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ORIGINAL PAPER

Effects of nutrient availability on performance and mortality of *Rumex obtusifolius* and *R. crispus* in unmanaged grassland

Michal Hejcman · Lukáš Strnad · Pavla Hejcmanová · Vilém Pavlů

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Abstract Several authors record high mortality of the weedy species *Rumex obtusifolius* in unmanaged grasslands, but there have never been any studies on how the performance and mortality of *R. obtusifolius* and *R. crispus* are affected by different nutrient availability in unmanaged temperate grasslands. To investigate this question, we established a seedlings transplantation and fertiliser experiment on unmanaged *Dactylis glomerata* grassland in Czech Republic. We monitored plant height, number of leaves per plant, fertility and mortality of transplanted *R. obtusifolius* and *R. crispus* plants, from 2008 to 2011, in an unfertilised (U) and a fertilised (F) treatment (manure applied in 2008 at the rate of 200 kg of nitrogen, 42 kg of phosphorus and 230 kg of potassium per ha). In 2010, taller plants of both species were recorded in treatment F than in treatment U, but there was no effect of treatment on the number of leaves. In 2010, fertility for both species was 50% in treatment F, but only 20% for *R. obtusifolius* and 10% for *R. crispus* in treatment U. Over 4 years, no mortality of *R. obtusifolius* in treatment F contrasted with 30% mortality in treatment U. In the case of *R. crispus*, mortality was 50% in treatment F and 28% in treatment U. An increase in nutrient availability can decrease mortality of *R. obtusifolius* plants in grasslands, but increase mortality of *R. crispus*. It seems that leaving grassland unmanaged for several years is only an effective strategy for control of *R. obtusifolius* under conditions of low nutrient availability.

Keywords Abandoned meadow · Broad-leaved and curled docks · Manure fertiliser experiment · Organic farming · Seedling transplantation · Weed control

Introduction

In recent decades, occurrence and control of *Rumex obtusifolius* ssp. *obtusifolius* L. (broad-leaved dock, hereafter referred to as *R. obtusifolius*) in temperate grasslands has received high research attention (Zaller 2004, 2006; Bayhan et al. 2006; Van Evert et al. 2009; Gilgen et al. 2010; Stilmant et al. 2010; Strnad et al. 2010; Hrdličková et al. 2011; Hann et al. 2012). This is because *R. obtusifolius* is considered to be one of the most troublesome grassland weedy species worldwide due to: (1) its general avoidance by grazing livestock, especially cattle and horses; (2) its negative effects on forage quality, as it induces health problems if eaten in high quantity and (3) its negative effects on biomass production of desired forage species by competition for space, water and nutrients (Zaller 2004). *R. obtusifolius* represents a serious problem, especially for organic farming because no herbicides can be used for its control (Zaller 2004, 2006; Stilmant et al. 2010). In Europe, *R. obtusifolius* is highly successful in grasslands supplied with high N, P and K application rates (Niggli et al. 1993; Humphreys et al. 1999; Hopkins and Johnson

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M. Hejcman (✉) · L. Strnad · V. Pavlů
Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 1176, 165 21 Prague 6, Suchdol, Czech Republic
e-mail: hejcman@fzp.czu.cz

M. Hejcman · V. Pavlů
Crop Research Institute, Drnovská 507, 161 06 Prague 6, Ruzyně, Czech Republic

P. Hejcmanová
Department of Forest Management, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Kamýcká 1176, 165 21 Prague 6, Suchdol, Czech Republic

2002; Strnad et al. 2010). Reduction in fertiliser supply and cutting frequency can be an effective strategy for *R. obtusifolius* control, especially on basic soils heaving a high Ca and Mg status (Hann et al. 2012).

Rumex crispus L. (curled dock), on the other hand, is considered to be one of the most detrimental weedy species on arable land often occurring in regularly disturbed ruderal and waste areas. In permanent grasslands, *R. crispus* possesses lower competitive ability than *R. obtusifolius* (Cavers and Harper 1964; Zaller 2004) because spreads mainly by seeds, although its regeneration from below-ground organs was also recorded (Pye and Andersson 2009; Pye et al. 2011). Similar to *R. obtusifolius*, *R. crispus* decreases yield of different crops by competition for space, water and nutrients, and considerably decreases the nutritive value of forage produced by fodder crops (Zaller 2004).

In twice-cut grasslands in Central Europe, mortality of *R. crispus* over 3 years was substantially higher than mortality of *R. obtusifolius*, especially in plots well-supplied with N, P and K (Strnad et al. 2012). It was concluded that *R. obtusifolius* is better adapted to growing in highly productive managed temperate grasslands than *R. crispus* because of its perennial character, larger plant size and higher fertility (Strnad et al. 2012). *R. obtusifolius,* in comparison with *R. crispus*, invests a higher proportion of assimilates into below-ground organs, and therefore better regenerates after removal of its above-ground organs (Pino et al. 1995). *R. crispus*, on the other hand, is more dependent on successful seed production because of its tendency to die after seed production (Weaver and Cavers 1980; Pye and Andersson 2009; Křišťálová 2010). It is generally believed that *R. crispus* is a short-lived species whose perennation in grasslands can be enabled by cutting performed before production of seeds (Bond et al. 2007; Strnad et al. 2012).

Rumex obtusifolius and *R. cripus* are both considered to be serious weeds, which differ substantially in their response to nutrient availability in grassland cut twice per year, as was recently noted by Strnad et al. (2012). In unmanaged grassland, low or no survival of *R. obtusifolius* plants over several years was recorded by Pavlů et al. (2008) and by Martinkova et al. (2009). However, the effect of nutrient availability in unmanaged temperate grasslands on the performance and mortality of both species has never been investigated. With respect to high mortality of *R. obtusifolius* in unmanaged grassland, we hypothesised that leaving grassland unmanaged for several years could be a possible strategy for *R. obtusifolius* control under conditions of organic farming.

The aim of this study was therefore to investigate performance and mortality of *R. obtusifolius* and *R. crispus* in unmanaged grassland with different nutrient availabilities by conducting a seedling transplantation and fertiliser experiment. We investigated how plant height, number of leaves per plant, fertility and mortality of transplanted *R. obtusifolius* and *R. crispus* plants are affected by organic fertiliser (manure) supply in unmanaged grassland. Results of this study were directly compared with a neighbouring seedling transplantation and fertiliser experiment performed on grassland cut twice per year (Strnad et al. 2012). Based on comparing these two studies, we asked how performance and mortality of *R. obtusifolius* and *R. crispus* differed in unmanaged and regularly cut grasslands.

Materials and methods

Study site

The experiment was established near Mšec village, 45 km north-west of Prague (50°12′24″N; 13°51′40″E). The study site was a flat meadow with a mean annual precipitation and temperature of 550 mm and 8°C, respectively. The altitude of the study site was 490 m a.s.l., and its soil was classified as Pararendzina (syn. Calcaric Leptosol). The meadow had been occasionally fertilised with farmyard manure before establishment of the experiment, and the average annual dry matter biomass yield under two-cut management was 3.5 t ha^{-1}. *Dactylis glomerata* was the dominant species before establishment of the experiment. In the upper 10 cm of the soil the pH (H_2O) was 6.4, concentrations of plant-available (Mehlich III) P, K, Ca and Mg were 152, 267, 1688 and 171 mg kg^{-1}, respectively, and the content of total (Kjeldahl) N was 2.3 g kg^{-1} before the start of the experiment. Plant available P and K concentrations are considered to be optimal for crops with high P and K requirements (Hrevušová et al. 2009; Černý et al. 2010).

Experimental design

The experiment was established on freshly cut meadow in June 2008. Seedlings were transplanted on a meadow directly after the last cut in June 2008, following which the meadow was unmanaged. The seedlings were grown from seeds of *R. obtusifolius* and *R. crispus* collected during autumn 2007. To ensure direct comparability of the results with results of two previous experiments [pot fertiliser experiment by Křišťálová et al. (2011) and field transplantation experiment by Strnad et al. (2012)], the same seeds were used. Seed material for seedling production was collected from three sites (roadside ditches and abandoned fields) close to Prague, Czech Republic. Five plants were randomly selected at each site, taking care not to favour tall or small plants. Seeds were sown in late March 2008 in a greenhouse and small seedlings with the first true leaf were transferred into individual pots in April and then, from the beginning of May, planted in open-air conditions. Seedlings with three true leaves were transplanted into experimental plots. All transplanted seedlings were marked with a plastic stick and watered with the same amount of water during the first 2 weeks after transplantation to prevent desiccation.

Seedlings were transplanted into ten lines, each 4.5 m long with five seedlings for each species (10 seedlings for both species together per line, 100 for the experiment). The experiment was therefore a grid of 100 plants arranged in a $4.5 \times 4.5\ m^2$ and with a distance of 50 cm between individual seedlings in the same line, as well as between the lines. In each odd-numbered line (line numbers 1, 3, 5, 7 and 9), five seedlings of *R. obtusifolius* were placed on the left-hand side and five seedlings of *R. crispus* were placed on the right-hand side. The case was vice versa for each even line (line numbers 2, 4, 6, 8 and 10). During the transplantation, we used cylindrical planting holes, each with 6 cm diameter and depth. Original grassland vegetation was removed only on the area of each planting hole. In lines 1–4, all transplanted seedlings on the left-hand side were fertilised with manure (fertilised treatment—F), and vice versa in the case of lines 5–10 (unfertilised treatment—U). Finally, 50 seedlings for each species were monitored—25 fertilised with manure in F treatment and 25 unfertilised in U treatment.

In the F treatment, individual plants were manually fertilised immediately after transplantation. We used 1-year-old goat manure which was put directly on the soil surface in an area with a radius of 10 cm around the transplanted plant. We used the application rate 200 kg N ha^{-1}, 42 kg P ha^{-1} and 230 kg K ha^{-1} for each fertilised plant. The application rate used was in accordance with common farming practices in Czech Republic. The amount of applied manure was based on results of manure chemical analysis. No naturally occurring seedlings of both *Rumex* species were recorded in the study area during the experiment.

Data collection

Plant height, the number of leaves per plant and the proportion of unassigned plants were recorded repeatedly during four vegetation seasons from 2008 to 2011. The proportion of fertile for living (=not unassigned) plants was recorded only in July 2010. We measured plant height, number of leaves per plant and proportion of fertile plants, as these characteristics of plant performance were used in our previous studies (Hrdličková et al. 2011; Křišťálová et al. 2011; Strnad et al. 2012) therefore making results of all studies directly comparable. We calculated mean values for each row and fertiliser treatment (five independent replications for each species and treatment). All recorded characteristics were obtained non-destructively, therefore enabling repeated measurements of undisturbed plants. Unassigned plants were: (1) living plants but without any green leaves; and (2) dead plants. The term “unassigned plants” was used because it was impossible to distinguish between living plants with no leaves and dead plants on some occasions. On the last sampling date, the number of unassigned plants corresponded to mortality, as no regeneration of stems was later recorded. The percentages of unassigned and fertile plants were calculated for five replicates in each treatment (five plants in five rows: one dead plant represented mortality of 20% in one row and mortality of 4% on the level of one species and treatment).

Data analysis

Repeated measures ANOVA was used to evaluate effects of time (date of data collection), species, treatment and their interactions on plant height, number of leaves per plant and proportion of unassigned plants. Factorial ANOVA was used to evaluate effect of species, treatment and their interaction on proportion of fertile plants. After obtaining results of repeated measures or factorial ANOVA, one-way ANOVA was used to identify significant differences among treatments for individual species in individual sampling dates. All analyses were performed using the STATISTICA 8.0 software (StatSoft, Tulsa, USA). ANOVA was used because all assumptions of normality (tested by Kolmogorov–Smirnov test) and homogeneity of variance were met.

Results

Calculated by repeated measures ANOVA, plant heights were significantly affected by time, but only a trend was recorded for the treatment effect (Table 1). The non-significance of interactions between species × time and species × treatment and the significant time × treatment interaction indicated that both species behaved similarly at different sampling dates and responded similarly to applied F and U treatments.

Calculated by one-way ANOVA separately for each species at each sampling date, plant heights of *R. obtusifolius* and *R. crispus* were significantly affected by treatment in one and two measurements from the five performed (Fig. 1). In all significant cases, plant heights were higher in F than in U treatment. The maximum recorded plant height was 85 cm for both species.

Calculated by repeated measures ANOVA, number of leaves per plant was significantly affected by time, species and the species × time interaction (Table 1). This indicated changes in number of leaves during the season,

Table 1 Results of repeated measures ANOVA analyses calculated for *R. obtusifolius* and *R. crispus* characteristics

Tested variable	Effect	DF	*F* value	*P* value
Plant height	Time	4	8.0	**0.034**
	Species	1	1.1	0.488
	Treatment	1	71.3	0.075
	Species × time	4	0.0	0.999
	Species × treatment	1	0.0	0.918
	Treatment × time	4	12.3	**0.016**
Number of leaves per plant	Time	4	16.0	**0.010**
	Species	1	36.5	**0.011**
	Treatment	1	0.0	0.771
	Species × time	4	97.0	**<0.001**
	Species × treatment	1	0.0	0.694
	Treatment × time	4	2.0	0.226
Proportion of fertile plants	Species	1	2.7	0.348
	Treatment	1	29.6	**0.004**
	Species × treatment	1	1.2	0.472
Proportion of unassigned plants	Time	4	16.0	**0.010**
	Species	1	7.1	0.235
	Treatment	1	54.0	0.086
	Species × time	4	21.2	**0.006**
	Species × treatment	1	1.1	0.531
	Treatment × time	4	30.0	**0.003**

Significant results are printed in bold

DF degrees of freedom

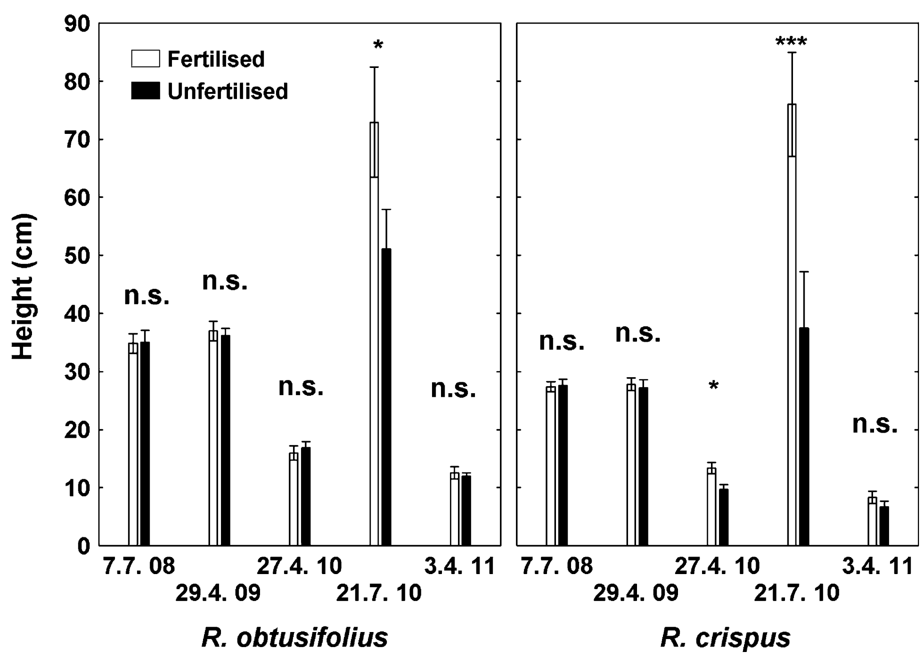


Fig. 1 Plant height of *R. obtusifolius* and *R. crispus* in fertilised and in unfertilised treatment over the study period from 2008 to 2011. *Bars* represent mean values. *Error bars* represent standard error of the mean (SE). Calculated by one-way ANOVA, effect of treatment in each sampling date was not significant (n.s.), significant on 0.05 (*) and on 0.001 (***) probability level

different number of leaves between species and different development of number of leaves in both species during the experiment.

Number of leaves per plant of *R. obtusifolius* and *R. crispus* was neither significantly affected by treatment considering all sampling dates together (result of repeated measures ANOVA), nor in any single measurement from the five performed (results of one-way ANOVA, Fig. 2). Numbers of leaves per plant were generally higher for *R. obtusifolius* than for *R. crispus*. The maximum number of leaves per plant of *R. obtusifolius* and *R. crispus* were nine and five, respectively.

Calculated by factorial ANOVA, the proportion of fertile plants was significantly affected by treatment, but not by species or the species × treatment interaction (Table 1). This indicated a similar response of both species to the applied treatments. In July 2010, proportions of fertile plants from living plants of *R. obtusifolius* and *R. crispus* were significantly higher in the F than in the U treatment (Fig. 3). The total proportion of fertile plants was slightly higher for *R. obtusifolius* than for *R. crispus*, but the effect of species as a factor was not significant (Table 1).

Calculated by repeated measures ANOVA, the proportion of unassigned plants was significantly affected by time, species × time and treatment × time. The effect of treatment was not significant, but a marked trend was recorded (Table 1). This indicated a specific development in the proportion of unassigned plants during the experiment for both species and treatments. The proportion of unassigned plants was substantially lower for *R. obtusifolius* than for *R. crispus* (Fig. 4). For *R. obtusifolius*, the highest proportion of unassigned plants was recorded in the U treatment and the effect of treatment was significant in three out of five measurements. For *R. crispus*, the proportion of unassigned plants gradually increased during the experiment. The highest proportion of unassigned plants was recorded in the F treatment and the effect of treatment was significant in two out of five measurements. Total mean mortality over both treatments and over the 4 years, as recorded at the last sampling date in April 2011, was 15% for *R. obtusifolius* and 38.8% for *R. crispus*. The mortality of 30% for *R. obtusifolius* in the U treatment contrasted with no mortality in the F treatment. In the case of *R. crispus*, the mortality was 49.6% in the F treatment and only 28% in the U treatment at the last sampling date (Fig. 4).

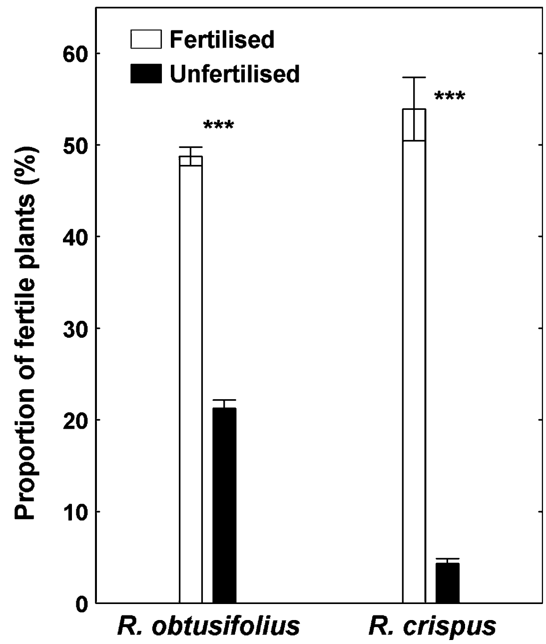
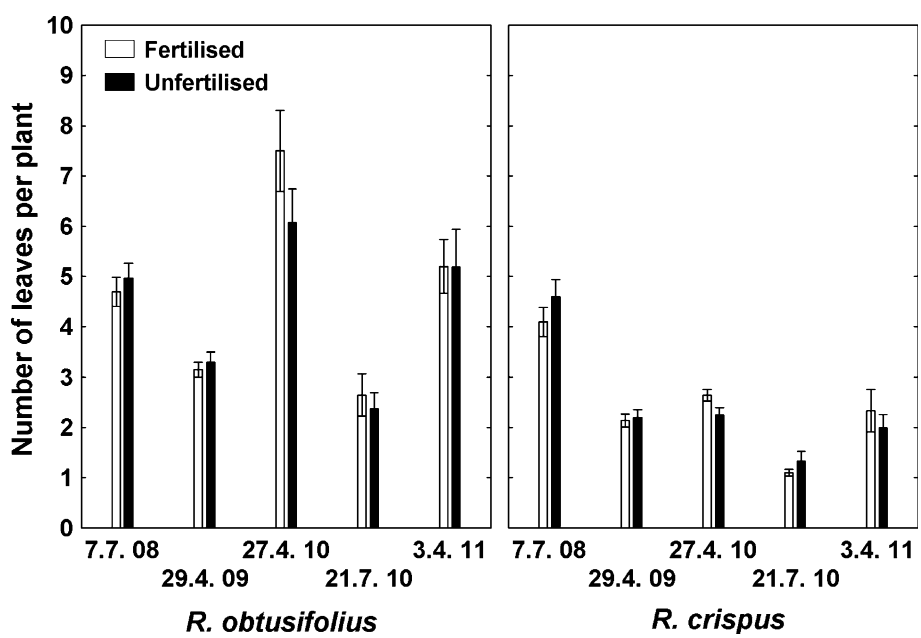


Fig. 3 Proportion of fertile plants from discovered living (= not unassigned) plants for *R. obtusifolius* and *R. crispus* in fertilised and in unfertilised treatment on 21st July 2010. *Bars* represent mean values. *Error bars* represent standard error of the mean (SE). Calculated by one-way ANOVA, effect of treatment on sampling date was significant on 0.001 (***) probability level

Discussion

Rumex obtusifolius and, in part, *R. crispus* are considered to require high nutrient supply (Grime et al. 1988; Humphreys et al. 1999; Jursík et al. 2008; Křišťálová et al.

Fig. 2 Number of leaves per individual plant of *R. obtusifolius* and *R. crispus* in fertilised and in unfertilised treatment over the study period from 2008 to 2011. *Bars* represent mean values. *Error bars* represent standard error of the mean (SE). Calculated by one-way ANOVA, effect of treatment on each sampling date was not significant on number of leaves per plant in each species



2011), but the effect of nutrient availability on performance and mortality of both species has been investigated only in cut grasslands (Niggli et al. 1993; Hopkins and Johnson 2002; Hann et al. 2012; Strnad et al. 2012). Cutting management can substantially affect life-span of individual plants, and therefore results from cut grasslands are not transferable into unmanaged grasslands (Martinkova et al. 2009). The advantage of our study is that results from the unmanaged grassland can be directly compared to results obtained from an experiment on cut grassland published by Strnad et al. (2012), as both experiments were located on the same meadow, performed over the same time period, and used transplanted plants of the same origin and age.

The small differences in height of plants and number of leaves in both species between the F and U treatments can be partly explained by: (1) high competition of transplanted plants for water and nutrients with well-established plants of *D. glomerata*; and (2) by the time of measurement within the vegetation season. Competition of surrounding vegetation was relatively high because the no-cutting regime increased the importance of competition for light. Competition for light was especially high in the F treatment because the advantage of increased nutrient availability was exploited not only by transplanted *Rumex* plants, but also by well-established plants of *D. glomerata*. According to our previous experience, the advantage of increased nutrient availability on plant height and number of leaves per plant can be best expressed in regularly cut grasslands (Strnad et al. 2012) or in monocultures with no competition from other species (Křišťálová et al. 2011).

Furthermore, three out of five measurements were performed in April (2009–2011), at this time in the year there had probably not been enough time since the start of the vegetation season for plants to take an advantage of improved nutrient availability in the F treatment. This is supported by the significantly taller plants of both species being recorded in the F treatment, in comparison to the U treatment, in July 2010. In July 2010, the taller plants in the F than in the U treatment were matched by a substantially higher fertility of transplanted plants in the F than in the U treatment. Plants of both *Rumex* species invested a substantially higher amount of energy into long stems, and therefore flowering, in the F treatment. In contrast to grassland with the cutting regime and therefore with more stems per plant, maximally one stem and several leaves per plant developed in both species in unmanaged grassland. Although no data were collected, we visually recorded a positive effect of F treatment on size of individual leaves. Therefore, leaf area, in addition to the number of leaves per plant, seems to be a suitable variable for future monitoring of effects of nutrient availability on plant performance.

The maximum number of leaves for *R. obtusifolius* was eight under the no-cutting regime, and 25 in cut grassland with high N, P and K availability (Strnad et al. 2012). Such a high difference in number of leaves was caused by no sprouting of transplanted plants under the no-cutting regime, but high sprouting in cut grassland. Because of no sprouting and low number of leaves per plant, there was no above-ground competition of transplanted plants for light, even though the distance between individual plants was only 50 cm. We

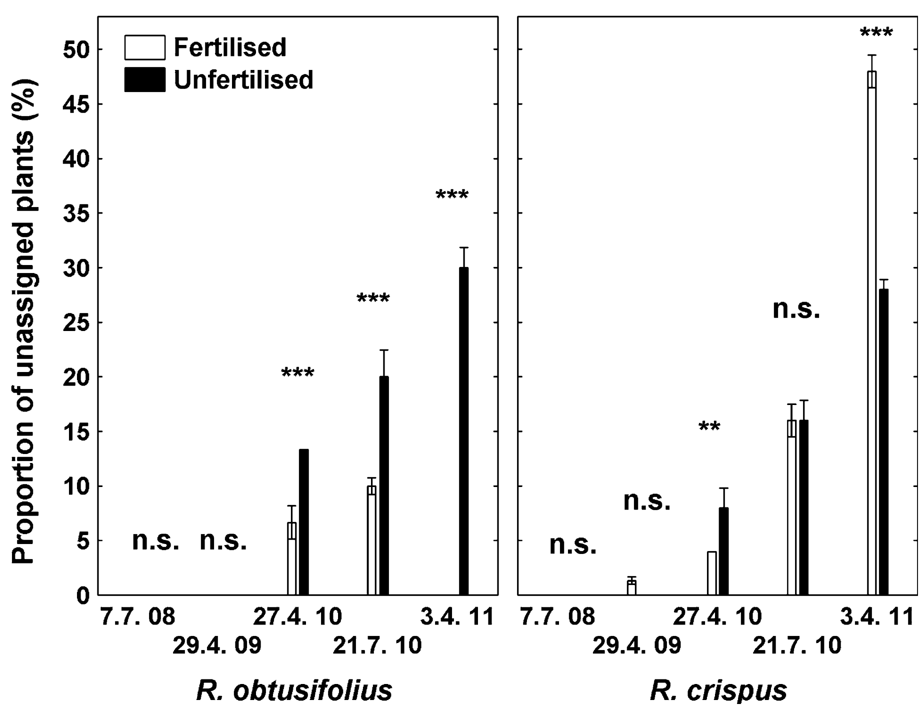


Fig. 4 Proportion of unassigned plants from total number of transplanted plants for *R. obtusifolius* and *R. crispus* in fertilised and in unfertilised treatment over the study period from 2008 to 2011. Proportion of unassigned plants on 3rd April 2011 represents cumulative mortality over 4 years. *Bars* represent mean values. *Error bars* represent standard error of the mean (SE). Calculated by one-way ANOVA, effect of treatment in each sampling date was not significant (n.s.), significant on 0.01 (**) and on 0.001 (***) probability level

concluded that *R. obtusifolius* is well-adapted to cutting management in productive grasslands, which decreases apical dominance and thus stimulate sprouting, and therefore supports the performance of individual plants. For *R. crispus*, the maximum number of leaves was five, and this was recorded at the start of the experiment. Since the second year, there were only two leaves. Such a decrease is consistent with results from cut grassland, where the number of leaves per plant sharply decreased from eight to two under high nutrient supply in the third and fourth year of the experiment (Strnad et al. 2012). This suggests *R. crispus* is not well-adapted to growing in closed grassland canopy, and rather prefers open cultivated fields or disturbed ruderal sites, as recorded by other authors (Pye and Andersson 2009; Pye et al. 2011).

The same maximum plant height recorded for both species (85 cm) in F treatment in unmanaged grassland, contrasts with results obtained for grassland cut twice per year with application of mineral N, P and K fertilisers: 100 and 80 cm for *R. obtusifolius* and *R. crispus*, respectively (Strnad et al. 2012). Growth potential of *R. obtusifolius* can be therefore better expressed under high N, P and K supply in grassland cut twice per year, than in unmanaged grassland. On the other hand, *R. crispus* performs better under no cutting because of its high sensitivity to removal of its above-ground organs (Hongo 1988).

An increase in nutrient availability in the F treatment significantly increased the proportion of fertile plants 2 years after the manure application. In 2010, the proportion of fertile plants of *R. obtusifolius* increased from 20% in the U to 50% in the F treatment; and the proportion of fertile *R. crispus* plants increased from 10% in the U to 50% in the F treatment, respectively. Such a long-lasting effect of manure application on fertility of plants can be explained by: (1) the plant "memory effect", by which a small advantage at the start of plant growth can be expressed after several years; and (2) the long-lasting positive effect of manure application on soil fertility, as the nutrients are released slowly from manure, but for several years (Gale et al. 2006). Long-lasting increase in soil organic matter content can also increase water holding capacity. Therefore, even a single application of fertiliser can change ecosystem functioning for many years (Spiegelberger et al. 2006; Hejcman et al. 2007). Fertility of *R. obtusifolius* was lower and fertility of *R. crispus* was higher in unmanaged than in the grassland cut twice per year (Strnad et al. 2012). This comparison also indicates rather the negative effect of no management on *R. obtusifolius* and the positive effect on *R. crispus*. Cutting management can substantially decrease or totally prevent seed production in *R. crispus*, but not in *R. obtusifolius*.

We used the proportion of unassigned plants, as to decide whether the plants were living without any leaves or dead was impossible in several cases. Therefore, in the F treatment, unassigned plants of *R. obtusifolius* on 27th April 2010 and 21st July 2010 were recorded as living in spring 2011. The unassigned plants of *R. obtusifolius* in the F treatment were heavily grazed by the beetle *Gastrophysa viridula* Degeer (Coleoptera: Chrysomelidae) during that time, but later regenerated.

Finally, no mortality of *R. obtusifolius* in the F treatment after 4 years contrasted with 30% mortality in the U treatment. Higher mortality under low nutrient availability and no mortality under high nutrient availability in unmanaged grassland are consistent with results from the cut grassland: 13% mortality in unfertilised treatment and no mortality in NPK treatment (Strnad et al. 2012). The mortality and life-span of individual *R. obtusifolius* plants is therefore highly affected by soil fertility in grasslands: this was also recorded by Humphreys et al. (1999) or by Hann et al. (2012). High mortality of *R. obtusifolius* recorded in unmanaged grassland by Martinkova et al. (2009) was thus probably at least partly connected with shortage of nutrients, most probably N and/or P. In addition, high mortality can also be connected with high soil Ca status, as was recorded by Hann et al. (2012). In the case of *R. crispus*, we recorded opposite results. Mortality was higher under increased nutrient availability: 28% in the U and 50% in the F treatment, respectively. This is consistent with results from cut grassland (Strnad et al. 2012). One possible explanation is the monocarpic character of *R. crispus* (Křišťálová 2010). In the F treatment, plants had enough resources to start flowering earlier and therefore died more quickly than in the U treatment. Although it is generally believed that perennation of *R. crispus* is enabled by cutting of stems before seed production (Bond et al. 2007; Strnad et al. 2012), some plants can also survive for longer when there is a no-cutting regime, especially under low nutrient availability.

The more than two times lower total mortality of *R. obtusifolius* (15%) than total mortality of *R. crispus* (39%) over 4 years indicates a substantially longer life-span of *R. obtusifolius* in permanent unmanaged grasslands than of *R. crispus*. The same results were also recorded in the grassland cut twice per year in Central Europe (Strnad et al. 2012), but not in the cold winter region of Hokkaido where the results were opposite (Hongo 1989). Thus, together with the number of leaves, mortality indicates better adaptation of *R. obtusifolius* than *R. crispus* to growing successfully in permanent grasslands in Central Europe, but not in regions with cold winters.

Conclusions

An increase in nutrient availability can decrease mortality of individual *R. obtusifolius* plants in temperate grasslands, but can increase mortality of *R. crispus*. The same effect of nutrient availability on mortality of both species was recorded both in unmanaged grassland and in grassland cut twice per year. It seems that no grassland management for several years can be an effective strategy for control of *R. obtusifolius* only under conditions of low nutrient availability in the soil. Further research is needed to evaluate the effect of grassland management and nutrient availability on performance and mortality of both species in temperate grasslands.

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