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Resprouting and life cycles of short-lived herbs

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RESPROUTING AND LIFE CYCLES OF SHORT-LIVED HERBS

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PHD. THESIS

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ANNOTATION

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Disturbance is a strong selective factor in plants causing a partial or total destruction of plant biomass. Even though resprouting has become a generally accepted strategy of tree species in highly disturbed habitats, in herbs it is still incorrectly claimed that short-lived ones occupying such habitats regenerate after damage of all aboveground biomass only from seeds. However, there is a significant number of short-lived herbs that survive removal of 100 % of aboveground biomass, and in spite of such severe injury to their body, resprout and finish the reproductive cycle. Nevertheless, resprouting in short-lived herbs has been neglected by plant ecologists so far, and only little information on this strategy is available.

The present thesis is composed of six original studies describing characteristics, ecological relationships and consequences of the ability of resprouting in the following short-lived herbs of highly disturbed habitats: *Rorippa palustris, Barbarea vulgaris, Oenothera biennis, O. issleri, O. fallax* and *O. glazioviana.*

DECLARATION

I hereby declare that this thesis has been fully worked out by myself and the named co-authors with the use of cited references.

Jana Martínková

České Budějovice, 9th September 2004

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CHAPTER I

General Introduction

GENERAL INTRODUCTION

Disturbance is one of the strongest selective factors in plant populations. It is defined as a mechanism causing partial or total destruction of plant biomass and influences almost every plant population (Silvertown & Lovett-Doust 1993, Grime 2001). The most disturbed habitats of Central Europe are areas under strong impact of man, e.g. industrial areas, belts along roads and railways, arable land, urban areas, or naturally disturbed habitats such as river alluvia. Disturbance influencing these areas acts frequently, unpredictably, very severely, and often kills whole plant populations. Moreover, in comparison to less disturbed habitats such as grasslands or pastures, in anthropogenic habitats and river alluvia, disturbance frequently erodes upper soil layers, and thus destroys all aboveground biomass or even fragmentises underground plant parts. Predominance of short-lived species, i.e. annuals, biennials and short-lived perennials, in highly disturbed habitats supports the view that individual vegetative regeneration is not of great value for natural populations under these conditions (Bellingham & Sparrow 2000, Grime 2001), and a rather short life cycle, fast maturation and a high production of small seeds are advantageous under these severe conditions (MacArthur & Wilson 1967, Grime 2001). However, it has been found that some short-lived species of severely disturbed habitats, i.e. anthropogenic areas, are able to survive total destruction of aboveground parts or body fragmentation and regenerate successfully (Irmisch 1857, Dubard 1903, Rauh 1937, Klimešová 2003). Therefore, it is plausible that some short-lived species of highly disturbed habitats do not rely only on the seed bank, so resprouting (vegetative regeneration) from the bud bank (Harper 1977) after a disturbance could play an important role in persistence of their populations (Fig. 1). However, the ability of resprouting of short-lived species has so far been overlooked and its factual importance in highly disturbed habitats is still unknown.

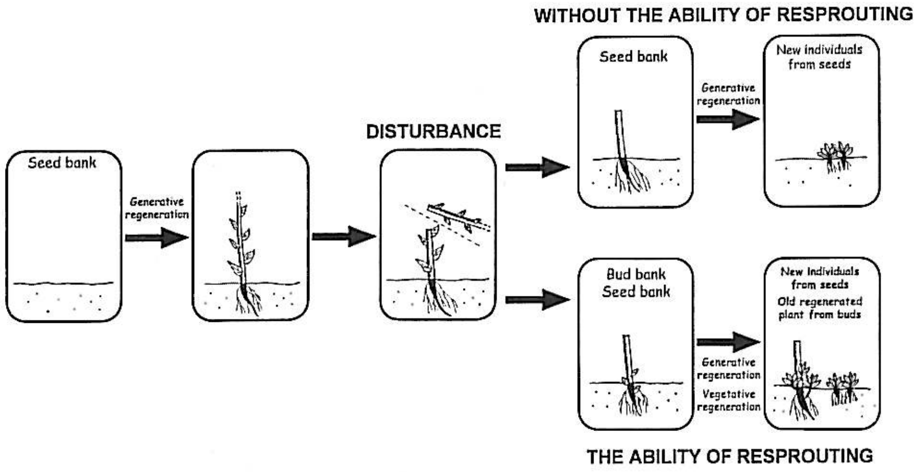
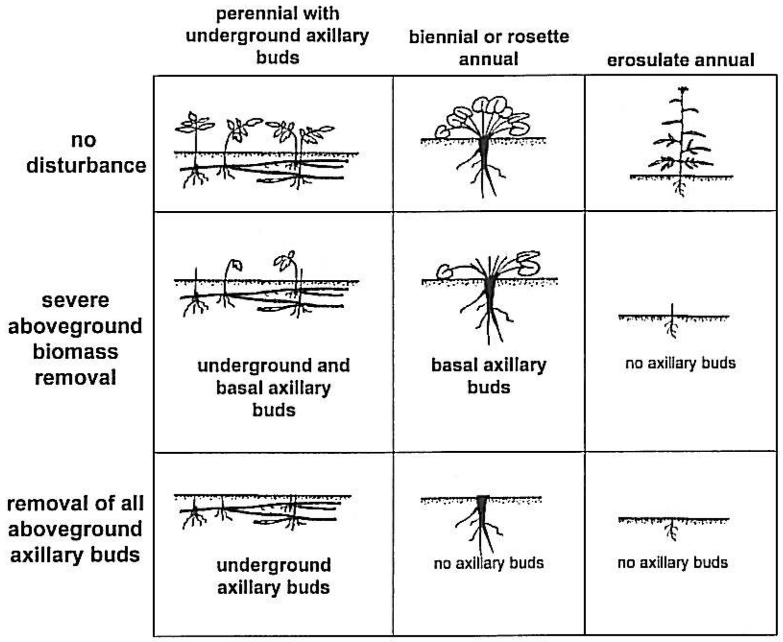


Fig. 1. Diagram comparing regeneration after disturbance of a short-lived species without the ability of resprouting – death of injured individual followed by generative regeneration of population from the seed bank (top pictures) – with a short-lived species with the ability of resprouting – vegetative regeneration of injured individual from the bud bank and generative regeneration of population from seeds (bottom pictures).

SEVERITY OF DISTURBANCE

All higher plants without exception – trees, herbs, perennials, short-lived plants, clonal and non-clonal ones – possess axillary buds in all leaf axils (Esau 1965, Bell 1991, Kerstetter & Hake 1997). Positioning and utilisation of these axillary buds define the plant's growth form, and thus the extent of tolerance to disturbance (Marquis 1996, Bellingham & Sparrow 2000, Del Tredici 2001, Huhta et al. 2003, Klimešová & Klimeš 2003) (Fig. 2). From this point of view, the most disturbance resistant species are perennial herbs with underground organs of stem origin such as rhizomes, stolons etc. After a severe disturbance removing all aboveground biomass or after fragmentation of underground parts, individuals of such species can easily regrow from preserved underground axillary buds. Short-lived herbs (e.g. rosette or semirosette annuals and biennials, Krumbiegel 1998, 1999), with axillary buds concentrated on stem bases frequently pulled under the ground, can survive only less severe disturbance when the stem basis is left intact, while removal of all aboveground biomass and fragmentation of underground parts are lethal to them. The most sensitive plants to a disturbance are those without either underground or basal axillary buds (e.g. trees or erosulate annuals). They tolerate only mild disturbances such as clipping of branches or apical meristem removal (Gill 1995, Huhta et al. 2003).

However, there are some species that survive even removal of all axillary buds and vegetativelly regenerate after such severe disturbance irrespective of their growth form (Klimešová & Klimeš 2003). This is enabled by the ability to form adventitious buds on roots and resprout from them (Fig. 3). The majority of root-sprouting species is found in those which have no underground axillary buds, i.e. in trees or short-lived species (Del Tredici 2001, Klimešová & Klimeš 2003). Therefore, it seems that adventitious buds on roots form an alternative bud bank to underground axillary buds in species without underground organs of stem origin.



	perennial with underground axillary buds	biennial or rosette annual	erosulate annual
no disturbance			
severe aboveground biomass removal	underground and basal axillary buds	basal axillary buds	no axillary buds
removal of all aboveground axillary buds	underground axillary buds	no axillary buds	no axillary buds

Fig. 2. Diagram comparing the bud bank, which can be used for regrowth after two different disturbance severities in a perennial plant, a biennial or rosette annual plant, and an erosulate annual plant. Drawings by J. Klimešová were adopted from Klimeš et al. (1997) and modified.

FREQUENCY OF DISTURBANCE

When not only severity of disturbance, but also its frequency is considered, growth rate and length of the vegetative phase of individuals become important as well (MacArthur & Wilson 1967, Kozlowski 1992, Grime 2001, Kudoh et al. 2002). Species with a short life cycle and fast maturation have a more favourable position than perennials in frequently disturbed habitats, because they are able to finish seed reproduction and form a seed bank before a next disturbance. On the other hand, a strong trade-off between allocation to vegetative and generative growth restricts the ability to regenerate vegetatively after an injury in short-lived species (King & Roughgarden 1982, Kozlowski & Wiegert 1986, Chapin et al. 1990, Reekie et al. 1997).

Nevertheless, energy allocation into vegetative and generative growth is not a single switch of the investment, but it is rather graded (King & Roughgarden 1982, Kudoh 2002), and thus a certain degree of vegetative regeneration is possible in short-lived species as well. It seems that short-lived species use resprouting from roots to compensate both for lower ability of vegetative regeneration determined by their growth form (in comparison to perennials with underground axillary buds) and for a longer vegetative phase (in comparison to annuals with fast maturation). Therefore, it is not surprising that the highest number of root-sprouting species among short-lived herbs is found in biennials (Klimešová & Klimeš 2003).

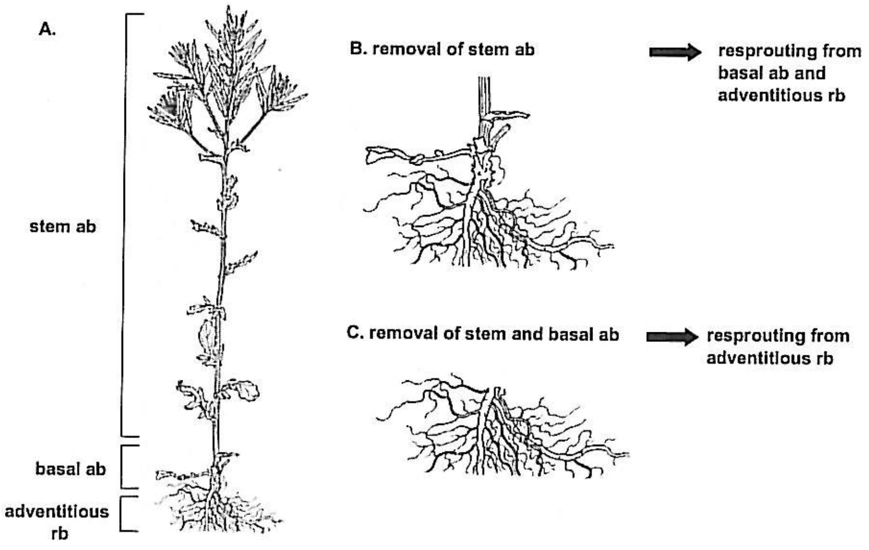


Fig. 3. A. A bud bank along the uninjured body of a short-lived herb with the ability of root-sprouting (*Barbarea vulgaris*). **B.** A bud bank of the same species after removal of stem axillary buds. **C.** A bud bank of the same species after removal of all axillary buds. ab – axillary buds, rb – root buds. Drawing by E. Smrčinová adopted from Dvořák (1992) and modified.

FACTORS INFLUENCING RESPROUTING

Even though some short-lived species can resprout after severe disturbance, it is highly probable that the extent of this ability varies among short-lived species and could be influenced by many factors: (i) internal ones such as age, life cycle phase and life cycle mode of a plant at the time of its injury, and by the amount of stored reserves, or (ii) by external factors such as severity of disturbance and nutrient availability. Unfortunately, information on the ecology of root-sprouting short-lived species is scarce and comes mostly from historical and rather descriptive or morphological literature (Irmisch 1857, Wittrock 1884, Rauh 1937). Nevertheless, the root-sprouting ability may play a crucial role in highly disturbed areas, and be of high importance and advantage for populations of short-lived species occurring there.

AIMS AND CONTENTS OF THIS THESIS

Consequently, the main aims of this thesis are: (i) to confirm the ability of root-sprouting in selected short-lived species of highly disturbed habitats, (ii) to analyse the effects of internal and external factors on the ability of resprouting in these species, and (iii) to describe possible consequences of the resprouting ability for their natural populations. To this purpose, several experiments and field observations were conducted and the following short-lived species with already documented ability to form adventitious buds on roots or species suspected of this ability were selected: the annual *Rorippa palustris*, the biennials *Oenothera biennis, O. issleri, O. fallax, O. glazioviana* and the short-lived perennial *Barbarea vulgaris.*

Chapter II of the thesis represents an experimental study assessing the ability of resprouting in three short-lived species. The study comprises a chamber experiment with *Rorippa palustris* and a field experiment with two *Oenothera* species (O. *biennis* and *O. issleri*). The effects of three factors on the resprouting ability were examined: timing of injury, severity of injury, and nutrient availability.

Chapter III represents an experimental study with juvenile plants of the model root-sprouting herb *Rorippa palustris.* In a chamber and greenhouse experiment, the effects of plant age, nutrient availability and severity of injury on the resprouting ability were tested.

Chapter IV represents a three-year garden experiment with *Barbarea vulgaris.* In this study, the effects of nutrient level, severity and timing of injury on resprouting and also on consequential seed production were assessed.

In **Chapter V**, the question how frequent resprouting is in natural populations of *Oenothera biennis* is answered based on field observations. In the same study, the effects of injury severity and life cycle phase on resprouting were experimentally tested in *O. biennis*, as well as in its closely related congeners, *O. fallax* and *O. glazioviana.*

In **Chapter VI,** it is shown on root-sprouting plants that growth forms intermediate between non-clonal and clonal plants can be used to ask questions about the functional ecology of clonality. A major part of the examples is based on results of experiments with short-lived root-sprouting species.

Chapter VII represents the contribution on *Rorippa palustris*, the model short-lived root-sprouting species, to Biological Flora Central Europe. The contribution reviews its taxonomic status and morphology, distribution, ecology, life cycle, population biology and genetics.

The main results of this thesis are summarised in **Chapter VIII.**

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CHAPTER II

Resprouting after disturbance:
an axperimental study with short-lived monocarpic herbs

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CHAPTER II

Resprouting after disturbance: an experimental study with short-lived monocarpic herbs

Abstract

We experimentally demonstrated the ability of three short-lived monocarpic species to vegetatively regenerate (resprout) from roots after severe disturbance. We assessed the relationship between resprouting ability and 1. timing of injury with respect to life-cycle stage (reproductive vs. vegetative plant), life-history mode (annual vs. winter annual) and phenological stage (flowering vs. fruiting plant), 2. nutrient availability, and 3. disturbance severity (removal of all axillary buds Yes/No).

In a chamber experiment with the annual or potentially winter annual *Rorippa palustris*, all injured plants resprouted in all nutrient levels and day-length regimes (day-length regimes simulated conditions of an annual and a winter annual cohort). The number of adventitious buds on roots was positively affected only by injury. The extent of regeneration and amount of regenerated biomass were higher at high nutrient level and long-day regime.

In a field experiment with the biennials, *Oenothera biennis* and *Oenothera issleri*, both species responded to injuries by resprouting from axillary buds, when at least only one axillary bud was still present. However, they were also able to resprout form adventitious buds on roots. Reproductive plants resprouted more frequently at the flowering stage than at the fruiting stage. Resprouting of vegetative plants (rosettes) was equal throughout the year and more frequent than in the reproductive plants.

Key-words: *day-length regime, injury, nutrient level,* Oenothera biennis, Oenothera issleri, Rorippa palustris, *vegetative regeneration*

INTRODUCTION

Disturbance provides areas of bare soil, where competitively less efficient species (i.e., annuals, biennials and short-lived perennials) are able to germinate, grow and survive (Harper 1977, Klemow & Raynal 1983, Löfgren et al. 2000, Grime 2001). However, disturbance is also the most significant factor causing early mortality in these species. Population survival of annual and biennial species depends only on their ability to produce numerous seeds (Harper 1977, Crawley 1986, Van der Meijden et al. 1992, Grime 2001). Nevertheless, some studies (Irmisch 1857, Wittrock 1884, Rauh 1937, Kočvarová 2002, Klimešová 2003) suggest a possibility of vegetative regeneration (resprouting) after severe disturbance in some short-lived species. Severe injury to plants may not be fatal in about 2% of annual species and 14% of the biennials in Central European flora, because they are able to form *de novo* adventitious buds on roots (Klimešová 2003). This ability enables them to rebuild the plant body and complete the reproductive cycle, i.e., to produce seeds (Dubard 1903, Rauh 1937). Regenerated individuals can postpone flowering and fruiting to the next vegetation season, or they do not die after seed production and flower more than once (van Tooren et al. 1987, Hautekeete et al. 2002, Klimešová 2003).

The presently available information on root sprouting in short-lived monocarpic species is scarce and comes mostly from descriptive and morphological literature (e.g. Dubard 1903, Rauh 1937, Klimešová 2003); hence a need to perform manipulative experiments is evident. For this study, we chose the biennial species *Oenothera biennis* with its already mentioned resprouting ability (Dubard 1903, Rauh 1937), its relative *Oenotheea issleri* and the annual species *Rorippa palustris*, both being able to resprout in the field (Klimešová 2003). The effect of three factors on the resprouting ability was examined.

(I) TIMING OF INJURY with respect to life-cycle stage (reproductive vs. vegetative plant), life-history mode (annual vs. winter annual) and phenological stage (flowering vs. fruiting plant). Because, the relative investment into the vegetative vs. reproductive growth varies during the life of a monocarpic plant (e.g. King & Roughgarden 1982, van Mierlo & van Groenendael 1991, Olejniczak 2001), we hypothesised that the timing of injury can change the relative investment into vegetative vs. seed reproduction.

(II) NUTRIENT AVAILABILITY. Nutrient stress may have a similar effect on the growth form of woody species as moderate disturbance (Bellingham & Sparrow 2000), i.e., nutrient stress stimulates resprouting. Perennial plants experiencing nutrient shortage form more adventitious buds on roots than plants at higher nutrient levels (Mc Intyre & Hunter 1975, Klimeš & Klimešová 1999). We assumed the same relationship between nutrient availability and ability to resprout in the short-lived monocarpic species.

(III) SEVERITY OF DISTURBANCE. Adventitious buds need to differentiate from root cells (Esau 1965, Burrows 1990) and they often develop only after injury (Peterson 1975), whereas axillary buds are formed during normal plant ontogenesis and they are already present on intact plants (Esau 1965, Harper 1977). Thus, we assumed that injured plants would "prefer" resprouting from axillary buds than resprouting from adventitious buds on roots.

MATERIAL AND METHODS

STUDY SPECIES

Rorippa palustris (L.) Besser (Brassicaceae) is a cosmopolitan species occurring mostly in naturally or anthropogenicaly disturbed habitats. This species is an annual, winter annual or short-lived perennial (Jäger & Werner 2002). Its seeds germinate throughout the vegetation season. The early spring cohorts behave as annuals and the late summer cohorts show a winter annual life-history mode (Sosnová 2003). More than one generation can be produced within one year.

Oenothera biennis L. and *Oenothera issleri* Rostanski (Onagraceae) are non-native species in Europe, which occur mostly in naturally or anthropogenicaly disturbed habitats. Both species are classically mentioned as being non-clonal, monocarpic and biennial species (Hall et al. 1988, Jehlík 1997). *Oenothera* individuals die immediately after seed production (Dietrich et al. 1997). Seeds of *Oenothera* species germinate only during the spring months (Baskin & Baskin 1994).

RORIPPA CHAMBER EXPERIMENT

A chamber experiment with plants of *Rorippa palustris* was performed in the winter of 2001/2002. The plants were grown in two standardised chambers (KLIMABOX ZVK E-008, Váha Kladno Czech Republic) for a total of 12 weeks from germination. Irradiance, day and night temperatures and day and night humidities were the same in both chambers (184 $\mu Em^{-2}s^{-1}$; PhAR 62 Wm^{-2}, light – 20 °C, dark – 8 °C; light - 70% humidity, dark – 90% humidity). Treatments consisted of two day-length regimes: LONG DAY = 15h light/9h dark, SHORT DAY = 9h light/15h dark; and two nutrient levels: HIGH NPK 0.21/0.1/0.15 [g/kg of substrate]; LOW NPK 0.05/0.025/0.03 [g/kg of substrate]. The long-day treatment induced an annual life-history mode and the short-day treatment created a winter annual life-history mode in the experimental plants. Low and high nutrient levels simulated nutrient shortage and normal nutrient conditions, respectively. Thirty replicates (one replicate = one plant in a pot) were used for each combination of treatments.

After eight weeks from germination, 15 plants exposed to each combination of treatments were injured at the rosette stage. All aboveground biomass was removed along with the root crown, i.e., all axillary buds were destroyed. As only the roots were left in place, resprouting was possible only from adventitious buds on the roots.

Growth characteristics such as number of leaves, rosette diameter and the amount of removed biomass were recorded at the time of injury. The number of regenerated individuals, number of adventitious buds on roots, root and shoot biomass, number of leaves and rosette diameter were recorded four weeks after injury. Two growth characteristics were calculated – root/shoot ratio (for injured individuals shoot biomass was calculated as the sum of removed and regenerated biomass) and the extent of regeneration (ratio of regenerated / removed biomass).

OENOTHERA FIELD EXPERIMENT

An experiment with natural populations of *Oenothera biennis* and *Oenothera issleri* was performed in the summer of 2001 at the railway station near České Budějovice, Czech Republic (49°3'N, 14°22'E).

Treatments consisted of two types of experimental injury (i) cutting of plants 5 cm above the root crown + defoliation (some axillary buds left in place), (ii) cutting of plants 1 cm below root crown (all axillary buds were removed, only roots left in place).

Those treatments were applied at two life-cycle stages of the species – to vegetative rosettes and reproductive plants, respectively, and at two different phenological phases (in June and August) – to both younger and older vegetative rosettes, and to flowering and fruiting plants.

Ten replicates were used for all treatments and both species. The diameter of rosettes, number of leaves and removed biomass were recorded for the rosettes (vegetative plants) at the time of their injury. Similarly, the length of stem, basal stem diameter, number of fruits or flowers and biomass were recorded for the reproductive plants. The type of regeneration (from adventitious buds on roots or axillary buds on stems) and the number of regenerated individuals were evaluated four weeks after experimental injury.

STATISTICAL ANALYSIS

All statistical analyses were done using the STATISTICA 5.5 package. Logarithmic transformation of the characteristics (biomass amounts and calculated ratios) was used. The *Rorippa* chamber experiment was evaluated by Main Effects 3-way ANOVA (effect of light regime, nutrient level and injury), 2-way ANOVA (effect of light regime and nutrient level) and simple regression in generalised linear models (Poisson or Gamma distribution, log link function). Main Effects 4-way ANOVA (effect of species, phenological stage, life-cycle stage and type of injury) and general customs design in generalised linear models (binomial distribution, logit link function) were used for evaluation of the *Oenothera* field experiment.

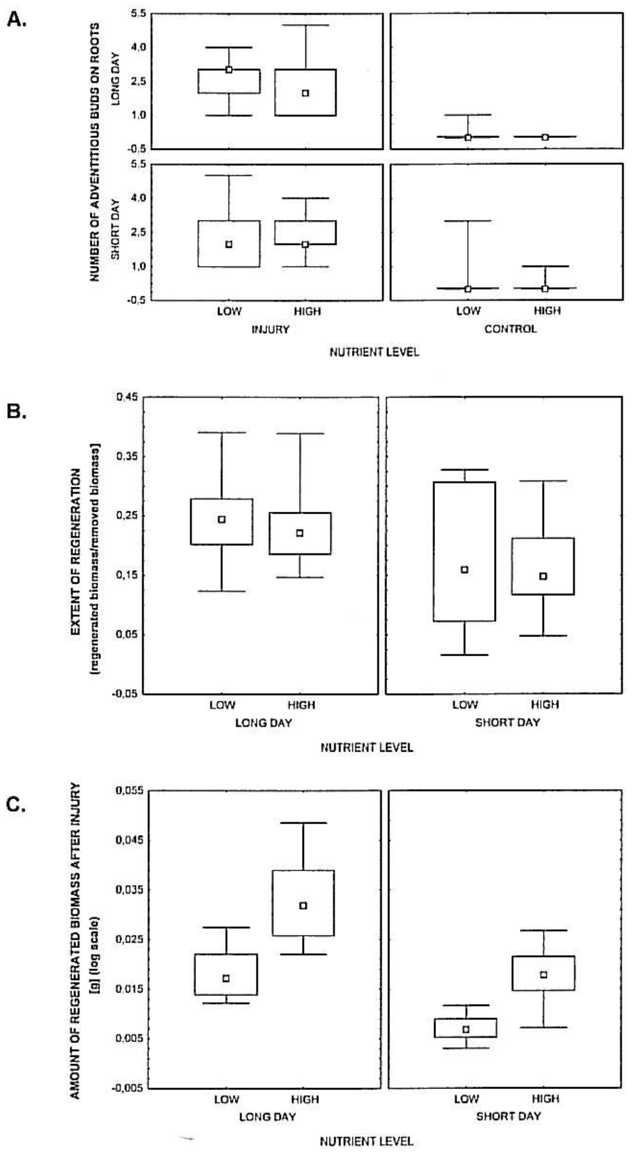

Fig. 2. Characteristics of resprouting in the chamber experiment with *Rorippa palustris*. A – number of adventitious buds on roots formed after injury; B – extent of regeneration expressed as a ratio of regenerated/removed biomass; C – amount of regenerated above-ground biomass after injury. CONTROL refers to individuals without injury. Low and high refer to nutrient levels. Median, quartiles and ranges are shown. For signifikance levels, Wald statistics, *n* and d.f. see Tab. 1.

RESULTS

RORIPPA CHAMBER EXPERIMENT

After two weeks, all injured individuals of *Rorippa palustris* resprouted from adventitious buds on roots in all experimental treatments of the chamber experiment (Fig. 1), even though the variability in the initial plant size was substantial at the time of injury. The number of new adventitious buds on roots was not related to initial plant size (rosette diameter: Wald statistics = 0.12, d.f.=1, n=120, $p>0.05$; number of leaves: Wald statistics = 0.262, d.f.=1, n=120, $p>0.05$). The number of new adventitious buds on roots was significantly affected only by injury (Wald statistics = 61.041, d.f. =1, n=120, $p<0.001$), even though some adventitious buds on roots were also formed in the control treatment. The day-length regime and nutrient level did not significantly affect the formation of adventitious buds (Fig. 2, Tab. 1). The amount of regenerated biomass was significantly affected by the day-length regime (Wald statistics = 98.433,d.f. =1, n=60, $p<0.001$) and also by nutrient level (Wald statistics = 84.38, d.f. =1, n=60, $p<0.001$). The amount of regenerated biomass was higher at the higher nutrient level and in the long-day light regime (Fig. 2, Tab. 1). Nevertheless, the extent of regeneration expressed as the ratio of regenerated/removed biomass was significantly affected only by the day-length regime (Wald statistics = 8.554, d.f.=1, n=60, $p<0.01$) (Fig. 2, Tab. 1). Rosette diameter and number of leaves were significantly affected by all factors (Tab. 1), but the reaction of these characteristics was different. Injury positively influenced rosette diameter and negatively affected the number of leaves. The amount of root biomass was also affected by all tested characteristics (Tab. 1). Injury, low nutrient level and short day-length regime reduced the amount of root biomass. Root/shoot ratio was significantly lower in the injury treatment than in the control and also in the long day-length regime, while nutrient level did not influence this characteristic (Tab. 1, Fig. 3). Rosette diameter measured at the time of injury was significantly affected by the nutrient level and day-length regime, but the number of leaves measured at the time of injury was affected only by the nutrient level, whereas the impact of day-length regime was non-significant (Tab. 1).

OENOTHERA FIELD EXPERIMENT

Both species *O. biennis* and *O. issleri* were able to resprout after injury from both axillary buds on stems and adventitious buds on roots equally (Fig. 1 and Fig. 5) (Wald statistics = 1.678, d.f.=1, n=160, $p>0.05$).

Vegetative and reproductive plants of both species regenerated more frequently in treatments where the root crown was left in place (Wald statistics = 11.031, d.f.=1, n=160, $p<0.001$) (Fig. 4). When the aboveground biomass of vegetative plants (rosettes) was removed and their root crown left in place, all injured individuals regenerated (Fig. 4).

Tab.1 Significance level and Wald statistics for all tested factors and measured characteristics in *Rorippa* chamber experiment. (***) $p<0.001$, (**) $p<0.01$, (*) $p<0.05$, n.s. = non-significant. D.f. = 1 for each test. Number of observations is 120, or 60 – in the case where effect of injury was not tested.

Measured characteristics	Factor	Wald statistics	p - level
Rosette diameter at the time of injury	Injury	-	-
	Nutrient level	93.53	***
	Day-length regime	28.34	***
Number of leaves at the time of injury	Injury	-	-
	Nutrient level	10.539	**
	Day-length regime	0.194	n.s.
Number of adventitious buds on roots	Injury	61.041	***
	Nutrient level	0.799	n.s.
	Day-length regime	0.0662	n.s.
Regenerated biomass	Injury	-	-
	Nutrient level	84.380	***
	Day-length regime	98.433	***
Extent of regeneration regenerated/removed biomass	Injury	-	-
	Nutrient level	0.755	n.s.
	Day-length regime	8.554	**
Rosette diameter after regeneration	Injury	7.483	**
	Nutrient level	63.535	***
	Day-length regime	62.357	***
Number of leaves after regeneration	Injury	138.021	***
	Nutrient level	24.665	***
	Day-length regime	5.613	*
Root biomass	Injury	194.39	***
	Nutrient level	106.14	***
	Day-length regime	60.00	***
Root/shoot ratio	Injury	15.950	***
	Nutrient level	2.551	n.s.
	Day-length regime	19.054	***

All these individuals regenerated only from axillary buds. On the contrary, not all treated reproductive plants resprouted (Fig. 4). The ability to resprout from adventitious buds on roots (treatment where all axillary buds were removed) was found in both injured vegetative and reproductive plants, but injured vegetative plants resprouted significantly more frequently (Wald statistics = 22.263, d.f.=1, n=80, $p<0.001$).

The number of regenerated individuals was affected by the time of injury only in reproductive plants (Wald statistics = 5.529, d.f.=1, n=80, p<0.05) and only the time of injury could be used as a predictor of resprouting probability (Wald statistics = 5.883, d.f.=1, n=80, p<0.05). The ability to regenerate was lower in August (fruiting plants) than in June (flowering plants). Vegetative plants (rosettes) were not significantly affected by the time of injury.

No relationship was found between the resprouting ability and measured characteristics at the time of injury (number of rosette leaves, number of fruits and length of stems, etc.).

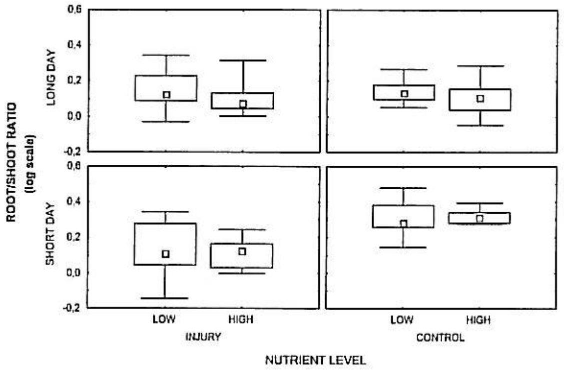

Fig. 3. Effects of injury, nutrient level and day-length regime on root/shoot ratio in *Rorippa* chamber experiment. Control refers to individuals without injury. Low and high refer to nutrient levels. Median, quartiles, ranges and significance levels are shown. For significance levels, Wald statistics, *n* and d.f. see Tab.1.

DISCUSSION

We found that the studied short-lived monocarpic species are able to cope with severe disturbance by resprouting from their roots. Thus, we confirm the historical studies of Irmisch (1857), Dubard (1903), Rauh (1937) etc., who described adventitious root sprouting in short-lived monocarpic species for the first time. Our results also suggest that the timing and severity of injury influence the extent of this ability, while the nutrient level have less important effect on resprouting in studied species.

TIMING OF INJURY

Experimental conditions (short vs. long day) influenced the growth of *Rorippa palustris.* Plants in the short-day treatment had prostrate rosettes with the apical meristem pushed into the ground, whereas plants cultivated in the long-day treatment had rosettes with erect leaves and aboveground apical meristem (Fig. 1). Geophyllous growth form of plants in the short-day treatment corresponded with our observations that plants germinating under late summer conditions behave as winter annuals. This fact was supported also by higher R/S ratio in plants cultivated in the short-day treatment, as this allocation is advantageous in spring regrowth (Hirose & Kachi 1982, Volenec et al. 1991, Bushway & Pritts 2002). Despite these growth differences, the resprouting ability was independent on life-history mode of experimental plants; injury was the most important factor causing an increase in the number of root buds in *Rorippa palustris.* The difference between plants of the same life-history mode was found only in the extent of regeneration (regenerated/removed biomass).

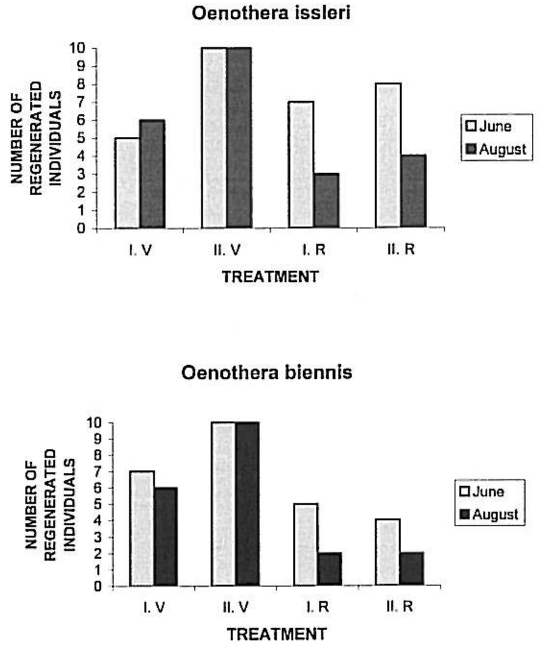

Fig. 4. Regenerated individuals for different injury treatments in a field experiment with *Oenothera* species. V-vegetative plants; R-reproductive plants; I. - removal of aboveground biomass together with the root crown; II. - removal of aboveground biomass, but the root crown left in place.

Plants cultivated under long-day conditions causing the annual life-history mode had more root biomass than winter annuals in our experiment, despite their lower R/S ratio. Higher root biomass may have caused more vigorous resprouting in annual plants than in winter annual plants. Aboveground biomass was probably also enhanced by more intensive compensatory photosynthesis in the long-day treatment (Meyer 1998). It seems that injury to the plant body is more risky for plants in the winter annual life-history mode that for plant in the annual life-history mode.

The life-cycle stage and phenological stage of treated *Oenothera* species had an important effect on their resprouting ability. Both vegetative and reproductive plants of *Oenothera* species were able to regenerate; nevertheless, vegetative plants resprouted more frequently than reproductive plants. This is in contradiction with the findings of Dubard (1903) and Rauh (1937), who reported successful resprouting of *Oenothera biennis* only in rosette stage and of the biennials *Alliaria officinalis* and *Bryonia dioica* only at the end of the first growing season. This pattern of resprouting ability throughout the life of monocarpic species can be interpreted as a consequence of accumulation of reserve assimilates during the vegetative phase and of their depletion during the reproductive phase of the life cycle (Dubard 1903, Chapin et al. 1990). Nevertheless, this allocation cannot be seen as a single switch of investment into vegetative and reproductive growth, but we can rather expect graded allocation as it was proposed earlier in trade-off models of monocarpic plants (King & Roughgarden 1982, Kudoh et al. 2002).

NUTRIENT AVAILABILITY

Nutrient level did not significantly affect the number of adventitious buds in our experiment. Plants at low nutrient level showed only a slight tendency to produce more adventitious buds on their roots than did plants at high nutrient level. These results were probably caused by the short duration of our experiment (8 weeks), because in another longer experiment (6 months) with *Rorippa palustris*, we found a significantly higher number of root buds in the nutrient stress treatment (Martínková et al., in press).

SEVERITY OF DISTURBANCE

As we expected, both *Oenothera* species definitely preferred resprouting from axillary buds, when at least only one axillary bud was still present. The growth of adventitious shoots from buds on roots was probably more energy demanding and resprouting from roots required a longer time. Alternatively, the presence of axillary buds could prevent adventitious root-sprouting in *Oenothera* species, because correlative inhibition of root buds can be broken by removal of the present leaves and apical or axillary buds (Horvath 1998, Horvath 1999).

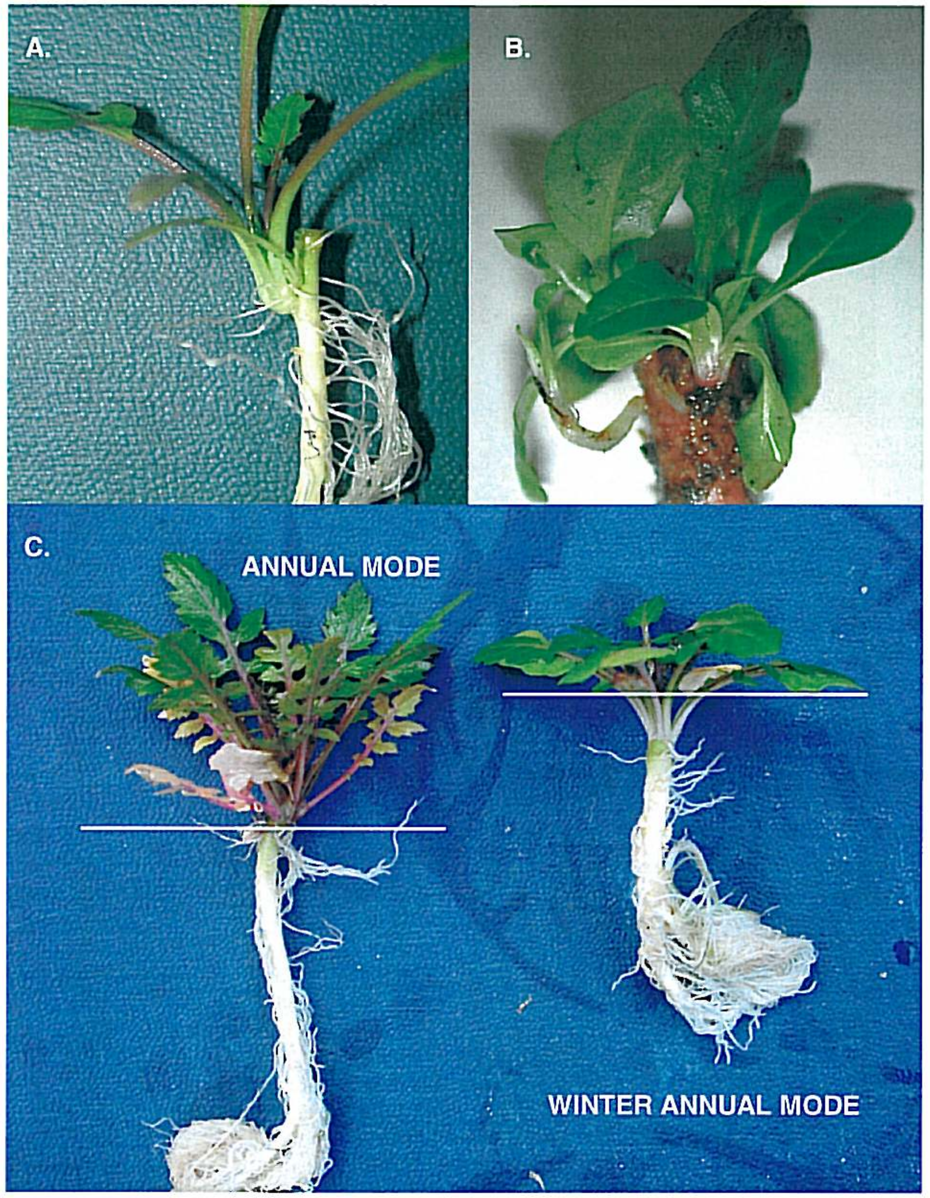

Fig. 1. Vegetative regeneration (resprouting) from adventitious buds on roots after experimental injury in *Rorippa palustris* (A) and *Oenothera issleri* (B). Different growth forms of non-injured plants of *Rorippa palustris* in annual and winter annual life-history mode (C) – ground level is shown.

Fig. 5. A – *O. issleri* individuals in different injury treatment of a field experiment. II.V – Removal of aboveground biomass of rosette without root crown (successful regeneration), I.R – removal of aboveground biomass of generative stem together with root crown (successful regeneration) II.R – removal of aboveground biomass of generative stem without root crown (regeneration fails); B – *Oenothera issleri* - spontaneous resprouting from adventitious buds on roots, plant was found in the field; C – *Oenothera biennis* – polycarpic individuum: I. – previous year generative stem, II. – current year generative stem, plant was found in the field.

Disturbance to plant body is a crucial event that may considerably influence the plant's future. In the case of short-lived monocarpic species with the ability to resprout from roots, the effect of disturbance is serious but not lethal. Even though the ability is influenced by many factors (e.g. nutrient level, timing of injury), root-sprouting species are still favored over injured species without this ability. Species forming a bud bank on roots are able to compensate for the loss of aboveground biomass by vegetative regeneration, and do not only rely on regeneration from seeds. Consequently, population extinction in root-sprouting species may be less probable than in other populations due to a higher survival of individuals with the ability of root-sprouting.

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CHAPTER III

Resprouting after disturbance in the short-lived herb *Rorippa palustris* (Brassicaceae): an experiment with juveniles

Martínková, J. Kočvarová, M. & Klimešová, J. (2004)
Acta Oecologica 25: 143–150

CHAPTER III

Resprouting after disturbance in the short-lived herb *Rorippa palustris* (Brassicaceae): an experiment with juveniles

Abstract

The impact of plant age, severity of injury and nutrient levels on the ability to resprout from roots was experimentally assessed in juveniles of the short-lived herb *Rorippa palustris* (L.) Besser.

In a chamber experiment, six cohorts of young plants (1–6 weeks old) were injured to obtain data on the threshold age for the ability to resprout from roots. We found that plant age was an important factor influencing resprouting ability: injured individuals older than 5 weeks were able to resprout, but not plants younger than 3 weeks.

The impact of injury severity (defoliation and removal of axillary buds) and nutrient levels on resprouting ability was assessed on juveniles in a greenhouse experiment. Injury induced growth of new shoots from root buds, while the number of adventitious buds on roots was not influenced by injury. Both injury treatments had a similar effect in this respect, and the amount of regenerated biomass and the extent of regeneration were not different among injury treatments. The number of new shoots produced after injury was higher at the high nutrient level, but the number of formed adventitious buds on roots was not influenced by nutrient level. Nutrient level also influenced the amount of regenerated biomass, but the extent of regeneration (regenerated/removed biomass) was not influenced.

The short-lived monocarpic species *R. palustris* is able to resprout from roots relatively easily. This ability seems to be advantageous in disturbed habitats and this idea is discussed throughout the paper.

Key-words: *adventitious bud on root, axillary bud, defoliation, injury,* Oenothera biennis, *root crown*

INTRODUCTION

Disturbance is one of the most important phenomena occurring in nature, and creates plant life strategies (e.g. Schippers et al., 2001). Short-lived monocarpic species (annuals, biennials and short-lived monocarpic perennials) are less efficient competitors and occur mostly in irregularly and unpredictably disturbed habitats (e.g. Grime, 2001). Disturbance generates areas of bare soil, where such species are able to germinate, grow and survive (Harper, 1977; Klemow and Raynal, 1983; Löfgren et al., 2000; Grime, 2001). However, disturbance is also the main factor causing early mortality or severe injury to these species. Short-lived monocarpic species solve this problem by minimisation of the vegetative phase, intensive seed production (Harper, 1977; Crawley, 1986; Van der Meijden et al., 1992; Grime, 2001) and also by vegetative regeneration (Klimešová, in press). However, information on vegetative regeneration (resprouting) after disturbance is scarce (Klimešová and Klimeš, 2003).

Resprouting of short-lived monocarpic species is realised through a bud bank (Harper, 1977). The majority of biennial and plurennial species have a bud bank along truncated stems with many axillary buds originating in overwintering rosettes (Krumbiegel, 1999). This truncated stem is usually pulled under the soil surface by contractile roots. When the whole aboveground biomass is destroyed or removed, plants are able to resprout from axillary buds located under the soil surface. In the case of severe disturbance penetrating the upper soil layer, loss of all axillary buds is highly possible and resprouting is threatened. However, some species are able to resprout from buds on roots in spite of such severe disturbance. Only 39 short-lived monocarpic species occurring in central Europe have been reported to be able resprout from adventitious buds on roots, e.g. *Barbarea vulgaris, Oenothera biennis* and *Rorippa palustris* (Klimešová, in press). Information about the ability to resprout from roots is incomplete, descriptive and mostly obtained from historical morphological literature (Wittrock, 1884; Dubard, 1903; Holm, 1925; Rauh, 1937), and the ecology of this phenomenon is largely unclear.

Although a large proportion of root-sprouting species are able to resprout from roots spontaneously (Rauh, 1937; Peterson, 1975), the formation of adventitious buds on roots and the growth of new shoots is frequently conditioned or stimulated by injury (Peterson, 1975). Removal and injury of aboveground biomass may facilitate the formation of adventitious root buds or break their dormancy (Rauh, 1937; Peterson, 1975). Defoliation, decapitation or removal of all axillary buds are examples of stimuli initiating the growth of adventitious buds (McIntyre, 1972; Horvath, 1999), but exact mechanisms remain unknown (Peterson 1975). In a previous study, Martínková et al. (in press) found that the number of newly initiated adventitious buds on roots is markedly supported by injury to vegetative plants of *Rorippa palustris*, and differ with severity of disturbance in *Oenothera* species.

The ability to resprout can vary during plant development, because the number of buds increases with plant age (Esau, 1965; Harper, 1977), availability of stored nutrients (Burns et al., 1997), and also with meristematic activity and secondary thickening (Esau, 1965). Peterson (1975) reported that the poorest sprouting from root buds occurs at the time of flowering, but these observations were probably only from perennial species. Rauh (1937) found that the biennial species *Oenothera biennis* is able to sprout from roots only during the rosette stage. However, Martínková et al. (in press) found that this species is also able to sprout from roots during the generative phase, but less successfully. This indicates that the ability to sprout from roots is related to plant life-history stage, and that plant age plays an important role in the ability to resprout.

An essential difference between adventitious buds on roots and axillary buds on stems is that adventitious buds need to differentiate from root cells (Esau, 1965; Peterson, 1975; Burrows, 1990), while axillary buds are fully differentiated during normal plant ontogenesis (Esau, 1965; Harper, 1977). Thus, we assume that resprouting from axillary buds is faster and, in terms of energy costs, more advantageous than resprouting from root buds.

Levels of nutrients also have an important effect on regenerative ability of injured plants (Bellingham and Sparrow 2000; Grime 2001), but the relationship between bud formation, shoot initiation and nutrient level is unclear. In one study, higher nutrient levels supported bud formation and initiation, broke down bud dormancy and facilitated shoot growth (Peterson, 1975). However, in another study with *Chondrilla juncea* an opposite effect was found (Kefford and Caso, 1972). McIntyre and Hunter (1975) found that root buds of *Euphorbia esula* are initiated more frequently on plants growing at low nutrient levels than at high nutrient levels and shoots develop from established root buds more frequently in environments with higher nutrient availability than at low nutrient levels. Similarly, Klimeš and Klimešová (1999) found that nutrient shortage facilitates the formation of root buds in *Rumex acetosella*, but that their growth and transition to shoots is stimulated at higher nutrient levels. Moreover, in the model of Iwasa and Kubo (1997), plants grown under low nutrient availability might have similar architecture as plants grown under low disturbance. This means that plants experiencing nutrient shortage or disturbance form shorter stems and higher numbers of stems than plants at higher nutrient levels or uninjured plants. Results from a previous study (Martínková et al., in press) suggest that in adult vegetative plants of the herb *Rorippa palustris* the number of newly initiated adventitious buds on roots after injury is not affected by nutrient level. Consequently, in the present study we aim to assess the following hypotheses using juveniles of the short-lived monocarpic species *Rorippa palustris* (L.) Besser.

(i) The ability to resprout from roots after injury is related to the age of juveniles. Older juveniles regenerate more successfully and sooner than younger juveniles.

(ii) Due to preserved axillary buds, plants under mild disturbance regenerate faster and more vigorously than plants under severe disturbance.

(iii) Experimental plants at low nutrient level form a greater number of adventitious buds on roots than plants growing at high nutrient level, but regenerative growth is suppressed under low nutrient conditions.

MATERIALS AND METHODS

STUDY SPECIES

R. palustris (L.) Besser (Brassicaceae) is a native European species occurring mostly in primary or secondary open habitats, i.e. river and pond beds, anthropogenic and other wet disturbed habitats (Tomšovic, 1992). Vegetative plants are characterised by a distinct rosette and generative plants by a semi-rosette with elongate fruiting stems. The species is an annual, winter annual or short-lived perennial species (Klimešová et al., in prep.). The life-history mode is determined by the time of germination; germination from April to June causes the annual life-history mode, while winter annuals germinate later in the vegetative season (Sosnová, 2003). Spontaneous polycarpy, repeated flowering induced by injury, and resprouting from adventitious buds on roots have been observed in this species (Klimešová, in press).

GROWTH CHAMBER EXPERIMENT

A growth chamber experiment with *Rorippa palustris* juveniles was performed in the early spring of 2002. Six different age groups (cohort) of juveniles were cultivated in a growth chamber (KLIMABOX ZVK E-008, Váha Kladno, Czech Republic) in a full-factorial design: Luminosity 184 $\mu Em^{-2}s^{-1}$; DAY 15 h / 23 °C / 70% humidity; NIGHT 9 h / 10 °C / 90% humidity. These conditions simulated climatic conditions of spring and induced the annual life-history mode in *R. palustris*. To obtain different age cohorts, seedlings were sown and germinated six times at exactly 7-day intervals. Germination conditions were identical to the conditions in the growth chamber. Immediately after germination (4 days after sowing) seedlings were transplanted into the pots (one seedling per pot, diameter 9 cm) and placed in the growth chamber. Herewith, we obtained six cohorts ranging from one to six weeks of age with 15 replicates for each cohort.

Plants of each cohort were randomly allocated to 3 groups. Plants of the first group were injured (REGENERATION), plants in the second one served as controls at the time of injury (CONTROL 1), and plants in the third group were controls for regeneration (CONTROL 2).

(i) REGENERATION – Plants were injured after one week from the transplantation into the pots. Thus, the oldest cohort was 6-week-old, and the youngest one was 1-week-old at the time of injury. All individuals were in the vegetative phase at the time of injury. All aboveground biomass of the juvenile plants was removed together with their root crown to simulate severe injury to the plant body. The root crown is at the base of the stem and contains many axillary buds, which are partly pulled into the soil by root contraction. When the root crown was removed, all axillary buds were destroyed and only the root was left in place, therefore only regeneration from adventitious buds on roots was possible. Aboveground biomass (removed by injury) of these injured juveniles was recorded.

(ii) CONTROL 1 – Plants were harvested at the time of injury to the first group to measure belowground biomass, number of leaves, rosette diameter and aboveground biomass of juveniles in all six cohorts.

(iii) CONTROL 2 – Plants were harvested at the end of the experiment (4 weeks after the injury) to measure the following characteristics of uninjured individuals: belowground biomass, number of leaves, rosette diameter, aboveground biomass and also height of flowering stems (in the case of plants which initiated the generative phase during the experiment).

The experiment was terminated and regeneration evaluated 4 weeks after the injury. The number of days from injury to the start of resprouting (shoot appearance on soil surface) was recorded. The number of leaves, rosette diameter, aboveground and belowground biomass, number of adventitious buds on roots and number of new shoots were evaluated on injured individuals. The extent of regeneration, based on the ratio of regenerated biomass to removed biomass and the number of adventitious meristems (adventitious root buds on roots + new shoots) was calculated.

GREENHOUSE EXPERIMENT

Seeds of *Rorippa palustris* were germinated in late August 2001 under greenhouse conditions, without any artificial temperature regulation (approximate day-night temperature fluctuation: 10–15 °C). Germination and consequential growth of plants in these late-summer conditions cause the winter annual life-history mode in this species. Four-day old seedlings were transplanted into pots (one seedling per pot, diameter 9 cm) with three different nutrient levels (high, medium and low). These three nutrient levels corresponded to the three soil mixtures that were prepared from pure washed sand and standard garden soil (N 400 mg/l, P_2O_5 300 mg/l, K_2O 400 mg/l, pH = 6.0). A volume ratio of 1:2 of garden soil and sand was mixed for the low nutrient level, a ratio of 1:1 for the medium nutrient level, and only garden soil was used for the high nutrient level. All pots were placed in the greenhouse in a full-factorial design with 15 replicates for each nutrient level.

Plants of each nutrient level were randomly allocated to 3 groups. Two different types of injury were applied on two groups after eight weeks from germination, one group served as control.

(i) The first group of plants was defoliated. All axillary and apical buds were left in place.

(ii) In the second group of plants, all aboveground biomass of vegetative rosettes was removed together with the root crown.

(iii) The third group acted as a control group without injury.

All individuals in the experiment were in the vegetative phase at the time of injury. The number of leaves and rosette diameter were evaluated on both injured and control plants at the time of injury. The aboveground biomass was measured only on injured individuals.

Immediately after injury, all pots including controls were transferred out of the greenhouse into a common garden and thus subjected to natural autumn/winter conditions. The plants were returned to the greenhouse in the beginning of March 2002 and resprouting ability was evaluated four weeks later. The number of leaves, rosette diameter, aboveground and belowground biomass, number of adventitious buds on roots and the number of new shoots were measured on all plants. The extent of regeneration (ratio of regenerated and removed biomass) was calculated only for injured individuals and the number of adventitious meristems (adventitious root buds + new shoots) was calculated for both control and injured plants.

STATISTICAL ANALYSES

All statistical analyses were carried out using the STATISTICA 5.5 package. Parameters were log transformed when necessary. The experiments were evaluated by one-way ANOVA (effect of the cohort), two-way ANOVA (effect of nutrient level and injury) both in the Generalised Linear Model (Poisson or Gamma distribution, log function) and by Single Regression also in the Generalised Linear Model (Poisson or Gamma distribution, log function). To estimate significance of individual factors in the Generalised Linear Models, the Wald statistics were used.

RESULTS

AGE (GROWTH CHAMBER EXPERIMENT)

Regeneration success was directly related to age of juveniles (Table 1). All five injured individuals in the oldest cohort (6-week-old) regenerated, i.e. resprouted from roots after injury. Four individuals resprouted in the 5-week-old cohort, and in the 4-week-old cohort only two out of five injured individuals regenerated. None of the 1–3-week-old plants regenerated after injury. Control plants without injury did not form any adventitious shoots. Two individuals flowered (one from the 6-week-old cohort and one from the 5-week-old cohort) in the control group at the end of experiment.

Older individuals regenerated significantly faster (approximately 7 days after injury) than younger plants, which regenerated 10 days after injury (Wald statistic = 439.28; $p<0.001$). All adventitious buds formed on roots were used for new shoot growth, thus the number of adventitious meristems was equal to the number of adventitious buds and also to the number of new shoots. A greater number of adventitious meristems on roots (approximately five) was found for the 6-week-old cohort than for younger individuals (approximately one in both the 5- and 4-week-old cohort) (Wald statistic = 11.31; $p<0.01$).

Growth characteristics of injured individuals were also related to their age (Table 1). Older injured plants had a higher number of leaves (Wald statistic = 46.34; $p<0.001$), greater rosette diameter (Wald statistic = 35.93; $p<0.001$), higher amount of regenerated biomass (Wald statistic = 89.72; $p<0.001$), higher amount of root biomass (Wald statistic = 30.15; $p<0.001$) and higher extent of regeneration (Wald statistic = 24.10; $p<0.001$) than younger plants. No resprouting was observed in injured seedlings with less than 10 leaves, a rosette diameter of less than 14 cm and with an aboveground biomass of less than 0.14 g at the time of treatment (Fig. 1).

Table 1. Growth characteristics for controls and injured plants measured on six cohorts of *R. palustris* juveniles at the time of injury (Control 1) or at the end of experiment, i.e. 4 weeks after the injury of treated plants (Control 2, injured plants). Means and standard errors of mean are shown. Number of reg. plants – number of regenerated plants, time to reg. – time to first new shoot appearance on soil surface, number of adv. merist. – number of adventitious meristems, reg. biomass - regenerated biomass, extent of reg. – extent of regeneration (regenerated / removed biomass)

Cohort	**Characteristics at the time of injury control 1**				**Characteristics at the end of the experiment control 2**			
Age at the time of injury [weeks]	Number of leaves	Rosette diameter [cm]	Root biomass [mg]	Shoot biomass [mg]	Number of leaves	Rosette diameter [cm]	Root biomass [g]	Shoot biomass [g]
CONTROL UNINJURED PLANTS								
1	1.0 ± 0.00	1.4 ± 0.24	0.88 ± 0.06	0.7 ± 0.09	13.8 ± 0.58	18.4 ± 1.04	0.12 ± 0.01	0.51 ± 0.04
2	4.8 ± 0.20	3.96 ± 0.13	2.84 ± 0.61	9.48 ± 0.73	18.4 ± 1.36	18.4 ± 1.03	0.37 ± 0.09	0.93 ± 0.13
3	7.4 ± 0.40	8.84 ± 0.79	10.08 ± 1.27	42.68 ± 4.44	22.2 ± 1.36	20.0 ± 1.45	0.63 ± 0.08	1.51 ± 0.13
4	10.4 ± 0.60	15.3 ± 1.47	43.12 ± 6.66	151.1 ± 22.91	26.4 ± 1.20	22.0 ± 0.32	1.02 ± 0.05	2.16 ± 0.18
5	15.4 ± 1.03	17.7 ± 1.41	130.54 ± 16.52	543.98 ± 80.00	29.5 ± 1.32	20.75 ± 0.75	1.26 ± 0.15	2.41 ± 0.30
6	21.6 ± 1.86	19.7 ± 0.98	209.11 ± 32.62	534.64 ± 63.41	30.75 ± 2.10	22.5 ± 1.26	1.52 ± 0.10	3.03 ± 0.15

Cohort	**Characteristics at the end of the experiment**							
Age at the time of injury [weeks]	Number of reg. plants	Time to reg. [days]	Number of adv. merist.	Number of leaves	Rosette diameter [cm]	Reg. biomass [g]	Root biomass [g]	Extent of reg.
REGENERATION OF INJURED PLANTS								
1	0	-	-	-	-	-	-	-
2	0	-	-	-	-	-	-	-
3	0	-	-	-	-	-	-	-
4	2	10.0 ± 3.00	1.0 ± 0.00	5.5 ± 1.50	7.0 ± 0.50	0.02 ± 0.01	0.03 ± 0.01	0.08 ± 0.05
5	4	10.5 ± 1.50	1.5 ± 0.50	9.6 ± 3.20	13.13 ± 1.82	0.16 ± 0.04	0.27 ± 0.03	0.38 ± 0.05
6	5	7.4 ± 0.40	5.2 ± 0.73	25.8 ± 2.24	16.5 ± 0.96	0.26 ± 0.04	0.43 ± 0.07	0.32 ± 0.05

NUTRIENT LEVEL AND SEVERITY OF INJURY (GREENHOUSE EXPERIMENT)

All injured individuals regenerated at all nutrient levels and in both types of injury. The number of adventitious buds on roots was different between nutrient levels (Table 2). Plants growing at a high nutrient level had a lower number of buds on their roots than plants at lower nutrient levels. There was no impact of injury severity on bud formation (Table 2), however the number of new shoots was significantly influenced by both nutrient level and injury severity (Table 2). In contrast to the trends for the number of adventitious buds, a higher number of new shoots was found in the high nutrient treatment. Nevertheless the sum of adventitious buds on roots and new shoots (number of adventitious meristems) did not differ either among nutrient levels or injury treatments (Table 2). Rosette diameter at the time of injury was significantly influenced by nutrient level, but the number of leaves at the time of injury was the same for all nutrient levels (Table 2). No correlation was observed between number of adventitious buds, number of new shoots and rosette diameter on the one hand and number of leaves at the time of injury on the other.

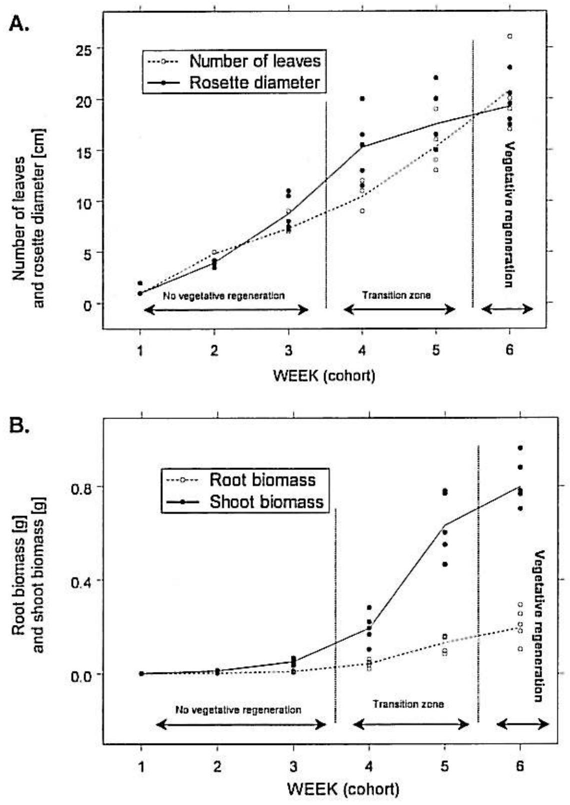

Fig. 1. Growth characteristics for six cohorts of juveniles of *R. palustris* at the time of injury. A. – number of leaves and rosette diameter (cm) were obtained from injured individuals. B. – shoot biomass (g) = removed biomass of injured plants and root biomass (g) was obtained from control 1. Values for individual plants are shown. The curves were fitted by local polynomial regression fitting (loess) using Friedman's super smoother. Three types of zones in each plot are given. No vegetative regeneration zone – no juveniles in these cohorts resprouted from roots after injury. Transition zone – some juveniles in these cohorts resprouted from roots after injury. Vegetative regeneration – all juveniles resprouted from roots after injury.

Both nutrient level and severity of injury significantly influenced rosette diameter during regeneration (Table 2), whereas only nutrient level had an impact on the number of leaves after regeneration (Table 2), with high nutrient levels resulting in larger rosettes and greater numbers of leaves. Defoliation led to a higher leaf number at the high nutrient level, while removal of the root crown led to a higher leaf number at the low nutrient level (Table 2, Fig. 2). Both severity of injury and nutrient level significantly influenced root biomass (Table 2). In the high nutrient treatment significantly more roots were produced

than in the medium and low level treatments and removal of aboveground biomass (injury) of plants resulted in decreased root biomass (Table 2).

Table 2. Growth characteristics of *R. palustris* juveniles in an experiment manipulating two factors – nutrient level and type of injury. Wald statistics and p-levels are shown for both factors. Means and standard errors of means are shown for each treatment. RC removal – removal of root crown, Control – uninjured plants. (***) p<0.001, (*) p<0.05, n.s. - non-significant.

CHARACTERISTIC	FACTOR		NUTRIENT LEVEL			INJURY	
	TREATMENT	LOW	MEDIUM	HIGH	DEFOLIATION	RC REMOVAL	CONTROL
Rosette diam. at the time of injury [cm]							
	Wald and p-level		16.08 ***			-	
	mean ± error	11.27 ± 0.47	12.86 ± 0.41	16.55 ± 0.53	-	-	-
Rosette diam. after regeneration [cm]							
	Wald and p-level		15.96 ***			7.82 *	
	mean ± error	7.57 ± 0.74	10.88 ± 0.69	11.37 ± 0.80	8.64 ± 0.92	9.54 ± 0.71	11.65 ± 0.76
Number of leaves at the time of injury							
	Wald and p-level		0.94 n.s.			-	
	mean ± error	10.26 ± 0.27	9.8 ± 0.24	10.93 ± 0.25	-	-	-
Number of leaves after regeneration							
	Wald and p-level		20.36 ***			2.06 n.s.	
	mean ± error	15.73 ± 1.54	17.13 ± 0.83	22.47 ± 1.25	17.2 ± 1.82	18.73 ± 1.13	19.4 ± 1.29
Number of adv. buds							
	Wald and p-level		6.98 *			0.19 n.s.	
	mean ± error	7.8 ± 1.80	6.13 ± 1.28	5.4 ± 0.1	6.27 ± 1.63	6.4 ± 1.15	6.67 ± 1.45
Number of new shoots							
	Wald and p-level		13.70 *			13.19 *	
	mean ± error	1.0 ± 0.45	1.27 ± 058	2.66 ± 0.80	2.07 ± 0.79	2.27 ± 0.52	0.6 ± 0.53
Number of adventitious meristems							
	Wald and p-level		0.37 n.s.			0.39 n.s.	
	mean ± error	8.8 ± 1.77	7.4 ± 1.32	8.07 ± 1.70	8.67 ± 1.31	8.33 ± 1.87	7.27 ± 1.60
Regenerated biomas [g]							
	Wald and p-level		7.11 *			0.001 n.s.	
	mean ± error	0.06 ± 0.01	0.1 ± 0.02	0.17 ± 0.04	0.21 ± 0.04	0.17 ± 0.02	-
Extent of regeneration							
	Wald and p-level		1.54 n.s.			0.13 n.s.	
	mean ± error	0.84 ± 0.43	0.79 ± 0.52	0.81 ± 0.42	0.79 ± 0.39	0.81 ± 0.42	-
Root biomass [g]							
	Wald and p-level		21.50 ***			6.93 *	
	mean ± error	0.14 ± 0.01	0.21 ± 0.03	0.31 ± 0.04	0.21 ± 0.04	0.17 ± 0.02	0.27 ± 0.03

The amount of regenerated biomass was higher at the high nutrient level than at medium and low nutrient levels (Table 2), but was not influenced by the severity of injury (defoliation vs. removal of root crown). The extent of regeneration expressed as the ratio between regenerated and removed biomass was not influenced by either nutrient level or severity of injury, so that the amount of regenerated biomass was directly related to the amount of biomass removed (Table 2).

DISCUSSION

Results of experiments with juvenile plants of the short-lived monocarpic species *R. palustris* showed that these plants are easily able to resprout from roots after injury. However, plants of this species are only able to resprout after reaching a certain age. The ability to resprout also varied with nutrient level and severity of disturbance, but these two factors affected resprouting ability less than plant age.

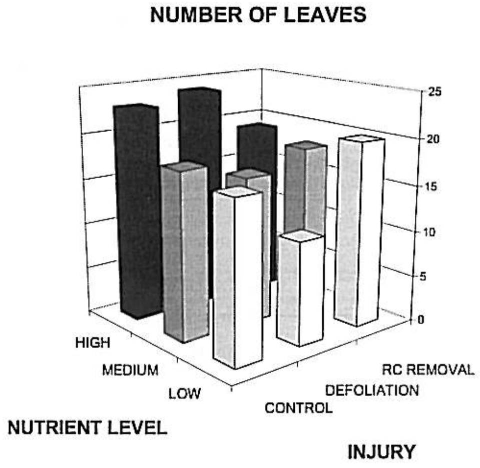

Fig. 2. Number of leaves regenerated after injury at different nutrient levels and injury treatments compared with leaves in the control treatment without injury. Data were obtained from the greenhouse experiment with juvenile plants of *R. palustris*. RC removal – removal of root crown.

PLANT AGE

Resprouting following injury in plants from the growth chamber experiment was dependent on the age of injured individuals. Only plants older than 3 weeks were able to resprout. According to this and the previous study of Martínková et al. (in press), we suggest that resprouting ability in the vegetative stage of the plant's life cycle is age-dependent and that plants must reach a certain age to be able to resprout from roots. Very young seedlings are unable to form adventitious buds on roots because the ability of root-sprouting is connected to sufficient assimilation and to the presence of activated meristem tissue (Esau, 1965; Peterson, 1975). Moreover, all adventitious buds formed on roots of these young injured plants were used for growth of new shoots, thus no dormant root buds were left in place for a possible next disturbance.

It also seems that plant size plays a less important role in resprouting ability than plant age. Injured plants from the growth chamber experiment reached values of plant size characteristics (number of leaves, rosette diameter) comparable to the values of plants in the greenhouse experiment. Nevertheless, younger plants from the chamber experiment were not so successful in resprouting as older plants in the greenhouse.

INJURY SEVERITY

Defoliation or removal of aboveground biomass with all axillary buds was applied to juvenile plants to simulate different severity of disturbance. All injured plants resprouted from roots regardless of the type of injury, whereas almost no new shoots were formed in control plants. Thus, we can conclude that injury induced growth of new shoots. These findings correspond to the literature (Rauh, 1937; Peterson, 1975). The number of adventitious meristems was the same in the two types of injury and the control group, indicating that *R. palustris* can produce preformed adventitious buds on roots without injury. Therefore, *R. palustris* produces adventitious buds on their roots, but do not resprout from them until injured. A similar strategy is described in *Rumex sanguineus* and *Geranium sanguineum* (Rauh 1937). Moreover, injured *Rorippa palustris* juveniles saved some preformed adventitious buds on roots for a possible next disturbance. This strategy of older juveniles is different from the strategy of younger juveniles, which used all available adventitious buds for regenerative regrowth. In contrast to *R. palustris*, the short-lived perennial species *Oenothera biennis* does not have preformed adventitious buds on its roots at all. In *Oenothera biennis*, root buds together with new shoots are produced exclusively after injury causing removal of all axillary buds (Martínková et al., in press). Thus, the extent of injury necessary for activation or formation of adventitious buds on roots is different among species.

Modelling of herbivore pressure and axillary buds behaviour suggests that species with highly dormant buds are adapted to strong herbivore pressure, while species with an easy and vigorous release of buds are adapted to weak disturbance (Tuomi et al, 1994; Lehtilä 2000). Applying this hypothesis to adventitious buds on roots, *O. biennis* is adapted to frequent disturbance, while *R. palustris* is adapted to relatively rare disturbance. Typical habitats of *Rorippa palustris* in the Czech Republic are river or pond banks, emerged bottoms of fishponds and wet fertile human-made habitats (Klimešová et al., in prep.), whereas typical habitats of *Oenothera biennis* are anthropogenic habitats along railways, railway yards, and other highly disturbed habitats. Therefore, *Oenothera biennis* is exposed to disturbance more frequently than *Rorippa palustris*, thus higher dormancy of adventitious buds on roots is an adaptive strategy in this species.

Neither the amount of regenerated biomass nor extent of regeneration was different between the two injury treatments. We hypothesised that defoliated plants still possessing axillary buds would show faster regrowth due to the preserved axillary buds than plants in which the root crown was removed together with all axillary buds. However, regeneration was equal in both treatments and plants compensated for loss of biomass to the same extent. This result can be explained by the presence of preformed adventitious buds, which are comparable to axillary buds, with which injured plants are able to respond rapidly to injury. This results suggests a hypothesis that the presence of preformed adventitious buds allowing rapid regeneration in *Rorippa palustris* individuals is undoubtedly favourable when all aboveground biomass is removed through injury.

NUTRIENT LEVEL

In the greenhouse experiment, numbers of adventitious buds, growth of new shoots, and the amount of regenerated biomass were influenced by the level of nutrients. Higher nutrient levels resulted in more vigorous plant growth, but did not increase values of regenerative characteristics. High nutrient level frequently causes more intensive branching in rhizomatous plants (Klimešová and Klimeš, 1997) leading to a higher number of axillary buds. However, we found no effect of nutrient level on the formation of adventitious meristems on roots. Thus the higher number of new shoots grown after injury at high nutrient levels was only due to the release of a greater proportion of adventitious buds. These results are consistent with findings obtained from perennial root sprouters (Peterson, 1975; McIntyre and Hunter, 1975; Klimeš and Klimešová, 1999). Therefore, our results support the hypothesis that a higher nutrient level facilitates the growth of new shoots. Although nutrient levels influenced the amount of regenerated biomass, the extent of regeneration (ratio of regenerated/removed biomass) was not influenced. The extent of regeneration was directly correlated to the amount of removed biomass, but tended to be higher at the high nutrient level. This is likely due to the fact that resources utilised for the production of new biomass are stored in roots and, since plants growing at a high nutrient level have a larger root system, more energy for regeneration after injury is available.

The ability of some short-lived monocarpic species to cope with severe disturbance by resprouting seems to be a crucial life-history trait in disturbed habitats. Accumulated resources and established genotypes are not lost if injured plants can survive, produce inflorescences and set seeds. These results indicate that populations of short-lived species with the ability to sprout from roots would be favoured over populations of short-lived monocarpic species without this ability in disturbed environments. Populations of short-lived monocarpic species have been reported as very unstable at the metapopulation level (Van der Meijden et al., 1992). The fact that some short-lived monocarpic species are able to survive severe disturbance and regenerate from roots should change our perception of such species and their populations. Our findings emphasise the importance of this strategy and its relation to distribution, coexistence and population dynamics of short-lived monocarpic species.

CONCLUSIONS

(i) Resprouting ability after injury is age-dependent in the vegetative stage. Therefore, *Rorippa palustris* juveniles have to reach a certain age to be able to resprout from roots after injury.

(ii) Severity of injury does not affect resprouting ability. The preference for resprouting from axillary or adventitious buds does not exist in *Rorippa palustris* due to preformed adventitious buds on roots.

(iii) Release of adventitious buds and growth of new adventitious shoots is facilitated at higher nutrient levels, however, the number of created adventitious meristems is not affected by nutrient level.

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CHAPTER IV

Resprouting after disturbance in the short-lived herb *Barbarea vulgaris* (Brassicaceae): effect of nutrient level, timing and severity of injury

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[manuscript]

CHAPTER IV

Resprouting after disturbance in the short-lived herb *Barbarea vulgaris* (Brassicaceae): effect of nutrient level, timing and severity of injury

Abstract

Barbarea vulgaris is a short-lived herb occurring in habitats exposed to frequent and severe disturbance, e.g. in man-made ones. In spite of a short life cycle, this species may resprout after a severe injury to its body, i.e. even after 100% removal of aboveground biomass, from preserved basal axillary buds or from adventitious buds on roots. Since resprouting is influenced by many factors, we carried out a three-year garden experiment to assess the effect of nutrient level, severity and timing of injury on resprouting and consequential seed production in this species.

Plants of *B. vulgaris* were cultivated at two nutrient levels (low *vs.* high), and two levels of injury severity were applied (high – removal of all aboveground biomass, only root left intact – *vs.* low – removal of aboveground biomass, but leaving basal axillary buds intact). Injuries were made in four life-cycle phases (young rosette, overwintered rosette, flowering plant, fruiting plant). All plants survived the experimental injury and resprouted irrespectively of life-cycle phase, severity of injury and nutrient availability. Nutrient level only marginally affected resprouting after injury and seed production. Injury significantly affected seed production and also the life-cycle of plants. Plants injured in the second year of life, i.e. in overwintered rosettes, flowering and fruiting phases, postponed reproduction to the third season (in the case of high injury) or their seed production markedly decreased (in the case of low injury). In plants injured in the first year of life, i.e. in the young rosette phase, seed production and life-cycle were not influenced.

The experiment showed that the short-lived herb *B. vulgaris* may efficiently utilise the whole bud bank, i.e. both axillary buds and adventitious buds on roots, for regrowth after a disturbance. The herb successfully copes with disturbance by resprouting and does not rely only on its seed bank.

INTRODUCTION

Based on studies concerning individual responses of trees to disturbance, two strategies were described: seeders and resprouters (Meney et al. 1997, Bellingham and Sparrow 2000, Sparrow and Bellingham 2001, Pausas 2001, Vesk and Westoby 2004). Seeders are killed by disturbance and their populations re-establish from the seed bank, while resprouters survive disturbance as individuals and resprout from preserved vegetative parts (Kruger et al. 1997, Bell 2001). In disturbed habitats exposed to serious aboveground biomass removal, allocation of woody species to the resprouting or seeding strategy depends on frequency of acting disturbance (Bellingham and Sparrow 2000). Seeders prevail in severely disturbed habitats with a low or moderate frequency of disturbance, while the sprouting strategy dominates in severely disturbed habitats with a high frequency of disturbance (Bellingham and Sparrow 2000, Vesk and Westoby 2004). Even though resprouting has become a generally accepted strategy of tree species in severely and frequently disturbed habitats (Bond and Midgley 2001, Hunter 2003, Pausas et al. 2004, Vesk and Westoby 2004), in herbs it is still incorrectly claimed that short-lived ones occupying such habitats, i.e. man-made ones or arable land, regenerate after damage of all aboveground biomass only from seeds (Harper 1977, Grime 2001). However, there is a significant number of annual, biennial and short-lived perennial herbs that survive removal of 100% of aboveground biomass or root fragmentation and which, in spite of such severe injury to their body, resprout and finish the reproductive cycle (Klimešová 2003, Martínková et al. 2004a and b, Klimešová and Martínková, in press). For resprouting after injury, woody species as well as short-lived herbs exploit the bud bank (Harper 1977), i.e. axillary buds on preserved parts of the stem and adventitious buds on roots.

Even though short-lived herbs possess the ability of resprouting after disturbance, there are several factors that may freeze this ability or negatively influence compensatory growth: severity of injury, plant's age, life-cycle phase, amount of stored reserves at the time of injury, and nutrient availability (McNaughton 1983, McAllister and Haderlie 1985, Chapin et al. 1990, Kays and Canham 1991, Sosnová 2003, Martínková et al. 2004a and b, Klimešová and Martínková, in press,). Short-lived herbs without the ability to form adventitious buds on roots do not survive removal of all axillary buds, even though less severe injury is not fatal to these species (Martínková et al. 2004a and b, Klimešová and Martínková, in press). Plants in more advanced life-cycle phases, i.e. during flowering and fruiting, have already depleted a substantial portion of reserves accumulated during the vegetative phase, hence resprouting of flowering and fruiting short-lived herbs is more difficult than resprouting of vegetative plants (Chapin et al. 1990, Martínková 2004a). Similarly, resprouting of plants growing at higher nutrient availability may be easier than regeneration of plants under nutrient stress (Peterson 1975, van der Meijden et al. 1988, Maschinski and Whitham 1989, Bímová et al. 2003, Martínková 2004a). Nevertheless, an interaction of all these factors can change formerly clear relationships (Maschinski and Whitham 1989, Huhta et al. 2000).

Since information on the ecology of short-lived herbs with a sprouting strategy is still insufficient, we established a three-year garden experiment to identify the effect of nutrient level, timing and severity of injury on the ability to resprout, survival and seed production of the short-lived herb *Barbarea vulgaris*. This model species often occurs in highly disturbed man-made habitats and is able to use both axillary buds and adventitious buds on roots for resprouting after injury (Klimešová 2003). In the present study, we have raised the question if resprouting after injury, survival and seed reproduction of *Barbarea vulgaris* are lowered by higher severity of injury, more advanced life-cycle phases of plant at the time of its injury, and at lower nutrient level.

MATERIAL AND METHODS

STUDY SPECIES

Barbarea vulgaris R. Br. (Brassicaceae) is a temperate species distributed worldwide (MacDonald and Cavers 1991). It prefers man-made (e.g. arable land, ruderal habitats, roadside ditches, railway banks etc.) or naturally disturbed habitats (e.g. river alluvia) (Dvořák 1992). It is generally a biennial or short-lived perennial herb reproducing once or repeatedly (MacDonald and Cavers 1991). During the first year of life, plants remain vegetative and rosettes overwinter to the next season. *Barbarea* has an obligate requirement for vernalisation (MacDonald and Cavers 1991), and seed production is initiated during the second year of life. In the beginning of the second year of life, vegetative rosettes transform into reproductive stems and former rosette leaves mostly defoliate. The species is capable of root-sprouting (Kott 1963, Klimešová 2003), i.e. adventitious buds on roots may develop on the main root or on root branches which lie near the soil surface, and a number of adventitious rosettes or stems may arise from these buds afterwards. Survival to the third season and polycarpy are connected with this ability in this species (MacDonald and Cavers 1991).

EXPERIMENT

In a three-year garden experiment, plants of *Barbarea vulgaris* were injured to establish the ability of resprouting and to assess the effect of injury on its seed reproduction. Plants were cultivated at two nutrient levels, two types of injury severity were applied, and the plant body was injured in four phases of the plant's life cycle.

YEAR 2001

Seeds of *Barbarea vulgaris* were made to germinate in moist sand under greenhouse conditions without temperature regulation at the beginning of April. Ten days after sowing, four-day old seedlings were transplanted into pots (18 cm diameter, 2.5 l volume) that were filled with a 5:1 sand-soil mixture. A total of 180 seedlings were transplanted into the pots (one seedling per pot) and placed outdoors in an experimental garden. In the garden, to protect frost killing of plants during overwintering, all pots were put into a shallow pit filled with a 5:1 sand-soil mixture. Two different nutrient levels, low and high, were created in the pots by adding tablet fertiliser immediately after seedling transplantation: NPK [g/pot] high level (90 pots): 1.1/0.5/0.8, low level (90 pots) 0.55/0.25/0.4. Pots with low and high nutrient levels were positioned randomly in the pit. To maintain stable nutrient levels, the tablet fertiliser was added from April to October (growing season) regularly every two months during the whole three-year experiment in the same amount as the first dose. During the first year of the experiment, no experimental injury was applied and plants were only cultivated.

YEAR 2002

In the second year of the experiment, another group of plants (60 in total) was made to germinate, transplanted and placed in the experimental garden according to the same method and time schedule as described above. These plants were also cultivated at low (30 pots) and high (30 pots) nutrient levels (see above).

From March to July, plants in four different life-cycle phases cultivated at both nutrient levels were injured (Table 1). The plants were injured in the following life-cycle phases: young vegetative rosettes, i.e. in the first year of the plants' life, and overwintered vegetative rosettes, flowering plants and fruiting plants, i.e. in the second year of plant's life. The applied severity of injury was either high, i.e. removal of all aboveground biomass together with all axillary buds, only root left intact, or low, i.e. removal of all aboveground biomass, but basal axillary buds left intact. Both types of injury were applied to plants in the flowering and fruiting phase, and only the high injury was applied to young and overwintered rosettes, as rosettes do not have a stem and low injury to rosettes causes only defoliation and no bud removal (Table 1). The control for seed reproduction was not injured. The number of replicates was 15 per treatment and nutrient level. Plants for individual treatment were selected randomly. Growth characteristics of plants were measured for each life-cycle phase and nutrient level at the time of injury: removed biomass and rosette diameter were measured on vegetative rosettes, removed biomass, plant height and number of stems were measured on flowering and fruiting plants.

Three months after injury (Table 1), all plants in the treatments were divided into two groups. The first group of plants (7 replicates) was harvested to determine (a) the capability of regeneration, i.e. the amount of regenerated aboveground biomass after injury, and (b) the extent of regeneration, i.e. the ratio of regenerated and removed biomass. The second group (8 replicates) was left in place until the next season.

During this year, the number of flowering plants, plants setting seed and the number of reproducing stems per plant in each treatment and nutrient level were also recorded. Seeds of plants able to finish the reproductive cycle within this year were trapped with a light, white cloth. Reproducing plants were wrapped in this cloth when all their flowers terminated flowering. Trapped seeds were weighed after their maturation. For each reproducing plant, 30 seeds in three replicates from the whole set of seeds were weighed. The average weight of one seed and total number of seeds per plant were calculated from the weight of all trapped seeds and from the average weight of 30 seeds.

Table 1 List of controls and experimental treatments with their descriptions. Dates of germination, injury and harvest of experimental plants in each treatment are shown. Severity of injury: high – removal of all aboveground biomass with all axillary buds, only root left intact; low – removal of aboveground biomass, but basal axillary buds left preserved. Additionally, plants in all treatments and controls were cultivated at low and high nutrient level. All injuries and harvests were carried out in year 2002. Characteristics of seed reproduction were assessed in the second year of the plant's life, i.e. for R1+ in 2003, for other treatments in 2002.

Year of germination	Treatment	Life-cycle phase	Severity of injury	Date of injury	Date of harvest
2001	**R2+**	**Overwintered rosettes**	high	29 MAR	28 JUN
2001	**FL +**	**Flowering plants**	high	7 MAY	5 AUG
2001	**FL -**		low	7 MAY	5 AUG
2001	**FR +**	**Fruiting plants**	high	1 JUN	29 AUG
2001	**FR -**		low	1 JUN	29 AUG
2002	**R1+**	**Young rosettes**	high	24 JUL	20 OCT
2001	**C2**	**control** of seed reproduction for all treatments, except for R1+			
2002	**C1**	**control** of seed reproduction for R1+ treatment			

YEAR 2003

In this year, low and high nutrient levels were still maintained. For plants that germinated in 2001 and were left in the experiment from the previous year, the number of plants that survived until the third vegetative season was recorded in late April 2003. For this group, the experiment was terminated at that time.

For plants that germinated in 2002 and also were left in the experiment from the previous year, the number of flowering plants, plants setting seed and the number of reproducing stems per plant in each injury treatment and nutrient level were recorded. Seeds were trapped according to the same method as in 2002. The number of seeds per plant and weight of one seed per plant were calculated (see above). Survival until the third vegetative season was estimated in November 2003 and the experiment was terminated for this group afterwards.

STATISTICAL ANALYSES

All statistical analyses were carried out in Generalised Linear Models. 1-way ANOVA with Poisson and Gamma distribution used to test differences in growth characteristics measured at the time of injury among life-cycle phases and between nutrient levels. 2-way ANOVA with Gamma distribution was used to test the effect of nutrient level and treatment on the amount of regenerated biomass and on the extent of regeneration. The effects of nutrient level and treatment on the number of flowering and fruiting plants and on survival until the third season were analysed with Main Effects ANOVA with Binomial distribution. Characteristics of seed reproduction were analysed with 2-way ANOVA using Poisson and Gamma distribution.

RESULTS

GROWTH CHARACTERISTICS AT THE TIME OF INJURY

The plants of both vegetative phases, young and overwintered rosettes, differed neither in rosette diameter nor aboveground biomass at the time of their injury (Table 2). Nutrient level did not influence these characteristics either. On the other hand, reproductive phases, flowering and fruiting plants significantly differed in all tested characteristics: aboveground biomass, the height of plants and the number of reproducing stems per plant were higher in fruiting plants than in flowering plants (Table 2). Low nutrient level statistically significantly lowered these characteristics, except plant height.

Table 2 Growth characteristics of plants measured at the time of injury. Means and standard errors are shown for plants in different life-cycle phases and at both nutrient levels. Wald statistics and significance levels are shown for nutrient level and vegetative and generative phases. R1 – young rosettes, R2 – overwintered rosettes, FL – flowering plant, FR – fruiting plant, n.s. – non-significant, * – $p<0.05$, *** – $p<0.001$.

	Vegetative phases				Reproductive phases			
Life-cycle phase	R1		R2		FL		FR	
Date of injury	24 JUL 2002		29 MAR 2002		7 MAY 2002		1 JUN 2002	
Nutrient level	high	low	high	low	high	low	high	low
Removed biomass [g]	6.60 ± 1.15	3.20 ± 0.73	7. 04 ± 1.22	5.40 ± 0.97	20.96 ± 2.14	10.69 ± 1.57	33.57 ± 4. 36	17.55 ± 2.56
Wald + p for nutrients	**5.01***		1.42 n.s.		**22.79*** **		**12.21*** **	
Wald + p for phases	1.46 n.s.				**13.12*** **			
Rosette diameter [cm]	22.89 ± 1.82	18.40 ± 1.88	20.00 ± 1.20	17.87 ± 1.23	not applicable			
Wald + p for nutrients	2.87 n.s.		1.55 n.s.					
Wald + p for phases	1.02 n.s.							
Height of plant [cm]	not applicable				47.39 ± 1.73	45.97 ± 1.28	56.43 ± 2.78	57.47 ± 2.13
Wald + p for nutrients					0.41 n.s.		0.07 n.s.	
Wald + p for phases					**21.55*** **			
Number of stems	not applicable				8.29 ± 0.69	5.17 ± 0.58	7.97 ± 0.83	4.87 ± 0.34
Wald + p for nutrients					**21.08*** **		**22.02*** **	
Wald + p for phases					0.61 n.s.			

REGENERATED BIOMASS

All injured plants resprouted after injury, i.e., no plant died after injury. However, the amount of aboveground biomass regenerated after injury and also the extent of regeneration were statistically significantly affected by the treatment (regenerated biomass: Wald statistics = 33.3, $p<0.001$, extent of regeneration: Wald statistics = 41.3, $p<0.05$) and also by nutrient level (regenerated biomass: Wald statistics = 63.8, $p<0.001$, extent of regeneration: Wald statistics = 4.4, $p<0.001$) (Fig. 1). At high nutrient level, severly injured young rosettes and flowering plants produced the lowest biomass, while plants in other injury treatments formed equal amounts of regenerated biomass (Fig. 1A). The low nutrient level had a statistically significant negative effect on regenerated biomass, but the regenerated biomass was equal among treatments at this nutrient level (Fig. 1A). When compensatory growth was expressed relatively as the extent of regeneration (regenerated/removed biomass), lower values were found for plants in treatments with higher injury and in more advanced life-cycle phases (Fig. 1B). This trend was not observed at low nutrient level. Nevertheless, in general the extent of regeneration was higher at low nutrient level, and only overwintered rosettes were able to fully compensate for lost aboveground biomass (Fig. 1B).

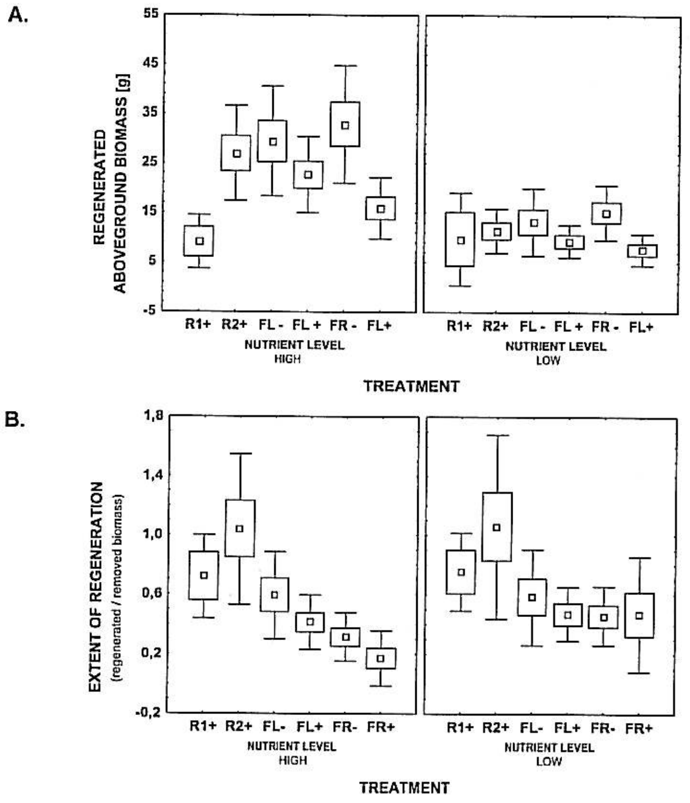

Fig. 1. Aboveground biomass regenerated after experimental injury (A) and the extent of regeneration (B) in all injury treatments and at low and high nutrient level. Injury was applied in different life-cycle phases (R1 – young rosettes, R2 – overwintered rosettes, FL – flowering plant, FR – fruiting plant), and at one or two severity levels (+ – high injury: removal of all aboveground biomass with all axillary buds, only root left intact, - – low injury: removal of aboveground biomass, but basal axillary buds left intact). Means, standard deviations and standard errors of mean are shown.

SEED REPRODUCTION AND SURVIVAL

The number of flowering individuals and number of plants that set seed in the year of injury were statistically significantly affected by the treatments (Table 3). The number of plants surviving until the third season was not influenced. Nutrient level did not influence a single characteristic (Table 3). Almost all plants in both control groups flowered and also set seed. In plants injured in the second year of life, i.e. in overwintered rosettes, flowering and fruiting phases, high injury caused prolongation of the vegetative phase. Low injury caused prolongation of the vegetative phase only in some plants injured in the second year of life (Table 3). In plants injured in the first year of life, i.e. in the young rosette phase, seed production and life-cycle were not influenced (Table 3).

Table 3 Number of flowering individuals, plants setting seed and plants surviving until the third growing season in individual treatments and at both nutrient levels. The maximum number, i.e. the number of replicates per treatment and nutrient level, is 15. Results of significance tests of both factors (nutrient level and treatment) are shown for all characteristics, n.s. – non significant, *** p<0.001. C1 – control for seed reproduction of plants germinated in 2002, C2 – control for seed reproduction of plants germinated in 2001, R1 – young rosettes, R2 – overwintered rosettes, FL – flowering plant, FR – fruiting plant. + – high injury: removal of all aboveground biomass with all axillary buds, only root left intact; - – low injury: removal of aboveground biomass, but basal axillary buds left intact.

Characteristics	Number of flowered individual		Number of plants setting seeds		Number of survived plants	
Nutrient level	high	low	high	low	high	low
Treatment						
C1	15	15	15	15	6	5
C2	15	15	14	15	6	4
R1	15	15	15	15	8	4
R2	2	0	1	0	2	6
FL -	11	9	10	6	5	5
FL +	0	1	0	1	6	5
FR +	9	5	0	0	7	6
FR -	0	0	0	0	7	6
	chi-square	p-level	chi-square	p-level	chi-square	p-level
Nutrient level	2.13	n.s.	2.97	n.s.	0.65	n.s.
Treatment	226.99	***	221.36	***	2.63	n.s.

CHARACTERISTICS OF SEED REPRODUCTION

The treatments significantly affected all tested characteristics of plant reproduction (number of reproducing stems: Wald statistics = 37.9, p<0.001, weight of seed: Wald statistics = 14.1, p<0.01, number of seeds per plant: Wald statistics = 96.9, p<0.001; Fig. 2). Overwintered rosettes and highly injured flowering and fruiting plants failed to reproduce in the year of injury (Table 3). The highest number of reproducing stems was found in injured young rosettes. Control plants produced the heaviest seeds (Fig. 2). The number of seeds per plant was lower in injured flowering and fruiting plants than in controls and young rosettes. The nutrient level statistically significantly affected only the number of reproducing stems (Wald statistics = 3.9, p<0.05); a higher number of reproducing stems was formed at high nutrient level.

DISCUSSION

SEVERITY OF INJURY

Severe injury to plant body that removes or damages most of the aboveground biomass is fatal to many species, especially to short-lived herbs, because they do not have underground organs of stem origin with axillary buds that can be used for recovery (Klimeš et al. 1997, Klimešová and Klimeš 2003). However, the probability of injury to their body is higher than in perennial species, because they mostly occupy habitats with frequent, unpredictable and severe disturbance (Fernández Alés et al. 1993, Grime 2001, Schippers et al. 2001). MacDonald and Cavers (1974) reported a high regenerative ability of the short-lived herb *Barbarea vulgaris* after clipping. In our experiment, it was demonstrated that this species possesses a very effective tool to cope with even more severe disturbance, i.e. with removal of 100% of the aboveground biomass. The adventitious buds on roots enabled successful compensation for lost of all axillary buds. Adventitious buds on roots form an efficient underground bud bank for recovery after disturbance and in the short-lived herb *Barbarea vulgaris* act as an alternative to missing underground axillary buds. Probably for similar reason, the highest number of root-sprouters is found in trees and short-lived herbs, i.e. in species without underground organs of stem origin (Del Tredici 2001, Klimešová and Klimeš 2003). Moreover, regenerated biomass after injury did not differ between treatments of injury severity in our experiment. This indicates that adventitious buds on roots and axillary buds were equally efficient in the ability to resprout in *Barbarea vulgaris*. Similar results were obtained for the annual species *Rorippa palustris* (Martínková et al. 2004a). Therefore, not only a higher number of "reserve meristems" on basal parts of the stem (Huhta et al. 2000), i.e. basal axillary buds, but also the formation of adventitious buds on roots increases tolerance to severe disturbance in short-lived species.

In our experiment, removal of all axillary buds caused prolongation of the vegetative phase in injured vegetative plants, and induced rejuvenation in injured reproducing plants. We found that such severe injury leads to a substantial change in the life cycle of *Barbarea vulgaris*. These findings correspond to the observations by Kott (1963) and MacDonald and Cavers (1991), and suggests that vernalisation of axillary buds is necessary for an initialisation of seed reproduction in *Barbarea vulgaris*. According to the present study, injury impact assessment within only one season is insufficient, and longer-time studies are necessary due to possible prolongation of life-cycle phases even in short-lived herbs.

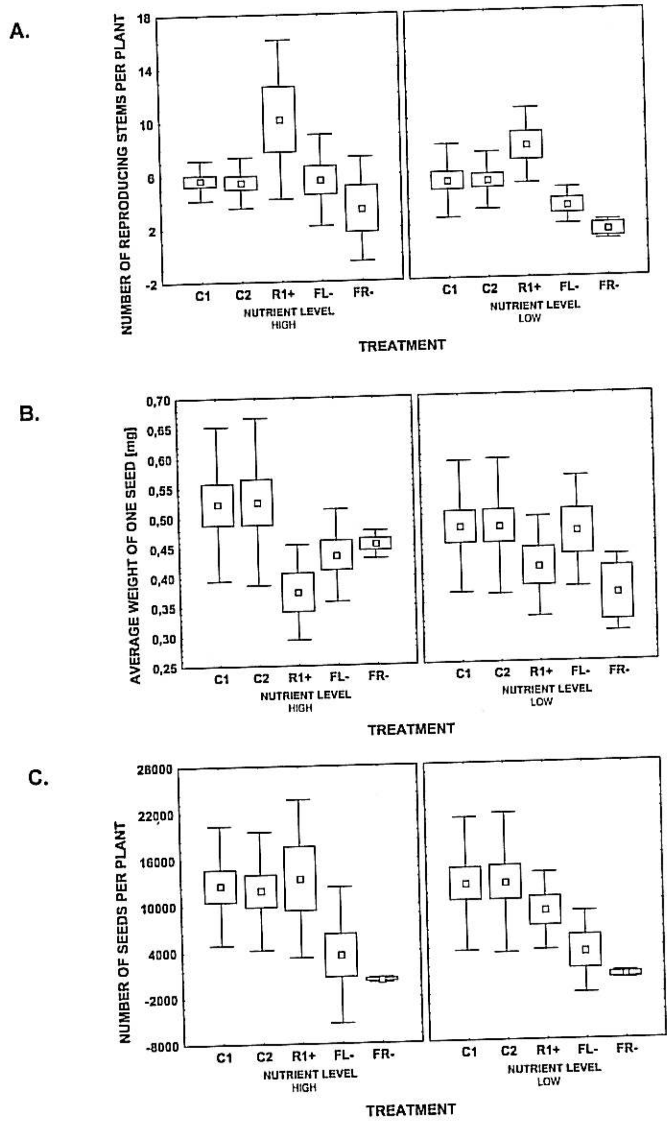

Fig. 2. Characteristics of seed reproduction of injured and control plants at low and high nutrient level. **A.** number of reproducing stems per plant. **B.** average weight of one seed. **C.** number of seeds per plant. Treatments in which more than three plants produced seeds are shown (C1 – uninjured control for seed reproduction of young rosettes, C2 – uninjured control of seed reproduction for other life-cycle phases, R1 – young rosettes, R2 – overwintered rosettes, FL – flowering plant, FR – fruiting plant, + – high injury: removal of all aboveground biomass with all axillary buds, only root left intact, - – low injury: removal of aboveground biomass, but basal axillary buds left intact). Means, standard deviations and standard errors of mean are shown.

TIMING OF INJURY

The ability to regenerate after injury is connected with the accumulation of reserves (Kays and Canham 1991, van der Heyden and Stock 1995, van der Meijden et al. 1988). In the case of removal or damage of all photosynthetic organs, a plant must utilise reserves stored in underground organs, e.g. in roots, bulbs, rhizomes etc., for its recovery. However, reserves can be also depleted by for example flowering, fruiting and regrowth after overwintering (Chapin et al. 1990, Suzuki and Stuefer 1999). When injury and one of the above mentioned events occur simultaneously, reserves can be exhausted and regeneration fails or becomes more complicated (Čížková et al. 1992). In our experiment, even though plants injured in different life-cycle phases differed in amount of removed biomass, they formed a similar amount of biomass after three months of regeneration. Therefore, results from our experiment support the theory of graded allocation between vegetative growth and seed reproduction in short-lived plants (King and Roughgarden 1982, Kudoh et al. 2002.), because the relative amount of regenerated biomass of injured plants decreased with the progress of life-cycle phase at the time of injury (Fig. 1B). A parallel hypothesis also arises: short-lived perennial plants are the most sensitive to injury in the reproductive phase similarly to annual or biennial species. Probably for this reason, short-lived species, similarly to biennial species, postpone the start of the reproductive phase and thus relatively decrease its duration in comparison to the vegetative phase (Kozlowski and Wiegert 1986, Kudoh et al. 2002). The greatest extent of regeneration occurring in overwintered rosettes in our experiment supports this hypothesis.

In our experiment, although injured plants produced lighter seeds than control plants in general, only plants injured in the young rosette phase produced a seed number equal to the control. Therefore, plants injured during the first year of their life compensated for the lower amount of reserves by decreasing seed weight to maintain a seed number comparable to uninjured individuals. Seed production of plants injured during the second year of life was postponed or decreased, but survival until the next season was equal to control groups. Therefore, reduction of seed production in the year of injury or its postponement to the next season is probably advantageous and can lead to a higher accumulation of reserves in the year of injury in comparison to uninjured individuals. Thus, seed production of injured plants can be in total equal or higher than in uninjured individuals. However, these hypotheses need more detail exploration.

NUTRIENT LEVEL

In many studies, it is shown that soil nutrient level influences a wide range of plant traits and significantly affects plant performance (Harper 1997, Grime 2001). Similarly in our experiment, some growth characteristics of plants measured at the time of injury differed between nutrient level treatments, and the same result was found for regenerated biomass as well as extent of regeneration. The low nutrient level erased differences caused by injury in different life-cycle phases and lowered the amount of regenerated biomass after injury. A similar result was found for the short-lived herb *Rorippa palustris*

(Martínková et al. 2004a), in which a release of adventitious buds and growth of new adventitious shoots were suppressed by a low nutrient level. However, the extent of regeneration was lower at a higher nutrition level. It seems that plants at a higher nutrient level compensate for loss of aboveground biomass relatively less than plants at a low nutrient level, especially when they are injured in the reproductive phase. This relationship could be associated with the fact that plants growing under nutrient shortage have a relatively higher proportion of stored reserves than plants that are not limited by nutrients (White 1973). Therefore, plants at a low nutrient level may build up relatively more reserves for regeneration after severe injury than plants growing under higher nutrient availability. However, nutrient availability must not be so limiting to prevent regeneration and growth of species at all (Maschinski and Whitham 1989, Iwasa and Kubo 1997, Grime 2001). Nevertheless, it seems that timing and severity of injury are more important factors influencing prosperity of short-lived plants after damage than nutrient availability, since characteristics of seed reproduction were not influenced by nutrient level in our experiment. Observed lower influence of nutrient level on resprouting corresponds to the findings of Martínková et al. (2004b) done on the short-lived herb *Rorippa palustris.*

CONCLUSIONS

The short-lived herb *Barbarea vulgaris* successfully copes with severe disturbance by resprouting and does not rely only on its seed bank. We suggest that the resprouting strategy may be a significant alternative to the seeding strategy in some short-lived herbs occupying severely and frequently disturbed habitats.

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CHAPTER V

Resprouting of biennial *Oenothera* congeners after disturbance: field observations and an experimental study

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[manuscript]

Resprouting of biennial *Oenothera* congeners after disturbance: field observations and an experimental study

Summary

1. It has been recently shown that some short-lived species of man-made habitats cope with severe disturbance by resprouting (vegetative regeneration) from their bud bank and do not only rely on regeneration from seeds. Nevertheless, information on the ecology of this phenomenon is rare. In a field study, we answered the question how frequent is resprouting from root buds in populations of the ruderal biennial herb *Oenothera biennis*, and how it is affected by habitat conditions. In an experiment, we tested the hypothesis that higher severity of injury and later life cycle phase of the injured plants suppress resprouting from both axillary and root buds in *O. biennis* and also in its closely related congeners *O. fallax* and *O. glazioviana*.
2. In 25 out of 29 studied ruderal populations of *O. biennis* severely injured individuals were found, however only half of these populations included injured individuals that resprouted from roots. Among these populations, the number of root-sprouting individuals varied highly (from 3 to 67% of injured individuals). The largest populations and the highest percentage of root-resprouting individuals were found in urban habitats with sandy/gravelly substrate, a low vegetation cover, and a high frequency of disturbance.
3. In the experiment with three *Oenothera* congeners, removal of aboveground biomass with all axillary buds largely led to the death of plants of all three species. When a portion of the basal axillary buds remained intact, individuals of *O. biennis* mostly failed to regenerate, whereas individuals of *O. fallax* and *O. glazioviana* survived and formed seeds. A higher severity of injury suppressed resprouting in *Oenothera* congeners. Since regeneration after removal of all axillary buds was exceptional in all life cycle phases and contradiction with literature arose, the relationship between life cycle phase and the ability to resprout remains unclear in *Oenothera* species.
4. Resprouting after severe injury seems to be an important feature of some short-lived species occurring in man-made habitats and represents an alternative strategy to regeneration from the seed bank.

Key-words: *adventitious buds, axillary buds, bud bank, injury, man-made habitat, ruderal species, vegetative regeneration*

INTRODUCTION

Disturbance is a strong selective factor causing a partial or total destruction of plant biomass (Grime 2001). Since the goal of every organism is to maximise fitness, species necessarily "invent" an appropriate strategy in order to cope with disturbance and minimise its negative effect (Silvertown & LovettDoust 1993). The selected strategy of species inhabiting highly disturbed habitats, e.g. man-made ones, is manifested by a short life cycle, fast maturation and high production of small seeds (MacArthur & Wilson 1967, Grime 2001). Predominance of annual and biennial species in man-made habitats supports the view that resprouting (vegetative regeneration) has no value for natural populations under these conditions (Bellingham & Sparrow 2000, Grime 2001). However, it has been found that some annual and biennial species occupying man-made habitats may survive practically total destruction of aboveground parts and resprout from their bud bank, i.e. from basal axillary buds and also from adventitious buds on roots (Klimešová 2003, Martínková et al. 2004a, b). Nevertheless, according to experimental studies, resprouting behaviour markedly differs among these short-lived species. In *Barbarea vulgaris* and *Rorippa palustris*, low injury or addition of nutrients trigger the resprouting from roots, whereas in *Oenothera biennis* and *O. issleri*, resprouting is induced primarily by severe disturbance removing all axillary buds (Martínková et al. 2004a, b). From the models by Tuomi et al. (1994) and Lehtila (2000), predicting a higher bud dormancy under strong herbivore pressure, we expect that populations of *Oenothera* spp. are exposed to more frequent and more severe disturbance than populations of *Rorippa palustris* and *Barbarea vulgaris*. Even though resprouting after disturbance was observed in the field in all above mentioned species, information on the importance and frequency of resprouting under conditions of man-made habitats is insufficient (Klimešová 2003, Martínková et al. 2004a).

Full compensation or overcompensation for lost biomass is considered as an adaptation to herbivory (Tuomi et al. 1994). In populations under herbivore pressure, only some individuals are harmed and those mostly survive herbivore attack and persist in impaired populations (Maschinski & Whitham 1989, Lennartsson et al. 1998, Paige 1999, Strauss & Agrawal 1999). However, in habitats affected by man-made disturbance that frequently and severely injures all individuals in populations, survival of as many plants as possible and any seed production are important for population persistence. In previous experiments with short-lived ruderal species, it was found that seed production of severely harmed plants, i.e. plants in which 100% of the aboveground biomass was removed, may be about as high as seed production of uninjured plants (*Barbarea vulgaris*), or even higher than in plants regenerated at the time of disturbance from the seed bank (*Rorippa palustris)* (Martínková et al. in press, Martínková et al., submitted). Thus, injured individuals of these root-sprouting species do not only cope with severe disturbance, but it may be even advantageous for them. On the other hand, there are some internal factors that can freeze resprouting after disturbance or decrease its rate (Dubard 1903, Rauh 1937, Peterson 1975, Martínková et al. 2004a, b, Martínková et al., submitted). It was found that a more advanced life-cycle phase at the time of injury and higher disturbance severity decrease either the probability of successful resprouting or regrowth in short-lived species (Martínková et al. 2004a, Martínková et al., submitted). Even though injured individuals successfully regenerate, their life cycle may differ from uninjured ones, i.e. the vegetative phase is prolonged, and polycarpy in monocarps is initiated (Sosnová 2003, Martínková et al. 2004a, Martínková et al., submitted).

We chose as a model for this study a common biennial species of man-made habitats, Oenothera *biennis*, in which ability of resprouting after a disturbance has been documented (Rauh 1937, Klimešová 2003, Martínková et al. 2004a), and its closely related congeners *O. fallax* and *O. glazioviana*, about which no literary information on their resprouting abilities existed. Since habitats occupied by all these *Oenothera* congeners are frequently disturbed, and thus not suitable for longer field experiments, manipulative garden experiments are necessary to evaluate the effects of timing and severity of injury on the resprouting ability in these *Oenothera* species. Nevertheless, field observations on the frequency of resprouting in ruderal populations could reveal the real ecological importance of resprouting. Consequential comparison of experimental results and field observations provides a more complex view of the vegetative regeneration of these biennials. Unfortunately, the number of natural populations of *O. fallax* and *O. glazioviana* is low in South Bohemia, and comparison of all three congeners can acquired only by experimental data. Nevertheless, we believe that this more comprehensive study provides useful information on resprouting as an alternative strategy to regeneration from the seed bank in short-lived species occupying man-made habitats.

In the present study we defined the following goals:

(i) to find out by means of field observations how frequent is resprouting from roots in ruderal populations of *Oenothera biennis*, and how it is affected by habitat characteristics and disturbance regime,

(ii) to experimentally assess how severity and timing of injury affect the probability of successful regeneration, seed production and life cycle in three biennial root-sprouting *Oenothera* congeners (*O. biennis, O. fallax* and *O. glazioviana)*

METHODS

STUDY SPECIES

Oenothera biennis L. and *Oenothera glazioviana* M. Micheli (Onagraceae) are non-native and invasive species in Europe (Hall et al. 1988, Mihulka & Pyšek 2001). *Oenothera fallax* Renner is a hybrid between *O. biennis* and *O. glazioviana* of European origin, showing an expansive behaviour (Mihulka & Pyšek 2001). All species prefer light sandy or gravelly substrates and man-made habitats, such as habitats along roads and railways, railway yards, urban areas etc. (Hall et al. 1988, Mihulka & Pyšek 2001). The species are non-clonal, monocarpic and biennial (Hall et al. 1988, Dietrich et al. 1997), with very long-lived seeds (Baskin & Baskin 1994). During the first year of their life, individuals of these *Oenothera* species form vegetative rosettes and after overwintering, reproducing stems are formed (Hall et al. 1988). All studied species may regenerate vegetatively (resprout) from axillary and adventitious buds, both after injury and also spontaneously (Klimešová 2003, Martínková et al. 2004a, Martínková, pers. obs.). Polycarpic individuals of all three species were observed in the field as well (Martínková et al., 2004a, Martínková, pers. obs.).

FIELD OBSERVATION OF O. BIENNIS POPULATIONS

Field observation of 29 ruderal populations of *O. biennis* was carried out in June and July of 2003 in South Bohemia near the town of České Budějovice, Czech Republic (Fig. 1), to describe the frequency of resprouting from roots under natural conditions. Selected populations were at a minimum distance of 200 m apart and included more than ten flowering individuals. For each population, besides the characteristics of resprouting from roots (spontaneous resprouting, resprouting after injury, polycarpy), characteristics describing habitat, disturbance regime and vegetation were recorded. A list of all characteristics and their descriptions is given in Table 1. Plants without any evident damage, i.e. apical meristem removal, stem removal, branch cutting etc., and with sprouts originated on roots were classified as spontaneously resprouting. Injured plants with sprouts originated on roots as resprouting from roots after injury, and plants with both a current-year stem and also preserved parts of a previous-year stem were classified as polycarpic.

In the course of the study, also data on *O. fallax* and *O. glazioviana* were recorded, however only five populations of *O.fallax* and two of *O. glazioviana* were found. Thus we decided to remove these data from further analyses for their insufficiency.

GARDEN EXPERIMENT

In a three-year experiment, plants of *Oenothera biennis, O. fallax* and *O. glazioviana* were injured to determine the ability of resprouting from axillary buds and also adventitious buds on roots after a disturbance. Two types of injury severity were applied and the plant body was injured in four different phases of the life cycle.

Year 2000 – Seeds of all three *Oenothera* species selected for the experiment were collected in a single population in South-Bohemia (Fig.1). The collected seeds overwintered in paper bags under laboratory conditions.

Year 2001 – The seeds were made to germinate in moist sand under greenhouse conditions without temperature regulation at the beginning of April. Fifteen days after sowing, five-day old seedlings were transplanted into small peat pots (5x5x5 cm, one seedling per pot) filled with a 5:1 sand-soil mixture. In total, 180 seedlings per species were transplanted to the pots placed in a greenhouse. After 3 weeks, at the beginning of May, peat pots with seedlings were placed outdoors to flowerbeds with a 2:3 sand-soil mixture. The peat pots served only for precultivation and decomposed after transplantation to the garden. During the first year of the experiment, no experimental injury was administered and plants were only cultivated.

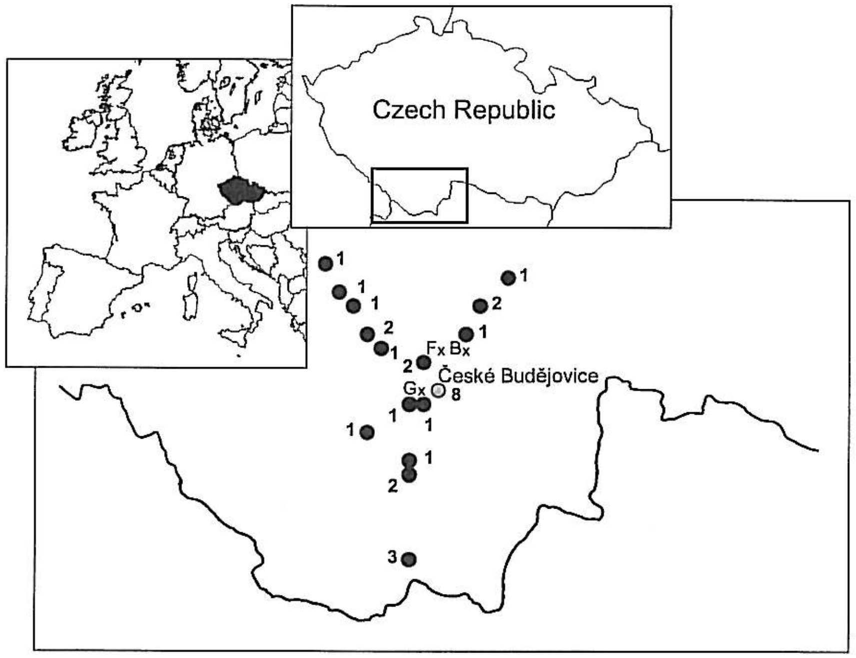

Fig. 1 Distribution map of the 29 studied populations of *Oenothera biennis* in the Czech Republic. Localities with studied populations are marked by black points (outside of the town of České Budějovice) or by gray points (in the town of České Budějovice), the number at the points identifies the number of studied populations within a locality. Crosses mark localities from which seeds of *O. biennis* (B), *O. fallax* (F), and *O. glazioviana* (G) were collected for the garden experiment

Year 2002 – In the second year of the experiment, another group of plants (60 per species) was made to germinate, transplanted and placed in the experimental garden, using the same method and time schedule as described above. From April to August, plants of all species were injured in four different life-cycle phases: young vegetative rosettes (in the first year of the plant's life), overwintered vegetative rosettes (in the second year of the plant's life), flowering plants and fruiting plants (both in the second year of the plant's life) (Table 2). Since plants did not reach the fruiting life-cycle phase at the same time, dates of injury differed among species for this life cycle phase (Table 2). The severity of injury was either high (removal of all aboveground biomass together with all axillary buds, only root left intact) or low (removal of all aboveground biomass without root crown – basal axillary buds and root left intact). Both these types of injury were applied on plants in flowering and fruiting phase, and the high injury severity was only applied to young and overwintered rosettes, as rosettes do not have a stem but only a root crown (Table 2). Two groups of plants served as a control and were not injured (Table 2). Plants were selected for individual treatments randomly at the beginning of the experiment. Originally, the number of replicates was 30 per treatment, however some plants died before injury, thus the number of replicates differed among the treatments (Table 2). The ability to regenerate after injury was assessed at the beginning of November when the following characteristics were recorded: number of regenerated and fruiting plants, and also number of branches, height of plant and number of fruits per plant. From 20 randomly selected fruits per species, the number of seeds per fruit was calculated. Seed production per plant was calculated from the number of fruits per plant and the number of seeds per fruit.

Table 1. List of characteristics that were used in the field study of *Oenothera biennis* populations. Characteristics describe habitats occupied by *O. biennis*, disturbance regimes occurring there, vegetation, and also population and resprouting abilities of *O. biennis*. Description of characteristics, possible values and short cuts used in the ordination diagram are given. Fuzzy and dummy variables used in the ordination analysis are specified. 0-1: in the case of characteristics coded as fuzzy variables, individual categories possess values from 0 to 1; *value*: characteristic possesses a factual, calculated or estimated number, units are shown, Yes/No – in the case of characteristics coded as dummy variables, individual categories possess the value Yes or No.

Characteristic	Category	Short cut	Value	Description
Habitat				
Type of habitat (fuzzy coding)	railway yard	rail-yard	0-1	adjacent areas of train stations
	railways	railways	0-1	between two train stations, out of the settlements
	road	road	0-1	along automobile roads, out of the train station areas
	urban areas	urban	0-1	in the town, out of the train station areas
Age of habitat (dummy variable)	< 3 years	< 3 years	Yes / No	number of years from the last high severe disturbance that reached upper substrate level and destroyed vegetation (estimation)
	3-10 years	3-10 years	Yes / No	
	>10 years	>10 years	Yes / No	
Substrate (fuzzy coding)	loam	loam	0-1	loamy substrate
	stones	stones	0-1	> 5 cm
	gravel	gravel	0-1	5 - 1 cm
	sand	sand	0-1	< 1 cm
Water availability		water	*value*	weight average of Ellenberg's values (Ellenberg 1988) of co-occurring species
Nitrogen availability		nitrogen	*value*	weight average of Ellenberg's values (Ellenberg 1988) of co-occurring species
Area		area	*value* m^2	(estimation)
Slope		slope	*value* °	(estimation)
Disturbance				
Frequency of disturbance		disturbance_interval	*value* years	number of years between two disturbances regardless their severity (estimation)
Severity of last disturbance (dummy variable)	high	high_disturbance	Yes / No	disturbance reached upper substrate level
	low	low_disturbance	Yes / No	aboveground disturbance
Vegetation				
Vegetation density (dummy variable)	low	low_density	Yes / No	biomass of all species covers < 25% of the ground
	middle	middle_density	Yes / No	covers 25 - 75%
	high	high_density	Yes / No	covers > 75%
Number of species		species_number	*value* m^{-2}	number of species co-occurring with *O. biennis* population
Species diversity		species / area	*value* m^{-2}	number of species / area
O. biennis population				
Number of individuals		individuals	*value*	number of all *Oenothera* individuals
Density of Oenothera individuals		*Oenothera*_ density	*value* m^{-2}	number of individuals / area
Ratio of fruiting / vegetative plants		fruiting / vegetative	*value*	number of fruiting / number of vegetative individuals
Number of injured flowering plants		injured_individuals	*value* %	% of injured flowering individuals from all flowering individuals
Number of spontaneously resprouted plants		spontaneous	*value* %	% of individuals resprouting without evident injury from all flowering individuals
Number of plants resprouted after injury		after_injury	*value* %	% of individuals resprouting after injury from all injured flowering individuals
Number of polycarpic individuals		polycarpic	*value* %	% of individuals possessing stems of both current and previous year from all flowering individuals

Year 2003 – In May, the number of plants that survived until the third vegetative season was recorded and the experiment terminated.

Table 2. Treatments used in the garden experiment with *Oenothera biennis, O. fallax* and *O. glazioviana*. Germination year of treated plants, treatment abbreviation, life-cycle phase at the time of injury and applied injury severity are specified for individual treatments. Dates of injury (all in year 2002) and number of replicates (n) are shown for each treatment and species. Severity of injury: high – removal of all aboveground biomass with all axillary buds, low – removal of aboveground biomass, but basal axillary buds left intact. - – not applicable.

				O. biennis		*O. fallax*		*O. glazioviana*	
Year of germination	Treatment	Life-cycle phase	Severity of injury	Date of injury	n	Date of injury	n	Date of injury	n
2001	C2	control for all, except R1+	-	-	26	-	30	-	30
2002	C1	control for R1+	-	-	30	-	30	-	30
2002	R1+	Young rosettes	high	20 AUG	30	20 AUG	30	20 AUG	30
2001	R2+	Overwintered rosettes	high	19 APR	28	19 APR	30	19 APR	29
2001	FL-	Flowering plants	low	27 JUN	27	27 JUN	30	27JUN	28
2001	FL+	Flowering plants	high	27 JUN	24	27 JUN	30	27 JUN	30
2001	FR-	Fruiting plants	low	24 JUL	19	14 AUG	30	27AUG	29
2001	FR+	Fruiting plants	high	24 JUL	26	14 AUG	30	27 AUG	30

RESULTS

FIELD OBSERVATION

In Table 3, the main results of the field study of 29 *Oenothera biennis* ruderal populations are summarised. Almost each population included injured individuals, but also plants spontaneously regenerated from roots. However, polycarpic individuals were found in eight populations only and some plants regenerated from roots after injury in thirteen populations only (Table 3). Nevertheless, within populations the percentage of spontaneously regenerated plants was much lower than the percentage of plants regenerated after injury. Polycarpy was exceptional.

Fig. 2 displays an ordination diagram of Principal Component Analysis (PCA) performed using Canoco (ter Braak & Šmilauer1998) on data from the field. The first two ordination axes explained 30.7% of the variability in the data set. Data were log-transformed, standardised and centered by species averages. The same characteristics were coded as dummy or fuzzy variables (Table 2) (Lepš & Šmilauer 2003). The ordination diagram shows the relationships among resprouting and habitat characteristics and disturbance regime. The first two axes separate the ordination space into four quadrants that represent the main *O. biennis* habitat types. A high disturbance, high species diversity, stony substrate and low habitat age characterise the top left quadrant. These habitats are mostly found close to railways. The top right quadrant represents urban habitats with a sandy and gravelly substrate, low vegetation cover, and high frequency of disturbance, and habitats where vegetative rosettes of *O. biennis* dominate over adult fruiting individuals. The bottom right quadrant includes large older localities with a loamy substrate, close to roads or at adjacent train station areas where last disturbance was low. And finally, a low disturbance frequency, high vegetation cover, and predominance of adult fruiting individuals over vegetative rosettes of *O. biennis* are typical characteristics of the bottom left quadrant. It is obvious from this diagram that *O. biennis* prefers habitats represented by the top right quadrant. The largest *O. biennis* populations occupy these habitats and thus the highest number of individuals spontaneously regenerated from roots, polycarpic individuals, and also individuals regenerated from roots after injury can be found there. According to the diagram, a high frequency of disturbance, and rather low severity of last disturbance also characterise these habitats.

Table 3. Population and resprouting characteristics of 29 natural populations of *Oenothera biennis*. Means ± standard errors, minimum and maximum values and frequency among populations are shown for each characteristic. Population frequency is the number of populations, in which spontaneously resprouted plants, injured plants, plants resprouted after injury and polycarpic individuals were observed. A more detailed description of individual characteristics is given in Table 1.

Characteristic	Mean	Min	Max	Frequency among populations
Number of individuals	288.8 ± 88.2	15	2300	
Number of spontaneously resprouting plants [%]	1.3 ± 2.4	1.0	3.3	21
Number of injured flowering plants [%]	23.2 ± 4.1	1.6	71.4	25
Number of plants resprouting after injury [%]	32.4 ± 5.9	3.3	66.6	13
Number of polycarpic individuals [%]	1.5 ± 0.5	0.1	4.1	8

GARDEN EXPERIMENT

The number of regenerated plants after injury significantly varied among treatments and also among species (treatment: Π^2 = 408.5, p<0.001, df = 5; species: Π^2 = 10.5, p<0.01, df = 2, treatment*species: Π^2 = 39.8, p<0.001, df = 9). In the treatment with low injury severity, *O. glazioviana* and *O. fallax* regenerated more frequently than *O. biennis* (Table 4). The same pattern was found for the number of fruiting plants in the year of injury (treatment: Π^2 = 348.8, p<0.001, df = 7; species: Π^2 = 21.4, p<0.001, df = 2, treatment*species: Π^2 = 19.5, p<0.05, df = 13) (Table 4). The number of regenerated plants was lower than the number of fruiting plants in *O. glazioviana* indicating that some individuals fail to finish the reproductive cycle in the year of injury, in spite of a successful regeneration. The number of survived plants until the third vegetative season did not differ among species, but varied among treatments (treatment: Π^2 = 57.03, p<0.001, df = 7; species: Π^2 = 3.6, n.s., df = 2, treatment*species: Π^2 = 15.1, n.s., df = 13). Nevertheless, surviving until the third vegetative season was infrequent (Table 4). Factorial ANOVA in Generalized Linear Models with Binomial Distribution tested these data. The number of branches, plant height, the number of fruits and the number of seeds recorded markedly decreased by injury, and more in injured fruiting plants than in injured flowering plants (Table 4).

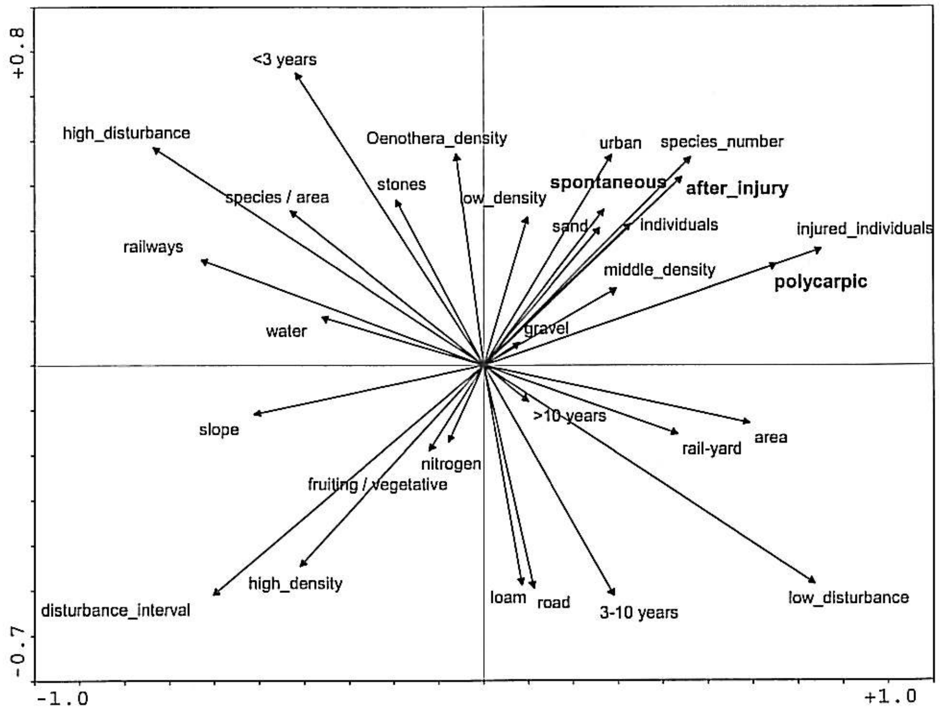

Fig. 2 PCA ordination diagram displays the relationship between the characteristics recorded in the field study of *Oenothera biennis* populations. Characteristics describe habitats occupied by *O. biennis* populations, disturbance regimes occurring there, local vegetation, population and resprouting abilities of *O. biennis*. The first two ordination axes are shown. For detailed description of characteristics and their full names see Table 1.

DISCUSSION

In our previous field experimental study with *Oenothera bienis* (Martínková et al. 2004a), we found that regeneration is high after both low and high injury severity. However, in the present garden experiment we found that *O. biennis* exhibits only very low ability to regenerate. Three possible explanations exist. Firstly, in the previous study (Martínková et al. 2004a) regeneration was evaluated already one month after injury, however in the present study it was assessed after at least three months. Thus, inconsistency between the results of these two studies could be caused by an increase of

Table 4. Results of garden experiment with *Oenothera biennis, O. fallax* and *O. glazioviana.* Number of regenerated individuals after injury, plants setting seed in the year of injury, and plants surviving until the next vegetation season (the second one in the case of C1, the third one in the case of all other treatments) are shown for each species. The number of plants in each treatment (n) is given. Means and the standard errors of mean of the characteristics are displayed. In case data were available only for a single plant, the factual value is given. If the number of fruiting plants is lower than the number of regenerated plants in a treatment, some regenerated plants remained vegetative, i.e. formed rosettes and did not produce any branch and stem. In the case of treatment C1, all plants were in a young vegetative rosette phase, could not produce any branches and plant height was not measured. Injury treatments: C2 – control for all treatments except R1+; C1 – control for R1+; R1+ – highly injured young rosettes; R2+ – highly injured overwintered rosettes; FL- – little injured flowering plants; FL + – highly injured flowering plants; FR- – little injured fruiting plants; FR + – highly injured flowering plants, high injury (+) – removal of all aboveground biomass with all axillary buds, low injury (-) – removal of aboveground biomass, but basal axillary buds left intact. - – not applicable.

Treatment	n	Number of regenerated individuals	Number of fruiting individuals	Number of branches	Plant height [cm]	Number of fruits	Number of seeds	Number of survived individuals
O. biennis								
C2	26	-	22	2.9 ± 0.8	79.9 ± 4.1	48.3 ± 6.7	7627.1 ± 1066.1	4
C1	30	-	-	-	-	-	-	22
R1+	30	0	0	0	0	0	0	0
R2+	28	2	1	14	9	10	1580	0
FL-	27	2	0	0	0	0	0	1
FL+	24	1	1	1	6	2	316	0
FR-	19	2	1	1	18	2	316	1
FR+	26	3	0	0	0	0	0	1
O. fallax								
C2	30	-	30	17.9 ± 1.3	143.0 ± 3.7	381.1 ± 46.7	46780.8 ± 5972.4	0
C1	30	-	-	-	-	-	-	24
R1+	30	0	0	0	0	0	0	0
R2+	30	2	1	3	140	55	7040	0
FL-	30	19	19	5.2 ± 0.5	44.0 ± 3.4	36.2 ± 4.9	4634.9 ± 629.2	4
FL+	30	1	0	0	0	0	0	1
FR-	30	13	12	2.5 ± 0.4	10.2 ± 2.6	3.2 ± 0.5	403.7 ± 69.3	0
FR+	30	0	0	0	0	0	0	0
O. glazioviana								
C2	30	-	29	17.7 ± 1.8	128.5 ± 4.8	312.1 ± 34.5	48688.1 ± 5385.9	1
C1	30	-	-	-	-	-	-	24
R1+	30	0	0	0	0	0	0	0
R2+	29	2	1	3	46	160	23400	0
FL-	28	23	19	4.8 ± 0.4	39.8 ± 5.0	33.5 ± 7.0	1833.0 ± 354.5	2
FL+	30	1	0	0	0	0	0	0
FR-	29	26	12	2.45 ± 0.6	6.9 ± 3.8	11.8 ± 2.3	611 ± 118.2	1
FR+	30	0	0	0	0	0	0	0

mortality in regenerated individuals appearing after the first month from injury. Secondly, a difference in substrate between these two experiments could have brought about this dissimilarity as well. Even though light is not essential for root bud formation (Peterson 1975), loamy soil probably restricted light penetration into the soil and newly formed sprouts in the present experiment were mostly unable to reach the soil surface and died. On the other hand, in the previous experiment observed resprouting was higher (Martínková et al. 2004a), because the gravelly substrate did not prevent light penetration and new sprouts could be formed. A third explanation concerns the specific genetic characteristics of *Oenothera* spp. (self-pollination and Renner's circles, Cleland 1972) that cause a high inter-population and a low intra-population genetic variability in *Oenothera* species. For this reason, a high variability in resprouting abilities among *O. biennis* populations can be expected. Therefore, a population with a lower ability to resprout from the bud bank was possibly chosen for the present experiment, whereas a population with a higher resprouting ability was studied in previous one. Present field observations support the last explanation, because a high variability in resprouting after injury was found among populations of *O. biennis*. Even though the majority of studied ruderal populations was disturbed, only half of the disturbed populations showed individual responding to injury by resprouting from roots. However, according to the field observations the second explanation is also likely, because the highest number of root-sprouting individuals was found in habitats with a light sandy/gravelly substrate.

FREQUENCY OF DISTURBANCE

In spite of injury, some *Oenothera* individuals finished their reproductive cycle and set seeds in present experiment. However, the reproductive characteristics were considerably lower in comparison to intact plants. Therefore, it seems that the ability of resprouting from the bud bank in *Oenothera* populations is advantageous in man-made habitats with a disturbance frequency of approximately once per 1–3 years. Because there is a high probability that plants without resprouting never finish the reproductive cycle and a seed bank cannot be formed there or will be depleted soon. Moreover, due a high longevity (Baskin & Baskin 1994), relatively high weight and poor dispersal of *Oenothera* seeds (Hall et al. 1988), it seems that *Oenothera* species prefer areas repeatedly disturbed, and thus habitats suitable for re-colonisation occur as "windows" in time and *Oenothera* species possess a "wait for" strategy (Schippers at al. 2001).

Klimešová & Klimeš (2003) found the highest accumulation of root-sprouters in biennial species in comparison to annuals and perennials. From the previous discussion on disturbance frequency, a possible explanation for their findings arises. A fast life cycle allows annual species to occupy habitats with a very high frequency of disturbance, where regeneration from the seed bank is more advantageous than vegetative regeneration from the bud bank. On the other hand, perennials occupy less frequently disturbed habitats, where efficient (but more "expensive") devices for vegetative regeneration, such as stolons, rhizomes etc., may be developed. Since biennial life cycle is a transition between an annual and a perennial life cycle, biennials probably occupy intermediate habitats from disturbance frequency point of view, and root-sprouting in biennials is a compensation for a longer life-cycle (in comparison to annuals) and a shorter time and smaller reserves for the formation of a special device for vegetative regeneration (in comparison to perennials). However, this hypothesis still needs to be tested.

DISTURBANCE SEVERITY

In our experiment, successful resprouting was less frequent than in the study by Martínková et al. (2004a). As shown above, this can be caused by a high genetic variability of tested populations. Nevertheless, it is still true that resprouting from preserved basal axillary buds on the stem is more frequent than resprouting from adventitious root buds. This is probably caused by a high energy cost of adventitious bud formation in *Oenothera* species in comparison to the "cheap", i.e. already present, axillary buds (Essau 1965, Peterson 1975, Martínková et al., 2004a). In contrast, this relation was not found in two other short-lived herbs of disturbed habitats, *Rorippa palustris* and *Barbarea vulgaris*, that do not prefer axillary buds to adventitious buds on roots for regeneration after injury, and easily resprout no matter whether all axillary buds are destroyed or not (Martínková et al. 2004b, Martínková et al. submitted). Even though highly injured plants of *Oenothera* species mostly failed to resprout in the present experiment, lower injury to the plant body and breaking down of apical dominance induced resprouting in these species (for resprouting and apical dominance see e.g. Peterson 1975, Horvath 1998, Anderson et al. 2001). Thus, we suggest that *Oenothera* species are able to cope with cutting of aboveground biomass by resprouting from basal axillary buds, and that the ability to resprout is advantageous under these conditions. On the other hand, *Oenothera* species fail to resprout in strongly disturbed habitats, where all axillary buds are regularly destroyed, and population recovery is realised via the seed bank there. Therefore, the hypothesis that higher bud dormancy is an adaptation to severe disturbance (Tuomi et al. 1994, Lehtila 2000) is not valid in *O. biennis*. Our field data also support this idea, because no resprouting population that survived severe large-area disturbance was found, while frequently but less severely disturbed habitats were preferred. On the other hand, characteristics of the habitats occupied by *O. biennis* suggest that *Oenothera* species avoid older successional phases with a lack of disturbance and higher competition that prevent population regeneration from the seed bank.

TIMING OF DISTURBANCE

Due to a high inter-population variability in resprouting abilities, Rauh (1937) probably found a successful regeneration from root buds only in vegetative plants of *O. biennis*. However, Martínková et al. (2004a) reported a relatively high probability of regeneration after injury from root buds in both vegetative and reproductive plants. In contrast, no young vegetative rosettes regenerated from roots, and regeneration of reproducing plants and overwintered rosettes was exceptional in the present experiment. Therefore, the relationship between life-cycle phase at the time of injury and the probability of successful resprouting remains unclear in *Oenothera* species, even though the characteristics of plants regenerated from axillary buds were poorer in plants injured in the fruiting phase than in those injured in the flowering phase.

LIFE-CYCLE OF INJURED PLANTS

We found in the field that injured plants may resprout from root buds if disturbance severity is low. It seems that breaking down of apical dominance and a lack of auxin may induce resprouting from roots, despite the presence of axillary buds (Horvath 1998, Anderson et al. 2001). This event could lead to the observed polycarpy in *Oenothera*, because new root-sprouts can remain vegetative during the year of formation, but they can survive reproduction of the main stem and reproduce next season after overwintering (Klimešová 2003, Martínková pers. obs). However, as shown above, the observed frequency of polycarpy was very low in ruderal populations and survival of experimental individuals until the third season was rare. Therefore, it seems that *Oenothera* species adhere to a biennial life cycle and injury to the plant body does not postpone their reproduction from the second to the third year of life, in a way similar to the short-lived root-sprouting herb *Barbarea vulgaris* (Martínková et al., submitted).

To summarise, resprouting from roots takes place in about a third of studied ruderal populations of *Oenothera biennis*. However, among these populations, root-sprouting abilities highly vary. The ability of the biennial species *O. biennis, O. fallax* and *O. galziovina* to regenerate after a disturbance from the bud bank is probably efficient only under a certain disturbance degree, i.e. when some axillary buds are preserved. Individuals of all tested species largely prefer resprouting from axillary buds, and resprouting from roots is less important, even though they are capable of doing so. How important is the role of the life-cycle phase at the time of injury for the probability of successful resprouting, is a question that still needs to be answered in *Oenothera* species. Even though *Oenothera* spp. are less efficient root-sprouters than so far studied *Rorippa palustris* and *Barbarea vulgaris*, it is still upheld that the ability to resprout from the bud bank after injury can be crucial for short-lived species in highly and frequently disturbed habitats.

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CHAPTER VI

Intermediate growth forms as a model for the study of plant clonality functitioning: an example with root sprouters

Klimešová, J. & Martínková, J.
Evolutionary Ecology [in press]

CHAPTER VI

Intermediate growth forms as a model for the study of plant clonality functioning: an example with root sprouters

Abstract

In this contribution we want to show that growth forms intermediate between non-clonal and clonal plants can be used to ask questions about the functional ecology of clonality. We discuss this idea on plants sprouting adventitiously from roots and accomplishing clonal growth via root spacers.

Based on extensive literature dealing with growth forms of root sprouting plants, we characterise forms functionally intermediate between clonal root-sprouters and non-clonal plants. We delimit them according to their potential ability to form adventitious shoots and horizontal roots. By reviewing experimental work with root sprouters, we identify the most important triggering factors and developmental constraints influencing these intermediate forms: plant age, life-history mode and life-history stage. Using this information we ask questions about the importance of root sprouting in (i) conditions of unpredictable disturbance, where root-sprouting ability may be viewed as a tool for vegetative regeneration, and in (ii) temporarily and spatially heterogeneous environment, where foraging by roots may serve as a way of exploiting patchy resources.

INTRODUCTION

Sachs (2002) has recently postulated that developmental processes distinguishing clonal plants from their non-clonal relatives might be understood as rearrangements and quantitative changes of overall morphology. Since more than one change is required for a non-clonal plant to become clonal, intermediate forms possessing only an incomplete set of clonal properties must also have certain ecological advantages allowing them to exist and to be "stepping stones" on the pathway from a non-clonal to a clonal plant. Sachs (2002) showed that such intermediate forms really could be found using the example of plants with stem spacers (i.e., new ramets of these plants are established by a plagiotropic shoot whose apex turns upwards and forms an erect stem at some distance from the mother plant). He distinguished branch differentiation, development of erect axes and formation of shoot-borne roots as developmental processes steering for clonality. Furthermore, he discussed developmental mechanisms operating during plant morphogenesis, and outlined possibilities for further research of functional aspects of clonality based on stem spacers by studying the intermediate forms.

Inspired by Sachs' paper, we want to do the same intellectual exercise with plants whose ramets are not formed at the apex of a horizontal stem (stoloniferous or rhizomatous plants), but along horizontal roots (root sprouters). As root sprouting is of polyphyletic origin (Groff and Kaplan, 1988; Klimešová and Klimeš, in prep.) and we have no data mapping the evolutionary pathways at least not in certain genera of root sprouting plants differing in architecture, we will not follow the attempt of Sachs to interpret the intermediate forms evolutionarily. Functionally intermediate forms allow us to study functional aspects of clonal versus non-clonal growth, and also to ask questions about the adaptability of clonal growth in root sprouters. Our goal is to integrate our knowledge about the growth forms, ecology and evolution of root sprouting plants and to build up hypotheses testable by using functionally intermediate growth forms. We try to achieve this goal in several steps: first, we describe what root sprouting is; second, we outline functionally intermediate growth forms; third, we review triggering mechanisms of root sprouting in different growth forms.

ROOT SPROUTING

In addition to buds formed during embryogenesis and in the axils of leaves, buds can also be found at other locations on the plant body: on leaves, stems, hypocotyl and roots (Kerstetter and Hake, 1997). Because of their unusual location apart from leaf axils they are called adventitious (Groff and Kaplan, 1988). While axillary buds develop exogenously during normal shoot growth from the apical meristem, adventitious buds often need special stimuli to be formed endogenously from more or less differentiated tissues (Esau, 1965). The ability to form adventitious buds on roots is limited to less than 10% of the Central European flora (Klimešová and Klimeš, in prep.). Formation of adventitious buds on roots is a typical feature of some plant families (*Podostemataceae*) or genera (*Euphorbia, Linaria, Rorippa*). On the other hand, it is unknown in Monocotyledons except *Orchidaceae* (Rauh, 1937). A typical clonal root sprouting plant has long horizontal roots creeping close to the soil surface, root sprouting is spontaneous, and the area covered by a genet may be enormous (e.g., aspen clones – Brodie *et al.*, 1995). Except for this typical form, many others also exist, either with less extensive clonal growth or with only facultative formation of root sprouts.

INTERMEDIATE GROWTH FORM OF ROOT SPROUTERS

REVIEW OF GROWTH FORM CLASSIFICATIONS

In spite of the small number of root sprouting plants, the diversity of their growth forms is enormous and has been subjected to detailed morphological study. Similar analyses covering all rhizomatous plants do not exist.

The first descriptions of root sprouters originate from the middle of 19th century (Irmisch, 1857, 1859; Reichardt, 1857). Already Wittrock (1884) listed 138 root sprouting species in Europe (including cultivated plants). Also the first attempt to typify root-borne shoots according to their biological significance dates back to Wittrock (1884). He recognised three types of root sprouting: (1) additive – root-shoots arise during normal plant ontogeny but are not necessary to complete the plant's life cycle; (2) necessary – root-shoots are necessary for flowering or over-wintering of the plant; (3) regenerative – root-shoots arise only after injury to a plant (Fig. 1).

A next growth-form analysis of root-sprouters was published by Rauh (1937). After detailed studies of many species, Rauh elaborated a growth form system, in which the main groups were classified as obligate root-sprouters, facultative root-sprouters and regenerative root-sprouters. These three categories correspond to some extent with the three types proposed by Wittrock (1884). Exceptions are plants which have a flowering main shoot and regularly sprout from roots. They are classified as additive root-sprouters after Wittrock and obligatory root-sprouters after Rauh (e.g., *Rumex acetosella*).

Moreover, Rauh's groups were further divided based on the fate of the main shoot (flowering vs. non-flowering) and the localisation of adventitious shoots (hypocotyl, main root, lateral roots) (Fig. 1). However, Rauh (1937) not only classified root-sprouters and described their ontogenetic development, but also noticed a correlation between the development of the main (primary) shoot and the spontaneity of root sprouting. Rauh outlined a series of growth forms of related taxa, ranging from species with adventitious buds restricted to the hypocotyl and arising only facultatively and with a potentially flowering main shoot (*Euphorbia helioscopia*, *E. segetalis*), through a type in which root-sprouts grow spontaneously from the hypocotyl and the main root, and in which the main shoot flowers (*E. gerardiana*), to species with spontaneous root sprouting from the main root and with non-flowering main shoots (*E. amygdaloides*) and eventually, to species with non-flowering main shoots and vigorous root sprouting from horizontal roots (*E. cyparissias* and *E. esula*) (Fig. 2). Rauh described a similar spectrum of growth forms also in the genus *Linaria* and related species. Whereas species with facultative root sprouting restricted to the main root and hypocotyl are short-lived, obligate root sprouters with non-flowering main shoots and horizontal roots ("Wurzel Ausläufer") are perennial in the two genera mentioned. Even though Rauh indicated the respective forms by such adjectives as "basic type" or "derived type", he did not interpret them explicitly in evolutionary terms.

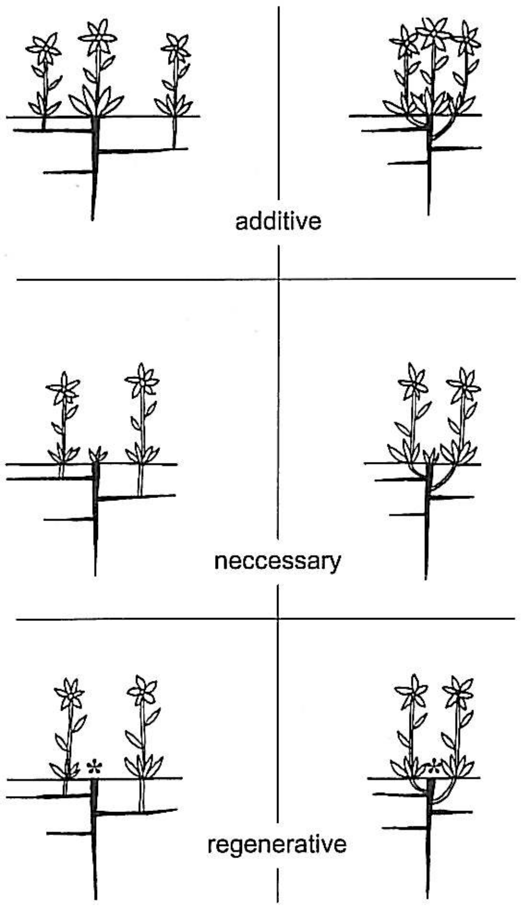

Fig. 1. The role of adventitious sprouting in the life of plants. Left icons – adventitious shoots are formed on horizontal roots; right icons – adventitious shoots are formed on main root and/or hypocotyl (according to Rauh 1937); additive – flowering and over-wintering of plants is not dependent on root-sprouting; necessary – flowering and over-wintering of plants is dependent on root-sprouting; regenerative – root-sprouting is triggered by injury to plant body; asterisk - injury (according to Wittrock 1884).

Replacement of the main shoot by adventitious shoots inspired Aeschimann and Bocquet (1980) to a new classification of root sprouters. Their classification is based on symmetry with the concept of allorhizy vs. homorhizy (Groff and Kaplan, 1988). They distinguished plants with either a primary root or a primary shoot only as representatives of allorhizy and allocauly, respectively. Plants with the main root substituted by shoot-borne roots (adventitious roots) were classified as representatives of homorhizy, and plants with the main shoot substituted by root-borne shoots (adventitious shoots) as representatives of homocauly. They further distinguished "accidentelles" (i.e., accidental), "supplémentaires" (i.e., additional) and "vicariantes" (i.e., replacing) adventitious roots and shoots (Fig. 1). These categories are similar to Wittrock's classification of root sprouters. A shortcoming of the classification is the implicit exclusion of the possibility that one plant can possess adventitious shoots and adventitious roots at the same time.

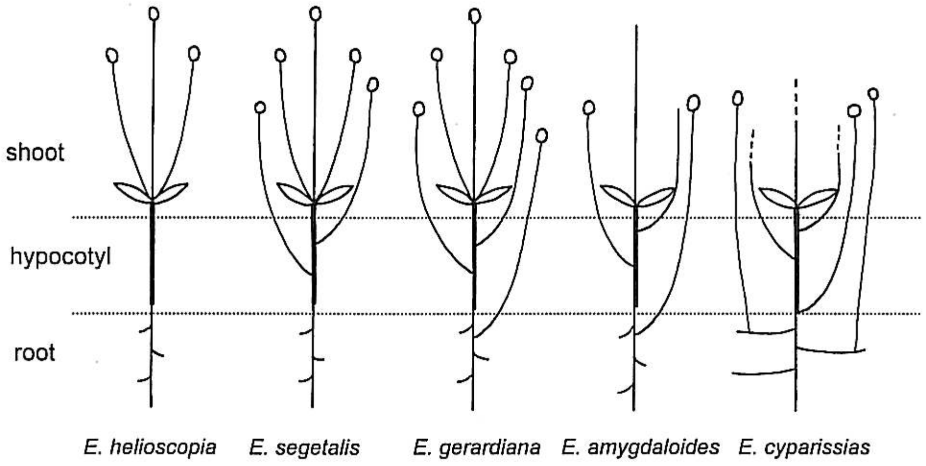

Fig. 2. Schema of growth of root sprouting members of the genus *Euphorbia*. Flowering shoots are marked by circles (according to Rauh 1937). For details see text.

The most complex system of relations between root and shoot systems in vascular plants (not only in root sprouters) was proposed by Groff and Kaplan (1988). They distinguished four "Structural classes". I – The plant has only a primary shoot system and primary root system, clonal growth is limited to vertical fragmentation. II – The plant forms adventitious roots, clonality is attained by fragmentation of the shoot system. III – The plant forms adventitious shoots, clonality is attained by fragmentation of the root system. IV – The plant forms both adventitious shoots and roots (Fig. 3), therefore three possibilities for clonal growth exist: (a) only the shoot system fragmentises, (b) only the root system fragmentises, or (c) both the root and shoot system fragmentise (Fig. 3). This classification covers all principal types of clonal and non-clonal plants. A shortcoming of this classification is that not all species capable of producing adventitious shoots or roots, or both, really display clonal growth (Rauh, 1937; Sachs, 2002). Moreover, it is not taken into consideration that clonal growth can be achieved by normal growth or by reiteration (*sensu* Hallé *et al.*, 1978).

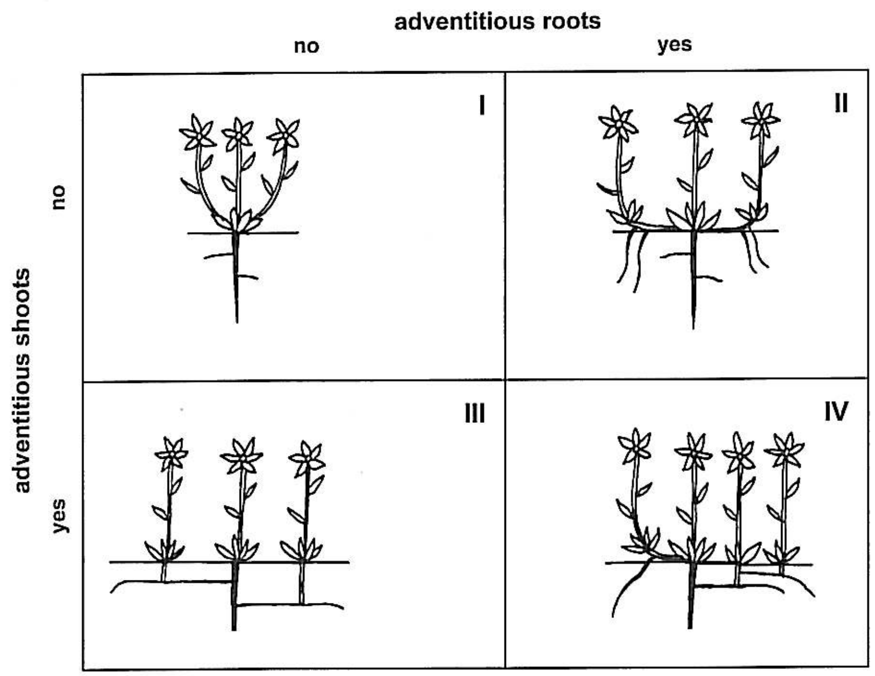

Fig. 3. Participation of adventitious roots and adventitious shoots in structural classes (I – IV) by Groff and Kaplan 1988. Structural classes embody possible ways how to achieve clonal growth.

Agnes Arber with her theory of partial shoot (Arber, 1930, 1941) stays apart from the positions of the German typology school represented by Rauh (1937), Aeschimann and Bocquet (1980) and Groff and Kaplan (1988). She views the ability of roots of some species to form adventitious shoots as a proof of the shoot and root not being distinct morphological categories, i.e., the potentiality to behave as a shoot lies dormant within the character of a root. This hypothesis of dynamic plant morphology has no phylogenetic implications (Classen-Bockhoff, 2001). This developmental point of view has the advantage of no boundaries being laid across the process, which is without doubt continuous. On the other hand, typology gives us a useful tool for the description of the observed pattern.

FUNCTIONALLY INTERMEDIATE GROWTH FORM

We delimited the following attributes necessary for extensive clonal growth: formation of root-borne shoots and plagiotropic growth of roots. On the other hand, formation of adventitious (shoot-borne) roots seems to be unnecessary because indefinitely growing lateral roots starting to decay from their older parts may serve extensive clonality well (Groff and Kaplan, 1988) (Fig. 3). Such attributes are found in plants that potentially sprout from roots and thus potentially grow clonally to some extent. Those plants may be classified as regenerative and additional root sprouters after Wittrock (1884) and facultative and regenerative root sprouters after Rauh (1937), and Aeschimann and Bocquet (1980).

We may find potential root sprouters among all life-forms. While perennial potential root sprouters are able to regenerate from root buds after injury and then continue the usual iterative growth, short-living monocarpic species (e.g. *Rorippa palustris, Oenothera biennis, Barbarea vulgaris*) often change their growth form or life history (prolongation of life span, prolongation of juvenile phase, polycarpy, extensive growth of lateral roots or adventitious roots) by triggering root sprouting (Dubard, 1903; Rauh, 1937; Kott, 1963; Klimešová, 2003).

As root systems are very plastic in relation to soil quality, horizontal roots may be observed in many plants both with and without root sprouting (Kutschera and Lichtenegger, 1992). However, it is reported that root sprouting may be a triggering factor for horizontal growth of roots. According to Wehsarg (1954) *Knautia arvensis* and *Centaurea scabiosa* growing on arable land may lose all aboveground parts and even the upper parts of their below-ground organs by ploughing. After such severe injury the plants regenerate from their fleshy main roots and produce several adventitious shoots compensating for the lost parts. On underground parts of adventitious stems, adventitious roots arise during the growing season, and from them adventitious shoots regrow. Root-borne shoots translocate assimilates preferentially to the apical end of the adventitious mother root (see also Troll, 1941; Fykse, 1974). This means that the original main root successively loses its dominant function and a unitary plant turns into a clonal root sprouter (Wehsarg, 1954). A similar trend can be observed in the short-lived herbs *Barbarea vulgaris* (Kott, 1963) and *Cirsium arvense* (Wehsarg, 1954). The plants start, after injury, to produce adventitious buds on their horizontal roots and support their lateral growth. But this is possible only in wet soil, where the plants have a richly branched main root. In dry or heavy soil the main root is poorly branched, and regeneration may therefore be restricted.

FACTORS TRIGGERING ROOT SPROUTING

The most studied factors triggering root sprouting are external factors such as disturbance (injury to plant body) and nutrient level. The interactions of these external factors with ontogeny are less examined, but they also play an important role in the ability of a plant to sprout adventitiously from roots.

EXTERNAL FACTORS

DISTURBANCE

Root sprouting is conditioned or stimulated by disturbance of plant integrity in the majority of plants (Peterson, 1975). Most trees do not begin sprouting from roots until the primary trunk has been injured (Del Tredici, 2001). Removal of or injury to aboveground plant parts facilitates and often enables the formation of new root buds, or break their dormancy and also facilitate growth of new root-borne shoots (Rauh, 1937; Peterson, 1975; Del Tredici, 2001; Martínková *et al.*, 2004a, b). Examples of such disturbances are defoliation, decapitation, cutting of branches or removal of all aboveground plant parts. For example, McIntyre (1972) found that decapitation of lateral branches of the perennial herb *Euphorbia esula* promotes elongation and growth of root buds. Horvath (1998, 1999) showed that leaves and growing meristems (apical or axillary buds) of the same species prevented root buds from breaking quiescence, or reduced the growth of root buds. In an experiment with juvenile plants of the short-lived herb *Rorippa palustris,* it was found that the number of initiated adventitious buds on roots was markedly enhanced by injury. On the other hand, the severity of injury (defoliation vs. removal of all leaves and stem parts) does not play any role in root-sprouting of this species (Martínková *et al.*, 2004b). This implies that *Rorippa palustris* produces preformed adventitious buds on its roots, but does not resprout from them until a plant is injured. Rauh (1937) found the same strategy in *Geranium sanguineum* and *Rumex sanguineus*. On the other hand, *Oenothera biennis* forms root buds and releases new root-borne shoots almost exclusively after injury (Martínková *et al.*, 2004a), even though these buds are not formed directly on the callus tissue as in some other species, e.g. *Trifolium alpestre, Centaurea scabiosa* (Rauh, 1937).

Injury to the plant body is the most essential factor triggering root-sprouting regardless of the fact that the extent of injury necessary for activation or formation of adventitious buds on roots varies among species.

NUTRIENT LEVEL

Mineral nutrition has an important effect on the growth of root sprouting plants (Peterson, 1975). Higher nutrient levels support bud formation, break down bud dormancy and facilitate growth of root-borne shoots in perennial species (Peterson, 1975). However, an opposite effect was also found: the number of adventitious buds on roots of *Chondrilla juncea* was not affected by the nutrient level, but the growth of new shoots was supported by low nutrient levels (Kefford and Caso, 1972). According to a study by McIntyre and Hunter (1975) on another perennial weed of arable land, *Cirsium arvense*, root buds are initiated more frequently on plants growing at lower nutrient levels than at higher ones. Nadeau and Van den Born (1990) found no effect of nitrogen addition on the number of emerged root buds. However, the majority of these buds remained dormant under these conditions and new shoots were developed from established root buds more frequently in environments with a higher nutrient availability (McIntyre and Hunter, 1975). Similarly, Klimeš and Klimešová (1999a) found that nutrition stress facilitated the formation of root buds in *Rumex acetosella*, but their growth and transition to shoots was enhanced at higher nutrient levels. Studies on *Rorippa palustris* (Martínková *et al.*, 2004a, b) showed that the number of adventitious buds formed on roots was not affected by nutrition level, but a higher nutrient level supported the release of buds and growth of new root-borne shoots.

The relationship between nutrient level and root sprouting is still uncertain, but it seems that both regenerative growth after disturbance and transition of root buds into shoots are facilitated at higher nutrient levels. The number of buds is either not affected by nutrient level, or supported by nutrient shortage.

INTERNAL FACTORS

PLANT AGE

Information on the relationship between plant age and root-sprouting ability is very scarce, but it is obvious that root sprouting following injury is dependent on plant age (Martínková *et al.*, 2004b). Very young seedlings are unable to form adventitious buds on their roots and to sprout, because the ability to root-sprout is connected with sufficient assimilation and the presence of activated meristem tissue (Esau, 1965; Peterson, 1975). Evetts and Burnside (1972) found that injured juveniles of *Asclepias syriaca* were able to sprout at the age of three weeks. Some injured juveniles of the short-lived herbs *Rorippa palustris* and *Barbarea vulgaris* were capable of root-sprouting at the age of four weeks, and 100% regeneration occurred already in six week old juveniles of these species (Martínková *et al.*, 2004b). However, all adventitious buds formed on roots were used for growth of new shoots after disturbance at this age, thus no dormant root buds were left in place for a possible later disturbance (Martínková *et al.*, 2004b). In contrast to this, eight week old plants of *Rorippa palustris* save some adventitious buds on their roots for possible later disturbance as an insurance for faster regrowth (Martínková *et al.*, 2004b). It also seems that plant age is more important for the root-sprouting ability than plant size. Injured plants of *Rorippa palustris* reached comparable values of plant size characteristics (number of leaves, rosette diameter, etc.) in two different experiments. Nevertheless, younger plants were not as successful in root sprouting after injury as older plants (Martínková *et al.*, 2004b).

LIFE-HISTORY MODE

The life-history mode of *Rorippa palustris* is determined by the time of germination; germination from April to June results in an annual life-history mode, while winter annual plants germinate later in the growing season (Sosnová, 2003). In a study by Martínková *et al.* (2004a), both life-history modes of this species were artificially induced by manipulating with day length in a growth chamber experiment. The root-sprouting ability was independent of the life-history mode of the experimental plants, only the extent of regeneration was influenced by it (Martínková *et al.*, 2004a). In a garden experiment with sequentially sown cohorts of *Rorippa palustris*, Sosnová (2003) found that plants behaving as (summer) annuals regenerated more successfully than plants in winter annual mode. According to these studies, it seems that injury to the plant body is more disadvantageous for plants in the winter annual life-history mode than for plants in the (summer) annual life-history mode. No study was found that would compare the root sprouting ability of short-lived plants with that of perennial plants.

LIFE-HISTORY STAGE

The poorest root sprouting of perennial species occurs at the time of flowering (Peterson, 1975). Dubard (1903) and Rauh (1937) noticed that the biennials *Alliaria officinalis* and *Bryonia dioica* were able to sprout from roots only at the end of the first growing season, and the short-lived herb *Oenothera biennis* was only in vegetative stage (rosette). According to a more recent study (Martínková *et al.*, 2004a), *Oenothera biennis* regenerates from roots also in the generative phase (fruiting stem), but less successfully than in the vegetative stage. The same result was found for the short-lived perennial *Barbarea vulgaris* (Martínková *et al.*, in prep.). Moreover, flowering plants of *Oenothera biennis* and *Barbarea vulgaris* regenerate more successfully than plants setting seeds (Martínková *et al.*, 2004a, b; Martínková *et al.*, in prep.). In some woody species sprouting ability increases with size to reach a maximum at adult stage. In other species, however, sprouting is common in juveniles whereas adults are unable to resprout (Del Tredici, 2001). This pattern of resprouting ability throughout a plant's life can be interpreted as a consequence of reserve accumulation capability and reserve allocation to growth or reproduction (Dubard, 1903; Chapin *et al.*, 1990; Bellingham and Sparrow, 2000; Bond and Midgley, 2001). On the other hand, no seasonal pattern was found in the presence of root buds in different times of the year in *Cirsium arvense* (McAllister and Haderlie, 1985).

In perennial plants, resprouting from roots often starts in the second year of their life, when the main shoot dies (Rauh, 1937). However, young plants of *Epilobium angustifolium* are able to sprout in the first year of their life if they are severely injured (Klimešová pers. obs.).

The observed relationships between root sprouting and ontogeny are not only direct but also more complicated, and all possible interactions of the known triggering factors together should be taken into account.

CONCLUSIONS

The proportion of root sprouters differ among growth forms. It was found to be the highest of all growth forms in biennials and trees (Klimešová and Klimeš, 2003). These forms do not possess clonal growth organs of stem origin (such as stolons or rhizomes) or a below-ground bud bank. Even root-sprouting perennial herbs usually do not posses any other organ that could be used for clonal growth (Irmisch, 1857; Klimeš and Klimešová, 1999b). It seems that the evolution of clonality via root spacers was probably not only independent of, but also alternative to that mediated by stem spacers (rhizomes and stolons).

Facultative root sprouters, especially biennials, seem to suit our demands for functionally intermediate forms between a unitary plant and a clonally growing root-sprouter. As injury is the main triggering factor of root sprouting in short-lived perennials, we cannot exclude the possibility that potential sprouting represents an adaptation to unpredictable disturbance. In experiments, where clonality is manipulated by severe injury to the plant body, we cannot, unfortunately, separate the advantage of bud bank formation from an initiation of clonal growth. An important question is, if root-sprouting biennials differ from non-sprouting ones in their allocation to growth, flowering and storage. Alternatively, one may ask whether root-sprouting biennials, after being injured at a specific age and ontogenetical stage of development, change allocation between structures of vegetative versus generative regeneration. It follows from studies on ontogenetical constraints on potential root sprouting that switching from exclusive investment into seed formation to the investment into vegetative growth declines with progressive exhaustion of reserves allocated to fruits.

Another promising direction of research can be seen in the manipulation with root sprouting by temporal heterogeneity in nutrient availability. Three main factors, enhanced formation of adventitious buds due to nutrient shortage, vigorous sprouting due to nutrient addition, and root proliferation in rich soil patches, may be responsible for opportunistic growth of root sprouting plants. Root sprouting is a form of foraging, since roots must grow for other reasons, so it could be an extremely efficient way of searching the environment for the best locations (see also Sachs 2002). This strategy may be advantageous in nutrient-limited habitats, such as xeric grasslands, where a relatively high proportion of root sprouters is found (Klimešová and Klimeš, in prep.).

We can conclude that clonality via root spacers may be viewed as a way of vegetative regeneration. We cannot exclude clonality attained by reiteration, because injury to plant body in habitats with unpredictable disturbance may become an important stimulus for repeated occurrence of intermediate forms between clonal and non-clonal plants in such habitats. On the other hand, obligate root sprouters among perennial plants may be regarded as typical representatives of a "sit and wait" strategy, as they possess prerequisites for foraging in temporarily heterogeneous environments.

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CHAPTER VII

Biological flora of Central Europe: *Rorippa palustris* (L.) Besser

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CHAPTER VII

Biological flora of Central Europe: *Rorippa palustris* (L.) Besser

Summary

Rorippa palustris is an autogamous short-lived polycarpic herb capable of sprouting from roots. It prefers open wet habitats, such as pond and river shores or emergent pond bottoms. Recently it has expanded into disturbed fertile wetlands. This article reviews the taxonomic status and morphology, the distribution, ecology, life cycle, population biology and genetics of this species.

Key words: Rorippa palustris, *ecology, species biology, Central Europe*

1. TAXONOMY AND MORPHOLOGY

1.1. TAXONOMY

Rorippa palustris (L.) Besser (Brassicaceae) – Gewönliche Sumpfkresse, marsh yellow-cress, bog yellow-cress

Synonyms:
Sisymbrium amphibium L. var. *palustre* L.
Sisymbrium palustre (L.) POLLICH
Sisymbrium terrestre WITH.
Nasturtium terrestre (WITH.) R. BR. non TAUSCH.
Nasturtium palustre (L.) DC.
R. islandica auct. non (OEDER ex MURRAY) BORBÁS

The formerly often used name *R. islandica* (OEDER ex MURRAY) BORBÁS applies to a diploid species occurring in Iceland, north west Europe, the Pyreneans, Alps, Apennines and in the Balkan peninsula. In Europe only subsp. *palustris* occurs. Other subspecies are reported from N. America and Asia (STUCKEY 1972; TOMŠOVIC 1992). Their value is, however, questionable (AL-SHEBAZ, in litt.).

1.2. MORPHOLOGY

A young plant consists of a main shoot with a rosette of leaves and a main root. The main shoot grows in the field into a leafy flowering shoot after 2–3 months of vegetative growth or after overwintering. In a growth chamber, under conditions without competition in full light with high availability of nutrients, the vegetative phase lasts 4 weeks only. At the time of flowering the rosette leaves die out. Adventitious buds appear on the main root after several weeks on cultivated plants (MARTÍNKOVÁ et al. 2004b) and remain dormant until injury or senescence of the primary stem. Axillary and adventitious buds that usually start to grow at the end of the season, producing new rosette of leaves, flower next season. Adventitious roots start to grow along with the formation of adventitious buds, however, the main root remains dominant throughout the first year of life. In the description of the root system of young *Rorippa palustris* growing on arable land, KUTSCHERA (1960) reported only a richly branched main root reaching a depth of 76 cm, and did not observe any adventitious roots. Similarly, KOPECKÝ (1961) pictured a young plant observed on sandy sediments of a river bed, possessing a main root growing to a depth of 70 cm with horizontal branches up to 40 cm long without adventitious roots and shoots.

The flowering stem is simple or branched, 5 cm – 14 dm tall, either glabrous or sparingly to densely hirsute on lower parts, upper parts of the stem are sparingly hirsute or glabrous. Leaves are sessile or more or less petiolate or auriculate. The leaf blade is pinnatisect to (lyrate) pinnatifid (rarely upper undivided), 4–20(30) cm long, 15(8) cm wide, glabrous or sparingly to densely hirsute. The terminal lobe apex is narrow to broadly acute. Lateral lobes are irregularly serrate, repand or variously pinnate-divided.

Racemes are terminal and axillary. The terminal raceme develops as the earliest one. The oldest siliques are located at the lowest position of the raceme. Sepals are greenish, 1.2–2.5 mm long, pellicular; petals are yellow, dry ones whitish, 1.5–2.5 mm long, shorter or equal to the sepals. Siliques are oblong, alternatively cylindrical, straight or slightly curved upwards, 4.5–7.0 mm long, 2.0–2.5 mm wide, mostly 2–4 times longer than wide. The valves are glabrous. Styles are 0.2–1.2 mm long; the pedicels ascending, divergent or slightly to strongly recurved, 3–10 mm long, shorter than or of the same length as fruits. Siliques contain more than 50 seeds. Seeds are 0.5–0.7 mm long, the weight of a single seed is about 0.07 mg. The surface of the seed is coarsely colliculate and of a light ochre colour (GRIME et al. 1988; TOMŠOVIC 1992; BENDOVÁ 2002).

R. palustris flowers from June to September (STUCKEY 1972; TOMŠOVIC 1992). After flowering of the main shoot, axillary shoots start to grow from its base, and flower. Seeds ripen from July to the end of the season. Flowers are hermaphrodite and autogamous.

2. DISTRIBUTION AND HABITAT REQUIREMENTS

2.1. GEOGRAPHICAL DISTRIBUTION

Rorippa palustris is the most widespread species of the genus. Its distribution is boreal circumpolar (Fig. 1) (HULTÉN & FRIES 1986). *R. palustris* reaches occasionally the Arctic zone, for example in Greenland, Arctic slopes of Alaska and in Arctic Siberia where it has probably been introduced recently (JONSELL 1968). In Europe, it has a continuous distribution between the 45th parallel and the north coast of the continent. In northern Fennoscandia, localities of *R. palustris* are rare but often regarded as indigenous (JONSELL 1968). It is missing on Iceland, Svalbard, the northernmost part of Scotland, and in the Mediterranean. In Asia, *R. palustris* has a continuous distribution area between the 50th and 60th parallels. Outside this area it occurs in the Himalayan region, in the Central Asiatic mountains and in the Caucasus. Plenty of specimens have been collected in China and Japan (JONSELL 1968). In North America, *R. palustris* occurs nearly continuously except for the warmest parts of the USA. Localities in Central America (Cuba, Haiti, Mexico), South America (Brazil, Colombia), Africa (Egypt, Ethiopia), Australia, New Zealand and Tasmania are probably secondary (JONSELL 1968). GARNOCK-JONES (1978) supposes *R. palustris* to be a native species in New Zealand, due to the existence of herbarium specimens from the beginning of British colonisation.

R. palustris is a lowland species and its distribution overlaps with the distribution area of *R. islandica*, which is an arctic and alpine species and occurs in Ireland, Scotland, Greenland, Alps, the Pyrenees and the Apeninnes (JONSELL 1968). Despite of localities where both species are present in Ireland, Wales and England (GOODWILLIE 1995; RICH & CHATER unpubl.), *R. palustris* usually occurs at lower altitude than *R. islandica*. At higher altitudes it is allochtonous, and plants are often small and rather sterile there (JONSELL 1968). There are records of *R. palustris* at 1800 m a.s.l. in the Pyrenees, from 1300 to 2600 m a.s.l. in the Alps, at 593 m a.s.l. in Storlien, Jämtland (Sweden) (JONSELL 1968) and at 1300 m a.s.l. in the Giant Mts. (Czech Republic) (TOMŠOVIC 1992).

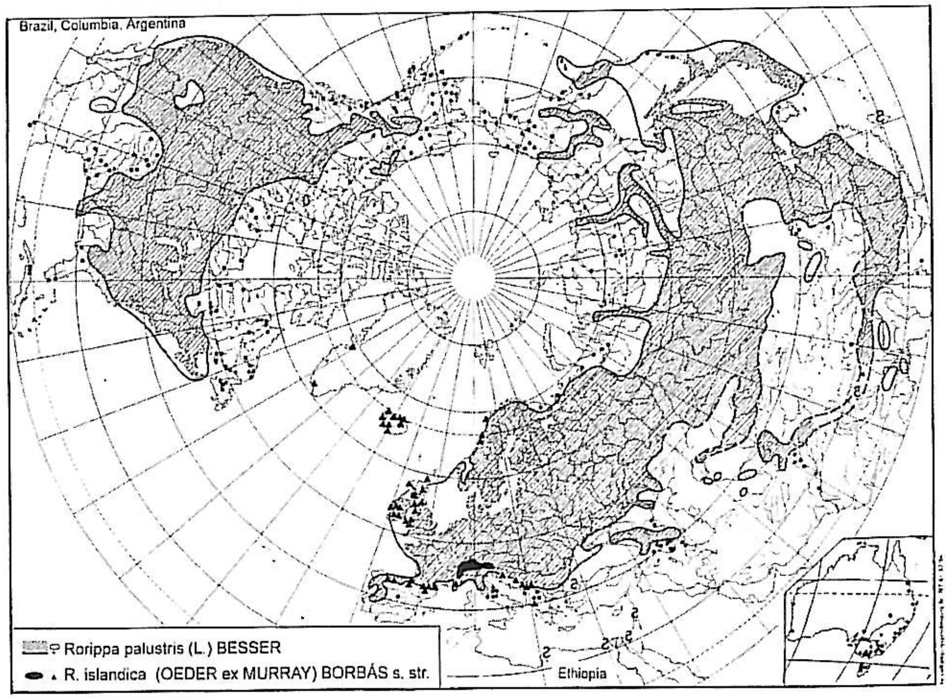

Fig. 1 Distribution map of *R. palustris*

2.2. HABITAT

R. palustris is reported from moist, both clayey and sandy soils. Soil pH is usually above 5.0 and nutrient concentration is high (TOMŠOVIC 1992; GRIME et al. 1998, KÄSTNER et al. 2001).

Plants of *Rorippa palustris* inhabiting a summer-drained bottom of the Starý Vdovec fishpond (South Bohemia, Czech Republic) were growing in the wettest parts of the moisture gradient (water table a few cm below the soil surface) (PRACH et al. 1987). The species covered about 25% of the soil surface there. In comparison with other sites in the area, the habitat where *R. palustris* occurred was characterised by the deepest sediment layer (0.7 to 0.9 m), the biomass of the stand was the highest (500 $g.m^{-2}$), the plants were the tallest (0.8 m) and the species diversity was the lowest. For the habitat of *R. palustris*, high concentrations of principal nutrients (N, K, Ca, P) were also characteristic. Chemical properties of the bottom sediment were as follows: $pH(H_2O)$ 6.6, PO_4-P 360.0 $mg.kg^{-1}$, NO_3-N 57.2 $mg.kg^{-1}$, NH_4-N 126 $mg.kg^{-1}$, Ca^{2+} 6600 $mg.kg^{-1}$, K^+ 210 $mg.kg^{-1}$, Na^+ 980 $mg.kg^{-1}$, Mg^{2+} 740 $mg.kg^{-1}$.

2.3. COMMUNITIES

Rorippa palustris occurs on semi-natural and anthropogenic habitats, such as field edges and wet terrain depressions filled by spring rainwater (vernal pools) for a short period. *R. palustris* typically occurs together with *Alopecurus geniculatus, Bidens tripartita, Lythrum hyssopifolia, Ranunculus flammula, Polygonum aviculare, Plantago uliginosa, Poa annua, Ranunculus repens* and *Juncus bufonius*. Stands of these species are classified as communities belonging to the class Isoëto-Nanojuncetea, the alliance Nanocyperion flavescentis (VALACHOVIČ et al. 2001).

In pioneer swards of nutrient-rich drained bottoms of periodic pools and fishponds, *R. palustris* occurs together with *Cyperus fuscus, Eleocharis acicularis, E ovata, Limosella aquatica, Lindernia procumbens, Schoenoplectus supinus, Riccia cavernosa, Carex bohemica, Coleanthus subtilis* etc. Communities of those habitats are classified into the class Isoëto-Nanojuncetea, alliance Elatini-Eleocharition ovatae (VALACHOVIČ et al. 2001).

Rorippa palustris can also be found in open stands of *Phalaris arundinacea* along banks of flowing waters together with *Rorippa amphibia, Persicaria hydropiper, Poa palustris, Ranunculus repens* and *Phalaris arundinacea* (ass. Rorippo-Phalaridetum arundinaceae, class Phragmito-Magnocaricetea) (KOPECKÝ 1961).

Rorippa palustris is a characteristic species of the phytosociological class Bidentetea tripartiti and can be found in habitats of the class Salicetea purpureae, and also in various wet man-made habitats of the classes Chenopodietea and Secalietetea. *Rorippa palustris* is a heliophilous crop weed indicating wet soil (TOMŠOVIC 1992), typically co-occurring with *Myosoton aquaticum* (KUTSCHERA 1960).

2.4. RESPONSE TO ABIOTIC FACTORS

Adventitious sprouting from roots after injury was assessed in relation to nutrient level, age of plant and life history mode. MARTÍNKOVÁ et al. (2004a) investigated the influence of day length on growth and resprouting after injury of vegetative plants of *R. palustris* in a chamber experiment. Plants growing in a short-day treatment (9 h light/15 h dark) had prostrate rosettes with the apical meristem hidden under the soil surface, thus prepared for overwintering (winter-annual life history mode). Plants cultivated in a long-day treatment (15 h light/9 h dark) had rosettes with erect leaves and the apical meristem aboveground, prepared for flowering (summer-annual life history mode). Plants in the short-day treatment had a higher R/S ratio and smaller rosette diameter than plants in the long-day treatment. Regeneration from roots as for the number of adventitious buds on roots after total removal of leaves and stem parts was not affected by the day length regime. Regeneration from roots as for newly produced biomass was higher under the long-day treatment (MARTÍNKOVÁ et al. 2004a). In the same experiment, a high nutrient level resulted in larger leaf rosettes of intact plants and a higher amount of regenerated biomass in injured plants of *R. palustris* than at low nutrient levels.

In a glasshouse experiment, ability of vegetative regeneration after injury was studied in overwintering rosettes of *R. palustris*. The plants cultivated at three levels of nutrient availability were subjected to two levels of injury (defoliation and decaulination) (MARTÍNKOVÁ et al. 2004b). In the experiment, standard garden soil was used (N 400 mg/l, P_2O_5 300 mg/l, K_2O 400 mg/l, pH = 6.0) mixed with sand (volume ratio 1:2 for low, 1:1 for medium and 1:0 for high nutrient level). In intact plants, the high nutrient level positively influenced rosette diameter, but the number of leaves was not affected. In injured plants, the high nutrient level caused a higher number of resprouted shoots. In the low nutrient treatment, the number of dormant adventitious buds on roots was higher than in the high nutrient treatment. The total number of adventitious meristems (shoots and buds together) was not affected by the level of nutrients (MARTÍNKOVÁ et al. 2004b).

The response of young plants of *R. palustris* to timing of injury was investigated in a chamber experiment (MARTÍNKOVÁ et al. 2004b). Six-week old plants resprouted after removal of all leaves and stems, but plants younger than three weeks did not. Juveniles with either < 10 leaves and < 14 cm rosette diameter or with dry aboveground biomass < 0.14 g failed to resprout after injury.

According to our field observations in the Třeboň basin (South Bohemia), *R. palustris* grows successfully under a fluctuating water table. It withstands repeated submersion lasting for several days. Even if *R. palustris* colonises preferably habitats without shading, we have observed stunted plants growing under a canopy of *Polygonum hydropiper* and *P. lapathifolium*. They were only about 10 cm high with a few green leaves on an unbranched stem, and flowered sparsely.

2.5. ABUNDANCE

Large populations of *R. palustris* with thousands of individuals are found at lower middle Elbe (Germany) (Bleeker unpubl.) or may exceptionally establish on summer drained bottoms of fishponds. Usually individuals of *R. palustris* are scattered within plant communities. Only once, we observed a dense population of stunted plants in an understory of a *Polygonum* stand on a shore of a fishpond in South Bohemia (Czech Republic) (see response to abiotic factors).

3. LIFE CYCLE AND BIOLOGY

3.1. LIFE CYCLE

R. palustris is usually a therophyte, but sometimes it may behave as a hemicryptophyte with overwintering rosette of green leaves, or geophyte. The last possibility is realised due to adventitious buds on roots (Fig. 2).

The annual life-history is observed mainly in ephemeral habitats, such as river shores, bottoms of summer drained fishponds or in wet depressions of arable land. Seeds germinate after flooding but established plants are often killed by raising water or may dry out (HEJNÝ 1960). Outside of these ephemeral habitats, *R. palustris* may perennate and flower repeatedly (Fig. 3). Plants of *R. palustris* germinating from April to June form seeds in the same year and behave as annuals, but plants germinating later in the season overwinter as a rosette and reproduce in the next year (SOSNOVÁ 2003). In natural populations, almost half of individuals flower repeatedly (KOČVAROVÁ in press). Seedlings, young sprouts from root fragments, one-year-old flowering plants and two-year-old flowering and vegetative plants may be found at one locality (KOČVAROVÁ in press). JONSELL (1968) cultivated plants of *R. palustris* and found that no plant survived more than 5 years. However, he also mentioned that populations from different regions differ in their ability to perennate (JONSELL 1968).

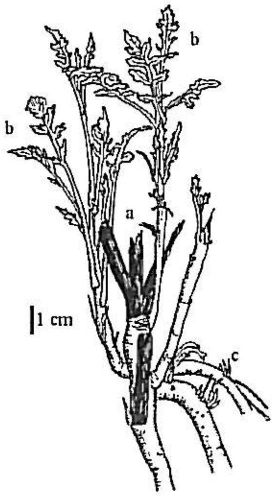

Fig. 2 Spring regrowth of *Rorippa palustris*. (a) last-year flowering shoot, (b) renewal axillary shoots with preformed inflorescences, (c) adventitious buds on roots. The plant was collected in March 2003 in the littoral zone of the Ratmírovský fishpond (Czech Republic).

3.2. SPATIAL DISTRIBUTION OF PLANTS WITHIN POPULATIONS

Individuals are usually easily recognisable due to very limited lateral growth and a persisting main root. However, in disturbed habitats clones may develop as a consequence of resprouting from fragments of aboveground stems or roots after disintegration of the plant body.

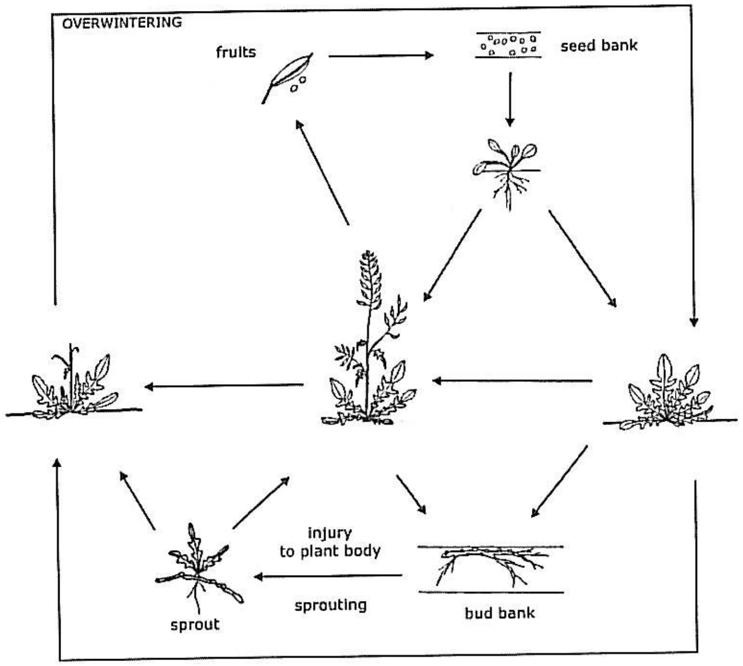

Fig. 3 Possible pathways of the life-cycle of *Rorippa palustris*.

3.3. Phenology

Early growth of young plants may be rather quick. A rosette with more than five leaves of about 3 cm long may be formed during three weeks of life in favourable conditions (Fig. 4). The phenology of plants in the first year of life differs according to their time of germination. Sosnová (2003) cultivated six cohorts of *R. palustris* in a pot experiment. Plants sown in April and May started to flower three months later and flowered and fruited the whole season. Plants sown in June flowered less than older plants but started to flower earlier, i.e. at the beginning of August. Plants sown in July, August and September flowered only rarely and overwintered as leaf rosettes. Successful overwintering of plants depended on the date of the plant's germination. Less than 20% of plants germinating in April, May and September overwintered successfully, whereas more then 50% plants germinating in June, July and August overwintered successfully. Overwintering plants start to flower next June (Sosnová 2003).

The first seeds ripen in June and because flowering continues up to the end of the season, seeds also ripen unitl the end of September. They are able to germinate immediately after being shed from the mother plant. Seedlings appear throughout the whole season with maximum occurrence in spring (Kästner et al. 2001).

3.4. Reproduction

Plants of *R. palustris* have small hermaphroditic autogamous flowers which are probably always self-pollinated (Jonsell 1968). Plants may produce a huge amount of seeds: small individuals about 200 medium individuals about 130,000 seeds (Grime et al. 1988). The reproductive capacity of a population (defined as the number of seeds produced by a species per 1 m^2 at its one-hundred-per-cent cover per one season) was estimated at 396,175 (n = 3, standard deviation = 129,559) (Šerá & Šerý 2004).

In Czech populations, seeds differ twofold in their size on a plant. Small seeds have a lower germinability and emerged seedlings have a higher mortality and lower relative growth rate than seedlings arising from big seeds (Kočvarová & Klimešová in prep.) (see Chapter 3.5 Germination).

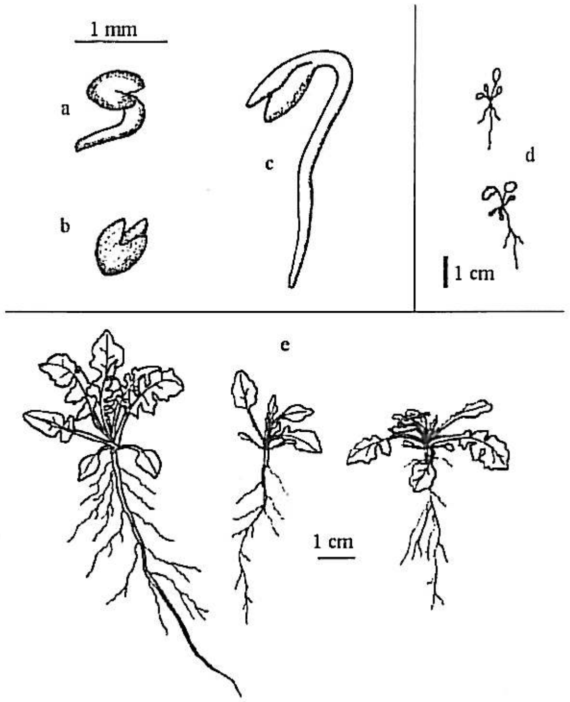

Fig. 4 Germination and seedling development during the first weeks of the life of *R. palustris*. (a), (b) germinating seed, (c) 3-day-old seedling, (d) 10-day-old seedling, (e) 3-week-old plants.

Seeds are easily spread by water as they have a water-repellent surface and their buoyancy is longer than one month (Bendová 2002). Due to their small size, dispersion by animals is potentially also efficient, as reported by Bendová (2002), who experimentally tested this ability using rabbit fur. She placed 10 seeds on the fur, which she moved into a vertical position, and counted seeds remaining attached to the fur. This procedure was repeated five times, always with new seeds. The average percentage of attached seeds for *R. palustris* was 100%. BENDOVÁ (op. c.) also experimentally tested seed traits promoting anemochory (time needed for a diaspore to fall from a height of 4.5 m), which was relatively poor for *R. palustris* in comparison with more than two hundred other species from different biotopes. However, due efficient hydrochory and epizoochory, *R. palustris* ended at the 14th position, after all tested species had been arranged according to their total dispersability potential (i.e. sum of individual dispersal abilities).

Vegetative spread is not so important in *R. palustris* in comparison with seed propagation. However, detached parts of vegetative shoots rapidly re-root (Fig. 5). This ability often allows re-anchoring of plant parts cut off by flooding. Exceptionally, main stems pushed into a muddy soil surface may produce adventitious roots and shoots along their entire length to form small clonal patches (GRIME et al. 1988).

MÖBIUS (1940) reported adventitious buds on leaves but this was not proved afterwards and STUCKEY (1972) explicitly rejected this possibility. Plantlets arising from root fragments can be found, especially in antropogenic habitats (Fig. 5) (KOČVAROVÁ 2002). JONSELL (1968) also observed adventitious sprouting from roots, but only occasionally. The first report of this ability comes from SERNANDER (1901, p. 350 – cited in JONSELL 1968).

Regeneration from the root fragments may provide important way of reproduction. In experiment where growth of plant originated from root fragments was compared with plants established from seeds, fruit production of root-sprouts was significantly higher (MARTÍNKOVÁ et al. in press).

3.5. GERMINATION

Germination is stimulated by light, large daily fluctuations of temperature, and scarification (GRIME et al 1988; ELLIS et al. 1989). Increasing photon flux density (from 10^{-5} mol.m^{-2}.d^{-1} to 4 mol.m^{-2}.d^{-1}) stimulated germination in *R. palustris* when the light period was shorter than 24 hours per day (1 min.d^{-1}, 1 hour.d^{-1}, 8 hours.d^{-1} – ELLIS et al. 1989). In continuous light, mean germination was equal to that obtained in shorter photoperiods at a photon dose of 10^{-2} mol.m^{-2}.d^{-1} and in the dark no germination was obtained at all (seeds in this experiment were dried at approx. 20 °C at 20% relative air humidity and then hermetically stored at -20°C).

According to KOČVAROVÁ (2002), lower temperature suppresses seed germination of *R. palustris.* Under a temperature of 25/10 °C (day/night) germination reached 80 to 100%, under 15/3 °C germination was 20% only. When the difference between minimal and maximal temperature was 5 °C (15/10 °C), germination was only 4% (Fig. 6). Day length affected the onset of germination: under long-day conditions, seeds started to germinate earlier than under short-day conditions. In the treatment "day 15 h, 15 °C / night 9 h, 3 °C", germination started 5 days earlier than in the treatment "day 9 h, 15 °C / night 15 h, 3 °C " (Fig. 6) (KOČVAROVÁ 2002). Seeds used in these experiments were harvested in September and stored under dry and dark conditions at about 5 °C for half a year.

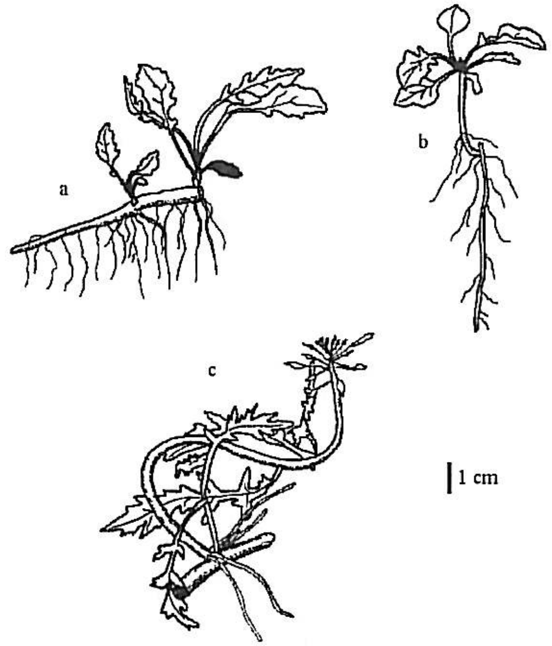

Fig. 5 Vegetative regeneration from root and stem fragments. (a), (b) adventitious shoots on root fragments, (c) adventitious roots on shoot fragment.

Seeds differing in their size differed also in germinability: germination of small seeds (0.4–0.6 mm in length) was 36% only, whereas 54% of large seeds (0.6–0.9 mm) germinated (seeds were collected in September and stored under dry and dark conditions at about 5 °C for half a year, germination conditions were: day 15 h, 25 °C / night 9 h, 10 °C) (KOČVAROVÁ & KLIMEŠOVÁ in prep.).

According to JONSELL (1968), seeds older than 5 years do not germinate, and already after about two years there was a considerable decrease in germination capacity, but nothing is known about storage conditions of the seeds in this experiment. In another germination experiment by KOČVAROVÁ (2002), seeds were harvested in three consecutive autumns and stored in a cool box (dry and dark conditions, about 5 °C). Differences in germination between seeds from the particular harvests were up to 40%. However, the percentage of germinated seeds did not correlate with age of the seeds, and was different in different treatments (KOČVAROVÁ 2002).

R. palustris has a permanent seed bank. Seeds may germinate immediately after they are shed from a plant (THOMPSON et al. 1997) throughout the season (GRIME et al. 1988). Across the whole range of localities inhabited by *R. palustris,* from pond shores to ruderal habitats, the number of germinable seeds in the soil ranged from 2,500 to 40,000 seeds/m^2 (sampled to the depth of 2 cm) (KOČVAROVÁ 2002). This is more than the value of 1,300 seeds/m^2 given by THOMPSON et al. (1997), sampling depth was 0–50 cm in this case. It was experimentally proved that seeds of *R. palustris* may be buried in the subsoil by digging beetles. Thus seeds found in deeper soil layer are not necessarily older then the seeds located at the soil surface (BERNHARDT 1995).

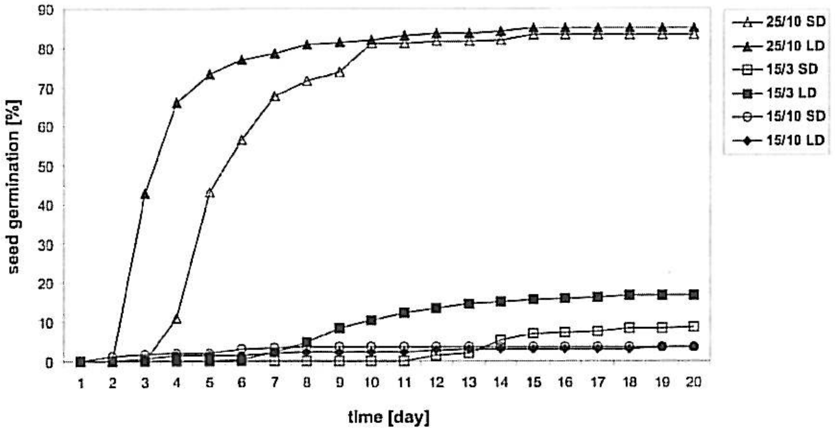

Fig. 6 Germination in different day-length and temperature treatments in a chamber experiment. LD - long day: 15/9 h, SD - short day: 9/15 h, temperature treatments: day/night temperature [°C]. Data from KOČVAROVÁ 2002.

3.6. RESPONSE TO COMPETITION AND MANAGEMENT

R. palustris is a weak competitor with high light requirements. It occupies open habitats with sparse vegetation. Due to its ability to sprout from roots, it is able to survive severe disturbance and persist on moist arable land.

3.7. HERBIVORES AND PATHOGENS

At localities in South Bohemia (Czech Republic), we found two herbivorous species feeding on *R. palustris*. One of them is the aphid *Brevicoryne brassicae* L. (det. J. Havelka). It is a herbivore feeding on plants of the whole Cruciferae family, causing deformation and yellowing of plant tissues. The second one is the gall midge *Centarinia nasturtii* Kiefer from the Cecidomyiidae family (det. J. Havelka). This species is also restricted to Cruciferae. Adults oviposite in meristematic regions of the stem and the growth of larvae causes swellings which stop apical growth of the plant (Fig. 7) (DEN OUDEN et al. 1987). Attacked plants are usually short and richly branched. The whole population usually suffers from an attack of this herbivore, especially in the second part of the growing season. Cultivated Cruciferae may be heavily attacked due to its concentration at one place. Pyrethroids are usually used for control of *C. nasturtii* in cabbage cultivation, but dipteras become rapidly resistant to it (DEN OUDEN et al. 1987). Alternatively Oppanol polyisobutylene polymers emulsion can be used for control. It causes a sticky surface which traps small flies and protect the plant from oviposition (DEN OUDEN et al. op.c.).

Similar syndromes as those observed in plants infested by *Centarinia nasturtii,* can be found in *R. palustris* infested by *Dasineura (Tipula) sisymbrii.* (http://www.kulak.ac.be/facult/wet/biologie/pb/kulakbiocampus/gallen/pagina's/moeraskes.htm).

From America, it has been reported that *R. palustris* is a host plant of larvae of the American red turnip beetle *Entomoscelis americana* (*Coleoptera: Chrysomelideae*) (GERBER 1984). These larvae are oligophagous, feeding successfully on a large number of *Cruciferae* from March to May (GERBER et OBADOFIN 1981; GERBER 1989).

From Australia, the caterpillar of *Pieris rapae* subsp. *rapae* L. has been reported as a herbivore feeding on *R. palustris.* Neither the butterfly nor the plant is native in Australia (www. usyd.edu.au/su/macleay/larvae/pier/rapae.html.).

We have no information about pathogens of *R. palustris.*

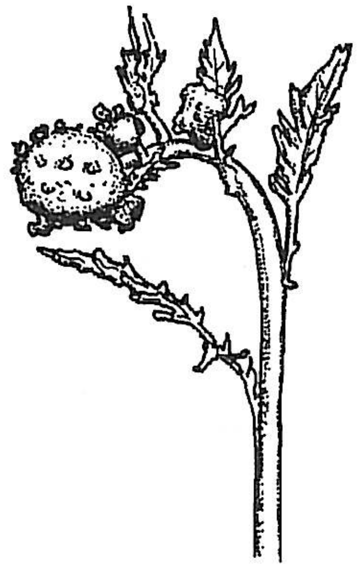

Fig. 7 Plant inflorescence deformed by the gall midge *Contarinia nasturtii* Kiefer.

3.8. MYCORRHIZA

Mycorrhizal association absent (HARLEY & HARLEY 1986).

3.9. Physiological data

No information available.

3.10. Biochemical data

No information available.

3.11. Genetic data

Phylogenetic relationships of *Rorippa* species were studied using sequences of two non-coding regions of chloroplast DNA (trnL intron, trnL/F spacer) (Bleeker et al. 2002). *R. palustris* is tetraploid (2n=32), closely related to the other tetraploid lowland species (*R. amphibia*, *R. sylvestris*) and also to the southeastern European diploids *R. brachycarpa* and *R. austriaca* and the eastern Asian *R. globosa* and *R. barbareifolia*. Surprisingly, *R. palustris* was not found to be closely related to the diploid *R. islandica*, as was earlier suggested (Jonsell 1968).

3.12. Hybrids

Hybrids of *R. palustris* are relatively rare in comparison with other *Rorippa* species, because of its autogamy, small flowers and early ripening pods (Jonsell 1968). Nevertheless, hybrids with *R. austriaca* (*R.* x *neogradensis* Borbás) and with *R. amphibia* (*R.* x *erythrocaulis* Borbás) have been described (Tomšovic 1992). In experimental hybridisation of common European *Rorippa* species, *R. palustris* was used only as a donor of pollen, and hybridised with *R. amphibia* (2n=16, 32), *R. amphibia* x *sylvestris* and *R. sylvestris* (2n=32, 2n=40). Only crosses with *R. amphibia* (2n=32) were successful and gave fertile progeny (Jonsell 1968).

Introgressive hybridisation between three *Rorippa* species (*R. amphibia*, *R. palustris* and *R. sylvestris*) was studied using isozymes and non-coding chloroplast DNA (trnL/F spacer) (Bleeker & Hurka 2001). *R. palustris* showed hybrid zones only with *R. amphibia* in an anthropogenic landscape in north-west Germany. Evidence was found for unidirectional introgression of *R. palustris* into *R. amphibia*. The *R. amphibia* introgressants grew in drainage ditches and anthropogenic factors play a major role in establishment and persistence of the hybrids (Bleeker 2001). The triploid hybrid *R.* x *erythrocaulis* Borbás (*R. amphibia* x *R. palustris*) was morphologically intermediate between the parent species and sterile (Bleeker 2001). Most hybrids of *R. amphibia* and *R. palustris* were tetraploid and not morphologically intermediate. For example, the upper cauline leaves were serrate instead of all upper cauline leaves being entire, typical of *R. amphibia* (Bleeker & Hurka 2001). In another hybrid, *R.* x *anceps* (Wahlenb.) Reichenb. (*R. amphibia* x *R. sylvestris*), allozymes of *R. palustris* were detected. That indicates a gene flow from *R. palustris* across *R. amphibia* to *R.* x *anceps*, and possibly also to *R. sylvestris* (Bleeker & Hurka 2001).

3.13. Status of the species

The typical habitats of *R. palustris* are river and lake shores with open vegetation. It expands to disturbed fertile wetlands, and also to wet arable fields frequently far away from water bodies. It is reported also from moist trampled ground, a shipwreck, roads, railways, harbours etc. (Jonsell 1968). The recent spread of *R. palustris* is connected with increasing human activities, which can be seen also from the fact that hardly any herbarium specimen was collected in Fennoscandia on arable land before 1900 (Jonsell 1968). In basins in South Bohemia, Czech Republic the species is more common in anthropogenic than in natural habitats. It is also common on temporally emerged bottoms of South-Bohemian fishponds. Spread of diaspores is ensured in such areas due to the network of natural and artificial water channels and streams (Prach 1991; Ferrero & Maggiore 1992). In similar habitats in northern Germany, breaking down distribution barriers allows hybridisation with *R. amphibia* and persistence of the hybrids (Bleeker 2001).

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CHAPTER VIII

Summary of Results

SUMMARY OF RESULTS

The main aims of the thesis were (i) to confirm the ability of root-sprouting in selected model short-lived species of highly disturbed habitats, (ii) to analyse the effects of internal and external factors on the ability of resprouting in these species, and (iii) to map resprouting abilities in their natural populations. The main results of this thesis are summarised as follows:

CONFIRMATION OF RESPROUTING ABILITIES IN SHORT-LIVED SPECIES

All studied short-lived species (*Rorippa palustris, Oenothera biennis, O. issleri, O. fallax, O. glazioviana* and *Barbarea vulgaris*) show the ability to resprout after 100 % aboveground biomass removal from both preserved basal axillary buds and also from adventitious buds on roots. Therefore, it is experimentally confirmed that short-lived species of highly disturbed habitats possess the ability of root-resprouting as was reported in the historical morphological literature. Moreover, spontaneous root-sprouting occurs in these species as well. Nevertheless, the efficiency to resprout from roots differs among these species.

Rorippa palustris does not require injury for the formation of adventitious root buds, but does not resprout from them until injured. Biomasss removal only breaks the dormancy of these preformed buds. Adventitious root buds and axillary buds are comparably efficient in the biomass recovery after injury. Spontaneous root-sprouting is sparse in this species. *Rorippa palustris* is a weaker root-sprouter than *Barbarea vulgaris*, but stronger than *Oenothera* species. (**Chapters II, III, V, VI and VII**)

Oenothera species require injury for initiation of adventitious bud formation. They prefer resprouting from axillary buds to root-sprouting, when at least only one axillary bud is present. Spontaneous root-sprouting is rare in these species. *Oenothera* species are weaker root-sprouters than *Rorippa palustris* and *Barbarea vulgaris.* (**Chapters II, VI and V**)

Barbarea vulgaris sprouts spontaneously very commonly and root-sprouting is a standard part of the life history of this species. However, spontaneous root-sprouting is not so vigorous as root-sprouting after biomass removal. This species is the most efficient root-sprouter of all studied species. (**Chapters IV and VI**)

EFFECTS OF INTERNAL AND EXTERNAL FACTORS ON RESPROUTING ABILITIES OF SHORT-LIVED SPECIES

Internal as well as external factors significantly influence resprouting of the studied short-lived species. These factors decide about the death or survival of injured individuals, and also, in the case of successful regeneration, about their consequential life cycle, the amount of regenerated biomass and seed production. The most important factor is severity of injury to the plant body. Injury is the primary stimulus inducing root-sprouting in short-lived species and its severity decides about the existence of injured plants. Another important factor is the timing of disturbance in respect to life-cycle phase of the injured plant. On the other hand, a less influential factor, i.e. nutrient availability, determines "only" the growth of regenerated plants. Moreover, these factors do not influence resprouting separately, but interact.

SEVERITY OF DISTURBANCE

Rorippa palustris reacts to the removal of either part of or all axillary buds in the same way and both injury severities are not fatal to this species; especially due to preformed adventitious buds on roots and a high ability to root-sprout. Moreover, differences in growth characteristics between injured and uninjured individuals are rapidly minimised. (**Chapters II, III, VI and VII**)

In *Oenothera* species, recovery after a disturbance is population-dependent. Removal of all aboveground biomass with all axillary buds largely leads to the death of *Oenothera* individuals, but *Oenothera* populations with higher abilities to regenerate from roots also exist. Regeneration of individuals originating in such populations is more successful. On the other hand, injury in which basal axillary buds are left intact is not so inauspicious for *Oenothera* individuals. However, a large difference in growth characteristics between injured and uninjured individuals persists. (**Chapters II, VI and V**)

Since root-sprouting is common in *Barbarea vulgaris* even without injury, the removal of either a part of or all axillary buds is not fatal to this species and the chance of regeneration is very high under both injury severities. However, the life cycle of injured plants is substantially changed by injury. Removal of all axillary buds causes prolongation of the vegetative phase or rejuvenation of reproducing plants. Removal of biomass, in which basal axillary buds are left preserved, markedly decreases seed production. (**Chapters IV and VI**)

TIMING OF INJURY

The root-sprouting ability of *Rorippa palustris* juvenile individuals is age-dependent in the vegetative phase. Plants must reach an age of at least four weeks to be able to resprout from roots. Vegetative individuals in annual and winter annual mode do not differ in root-sprouting abilities. (**Chapters III, VI and VII**)

In one study, vegetative plants of *Oenothera* species resprouted more frequently than reproductive plants (**Chapter II**). In another study, an opposite relationship was found (**Chapter V**). Therefore, the relationship of resprouting ability and timing of injury is still unclear in *Oenothera* species.

Plants of *Barbarea vulgaris* successfully resprout irrespective of timing of injury. However, seed production of plants injured during the second year of life was postponed to the third growing season or decreased. On the other hand, *Barbarea vulgaris* plants injured during the first year of their life maintain a seed number comparable to uninjured individuals. (**Chapters IV and VI**)

NUTRIENT AVAILABILITY

In *Rorippa palustris*, the formation of adventitious buds on roots is not affected by nutrient level. However, a greater proportion of adventitious buds is released and their growth is facilitated at a high nutrient level (**Chapters II, III, VI and VII**)

In *Barbarea vulgaris*, a low nutrient level erases differences caused by injury in different life-cycle phases and reduces the amount of regenerated biomass. Plants at a high nutrient level compensate for loss of aboveground biomass relatively less than plants at a low nutrient level, especially when they are injured in the reproductive phase. (**Chapters IV and VI**)

RESPROUTING IN NATURAL POPULATIONS OF SHORT-LIVED SPECIES

Individuals of all studied species resprout both after injury and spontaneously in the field. Resprouting from roots takes place in about a third of the studied ruderal populations of *Oenothera biennis*. However, among these populations root-sprouting abilities highly vary. The largest populations and the highest percentage of root-sprouting individuals were found in urban habitats with sandy/gravelly substrate, a low vegetation cover, and a high frequency of disturbance. (**Chapter V**)

CONCLUSIONS

It was demonstrated in this thesis that some short-lived species cope with severe disturbance by resprouting from the bud bank and do not rely only on seeds buried in the soil. Resprouting was found to be a significant strategy of some short-lived species occurring in highly disturbed habitats, where their biomass is frequently and unpredictably destroyed. Therefore, the resprouting strategy may be considered as a valuable alternative to the seeding strategy in some short-lived herbs of highly disturbed habitats. This fact should change our perception of short-lived species and their populations, especially in respect of their population dynamics and persistence.