# A Method for inducing germination of Running Buffalo Clover Seeds

## *(Trifolium stoloniferum)*

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## INTRODUCTION

Running buffalo clover *(Trifolium stoloniferum)* is a state and federal endangered species. Little is known about this plant's biology or reproductive habits. It is believed that the primary mode of reproduction of running buffalo clover is vegetative but knowledge concerning reproduction of this plant by seed is limited. The U.S. Fish and Wildlife Service (1989) found that seed germination is between 90-100% following scarification but little or no germination occurs in the absence of scarification even after overwintering on moist cold soils.

The author conducted two germination experiments using *T.s.* seeds. In the first experiment, seventy *T.s.* seeds were scarified by gently scratching the seed coats with a kitchen knife. The seeds were then scattered over two flats of sterilized soil, placed in a greenhouse (70-90°C) and watered daily. The results are as follows. 16 days after the seeds were placed in the greenhouse one seed had germinated, 26 days after, two seeds had germinated, and 59 days after, three seeds had germinated. A germination rate of 4% has been achieved after two months. The experiment is still being conducted.

At the suggestion of Dr. Miller McDonald, Professor of Seed Biology at The Ohio State University I conducted another seed germination experiment using a chemical as the scarifying agent. The methods and results of this experiment are given in detail below.

## MATERIALS AND METHODS

Seeds for this experiment were collected from a natural *Trifolium stoloniferum* population in July 1994 and stored in a paper envelope wrapped in Saran wrap at approximately 40°F for one year. It is not known what affect this pretreatment has on the germination of *T.s.* seeds.

Fifty *T.s.* seeds were used in this experiment. Other materials needed include five small beakers, five petri dishes (size is not important), ten pieces of Watman's filter paper which are the same size as the petri dishes, a tea leaf strainer with a handle, one large 500 ml beaker, and approximately 40 ml of concentrated sulfuric acid.

Ten seeds were placed in each of five 200 ml beakers. The beakers were labeled 0, 5, 10, 20, and 40 minutes to correspond with the amount of time they should be immersed in concentrated sulfuric acid. The seeds in the beaker labeled "0" are the control. 10 ml of concentrated sulfuric acid were poured in each of the beakers except for the control. The control seeds were transfered to the tea leaf strainer and then flushed with 500 ml of tap water. It is important that the entire amount of water is poured over the seeds all at once. These seeds were then placed on a paper towel and allowed to dry. Following five minutes of immersion, the contents of the five minute beaker were transfered to the tea leaf strainer and then flushed with 500 ml of tap water. These seeds were also placed on a paper towel and allowed to dry. This procedure was repeated after 10, 20 and 40 minutes of immersion for each of the respective beakers. All seeds were allowed to dry for 28 hours.

Two pieces of Whatman's #1 9cm filter paper were placed in each of five 9cm petri dishes. 7 ml of distilled water were added to each petri dish. The petri dishes were then

labeled and the appropriate seeds were placed in each dish. Because legumes are not light sensitive with respect to germination, the petri dishes were not placed in a light controlled environment.

## RESULTS

The results demonstrated positive correlation between immersion time and germination rate. Table 1 summarizes *T.s.* germination up to eight days after the seeds were placed in the petri dishes.

Table 1. *Trifolium stoloniferum* seed germination up to eight days (192 hours) after they were placed in the petri dishes.

| | 43 hrs | 64 hrs | 136 hrs | 192 hrs |
|---|---|---|---|---|
| 0 min | 0 germinated | 0 germinated | 0 germinated | 0 germinated |
| 5 min | 1 germinated | 1 germinated | 2 germinated | 2 germinated |
| 10 min | 4 germinated | 4 germinated | 5 germinated | 5 germinated |
| 20 min | 8 germinated | 8 germinated | 8 germinated | 8 germinated |
| 40 min | 9 germinated | 9 germinated | 9 germinated | 9 germinated |

## DISCUSSION

After considering the results of this experiment and the unsuccessful attempts by others to achieve germination without scarification (USFWS 1989) or with little scarification, it is evident that *Trifolium stoloniferum* seeds require substantial scarification to obtain a relatively high germination rate. Scarification similar to that which was produced in the experiment described above could occur naturally while

passing through the digestive tracts of mammals. A recommendation for further research would be to conduct an experiment where a known number of *T.s.* seeds are fed to a cow or some other mammal and then to monitor these seeds for germination. It would be interesting to compare the germination rate of *T.s.* seeds which have been digested to the rates which were achieved by acid scarificaiton.

## LITERATURE CITED

U.S. Fish and Wildlife Service 1989. *Trifolium stoloniferum* Recovery Plan. U.S. Fish and Wildlife Service, Twin Cities, MN.