.8	0.02	0.4	0.67
Sp × So × In	2	0.3	0.75	0.2	0.84	0.9	0.39

ment (Bonser & Aarssen 2001). Thus, the greater number of leaves in the case of *C. jacea* during the first year may represent a long-term-oriented investment to growth and reproduction in a community where most of the species are perennial (cf. Kull & Zobel 1991).

At the same time, CG inoculated *C. scabiosa* plants showed higher root biomass than AO inoculated plants. Thus, when biomass is taken into account, the working hypothesis about the stronger positive response of *C. jacea* to grassland inoculum was not confirmed. For perennial plants, storage of resources in special organs like rootstocks or rhizomes is an important strategy for performing successfully in stressful conditions (Grime 2001). In the case of *C. scabiosa*, inoculation with root-associated microbial community from intact grassland enhanced storage to below-ground organs.

We also hypothesised that if *C. jacea* shows a positive response to grassland inoculum, this response will be more pronounced at low fertility levels. This hypothesis was not confirmed, since the opposite pattern was observed: CG inoculum increased the number of leaves of *C. jacea* at high soil fertility levels.

Based on our results it can be concluded that root inoculum from intact grassland resulted in such behaviour, as one might have expected on the basis of their previous life strategy according to Grime *et al.* (1988, 1997) and Grime (2001). A predominant species of calcareous grassland communities, *C. jacea*, increased its leaf number, which may result in competitive advantage in the future. A subordinate species of calcareous grassland communities, *C. scabiosa*, increased allocation to storage organs. Interestingly, both responses were stronger in the case of a surplus of soil resources.

At the same time, the inoculum from a ruderal old-field site resulted in a lower number of leaves in *C. jacea* and lower below-ground biomass in *C. scabiosa*. It is known that cultivation history may lead to significant impoverishment of soil microbial communities like those of arbuscular mycorrhizal fungi (Johnson 1993, Helgason *et al.* 1998). Despite the fact that roots of plants from OF inoculated treatments were extensively colonised by AM fungi, inoculation had no positive net effect. It is known that
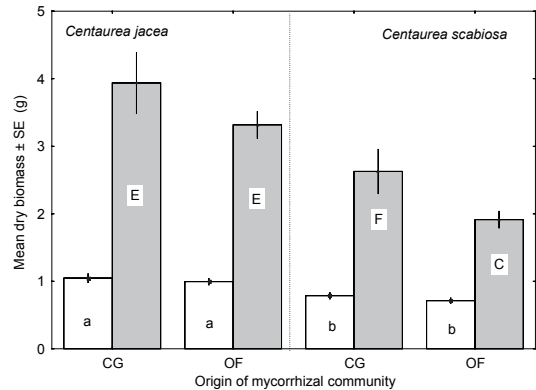

Fig. 2. Means (± SE) of the shoot (open bars) and root (filled bars) biomass of *Centaurea jacea* and *C. scabiosa* plants growing with root inoculum from calcareous grassland (CG) and acidic old-field (OF). Bars bearing the same letters are not significantly different (*P* < 0.05, Tukey test).

the species composition of the whole microbial community may not result in any net positive effect on plant performance (Klironomos 2003), which was evidently also the case in our OF inoculated plants.

The theory of plant strategies is mostly focussed on plant allocation and performance in different nutrient conditions (Grime *et al.* 1988, Grime 2001). The response to root-associated microbial communities is rarely considered within the context of the strategy theory. The different response of the two *Centaurea* species to grassland and old-field inocula at different soil fertility levels shows that resource allocation in plants may be dependent on the particular root-associated microbial community.

We also observed an interesting allocation pattern in experimental plants: the shoot to root ratio of both species decreased along the fertility gradient. Plants usually respond to low nutrient availability by shifting the allocation of carbohydrates to below-ground organs, and to low light availability by the allocation of more biomass above ground (Grace 1997, Shipley & Meziane 2002). The opposite allocation pattern in both of our *Centaurea* species may represent an 'element of stress-tolerating behaviour', which may be characteristic even to plant species that are usually classified as competitors. This 'element' — increased storage to below-ground in the case

of surplus resources — may be more common among perennial herbaceous plant species than usually recognized.

Acknowledgements

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