Available online at www.sciencedirect.com
SCIENCE DIRECT®
Flora 201 (2006) 287–297
FLORA
www.elsevier.de/flora

Vegetative regeneration of biennial *Oenothera* species after disturbance: Field observations and experiment

Jana Martínková[a,*], Jitka Klimešová[a], Stanislav Mihulka[b,c]

[a]*Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, CZ – 379 82 Třeboň, Czech Republic*
[b]*Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, CZ – 370 05 České Budějovice, Czech Republic*
[c]*Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ – 252 43 Průhonice, Czech Republic*

Received 24 February 2005; accepted 21 August 2005

Abstract

It has been recently shown that some annual and biennial 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*.

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.

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 in this experiment. However, the relationship between life-cycle phase and the ability to resprout remains unclear in *Oenothera* species.

This study showed that resprouting after severe injury is an important feature of *Oenothera* individuals occurring in man-made habitats and may represent an alternative strategy to regeneration from the seed bank under disturbance conditions.

Keywords: Adventitious buds; Axillary buds; Bud bank; Injury; Man-made habitat; Vegetative regeneration

*Corresponding author.
E-mail address: martinkova@butbn.cas.cz (J. Martínková).

0367-2530/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2005.08.005

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 and Lovett Doust, 1993). Highly disturbed habitats, including man-made ones, select for short life cycle, early maturity and production of many seeds (Grime, 2001; MacArthur and Wilson, 1967). 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 and 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; Klimešová et al., 2004; Martínková et al., 2004a, b). Nevertheless, according to experimental studies, resprouting behaviour markedly differs among these species. In *Barbarea vulgaris* and *Rorippa palustris*, the resprouting from roots is triggered even by low injury severity or it can be spontaneous, whereas in *Oenothera biennis* and *O. issleri*, resprouting is induced by severe disturbance removing all axillary buds (Martínková et al., 2004a, b). 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).

Individual full compensation or overcompensation for lost biomass is considered as an adaptation to herbivory (Tuomi et al., 1994). In population under herbivore pressure, only some individuals are harmed and those mostly survive herbivore attack and persist in impaired populations (Lennartsson et al., 1998; Maschinski and Whitham, 1989; Paige, 1999; Strauss and Agrawal, 1999). However, in habitats affected by disturbance that injures all individuals in population, survival of as many plants as possible and any seed production are important for population persistence. In previous experiments with 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 (*B. vulgaris*), or even higher than in plants regenerated at the time of disturbance from the seed bank (*R. palustris*) (Martínková et al., 2005; Martínková et al., in prep.). Injured individuals of these root-sprouting species successfully cope with severe disturbance by resprouting, and injury to plant body can be beneficial for them as it brakes apical dominance (Aarsen, 1995; Lortie and Aarsen, 2000) or liberates stored resources (Van der Meijden, 1990). However, there are some internal factors that can freeze resprouting after disturbance or decrease its rate (Dubard, 1903; Martínková et al., 2004a, b; Martínková et al., in prep.; Peterson, 1975; Rauh, 1937). 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 annual and biennial species (Martínková et al., 2004a, Martínková et al., in prep.). 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 (Martínková et al., 2004a; Martínková et al., in prep.; Sosnová, 2003).

We chose as a model for this study a common biennial species of man-made habitats, *O. biennis*, in which ability of resprouting after a severe disturbance has been documented (Klimešová, 2003; Martínková et al., 2004a; Rauh, 1937), and its closely related congeners *O. fallax* and *O. glazioviana*, about which no literature 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 be acquired only by experimental data.

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 *O. biennis*, and how it is affected by habitat characteristics and disturbance regime, and
(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 *O.* congeners (*O. biennis, O. fallax* and *O. glazioviana*).

Materials and methods

Study species

O. biennis L. and *O. glazioviana* M. Micheli (Onagraceae) are non-native and invasive species in Europe

(Hall et al., 1988; Mihulka and Pyšek, 2001). *O. fallax* Renner is a hybrid between *O. biennis* and *O. glazioviana* of European origin, showing an expansive behaviour (Mihulka and 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 and Pyšek, 2001). The species are non-clonal, monocarpic and biennial (Dietrich et al., 1997; Hall et al., 1988), with very long-lived seeds (Baskin and 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 10 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.

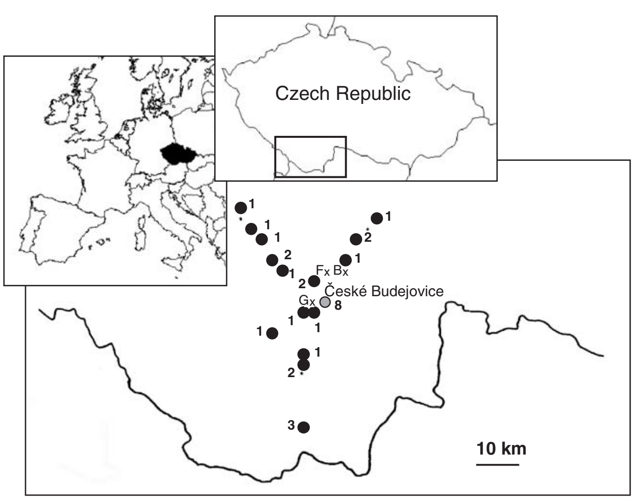

Fig. 1. Distribution map of the 29 studied populations of *Oenothera biennis* in the Czech Republic. Localities with studied populations are marked by black points (outside of the town of České Budějovice) or by grey points (in the town of České Budějovice), the number at the points identifies the number 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.

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 3-year experiment, plants of *O. 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, 5-day-old seedlings were transplanted into small peat pots (5 × 5 × 5 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.

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).

Table 1. List of characteristics that were used in the field study of *Oenothera biennis* populations

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* (deg)	(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

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.

Table 2. Treatments used in the garden experiment with *Oenothera biennis, O. fallax* and *O. glazioviana*

Year of germination	Treatment	Life-cycle phase	Severity of injury	*O. biennis*		*O. fallax*		*O. glazioviana*	
				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	27 JUN	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	27 AUG	29
2001	FR+	Fruiting plants	High	24 JUL	26	14 AUG	30	27 AUG	30

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.

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, and 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.

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

Statistical analysis

To analyse data from the field observations, we applied multivariate statistical methods – principal component analysis (PCA) and redundancy analysis (RDA) – using Canoco for Windows package (Ter Braak and Šmilauer, 1998). PCA method was used to describe real situation in a field and to show relationships between habitat conditions and resprouting characteristics of studied *O. biennis* populations. In PCA, all characteristics listed in Table 1 were included in primary data set. In RDA method, characteristics of *O. biennis* population (Table 1) were included in primary data set and other characteristics acted as explanatory variables. Monte Carlo permutation test (999 permutations, $\alpha = 0.05$) was used to assess the significance of the relationships found in RDA. Data were log-transformed, standardised and centred by species averages in both analyses. Some characteristics were coded as dummy or fuzzy variables (Table 1) (Lepš and Šmilauer, 2003).

Statistical analysis of all data from the greenhouse experiment was done by STATISTICA 5.5 package. The effect of treatment, species and treatment × species interaction on the number of regenerated plants after injury, the number of fruiting plants in the year of injury, and the number of plants surviving to the third season were tested using factorial ANOVA in Generalised Linear Models with Binomial Distribution and logit function.

Results

Field observation

Almost all 29 *O. biennis* ruderal populations included injured individuals, but also plants spontaneously regenerated from roots (Table 3). However, polycarpic individuals were found in eight populations only and some plants regenerated from roots after injury in 13 populations only (Table 3). Nevertheless, within populations the percentage of spontaneously regenerated

Table 3. Population and resprouting characteristics of 29 natural populations of *Oenothera biennis*

Characteristic	Mean (%)	Min (%)	Max (%)	Frequency among population
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

Means±standard errors, minimum and maximum values and frequency among populations are shown for each characteristic in percentage. Frequency among populations 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.

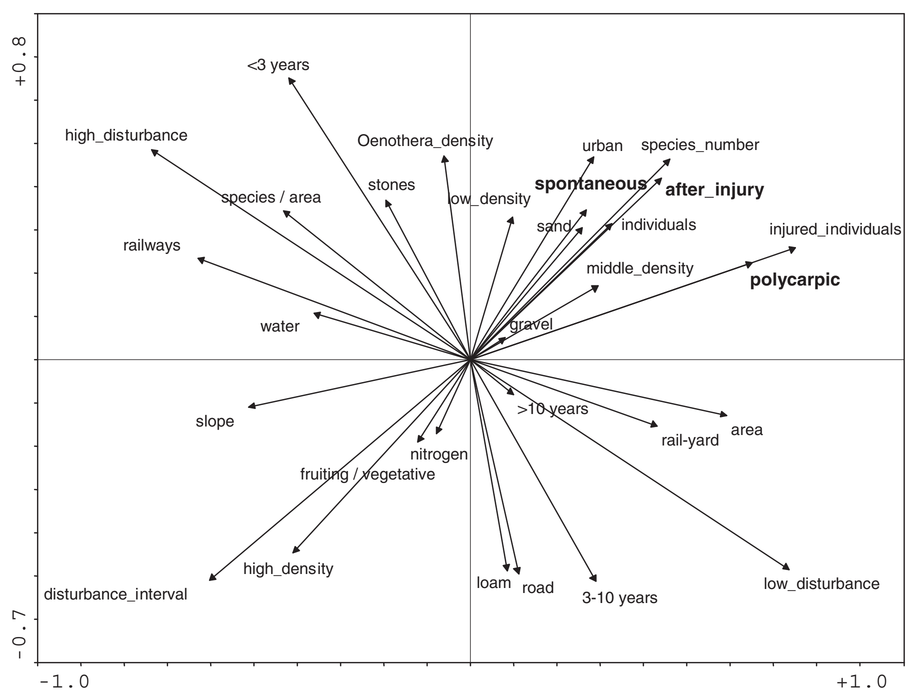

Fig. 2. PCA ordination diagram displays the relationship between the characteristics recorded in the field study of *Oenothera biennis* populations. Characteristics describe habitats occupied by *O. biennis* populations, disturbance regimes occurring there, local vegetation, population and resprouting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

plants was much lower than the percentage of plants regenerated after injury. Polycarpy was exceptional.

The ordination diagram of PCA describes the relationships among resprouting, habitat characteristics, and disturbance regime (Fig. 2). The first two ordination axes in this analysis explain 30.7% of the variability in the data set and separate the ordination space into four quadrants that represent the main *O. biennis* habitat types, which were found in a field. A high disturbance, high species diversity, stony substrate and low habitat age characterise the top left quadrant. These habitats were 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. From the diagram, it is evident that the highest portion of individuals spontaneously regenerated from roots, polycarpic individuals, and also individuals regenerated from roots after injury was found in urban habitats with a high frequency of disturbance. In the same type of habitat, the largest populations of *O. biennis* were found as well. The number of individuals spontaneously regenerated from roots, polycarpic individuals, individuals regenerated from roots after injury, and the number of all *Oenothera* individuals were not correlated with severity of last disturbance.

First two ordination axes of RDA explained 27.4% variability in primary data; both axes were significant ($p = 0.05$). On the base of the forward selection of explanatory variables using Monte Carlo permutation test, only area of habitat, gravely substrate and frequency of disturbance turn to have a significant effect on the characteristics of *O. biennis* populations.

Garden experiment

The number of regenerated plants after injury significantly varied among treatments and also among species (treatment: $\chi^2 = 408.5$, $p<0.001$, df $= 5$; species: $\chi^2 = 10.5$, $p<0.01$, df $= 2$; treatment × species: $\chi^2 = 39.8$, $p<0.001$, 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: $\chi^2 = 348.8$, $p<0.001$, df $= 7$; species: $\chi^2 = 21.4$, $p<0.001$, df $= 2$; treatment × species: $\chi^2 = 19.5$, $p<0.05$, 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: $\chi^2 = 57.03$, $p<0.001$, df $= 7$; species: $\chi^2 = 3.6$, n.s., df $= 2$; treatment × species: $\chi^2 = 15.1$, n.s., df $= 13$). Nevertheless, surviving until the third vegetative season was infrequent (Table 4). 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).

Discussion

Even though the view that vegetative regeneration has no value for annual and biennial species in disturbed habitats is widely accepted (Bellingham and Sparrow, 2000; Grime, 2001), the present study with *Oenothera* species is another example showing that resprouting (vegetative regeneration) from the bud bank after severe injury to plants may be a crucial feature of biennial species occupying highly disturbed habitats (Klimešová and Klimeš, 2003; Martínková et al., 2004a, b, 2005). Resprouting allows injured individuals survive severe harm to their body, and successfully finish reproductive cycle in order to set seeds. However, the ability of resprouting is frequently influenced by regime of disturbance, and among *Oenothera* populations, seems to be highly variable.

Population variability in resprouting abilities

Based on our experimental results and field observations, we suggest a high variability in resprouting abilities among *O. biennis* populations. In the present garden experiment, *O. biennis* exhibited only very low ability to regenerate after a disturbance. However, in a previous field experimental study with an *O. bienis* population (Martínková et al., 2004a), it was found that regeneration is high after both low and high injury severity. An explanation of the inconsistency between the experimental results may be a high inter-population variability in resprouting abilities, since *Oenothera* spp. possess specific genetic characteristics (self-pollination and Renner's circles, Cleland, 1972; Dietrich et al., 1997) causing a high inter-population and a low intra-population variability in this taxon. Therefore, a population with a lower ability to resprout from the bud bank was probably chosen for the present experiment, whereas a population with a higher resprouting ability was studied in the previous one (Martínková et al., 2004a). Results of present field observations support the hypothesis of a high inter-population variability in resprouting abilities as well. In 25 out of 29 studied ruderal populations of *O. biennis*, we found severely injured individuals; however, only half of these populations included injured individuals that resprouted from roots. Moreover, among these populations, the proportion of injured root-sprouting individuals varied highly. Nevertheless, only an experiment comparing resprouting ability of several *Oenothera* populations could reveal real inter-population variability in this characteristic.

An alternative explanation of the observed high variability in resprouting abilities in *O. biennis* concerns a difference in substrates. In the field observations, the highest number of root-sprouting individuals was found in habitats with a light sandy/gravelly substrate and the lowest at loamy substrates. Plants of *O. biennis* in the present garden experiment, which were cultivated on 2:3 sand–soil mixture, mostly died after injury. In the previous study (Martínková et al., 2004a), in which the observed resprouting was higher, the studied population grew on stony/gravelly substrate. Even though light is not essential for root bud formation (Peterson, 1975), it seems that loamy soil could restrict light penetration into deeper soil layers and newly formed sprouts are unable to reach the soil surface and died. However, to verify this idea it is necessary to dig up injured plants growing on loamy substrates and to check if they form root buds.

Table 4. Results of garden experiment with *Oenothera biennis*, *O. fallax* and *O. glazioviana*

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	48780.8±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

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.

Frequency of disturbance

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 2 years, i.e. once per their biennial life cycle. In the present experiment, *Oenothera* individuals finished their reproductive cycle and set seeds in spite of severe injury to their body; however, the reproductive characteristics were considerably lower in comparison to intact plants. Therefore, the ability of resprouting is advantageous only under such a disturbance, if there is a high probability that plants without resprouting never finish the reproductive cycle and a seed bank cannot be formed there or it will be depleted soon. Moreover, due a high longevity (Baskin and 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 et al., 2001).

Disturbance severity

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 disturbances, which neither reach soil surface nor destroy all axillary buds. Because in our experiment, it was found again that resprouting from preserved basal axillary buds on the stem is more frequent than resprouting from adventitious root buds (Martínková et al., 2004a). 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 (Esau, 1965; Martínková et al., 2004a; Peterson, 1975; Vesk and Westoby, 2004). In contrast, this relation was not found in two other herbs of disturbed habitats, *R. palustris* and *B. vulgaris*, that do not prefer axillary buds over 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, in prep.). 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. Aarsen, 1995; Horvath, 1998; Lortie and Aarsen, 2000; Peterson, 1975).

Timing of disturbance

The relationship between life-cycle phase at the time of injury and the probability of successful resprouting remains unclear in *Oenothera* species. In the present experiment, no young vegetative rosettes regenerated from roots, and regeneration of reproducing plants and overwintered rosettes was exceptional. Rauh (1937) found a successful regeneration from root buds only in vegetative plants of *O. biennis*. Martínková et al. (2004a) reported a relatively high probability of regeneration after injury from root buds in both vegetative and reproductive plants of *O. biennis*. This inconsistency of results can be caused by a high inter-population variability in resprouting abilities as is mentioned above and only more detailed experiments with several populations can reveal the relationship between timing of injury and the ability of resprouting in these species.

Life-cycle of injured plants

According to the present results, *Oenothera* species adhere to a biennial life cycle and injury to the plant body does not postpone their reproduction from the second to a third year of life, in a way similar to the short-lived root-sprouting herb *B. vulgaris* (Martínková et al., in prep.). 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 (Aarsen, 1995; Horvath, 1998). 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, the observed frequency of polycarpy was very low in ruderal populations and survival of experimental individuals until the third season was rare.

To summarise, resprouting from roots takes place in about a third of the studied ruderal populations of *O. 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 from the bud bank after a disturbance is probably efficient only under a certain degree and frequency of disturbance, i.e. when some axillary buds are preserved and frequency of disturbance is approximately once per 2 years. 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. Even though *Oenothera* spp. are less effective root-sprouters than *R. palustris* and *B. vulgaris*, our results support the idea that the ability to resprout from the bud bank after injury is a useful tool for persistence of short-lived species populations in highly and frequently disturbed habitats.

Acknowledgements

This research project was supported by grants nos. 206/01/1039, 206/03/P155 and 206/03/H034 from the Grant Agency of the Czech Republic, and also by AV0Z6005908 from the Grant Agency of the Academy of Sciences. We are grateful to L. Klimeš for valuable comments on the manuscript and J.W. Jongepier for improving our English.

References

Aarsen, L.W., 1995. Hypotheses for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. Oikos 74, 149–156.

Baskin, C.C., Baskin, J.M., 1994. Germination requirements of *Oenothera biennis* seeds during burial under natural seasonal temperature cycles. Can. J. Bot. 72, 779–782.

Bellingham, P.J., Sparrow, A.D., 2000. Resprouting as a life history strategy in woody plant communities. Oikos 89, 409–416.

Cleland, R.E., 1972. *Oenothera* Cytogenetics and Evolution. Academic Press, London.

Dietrich, W., Wagner, W.L., Raven, P.H., 1997. Systematics of *Oenothera* subsection *Oenothera* (Onagraceae). Syst. Bot. Monogr. 50, 1–234.

Dubard, M., 1903. Recherches sur les plantes a bourgeons radicaux. Ann. Sci. Nat. Bot. Biol.e Vég. 17, 109–225.

Ellenberg, H., 1988. Vegetation of Central Europe. Cambridge University Press, Cambridge.
Esau, K., 1965. Plant Anatomy. Wiley Eastern Ltd., New Delhi.
Grime, J.P., 2001. Plant Strategies, Vegetation Processes and Ecosystem Properties. Wiley, Chichester.
Hall, I.V., Steiner, E., Threadgill, P., Jones, R.W., 1988. The biology of Canadian weeds. 84. *Oenothera biennis* L. Can. J. Plant Sci. 68, 163–173.
Horvath, D.P., 1998. The role of specific plant organs and polar auxin transport in correlative inhibition of leafy spurge (*Euphorbia esula*) root buds. Can. J. Bot. 76, 1227–1231.
Klimešová, J., 2003. Monocarpic plants surviving severe disturbance [in Czech]. Zpr. Česk. Bot. Spol. Mater. 19, 37–48.
Klimešová, J., Klimeš, L., 2003. Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham–Sparrow′s model? Oikos 103, 225–229.
Klimešová, J., Martínková, J., Kočvarová, M., 2004. Biological flora of Central Europe: *Rorippa palustris* (L.) Besse. Flora 199, 453–463.
Lennartsson, T., Nilsson, P., Tuomi, J., 1998. Induction of overcompensation in the field gentian, *Gentianella campestris*. Ecology 79, 1061–1072.
Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data Using Canoco. Cambridge University Press, Cambridge.
Lortie, C.J., Aarsen, L.W., 2000. A test of the reserve meristem hypothesis using *Verbascum thapsus* (Scrophulariaceae). Am. J. Bot. 87, 1789–1792.
MacArthur, R., Wilson, E.O., 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, NJ.
Martínková, J., Klimešová, J., Mihulka, S., 2004a. Resprouting after disturbance: an experimental study with short-lived monocarpic herbs. Folia Geobot. 39, 1–12.
Martínková, J., Kočvarová, M., Klimešová, J., 2004b. Resprouting after disturbance in the short-lived herb *Rorippa palustris* (Brassicaceae): an experiment with juveniles. Acta Oecol. 25, 143–150.
Martínková, J., Sosnová, M., Kociánová, A., Klimešová, J., 2005. An effect of injury on seed production of short-lived root-sprouting species [in Czech]. Zpr. Česk. Bot. Spol. 40 Mater. 20, 87–101.
Martínková, J., Klimešová, J., Mihulka, S. Resprouting after disturbance in the short-lived herb *Barbarea vulgaris* (Brassicaceae): effect of nutrient level, timing and severity of injury, in preparation.
Maschinski, J., Whitham, T.G., 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability and timing. Am. Nat. 134, 1–19.
Mihulka, S., Pyšek, P., 2001. Invasion history of *Oenothera* congeners in Europe: a comparative study of spreading rates in the last 200 years. J. Biogeogr. 28, 597–609.
Paige, K.N., 1999. Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. Oecologia 118, 316–323.
Peterson, R.L., 1975. The initiation and development of root buds. In: Torrey, J.G., Clarkson, D.T. (Eds.), The Development and Function of Roots. Academic Press, London, pp. 125–161.
Rauh, W., 1937. Die Bildung von Hypocotyl- und Wurzelsprossen und ihre Bedeutung für die Wuchsformen der Pflanzen. Nova Acta Leopold. 4, 395–553.
Schippers, P., Van Groenendael, J.M., Vleeshouwers, L.M., Hunt, R., 2001. Herbaceous plant strategies in disturbed habitats. Oikos 95, 198–210.
Silvertown, J.W., Lovett Doust, J., 1993. Introduction to Plant Population Biology. Blackwell, Oxford.
Sosnová, M., 2003. Vegetative regeneration of *Rorippa palustris* (L.) BESSER. B.Sc. Thesis, University of South Bohemia, České Budějovice (in Czech).
Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185.
Ter Braak, C.F.J., Šmilauer, P., 1998. Canoco Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer power, Ithaca.
Tuomi, J., Nilsson, P., Åstrom, M., 1994. Plant compensatory responses: bud dormancy as an adaptation to herbivory. Ecology 75, 1429–1436.
Van der Meijden, E., 1990. Herbivory as a trigger for growth. Funct. Ecol. 4, 597–598.
Vesk, P.A., Westoby, M., 2004. Sprouting ability across diverse disturbances and vegetation types worlwide. J. Ecol. 92, 310–320.