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## Effective Treatments for the Induction of Germination in Mountain Rangeland Species

Many species from mountain rangeland produce seeds that are dormant or otherwise difficult to germinate because of specialized requirements. The development of laboratory methods for the germination of such seeds is a prerequisite for studies of subsequent growth and development. Early research on this subject (e.g., Griswald, 1936) was handicapped by lack of control of important environmental factors such as temperature, and by the unavailability of potent growth regulators such as gibberellic acid. Except for a survey of germination of forest range species from British Columbia (McLean, 1967), recent studies have been concerned with individual species or genera, or have dealt largely with factors that modify germination or influence seedling emergence. Since a need exists for a survey of treatments that will yield high germination in the more important mountain rangeland species, the present study of 44 species from sub-alpine rangeland in Utah and Montana was undertaken. This number of species seemed to constitute a sufficiently large sample to indicate both the prevalence of dormancy and effective laboratory means of relieving it in mountain rangeland species.

### Methods

Collections of seeds and fruits were made during the summers of 1965 and 1966 from aspen and grass-forb subalpine rangeland on the Wasatch and Uinta Mountains of Utah and the Centennial Mountains of Montana. Elevations in the areas of collection ranged from 2,000 to 3,100 meters. Selection was based on availability of seeds during visits to the collecting areas. A list of the 44 species studied is given in Table 1. Plant nomenclature is taken from Hitchcock, *et al.* (1959), except for grasses (Hitchcock, 1950), *Ligusticum porteri* (U.S. Dep. Agr., 1937), *Polemonium foliosissimum* (Davis, 1952), and *Carex hoodii* (Lewis, 1956).

Collections were cleaned and stored at 2°C in vapor-tight bottles until used in experiments. Seeds collected in 1966 were processed within one to four days; those collected in 1965 (*Achillea*, *Actaea*, *Agastache*, *Bromus*, *Carex*, *Clematis*, *Elymus*, *Glaucus*, *Ligusticum filicinum*, *Melica*, *Polemonium*, *Potentilla glandulosa*, *Rumex*, and *Sambucus*) remained under room conditions for three months before being processed.

Treatments to induce germination included variations in temperature and medium of incubation, light, leaching of seeds, and removal or modification of external parts of

<sup>1</sup> Stationed at Forestry Sciences Laboratory, Logan, Utah, maintained in cooperation with Utah State University.

TABLE 1. Final germination percentages of seeds imbibed in water or GA<sub>3</sub> at alternating temperatures. For percentages of 20 or more, numbers in parentheses indicate days required to reach half the final germination percentage.

Species	H <sub>2</sub> O			GA <sub>3</sub> <sup>1</sup>
	32°/22°C	22°/17°C	17°/12°C	22°/17°C
<i>Actaea rubra</i>	0	0	0	---
<i>Achillea millefolium</i>	91(3)	92(3)	96(4)	NR
<i>Agoseris glauca</i>	1	14	8	---
<i>Agastache urticifolia</i>	12	48(5)	68(7)	93(4)
<i>Antennaria rosea</i>	9	48(4)	67(5)	---
<i>Aquilegia coerulea</i>	70(10)	60(12)	47(14)	96(10)
<i>Arabis glabra</i>	35(4)	42(4)	90(4)	NR
<i>Berberis repens</i>	0	0	0	---
<i>Bromus polyanthus</i>	42(8)	65(9)	57(7)	---
<i>Carex hoodii</i>	1	59(16)	48(18)	---
<i>Camelina microcarpa</i>	10	98(2)	98(2)	NR
<i>Chrysothamnus viscidiflorus</i>	21(6)	98(7)	93(8)	NR
<i>Cirsium foliosum</i>	11	10	11	69(5)
<i>Clematis birsutissima</i>	0	1	4	---
<i>Collomia linearis</i>	1	78(5)	98(3)	NR
<i>Descurainia pinnata</i>	0	4	11	---
<i>Elymus cinereus</i>	10	14	36(10)	60(5)
<i>Elymus glaucus</i>	14	46(6)	52(8)	---
<i>Frasera speciosa</i>	0	0	0	---
<i>Geranium viscosissimum</i>	38(11)	24(13)	13	---
<i>Grindelia squarrosa</i> (central) <sup>2</sup>	98(3)	98(3)	97(4)	NR
<i>Grindelia squarrosa</i> (peripheral) <sup>2</sup>	68(12)	81(12)	67(14)	NR
<i>Hesperochloa kingii</i>	0	23(9)	24(7)	75(9)
<i>Heracleum lanatum</i>	0	0	1	---
<i>Ligusticum filicinum</i>	0	0	0	---
<i>Ligusticum porteri</i>	0	1	0	---
<i>Lupinus argenteus</i>	64(4)	80(4)	76(6)	NR
<i>Madia glomerata</i>	0	0	0	---
<i>Melica bulbosa</i>	0	0	0	---
<i>Osmorhiza occidentalis</i>	0	0	0	---
<i>Phleum alpinum</i>	91(7)	88(8)	98(10)	NR
<i>Pedicularis parryi</i>	0	8	18	97(3)
<i>Penstemon rydbergii</i>	0	0	4	94(11)
<i>Poa nevadensis</i>	0	60(8)	68(10)	94(7)
<i>Polemonium foliosissimum</i>	21(7)	61(8)	46(8)	98(6)
<i>Potentilla glandulosa</i>	87(4)	84(4)	73(5)	NR
<i>Potentilla gracilis</i>	96(4)	67(4)	72(7)	---
<i>Rumex crispus</i>	100(3)	98(3)	82(5)	NR
<i>Rudbeckia occidentalis</i>	86(4)	93(3)	1	NR
<i>Sambucus racemosa</i>	0	0	0	---
<i>Senecio integerrimus</i>	0	0	1	---
<i>Senecio serra</i>	3	20(12)	14	43(5)
<i>Taraxacum officinale</i>	98(4)	98(3)	96(4)	NR
<i>Tbalictrum fendleri</i>	71(10)	68(9)	22(12)	97(6)
<i>Tragopogon dubius</i>	80(10)	98(6)	98(5)	NR

<sup>1</sup> GA<sub>3</sub> had no promotive effect (dash) or this treatment was not run (NR).

<sup>2</sup> Two distinct morphological types of akenes occupy central and peripheral portions of the capitulum.

<sup>3</sup> Seeds were incubated at 17°/12°C.

the dispersal unit. The objective was to obtain nearly complete germination by particular treatments or combinations. Preliminary work with the collections of 19 was used as a basis for determining the choice of treatment variations, and duration of application in the final experiments described and reported below.

Seeds were imbibed in 4ml of distilled water or 10<sup>-3</sup> M solution of gibberellic acid (GA<sub>3</sub>) on double layers of filter paper in 9-cm petri-dishes. Duplicate dishes with 100 seeds per dish were used for each treatment, and experiments were run two

The seeds were incubated for 28 days under controlled temperature alternations in the range 32°–12° C, or were stratified for 12–16 weeks at 2° C before or after incubation at higher temperatures. In one experiment only, imbibed but ungerminated seeds were transferred from stratification to higher temperatures. The higher temperature of an alternation was applied during an eight-hour photo-period (e.g., 32°/22°C) at an average illuminance of 50 lux, and seeds were examined on alternate days for germination.

External parts of the dispersal units interfered with germination of certain species—the perigynium (*Carex*), perianth (*Rumex*), and pericarp (*Clematis*, *Thalictrum*). These parts were removed before imbibition, except that for *Clematis* only a small portion of the pericarp at the embryonic root end was clipped off. The hard seed coats of *Lupinus* were made permeable by a 4-min submergence in sulfuric acid. *Potentilla glandulosa* required the same treatment for 11 minutes. Acid-treated seeds were washed in running tapwater for one-half hour to eliminate all traces of acid.

Imbibing seeds of *Thalictrum* released into the filter paper a brownish substance that delayed germination and reduced the final percentage in preliminary work. This substance was removed from the seeds by a 4-hr wash in running tapwater. Leachable substances were apparent also in *Heraclium*, *Ligusticum filicinum*, *L. porteri*, and *Osmorbiza*. Seeds of these species were leached similarly; whether these leachates were inhibitory was not determined.

Germination was identified by visible root growth in all species except *Carex*, where root and shoot growth was approximately simultaneous, and *Potentilla glandulosa*, where approximately half of the seeds germinated by an initial growth of the shoots. Final germination percentages are presented as the means of four determinations, and germination rates as the number of days required to reach half of the final percentage. For certain comparisons of percentages resulting from two treatments, a t-test (Bailey, 1959) was used to determine the statistical significance of differences at the 5 per cent level.

## Results

With water-imbibed seeds, germination in excess of 50 per cent was obtained in 23 species under at least one of the temperature alternations of Table 1, whereas 21 species showed little or no germination under these conditions (Table 1, H<sub>2</sub>O). The number of species yielding highest germination percentage at one temperature alternation declined from 16 to 9 to 7 with increasing temperatures. There was appreciable variation in germination rates among species, and a trend within species toward reduced rates at lower temperatures.

When seeds of species with germination percentages of 70 or less were imbibed in 10<sup>-3</sup> M GA<sub>3</sub> at 22°/17° C (17°/12° C for *Penstemon*), there was a significant improvement in the germination of *Agastache*, *Aquilegia*, *Cirsium*, *Elymus cinereus*, *Hesperochloa*, *Pedicularis*, *Penstemon*, *Poa*, *Polemonium*, *Senecio serra*, and *Thalictrum* (Table 1, GA<sub>3</sub>). Rate of germination was increased in six species in which this comparison was possible.

Seeds of 22 species that yielded germination percentages of 70 or less under alternating temperatures were imbibed in water or GA<sub>3</sub> and were held at 2° C for 16 weeks. All but three of these species germinated to varying extents while at 2° C (Table 2, 2° C). The germination percentages were significantly higher in GA<sub>3</sub>-imbibed seeds

for all species except *Bromus*, *Clematis*, *Frasera*, *Geranium*, *Heraclium*, and *Osmorbiza*. When seeds ungerminated at 2° C were transferred from water and GA<sub>3</sub> to water moistened filter paper at 22°/17° C, germination began in *Antennaria*, *Carex*, and *Madia*, and in seeds of *Actaea* and *Sambucus* imbibed in water at 2° C. Upon transfer the remaining species showed additional germination beyond that occurring at 2° C. The water-to-water transfers gave much higher germination percentages than occurred under higher incubation temperatures alone in all species except *Elymus glaucus* (22°, 17° C, Tables 1 and 2). Rate of germination was increased also in those species in which comparisons were possible.

High germination percentages in water-imbibed seeds of *Actaea*, *Frasera*, and *Melica* could be obtained if the seeds were held at 22°/17° C for three weeks before transfer to 2° C for 12 weeks. Under these conditions, the mean germination percentages (± standard deviations) were 87 ± 4, 94 ± 2, and 97 ± 2, respectively. The germination occurred at 2° C in *Actaea* and *Melica*; and in *Frasera*, in part upon transfer to 22°/17° C.

TABLE 2. Germination percentages after 16 weeks at 2° C for seeds imbibed in water or GA<sub>3</sub> (2° C), and the percentage germination that followed transfer to water at alternating temperatures of 22°/17° C (2° to 22°/17° C). For percentages of 20 or more at 22°/17° C, numbers in parentheses indicate days required to reach half the final germination percentage.

Species	2° C		2° to 22°/17° C <sup>a</sup>	
	H <sub>2</sub> O	GA <sub>3</sub>	H <sub>2</sub> O	GA <sub>3</sub>
<i>Actaea rubra</i>	0	51	24(4)	32(3)
<i>Agoseris glauca</i>	60	98	98(2)	NR
<i>Antennaria rosea</i>	0	0	99(2)	97(2)
<i>Berberis repens</i>	30	79	10	28(3)
<i>Bromus polyanthus</i>	93	92	NR	NR
<i>Carex hoodii</i>	0	0	99(7)	98(6)
<i>Cirsium foliosum</i>	70	93	26(2)	NR
<i>Clematis hirsutissima</i>	23	19	80(6)	69(8)
<i>Descurainia pinnata</i>	11	20	82(3)	79(3)
<i>Elymus cinereus</i>	11	70	75(1)	98(1)
<i>Elymus glaucus</i>	34	92	40(3)	NR
<i>Frasera speciosa</i>	19	14	23(4)	24(3)
<i>Geranium viscosissimum</i>	28	31	96(3)	94(3)
<i>Heraclium lanatum</i>	28	34	96(3)	93(4)
<i>Ligusticum filicinum</i>	20	53	38(5)	80(7)
<i>Ligusticum porteri</i>	63	81	88(2)	90(2)
<i>Madia glomerata</i>	0	0	97(2)	94(2)
<i>Melica bulbosa</i>	30	83	11	99(3)
<i>Osmorbiza occidentalis</i>	81	83	100(2)	97(2)
<i>Sambucus racemosa</i>	0	8	13	72(1)
<i>Senecio integerrimus</i>	84	97	99(3)	NR
<i>Senecio serra</i>	46	75	89(4)	97(3)

<sup>a</sup>NR indicates that the transfer to 22°/17° C was not made if germination at 2° C exceeded 90 per cent.

## Discussion

With regard to germination percentages and rates, the species are divisible into two major groups: those that germinate readily at alternating temperatures (Table 1), and those that require a low-temperature treatment of the imbibed seeds (Table 2). Species of both groups show a requirement for GA<sub>3</sub> for maximum germination (Tables 1 and 2, GA<sub>3</sub>). Induction of germination by GA<sub>3</sub> is an artificial treatment, in contrast

stratification and favorable higher temperatures. However, it is known that gibberellins are naturally occurring growth regulators that are synthesized in various plant parts, including seeds, in response to particular levels of environmental factors (Paleg, 1965); these levels presumably forecast conditions favorable for growth.

If the species examined constitute a fair representation of the range of variability among all species of mountain rangeland with respect to germination requirements, then the treatments and combinations used in this study may have wider applicability for inducing rapid and nearly complete germination. In particular, if a species fails to respond to photoperiodic and alternating temperature treatments, and if scarification, leaching, or removal of certain accessory parts is not indicated, then a successful treatment may include stratification of seeds in water or GA<sub>3</sub> for three to four months before a return to higher temperature. A modification of this treatment, whereby the imbibed seeds are transferred from a higher temperature to 2°C, also may be considered.

#### Acknowledgments

The assistance of Paul Conrad and Verl Matthews of the Forest Service and Hayle Buchanan of Weber State College in making collections of seeds is appreciated.

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Accepted for publication September 16, 1968

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## Radionuclide Deposition and Elimination in a Darkling Beetle<sup>1</sup>

The introduction of man-made radioactive materials into the biosphere has prompted the study of food chains to identify the kinds of organisms that accumulate radionuclides. Selected radionuclides are useful in ecological studies to aid in the identification of animal food sources and to determine rates of food consumption (Wiegert, Odum and Schnell, 1967).

This paper reports on the elimination rate (biological half-time) and gross pattern of body distribution of <sup>134</sup>Cs, <sup>85</sup>Sr, <sup>65</sup>Zn, and <sup>22</sup>Na in a darkling beetle, *Eleodes hispilabris*, one of the conspicuous ground dwelling insects of desert steppe ecosystems.

#### Materials and Methods Employed

Field trapped beetles were brought indoors and accommodated to a diet of dry oatmeal flakes and water supplied ad libitum. Each beetle, maintained separately in a pasteboard carton, was fed oatmeal flakes containing about 2 microcuries of radionuclide. After ingesting the treated flakes, the beetles were supplied a radionuclide-free diet. The rate of elimination of radionuclide was determined by periodically counting each beetle for radioactivity over a four- to five-week period. For counting purposes each beetle was placed inside a test tube which in turn was inserted into a 3 x 3 inch well-type NaI(Tl) gamma ray detector crystal. The influence of environmental temperature upon radionuclide retention was studied by maintaining beetles in incubators held at temperature regimes of 23° C and 32° C. Relative humidity was held at 80 per cent. Experiments with <sup>22</sup>Na and <sup>65</sup>Zn were conducted under room conditions.

Samples of voluntary muscle, exoskeleton and internal organs were taken four weeks after radioisotope administration to obtain information concerning the body distribution of <sup>85</sup>Sr and <sup>134</sup>Cs.

In this investigation, biological half-time ( $T_{1/2}$ ) is regarded as the amount of time required for a particular loss component to decrease by a factor of two.

The elimination component half times were obtained by fitting lines to the retention curves by the least squares method (Snedecor, 1956). The line fitting technique was begun on the slowest phase component to allow for a correction of its contribution to radioisotope loss during the initial rapid loss phase (or phases).

<sup>1</sup>This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)1830.