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Effects of Scarification and Stratification on Seed Germination and Dormancy of *Turgenia Latifolia*, *Cuscuta Sp* and *Sophora Alopecuroides* in Different Temperature Regimes

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ABSTRACT- One of the major problems with effective weed control is the presence of dormancy in weed seeds. In this study, the effects of stratification and scarification on seed germination and dormancy of *Turgenia latifolia, Cuscuta sp* and *Sophora alopecuroides* seeds in different temperature regimes were investigated. None of *Turgenia latifolia* hulled seeds germinated in any of the temperature regimes, whereas dehulled seeds germinated at 15 and 20°C. Also, scarification by acid sulfuric as a means of promoting germination significantly increased germination percentages of *Cuscuta sp* and *Sophora alopecuroides* seeds. Stratifying intact dormant seeds for 2, 4, 6, and 8wk increased germination rates dramatically. Best *Turgenia latifolia* seed germination was achieved with 8 wk stratification at 15 and 20°C (60 and 64%, respectively). Stratification did not stimulate the germination of *Cuscuta* seeds, whereas 8-wk stratification of *Sophora alopecuroides* seeds increased germination up to 40% regardless of the germination temperature. The results showed that both seed coat-imposed and internal dormancy were the main causes of *Turgenia latifolia* seed dormancy, whereas only seed coat-imposed dormancy was the main cause of *Cuscuta sp* and *Sophora alopecuroides* seeds.

Keywords: Dormancy, Seed germination, *Turgenia latifolia, Cuscuta sp.*, *Sophora lopecuroides*

INTRODUCTION

Dormancy is a physiological state in which a viable seed fails to germinate when provided with water and environmental conditions normally favorable to germination (16). Dormancy is a property of many weed seeds that enables them to survive conditions hazardous to plant growth and to germinate at some later time or in some other place (6). Weed seeds, and their germination requirements, are very diverse. The exact range of requirements for germination is peculiar to each species and even to each seed sample (12).

Turgenia latifolia, bur parsley, from the Apeaceae family is a summer annual weed. This weed is common in wheat fields of Iran and the Middle East and often

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emerges in late winter or early spring. Hamidi and Ghadiri (9) conducted experiments to determine the effect of eight constant temperatures on germination of bur parsley hulled and dehulled seeds under dark conditions. In general, after 28 days, the accumulated germination percentage of dehulled seeds was higher than the hulled seeds at all temperature regimes. The best germination was achieved at 15 and 20°C in the dark (9). Seed dormancy in bur parsley seeds could be attributed to the presence of inhibitory substances in the seed coat (8).

Small seed lots are sometimes scarified by chemically treating the seed with strong acids or bases (3). Concentrated sulfuric acid has been used experimentally for many years with considerable success on many species (14). Numerous experiments were conducted to break seed dormancy by chemical and mechanical scarification treatments. Artificial softening is the quickest and most practical means to reduce hardseededness in seed lots.

Cuscuta attenuata, dodder, from the Cuscutaceae family, is a parasite weed. Scarification of this seeds in concentrated sulfuric acid for 15, 30, 45 or 60 minutes increased germination rates dramatically (13). Hardseedness is also common in other species.

Sophora alopecuroides, pagoda tree, from the Fabaceae family, is an annual weed. The prevention of water uptake into the seeds that have impermeable seed coats is a very effective dormancy mechanism occurring in weedy members of several families, particularly those of the Fabaceae, Malvaceae, Chenopodiaceae, and Solanaceae (17).

Embryo dormancy is another reason for low germination in some weed seeds. Seeds with embryo dormancy usually require a low-temperature pretreatment before germination can occur. Such a treatment is known as stratification. The moistened seeds are usually preconditioned at temperatures between 3-10°C although the specific temperatures and lengths of exposure may vary. For some species, low temperature stratification is an absolute requirement for germination; for others, it may only hasten germination and increase the speed of growth. In a research on *Eriochloa villosa*, woolly cupgrass, the results showed that the seed type and stratification treatment affected seed germination. Dehulling dormant seeds partially released dormancy, resulting in 62% germination in the absence of stratification. Stratifying dehulled dormant seeds for 2wk increased germination to 98%, regardless of the germination temperature. In contrast, intact dormant seeds required an 8wk stratification period to completely afterripen (2).

In the present study, the effects of stratification and scarification on seed germination and dormancy of bur parsley, dodder and pagoda tree seeds in different temperature regimes were investigated.

MATERIALS AND METHODS

General Procedures

Bur parsley, dodder and pagoda tree seeds were harvested at physiological maturity during July and September 2001 from an indigenous population at Bajgah valley, 18 km north of Shiraz, and at Sepidan 75 km north west of Shiraz. Seeds were cleaned immediately after harvesting, and stored in darkness at room temperature (25-30°C) until used. Seed viability was nearly 100% as determined by tetrazolium (TZ) tests. All

seeds were surface sterilized with a 5% (v/v) sodium hypochlorite (NaOCl) solution for 5 min and rinsed repeatedly with distilled water prior to use. Four replications with 50 seeds each were placed on Whatman No. 2 filter paper moistened with 5 ml distilled water in 9-cm petri dishes that had been sterilized in an oven at 110°C for 2h. Germination was checked daily by visible radicle protrusion for 2 wk. Germinated seeds were removed from petri dishes every day and after 14 days, germination percentage was calculated.

Stratification Tests

Intact dormant seeds were prepared as previously described, then stored in a refrigerator (6±2°C) for 2, 4, 6 and 8 wk. After stratification, seeds were incubated at 10, 15, 20, 25, 30 and 35°C in Conviron growth chamber (CMP 4030 model, Canada).

Scarification Tests

Concentrated sulfuric acid (98%) was used to soak seed samples for 5, 10, 15, 20, 30, 40 and 60 minutes. After soaking, the seeds were washed in running water for 5 minutes and then rinsed with distilled water prior to use. Petri dishes were incubated in growth chamber in different temperature regimes (10, 15, 20, 25, 30 and 35°C) in darkness. Since even 5-min scarification of bur parsley seeds with sulfuric acid (98%) caused seed damage, these seeds were dehulled (by hand, without sulfuric acid) and were placed in different temperature regimes.

Statistical analysis

All experiments were arranged as factorial in a completely randomized design with 4 replications. All data were subjected to analysis of variance and means were compared using standard errors and Duncan's new multiple range test. In all experiments, percentage germination data were transformated using the arcsin.

RESULTS AND DISCUSSION

Bur parsley

Hulled and dehulled bur parsley seeds. None of hulled bur parsley seeds germinated at different temperature regimes, whereas dehulled seeds germinated significantly as compared to hulled seeds (Fig. 1). After 14 days, germination of dehulled seeds was 32, 23, and 12% at 15, 20 and 10°C, respectively. The results indicated that low temperatures (10°C to 20°C) are more favorable for germination of bur parsley seeds than high temperatures (25 to 35°C). These results are similar to the results obtained by Hamidi and Ghadiri (9). Two factors can be responsible for germination failure of hulled as compared to dehulled seeds: I) fruit coat (seed coat) which is an important barrier to the penetration of water and other essential elements such as oxygen into the seed (6); and ii) the presence of inhibitory substances in the seed coat (8). Many seeds (including *T. latifolia)* either fail to germinate or show less than l00% germination in response to a single dormancy-breaking treatment. In such cases, high germination often results from the use of two distinct dormancy-breaking treatments applied either simultaneously or successively (4).

Stratification. Intact dormant seeds required an 8wk-stratification period (or more) to germinate (Table 1). Stratifying intact dormant seeds for 8wk increased germination to 31%. A stratification period of less than 8wk significantly reduced the germination percentage of intact seeds, regardless of the germination temperature. Maximum germination was observed in seeds placed at 20 and 15°C (Table 1). Temperatures below 15°C and above 20°C significantly reduced germination percentages of these seeds. There was a significant interaction between stratification and temperature.

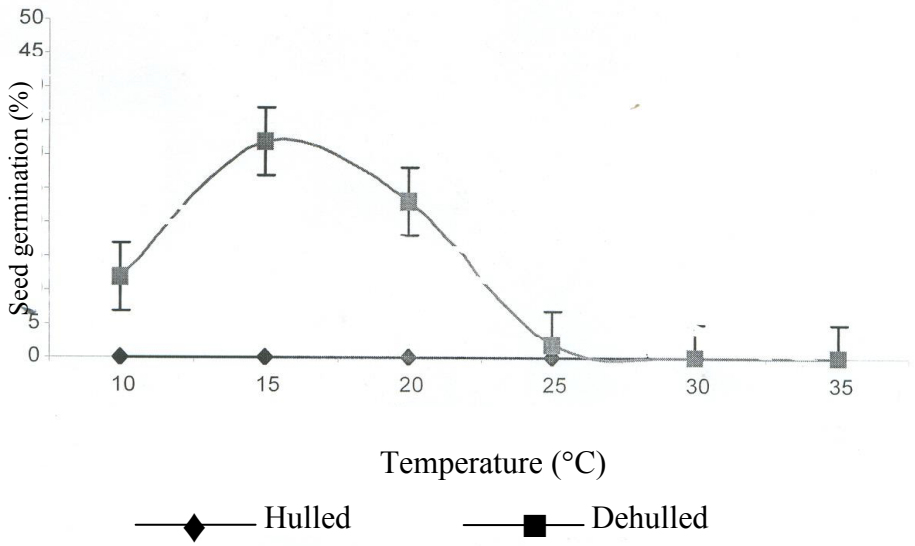

Fig. 1. Effect of temperature on bur parsley seed germination in hulled and dehulld seeds

The results of this study suggest that factors responsible for low germination percentage of bur parsley seeds under stratification treatment could be: i) seed coat which is an important barrier to the penetration of water and other essential elements such as oxygen into the seed; ii) the presence of some inhibitory substances in the seed coat; and iii) the seeds requirement for a long stratification period at low temperature (more than 8wk) for breaking embryo dormancy.

The presence of endogenous substances such as ferulate, coumarin-like substances, benzoate and ABA in seed coverings and embryos prevent the commencement of germination (4). The results of this study showed that stratifying bur parsley seeds for a long time possibly permitted the inhibitor to break down or to be leached out. The results obtained from this study also showed that bur parsley seeds had a double dormancy (mechanical and physiological dormancy). Mechanical dormancy was removed by dehulling seeds. In a study on beneh (*Pistacia mutica* F. & M.) the results showed that scarification with 98% cold sulfuric acid (90 min) plus 20 d stratification at 5±1°C increased seed germination from 27.7 to 62.1 % (1). They indicated that inhibitors extracted from beneh seeds had properties similar to abscisic acid (ABA), and a hard endocarp and internal dormancy were the main causes of seed dormancy. In addition, the physiological dormancy in seeds of some plants is dependent

on the ratio of the levels of the growth inhibitor abscisic acid (ABA) and the growth promoter GA. The seed remains dormant if the level of ABA is higher than that of GA, whereas it germinates if the GA level is higher than that of ABA (11). Physiological dormancy can be broken by either exogenous GA (data not shown) or a treatment which results in the synthesis of endogenous GA (such as 8 wk chilling at 6±2°C followed by transfer to higher temperature).

Table1. Cumulative germination percentage of dormant bur parsley seeds as affected by temperature regimes and stratification period

Stratification (wk)	Temperature (°C)						
	10	15	20	25	30	35	Mean
0	0 Ca†	0 Ca	0 Da	0 Ca	0 Ca	0 Aa	0 D
2	0 Ca	1 Ca	2 Da	0 Ca	0 Ca	0 Aa	0.5 D
4	0 Cb	0 Cb	34 Ca	0 Cb	0 Cb	0 Ab	5.67 C
6	18 Bb	50 Ba	51 Ba	16 Bb	4 Bc	0 Ad	23.2 B
8	30 Ab	60 Aa	64 Aa	22 Ac	8 Ad	0 Ae	30.67 A
Mean	9.6 c	22.2 b	30.2 a	7.6 d	2.4 e	0 f	

†-Means within each column followed by the same capital letters are not significantly different at the 5% level according to Duncan's multiple range test.
Means within each row followed by the same small letters are not significantly different at the 5% level according to Duncan's multiple range test.

Dodder

Scarification. Scarification increased germination rates dramatically (Table 2). None of unscarified seeds germinated at different temperature regimes, whereas 88% germination was achieved with 60-min scarification of seeds with sulfuric acid. Temperature also had an impact on germination. The best temperature for seed germination was 20 to 35°C. Temperatures below 20°C significantly reduced germination (Table 2). There was a significant interaction between temperature and scarification.

Table 2. Cumulative germination percentage of dormant bodder seeds as affected by temperature regimes and stratification treatment

Stratification (wk)	Temperature (°C)						
	10	15	20	25	30	35	Mean
0	0 Da†	0 Da	0 Ea	0 Da	0 Ea	0 Ea	0 E
5	0 Da	1 Da	4 Ea	4 Da	2 Ea	3 Ea	2.3 E
10	8 Cc	44 Cb	64 Da	62 Ca	66 Da	60 Da	50.67 D
15	7 Cc	44 Cb	77 Ca	72 Ba	72 CDa	81 Ca	58.8 C
20	30 Bc	88 Bab	90 Ba	94 Aa	91 ABa	85 BCb	79.7 B
30	34 Bc	88 Bb	99 Aa	96 Aa	96 Aa	85 BCb	83 AB
40	39 ABd	89 Bbc	98 Aa	96 Aa	92 ABab	90 ABb	84 AB
60	46 Ad	97 Aa	100 Aa	98 Aa	90 Bc	94 Aab	87.5 A
Mean	20.5 c	56.4 b	66.5 a	65.3 a	63.6 a	62.3 a	

†Means within each column followed by the same capital letters are not significantly different at the 5% level according to Duncan's multiple range test.
Means within each row followed by the same small letters are not significantly different at the 5% level according to Duncan's multiple range test.

The results of this study clearly showed that a high hard seed percentage is a characteristic of seed lots of dodder (*Cuscuta* sp.) and with increase in soaking time of seeds in concentrated sulfuric acid (98%) from 5 to 10, 15, 20, 30, 40 and 60 minutes, seed germination increased from 2 to 50, 59, 80, 83, 84% and 87% respectively, without causing damage to seeds. Sulfuric acid treatment is a well recognized effective method of softening impermeable seed coats. It is quick, and does not need complex equipment. A period of sixty minutes was effective time for obtaining maximum dodder seed germination with concentrated sulfuric acid. However, hardseededness was considered as the most likely cause of this dormancy. Therefore, coat-imposed dormancy prevented seed germination by blocking water entry to the embryo. Lyshede (10) showed that the main cause of seed dormancy in *Cuscuta* is the impermeability of the seed coat. Scarification results in high germination percentages of seeds of many *Cuscuta* species. Thus seeds have physical dormancy and in this type of seed dormancy seed coat impermeability is usually associated with the presence of one or more layers of impermeable palisade cells of lignified cells. For example, impermeable seed coats in *Cuscuta pedicellata* and C. *campestris* (Convolvulaceae) have two palisade layers; only the inner one is impermeable.

Stratification. None of the dodder (*Cuscuta* sp) seeds germinated at different levels of stratification (2, 4, 6 and 8wk). In general, stratification was unable to release seeds from dormancy (data not shown). Results of this study clearly showed that 8wk of stratification did not overcome the dodder seeds coat-imposed dormancy. These seeds, called hard seeds, did not respond to stratification of the seeds by imbibing water and germinating.

Pagoda tree

Scarification. Scarification increased germination rates dramatically (Table 3). The highest germination percentages were observed in seeds exposed to 30, 40 and 60 min scarification with sulfuric acid, regardless of the germination temperature. Temperatures below 30°C significantly reduced germination percentages of seeds. There was a significant interaction between scarification and temperature.

Table 3. Cumulative germination of dormant pagoda tree seeds as affected by temperature regimes and stratification treatment

Stratification	Temperature (°C)						
(wk)	10	15	20	25	30	35	Mean
0	0 Be†	18 Ed	28 Fc	49 Eb	64 Ea	64 Ea	37.2 F
5	0 Bd	22 Ec	54 Eb	53 Eb	63 Ec	66 Ea	43 E
10	2 Ac	37 Db	72 Da	74 Da	76 Da	76 Da	56.4 D
15	2 Ac	43 Cb	82 Ca	81 Ca	84 Ca	86 Ca	63 C
20	4 Ac	63 Bb	89 Ba	87 Ba	90 Ba	92 Ba	70.8 B
30	5 Ab	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	84.2 A
40	6 Ab	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	84.3 A
60	6 Ab	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	84.3 A
Mean	3.6 d	60.4 c	78 b	80.5 b	84.6 a	85.5 a	

†Means within each column followed by the same capital letters are not significantly different at the 5% level according to Duncan's multiple range test.
Means within each row followed by the same small letters are not significantly different at the 5% level according to Duncan's multiple range test.

A period of thirty minutes was the minimum effective time to break hard seeds with concentrated sulfuric acid. At this time, nearly the whole hard seed coat was broken. The results clearly showed that in seeds of *S. alopecuroides,* coats or coverings impose dormancy over the embryo. In general, according to the results obtained from the present study, scarification of the pagoda tree seed is an effective means of reducing hardseedness in these seeds because impermeability of seed coats was the main cause of seed dormancy. Seed coat impermeability is usually associated with the presence of one or more layers of impermeable palisade cells. In addition to the development of impermeable layers in the seed (or fruit) coats, all the natural seed openings, including the micropyle, hilum, and chalazal area, also become impermeable to water.

Stratification. Maximum germination occurred in an 8-wk stratification (40%), whereas nonstratified seeds (control) germinated about 37% (Table 4). This indicated that seed stratification of the pagoda tree for a short time was unable to increase the germination percentage (Table 4). The best temperature for germination was 35°C. Temperatures below 35°C significantly reduced germination percentage of seeds. A significant interaction existed between temperature and stratification. A high hard seed percentage is a characteristic of seed lots of *S. alopecuroides*.

Results of this study showed that hardseededness reduced the germination of seeds. Stratification was not a good means of reducing hardseededness, whereas artificial softening, via scarification by sulfuric acid, was shown to be the quickest and most practical means of reducing hardseededness in seed lots. In general, stratification was unable to stimulate the germination of *S. alopecuroides* seeds.

Table 4. Cumulative germination percentage of dormant pagoda tree seeds as affected by temperature regimes and stratification treatment

Stratification (wk)	Temperature (°C)						
	10	15	20	25	30	35	Mean
0	0 Be	18 Ad[†]	28 Bc	49 Ab	64 Aa	64 Aa	37.2 B
2	0 Be	8 Bd	34 ABc	50 Aab	42 Cb	57 Ba	31.8 D
4	0 Bd	12 ABc	35 Ab	40 Bb	51 Ba	48 Ca	31 D
6	0 Be	14 Ad	36 Ac	42 Bc	51 Bb	64 Aa	34.5 BC
8	8 Af	16 Ae	40 Ad	50 Ac	60 Ab	68 Aa	40.3 A
Mean	1.6 f	13.6 e	34.6 d	38.5 c	53.6 b	60.2 a	

[†]**Means within each column followed by the same capital letters are not significantly different at the 5% level according to Duncan's multiple range test.**
Means within each row followed by the same small letters are not significantly different at the 5% level according to Duncan's multiple range test.

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