American Journal of Environmental Sciences 2 (2): 74-78, 2006
ISSN 1553-345X

Survival and Changes in Germination Response of *Rumex obtusifolius* *Polygonum longisetum* and *Oenothera biennis* during Burial at Three Soil Depths

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Abstract: To investigate patterns in seed germination and survival at three different soil depths over a long time span, seeds of *Rumex obtusifolius*, *Polygonum longisetum* and *Oenothera biennis* were examined as a function of season (spring, autumn) and soil depth (3, 10, 30 cm). Buried seeds were exhumed after 3 years and subjected to two different temperature treatments. These were gradually increasing and decreasing temperatures, starting at 5 and 35°C, respectively. Treatments commenced after a 5-day acclimation to the initial starting temperature with light. With increasing burial depth, diurnal temperature fluctuations decreased and seed survival increased. Seed dormancy patterns changed with season and burial depths for all the species. *Polygonum* seeds germinated between 15 and 35°C in spring, but not in autumn. *Rumex* seeds germinated between 15 and 25°C in both spring and autumn. The optimal germination temperature for *Oenothera* seeds differed between spring (>30°C) and autumn (ca 30°C). Seed dormancy and survival are controlled by soil temperature fluctuations that differ with soil depth and seeds appear to have season-sensing and burial-depth detecting mechanisms based upon temperature fluctuations.

Key words: Burial depth, seasonal change, seed, germination, seed survival, soil temperature fluctuation, weed species

INTRODUCTION

Seed dormancy has an important role in plant survival via the alteration of seed germination patterns and various types of seed dormancy have been reported, in particular for weeds[1,2]. Since light does not usually penetrate the soil beyond a few centimeters, the seed dormancy cycle is mostly determined by diurnal and seasonal temperature fluctuations[3,4]. The magnitude of diurnal temperature fluctuations differs with soil depth[5] and therefore seed dormancy cycles also differ with the depth at which the seed is buried.

Diurnal temperature fluctuations often break dormancy in many species and decreases with increasing soil depth[6]. Due to decreasing diurnal temperature fluctuations, the percentage of seeds that are dormant increases with increasing depth of burial[1,7]. In addition, seedling emergence is not possible when the seeds are buried deeply[8,9]. Burial depth may influence seedling establishment by restricting seedling emergence and by changing the pattern of seed dormancy. To investigate changes in seed survival and seed germination patterns at different burial depths and in different seasons, I studied the temperature treatments required for seed germination in three common weed species. Three common species were selected: *Rumex obtusifolius* L. (perennial, exotic), *Polygonum longisetum* De Bruyn (annual, native) and *Oenothera biennis* L. (biennial, exotic). *R. obtusifolius* is European origin and *O. biennis* is North American origin. Nowadays both species are widespread in temperate zones in the Northern Hemisphere. For example, *O. biennis* is invasive on Europe in the present[9]. *P. longisetum* is commonly distributed in Asian Continent. The flowering periods of those three species are usually from June to October in northern Japan.

MATERIALS AND METHODS

The species used were *Rumex obtusifolius*, *Polygonum longisetum* and *Oenothera biennis*. Seeds were collected from arable lands in Niigata City (37°58'N, 138°87N, 10-30 m in altitude), Japan, in November, 1991. For each species, more than 10 individuals, that were ripe and produced mature seeds, were selected for the seed collection. When the seeds were sorted, only sound seeds were selected.

Four holes (1 mm in diameter) were drilled on the side of each transparent plastic tube (23 mm in height, 13 mm in diameter) to maintain a moisture balance with the surrounding soil. Seeds were placed into each plastic tube containing washed sea sand (20-35 mesh, Wako, Osaka). In each tube, 100 seeds of each species were set up. Two 50 cm × 50 cm plots were established near the collection sites on November 9, 1991.

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Six tubes were buried 3, 10 and 30 cm deep in each plot for each species. The soil type was in sandy soil, of which major texture ranged from 0.05 mm to 2.00 mm in any depths. Because the sandy soils had high water percolation rate (ca 40 ml/min), soil moisture was stable (range: 10-25%) in any soil depths[10]. The site for seed burial was weakly shaded because of a few tree canopies. Mean annual temperature is 13.5°C and total annual precipitation is 1776 mm[11]. A soil horizon was lacked, indicating that the nutrient is poor.

Soon after the seed collections, seed viability was estimated from the extent to which they were firm and intact[12,13]. Seeds were considered to dead if the seed albumen when crushed by a needle was not moist and/or was brown. Germination tests were conducted two times. The first and second seed germination tests started directly after exhuming seeds from a plot on April 7, 1994 (spring) and September 28, 1994 (autumn). Seeds in each tube were separated into two subsamples, i.e., 50 seeds each, to be subjected to germination tests.

A thermal screening system was used to determine seed germination patterns[14]. Temperature manipulation started with 5 days at 5°C or 5 days at 35°C for the increasing and decreasing temperature regimes, respectively, in light. Afterwards, the respective temperatures increased from 5°C (low to high, L-H) and decreased from 35°C (high to low, H-L) at 1°C intervals each day. This gradual warming or cooling continued for 30 days. The accuracy of the temperature was ± 0.3°C within a day in each incubator (Sanyo, Type MIR-152). Then, seed germination tests with temperature fluctuations with 5°C/35°C (12h/12h) were conducted until no more germination occurred for 2 weeks.

After each germination test, viability of the ungerminated seeds was checked by the same way described above. The final seed survival percentages were based on the total number of viable seeds. Differences between germination percentages between burial depths and between seasons were tested by two-way ANOVA for each species.

Air and soil temperature at the three soil depths (3, 10 and 30 cm deep) were taken from October 1, 1992, to September 30, 1993, at hourly intervals by automatic temperature recorders (Kadec-U, Kona System, Sapporo).

RESULTS

Soil temperatures: Over the period of temperature measurements, the mean monthly air temperature ranged from 4.5°C in February to 23.3°C in August. Mean monthly temperature at all depths fluctuated with air temperature. However, throughout the year the diurnal soil temperature fluctuations decreased with increasing soil depth, i.e., average maximum temperature differences in a day were 6.7, 4.9, 2.1 and 0.4°C in air and at depths of 3, 10 and 30 cm, respectively. Thus, the seeds at different depths were subjected to very different diurnal temperature fluctuations.

Seed survival and germination: More than 70% of the seeds of *Rumex obtusifolius* and *Oenothera biennis* germinated within 5 days after sowing in the H-L tests performed immediately following seed collection in autumn 1991. In the L-H experiments, seeds of both species began to germinate at ca 25°C. No seeds of *Polygonum longisetum* germinated in either the H-L or L-H tests. Seed viabilities based upon piercing the albumen, of *Polygonum*, *Oenothera* and *Rumex* were 90, 99 and 96%, respectively.

Percentage seed survival differed among the species three years after burial (F = 28.22, significant at P < 0.001, Table 1). Only 15% of the *Polygonum* seeds survived a depth of 3 cm; most had germinated before they were exhumed. On the other hand, more than 70% of the *Oenothera* and *Rumex* seeds survived at this depth. For all three species, the combined percentage seed survival increased with increasing soil depth (F = 4.25, significant at $P<0.01$), but did not differ between spring and autumn (F = 0.29, not significant). Final percent germination after 5/35°C did not differ between the H-L and L-H tests (F=2.43, not significant).

Polygonum longisetum: Final percentage germination did not differ significantly between the L-H and H-L tests at any burial depth in spring (Fig. 1). Since the seeds began to germinate at 15°C in the L-H test in spring and most seeds ceased to germinate at 35°C during the first 5 days in the H-L tests, optimal temperatures for seed germination were higher than 24°C. Seeds germinated in neither the L-H nor H-L tests in autumn (Fig. 1), but ca 60% of them germinated when 5/35°C diurnal temperature fluctuations were given after the germination tests.

Oenothera biennis: Most seeds failed to germinate in either the spring or autumn in the L-H tests (Fig. 1), suggesting that the seeds of this species germinate more in spring condition throughout the year. Although final percent germination did not differ between the two seasons, the germination patterns were different. Most seeds had germinated during 35°C treatments for the first 5 days in the H-L tests in spring, while seed germination ceased at 25°C in autumn. A few seeds germinated at temperatures > 27°C in both spring and autumn in the L-H tests, but did not germinate below this temperature.

Rumex obtusifolius: Except for seeds buried 10 cm in autumn, few germinated in the H-L tests (Fig. 1).

Table 1: Percent seed survival after a three-year burial period in soils at different depths, based on the final germination and viability check. ANOVA indicates that significant differences are observed between burial depths for all species and between the seasons for *O. biennis* ($P < 0.05$). H-L: temperatures decreased 1°C each day from 35°C to 5°C. L-H: temperatures increased 1°C each day from 5°C to 35°C. Each numeral shows mean with standard error

Species		*Rumex obtusifolius*		*Polygonum longisetum*		*Oenothera biennis*	
Temperature type		H-L	L-H	H-L	L-H	H-L	L-H
Spring	3 cm	84 ± 2	71 ± 2	14 ± 2	14 ± 2	87 ± 1	88 ± 1
	10 cm	87 ± 1	97 ± 0	60 ± 1	55 ± 2	95 ± 1	95 ± 1
	30 cm	90 ± 1	88 ± 0	75 ± 1	80 ± 1	94 ± 0	97 ± 1
Autumn	3 cm	73 ± 3	79 ± 3	18 ± 1	14 ± 2	80 ± 2	82 ± 2
	10 cm	90 ± 1	88 ± 1	39 ± 3	56 ± 2	84 ± 1	85 ± 2
	30 cm	94 ± 0	90 ± 1	68 ± 1	63 ± 1	96 ± 1	93 ± 1

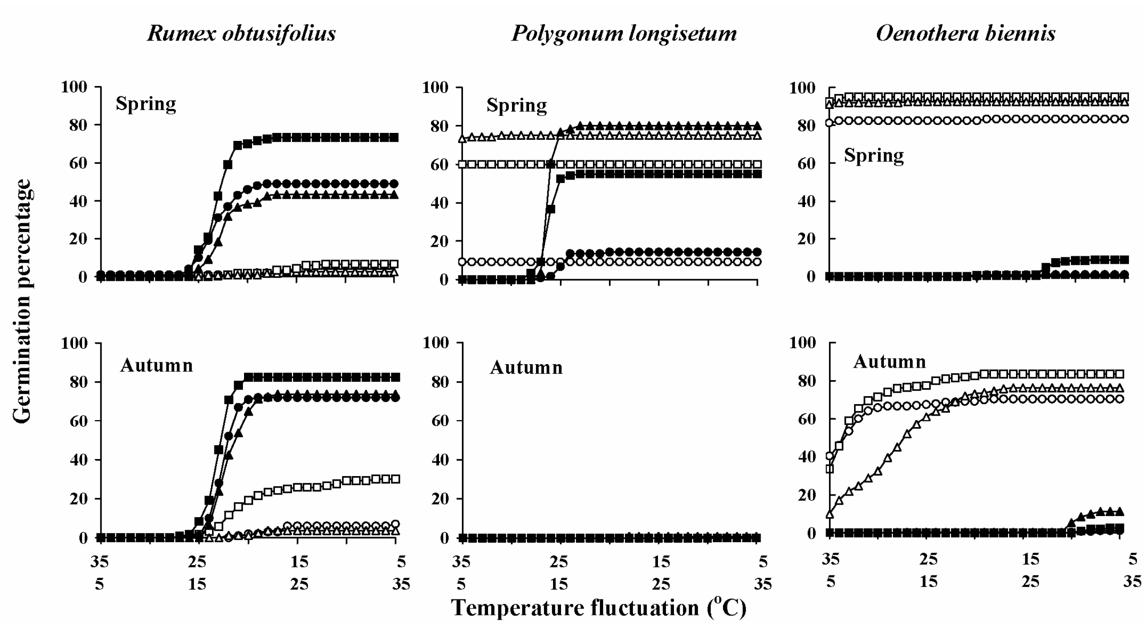

Fig. 1: Seed germination curves for three species buried at three soil depths (3, 10 and 30 cm). Mean germination percentage on six replicates was shown. All standard errors are less than 4 and are not shown in the figure, Closed symbols indicate temperature changes from low to high (L-H) and open symbols from high to low (H-L). Circles, squares and triangles are for seeds buried at depths of 3, 10 and 30 cm, respectively

In contrast, in the L-H tests most seeds germinated between 15°C and 20°C in both spring and autumn. Therefore, the seeds of this species should germinate more in autumn. Percent seed germination was higher in autumn than in spring in the L-H tests and was highest for seeds buried 10 cm deep in both seasons.

DISCUSSION

Burial depth and seed dormancy: Seed survival rates did not differ for about half year, i.e., between spring and autumn in the present study, while seed survival rates generally decrease exponentially soon after burial[2]. Seeds died and/or germinated in the first two years and remainders might be strongly dormant, in particular, for the seeds buried deeper in soil.

The *Polygonum longisetum* seeds buried 3 cm deep showed a dramatic decrease in survival, but this was because they had germinated. For the other two species, seed survival was slightly higher with increasing burial depth. Percent seed survival is increasingly higher with increasing burial depth[7,6]. Light requirement for seed germination has been reported for *Oenothera biennis*[3] and many other weeds[1]. No seeds of *Oenothera* and *Rumex obtusifolius*, buried at 3 cm, germinated in the field, indicating that little light penetrated to this depth. These results suggested that these species should form a persistent seedbank when buried in deeper soils. In fact, the seeds of *P. longisetum* and *O. biennis* survived > 10 years and *R. obtusifolius* survived > 20 years beneath ca 1 m volcanic deposits on Mount Usu, northern Japan[13] and phylogenetically related species can survive for several decades under experimental conditions[15]. However, seedling emergence is prevented by deep burial[8,9]. Consequently, the depth of seed burial may have two roles in the development of a seedbank: the maintenance of seed dormancy and adjustment in the timing or the prevention of seedling emergence.

Diurnal temperature fluctuations and seed germination: Temperature fluctuations with various time scales, from day to season, initiates germination in certain plant species, but the amplitudes required for full germination differ between species[16,17]. Temperature increased or decreased 1°C/day, therefore, all the seeds received 1°C diurnal temperature fluctuations with ±0.3°C error. *Polygonum* seeds did not germinate in autumn during either the L-H or H-L test and *Oenothera* seeds did not germinate in either the L-H tests in spring or autumn, although most of the seeds germinated in the 5/35°C regime. For *Oenothera*, the steady increase temperature induced different effects from the decrease temperature on seed germination in both spring and autumn. *Rumex* seeds germinated with 1°C temperature fluctuations in the L-H tests, but few germinated in the H-L tests. This species requires a shift to higher temperatures for induction of germination[5] and the seeds changed seed germination pattern little with different seasons.

Seasonal changes of dormancy patterns with different burial depths: Changes in seed dormancy are observed for the three species with different patterns. Seedling emergence strategies are more critical for annuals than for perennials, since annuals must be extinct if all seeds germinate at the same time with hazardous environments such as drought conditions[18]. Thus, dramatic changes in seed dormancy patterns occurred for the annual *Polygonum longisetum* between the different seasons and burial depths.

The temperature required for germination of *Oenothera* seeds narrowed in autumn, although their germination temperatures were more than 24°C in spring. Exposure to low temperatures induces physiological dormancy in *Oenothera* seeds. Dormancy in this species is conditional in autumn since the seeds germinated at high temperatures while seeds are nondormant in spring[3]. A few seeds for *Oenothera* germinated in the L-H tests between 5°C and 24°C and seems to be still in conditional dormancy in spring. The three species used showed at least some conditional seed dormancy patterns in both seasons.

The highest percent germination on *Rumex* was for seeds buried in 10 cm deep in both spring and autumn. In addition, seeds buried 10 cm deep germinated in autumn in the H-L tests, while only a few seeds germinated in the other H-L tests. Optimal burial depth for the germination of *Rumex* seeds was about 10 cm and this may has an important role for seedbank dynamics and seedling establishment.

CONCLUSION

Patterns of seed dormancy and survival were determined by soil temperature fluctuation patterns which decreased with increasing soil depth.

Seeds have season-sensing mechanisms that information is shift from low to high or from high to low temperatures. Deep burial decreases the action of season-sensing mechanisms, due to narrow temperature fluctuations in a day and in a year. Seed burial depths are of prime importance for seed survival and seedling emergence.

ACKNOWLEDGMENTS

The author thanks S. Yoshida and A. Suzuki for useful suggestions, M. Hayama for his help with the laboratory experiments, Y. Tsuyuzaki for her help with seed collection and J.H. Titus for his critical reading of the manuscript. This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan.

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