DEPARTMENT OF ECOLOGY

Ecological requirements of hybrid dock (*Rumex patientia* L. x *Rumex tianschanicus* A. Los.) and its potential as a weedy species in comparison with common dock species (*Rumex crispus* L. and *Rumex obtusifolius* L.)

(Ekologické nároky šťovíku (Rumex patientia L. x Rumex tianschanicus A. Los.) a jeho potenciál jako plevelné rostliny ve srovnání s nejčastěji se vyskytujícími širokolistými šťovíky (Rumex crispus L. a Rumex obtusifolius L.))

/PhD Thesis/

Renata Hujerová

Supervisor: Prof. Dr. Vilém Pavlů
Advisor: Ing. Lenka Pavlů Ph.D.

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I thereby declare that I wrote the Ph.D. thesis by myself using the results of my own work or collaborative work with my colleagues and with help of other publication resources which are properly cited.

Prohlašuji, že jsem vypracovala disertační práci samostatně, s využitím výsledků mé vlastni práce, výsledků spolupráce s mými kolegy a dalších publikovaných zdrojů, které jsou náležitě citovány.

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Content

Chapter 1

1. Introduction

The energy demand continues to grow and fossil fuels and natural gas are gradually decreasing worldwide. Therefore, in the most developed countries around the world intensifies efforts to find and to use renewable energy sources, where using biomass could represent the possibility. This trend has been joined also by the Czech Republic. Numerous abandoned agricultural lands have occurred after a significant decrease of livestock in the Czech Republic. It is a new challenge for a production of energy crops. A new *Rumex* crop hybrid of *R. patienta* x *R. tianschanicus*, registered as *Rumex* cv. OK-2, has been developed for use as a forage and energy (biofuel) crop. Hereinafter it is referred as *Rumex* OK-2, but is also known as 'Uteusha' in the Czech Republic (Usťak, 2007) after its Ukrainian breeder, Prof. Uteusha. *Rumex* OK-2 has also recently been introduced into several other European countries, including Germany, Slovakia, Bulgaria and Norway. It has been recorded as having escaped from arable fields into surrounding grassland and has the potential to become a new invasive weedy species (Hujerová, 2010; Pyšek et al., 2012; Hujerová, 2013) (**Chapter 2**).

The broad leaves *Rumex* species such as *Rumex obtusifolius* and *Rumex crispus* are considered to be among the most widely distributed weedy species (Holm et al., 1977; Grime et al., 1988; Zaller, 2004; Stilmant et al., 2010; Hrdličková et al., 2011). *R. obtusifolius* is one of the most troublesome weedy species in temperate grasslands because of its avoidance by livestock, high biomass and seed production, perennial character, persistent soil seed bank and its high ability to regenerate from fragmented underground organs (Carves and Harper, 1964; Zahler, 2004) whereas *R. crispus* is considered to be a serious weed on arable land and in highly disturbed areas because of its short life span and rather monocarpic character (Carves & Harper, 1964; Hejcman et al., 2012a).

2. Species

2.1. *Rumex crispus*

Rumex crispus is a perennial herb with a short, poorly branched root, main root and numerous long branched side roots. Stems are 30-100 cm tall, erect, often brownish, usually branched only in inflorescence. Basal leaves up to 35 cm long and 8 cm wide, oblong-lanceolate with curly margines and long petioles, upper stem leaves similar shape, smaller, usually less curly. Inflorescence is branched panicle. Perianth is usually rounded triangular, 3.5 to 5.0 (6.5) mm long, 3-6 mm wide, entire or only with very small teeth, with tubercle on one or all of the perianth (usually more then one). Achenes are 2-3 mm long, brown. It blooms from June to August (Hejný and Slavík, 1990). *Rumex crispus* has a lower competitive ability in permanent grasslands than *R. obtusifolius* due to its dependence on regular regeneration from seeds and its high sensitivity to regular cutting management (Hejcman et al., 2012a,b; Strnad et al., 2012). Vitality of *R. crispus* plants can be reduced by a high frequency of mowing or by intensive grazing (Stilmant, 2010). However, over the intensity of management (grazing, using heavy machinery), particularly in conjunction with drought, can lead to exposed sites that are sprouting new opportunities for individual plants (Grossrieder, 2004). According to Hongo (1988) and Bond et al. (2007) poor regrowth of *R. crispus* after cutting, compared with the more rapid and highly intensive regrowth of *R. obtusifolius*, it is connected to its lower investment into below-ground organs.

2.2. Rumex obtusifolius

Rumex obtusifolius is a highly variable species and many forms, varietes and subspecies have been described. It is an erect perennial herb. Stems are 40-150 cm hight, deeply furrowed and oftten reddish. Basal leaves a rosette, panicle leaves alternate; basal and lower stem leaves 13-30 cm long and 8-15 cm wide ovate-oblong with long petiolate, cordate at base, top rounded, on the reverse side usually scattered hairy; upper leaves smaller with short petiolate, ovate-lanceolate to lanceolate at the top with acute apex. Inflorescence is branched panicle. Perianth is about 2.5-6 mm long and 1.5-4 mm wide, broadly triangular or ovate frequently at the base big-toothed with one or more tubercle. Perianth is green at flowering and turning to brown when ripe (Carves & Harper, 1964; Hejný and Slavík, 1990). *Rumex obtusifolius* creates a deep taproot with high storage capacity for assimilates and nutrients. It develops a root-collar with high regeneration ability following disturbance and with a high potential for clonal reproduction (Pino et al., 1995; Strnad et al., 2010). In addition to generative reproduction, *R. obtusifolius* can expand through a phalanx clonal growth strategy, resulting in a dense nest of ramets that can occupy an area of several tens of square meters around the mother plant (Pino et al., 1995). Individual plants can survive in the grassland sward for more than eight years, although a high proportion can die within five years under conditions of low N, P and K availability and no grassland management (Pavlů et al., 2008; Martinková et al., 2009; Hujerová et al., 2011; Hann et al., 2012; Hejcman et al., 2012a).

2.3. *Rumex* OK-2

It is a hybrid of *Rumex patientia* L. (maternal line) and *Rumex tianschanicus* A.Los. (paternal line), which was bred by multiannual selection (Usťak, 2007). The hybrid *Rumex* OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness and high tolerance to salt stress and soil moisture (Kosakivska et al., 2008). It starts regenerated (growing) in March, simultaneously with the melting of snow (Usťak, 2007). Stems are up to 2 m tall, straight, bottom round and juicy. Stem diameter at the basal portion (at 15 cm) of 15-24 mm. The number of internodes is from 25 to 50. The plant produces on average 4-6 vegetative shoots. The lower leaves are 45-60 cm. Upper stem leaves are 24-30 cm long and 9-12 cm wide. The leaves are juicy, ovate-lanceolate shaped. Leaf margins are entire or slightly toothed. Petioles are 15-30 cm long. Inflorescence is panicle, 90-130 cm long (sometimes up to 180 cm), consisting of 10 to 20 branches of the first order. Flowers are small, pink hue. Triangular achenes are light brown, shiny. Weight 1000 seeds is 3.02 (from 2.8 to 3.3) g. The lower leaves are placed on the long tunnel-shaped petioles. Petioles of lower leaves and flowers have a reddish color. *Rumex* OK-2 has root reminds form and size of the root of parsley in the first years of vegetation. Older plants have strong and branched roots, some sectors of the roots can reach of 1.5 to 2.0 m depth. It is highly fertile plants. Seed maturation occurs evenly (Usťak, 2007). Although *Rumex* OK-2 has been planted since 2001 in the Czech Republic, its detailed ecological requirements and its potential to become a new weedy species has never been investigated. In addition, there is no information about its basic ecological requirements.

3. Germination and growth of *Rumex* species

R. crispus and *R. obtusifolius* are success weed thanks to the following factors:

1) ability to flowering several times a year;

2) production of large number of seeds, which remain viable in the soil many years;

3) fast germination of seeds;

4) germinate whenever the environmental conditions are appropriate (Carves and Harper, 1964).

Germination of *R. crispus* after ten years can be even 76% (Toole and Brown, 1946), after fifty years 50% (Kivillan and Bandurski, 1981), after eighty years 2% (Darlington and Steinbauer, 1961), similarly high germination (83%) of *R. obtusifolius* was found after 21 years (Toole and Brown, 1946). Seed germination is regulated by light intensity and mainly by temperature changes, so germination is the highest in the spring and in the autumn (van

Assche and Vanlerberghe, 1989). Carves and Harper (1966) reported that the germination is significantly affected by the removal of perianth of seed. Germination of the seeds, from which perianth was not removed, is significantly lower than seeds without perianth and seeds are more susceptible to fungal infections. Many studies indicate that the percentage of germination can be increased substantially by temperature fluctuations, especially in total darkness (Carves and Harper, 1966; Thompson and Grime, 1983). The variation between studies concerning dock weeds germination differs significantly. For example germination of one year old seeds of *R. crispus* in the dark at 20 ° C was 0% in Steinbauer and Grisbyho (1957) experiments and 69% in Townley's study (Townley, 1955). These differences in the behavior of seeds identified by different observers may be caused by the following factors:
(1) different experimental techniques;
(2) differences between the behavior of geographically or ecologically distinct populations of the same species;
(3) variability in the behavior of seeds from individual plants of the same species;
(4) variability in behavior of seeds from various parts of the inflorescence;
(5) differences in seed maturity and storage conditions (Carves and Harper, 1966).
The seeds are the main source of plant regeneration (Pino et al., 1995). However, the seeds rarely germinate in dense grass sward. Their development is limited by the presence of areas with bare soil because as a seedlings they have low competitive ability (Carves and Harper, 1967). In this thesis was evaluated the ability of *Rumex* OK-2 to grow and survive in the grassland under different managements (**Chapter 3**).
Vegetative reproduction of the *Rumex* species plays not so important role in the expansion, but the underground system of the plant incurred in the management field (ploughing, disking) can regenerate in new individual plants (Carves and Harper, 1964; Hongo, 1989). The root collar is the most important part of plant for regeneration after mechanical damage (Pino et al., 1995).

4. Forage quality of Rumex species

The herbage of *R. obtusifolius* exhibits low dry matter (DM) content, crude protein and fibre contents comparable with red clover (Hejduk and Doležal, 2004). Herbage quality of *R. crispus* is not different of *R. obtusifolius* too much and the two species can therefore be assessed together (Weissbach, 1998). Quality of silages made of *Rumex* species is good but the silages show significantly lower contents of lactic acid, acetic acid and higher pH values as compared with the grass silage. Silages made of dock do not contain butyric acid and exhibit lower rates of proteolysis. The presence of broad leaves dock in herbage poses a danger of slow wilting and low production of fermentation acids (Hejduk and Doležal, 2004). *Rumex* OK-2, has been developed for use as a forage and energy (biofuel) crop (Usťak, 2007). Although it was originally breed as a valuable forage, there is only a few information about its quality. There are only several remarks at methodology published by Usťak (2007). This plant has the highest feeding quality in early stages of growth (from leaf formation to the beginning of the stem formation). *Rumex* OK-2 excel as a forage crop extremely early maturity (first harvesting in late April - early May) and high content of crude protein in the early stages of growth. Biomass of *Rumex* OK-2 using as forage or for the production of biogas is reaping with content of dry matter about 18-22 %. It is usually at the stage of beginning of the stem formation until early ripening of the fruit. In this case, can be harvested 2 to 3 times per vegetation season and green mass yields are approximately 30-50 tons per 1 ha. The quality of silage hybrid dock is similar to alfalfa silage. Preparation of silage is particularly suitable from a mixture of *Rumex* OK-2 and grasses - in this case *Rumex* OK-2 can by harvested in more early stages, i.e., at a lower dry matter (12-16 %) and this mixture has better forage quality (Usťak, 2007).

5. Control of *Rumex* species

In the conventionally managed grasslands, *Rumex* species can be controlled by a selective herbicides but the results are not straightforward and require repeated treatment (Niggli et al., 1993). However, under conditions of organic farming, the use of herbicides is prohibited. Fear of infestation of grassland and arable land by *Rumex* species discourage many farmers from the transition from conventional to organic farming (Zaller, 2004). Under organic farming only biological or mechanical methods of weeding are allowed. Biological methods of *Rumex* control include the use of specific insects e.g. *Gastrophysa viridula* (Hatcher et al., 1997; Honěk and Martinková, 2004), pathogenous fungi *Uromyces rumicis* (Keary and Hatchler, 2004) or specific grazers such as goats (Hejcman et al., 2014). Nevertheless, their application is still problematic (Strnad et al., 2010). Mechanical methods include no grassland management, variable intensity of defoliation, heating and digging. Frequent cutting of *R. obtusifolius* reduced vigour of the aboveground organs, particularly decreased number of leaves, size of the largest leaf and also herbage production (Stilmant, 2010). *Rumex* OK-2 is a perennial crop, characterized by high ecological plasticity, cold and winter hardiness, and tolerance to salt and increased humidity (Kosakivska et al., 2008) with strong adaptability to environmental changes (Hou et al, 2014). *R. obtusifolius* can tolerate a high cutting frequency for several years. Therefore, neither two nor three cuts per year are sufficient for its elimination from grassland (Niggili et al., 1993; Hopkins & Johnson, 2002; Stilmant et al., 2010; Hann et al., 2012; Strnad et al., 2012). *R. crispus* has a lower competitive ability in permanent grasslands than *R. obtusifolius* due to its dependence on regular regeneration from seeds and its high sensitivity to regular cutting management (Hejcman et al., 2012a,b; Strnad et al., 2012). Production of belowground and aboveground biomass of *Rumex* OK-2 under different frequencies of defoliation was compared with *R. crispus*, *R. obtusifolius* and *R. alpinus* (**Chapter 4**).

The mature belowground system of *R. obtusifolius* is typified by a stout taproot and a branched stem system above the root collar with a high potential to clonal reproduction (Pino *et al*., 1995). *R. crispus* have short, poor branched root collar, main taproot and some long and branched secondary roots (Hejný & Slavík, 1990). In the seedling year, roots of *Rumex* OK-2 have similar shape and size like parsley. For multi-annual crop, roots are strong, branched and some sectors of the roots can reach a depth of 1.5 to 2.0 m (Usťak, 2007). Production and distribution of belowground biomass at different depths was compared with *R. crispus* and *R. obtusifolius* during vegetation season (**Chapter 5**).

Suitable conditions for growth of *Rumex* species are high nutrient availability in the soil interacting with reduced competition from other sward components, such as occurs by regular defoliation management or by sward disturbances (Gaisler et al., 2010; Křišťálová et al., 2011; Hejcman et al., 2012a). *Rumex obtusifolius* creates a deep taproot with high storage capacity for assimilates and nutrients. It develops a root-collar with high regeneration ability following disturbance and with a high potential for clonal reproduction (Pino et al., 1995; Strnad et al., 2010). In addition to generative reproduction, *R. obtusifolius* can expand through a phalanx clonal growth strategy, resulting in a dense nest of ramets that can occupy an area of several tens of square meters around the mother plant (Pino et al., 1995). Individual plants can survive in the sward for more than eight years, although a high proportion can die within five years under conditions of low N, P and K availability and no grassland management (Pavlů et al., 2008; Martinková et al., 2009; Hujerová et al., 2011; Hann et al., 2012; Hejcman et al., 2012a). *R. obtusifolius* well regrows after cutting (Martinková and Honěk, 2001) and therefore does not suffer under a management system of cutting performed twice per year

(Strnad et al., 2012). **Chapter 6** is focused on mechanical weeding of *R. obtusifolius*. This study can be a first step for future comparison of similarities for weeding of *Rumex* OK-2.

6. Research questions

The objective of the thesis is to answer the following question:
Is *Rumex* OK-2 able to spread outside spontaneously from former field into countryside? What is the dynamic of this spreading?

What is the competition ability of *Rumex* OK-2: germination, emergence and growing under different grassland management?

What are differences in basic growing characteristics between *Rumex* OK-2 and weeds *Rumex obtusifolius*, *R. crispus*, *R. alpinus* under different cutting regimes?

What are differences in aboveground and belowground biomass production between *Rumex* OK-2 and other broad leaves dock weeds *(R. obtusifolius*, *R. crispus*)?

Are there differences of growing dynamics and allocation of belowground biomass in 2 m depth soil profile between *Rumex* OK-2, *R. obtusifolius* and *R. crispus*?

The comparison of growing parameters of *Rumex* OK-2 with the other broad leaves *Rumex* species could help to assess its potential to become a new weedy species. For that reason, we investigated the possibility of *Rumex obtusifolius* mechanical control in grasslands, to answer to following questions: i) How effective is digging of *R. obtusifolius* in 5 and 15 cm performed once or twice for its control in *Agrostis capillaris* grassland? ii) Is no grassland management over five years an effective method for *R. obtusifolius* control?

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Chapter 2

Hybrid of *Rumex patientia* and *Rumex tianschanicus* (*Rumex* OK-2) as a potentially new invasive weed in Central Europe

Renata Hujerová, Jan Gaisler, Bohumil Mandák, Lenka Pavlů and Vilém Pavlů

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Hybrid of *Rumex patientia* × *Rumex tianschanicus* (*Rumex* OK-2) as a potentially new invasive weed in Central Europe

Hujerová R. [1]**, Gaisler J.** [2]**, Pavlů L.** [1]**, Pavlů V.** [1,2]**, Mandák B.**[1,3]

[1]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, CZ-165 21, Prague 6 - Suchdol, Czech Republic

[2]Crop Research Institute Prague, Department of Plant Ecology and Weed Sciences, CZ-460 01, Liberec, Czech Republic

[3]Institute of Botany, Academy of Sciences of the Czech Republic, CZ-25243 Průhonice, Czech Republic

Email: hujerova@fzp.czu.cz

Abstract

Since 2001 a hybrid of docks *Rumex patientia* × *Rumex tianschanicus* (*Rumex* OK-2) has been grown as a new energetic crop in the Czech Republic. It was originally bred as a forage crop plants in Ukraine and then introduced for the same reason to the Czech Republic. In the past five years a successive spreading in the surroundings of the fields of the original plantation was observed. This paper evaluates the results of the two monitoring years (2011–2012) at the eastern edge of Prague where *Rumex* OK-2 on arable land were established. Each plant of the *Rumex* OK-2 was located by geodetic GPS equipment in the study area during the year 2011. Next year, the presence of the plants *Rumex* OK-2 was verified and some newly discovered plants were recorded. By comparison of the two successive years, we have shown successive spreading of *Rumex* OK-2 mainly in man-made habitats. It seems that *Rumex* OK-2 could have an invasive potential and further detailed study of its biology and ecology is needed.

Keywords: energetic crop, weed, spreading, *Rumex* OK-2

Introduction

Many broad leaves *Rumex* species are considered to be the most troublesome weeds in grasslands and arable land worldwide (Zaller, 2004). New forage and energetic crop hybrid *R. patienta* × *R. tianschanicus* registered as c.v. *Rumex* OK-2 (thereafter referred as *Rumex* OK-2) also known as "Uteush" (according to its breeder Prof. Uteush from Ukraine) was introduced to the Czech Republic about ten years ago (Usťak, 2007). This taxon has, moreover, recently been introduced into other European Countries (Bulgaria, Germany, Norway). It can potentially become a new invasive weed species, because the escape of *Rumex* OK-2 plants from cultivation into surrounding grassland has been recorded (Hujerová, 2010).

Rumex OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness, tolerance to salt stress and increased humidity (Kosakivska *et al*., 2008). Although *Rumex* OK-2 has been planted for ten years in the Czech Republic, its detailed ecological requirements, spatial distribution, possibility to hybridize with native species, and its potential to become a new weedy species has never been investigated.

In this study we present preliminary results of *Rumex* OK-2 spreading within two monitoring years in the vicinity of the former field where *Rumex* OK-2 was experimentally grown. Especially we would like to show whether the taxon is able to spontaneously spread in the countryside.

Materials and Methods

Monitoring was conducted over the years 2011 and 2012 in the eastern margin of Prague where *Rumex* OK-2 was experimentally grown about ten years ago.The ditches along the both sites of the roads (± 3 m) were monitored it total length about 20 km (Fig. 1). Each plant of *Rumex* OK-2 was located (± 3 cm) using geodetic GPS equipment ProMark 200 and its distribution was recorded in a special map.

Results and Discussion

In the first monitoring year (2011) 375 plants were found in the study area. In 2012, the second monitoring year, 264 of them (70.4%) were verified again. Furthermore, 275 additional plants were discovered. By comparing the two successive years we have shown successive spreading of *Rumex* OK-2 in the study area (Fig. 1). Majority of recorded plants were presented on the edges of fields and in grasslands along the roads. Although Usťak (2007) characterized *Rumex* OK-2 as a competitively weak plant that disappears from grassland vegetation after 2–3 years, our previous monitoring (Hujerová 2010) showed that it is able to persist in grassland for a much longer time.

Conclusion

The preliminary results of two monitoring years (2011–2012) showed the expansive spreading of the *Rumex* OK-2 from former field especially along roadside ditches. It seems that *Rumex* OK-2 could have an invasive potential and further detailed study of its biology, ecology, and distribution strategy in landscape is needed.

Acknowledgments

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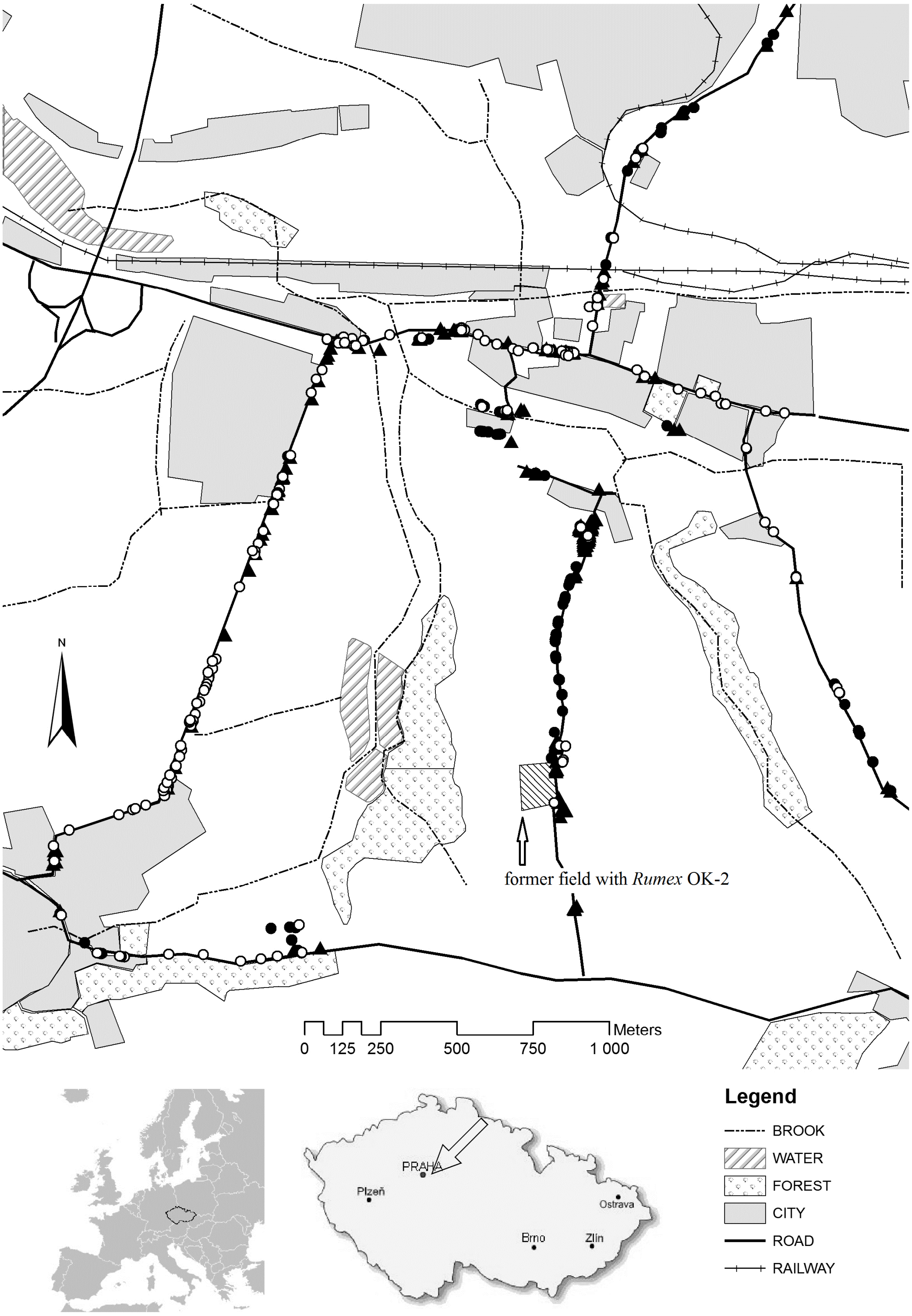

Figure 1. Map showing the distribution of *Rumex* OK-2 in the vicinity of Prague where a experimental field of the *Rumex* OK-2 was established about ten years ago. ▲ plants recorded in 2011 and 2012, ● plants recorded only in 2011, ○ plants recorded only in 2012.

Chapter 3

Emergence and survival of *Rumex* OK-2 (*Rumex patientia* x *Rumex tianschanicus*) in grasslands under different management conditions

Renata Hujerová, Jan Gaisler, Lenka Pavlů, Vilém Pavlů and Michal Hejcman

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Emergence and survival of *Rumex* OK-2 (*Rumex patientia x Rumex tianschanicus*) in grasslands under different management conditions

Hujerová R. [1], **Gaisler J.** [2], **Pavlů L.** [1], **Pavlů V.** [1,2] **and Hejcman M.** [1,2]

[1]*Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, CZ-165 21, Prague 6 - Suchdol, Czech Republic*

[2]*Crop Research Institute Prague, Department of Plant Ecology and Weed Sciences, CZ-460 01, Liberec, Czech Republic*

Email: hujerova@fzp.czu.cz

Abstract

Emergence and survival of *Rumex* OK-2 was studied in the north of the Czech Republic (in experimental garden in Liberec town) in 2013. There were applied three frequencies of cutting: no (0C), one (1C) and three (3C) cuts per year. Seeds of *Rumex* OK-2 were sown into the sward with different microsite conditions (no gap – gap; fertilizers application and no fertilizers application) in each treatment. The following plant characteristics were measured: number of emerged plants, number of surviving plants, plants height and numbers of leaves. Measurements were made three times per vegetation season (middle of June, end of July and end of September) before cutting. Plants of *Rumex* OK-2 emerged more in the treatments with gap. Surviving of *Rumex* OK-2 plants was connected with treatments with gap, especially in the second and the third cutting date.

Keywords: weeds, cutting frequency, competition, fertilizers application, gap

Introduction

Many broad leaves *Rumex* species are considered to be the most troublesome weeds in grasslands and arable land worldwide (Zaller, 2004). These plants often colonise grasslands as well as permanent agricultural crops (Novák, 1994; Brant *et al.*, 2006), where they can survive for a long time (Martinková *et al*., 2009). New forage and energetic crop hybrid *R. patienta* × *R. tianschanicus* registered as c.v. *Rumex* OK-2 (thereafter referred as *Rumex* OK-2) was introduced to the Czech Republic about ten years ago (Usťak, 2007). *Rumex* OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness, tolerance to salt stress and increased humidity (Kosakivska *et al*., 2008). Before the introduction to the culture it was presented as a competitive species with low possibility of invasibility (Usťak, 2007). However, it behaves as an invasive weed species especially in road ditches covered by grasslands in the vicinity of the field where it was previously grown (Hujerová, 2013a). Response of mature plants on different cutting frequency of *Rumex* OK-2 is very similar to *Rumex crispus* (Hujerová 2013b). In the view of the abovementioned knowledge we established manipulative experiment where emergence and survival of *Rumex* OK-2 in grasslands under different management conditions were studied.

Material and methods

The plot experiment was conducted in 2013 at the experimental garden of the Crop Research Institute, Grassland Research station Liberec, northern part of the Czech Republic under conditions of natural rainfall, temperature and daylight. Twenty seeds of *Rumex* OK-2 were sown into the sward in May 2013. Twelve factorial treatments were applied: i) three frequencies of cutting- no (0C), one (1C) and three (3C) cuts per year; ii) two levels of disturbance - gap and no gap; iii) two levels of nutrients - fertilizers application and no fertilizers application. The experiment was arranged in four complete randomised blocks with individual plot sizes of 0,5 m × 0,5 m. NPK fertilizer was applied in amount of 100 $kgN.ha^{-1}$ 52 kg $K.ha^{-1}$and 27 kg $P.ha^{-1}$in 0,15 m x 0,15 m areas allocated in the middle of each plot. Seeds were sown in the same area. We recorded number of emerged plants, number of surviving plants, plants height and numbers of leaves. Measurements were made three times per season (middle of June, end of July and September) before cutting. In the first cutting term the *Rumex* plants were not defoliated, because they were smaller than cutting height. One way ANOVA and repeated measures ANOVA were used to evaluate number of emerged plants, number of surviving plants, plants height and numbers of leaves.

Results and discussion

The number of emerged *Rumex* OK-2 plants was significantly divided into two groups according to disturbance. In the treatments without gap there was found up to one plant per plot whereas in plots with gap there were from seven to nine plants after one and a half month after sowing date (Fig.1).

There were not found emerged plants in no gap, non-fertilised, no cutting treatment (NGaNFC0). On the other hand the highest number of emerged plants was in gap, non-fertilised, no cutting treatment (GaNFC0). It confirmed results of Carves and Harper (1964) for *Rumex crispus* and *obtusifolius* that seed germination is possible when a gap occurs in the established sward. In the first cutting term there were only a few surviving plants in treatments without gaps but several times more of them in treatments with gaps (Fig.2). However, number of surviving *Rumex* OK-2 plants significantly decreased in the second cutting term because of high competitive ability of the existing sward. After the third cut only a few of *Rumex* OK-2 plants survived in gap treatments. However due to its fast spring growing and similar tolerance to cut as *Rumex crispus* has (Hujerová 2013b) we can expect its surviving, possible flowering and consequent seed production in the next vegetation.

Conclusion

The sward disturbance is the main factor for *Rumex* OK-2 infestation into the existing grasslands. Although in the course of vegetation season the plants of *Rumex* OK-2 are exposed by the high competitive pressure of existing sward, still some of them were revealed in the end of vegetation season. These plants in the next vegetation seasons can be important source of seeds and can support its expansion into surroundings. *Rumex* OK-2 has similar behaviour as other broad leaves docks in Central Europe so we can expect its further spreading.

Acknowledgments

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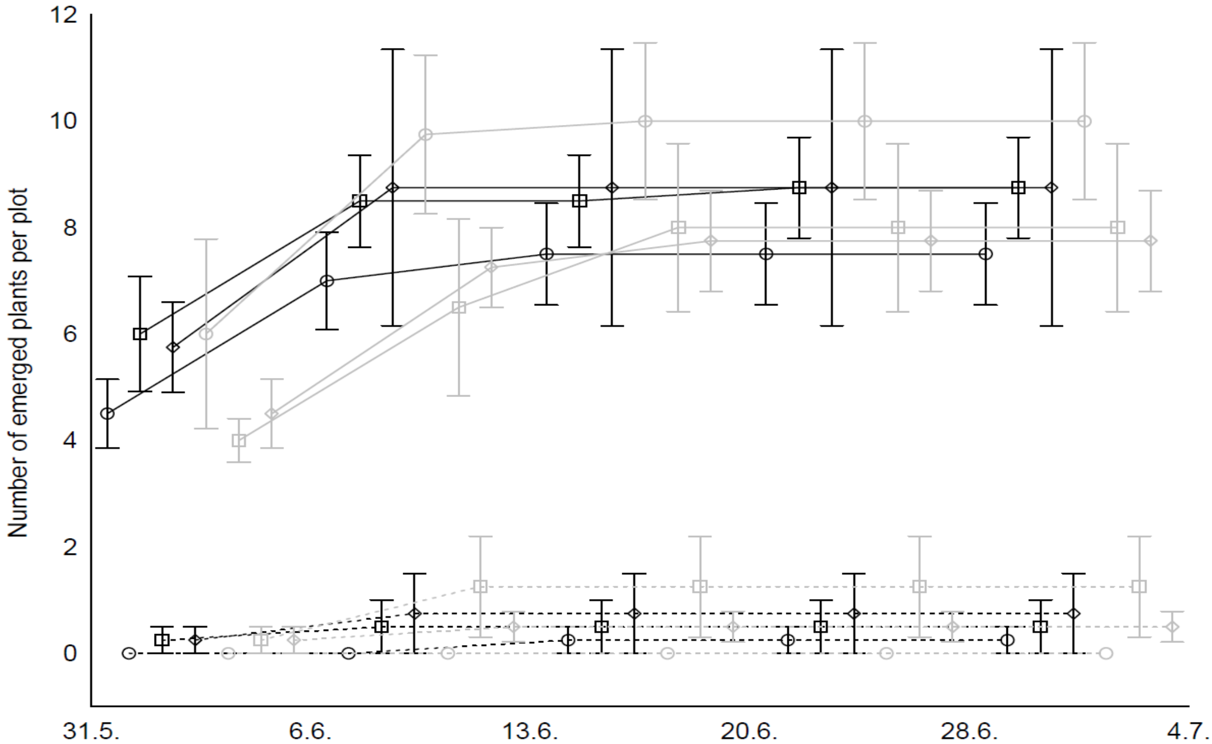

Figure 1. Development of emerged *Rumex* OK-2 plants during one and half month after sowing. Legend: ▬ fertilised, ▬ non-fertilised, ○ 0C, □ 1C, ◇ 3C, — gap, --- no gap.

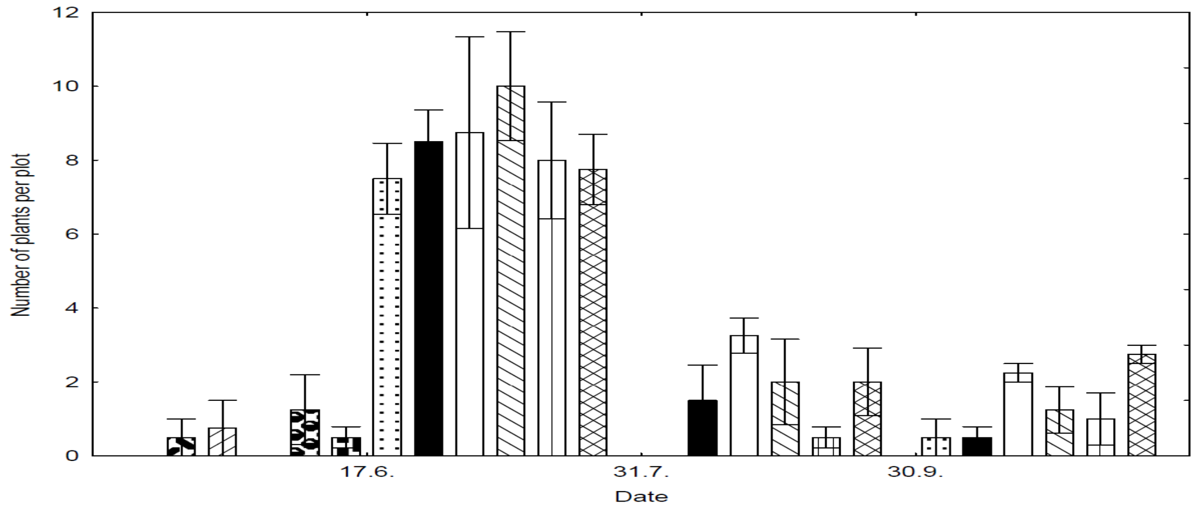

Figure 2. Number of survived plants *Rumex* OK-2 in three cutting terms. NGaFC1, NGaFC3, NGaNFC1, NGaNFC3, GaFC0, GaFC1, GaFC3, GaNFC0, GaNFC1, GaNFC3.

Chapter 4

Effect of cutting frequency on above- and belowground biomass production of *Rumex alpinus*, *R. crispus*, *R. obtusifolius* and the *Rumex* hybrid (*R. patienta* × *R. tianschanicus*) in the seeding year

Renata Hujerová, Vilém Pavlů, Michal Hejcman, Lenka Pavlů and Jan Gaisler

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Effect of cutting frequency on above- and below-ground biomass production of *Rumex alpinus, R. crispus, R. obtusifolius*, and the *Rumex* hybrid (*R. patienta* x *R. tianschanicus*) in the seeding year

R HUJEROVÁ[*], V PAVLŮ[*†], M HEJCMAN[*†], L PAVLŮ[*†] and J GAISLER[†]

[*]*Faculty of Environmental Sciences, Czech University of Life Sciences Prague,* Czech Republic, and [†]Grassland Research Station, Institute of Crop Research Institute Prague, Czech Republic

Correspondence: Vilém Pavlů, Grassland Research Station, Crop Research Institute Prague, Rolnická 6, CZ-460 11, Liberec, Czech Republic; Tel: +420 733 536 304; E-mail: pavlu@vurv.cz

Summary

Rumex species are important weeds in grasslands and on arable land. The *Rumex* hybrid (*R. patienta* x *R. tianschanicus;* cv. OK-2, Uteusha) has been planted as a forage and energy crop since 2001 in the Czech Republic, but its ecological requirements and its potential to become a new weedy species have never been investigated. In 2010 and 2011, we performed a pot experiment to investigate the effect of no (0C), one (1C) and two (2C) cuts per year on biomass production of *Rumex* OK-2 and common broad leaves *Rumex* species (*Rumex obtusifolius*, *R. crispus* and *R. alpinus)*. The higher cutting frequency can reduce the belowground biomass but no effect on the aboveground biomass was detected. Flowering in the seeding year was recorded in only 50% of *R. obtusifolius* plants. Non-flowering *R. obtusifolius* plants produced significantly more belowground biomass than flowering plants under 0C and 1C treatments. The growth response of *Rumex* OK-2 to different cutting treatments was very similar to *R. crispus.* These similarities indicate the weed potential of the hybrid to become a troublesome weedy species as *R. crispus.*

Keywords: dock weeds, grassland management, mowing frequency, *Rumex* OK-2

Introduction

Rumex obtusifolius subsp. *obtusifolius* (hereafter referred as *R. obtusifolius*) and *Rumex crispus* are considered to be among the most widely distributed non-cultivated weedy species in temperate zones worldwide (Holm *et al*., 1977; Grime *et al*., 1988; Zaller, 2004; Stilmant *et al*., 2010; Hrdličková *et al.*, 2011; Hejcman *et al.*, 2012c). *Rumex obtusifolius* is a very important perennial weed in grasslands (Strnad *et al.*, 2010) whereas *R. crispus* is considered to be a serious weed on arable land and in highly disturbed areas because of its short life span and rather monocarpic character (Carves & Harper, 1964; Hejcman *et al.*, 2012a). In addition to the above-mentioned species, *R. alpinus* is a troublesome perennial weed in high mountains of western, central and eastern Europe, and is common in abandoned grasslands (Šťastná *et al*., 2010). A new *Rumex* crop hybrid of *R. patienta* x *R. tianschanicus*, registered as *Rumex* cv. OK-2, has been developed for use as a forage and energy (biofuel) crop. In this paper it is referred to as *Rumex* OK-2, but is also known as 'Uteusha' in the Czech Republic (Usťak, 2007) after its Ukrainian breeder, Prof. Uteusha. *Rumex* OK-2 has also recently been introduced into several other European countries, including Germany, Slovakia, Bulgaria and Norway. It has been recorded as having escaped from arable fields into surrounding grassland and has the potential to become a new invasive weedy species (Hujerová, 2010; Pyšek *et al*., 2012).

Rumex obtusifolius creates a deep taproot with high storage capacity for assimilates and nutrients. It develops a root-collar with high regeneration ability following disturbance and with a high potential for clonal reproduction (Pino *et al*., 1995; Strnad *et al*., 2010). In addition to generative reproduction, *R. obtusifolius* can expand through a phalanx clonal growth strategy, resulting in a dense nest of ramets that can occupy an area of several tens of square meters around the mother plant (Pino *et al*., 1995). Individual plants can survive in the grassland sward for more than eight years, although a high proportion can die within five years under conditions of low N, P and K availability and no grassland management (Pavlů *et al*., 2008; Martínková *et al.*, 2009; Hujerová *et al*., 2011; Hann *et al*., 2012; Hejcman *et al.*, 2012a). *Rumex obtusifolius* well regrows after cutting (Martinková and Honěk, 2001) and therefore does not suffer under a management system of cutting performed twice per year (Strnad *et al.*, 2012). Suitable conditions for its growth are high nutrient availability in the soil interacting with reduced competition from other sward components, such as occurs by regular defoliation management or by sward disturbances (Gaisler *et al*., 2010; Křišťálová *et al*., 2011; Hejcman *et al*., 2012a). *Rumex obtusifolius* can tolerate a high cutting frequency for several years. Therefore, neither two nor three cuts per year are sufficient for its elimination from grassland (Niggili *et al*., 1993; Hopkins & Johnson, 2002; Stilman *et al*., 2010; Hann *et al*., 2012; Strnad *et al.*, 2012).

Rumex crispus has a lower competitive ability in permanent grasslands than *R. obtusifolius* due to its dependence on regular regeneration from seeds and its high sensitivity to regular cutting management (Hejcman *et al*., 2012a,b; Strnad *et al*., 2012). According to Hongo (1988) and Bond *et al*. (2007) poor regrowth of *R. crispus* after cutting, compared with the more rapid and highly intensive regrowth of *R. obtusifolius*, is connected to its lower investment into below-ground organs.

Rumex alpinus is a rhizomatous perennial species and is typically associated with grassland on mountainous sites that have either no or extensive defoliation management and high nutrient availability (Bohner, 2005; Šťastná *et al*., 2010). Its horizontal monopodial rhizome is formed by a shortened base of a shoot and grows at a depth of up to 5 - 10 cm (Klimeš, 1992). A new segment of the rhizome develops each year. Therefore, the growth of the plant and its age, which can be as much as several decades, can be determined from the number of segments (Šťastná, 2012). Although *R. alpinus* is able to regrow after being cut, it cannot withstand long-term and regular cutting management and it seldom occurs in regularly cut grasslands (Šťastná, 2010).

The hybrid *Rumex* OK-2 is described as a perennial (up to 10 years) stress-tolerant plant, characterized by high ecological plasticity, with cold and winter hardiness and high tolerance to salt stress and soil moisture (Kosakivska *et al*., 2008). Although *Rumex* OK-2 has been planted since 2001 in the Czech Republic, its detailed ecological requirements and its potential to become a new weedy species has never been investigated. In addition, there is no information about its basic ecological requirements.

Therefore, the present study addresses the following research question: Are there differences in basic growing characteristics between *Rumex* OK-2 and weeds *Rumex obtusifolius*, *R. crispus*, *R. alpinus* under different cutting regimes? Using a pot experiment, we tested differences in aboveground and belowground biomass production and in the ratio of belowground and aboveground biomass in the first year of growth. The comparison of growing parameters of *Rumex* OK-2 with the other broad leaves *Rumex* species could help to assess its potential to become a new weedy species.

Materials and methods

Study site and design of the pot experiment

The pot experiment was conducted in 2010 and 2011 at the experimental garden of the Crop Research Institute, Grassland Research station Liberec, northern Czech Republic (50°46'33.213"N, 15°2'20.207"E; altitude 366 m a.s.l.) under conditions of natural rainfall, temperature and daylight. Mean annual temperature at the study site was 7.5 °C and average annual precipitation was 800 mm. Monthly rainfall and mean air temperature at Liberec Meteorological Station during the vegetation seasons 2010-2011 are given in Table 1. We compared *Rumex* OK-2 with three *Rumex* species (*Rumex obtusifolius*, *R. crispus* and *R. alpinus*) under three cutting frequencies: no cut (0C), one cut in July (1C) and two cuts per vegetation season in July and August (2C). The frequency and timing of cutting treatments were based on the typical agricultural practice in the area. Each treatment was replicated five times (thus, 60 pots in total). The same type of soil and pots were used in both study years as the experiment was established two times, in spring 2010 and again in spring 2011.

Seeds of *R. obtusifolius*, *R. crispus* and *R. alpinus* were collected from plants in grassland near Liberec, abandoned fields near Prague and in abandoned grasslands in the Jizera Mts., respectively, in autumn 2008. Seeds of *Rumex* OK-2 were obtained as a commercial product (Crop Research Institute, Centre for Revitalization and Sustainable Development of North Bohemia, Chomutov). Tested germination was about 90% for all studied species. The one-month old seedlings (one per pot) were transplanted into pots at the end of May 2010 and 2011. We used 40 cm diameter conical pots (30 cm diameter at base) and the pot volume was 30 litres. In both years we used the same cambisol in the experiment as this is the most common soil type on the sites where seeds of the *Rumex* had been collected. The soil was collected in 2010 and it had the following chemical properties: pH_{KCl} = 5.5, P = 122 mg kg^{-1}, K = 234 mg kg^{-1}, Mg = 91 mg kg^{-1}. The pots were refilled with fresh soil in year 2011. The P, K and Mg were extracted by Mehlich III reagent to determine plant-available concentrations. During the vegetation season *Rumex* plants were watered when necessary to avoid any water stress. The position of individual pots was changed at weekly intervals to avoid any site and edge effects.

Data collection

At each cutting date the aboveground biomass of *Rumex* plants (shoot, leaves) was cut to 2 cm height above soil surface, then dried at 60ºC for 48 h and weighed. Finally, the total aboveground and belowground biomass was taken in all treatments in September 2010 and again in 2011 and then weighed. Aboveground biomass was collected in the same way as the regular cuts. In addition, we recorded presence of inflorescences in individual plants. Belowground biomass was determined after rinsing the roots free of soil using a water spray. The roots were then dried at 60ºC for 48 h and weight of dry matter was determined. The belowground/aboveground biomass ratio was calculated to determine the response of biomass allocation under the different cutting treatments.

Data analyses

Factorial ANOVA was used to test the effects of year, species, cut and their interactions on the aboveground biomass, belowground biomass, and belowground/aboveground biomass ratio. We tested the following effects: year, species, cut, and their interactions. One-way ANOVA was then used to test differences among species for particular cutting frequencies and among cutting frequencies for individual species. Calculation was conducted separately for each year. Post hoc comparison using the Tukey HSD test was applied to identify significant differences between species and individual treatments. The data met all requirements of ANOVA analysis. The relation between aboveground and belowground biomass was evaluated using the Pearson's correlation coefficient.

Results

Aboveground biomass

Effect of year, and interaction of species x year, and cut x year were significant on aboveground biomass production (Table 2). In contrast, there were no significant effects of cut, species, and of the species x cut interaction on aboveground biomass production. The aboveground biomass production of all the studied species under all cutting frequencies was higher in the second year than in the first year of the experiment (Fig. 1).

In the first year there were only small differences between the species in their aboveground production. *Rumex alpinus* was the only species that constantly tended to have the lowest amount of shoot growth under all cutting frequencies (Fig. 1). In the second year, *Rumex* OK-2 tended to have the weakest shoot growth in all treatments. Its aboveground biomass production was significantly lower than that of *R. obtusifolius* in all treatments.

Belowground biomass

The effect of year, cut and the interaction of species x cut and species x year were significant on belowground biomass production (Table 2). In contrast, there was no significant effect of species and cut x year interaction on belowground biomass production.

The belowground biomass production of all the studied species under all cutting frequencies was higher in 2011 than in 2010 (Fig. 1). The highest belowground biomass from all studied species and cutting frequencies was recorded for *Rumex* OK-2 followed by *R. crispus* under no cutting management in 2010, the same trend was observed in 2011 (Fig. 1). On the other hand, the lowest values of belowground biomass under the no-cutting management were recorded for *R. obtusifolius* and *R. alpinus* in 2010, and a similar tendency was recorded in 2011. Although *Rumex* OK-2 and *R. crispus* produced the greatest amounts of belowground biomass under the no-cutting management, there was a strong negative effect of both the one-cut and two-cuts management on their belowground biomass production (Fig. 1). This negative effect contrasted with the absence of an effect of one-cut or two-cuts management on belowground biomass production of *R. obtusifolius* in both years, and on belowground biomass production of *R. alpinus* in the second year.

There were no differences between *Rumex* species under the one-cut management in either year (Fig. 1) or under the two-cut management in the first year of the experiment. In the second year, belowground biomass was highest for *R. crispus*, followed by *R. obtusifolius*, and lowest for *Rumex* OK-2, followed by *R. alpinus* under the two-cut management.

Belowground/aboveground biomass ratio

Effects of species x year, cut x year, and species x cut interactions on the belowground/aboveground biomass ratio were significant (Table 2). In contrast, there was no significant effect of cut, year and species, on belowground/aboveground biomass ratio. The ratio generally tended to be highest in the *Rumex* OK-2, particularly in the second year of the experiment, when it was significantly higher under no-cut and on-cut management than in all other species (Table 3). Decreasing ratios between below- and aboveground biomass under increasing cutting frequencies, as measured for *Rumex* OK-2 and *R. crispus* was also recorded for *R. alpinus*,

With the exceptions of *R. obtusifolius* under no-cut and one-cut management and *Rumex* OK-2 under the no-cutting management, there was clearly a linear and highly positive relationship between belowground and aboveground biomass production for all *Rumex* species and under all cutting frequencies (Fig. 2). In the seeding year, flowering was recorded only in plants of *R. obtusifolius*. Over both years, the proportion of flowering plants of *R. obtusifolius* was 60%, 40% and 50% under management of no cutting, one-cut and two-cuts, respectively. Flowering plants produced almost the same amount of aboveground biomass as non-flowering plants, but they produced substantially lower amounts of belowground biomass particularly under no-cutting and on-cutting management (Fig. 3). Flowering thus substantially decreased the belowground/aboveground biomass ratio in *R. obtusifolius* plants (Table 4).

Discussion

There were large differences in the biomass production of all *Rumex* species between the two years of the experiment, despite the pots having been watered in both years to avoid water stress. It is possible that surplus precipitation may have affected the biomass production of *Rumex* species in year 2011.

Aboveground biomass

The ability of *R. obtusifolius* to flower in the seeding year and to produce sterile and flowering plants is consistent with results of other pot or field experiments performed in the Czech Republic (Křišťálová *et al*., 2011; Hrdličková *et al*., 2011; Hejcman *et al*., 2012b; Strnad *et al*., 2012). In contrast, *R. crispus* was not able to produce any seeds in the seeding year. This finding supports the results of several experiments in the Czech Republic which showed that the absence of flowering in the seeding year was a matter of genetics, rather than a consequence of insufficient nutrient availability (Křišťálová *et al*., 2011; Hejcman *et al*., 2012a,b; Strnad *et al*., 2012).

Rumex crispus is thus substantially more sensitive to unpredictable environmental conditions than *R. obtusifolius* as it needs at least two years to produce seeds. The absence of flowering and seed production in the seeding year might be one of several reasons that explain why *R. crispus* is a substantially less common species than *R. obtusifolius* in Central Europe.

From all the studied *Rumex* species, the highest year-to-year variability in biomass production was recorded for *R. alpinus*. Lower biomass production in 2010 (7.2 g for 0C, 3.8 for 1C and 9.0 for 2C) than in 2011 (34.2 g for 0C, 39.5 for 1C and 25.8 for 2C) was probably caused by heat stress as there were fewer cloudy and rainy days and temperature was higher in summer 2010 than in 2011. High sensitivity of *R. alpinus* to heat stress might be a reason why this species is common at high altitudes and generally missing in the lowlands (see also Šťastná *et al*., 2010).

Rumex OK-2 did not show the highest amount of aboveground biomass production, despite its recommended use as a forage and energy crop on the basis of its high herbage production (Usťak, 2007). Over the short duration of the investigation reported here, the growth potential of *Rumex* OK-2 was not different to that of common *Rumex* weedy species under low or medium nutrient availability in the soil. The aboveground biomass production of *Rumex* OK-2 might be higher under high soil nutrient availability, but this was not investigated in this experiment. Furthermore, one vegetation season may have been insufficient for the *Rumex* OK-2 to have reached a growth stage that would have allowed its production to proceed. Under adequate growing conditions, its relatively high belowground allocation in the establishment phase might be an advantageous strategy in the long term.

Although Hopkins and Johnson (2002) recorded the highest aboveground biomass production of *R. obtusifolius* under three cuts, in comparison with one, two and five cuts, we recorded no significant effect of cutting frequency on the aboveground biomass production neither in 2010 nor in 2011. We found differences in the aboveground biomass production between years. It seems that year-to-year variability in environmental conditions, especially precipitation, can highly affect biomass production of *Rumex* species under different cutting frequencies.

Belowground biomass

Some differences in belowground biomass production between the studied species were recorded, but these differences were expressed under different cutting managements in different years. With the exception of flowering *R. obtusifolius*, all the studied species tended to produce more belowground than aboveground biomass.

The constant belowground biomass production of *R. obtusifolius* under all cutting frequencies was very interesting. This is in contrast with results reported by Hongo (1989) and by Stilmant *et al*. (2010) who recorded a reduction in belowground biomass under intensive defoliation. Different results can be explained by the use of more frequent cutting in the above-mentioned studies than in our study. A decrease in belowground biomass production of *R. obtusifolius* can probably be recorded if at least three cuts are applied per vegetation season. The constant belowground biomass production under increasing cutting frequencies was recorded only for *R. obtusifolius* and it is probably connected to its high tolerance to intensive grassland management in comparison to the other *Rumex* species. A high tolerance of *R. obtusifolius* to different cutting frequencies was also recorded in the field experiment reported by Hopkins and Johnson (2002).

Rumex crispus and the *Rumex* OK-2 showed a significant decrease in belowground biomass with increasing cutting frequency in both years. *Rumex crispus* is well known for its poor regeneration after cutting and therefore for its low competitive ability in permanent grassland (Strnad *et al.*, 2012). The low tolerance of *R. crispus* to cutting management was consistent with the results of our study. It can be linked to a decreased energy investment into belowground organs after a loss of aboveground organs due to regular defoliation. Some authors (Hongo, 1988; Bond *et al*. 2007) have proposed that *R. obtusifolius* invests more energy into belowground biomass than *R. crispus*. It seems that the higher investment of energy by *R. obtusifolius* into belowground organs, compared to that of *R. crispus*, may be valid only in regularly cut grasslands. The reverse situation can be recorded in unmanaged grasslands. As *Rumex* OK-2 produced substantially less belowground biomass under cutting management than under no cutting, it will probably suffer from the effects of intensive grassland management, similarly to *R. crispus*. *Rumex alpinus* has already been recorded to be sensitive to regular and frequent mowing under field conditions (Šťastná *et al.*, 2010). The decrease of *R. alpinus* belowground production under increasing mowing frequencies in the first year, indicates more negative effects of cutting management on *R. alpinus* than on *R. obtusifolius.* On the other hand, the decrease in belowground biomass of *R. alpinus* was not as strong and constant as in the case of *R. crispus*. Therefore, it seems that the sensitivity of *R. alpinus* to cutting management is probably lower than sensitivity of *R. crispus*. The decreased belowground biomass production of flowering *R. obtusifolius* might be the reason for reduction of *R. obtusifolius* under no or low management (Martínková & Honěk, 2001; Pavlů *et al.,* 2008; Martinková *et al.*, 2009; Hann *et al.*, 2012).

Belowground/aboveground biomass ratio

Except for *R. obtusifolius* (0C, 1C) and *Rumex* OK-2 (0C), belowground and aboveground biomass showed a strong positive linear relationship. This was because *R. obtusifolius* was the only species that flowered in the seeding year and its plants invested energy into flower and seed production, rather than into belowground biomass. On the other hand the strategy of *Rumex* OK-2 under no cut was to invest into belowground biomass regardless the amount of aboveground biomass. Probably it is preparation for successful flowering and seed production in subsequent vegetation seasons.

Conclusions

A higher cutting frequency can reduce the belowground biomass but no effect on the aboveground biomass was detected. The growing response of *Rumex* OK-2 to different cutting treatments was not very different from other studied *Rumex* species. The belowground biomass production of *Rumex* OK-2 was most similar to *R. crispus* under low cutting frequencies. These similarities indicate that also the weed potential of *Rumex* OK-2 might be corresponding to that of *R. crispus.* As the results of three years of observations at the landscape level have revealed its fast expansion into surrounding grasslands in the area of its former planting, its high potential to become a new troublesome weedy species has already been confirmed (Hujerová *et al.*, 2013).

The results of the pot experiment can only be the first step to investigate the ecological requirements of a hybrid. The future investigations should be focused on response of *Rumex* OK-2 to different mowing frequencies subdued to competition by other plants under field conditions.

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Table 1 Monthly precipitation sum and mean air temperature at Liberec meteorological station during the experimental period and 30-year mean values.

	Rainfall (mm)			Air temperature (°C)		
			Average			Average
Month	2010	2011	1966-1995	2010	2011	1966-1995
June	81	75	85	15.6	16.3	15.5
July	296	149	88	19.7	15.9	16.1
August	87	376	88	16.5	17.6	16.2
September	65	170	65	14.5	14.5	12.7

Table 2 Results of factorial ANOVA analyses (Year was random factor) calculated for belowground biomass, aboveground biomass and belowground /aboveground biomass ratio.

Tested variable	Effect	DF	*F* value	*P* value
Aboveground biomass	Year	1	10.74	*0.026*
	Species	3	1.50	0.374
	Cut	2	0.11	0.902
	Species x Year	3	13.11	< *0.001*
	Species x Cut	6	1.01	0.426
	Cut x Year	2	6.65	*0.002*
Belowground biomass	Year	1	24.99	*0.015*
	Species	3	3.48	0.167
	Cut	2	31.65	*0.031*
	Species x Year	3	3.03	*0.033*
	Species x Cut	6	6.92	< *0.001*
	Cut x Year	2	1.63	0.200
Belowground /aboveground biomass ratio	Year	1	201.5	0.286
	Species	3	19.7	0.273
	Cut	2	0.73	0.086
	Species x Year	3	13.11	*0.002*
	Species x Cut	6	1.01	*0.031*
	Cut x Year	2	6.645	< *0.001*

DF degrees of freedom.

Significant results are in italics.

Table 3 Belowground/aboveground biomass ratio of *Rumex* species under different treatments in 2010 and 2011. Numbers represent average of five replicates, ± standard error of the mean (SE). Significant differences ($P < 0.05$) between treatments for each *Rumex* species according to the Tukey's post hoc test are indicated by different capital letters in row and significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters in column.

Year		Treatment		
		No cut	One cut	Two cuts
2010	*Rumex* OK-2	7.29 ± 1.35 a A	3.60 ± 1.21 ab AB	1.45 ± 0.09 a B
	R. obtusifolius	1.97 ± 0.52 b A	2.26 ± 0.56 ab A	1.16 ± 0.45 a A
	R. crispus	5.29 ± 0.48 ab A	1.68 ± 0.14 b B	1.19 ± 0.03 a B
	R. alpinus	5.43 ± 1.58 ab A	4.74 ± 0.63 a A	2.36 ± 0.67 a A
2011	*Rumex* OK-2	5.99 ± 0.78 a A	2.82 ± 0.14 a B	1.52 ± 0.15 ab B
	R. obtusifolius	1.97 ± 0.57 b A	1.47 ± 0.41 b A	1.40 ± 0.17 b A
	R. crispus	3.50 ± 0.08 b A	2.48 ± 0.15 ab B	1.99 ± 0.11 a
	R. alpinus	2.02 ± 0.18 b A	1.58 ± 0.25 b AB	1.25 ± 0.14 b B

Table 4 Belowground/aboveground biomass ratio of flowering and non-flowering *Rumex obtusifolius* under different treatments in 2010 and 2011. Numbers represent average, ± standard error of the mean (SE). Significant differences ($P < 0.05$) between treatments in each flowering category according to the Tukey's post hoc test are indicated by different capital letters in row and significant differences ($P < 0.05$) between treatments in each flowering category according to the Tukey's post hoc test are indicated by different small letters in column.

	Treatment		
Belowground/aboveground ratio	No cut	One cut	Two cuts
Flowering	0.96 ± 0.24 b A (n=7)	0.75 ± 0.21 b A (n=4)	0.69 ± 0.20 b A (n=5)
No flowering	3.15 ± 0.29 a A (n=4)	2.61 ± 0.29 a AB (n=6)	1.83 ± 0.21 a B (n=5)

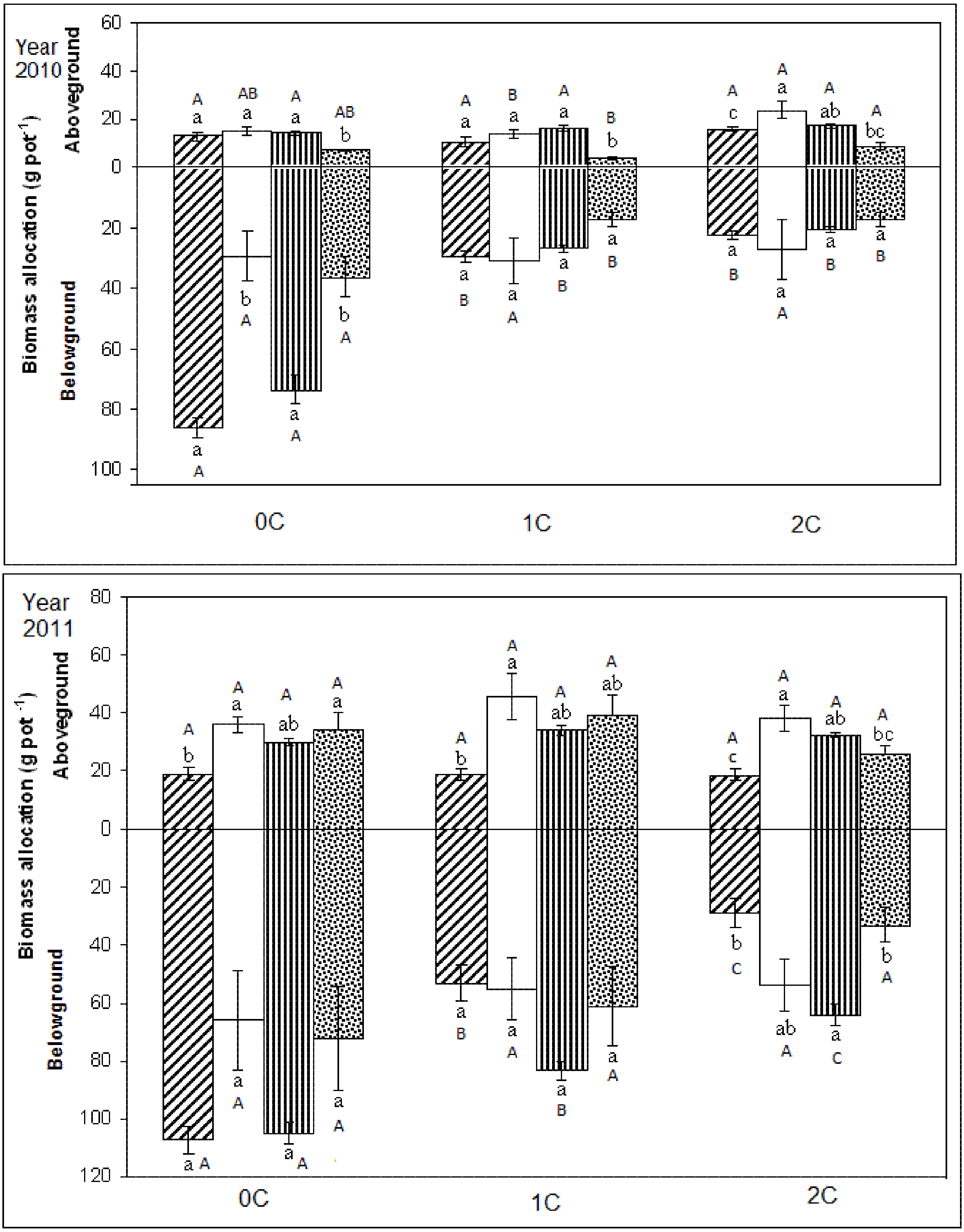

Fig. 1 Biomass dry matter allocation (g per plot) of *Rumex* species (*Rumex* OK-2, *R. obtusifolius*, *R. crispus*, *R. alpinus*) under different treatments (0C-no cut; 1C-cut once; 2C-cut two times) in years 2010 and 2011. Significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters and significant differences ($P < 0.05$) between treatments for each *Rumex* species according to the Tukey's post hoc test are indicated by different capital letters.

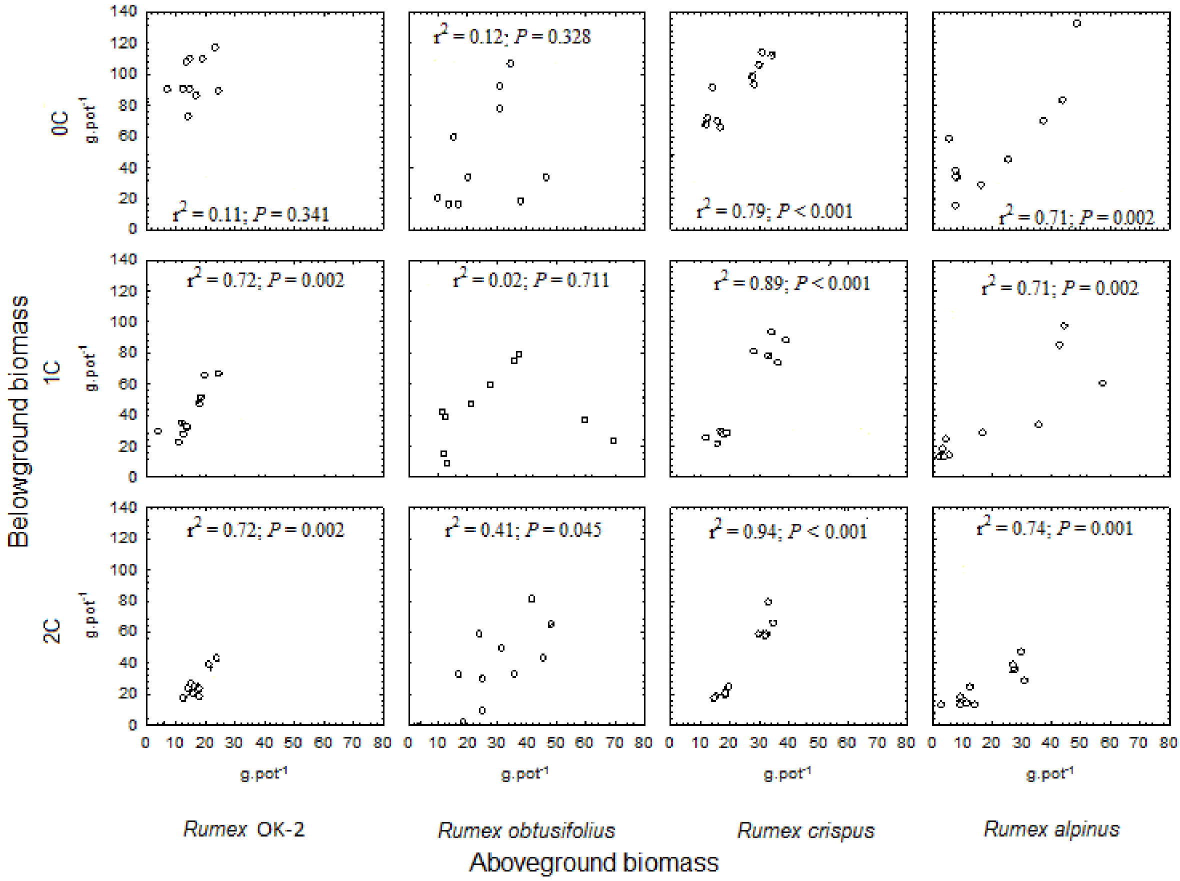

Fig. 2 Relationship between belowground and aboveground biomass of *Rumex* species under different treatments (0C-no cut; 1C-cut once; 2C-cut two times) during the experiment. Pearson's correlation coefficients and probability levels are presented inside each subfigure.

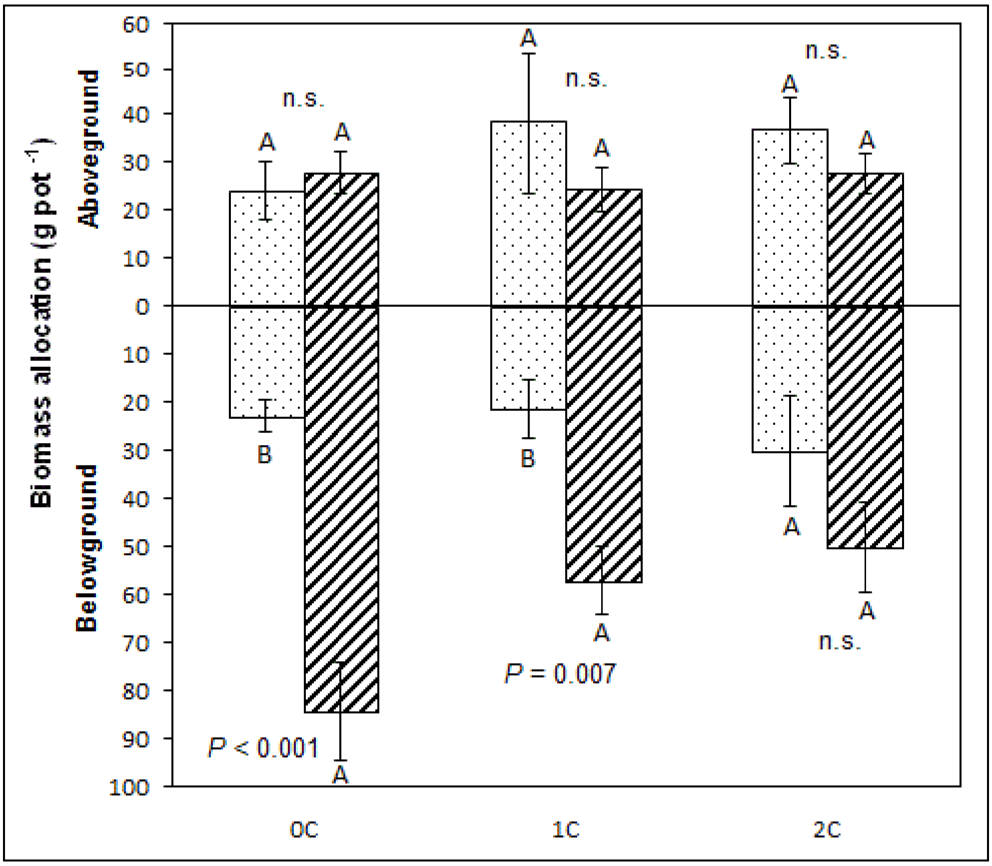

Fig. 3 Biomass dry matter allocation (g per plot) of *Rumex obtusifolius* with flowering (▒) and *R. obtusifolius* without flowering (▨) under different treatments (0C-not cut; 1C-cut once; 2C-cut two times). Number of observation (n) for flowering plants 0C n=7, 1C n=4, 2C n=5 and plants without flowering 0C n=4, 1C n=6, 2C n=5. Significant differences (P < 0.05) according to the Tukey's post hoc test are indicated by different capital letters in treatment. Error bars represent standard errors of the mean.

Chapter 5

The growth dynamics of belowground biomass of *Rumex crispus*, *R. obtusifolius*, and the *Rumex* hybrid cv. OK-2 (*R. patienta* x *R. tianschanicus*) in the seeding year

Renata Hujerová, Lenka Pavlů, Vilém Pavlů, Michal Hejcman and Jan Gaisler

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The growth dynamics of belowground biomass of *Rumex crispus*, *R. obtusifolius*, and the *Rumex* hybrid cv. OK-2 (*R. patienta* x *R. tianschanicus*) in the seeding year

R HUJEROVÁ*, L PAVLŮ*, V PAVLŮ*†, M HEJCMAN*†& J GAISLER†

*Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic.

†Department of Weeds and Vegetation of Agroecosystems, Crop Research Institute Prague, Liberec, Czech Republic.

Correspondence: Vilém Pavlů, Grassland Research Station, Crop Research Institute Prague, Rolnická 6, CZ-460 11, Liberec, Czech Republic; Tel: +420 484 800 562; E-mail: pavlu@vurv.cz

Summary

Broad leaves *Rumex* species are serious weeds on arable land and in permanent grasslands where they can persist on the same area for a long times especially due to well-established belowground systems. The *Rumex* hybrid (*R. patienta* x *R. tianschanicus*; cv. OK-2, Uteusha) has been planted as a forage and energy crop since 2001 in the Czech Republic and it has become a new weedy species, but its ecological characteristics are unknown. In 2010 and 2011 we performed a tube pot experiment to investigate growth dynamics and belowground biomass allocation of *R. crispus*, *R. obtusifolius* and *Rumex* OK-2 during the vegetation season. Biomass production of all *Rumex* species tend to grow from July to September. In the seeding year, flowering was recorded only in one plant of *Rumex* OK-2 and 27.5% of *R. obtusifolius* plants. The proportion of belowground biomass of *Rumex* species in upper 30 cm was about 70-80% whereas only 20-30 % was allocated deeper than 30 cm. The growth dynamics and allocation of belowground biomass of *Rumex* OK-2 was more similar to *R. crispus* than to *R. obtusifolius*. These similarities denote that the weed potential of *Rumex* OK-2 to become a troublesome weedy species, similar to *R. crispus*.

Keywords: broad-leaved dock, curled dock, *Rumex* OK-2, depth, biomass allocation

Introduction

Most of broad leaves *Rumex* species are troublesome weeds (Hongo, 1989; Pino *et al*., 1995). They produce a lot of seeds per plant (Hrdličková *et al*., 2011; Hejcman *et al*., 2012) with long term persistence in the soil seed bank (Honěk & Martinková, 2002). Vegetative regeneration plays a minor role in *Rumex* establishment, although belowground system fragments produced in field operations such as ploughing or rotavating are able to regenerate new individuals (Carves & Harper, 1964; Hongo, 1989). The thorough knowledge of the root system of *Rumex* is imperative for the development of sustainable control strategies against these species (Zaller, 2004). We focused our study to *Rumex crispus*, *R. obtusifolius* and *Rumex* OK-2.

R. crispus L. (curled dock) is a short-lived perennial or, more rarely, as an annual weed mostly on arable land, along the roads and permanent grasslands (Grime *et al*., 1988). In contrast to *R. obtusifolius*, *R. crispus* usually not flower in the seedling year and have low winter mortality (Hongo, 1989; Křížťálová *et al*., 2011; Strnad *et al*., 2012). *R. crispus* have short, poor branched root collar, main taproot and some long and branched secondary roots (Hejný & Slavík, 1990).

R. obtusifolius L. (broad-leaved dock) is a perennial weed mostly in grasslands and pastures (Cavers & Harper, 1964; Hopkins & Johonson, 2002; Stilmant, 2010). The mature belowground system of *R. obtusifolius* is typified by a stout taproot and a branched stem system above the root collar with a high potential to clonal reproduction. The region above the root collar was the only part able to regrow after damage. *R. obtusifolius* can expand through a phalanx clonal grow strategy resulting in a dense population that occupies an increasing area (Pino *et al*., 1995). *R. obtusifolius* often flowering in the seedling year in Central Europe conditions (Křížťálová *et al*., 2011; Hujerová *et al*., 2013a) and have high field winter mortality (Martinková *et al*., 2009; Křížťálová *et al*., 2011).

Rumex OK-2 is a hybrid dock of *R. patientia* L. and *R. tianschanicus* A. Los. It was bred in Ukraine by prof. Uteush for forage and energetic use (Usťak, 2007). *Rumex* OK-2 is a perennial crop (up to 10 years), characterized by high ecological plasticity, cold and winter hardiness, and tolerance to salt and increased humidity (Kosakivska *et al*., 2008). In the seedling year, roots of *Rumex* OK-2 have similar shape and size like parsley. For multi-annual crop, roots are strong, branched and some sectors of the roots can reach a depth of 1.5 to 2.0

m (Usťak, 2007). In the Czech Republic, *Rumex* OK-2 has been planted since 2001 mostly like an energetic crop (biofuel), but there was revealed its invasive spreading and similar behaviour as other broad leaves *Rumex* species (Hujerová, 2010; Pyšek *et al*., 2012; Hujerová *et al*., 2013b) but knowledge about its ecological characteristics are still very pure. In our previous study (Hujerová *et al*., 2013a) it was concluded that both (aboveground and belowground) biomass responses to cutting were very similar to *R. crispus*. Because of depth limitation of this previous pot experiments the present study was focused on comparison of belowground growing dynamic in 2 m tube pots.

Therefore the aim of this study was to answer to following question: Are there differences of allocation of belowground biomass in 2 m depth soil profile between *Rumex* OK-2, *R. obtusifolius* and *R. crispus*? Using a pot experiment, we tested especially differences in belowground biomass production a in the first year of growth. This comparison of growing parameters of *Rumex* OK-2 with the other broad leaves *Rumex* species could help to assess ecological characteristics this new weedy species.

Materials and methods

Study site and design of the pot experiment

The pot experiment was conducted in 2010 and 2011 at the experimental garden of the Crop Research Institute, Research Station Liberec, northern Czech Republic (50°46'33.213"N, 15°2'20.207"E; altitude 366 m a.s.l.) under conditions of natural rainfall, temperature and daylight. Mean annual temperature at the study site was 7.5 °C and average annual precipitation was 800 mm. Monthly rainfall and mean air temperature at Liberec Meteorological Station during the vegetation seasons 2010 - 2011 are given in Table 1.

We compared growth dynamics of belowground biomass of *Rumex* OK-2 with two *Rumex* species (*R. crispus* and *R. obtusifolius*) in four harvesting dates: at the end of July (1); at the end of August (2); at the end of September (3); at the end of October (4). Each treatment was replicated five times (thus, 60 pots in total). The same type of soil and pots were used in both study years as the experiment was established two times, in spring 2010 and again in spring 2011.

Seeds of *R. obtusifolius*, *R. crispus* were collected from plants in grassland near Liberec and abandoned fields near Prague respectively, in autumn 2008. Seeds of *Rumex* OK-2 were obtained as a commercial product (Crop Research Institute, Research Station

Chomutov). Tested germination was about 90% for all studied species. The one-month old seedlings (one per pot) were transplanted into pots at the end of June 2010 and 2011. There were used tube pots of 19.5 cm diameter and height of 150 and 200 cm. In both years we used the same cambisol in the experiment as this is the most common soil type on the sites where seeds of the *R. obtusifolius* had been collected. Due to better manipulation with root biomass at harvesting date a polyethylene sleeve was inserted into each tube pot before it was fill up by soil. The soil was collected in 2010 and it had the following chemical properties: pH_{KCl} = 5.5, P = 122 mg kg^{-1}, K = 234 mg kg^{-1}, Mg = 91 mg kg^{-1}. The P, K and Mg were extracted by Mehlich III (Mehlich, 1984) reagent to determine plant-available concentrations. During the vegetation season *Rumex* plants were watered when necessary to avoid any water stress.

Data collection

In each harvesting date the aboveground biomass of *Rumex* plants (shoot, leaves) was cut above soil surface, then dried at 60°C for 48 h and weight of dry matter was determined. At the same time the belowground biomass was taken out of pots and cut to 10 cm long parts. Belowground biomass was determined after rinsing the roots free of soil using a water spray in these parts. The roots were then dried at 60ºC for 48 h and weight of dry matter was determined. The belowground/aboveground biomass ratio was calculated to determine the response of biomass allocation under the different harvesting treatments.

Data analyses

One-way ANOVA was used to test differences among species for particular harvesting date and among harvesting date for individual species and calculation was conducted separately for each year. Post hoc comparison using the Tukey HSD test was applied to identify significant differences between species and individual treatments. Before analyse the ratio data were log transformed to meet the assumption of ANOVA.

Results

Aboveground biomass

The aboveground biomass production was higher in the second year than in the first year of the experiment of all studied species under all harvesting dates (Figs. 1, 2).
In the first year, there were only small differences between *R. crispus* and *Rumex* OK-2 in their aboveground production (Fig.1). *R. obtusifolius* has significantly higher production of aboveground biomass than other species in first, second and fourth harvesting date. Amount of aboveground biomass of all three species tended to grow from first to third harvesting date.
In the second year, there were not found any significant differences between *Rumex* species in their aboveground production (Fig.2). Amount of aboveground biomass *R. crispus* and *R. obtusifolius* tend to grow from first to third harvesting date, whereas no significant differences in aboveground biomass were found for *Rumex* OK-2 (Fig.2).

In the seeding year, flowering was recorded for several *R. obtusifolius* plants and for one plant of *Rumex* OK-2. Over both years, the proportion of flowering plants of *R. obtusifolius* was 20%, 30%, 50% and 10% for the first, second, third and fourth harvesting date, respectively. Flowering plants of *R. obtusifolius* produced significantly higher amount of aboveground biomass as non-flowering plants (Fig. 3). Non flowering plants of *R. obtusifolius* had similar amount of aboveground biomass in all harvesting dates.

Belowground biomass

The belowground biomass production was higher in 2011 than in 2010 for all the studied species under all harvesting dates (Figs. 1, 2). There was recorded the highest belowground biomass for *Rumex* OK-2 in the third harvesting date followed by *R. obtusifolius* under second harvesting date in 2010 (Fig.1). The similar trend was observed for *Rumex* OK-2 under fourth harvesting date and for *R. obtusifolius* under fourth harvesting date in 2011 (Fig.2).

There were no significant differences between *Rumex* species under the first and third harvesting dates in 2010 but amount of belowground biomass *R. crispus* and *Rumex* OK-2 tend to grow from first to third harvesting date (Fig.1). *R. obtusifolius* had a less amount of belowground biomass in third harvesting date than in second harvesting date in both experiment years (Figs. 1, 2).

In the second experimental year 2011, amount of belowground biomass of *Rumex* OK-2 and *R. crispus* tend to grow from first to third harvesting date. The highest belowground biomass was revealed for *Rumex* OK-2 and *R. obtusifolius* under fourth harvesting dates (Fig.2), whereas for *R. crispus* under third harvesting date.

Except the first harvesting date flowering plants of *R. obtusifolius* produced lower amounts of belowground biomass than plants of *R. obtusifolius* without flowering (Fig. 3).

Allocation of belowground biomass

The belowground biomass subsequently decreased with depth (Figs. 4, 5). Majority of the belowground biomass was stored in the upper 30 cm of the soil profile in all harvesting dates in both experimental years. In upper 10 cm, *R. crispus*, *R. obtusifolius* and *Rumex* OK-2 had 45-48%, 34-42% and 29-34% of belowground biomass, respectively. In top 20 cm, *R. crispus*, *R. obtusifolius* and *Rumex* OK-2 had 72%, 57-67% and 62-67% of belowground biomass, respectively. In upper 30 cm, *R.crispus*, *R. obtusifolius* and *Rumex* OK-2 had 81%, 68-78% and 77% of belowground biomass, respectively. Generally only 19-32 % of total belowground biomass for all *Rumex* species was deeper than 30 cm. *Rumex* OK-2 had mostly the biggest amount of belowground biomass in these upper three layers in all harvesting date in 2011 followed by *R. crispus* and *R. obtusifolius*. Generally *Rumex* OK-2 had the most of belowground biomass in layer of 10-20 cm deep in third harvesting date in 2010 from all *Rumex* species (Fig. 4C), the same tendency was recorded in fourth harvesting date in 2011 (Fig. 5D).

Belowground/aboveground biomass ratio

The ratio generally tended to be highest in the *Rumex* OK-2, particularly in the first year of the experiment, when it was significantly higher under first, second and fourth harvesting date (Table 2). In the second years of the study the ratio for *Rumex* OK-2 was the highest in the first and fourth harvesting date. This ration for *Rumex* OK-2 was more similar to *R. crispus* than to *R. obtusifolius.*

Discussion

Biomass production of all *Rumex* species was lower in 2010 than in 2011. Although pots were watered in both years to avoid drying stress different distribution of natural precipitation and temperature may have affected the biomass production of *Rumex* species (Table 1).

Aboveground biomass

Rumex obtusifolius is less sensitive to unpredictable environmental conditions (rainfall, temperature) than *R. crispus* and this could cause that *R. obtusifolius* had the most amount of aboveground biomass from all study species in the first year of the experiment. *Rumex* OK-2 and *R. crispus* had similar proportion of aboveground biomass similarly as results from the previous experiment (Hujerová *et al*., 2013a). Biomass production of all *Rumex* species tended to grow during the vegetation season till to third harvesting date only. Generally in the fourth harvesting dates, there were revealed a decrease of biomass productions. This decrease can be explained by the seasonal pattern of biomass growth in Central European conditions (Pavlů, 2006; Ellenberg, 2009). In comparison with previous study (Hujerová *et al*., 2013a), had *Rumex* species higher production of aboveground biomass in this experiment.

Our results confirmed results from several studies that *R. crispus* usually does not flower in the seedling year (Hongo, 1989; Křišťálová *et al*., 2011; Strnad *et al*., 2012). Although Usťak (2007) argued that *Rumex* OK-2 similarly as *R. crispus* is not able to produce seeds in seeding year we observed one flowering plants in year 2011. On the other hand *R. obtusifolius* is known to be able to flower in the seedling year (Hrdličková *et al*. 2011; Křišťálová *et. al*, 2011; Hujerová *et al*., 2013a) and as it was confirmed in this experiment where the rate of flowered *R. obtusifolius* plants was abaut 27%. This has consequence for possible crossbreeding *Rumex* OK-2 with the other broad leaves *Rumex* species, especially they can meet with *R. obtusifolius* and are able to create hybrids even in seeding year (unpublished results). There was confirmed our preliminary results (Hujerová *et al*., 2013a), that flowering plants of *R. obtusifolius* produced more aboveground biomass than plants without flowering. It was because flowering plants invested more energy to reproduction (flowering stem and seeds) on the other hand non flowering plants invested more energy to establish strong belowground system which is necessary to plant survive.

Belowground biomass

All studied species tended to produce more belowground than aboveground biomass with the exception third harvesting date for *R. obtusifolius* in 2011. In comparison with previous study (Hujerová *et al*., 2013a) *Rumex* species had lower production of belowground biomass in this experiment. Although we were able to reveal belowground biomass allocation in our experiment in different depth probably small diameter did not allowed full root development, because structure of belowground organs of *Rumex* species is wide (Pino *et al*., 1995). Generally all *Rumex* species were able to have belowground biomass in one meter deep after one month after seedling planting. It should be noted that some plants of *Rumex* OK-2 and *R. crispus* had belowground biomass even in 1.5 m depth. In fourth harvesting date all species had belowground biomass in 1.5 and 1.7 m depth in 2010 and 2011, respectively. *Rumex* OK-2 had the biggest belowground biomass production from all study species in third harvesting date in 2010 and in all harvesting dates in 2011. It confirms results from previous study (Hujerová *et al*., 2013a) in which had *Rumex* OK-2 biggest belowground biomass in treatment without cutting. In fourth harvesting date, *Rumex* OK-2 had not the highest belowground biomass production in 2010 probably because this crop is sensitive to drying stress (Usťak, 2007) and rainfall was strongly under long-term mean in October (fourth harvesting date) in Liberec. *Rumex* OK-2 is used as a forage and energy crop because it has high herbage production but no in the seedling year. During the first season it needs to establish strong belowground system (Usťak, 2007). This is the reason why *Rumex* OK-2 had not the biggest amount of aboveground biomass but its production of belowground biomass was the biggest.

Production of belowground biomass of *R. obtusifolius* decreased in third harvesting date. This could be due to the fact that 50% of plants flowered in this harvesting date and flowering plants of *R. obtusifolius* invested more energy to producing aboveground biomass.
Although some studies (Hongo, 1988; Bond *at al*., 2007) argued that *R. crispus* has lower investment into belowground biomass than *R. obtusifolius,* in present study and previous study Hujerová *et al*. (2013a) we revealed the opposite results. This contrast could be caused

by the fact that *R. crispus* does not flower in seedling year and invested into belowground biomass.

Allocation of belowground biomass

The majority (70-80%) of belowground biomass of all *Rumex* species was situated in upper 30 cm of soil. This is consistent with results of other studies about belowground biomass distribution of many plants species (e.g. Wyatt *at al*., 1980; Casper & Jackson, 1997; Jiang W *et al*., 2013). The allocation of belowground biomass of studied *Rumex* species is similar with the average global belowground biomass profile approximately 30% of roots biomass was in the top 10 cm, 50% in top 20 cm, and 75% in the top 40 cm (Jackson *at al*., 1996).

Belowground/aboveground biomass ratio

We confirmed preliminary results from our previous experiment (Hujerová *et al*., 2013a) that *Rumex* OK2 in contrast to *R. obtusifolius* invest into belowground biomass for preparation for successful flowering and seed production in subsequent vegetation seasons.

Conclusions

Both aboveground and belowground biomass production of *Rumex* species tend to grow from July to September. The proportion of belowground biomass of *Rumex* species in upper 30 cm was about 70-80% whereas only 20-30 % was allocated deeper than 30 cm. Besides flowering in the seedling year typical for *R. obtusifolius* plants there were revealed one flowering plant of *Rumex* OK-2. The growth dynamics and allocation of belowground biomass of *Rumex* OK-2 was more similar to *R. crispus* than to *R. obtusifolius*. These similarities indicate that the weed potential of *Rumex* OK-2 might correspond with that of *R. crispus*.

Acknowledgments

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Table 1 Monthly precipitation sum and mean air temperature at Liberec meteorological station during the experimental period and 30-year mean values.

	Rainfall (mm)			Air temperature (°C)		
			Average			Average
Month	2010	2011	1961-1990	2010	2011	1961-1990
July	149	296	89	19.7	15.9	15.7
August	376	87	89	16.5	17.6	15.2
September	170	65	66	11.2	14.5	11.6
October	12	50	61	6.3	8.4	7.3

Table 2 Belowground/aboveground biomass ratio of *Rumex* species under different harvesting detes in 2010 and 2011. Numbers represent average of five replicates, ± standard error of the mean (SE). Significant differences ($P < 0.05$) between treatments for each *Rumex* species according to the Tukey's post hoc test are indicated by different capital letters in row and significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters in column.

Year	Species	Treatment			
		1	2	3	4
2010	*Rumex crispus*	0.83 ± 0.04 b B	1.54 ± 0.07 b AB	2.27 ± 0.42 a A	2.35 ± 0.38 ab A
	R. obtusifolius	0.90 ± 0.10 b A	1.36 ± 0.22 b A	0.95 ± 0.29 a A	1.73 ± 0.08 b A
	Rumex OK-2	1.35 ± 0.08 a B	2.73 ± 0.40 a AB	2.23 ± 0.47 a AB	5.33 ± 1.90 a A
2011	*Rumex crispus*	2.47 ± 0.25 a A	1.67 ± 0.25 a AB	1.41 ± 0.20 a B	2.06 ± 0.18 ab AB
	R. obtusifolius	1.33 ± 0.18 b A	1.51 ± 0.34 a A	1.02 ± 0.41 a A	1.79 ± 0.37 b A
	Rumex OK-2	2.11 ± 0.37 ab A	3.20 ± 0.75 a A	1.74 ± 0.36 a A	3.34 ± 0.24 a A

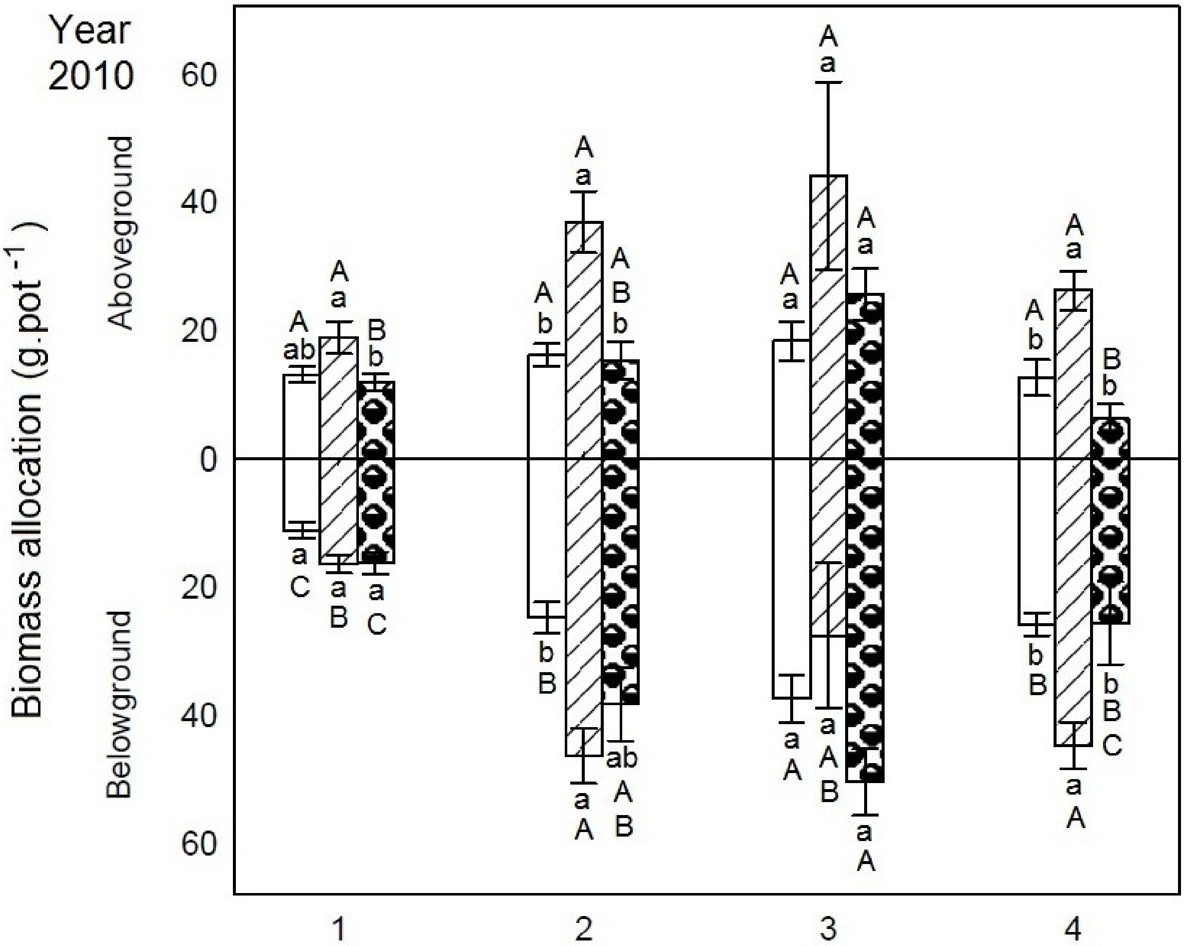

Fig. 1 Biomass dry matter allocation (g per plot) of *Rumex* species (☐ *Rumex crispus*, ▨ *R. obtusifolius*, ▩ *R.* OK-2) under different harvesting dates in year 2010. Significant differences (P < 0.05) between *Rumex* species in each harvesting date according to the Tukey's post hoc test are indicated by different small letters and significant differences (P < 0.05) between harvesting dates for each *Rumex* species according to the Tukey's post hoc test are indicated by different capital letters.

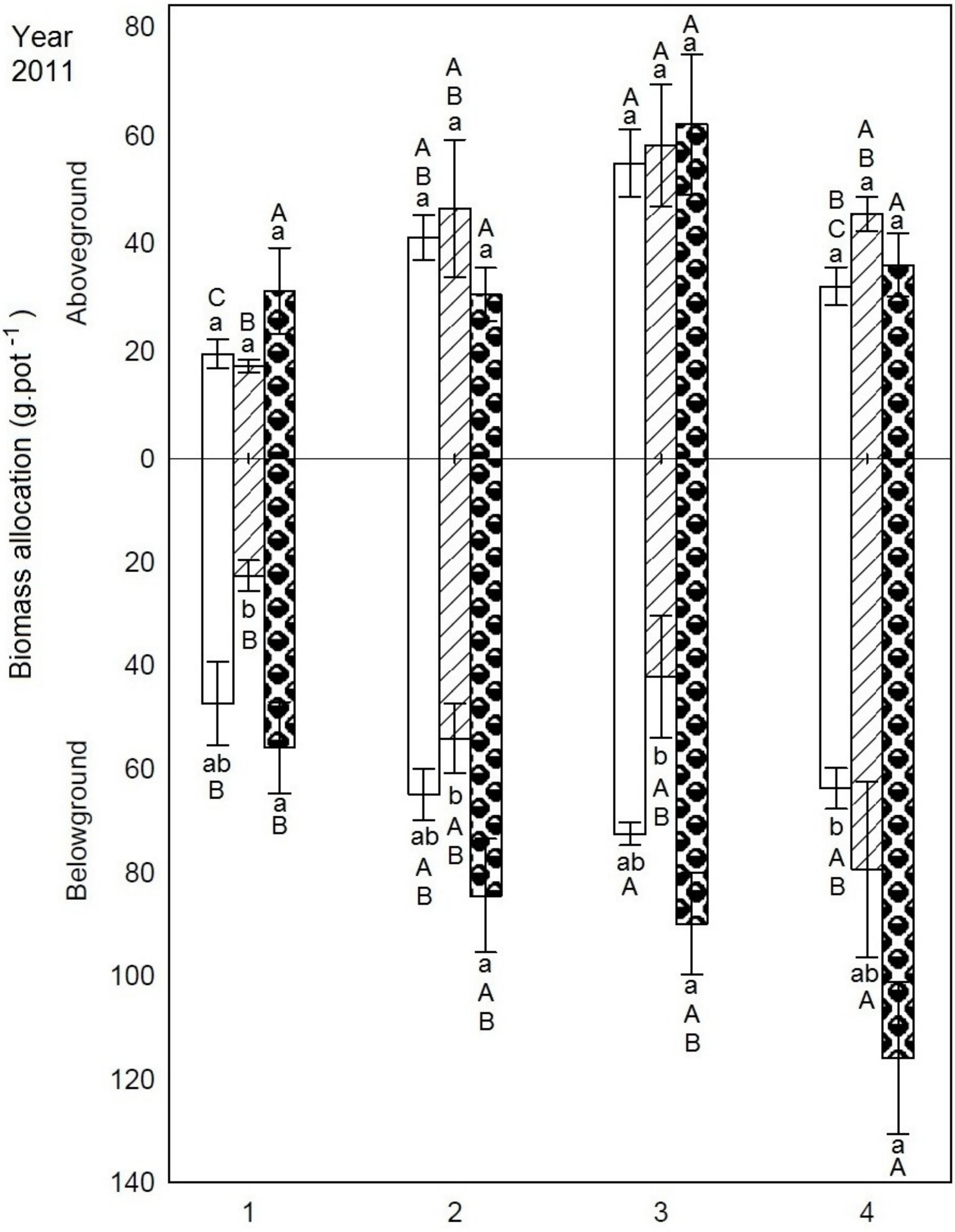

Fig. 2 Biomass dry matter allocation (g per plot) of *Rumex* species (□ *R. crispus*, ▨ *Rumex obtusifolius*, ▩ *R.* OK-2) under different harvesting dates in year 2011. Significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters and significant differences ($P < 0.05$) between treatments for each *Rumex* species according to the Tukey's post hoc test are indicated by different capital letters.

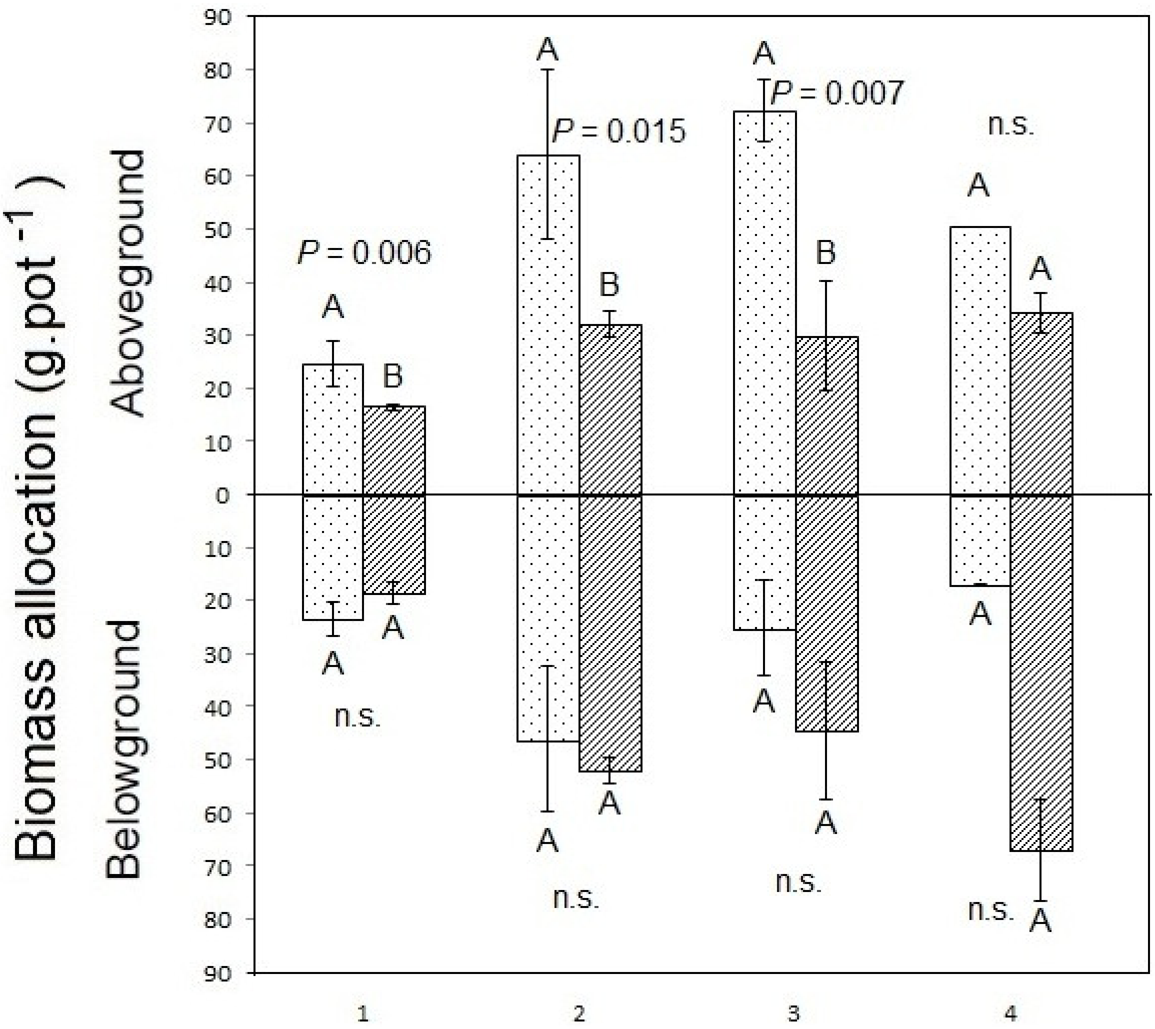

Fig. 3 Biomass dry matter allocation (g per plot) of *Rumex obtusifolius* with flowering (▒) and *R. obtusifolius* without flowering (▨) under different harvestig date. Number of observation (n) for flowering plants 1 n=2, 2 n=3, 3 n=5, 4 n=1 and plants without flowering 1 n=8, 2 n=7, 3 n=5, 4 n=9. Significant differences ($P < 0.05$) according to the Tukey's post hoc test are indicated by different capital letters in treatment. Error bars represent standard errors of the mean.

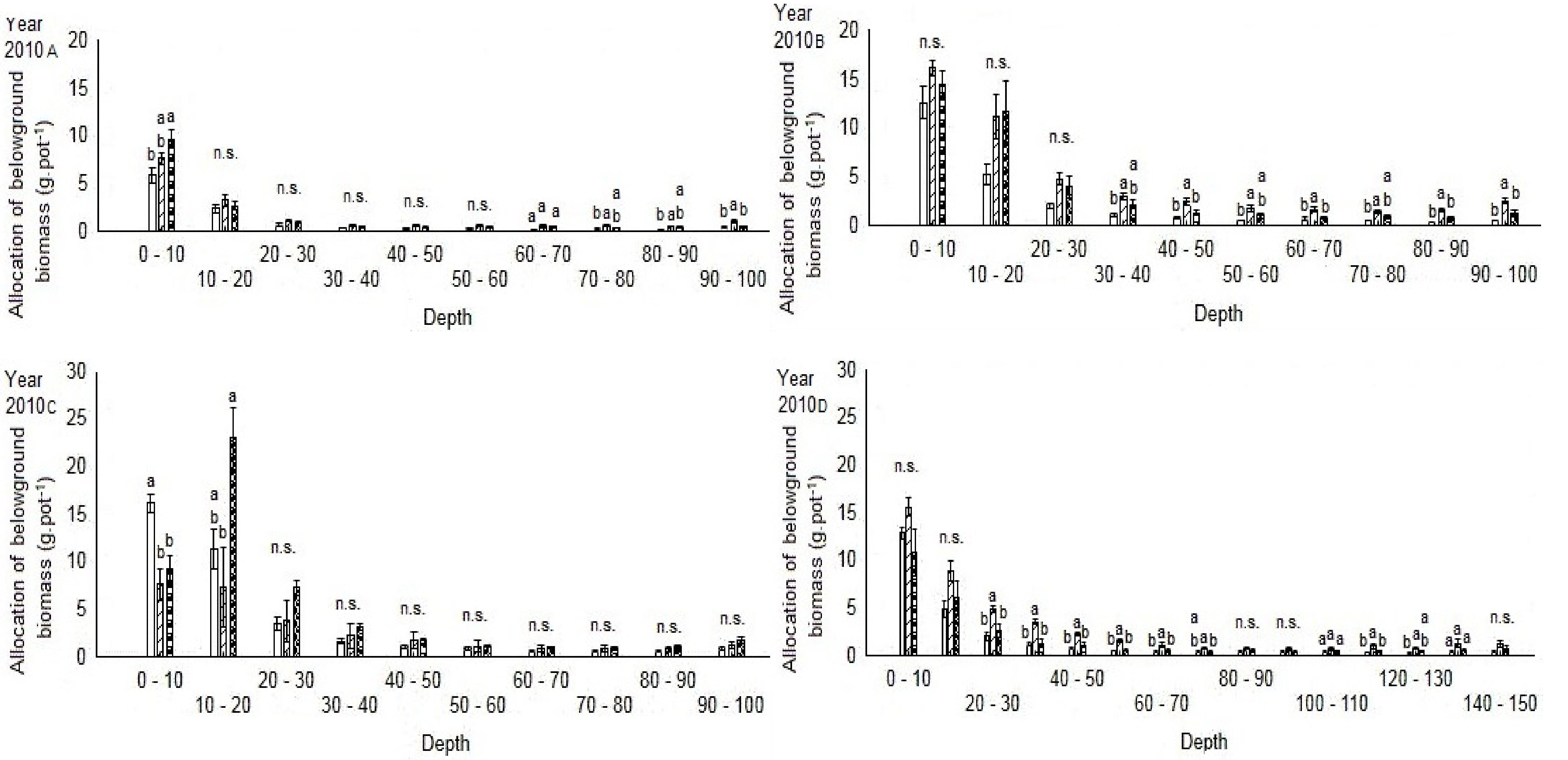

Fig. 4 Biomass dry matter allocation (g per plot) of *Rumex* species (□ *R. crispus*, ▨ *Rumex obtusifolius*, ▩ *R.* OK-2) in 2010; A - first harvesting date; B – second harvesting date; C – third harvesting date; D – fourth harvesting date. Significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters.

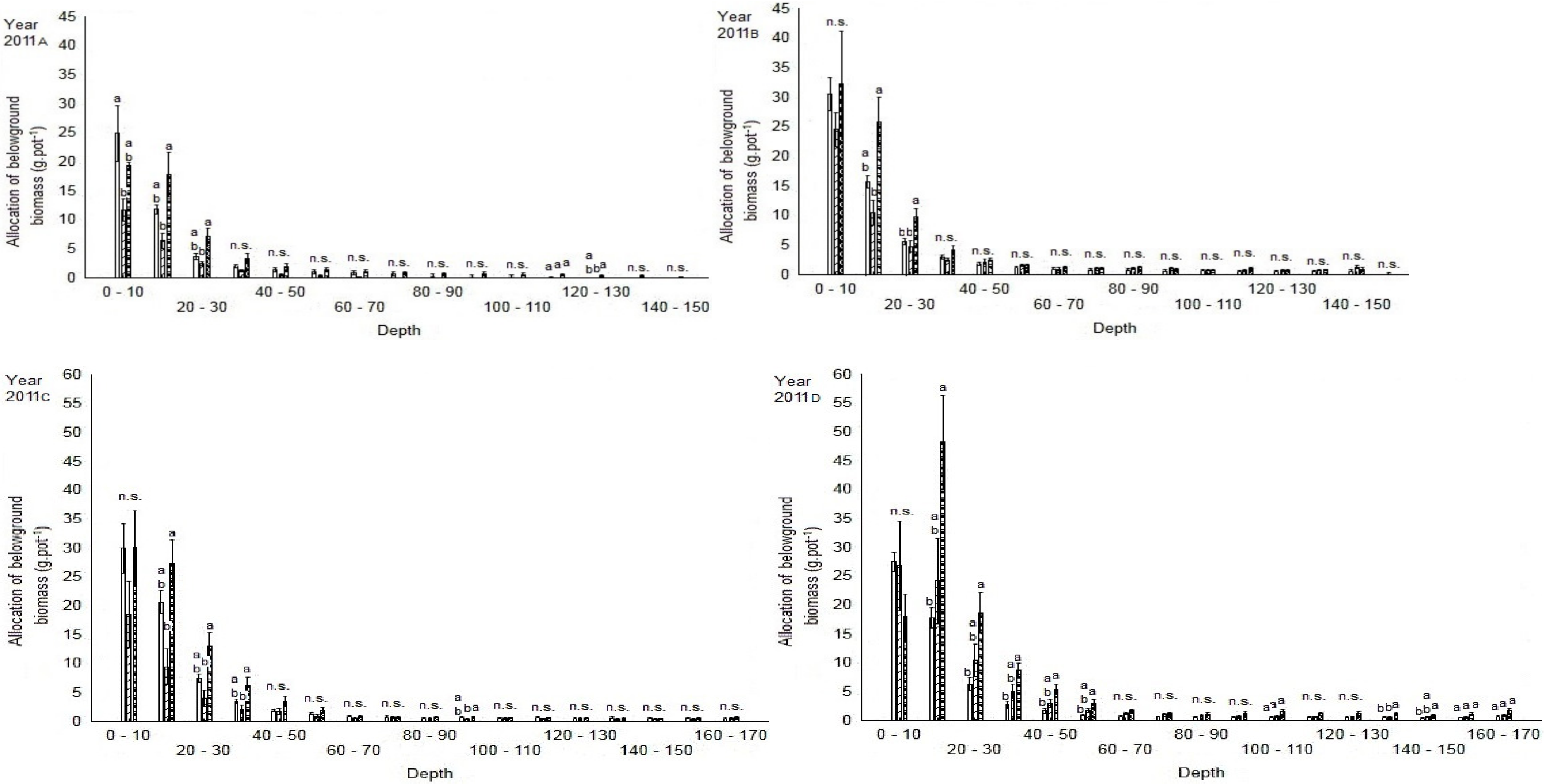

Fig. 5 Biomass dry matter allocation (g per plot) of *Rumex* species (□ *R. crispus*, ▨ *Rumex obtusifolius*, ▩ *R.* OK-2) in 2011; A - first harvesting date; B – second harvesting date; C – third harvesting date; D – fourth harvesting date. Significant differences ($P < 0.05$) between *Rumex* species in each treatment according to the Tukey's post hoc test are indicated by different small letters.

Chapter 6

Mechanical weeding of *Rumex obtusifolius* and its effect on plant species composition in *Agrostis capillaris* grassland under conditions of organic farming

Renata Hujerová, Lenka Pavlů, Vilém Pavlů, Jan Gaisler, Michal Hejcman and Vendula Ludvíková

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Mechanical weeding of *Rumex obtusifolius* and its effect on plant species composition in *Agrostis capillaris* grassland under conditions of organic farming

Hujerová R.[1], Pavlů L.[1], Pavlů V.[1,2]*, Gaisler J.[2], Hejcman M.[1,2], Ludvíková V.[1]

[1]Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, CZ-16521, Prague, Czech Republic.

[2]Department of Weeds and Vegetation of Agroecosystems, Grassland Research Station, Research Institute of Crop Production Prague, Rolnická 6, CZ-46011, Liberec, Czech Republic.

*Corresponding author: E-mail address: pavlu@vurv.cz (V. Pavlů), Tel.: +420 484 800 562

Abstract

In grasslands under organic farming, mechanical weeding can be used to eradicate troublesome weed *Rumex obtusifolius.* So far, effectiveness of mechanical weeding together with its effect on plant species composition in *Agrostis capillaris* pasture has never been investigated.

In 2007 on pasture heavily infested by *R. obtusifolius* were established following treatments: grazing by cattle with digging of taproots at the depth of 5 and 15 cm in August 2007 (GD_5O and $GD_{15}O$) and twice in August 2007 and May 2008 (GD_5T and $GD_{15}T$), grazing without weeding (GND) and unmanaged pasture (UND). Ungrazed patches were cut in all treatments with grazing. Cover of vascular plants, species richness and number of *R. obtusifolius* plants were monitored over five years.

In 2011, we recorded 3%, 26%, 28%, 33%, 34% and 77% of *R. obtusifolius* plants from initial number in 2007 in $GD_{15}T$, $GD_{15}O$, GD_5O, UND, GD_5T and GND treatments, respectively. Empty space after removed plants were replaced by nutrient demanding grasses (*Poa pratensis, Poa trivialis, Festuca pratensis* and *Lolium perenne*) and forbs (*Trifolium repens* and *Taraxacum* sp.). No management reduced *R. obtusifolius* plants but allowed seed production and spreading of tall weedy species (*Urtica dioica*, *Galium album* and *Elytrigia repens*). There was no effect of weeding on species richness, but species richness decreased from 15 to 9 per 4 m^2 under no grassland management. Repeated digging out at depth 15 cm

together with cutting of ungrazed patches is an effective method for *R. obtusifolius* control in permanent grasslands under conditions of organic farming.

Key words: broad-leaved dock, taproot, digging out, pasture, weed

Introduction

Rumex obtusifolius L. (broad-leaved dock) is one of the most troublesome weedy species in temperate grasslands because of its avoidance by livestock, high biomass and seed production, perennial character, persistent soil seed bank and its high ability to regenerate from fragmented underground organs (Carves and Harper 1964; Zahler 2004). *Rumex obtusifolius* is able to regenerate via buds on strong underground stem system above the root collar (Pino et al. 1995). *R. obtusifolius* plants often colonise grasslands as well as permanent agricultural crops (Novák 1994; Brant et al. 2006), where they can survive for many years (Martinková et al. 2009). In conventionally managed grasslands, *R. obtusifolius* can be controlled by selective herbicides but results are not straightforward and require repeated treatment (Niggli et al. 1993). However, under conditions of organic farming, the use of herbicides is prohibited and only biological or mechanical methods of weeding are allowed. Biological methods of *R. obtusifolius* control include the use of specific insects e.g. *Gastrophysa viridula* (Hatcher and Paul 1997; Honěk and Martinková 2004), pathogenous fungi *Uromyces rumicis* (Keary and Hatchler 2004) or specific grazers such as goats (Hejcman et al. 2014). Nevertheless, their application is still problematic (Strnad et al. 2010). Mechanical methods include no grassland management, variable intensity of defoliation, heating and digging. Frequent cutting of *R. obtusifolius* reduced vigour of the aboveground organs, particularly decreased number of leaves, size of the largest leaf and also herbage production (Stilmant et al. 2010). However *R. obtusifolius* is well adapted to defoliation and does not suffer from cutting performed twice per year (Strnad et al. 2010). In addition, *R. obtusifolius* can tolerate high cutting frequency for several years therefore three cuts per year are not sufficient for its elimination from the grassland (Niggili et al. 1993; Hopkins and Johnson 2002; Stilmant et al. 2010; Hann et al. 2012). According to results of several authors (Pavlů et al. 2008; Martinková et al. 2009; Hejcman et al. 2012b), *R. obtusifolius* can suffer more from no grassland management than from two or three cuts per year. No grassland

management could be therefore investigated as a promising method for control of *R. obtusifolius*.
After establishment in grasslands, *R. obtusifolius* creates a deep taproot with high storage capacity for assimilates and nutrients and root-collar with high regeneration ability after disturbance and with high clonal reproduction potential (Pino et al. 1995; Strnad et al. 2010). Buds are common on dock crowns in the upper 10 cm soil layer, therefore digging plants out from less than 10 cm below the soil surface is not an effective method for *R. obtusifolius* control (Dierauer 1993; Bond et al. 2007; Strnad et al. 2010). However, how is digging in 5 and 15 cm depth performed once or twice effective method for *R. obtusifolius* control on upland pasture dominated by *Agrostis capillaris* has never been investigated. Furthermore, there has been no study looking for effects of weeding on plant species composition and species richness of permanent grasslands.
The aim of our study was therefore to answer to following questions i) How effective is digging of *R. obtusifolius* in 5 and 15 cm performed once or twice for its control in *Agrostis capillaris* grassland? ii) Is no grassland management over five years an effective method for *R. obtusifolius* control? iii) Which plant species replace *R. obtusifolius* after digging? iv) How different weeding treatments affect species richness of vascular plants?

Material and methods

Study site

The experimental grassland was in Krásná Studánka (50°48'29.244"N, 15°2'17.56"E, and altitude 394 m a.s.l.) part of the town Liberec, Czech Republic. The bedrock is biotic granite covered by Cambisol with a pH (KCl) 5.45 and an organic C content 4.53%. Content of plant available (Mehlich III; Mehlich 1984) P, K, Ca and Mg was 28, 67, 1728 and 58 mg kg^{-1}, respectively. Mean annual temperature is 7.2°C and mean annual precipitation is 803 mm (Liberec meteorological station). The grassland was classified as upland hay mesophile meadow (alliance *Arrhenatherion*) (Chytrý 2007). Initial dominant vascular plant species before management introduction were *Agrostis capillaris* (cover 65%), *Trifolium repens* (14%), *Taraxacum* sp. (15%) and *R. obtusifolius* (7%). At the beginning of the experiment, the mean density of *R. obtusifolius* was about two plants per 1 m^2. The experimental pasture of 8 ha was managed under conditions of organic farming and rotationally grazed by Gasconne mother cows with their calves during four grazing cycles per vegetation season. To reduce inflorescence of *R. obtusifolius* plants, ungrazed sward patches were cut two times (after second and forth grazing cycle) per vegetation season.

Experimental design

The experiment was establish in the centre 8 ha pasture in four complete randomised blocks with individual plot sizes 3 m × 3 m. There were applied six treatments: i) grazing with digging taproot of *R. obtusifolius* out from a depth of 15 cm below ground twice ($GD_{15}T$); ii) grazing with digging taproot of *R. obtusifolius* out from a depth of 15 cm below ground once ($GD_{15}O$); iii) grazing with digging taproot of *R. obtusifolius* out from a depth of 5 cm below ground twice (GD_5T); iv) grazing with digging taproot of *R. obtusifolius* out from a depth of 5 cm below ground once (GD_5O); v) grazing without digging taproot of *R. obtusifolius* (GND - conventional farm management as a control); vi) unmanaged pasture without digging taproot of *R. obtusifolius* (UND). Digging of *R. obtusifolius* taproots was performed once or twice manually with a special narrow hoe. The first weeding was done in August 2007 and the second in May 2008. There was no weeding in years 2009 – 2011.

Data collection

Percentage cover of vascular plant species and numbers of broad-leaved dock plants were monitored in 2 m x 2 m plots located in the middle of each 3 x 3 m experimental plot from 2007 to 2011. The cover of all presented vascular plant species was assessed in spring (from end of May to beginning of June) whereas the numbers of plants *R. obtusifolius* were counted in spring (from end of May to beginning of June) and autumn (from end October to beginning of November) during five years of the experiment. One plant of *R. obtusifolius* was defined as a visible shoots growing from buds in the circle around 30 cm of the main shoot. Young seedlings were counted if leaf length was at least of 5 cm.

Statistical analyses

One way ANOVA was used to evaluate the number of *R. obtusifolius* plants (recalculated per $1m^2$) in spring and autumn of particular year and repeated measures ANOVA was used to evaluate the cover of dominant plant species and species richness. The success rate of mechanical weeding were expressed as ratio of final (year 2011) to initial (year 2007) number of *R. obtusifolius* plants and it was evaluated by one way ANOVA. Before analyse the ratio data were log transformed to meet the assumption of ANOVA. Linear regression was used to evaluate the relationship between *R. obtusifolius* plant density and its cover. All univariate analyses were performed using STATISTICA 12 software (StatSoft, Tulsa, USA) The community response was analysed by constrained ordinations. Redundancy analysis (RDA) in the CANOCO 5 program (terBraak and Šmilauer 2002) was used to evaluate multivariate plant species composition data in particular years. The blocks were used as covariables in all analyses to restrict permutations into blocks. Cover data in RDA were logarithmically transformed [y = log 10 (y + 1)] to down-weight dominant species and 999 permutations were used in all performed analyses. An ordination diagram, constructed by the CANOCO 5 program, was used to visualise the results of the RDA analysis of data collected in 2011.

Results

There were no significant differences in *R. obtusifolius* numbers among treatments after first digging in August 2007 (Fig. 1). The first significant differences in *R. obtusifolius* numbers among treatments appeared after the second digging in May 2008. Since spring 2008, there were recorded strong diversifications in *R. obtusifolius* numbers according to applied treatments. Generally the lowest number of *R. obtusifolius* plants was found in treatments where mechanical weeding was applied twice (GD_5T, $GD_{15}T$) and only slight reduction was recorded in treatments with one digging and in UND treatment. Commonly in spring higher number of *R. obtusifolius* plants was recorded under UND treatment. The cover of *R. obtusifolius* was significantly and positively correlated with its density (Fig. 2). In 2011, we recorded 3%, 26%, 28%, 33%, 34% and 77% of *R. obtusifolius* plants from initial number in 2007 in $GD_{15}T$, $GD_{15}O$, GD_5O, UND, GD_5T and GND treatments, respectively (Fig. 3). Although at the end of the experiment, 23% reduction in number of *R. obtusifolius* plants was recorded in GND treatment, there was high variation caused by differences among individual plots and in several cases, numbers of *R. obtusifolius* plants were higher in 2010 than in 2007. No significant effect of treatment on plant species composition calculated by RDA was recorded in 2007 (Table 1). The variability of plant species composition explained by treatments subsequently increased from initially 47% in 2008, to more than 50% after five years of the experiment in 2011. Four groups of treatments with similar plant species composition were recognised on the ordination diagram based on RDA analysis of data collected in 2011 (Fig. 4): GD_5T, $GD_{15}T$ and $GD_{15}O$ treatments as the first group, GND and GD_5O treatments as the second group, UDN as the third group. The association of individual species with particular management treatments is clearly visible from the ordination diagram. In GD_5T, $GD_{15}T$ and $GD_{15}O$ treatments, there was low cover of *R. obtusifolius* which was replaced particularly by *Poa pratensis*. Tall weedy forbs *Urtica dioica*, *Galium album* and tall grasses *Elytrigia repens, Dactylis glomerata* were associated with UND treatment. The GND and GD_5O treatments support productive grasses *Poa trivialis, Festuca pratensis,* and *Lolim perenne.* All grazed treatments were favourable for prostrate forbs *Trifolium repens* and *Taraxacum* sp. The majority of dominant plant species in sward (*Agrostis capillaris*, *Dactylis glomerata*, *Elytrigia repens*, *Festuca pratensis*, *Galium album, Lolium perenne, Poa pratensis* and *Trifolium repens)* were significantly affected by time and treatment but not by their interactions (Figs. 5 and 6, Table 2). *Rumex obtusifolius, Taraxacum* sp. and *U. dioica* were only three dominant species whose cover was affected not only by time and treatment but also by their interactions (Fig. 6, Table 2). No effect of time, treatment and their interactions was revealed for *Veronica chamaedrys.*

Species richness of vascular plants was significantly affected by time, treatment and their interaction (Table 2). Species richness was stable in all managed treatments, but decreased from 15 to 9 species per 4 m^2 in UDN treatment (Fig. 7).

Discussion

The main message of this study is that all treatments based on mechanical weeding as well as treatment with no management significantly reduced density of *R. obtusifolius* plants in *Agrostis capillaris* dominated grassland. The most successful treatment for *R. obtusifolius* control was repeated digging at depth 15 cm after which 97% of plants were eradicated.

Similarly, this depth was also recommended by Pötsch and Griesebner (2007) for rotational destroying of *R. obtusifolius* plants in Austrian alpine pastures. Likewise Dierauer (1993) and Bond et al. (2007) concluded that, under conditions of organic farming, the only effective method for control of *Rumex obtusifolius* is removal of the entire root or to cut the root at least 10 cm below ground. It means that it is necessary to remove majority of buds, which are responsible for vegetative reproduction of *R. obtusifolius* (Pino et al. 1995). However the mechanical weeding at the depth 5 cm could still reduced the number of *R. obtusifolius* plants considerably which also remarked Dierauer (1993). On the contrary Strnad et al. (2010) revealed that mechanical weeding by digging the plants out from 5 cm below the ground is not a sufficient method for *R. obtusifolius* control even when applied eight times during three vegetation seasons. The differences in results between these studies were probably caused by different content of plant available nutrients in the soil. For example in Strnad et al. (2010) study, there was N fertilisers applications and five and four times higher plant available P and K contents in soil in comparison to our study. It seems that mechanical weeding is much more efficient under low availability of nutrients, especially N, P and K. These nutrients are known to be responsible for broad leaves *Rumex* growth and reproduction (Hejcman et al. 2012a; Hejcman et al. 2012b). On the other hand in soil with high Ca and Mg availability, the density of *R. obtusifolius* plants was slightly positively related to K availability but with no relation to P availability (Hann et al. 2012).

R. obtusifolius plants were especially replaced by nutrient demanding grasses (*P. pratensis, P. trivialis, F. pratensis* and *L. perenne*) and also by prostrate forbs (*T. repens* and *Taraxacum* sp.) which were able quickly colonised bareground gaps after digging. These plants highly valuable for grazing livestock because of their high forage value (Frame 1992). There was nearly no regeneration of *R. obtusifolius* plants via seedlings in all managed treatments (personal observation, Pavlů). Repeated defoliation of ungrazed patches did not allow seed production and generative reproduction of *R. obtusifolius.* If there were some young seedlings of *R. obtusifolius*, they were grazed or damaged by cutting or by hoofs of the cattle during the grazing. Similarly Stilmant et al. (2010) recorded reduction in the vigour of the aerial parts of *R. obtusifolius* under grazing treatment, associated with a reduction in the number of leaves, in the size of the largest leaf and in the herbage mass. In spring we revealed higher number of *R. obtusifolius* plants in unmanaged treatments due to presence of seedlings close to mother plants (personal observation, Pavlů). However these seedlings did not survive till autumn of particular year as they were suppressed by overgrowing tall grasses and forbs. This confirmed low competitive ability of seedlings in close grassland communities (Weaver and Cavers 1979). Furthermore the high density of *R. obtusifolius* was never recorded under its low cover, which indicated generally very low seedling proportion in grassland during the study. Final low presence of *R. obtusifolius* plants in unmanaged treatment is in accordance with previous studies (Pavlů et al. 2008; Martinková et al. 2009; Hejcman et al. 2012b) in which high mortality of *R. obtusifolius* plants was recorded under several years unmanaged grassland. However under no management, sparse mature *R. obtusifolius* plants produce high amount of germinable seeds which can survive in the soil seed bank for many years (Toole & Brown 1946). As well no management supported tall weedy species with high nutrient requirements such as *U. dioica, G. album* and *E. repens* which are not desirable plant species in grasslands (Frame 1992).

Conclusion

Mechanical weeding by digging out together with cutting of ungrazed patches is the effective method for *R. obtusifolius* control in upland grassland dominated by *A. capillaris*. Two times performed digging of *R. obtusifolius* at the depth of 15 cm was the most successful method which eliminated 97% of *R. obtusifolius* plants. Bare ground gaps created by digging were colonized by nutrient demanding grasses and forbs with high forage value. Although no grassland management reduced number of *R. obtusifolius* plants in sward, it allowed seed production of surviving plants and supported tall weedy species in the sward and considerably reduced species richness of vascular plants.

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Table 1

Results of RDA analyses of cover estimates performed separately for each year. Applied treatments were $GD_{15}T$ grazing with digging taproot of *R. obtusifolius* out from a depth of 15 cm below ground twice; $GD_{15}O$ grazing with digging taproot of *R. obtusifolius* out from a depth of 15 cm below ground only once; GD_5T grazing with digging taproot of *R. obtusifolius* out from a depth of 5 cm below ground twice; GD_5O grazing with digging taproot of *R. obtusifolius* out from a depth of 5 cm below ground only once; GND grazing without digging taproot of *R. obtusifolius* out (conventional farm management as a control); UND unmanaged treatment; % expl. var.: species, variability explained by one (all) ordination axis (measure of explanatory power of the explanatory variables); F-ratio: F statistics for the test of particular analysis; P-value: probability value obtained by the Monte Carlo permutation test. Tested hypothesis: is there any effect of treatment on plant species composition in any particular year?

Year	Explanatory variables	Covariables	% expl. var. 1st axis (all axes)	F-ratio 1st axis (all axes)	P-value 1st axis (all axes)
2007	GD_5O, GD_5T, $GD_{15}T$, $GD_{15}O$, UND, GND	blocks	15.74 (28.93)	2.8 (1.2)	0.094 (0.192)
2008	GD_5O, GD_5T, $GD_{15}T$, $GD_{15}O$, UND, GND	blocks	35.01 (47.67)	8.1 (2.7)	0.001 (0.001)
2009	GD_5O, GD_5T, $GD_{15}T$, $GD_{15}O$, UND, GND	blocks	32.09 (43.84)	7.1 (2.3)	0.006 (0.004)
2010	GD_5O, GD_5T, $GD_{15}T$, $GD_{15}O$, UND, GND	blocks	36.92 (51.16)	8.8 (3.1)	0.004 (0.002)
2011	GD_5O, GD_5T, $GD_{15}T$, $GD_{15}O$, UND, GND	blocks	39.42 (53.84)	9.8 (3.5)	0.007 (0.002)

Table 2

Results of repeated measurements ANOVA (time, treatment, time x treatment) of dominant plant species and number of vascular plant species. *Df*: degree of freedom, *F*: value derived from F statistics in repeated measurements ANOVA, and *P*: probability value.

	Effect					
	Time; *Df*= 4		Treatment; *Df* = 5		Time × treatment; *Df* = 20	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Agrostis capillaris	46.65	<0.001	6.32	<0.001	1.39	0.15
Dactylis glomerata	3.94	0.01	5.60	<0.001	1.09	0.37
Elytrigia repens	7.17	<0.001	2.87	0.02	1.40	0.14
Festuca pratensis	8.90	<0.001	4.92	<0.001	1.31	0.19
Galium album	2.68	0.04	4.34	0.001	0.60	0.90
Lolium perenne	5.67	<0.001	2.81	0.02	1.07	0.40
Poa pratensis	8.80	<0.001	2.40	0.04	0.79	0.72
Rumex obtusifolius	37.12	<0.001	16.70	<0.001	3.55	<0.001
Taraxacum sp.	4.92	0.001	8.33	<0.001	2.50	0.002
Trifolium repens	12.01	<0.001	5.43	<0.001	1.40	0.14
Urtica dioica	2.54	0.05	13.61	<0.001	2.18	0.007
Veronica chamaedrys	1.49	0.21	2.06	0.08	0.76	0.75
Nuber of plant species	3.67	0.01	14.68	<0.001	3.26	<0.001

Fig. 1.

Density of *Rumex obtusifolius per* 1 m^2 under different treatments for the years 2007-2011. *P* represents probability value obtained by one-way ANOVA for each year, n.s. – non-significant result. Using the Tukey *post hoc* test, treatments with the same letter were not significantly different on 0.05 probability value. Error bars represent standard error of the mean. Treatment abbreviations (GD_5O ,GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Table 1.

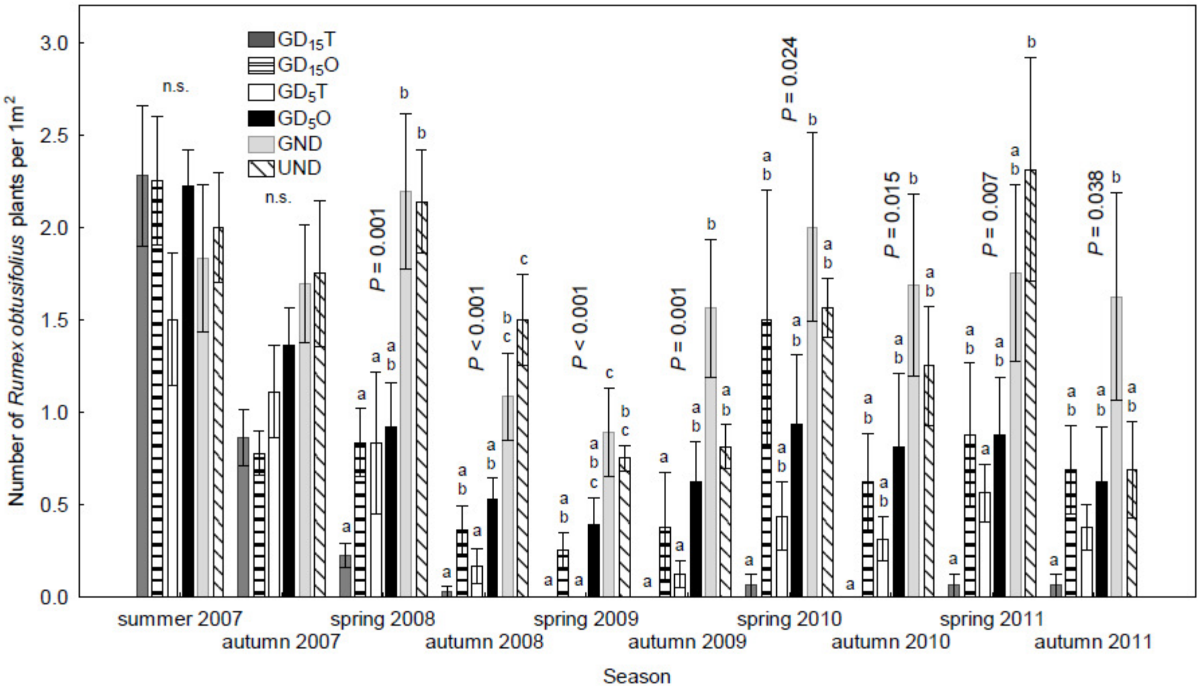


Fig. 2.

Relationship between cover (%) and plant density of *Rumex obtusifolius.*

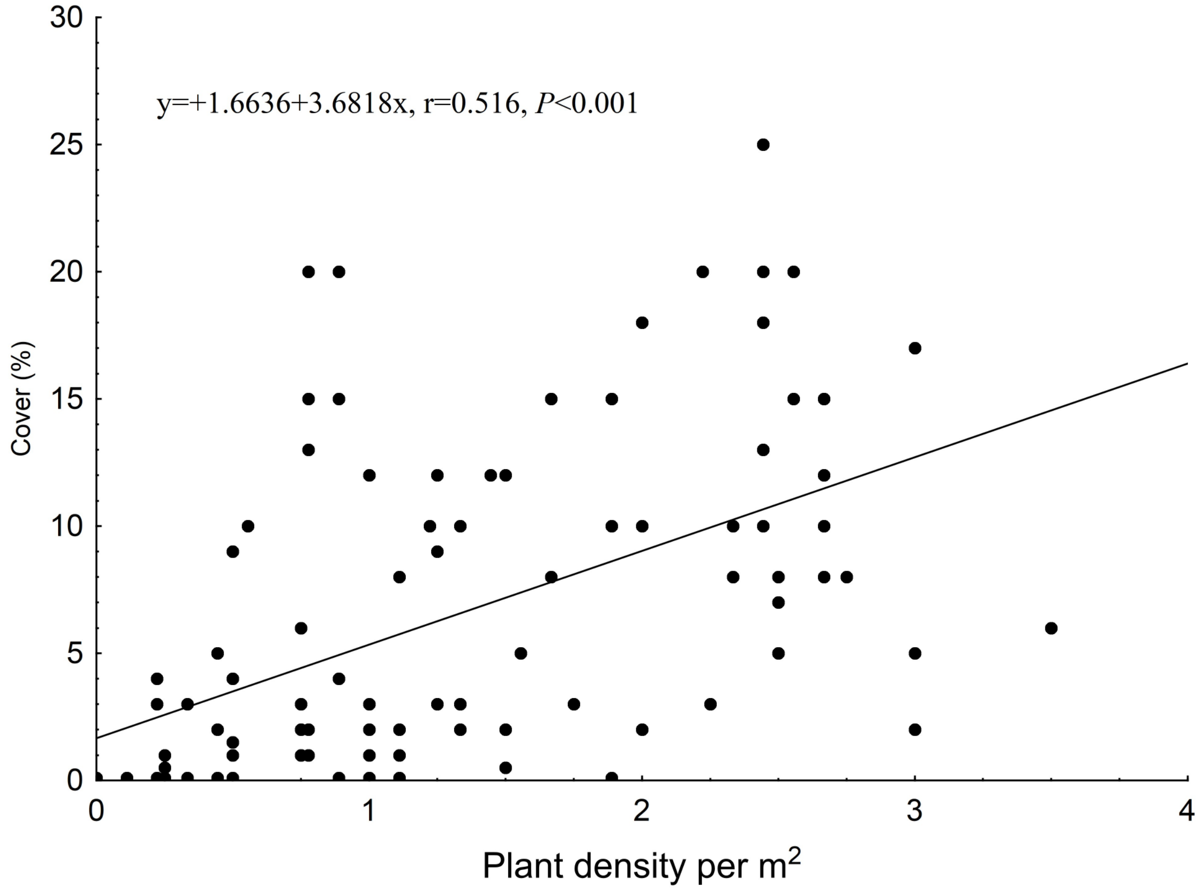


Fig. 3.

Survival rate of *R. obtusifolius* in autumn 2011 referred to the initial number of plants. Significant differences (P<0.05) according to the Tukey *post hoc* test are indicating by different letters. Error bars represent standard error of the mean. Treatment abbreviations (GD_5O, GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Table 1.

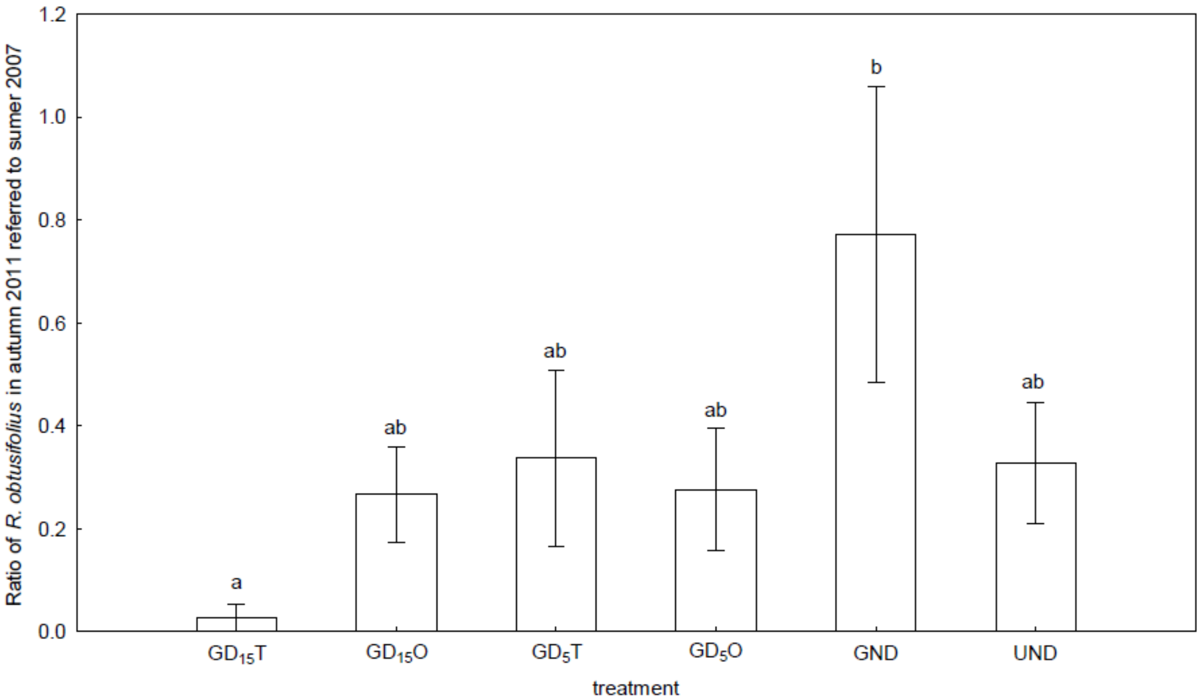


Fig. 4.

Ordination diagram showing results of RDA analysis of plant species composition data collected after five years of the experiment in 2011 (see Table 1 for details). Treatment abbreviations (GD_5O ,GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Tab. 1. Species abbreviations: *Alchsp: Alchemilla sp., Alopra: Alopecurus pratensis, Cerhol: Cerastium holosteoides, Dacglo: Dactylis glomerata, Elyrep: Elytrigia repens, Fespra: Festuca pratensis, Galalb: Galium album, Lolper: Lolium perenne, Poapra: Poa pratensis, Ranrep: Ranunculus repens, Rumobt: Rumex obtusifolius, Stegra: Stellaria graminea, Tarsp.: Taraxacum sp., Trirep:Trifolium repens, Urtdio: Urtica dioica, Verarv: Veronica arvensis, Vercha: Veronica chamaedrys and Viccra: Vicia cracca.*

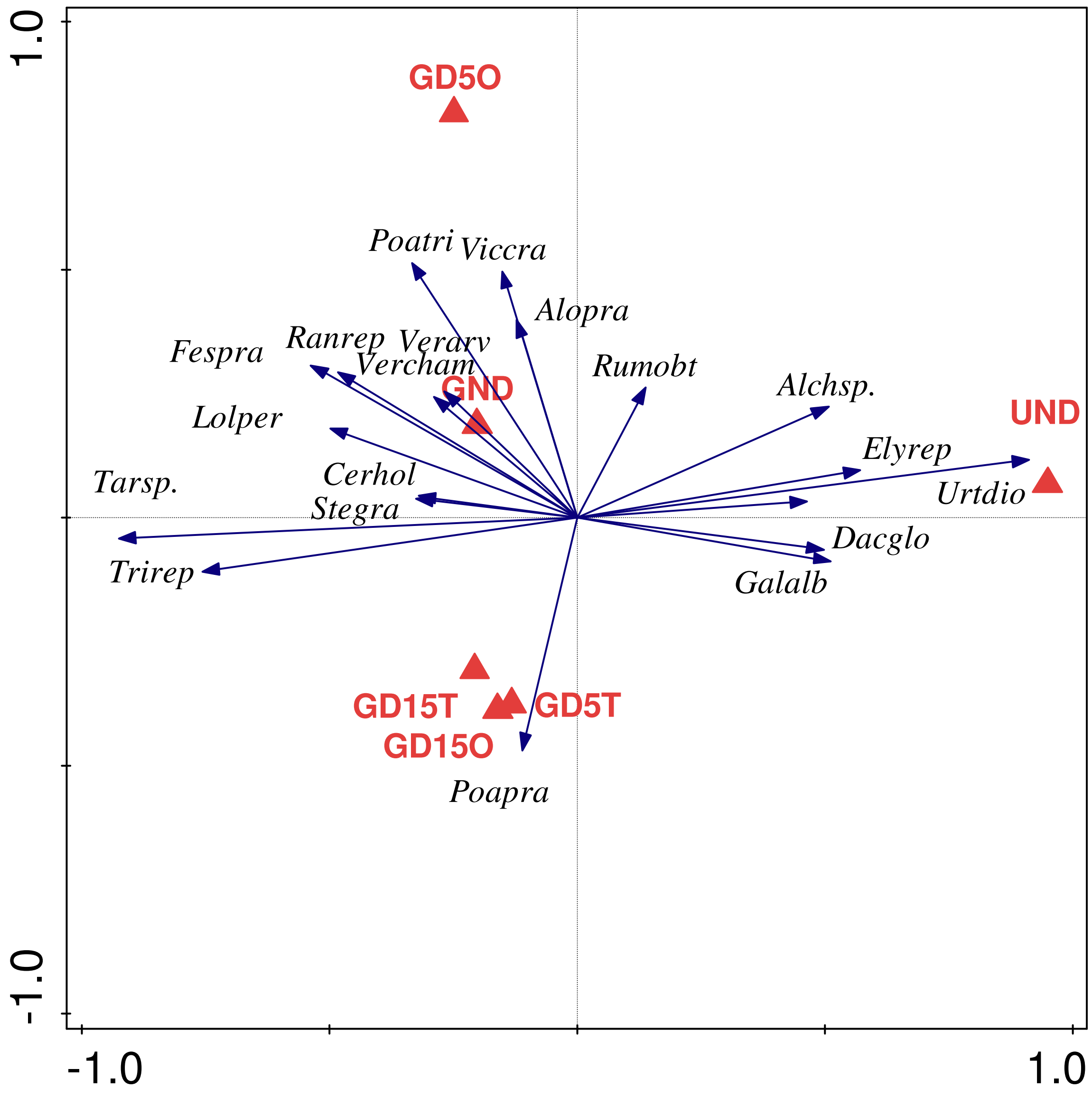


Fig. 5.

Cover (%) of the dominant grass species under different treatments for the 2007-2011. Error bars represent standard error of the mean. Treatment abbreviations (GD_5O, GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Table 1.

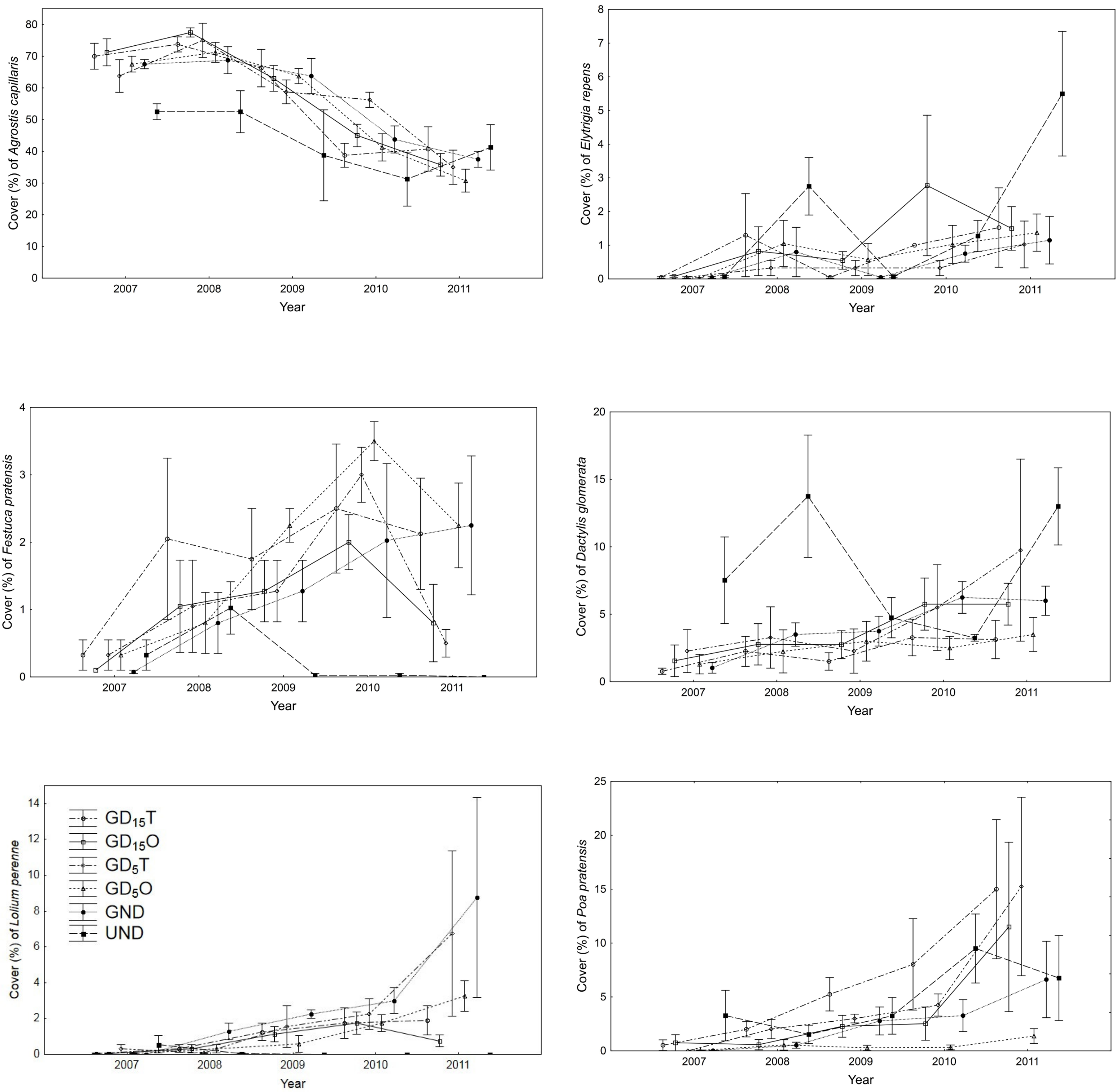


Fig. 6.

Cover (%) of dominant forb species under different treatments for the years 2007-2011. Error bars represent standard error of the mean. Treatment abbreviations (GD_5O, GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Table 1.

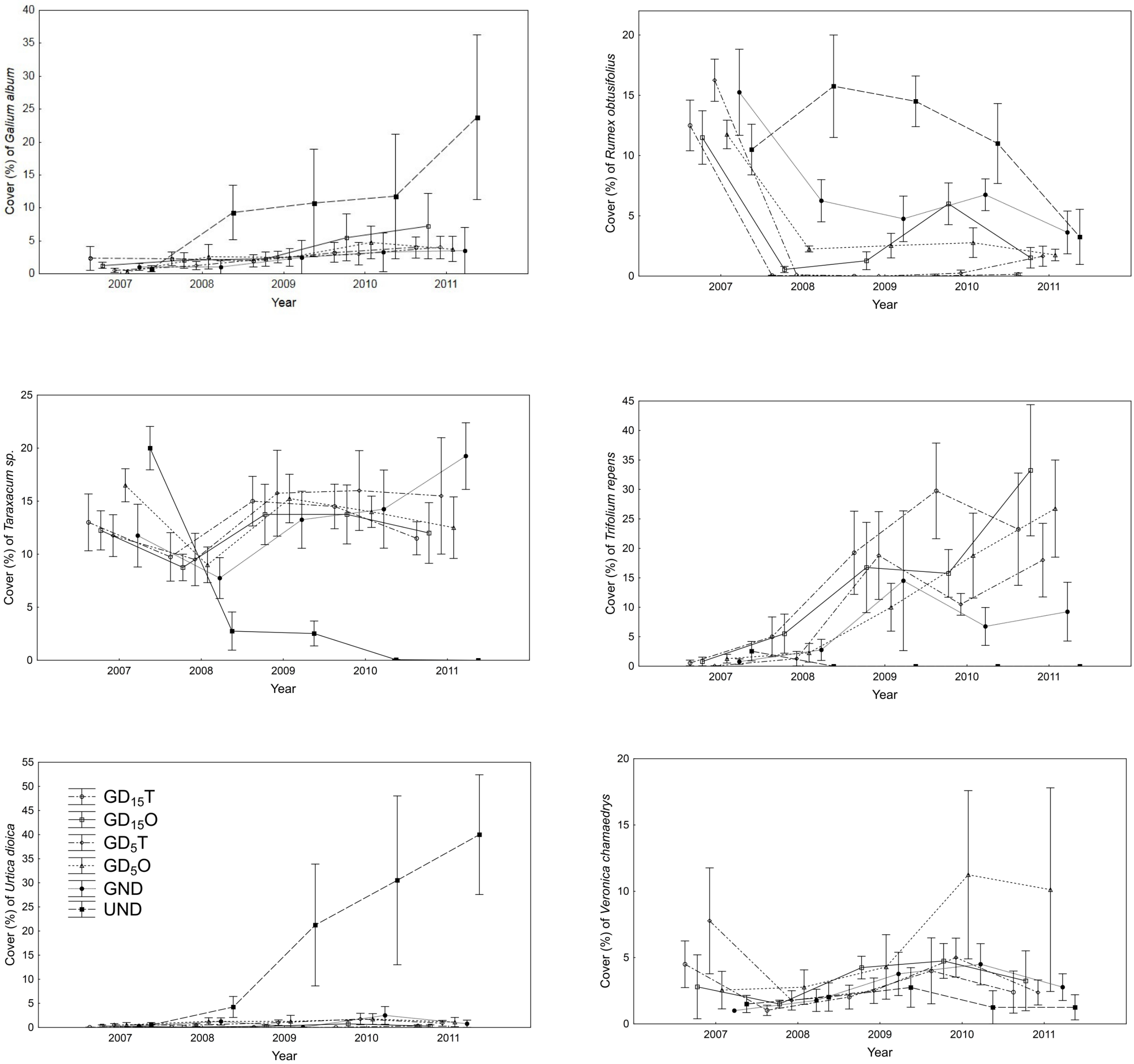
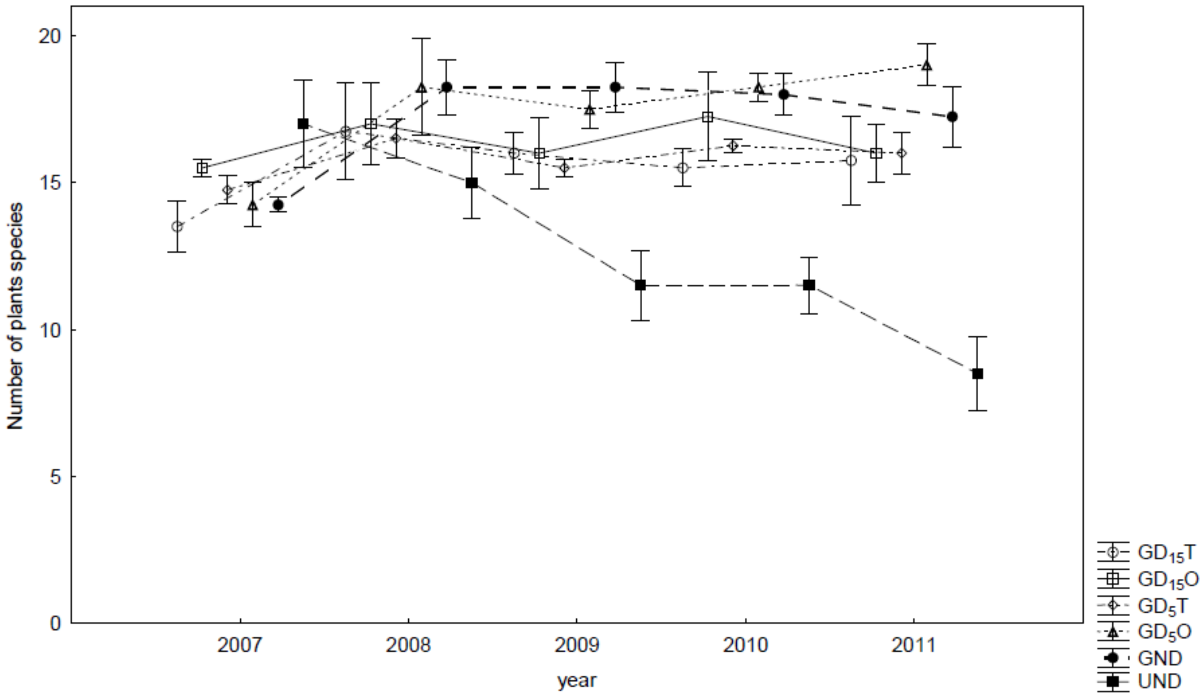


Fig. 7.

Species richness of vascular plants (per 4 m^2) under different treatments fotr the years 2007-2011. Error bars represent standard error of the mean. Treatment abbreviations (GD_5O, GD_5T, $GD_{15}T$,$GD_{15}O$, UND, GND) are explained in Table 1.



Chapter 7

Principal conclusions of the thesis

The PhD thesis is focused on the study of ecological characteristics of *Rumex* OK-2 and its potential as a weedy species in comparison with common dock species. *Rumex* OK-2 was breed in Ukraine for a forage use and for the same reason was introduce to the Czech Republic, where began to grow more for energy use (fuel). Its cultivation was initiated despite the very limited knowledge about the ecology and behavior. Many species of broad leaves dock are very problematic weeds in arable land and grassland. We tried to reveal if *Rumex* OK-2 has different ecological requirements than they have or not. **Chapter 1** is a general introduction to the topic of weedy broad leaves *Rumex* species. Further it was evaluated the ability of *Rumex* OK-2 to spread across the countryside from the former fields (**Chapter 2**) and grow and survive in the grassland (**Chapter 3**). Production of belowground and aboveground biomass of *Rumex* OK-2 under different frequencies of defoliation was compared with *R. crispus*, *R. obtusifolius* and *R. alpinus* (**Chapter 4**). Consequently production and distribution of belowground biomass at different depths was compared with *R. crispus* and *R. obtusifolius* during vegetation season (**Chapter 5**). **Chapter 6** is focused on mechanical weeding of *R. obtusifolius*. This study can be a first step for future comparison of similarities for weeding control of *Rumex* OK-2.
The contribution of the thesis for the current stage of scientific knowledge is evident from the individual papers mentioned above. Following there is very briefly summarizing the main benefit of each paper.

Chapter 2: The preliminary results of two monitoring years (2011–2012) showed the invasive spreading of the *Rumex* OK-2 from former field especially along roadside ditches. It seems that *Rumex* OK-2 could have an invasive potential and further detailed study of its biology, ecology, and distribution strategy in landscape is needed.

Chapter 3: The sward disturbance is the main factor for *Rumex* OK-2 infestation into the existing grasslands. Although in the course of vegetation season the plants of *Rumex* OK-2 are exposed by the high competitive pressure of existing sward, still some of them were revealed in the end of vegetation season. These plants in the next vegetation seasons can be important source of seeds and can support its expansion into surroundings. *Rumex* OK-2 has similar behaviour as other broad leaves docks in Central Europe so we can expect its further spreading.

Chapter 4: A higher cutting frequency can reduce the belowground biomass but no effect on the aboveground biomass was detected. The growing response of *Rumex* OK-2 to different cutting treatments was not very different from other studied *Rumex* species. The belowground biomass production of *Rumex* OK-2 was most similar to *R. crispus* under low cutting frequencies. These similarities indicate that also the weed potential of *Rumex* OK-2 might be corresponding to that of *R. crispus.*

The results of the pot experiment is only be the first step to investigate the ecological requirements of a hybrid. The future investigations should be focused on response of *Rumex* OK-2 to different mowing frequencies and competition by other plants under field conditions.

Chapter 5: Both aboveground and belowground biomass production of *Rumex* species tend to grow from July to September. The proportion of belowground biomass of *Rumex* species in upper 30 cm was about 70-80% whereas only 20-30% was allocated deeper than 30 cm. Besides flowering in the seedling year typical for *R. obtusifolius* plants there were revealed one flowering plant of *Rumex* OK-2. The growth dynamics and allocation of belowground biomass of *Rumex* OK-2 was more similar to *R. crispus* than to *R. obtusifolius*. These similarities indicate that the weed potential of *Rumex* OK-2 might correspond with that of *R. crispus*.

Chapter 6: Mechanical weeding by digging out together with cutting of ungrazed patches is the effective method for *R. obtusifolius* control in upland grassland dominated by *A. capillaris*. Two times performed digging of *R. obtusifolius* at the depth of 15 cm was the most successful method which eliminated 97% of *R. obtusifolius* plants. Bare ground gaps created by digging were colonized by nutrient demanding grasses and forbs with high forage value. Although no grassland management reduced number of *R. obtusifolius* plants in sward, it allowed seed production of surviving plants and supported tall weedy species in the sward and considerably reduced species richness of vascular plants.

Chapter 8

Recommendation for further research

With respect to lack of knowledge of ecological requirements of *Rumex* OK-2, further research should be focused on following issues:

Field survey for getting the knowledge of its distribution:

Monitoring of *Rumex* OK-2 spreading in the vicinity of former and current field in the Czech Republic

Small scale experiments (climate chamber and greenhouse) for study of its ecological characteristics:

Germination of *Rumex* OK-2 under different temperatures and levels of nutrients in comparison with the other broad leaves weedy *Rumex* species

Pot and field manipulative experiments for testing of its growing characteristics and control:

Growing characteristics of *Rumex* OK-2 under different nutrients status, disturbance level and competition conditions

Chemical, biological and mechanical control of *Rumex* OK-2

Testing of its genetics properties:

Possibility of hybridization of *Rumex* OK-2 with the other broad leaves weedy *Rumex* species

Chapter 9

Souhrn (Summary in Czech)

Disertační práce je zaměřena na studium biologických, ekologických vlastností šťovíku *Rumex* OK-2 ve srovnání s vybranými plevelnými širokolistými druhy rodu *Rumex*. Tento hybridní druh byl vyšlechtěn na Ukrajině pro krmivářské účely, za stejným účelem byl dovezen i do České republiky, kde se však velmi rychle začal více pěstovat pro energetické účely (palivo). Jeho pěstování bylo zahájeno i přes velmi omezené znalosti o vlastnostech a chování tohoto druhu. Vzhledem k faktu, že mnohé širokolisté druhy šťovíků jsou velmi problematickými plevely orné půdy i travních porostů, snažili jsme se zjišťovat, zda se od nich šťovík *Rumex* OK-2 svými nároky a chováním liší. **Kapitola 1** je obecným úvodem k problematice širokolistých plevelných druhů rodu *Rumex*. V této práci byly vyhodnoceny schopnosti šťovíku *Rumex* OK-2 uchytit se a přežívat v travním porostu (**Kapitola 3**) a šířit se krajinou od ploch jeho původního pěstování (**Kapitola 2**). Produkce nadzemní i podzemní biomasy šťovíku *Rumex* OK-2 za různé frekvence defoliace byla porovnána s produkcí plevelných druhů šťovíku kadeřavého, š. tupolistého a š. alpského (**Kapitola 4**). Rovněž tvorba a hlavně rozložení podzemní biomasy v různých hloubkách v průběhu vegetační sezóny bylo porovnáváno s druhy š. tupolistým a š. kadeřavým (**Kapitola 5**). **Kapitola 6** je zaměřena pouze na š. tupolistý a jeho mechanické odstranění z pastviny. Tato studie může posloužit jako první krok pro následné zkoumání podobnosti reakce se šťovíkem *Rumex* OK-2 v obdobném experimentu.

Kapitola 2: Předběžné výsledky za dva monitorované roky (2011-2012) ukazují ivazivní šíření šťovíku *Rumex* OK-2 z původních polí. K největšímu šíření dochází v příkopech podél komunikací a při okraji polí. Z této studie vyplývá, že by šťovík *Rumex* OK-2 mohl mít invazní potenciál a proto je nutné další studium jeho biologických a ekologických vlastností a jeho strategie rozšiřování a přežívání v krajině.

Kapitola 3: Narušení travního drnu je hlavním faktorem pro uchycení a následné zaplevelení existujícího travního společenstva šťovíkem *Rumex* OK-2. Ačkoli byly rostliny *Rumex* OK-2 v průběhu vegetační sezóny vystaveny vysoké konkurenci existujícího travního drnu, přesto několik z nich bylo zaznamenáno i na konci vegetační sezóny. Tyto rostliny mohou být v příštích vegetačních sezónách důležitým zdrojem semen a mohou podpořit jeho rozšíření do okolí. *Rumex* OK-2 má podobné chování jako ostatní druhy širokolistých šťovíků střední Evropy, proto se dá předpokládat jeho další šíření.

Kapitola 4: Vysoká frekvence sečení může redukovat množství podzemní biomasy, ale vliv na nadzemní biomasu zaznamenán nebyl. Růstová odezva šťovíku *Rumex* OK-2 na různé frekvence seče nebyla příliš odlišná od ostatních studovaných druhů rodu *Rumex*. Při nízké frekvenci seče se produkce podzemní biomasy šťovíku *Rumex* OK-2 nejvíce podobala produkci šťovíku kadeřavého (*R. crispus*). Tyto podobnosti ukazují, že *Rumex* OK-2 by mohl mít potenciál stát se plevelným druhem odpovídající šťovíku kadeřavému. Výsledky nádobového pokusu jsou prvním krokem ke zkoumání ekologických vlastností a nároků šťovíku *Rumex* OK-2. Budoucí výzkum by mohl být zaměřen na reakci *Rumex* OK-2 na různou frekvenci seče v souvislosti s konkurencí s jinými rostlinami v polních podmínkách.

Kapitola 5: Produkce nadzemní i podzemní biomasy od července do září u všech sledovaných druhů rodu *Rumex* vykazovala vzrůstající tendenci. Ve vrchních 30 cm půdy bylo situováno 70 až 80% podzemní biomasy, zatímco pouze 20 až 30% podzemní biomasy se nacházelo ve vrstvách hlubších než 30 cm. Kromě kvetoucích rostlin š. tupolistého, pro něhož je kvetení v první sezóně typické, byla zaznamenána i jedna kvetoucí rostlina šťovíku *Rumex* OK-2. Dynamika růstu a rozdělení podzemní biomasy šťovíku *Rumex* OK-2 bylo více podobné š. kadeřavému než š. tupolistém. Tyto podobnosti naznačují, že plevelný potenciál šťovíku Rumex OK-2 by mohl odpovídat š. kadeřavému.

Kapitola 6: Mechanické vyrývání společně se sečením nedopasků je efektivní metodou pro regulaci šťovíku tupolistého (*R. obtusifolius*) v podhorských travních společenstvech s dominantou *Agrostis capillaris*. Dvakrát provedené vyrytí šťovíku tupolistého do hloubky 15 cm bylo nejúspěšnější metodou, která snížila počet rostlin šťovíku tupolistého o 97%. Plošky obnažené půdy vytvořené vyrýváním byly obsazeny na živiny náročnými trávami a bylinami s vysokou pícninářskou hodnotou. Ačkoli varianta ponechání ladem snížila počet rostlin šťovíku tupolistého, umožnila produkci semen na přežívajících rostlinách, podpořila rozvoj vysokých plevelných druhů a značně omezila druhovou bohatost cévnatých rostlin.

List of publications

Papers in scientific journals with impact factor:

Hujerová R., Pavlů L., Pavlů V., Hejcman M. and Gaisler J. (2015) The growth dynamics of belowground biomass of *Rumex crispus*, *R. obtusifolius*, and the *Rumex* hybrid cv. OK-2 (*R. patienta* x *R. tianschanicus*) in the seeding year. Weed Research. Submitted paper

Hujerová R., Pavlů L., Pavlů V., Gaisler J., Hejcman M., Ludvíková V. (2015) Mechanical weeding of *Rumex obtusifolius* and its effect on plant species composition in *Agrostis capillaris* grassland under conditions of organic farming. Journal of Pest Science. Submitted paper

Hujerová R., Pavlů V., Hejcman M., Pavlů L. and Gaisler J. (2013) Effect of cutting frequency on above- and belowground biomass production of *Rumex alpinus*, *R. crispus*, *R. obtusifolius* and the *Rumex* hybrid (*R. patienta* × *R. tianschanicus*) in the seeding year. Weed Research 53, 378–386.

Papers in other scientific journals:

Hujerová R., Gaisler J., Pavlů L., Pavlů V. and Hejcman M. (2014) Emergence and survival of *Rumex* OK-2 (*Rumex patientia* x *Rumex tianschanicus*) in grasslands under different management conditions. Grassland Science in Europe 19: 327 - 329

Hujerová R., Gaisler J., Mandák B., Pavlů L. and Pavlů V. (2013) Hybrid of *Rumex patientia* and *Rumex tianschanicus* (*Rumex* OK-2) as a potentially new invasive weed in Central Europe. Grassland Science in Europe 18: 466 - 468

Hujerová R., Gaisler J., Pavlů L. and Pavlů V. (2011) Mechanical weeding of *Rumex obtusifolius* in organically managed grassland. Grassland Science in Europe, 16: 208-210.

Practical methodologies:

Pavlů V., Hejcman M., Gaisler J., Pavlů L. and Hujerová R. (2011) Možnosti regulace širokolistých šťovíků v travních porostech v systému ekologického zemědělství. Certifikovaná metodika, VÚRV, v.v.i. Praha.