Effect of fungicide on Wyoming big sagebrush seed germination

Robert D Cox, Lance H Kosberg, Nancy L Shaw, and Stuart P Hardegree

ABSTRACT

Germination tests of Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young [Asteraceae]) seeds often exhibit fungal contamination, but the use of fungicides should be avoided because fungicides may artificially inhibit germination. We tested the effect of seed-applied fungicides on germination of Wyoming big sagebrush at 2 different water potentials (–0.033 and –0.7 MPa) and found that treating test seedlots with fungicide reduced the germination percentage by up to half in some treatments. This effect was greatest at the lower water potential. We found that the fungicides were successful at delaying infection of the seeds or test media with fungi, but that the costs of reduced germination related to fungicide application make this practice undesirable. Because Wyoming big sagebrush is becoming a common species for revegetation and restoration activities, germination tests of this species are needed to increase understanding of proper seed storage and seeding methods. We recommend that those conducting germination trials with Wyoming big sagebrush either test untreated seed and accept some level of fungal contamination, or explore other methods of seedcoat sterilization that may have less impact on total germination. Any treatment for reducing fungal infection, however, should first be evaluated for potential effects on germination percentage.

Cox RD, Kosberg LH, Shaw NL, Hardegree SP. 2011. Effect of fungicide on Wyoming big sagebrush seed germination. Native Plants Journal 12(3):263–267.

KEY WORDS

Artemisia tridentata, Asteraceae, Captan, fungus, germination, Thiram

NOMENCLATURE

USDA NRCS (2011)

Figure 1. Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young [Asteraceae]) seeds, magnified approximately 20X. Photo by Robert D Cox

In germination experiments, fungal contamination of seeds or germination media can hinder observation of radicle emergence and growth, and may also destroy otherwise viable seeds, thus lowering the observed germination percentage (Chambers and MacMahon 1994). Such fungal contamination may be carried by the seeds themselves (Crist and Friese 1993), making it difficult to eliminate without surface sterilization or use of fungicidal chemicals. Therefore, surface sterilization and chemical control are often cited as pre-germination seed treatments, and the use of fungicides is generally allowable under official seed testing rules (Williams and others 2003; AOSA 2009; Morris and Schupp 2009).

Using fungicides to prevent fungal growth, however, has altered the outcome of some germination studies (Burgess and others 2005; Mitschunas and others 2009; Derbel and others 2010), which could bias the results of tests that seek to understand germination response to other factors. Because the effects of fungicide treatment on germination depend on both the plant species and the type of fungicide applied (Mitschunas and others 2009), it is risky, at best, to draw conclusions from germination experiments that include fungicidal treatments. Because government and private groups have increasing interest in revegetation and restoration using native species, germination studies of native species are likely to become more common.

For example, Wyoming big sagebrush (*Artemisia tridentata* Nutt. spp. *wyomingensis* Beetle & Young [Asteraceae]) is a woody shrub native to the Intermountain West that is a landscape dominant in the Great Basin and Columbia Plateau (Davies and others 2009) and that has been heavily impacted by invasive species and increasing wildfires across much of its range (Suring and others 2005). For this reason, it has recently become a species of interest for revegetation efforts (Shaw and others 2005). Such efforts, however, have had limited success due to a lack of knowledge about how Wyoming big sagebrush seeds are best stored (Lambert 2005), properly planted (Lysne and Pellant 2004), and optimally germinated (Meyer and others 1990; Meyer and Monsen 1992).

Incidental to another study investigating the germination requirements of Wyoming big sagebrush, we observed significant fungal contamination on and around germinating sagebrush seeds (Figure 2). No protocols are available for the use of fungicides during Wyoming big sagebrush germination experiments, although fungal infection has been noted in other studies (McDonough and Harniss 1974; Crist and Friese 1993). Our study objective was to determine whether fungicidal treatments would prevent fungal contamination and otherwise influence total germination response of Wyoming big sagebrush seeds.

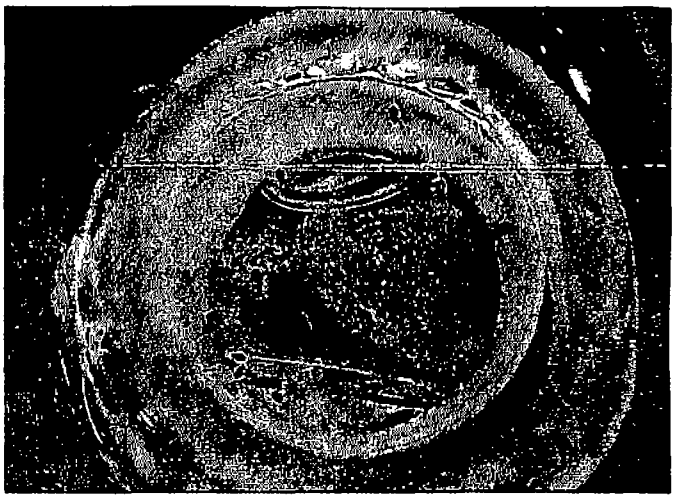

Figure 2. Fungal infection of Wyoming big sagebrush seeds and growth media. Photo by Lance Kosberg

MATERIALS AND METHODS

We utilized a completely randomized design, with seeds germinated at 2 water potentials and treated with one of 4 fungicidal treatments, or not treated, as a control. Seeds were germinated using the water potential control system described by Hardegree and Emmerich (1992). Water potential control was obtained with solutions of polyethylene glycol 8000 (PEG) mixed according to the calibration equations suggested by Hardegree and Emmerich (1990). Seeds were separated from the solution by a cellulose membrane that allowed the passage of water but prevented migration of PEG to the germinating seeds. We prepared 2 PEG/deionized water (DI) solutions for use in these germination vials: -0.033 and -0.7 MPa.

Fungicidal treatments were 1) Captan 50% (n-trichloromethylthio-4-cyclohexene-1,2-dicarboximide formulated at 48% active ingredient [a.i.]); 2) Thiram 50% (tetramethylthiuram disulfide formulated at 50% a.i.); 3) Thiram 97% (tetramethylthiuram disulfide formulated at 97% a.i.); 4) sulfur 90%; and 5) a control of no fungicidal treatment. All fungicides were in the form of wettable powders and were applied to the seeds by tapping a fine dusting of the fungicide from a small paintbrush that had been dipped in the powdered fungicide.

Seeds from 5 different seedlots of Wyoming big sagebrush were tested. Seedlots, origins, and vendors are listed in Table 1. Seeds were randomly selected and units of 30 seeds each were randomly assigned to each combination of experimental treatment. Each experimental unit—seedlot (5 levels), water potential (2 levels), and fungicide (6 levels because the control of no fungicide was performed twice)—was replicated 6 times for a total of 360 germination vials. Treatments were assigned randomly to each vial. All tests were conducted at room temperature (22 °C [72 °F]) on the laboratory bench.

TABLE 1

Origin and Utah vendors for Wyoming big sagebrush seeds used in this germination trial.

Origin	Vendor
Elko and Humoldt counties, Nevada	Maple Leaf Seed, Ephraim
Lassen County, California	Native Seed, Park City
Salt Lake County, Utah	Native Seed, Park City
USDI Bureau of Land Management, Idaho Falls District, Idaho	Native Seed, Park City
USDI Bureau of Land Management, Shoshone District, Idaho	Native Seed, Park City

Each vial was loaded with 30 seeds from one of the 5 seedlots. Seeds were placed on the membrane surface of the germination cup (inside the vials) and dusted with the fungicide that was randomly selected for that vial. No additional fungicide was applied during the course of the experiment. Vials were opened and inspected for fungal appearance on Monday, Wednesday, and Friday of each week for a total of 24 d. Germination was considered to have occurred when the radicle was seen to extend ≥ 2 mm (0.08 in) from the seedcoat. Germinants were removed and counted, and the appearance of fungal mycelia within the vial noted.

Data consisted of the number of days that each treatment combination remained free of fungal infection as well as total germination percentage for each treatment combination. Data were analyzed using Proc GLM in SAS (SAS Institute 2009) to test the effect of water potential and fungicide on total seed germination and on the number of days that germination vials remained free of fungi. Post hoc mean separations were performed using the Tukey's test (SAS Institute 2009) at the 0.05 level.

RESULTS

Untreated seedlots displayed similar germination of about 35%, with the exception of the seedlot originating from Humboldt and Elko counties, Nevada (HE), which germinated at about 29%. All chemical fungicide treatments delayed and reduced fungal infection of the germinating seeds. For example, Captan 50% and both Thiram treatments delayed fungal infection by approximately 6 d for most seed treatments (Figure 3). Because seeds were allowed to germinate for a total of only 24 d, many treated vials never developed fungal infection. In contrast, the sulfur treatment did not significantly delay the appearance of fungal mycelia when compared with the controls (Figure 3).

The interaction of water potential with fungicide type significantly influenced germination percentage. This interaction was especially striking at –0.7 MPa, where all chemical fungicides reduced total germination of the seeds tested when compared with either the untreated control or the sulfur application. At this water potential, control and sulfur-treated seeds displayed up to 34% germination, compared to less than 22% for seeds treated with Captan or Thiram, regardless of the concentration of fungicide (Figure 4). Seeds tested at –0.033 MPa, however, displayed greater total germination and smaller overall

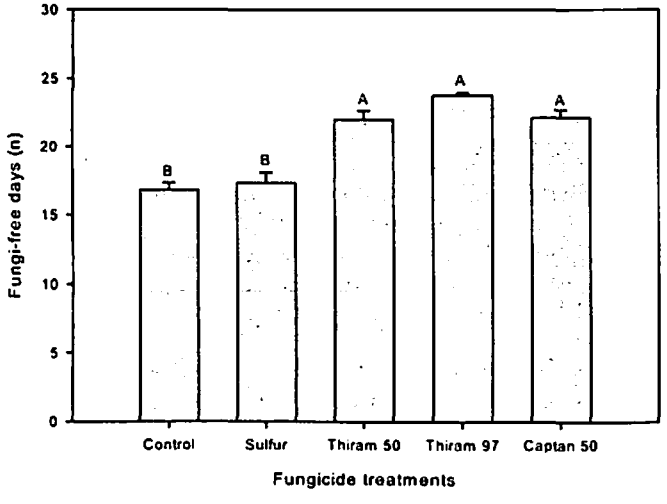

Figure 3. Fungi-free days for Wyoming big sagebrush seeds during germination trials. Fungicide treatments were Control (no treatment), sulfur (seeds dusted with pure sulfur fungicide), Captan 50 (50% formulation of Captan fungicide), Thiram 50 (50% formulation of Thiram fungicide), and Thiram 97 (97% formulation of Thiram fungicide). Bars represent standard error. Upper case letters represent significant differences at the $P = 0.05$ level.

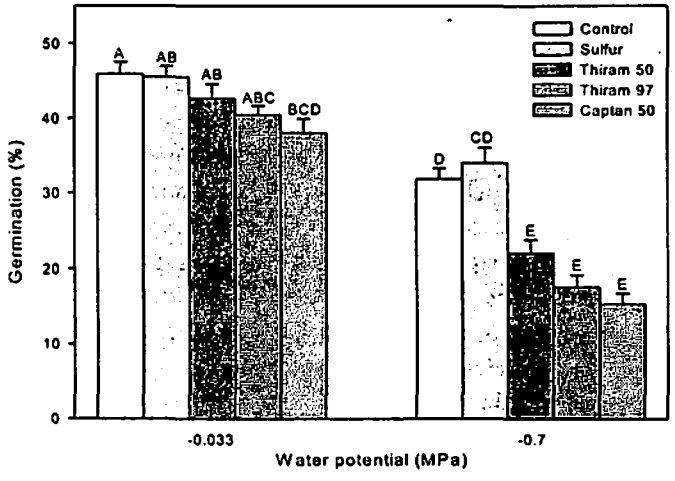

Figure 4. Germination percentage of Wyoming big sagebrush by water potential and fungicide application. Fungicide treatments were Control (no treatment), sulfur (seeds dusted with pure sulfur fungicide), Captan 50 (50% formulation of Captan fungicide), Thiram 50 (50% formulation of Thiram fungicide), and Thiram 97 (97% formulation of Thiram fungicide). Bars represent standard error. Upper case letters represent significant differences at the $P = 0.05$ level.

differences among the fungicidal treatments. At this higher water potential, only the Captan treatment (38% germination) was significantly lower than the control (45% germination) (Figure 4).

DISCUSSION

Fungal infection is common in seed germination studies and is often treated with chemical fungicides. We observed, however, that such treatments actually reduced overall germination of Wyoming big sagebrush seeds, potentially biasing results. This effect was especially pronounced at –0.7 MPa. Although the effect of Thiram at a water potential of –0.033 MPa was not significant, it does appear strong enough to indicate an unacceptable level of uncertainty; we recommend fungicides not be applied when Wyoming big sagebrush is tested for germination. Such fungicides may be successful, as in this experiment, at delaying or even preventing infection of the seeds or test media with fungi; nonetheless, the costs of reduced germination related to fungicide application make this practice undesirable.

Other species may also exhibit reduced germination when exposed to fungicides. For example, Mitschunas and others (2009) found that in a seed burial study, the fungicide Captan inhibited germination of lesser knapweed (*Centaurea nigra* L. [Asteraceae]) seeds. Our study also implicates Captan as being significantly detrimental to germination of sagebrush seeds, and we suggest that it should not be used when studying germination responses of this species. The other chemical fungicide used in this study, Thiram, also reduced germination and should be avoided, especially when seeds are germinated at low water potentials.

Wyoming big sagebrush is increasingly a species of interest for revegetation efforts in the Great Basin (Shaw and others 2005), which means germination experiments with this species are likely to continue and even increase. Fungal contamination may not always have detrimental effects on germination and seedling establishment, but it can be a problem with sagebrush seeds (McDonough and Harniss 1974; Crist and Friese 1993): be careful when contemplating the use of fungicides during germination experiments and tests. At least 2 chemical herbicides, Captan and Thiram, have the ability to depress germination in Wyoming big sagebrush. Other fungicides may have similar or different effects, so if used, test to determine if results are consistent with controls. Such fungicides could further alter natural germination responses in Wyoming big sagebrush or other native species and create interactions of factors being tested that are different from what might occur in nature. We recommend methods of fungi control other than fungicides, such as seedcoat sterilization, bleach, or running water (Dumroese and others 1988); also ensure proper germination conditions. In this regard, the differences in germination at different water potentials may be important, and researchers or practitioners conducting germination tests should consider optimum water potentials for germination as well as how these may interact if any fungicides are used.

REFERENCES

[AOSA] Association of Official Seed Analysts. 2009. AOSA rules for testing seeds. P. 6-3. Ithaca (NY): Association of Official Seed Analysts.

Burgess TL, Blazich FA, Nash DL, Randall-Schadel B. 2005. Influence of selected surface disinfectants, fungicides, and temperature on seed germination and initial growth of southern seaoats (*Uniola paniculata*). Journal of Environmental Horticulture 23:4–8.

Chambers JC, MacMahon JA. 1994. A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annual Review of Ecology and Systematics 25:263–292.

Crist TO, Friese CF. 1993. The impact of fungi on soil seeds: implications for plants and granivores in a semiarid shrub-steppe. Ecology 74:2231–2239.

Davies KW, Bates JD, Johnson DD, Nafus AM. 2009. Influence of mowing *Artemisia tridentata* ssp. *wyomingensis* on winter habitat for wildlife. Environmental Management 44:84–92.

Derbel S, Touzard B, Triki MA, Chaieb M. 2010. Seed germination responses of the Saharan plant species *Ephedra alata* ssp. *alenda* to fungicide seed treatments in the laboratory and the field. Flora 205:471–474.

Dumroese RK, James RL, Wenny DL, Gilligan CJ. 1988. Douglas-fir seed treatments: effects on seed germination and seedborne organisms. In: Landis TD, technical coordinator. Proceedings: combined meeting of the western forest nursery associations. Fort Collins (CO): USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-167. p 155–160.

Hardegree SP, Emmerich WE. 1990. Effect of polyethylene glycol exclusion on the water potential of solution-saturated filter paper. Plant Physiology 92:462–466.

Hardegree SP, Emmerich WE. 1992. Effect of matric-priming duration and priming water potential on germination of four grasses. Journal of Experimental Botany 43:233–238.

Lambert SM. 2005. Seeding considerations in restoring big sagebrush habitat. In: Shaw NL, Pellant M, Monsen SB, compilers. Sage-grouse habitat restoration symposium proceedings. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38. p 75–80.

Lysne CR, Pellant M. 2004. Establishment of aerially seeded big sagebrush following southern Idaho wildfires. Boise (ID): USDI Bureau of Land Management, Idaho State Office. Technical Bulletin 2004-01. 14 p.

McDonough WT, Harniss RO. 1974. Effects of temperature on germination in three subspecies of big sagebrush. Journal of Range Management 27:204–205.

Meyer SE, Monsen SB. 1992. Big sagebrush germination patterns: subspecies and population differences. Journal of Range Management 45:87–93.

Meyer SE, Monsen SB, McArthur ED. 1990. Germination response of *Artemisia tridentata* (Asteraceae) to light and chill: patterns of between-population variation. Botanical Gazette 15(1):176–183.

Mitschunas N, Filser J, Wagner M. 2009. On the use of fungicides in ecological seed burial studies. Seed Science Research 19:51–60.

Morris C, Schupp EW. 2009. Comparison of emergence speed and sterility in two sterile annual hybrid cereal grasses developed for use in restoration. Restoration Ecology 17:678–685.

SAS Institute. 2009. SAS/STAT 9.2 users guide, 2nd ed. Cary (NC): Statistical Analysis Software. 7869 p.

Shaw NL, DeBolt AM, Rosentreter R. 2005. Reseeding big sagebrush: techniques and issues. In: Shaw NL, Pellant M, Monsen SB, compilers. Sage-grouse habitat restoration symposium proceedings. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-38. p 99–108.

Suring LH, Rowland MM, Wisdom MJ, Schueck L, Meinke CW. 2005. Vegetation communities. In: Wisdom MJ, Rowland MM, Suring LH, editors. Habitat threats in the sagebrush ecosystem. Lawrence (KS): Alliance Communications Group. p 94–113.

[USDA NRCS] USDA Natural Resources Conservation Service. 2011. The PLANTS Database. URL: http://plants.usda.gov (accessed 10 Mar 2011). Greensboro (NC): National Plant Data Team.

Williams PR, Congdon RA, Grice AC, Clarke PJ. 2003. Fire-related cues break seed dormancy of legumes in tropical eucalypt savannas of north-eastern Australia. Austral Ecology 28:507–514.

AUTHOR INFORMATION

Robert D Cox
Assistant Professor
Department of Natural Resources Management
Texas Tech University
Lubbock, TX 79409
robert.cox@ttu.edu

Lance H Kosberg
Research Technician
lancekosberg@gmail.com

Nancy L Shaw
Research Botanist
nshaw@fs.fed.us

USDA Forest Service
Rocky Mountain Research Station
322 East Front Street, Suite 401
Boise, ID 83702

Stuart P Hardegree
Research Plant Physiologist
USDA ARS Northwest Watershed Research Center
800 Park Boulevard, Suite 105
Boise, ID 83712
stuart.hardegree@ars.usda.gov