Variation in the Germination of Seeds of *Rumex obtusifolius* in the Czech Republic*

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Abstract

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In 1995–1998 we investigated the variation in the germination of 215 seed accessions of *Rumex obtusifolius* L. collected at various sites of Bohemia (Western Czech Republic). Seeds of 5–15 randomly selected plants were harvested at each site and 100 or 150 seeds of each plant were germinated at constant 25°C and 4 h light daily. The average proportion of germinating seeds was 12.7%. At some sites there was a significant variation between plants as well as between fertile shoots within plants. At many other sites the proportion of germinating seeds was low in all plants. As a consequence, the variation between sites was significant in all years except 1998. By contrast, in all years the average germination did not differ between samples collected in October–December, January–March and April–June. There was no correlation between plant stature and germination percentage. The variation in germination percentage was probably caused by differences in the occurrence of primary seed dormancy, and controlled by genetic or maternal differences between plants but only slightly influenced by seasonal or annual variation of the weather.

Key words: *Rumex obtusifolius;* seed dormancy; germination; geographic variation; annual variation; weather; plant size; achenes

In central Europe, seeds of *Rumex obtusifolius* L. mature in August to October. The dispersal of propagules from dry standing fertile shoots continues over a long period and some seeds may remain on dry shoots until early June of the next year. Before dispersal, seed samples contain a variable proportion of seeds which do not germinate. The absence of germination is largely due to innate seed dormancy. The experiments with materials whose dormancy was terminated revealed that the proportion of non-germinating and probably dead seeds was low, mostly below 5% (e.g., TOTTERDELL & ROBERTS 1979). The proportion of primary dormancy could by revealed by germination experiments at constant temperatures, under light conditions (MILBERG 1997) where non-dormant seeds germinate. By contrast, experimental exposure of imbibed dormant seeds to fluctuating temperatures resulted in very rapid termination of dormancy (TOTTERDELL & ROBERTS 1979). Temperature fluctuations also affect termination of dormancy in the open (VAN ASSCHE & VANLERBERGHE 1989). The variation of the percentage of primary seed dormancy was the objective of several studies (CAVERS & HARPER 1966, 1967; TERAI 1994; KOHOUT & VOBORNÍKOVÁ 1997; MARTINKOVÁ & VOBORNÍKOVÁ 1998). In the UK the proportion of dormant seeds varied between plants at the same locality, as well as between localities. HARPER (1977) concluded that variation between plants is generally more important than locality-specific variation. The variation is influenced by conditions during the period of vegetative growth, flowering and seed maturation. Defoliation at anthesis caused the seeds to be smaller and to germinate more readily (MAUN & CAVERS 1971a) while partial removal of flowers caused the seeds produced by the remaining flowers to be larger but to germinate less readily (MAUN & CAVERS 1971b). MARTINKOVÁ *et al.* (1999) established a contrary trend in a part of seed materials collected from intact plants in the open and concluded that the incidence of primary seed dormancy is controlled by several factors including, besides extrinsic influences at the time of seed maturation, also genetic and maternal variation between the plants (HONĚK & MARTINKOVÁ in prep.).

As the proportion of germinating seeds varies geographically, we studied this variation in central Europe, where

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a detailed investigation has not been made until now. The extent of variation between individual plants at the same site and between sites were investigated. As the study extended over four years (1995–1998) the variation between years and the differences which may appear between seeds collected at different seasons were also considered. We also studied the importance of plant size on germinability of seed.

MATERIAL AND METHODS

Seed Collection. Seed materials were collected at localities of the Western Czech Republic, Bohemia (49°30'–50°47'N, 13°15'–15°47'E), at altitudes of 160–800 m a.s.l. (Fig. 1). Every year the sampling was done between October and June of the next year. The collection sites were located in meadows, abandoned fields, roadside ditches and forest clearings. Some stands were cut in the vegetational season and regenerated before the onset of autumn, others remained intact. It was impossible to discriminate between previously cut and uncut stands at the time of seed collection. Some sites were sampled repeatedly in successive years but sampling was done on different plants. In total we investigated 215 "seed materials". Each "seed material" was a set of samples of seeds of different plants collected at one site and date. Each seed material was collected from a group of plants growing on an area of ca. 30 × 30 m. The distance between sampling sites usually was >1 km, at least 200 m. Five plants were selected randomly at each site, taking care not to prefer tall plants or those with many shoots. The achenes were collected from all standing shoots, collecting from broken shoots was avoided. Between-plant variation was studied in material collected at Kaliště (49°40'N, 14°41'E, 580 m a.s.l.), on January 26, 1997. The seeds were collected separately from the tallest, medium and shortest fertile shoots of 15 plants. In 1996, the number of fertile shoots and the plant height were recorded for a number of sampled plants. The collected achenes were dried and stored at room conditions (21–23°C, 40% relative humidity) until germination determination 10–40 d after seed collection.

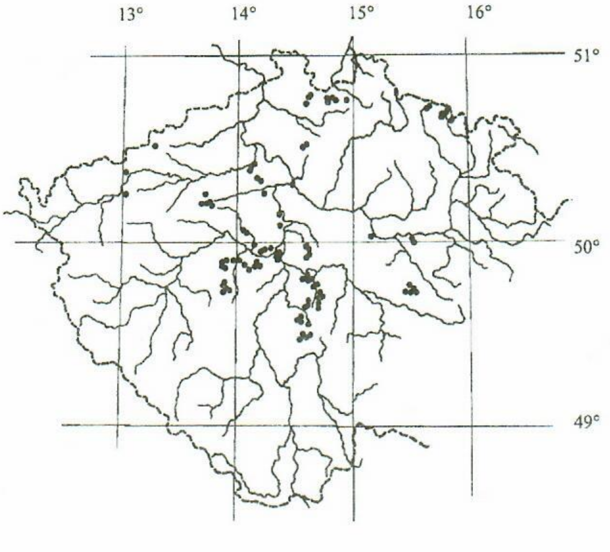


Fig. 1. The localities of collection of *R. obtusifolius* seeds. Some points represent several closely adjacent sampling sites

Germination. One week before germination was determined the seeds were freed from the perianths by hand rubbing. Three samples of 50 seeds or one sample of 100 seeds were established from each plant. For the study of between-plant variation (material of Kaliště) five samples of 50 seeds were used from each shoot. For the germination experiments each seed sample was placed on a filter paper in a 10 cm diameter Petri dish and 5 ml tap water were added. The Petri dishes were kept at 25°C at a 4 h light and 20 h dark regime. Germinated seeds were counted and removed in 2 d intervals until no further germination occurred within 4 days.

Statistical Evaluation. The germination percentages were arcsin transformed. To test for geographic, annual and seasonal differences the transformed values were subjected to one-way ANOVA. The within-years variation between sites was tested by using site as a factor and germination percentages of individual plants as replicates. To test variation in time, average germination percentages for different sites were calculated to prevent the effect of repeated measure data. These data were then grouped: (i) Across the years according to the season when the seed materials were collected: October–December, January–March, and April–June. (ii) Across the seasons according to the years of collection – winters 1995–1996 to 1998–1999 (the collection periods are further indicated by the year when they started, i.e., 1995–1996 as 1995). The existence of differences was tested by taking the season or the year as factors and average germination percentages at sites as replicates. The significance of seasonal and annual differences was then tested by Scheffé test. The between-plant and between-shoot variation (materi-

Table 1. Factors of between-plant variation in the percentage of seed germinability at the locality Kaliště, 26.1.1997. Nested 2-way ANOVA (shoots within plants) with random effects

Factor	Effect			Error			F	p
	SS	df	MS	SS	df	MS		
Plant	65.3720	14	4.6694	8.6164	30	0.2872	16.2577	0.0000
Shoot	8.6164	30	0.2872	9.6115	173	0.0556	5.1696	0.0000

al of Kaliště, 26.1.1997) was tested by nested 2-way ANOVA (shoots nested within plants) with random effects. The relationship between dormancy and plant stature was evaluated in 1996, by calculating regression of germination percentages of seeds of particular plants on height or numbers of fertile shoots. Statistical calculations were made using STATISTICA® for Windows (STATSOFT 1994).

RESULTS

Variation between Plants. At the locality of Kaliště (Table 1) there were significant differences in the average germination percentage between 15 investigated plants. The germination percentage ranged from 1.0 to 85.5%. The average germination was 18.2%, the interval of the mean ±SD was 1.7–49.9, being asymmetrical due to the angular transformation of the percentage data. The between-plant variation explained 70.9% of total variance in germination percentage. The variation between shoots within plants explained 9.3% of total variance in germination percentage but was still highly significant.

Effect of Plant Stature. In 1996, there was no correlation ($p > 0.05$) between plant height or number of fertile shoots per plant and germination percentage (Fig. 2). Both characters which contribute to whole plant size were significantly but only loosely correlated (N shoots = 0.056 × plant height – 0.936, $R^2 = 0.0736$, df = 176, $F = 13.98$, $p < 0.005$). We therefore calculated their product to obtain the overall index of plant size. However, neither this product was significantly correlated with percentage of seed germination (arcsin germination = 0.002 × plant height + 0.142, $R^2 = 0.0091$, df = 176, $F = 1.620$, $p > 0.05$). Plant stature thus was not related to percentage of seed germination.

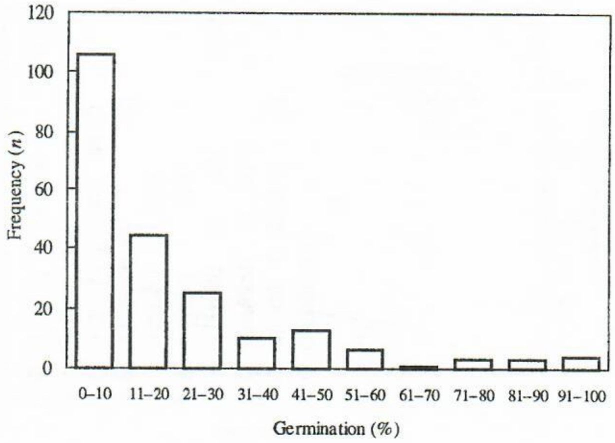


Fig. 2. The frequency of average percentage of germination in seed materials collected at 215 sites of Bohemia in 1995–1998

Variation between Sites. The variation between sites (Table 2) was highly significant in all years exept 1998. At some sites there was a high average germination, usually accompanied by a great between-plant variation. By contrast, at many sites the average proportion of germination and between-plant variation was low. The average germination at different sites varied between 0.0–99.5%. The distribution of the average germination percentage was left-skewed (Fig. 3), being < 3% at 59 sites (27% of the total of 215 seed materials).

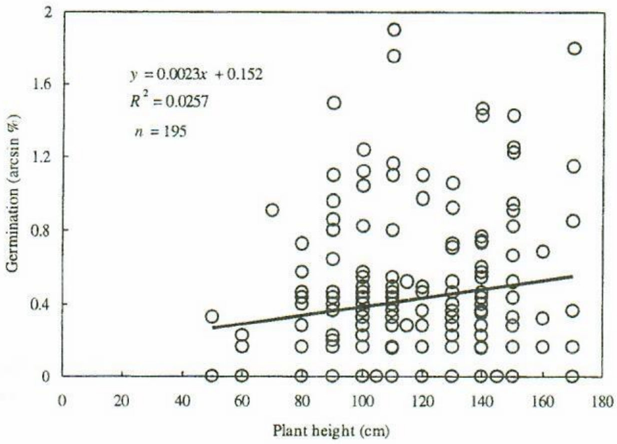
Seasonal Variation. The variation of proportions of dormant seeds in materials collected at different sections of the period during which mature seeds overwintered on dry standing shoots was not significant (Table 3). The average germination of seed materials collected in the October–December, January–March and April–June periods were 16.2%, 10.4% and 12.9%, respectively (Table 4). The mean total proportion of germination was 12.7% (SD 1.2–35.9%). The average proportion of germination re-

Table 2. The significance of between-site differences in the percentage of seed germinability within years of observation. 1-way ANOVAs of sets of samples collected in different years (1995–1998)

Factor	Effect			Error			F	p
	SS	df	MS	SS	df	MS		
1995	44.5759	17	2.6221	5.8843	158	0.0372	70.4062	0.0000
1996	146.8645	93	1.5792	24.3883	401	0.0608	25.9655	0.0000
1997	91.6032	80	1.1450	67.2794	341	0.1973	5.8035	0.0000
1998	6.3616	18	0.3534	15.2185	71	0.2143	1.6488	0.0709

Table 3. The significance of annual (data of Table 5) and seasonal (data of Table 4) differences in the percentage of seed germinability. 1-way ANOVA of all seed materials grouped along the years across the seasons or along the seasons across the years

Factor	Effect			Error			F	p
	SS	df	MS	SS	df	MS		
Year	1.6528	3	0.5509	51.4502	211	0.2438	2.2594	0.0826
Season	1.0533	2	0.5267	52.0497	212	0.2455	2.1451	0.1196



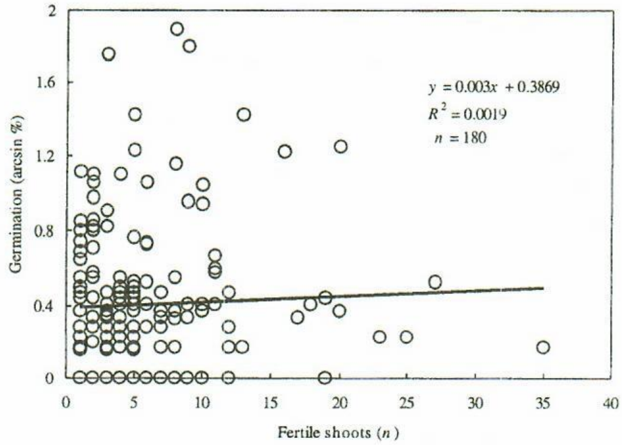


Fig. 3. The regression of germination percentage of seed materials of particular plants collected in 1996 at different sites on plant height (above) and number of fertile shoots (below)

Table 4. Seasonal differences in percentage of dormant seeds (all seed materials grouped along seasons across the years) and the test of statistical significance of the results (probability of null hypothesis that the means of the tested sets of data are equal)

Season	n	Mean	SD	Scheffé test	
				Autumn	Winter
Autumn	69	16.2	1.4–45.7	*	*
Winter	97	10.4	0.9–29.9	0.1199	*
Spring	49	12.9	1.8–33.6	0.6330	0.7034
Total	215	12.7	1.2–35.9		

Table 5. Annual differences in percentage of dormant seeds (all seed materials grouped along the years across the seasons) and the test of statistical significance of the results (probability of null hypothesis that the means of the tested sets of data are equal)

Season	n	Mean	SD	Scheffé test		
				1995	1996	1997
1995	18	24.6	5.3–55.7	*	*	*
1996	94	11.2	0.5–35.0	0.0861	*	*
1997	84	12.2	1.2–34.2	0.1501	0.9813	*
1998	19	13.0	4.8–25.0	0.4122	0.9808	0.9987
Total	215	12.7	1.2–35.9			

mained similar over the whole period of seed persistence on dry shoots.

Annual Variation. The variation of the percentage of germinating seeds between years was not significant (Table 3). The average proportion of germinating seeds was 16.2%, 11.2%, 12.2% and 13.0% in 1995–1998, respectively (Table 5).

DISCUSSION

The study has revealed a large variation in germination percentage between seed materials of different plants and sites. A large proportion of this variation was apparently due to differences in the percentage of innate seed dormancy, and thus reveal individual and geographic variation in the occurrence of this character. The between-plant variation in the percentage of germination was large, similar to Western Europe (CAVERS & HARPER 1966, 1967) and Japan (TERAI 1994). The study showed that regardless of a large variation between individuals, the variation between sites was still significant. This was due to the uneven distribution of the variation of germination between sites. At the majority of sites the percentage of germination was generally low and the between-plant variation was limited. Some sites, however, contained a mixture of plants with low and high seed germination. This variation caused a significant between-site variation established in all years when a large number of seed materials was available.

The study demonstrated that, in natural populations, seed germinability was not correlated with plant stature. This may appear to contradict published results on experimental manipulation of *Rumex* growth whose consequences also modified seed germinability. The absence of a correlation may be due to a multiplicity of factors affecting the induction of primary seed dormancy. These factors include genetic, maternal and epigenetic causes. We have demonstrated (HONĚK & MARTINKOVÁ in prep.) that germination percentage is correlated with the time of sprouting of fertile shoots in the spring. Early and late sprouting was specific for individual plants during a three years study. Further important factor of seed germinability is the cutting of shoots during the vegetational season which substantially affects the germinability of seeds harvested from regenerated fertile shoots. Cutting decreases not only the proportion of germinable seeds but also the between plant variation that would appear in uncut plants. Herbivore grazing on growing plants, arthropods as well as mammals, also influenced germinability (MAUN & CAVERS 1971a, b). These epigenetic effects largely modify the intrinsic tendency of seed germinability. At the period of seed dispersal it was impossible to establish the effects of earlier cutting or grazing. Therefore, we refrained from determining geographic (altitudinal or latitudinal) trends which might appear if the plants had remained intact.

An interesting result was the absence of annual variation in the percentage of germinating seeds. This variation may be expected if weather at the time of seed maturation were a factor of seed germinability. In 1995–1998, we did not observe a significnt annual variation of seed germinability, although differences in average July–September (period of seed maturation) temperatures were greater than 2.5°C (temperatures of Prague-Ruzyně). The absence of annual variation in the percentage of seed germinability indicated that weather had probably little influence on the induction of innate seed dormancy.

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Souhrn

MARTINKOVÁ Z., HONĚK A. (2000): **Variabilita klíčivosti nažek šťovíku tupolistého,** *Rumex obtusifolius* L., **v České republice.** Plant Protect. Sci., **36**: 101–106.

V letech 1995–1998 jsme zkoumali variabilitu klíčivosti nažek šťovíku tupolistého, *Rumex obtusifolius* L., v přírodních populacích ze severních Čech (49°30'–50°47' N, 13°15'–15°47' E). Materiály nažek byly sklízeny od října do června následujícího roku ze suchých mateřských rostlin. Na každé lokalitě byly odděleně sebrány nažky z 5–15 náhodně vybraných rostlin, celkem 215 vzorků z různých lokalit. Sebrané nažky byly uchovány v pokojových podmínkách. Během 10–40 dnů po sběru bylo z každého vzorku 3 × 50 nebo 1 × 100 nažek ponecháno klíčit za přístupu světla při konstantní teplotě 25 °C. Průměrný podíl klíčivých nažek na jednotlivých lokalitách byl 12.7 % s intervalem ±SD 1.2–35.9 % (asymetrie způsobena angulární transformací procentních dat). Na lokalitě Kaliště byla zjištěna statisticky významná variabilita procenta klíčivosti mezi jednotlivými rostlinami a menší i když rovněž statisticky významná variabilita mezi odnožemi na téže rostlině. Na řadě jiných lokalit však procento klíčivosti nažek bylo všeobecně nízké nebo vysoké a variabilita mezi semennými materiály z jednotlivých rostlin byla malá. Následkem toho byla zjištěna statisticky významná variabilita mezi lokalitami ve všech sledovaných letech kromě roku 1998. Variabilita klíčivosti nažek je zřejmě kontrolována faktory specifickými pro jednotlivé rostliny nebo lokality. Nebyla však zjištěna žádná korelace mezi velikostí nebo počtem odnoží mateřské rostliny a procentem klíčivosti nažek. Rovněž nebyly zjištěny významné rozdíly v klíčivosti mezi vzorky sebranými v jednotlivých letech nebo mezi vzorky sebranými na podzim, v zimě a na jaře. Z toho lze učinit závěr, že variabilita klíčivosti nažek šťovíku tupolistého je málo ovlivňována sezonními nebo meziročními změnami počasí.

Klíčová slova: *Rumex obtusifolius*; šťovík tupolistý; dormance semen; klíčení; geografická variabilita; meziroční variabilita; počasí; velikost rostliny

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