Evaluating Acid Scarification Effects on Dormant *Arctostaphylos nevadensis* Seeds

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Tetrazolium (TZ) staining was used to indicate embryo viability of dormant *Arctostaphylos nevadensis*seeds following acid scarification in concentrated sulfuric acid for 0 to 45 min. Embryo viability did not notably decrease even after 45 min in acid, although in a few seeds the endosperm tissues appeared slightly glassy and water-soaked around the micropylar opening where the acid had penetrated through the soft "micropyle plug". Other than penetrating the plug tissue, acid did not affect the seed coat even after 45 min. As in earlier trials, 25 min in acid was adequate time for the plug to be eroded, without any apparent damage to embryo or the endosperm. In most cases, viable embryos stained evenly in shades ranging from light pink to red, while most endosperm remained white or very light pink. Thus a glassy, water-soaked appearance of endosperm rather than TZ uptake may be the best indicator of over-treatment in acid. Seed coat thickness was not reliable as a guide in determining optimum acid scarification times in *A. nevadensis* seeds.

INTRODUCTION

Chemical scarification by soaking dormant seeds in concentrated sulfuric acid is recommended for many species of tree, shrub, and forb seed. Since the optimum treatment timing varies for different seed lots of the same species, the usual recommendation is to test a range of acid soaking times and then to observe the extent of thinning out or surface pitting of the testa by cutting and examining the seed coat. For some species, however, this may not provide an accurate assessment of the effects of scarification on inner seed tissues. For example, in seeds of *Arctostaphylos nevadensis*, the micropyle plug tissue is eroded well before the testa is affected (Schopmeyer, 1974). For this and many other species, additional and often lengthy stratification time is required to break endogenous dormancy. For A. *nevadensis*,even after scarification and lengthy stratification regimes, low germination rates (often 1% to 2%) are commonly reported (Carlson and Sharp, 1975). Schopmeyer (1974) reported varying success rates in other *Arctostaphylos* species by pretreating with acid for 3 to 15 h, followed by stratification to achieve up to 50% germination in*A. uva-ursi*. Others working with*A. nevadensis*,however, have found that acid soaking for 2 to 4 h degraded the seeds to mush, while shorter soaking times did not appreciably thin the seed coat or enhance germination (Colleen Archibald and Frank Callahan, personal communication).

Unfortunately, initial and post-treatment viabilities in some of the earlier studies were not reported. Knowing the viability and thus the maximum potential germination of a seed lot would be useful in evaluating the relative success of different treatments. Also, little is known about seed longevity for some wild shrub and forb

seeds either in controlled storage or natural habitat.

Fortunately, several methods of detecting viability in dormant seeds are available and in use in the seed-testing industry. Briefly, the basic methods include tetrazolium (TZ) staining to indicate enzyme activity and thus viability in hydrated seed tissues, root growth testing using a hydrogen peroxide presoak to initiate growth, incubation of excised embryos, and X-ray testing. Bonner et al. (1994) provide a brief outline of the methods, advantages and limitations of each type of test. X-ray analysis requires expensive equipment and training, but each of the other methods are readily adapted to small-scale, bench-top operations. Seed analysts familiar with the tests can often provide guidance in choosing and adapting the procedure best suited to a particular seed lot. Standardized procedures for some species are outlined in the International Seed Testing Association (ISTA) Rules Annexes (1985). Procedures for other species are continually added in the journals of professional seed analysts and testing associations. Vivrette (1995) recently described a technique of presoaking seeds in gibberellic acid (GA_3) to promote staining of tissues in deep dormancy that might respond slowly or not at all to TZ alone.

PROCEDURE

A study planned by the Plant Materials Center in 1993 and conducted at the Oregon State University Seed Lab determined that a 25-min soak in concentrated H_2SO_4 effectively eroded the micropyle plug without apparent damage to the embryonic tissues within. Tetrazolium viability testing of the seed lot before acid treatment showed 60% viability. However, viability tests were not repeated after acid treatments. We felt it would be useful to repeat the acid treatments, testing the seed after as well as prior to acid scarification.

In 1995, acid scarification and stratification procedures were repeated on *A. nevadensis* seed harvested in 1991 and 1993 from 1980-m elevation at Crater Lake National Park in Oregon. Air-dried seeds had been stored in the dark in cool, ambient air at 5 to 15C. Acid scarification was performed according to the methods outlined by King (1990). Acid treatment times ranged from 10 to 45 min. A 1% TZ solution was prepared according to the method in the ISTA (International Rules for Seed Testing Annexes, 1985). Seeds were bisected longitudinally to expose the embryonic axis and soaked in TZ stain for 24 h. Samples (n=25) of treated and stained seeds were examined by stereomicroscope to observe the extent of acid penetration and any effects on the viability of internal tissues. Stained seeds were stored in 85% lactic acid to preserve them for observation and photography. We also started several stratification regimes, varying the duration of both warm and cold periods. These seeds are due for germination checks in late fall of 1995.

OBSERVATIONS

Initial viability as measured by TZ staining for 24 h was estimated at 60% and 50% for the 1991 and 1993 lots, respectively. This estimate did not take into account seed that may have been in a state of deep dormancy because we did not pretreat seed in GA_3; in the case of coalesced units consisting of more than one nutlet, only one embryo was treated and observed. For purposes of this trial, seeds were counted as "normal" if the entire embryo was evenly stained from light pink to red. In most seeds, the endosperm did not stain. No instance of stained endosperm but non-staining embryos was encountered. Several embryos were so lightly stained they

were difficult to classify. In retrospect, the use of GA_3 might have enhanced the clarity of the results.

The most notable result was that even after 45 min in acid, nearly twice as long as the previously determined optimum time, little damage was observed to the endosperm and embryo of most treated seeds. Injury seen in a few of these seeds appeared as glassy or watery endosperm tissue at the micropylar end. Of these seeds, only three contained incompletely stained embryos and one of these abnormal embryos lacked staining at the cotyledon end rather than the radicle. While the small sample size of these preliminary tests and the presence of some lightly stained tissues precludes the use of statistical analysis, information from this trial indicates a window of time for effectively treating the seed without destroying internal seed tissues.

A question arises whether acid scarification is, in fact, advisable for this species. It is not known how long seed viability persists once the protective plug is eroded. The seed will have to fend off pathogenic organisms during the period of incubation to overcome endodormancy. To answer this question, TZ testing of non-germinating seeds is planned following stratification and greenhouse seedling emergence trials.

ACKNOWLEDGEMENTS

The author wishes to extend special thanks to Jack Peters of the Oregon State University Seed testing Laboratory for advice and encouragement on this project. Any errors are solely the responsibility of the author.

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