From Forest Nursery Notes, Summer 2011

16. Germination and viability of wild sunflower species achenes stored at room temperature for 20 years. Seiler, G. J. Seed Science and Technology 38:786-791. 2010.

Seiler, G.J. (2010). *Seed Sci. & Technol.*, **38**, 786-791

Research Note

Germination and viability of wild sunflower species achenes stored at room temperature for 20 years

G.J. SEILER

USDA-Agricultural Research Service[1], Northern Crop Science Laboratory, 1605 Albrecht Blvd N., Fargo, ND 58102, USA (E-mail: gerald.seiler@ars.usda.gov)

(Accepted April 2010)

Summary

Storage times and conditions affect the longevity of stored seeds. Achenes of two wild annual sunflower species, *Helianthus annuus* (common wild sunflower) and *H. petiolaris* (prairie sunflower), stored at room temperature (20 to 22°C) at a relative humidity of approximately 22% in a bell jar in the laboratory for 20 years were evaluated for germination and viability. The efficacy of using gibberellic acid (GA_3) as a germination medium to overcome dormancy was also tested. Germination of wild *H. annuus* achenes stored for 20 years was 13%, while *H. petiolaris* had only 1.5% germination. Germination of fresh achenes at harvest was 34.7% for *H. annuus*, and 18.5% for *H. petiolaris*. Stored wild *H. annuus* achenes had 90% positive staining using the tetrazolium viability test, while *H. petiolaris* had 80.2% indicating that the achenes were alive, but dormant. Treating scarified wild achenes with 1 mM GA_3 for one hour increased germination of the stored wild *H. annuus* achenes from 13% to 88%, and *H. petiolaris* from 1.5% to 85%. This indicates that the wild sunflower achenes stored at less than optimal conditions for long periods are dormant and should not be discarded as dead. This information will be useful for curation of wild sunflower germplasm.

Experimental and discussion

Genebanks have maintained genetic resources as seeds for over decades or centuries. Storage of seeds in genebanks has been the most common technique for *ex situ* conservation of plant genetic resources since the 1920s (Pita *et al.*, 2005; Pérez-García *et al.*, 2009). In most base genebank collections, 3 to 7% seed moisture and -18°C or lower storage temperature theoretically assure seed viability, vigour, and genetic integrity for thousands of years (Ellis and Roberts, 1980).

Hypotheses relating to chemical constituents of seed have been put forward to explain the variation of longevity among species. Walters *et al.* (2005) concluded that there is no apparent trend between seed storage reserves and seed longevity. Cultivated sunflower

[1]Mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

(*Helianthus annuus* L.) has a high oil content in achenes of 440 to 480 g/kg (Robertson *et al.*, 1979). Wild *Helianthus* populations generally have oil concentrations of 250 to 350 g/kg (Seiler, 1985). Storage of cultivated sunflower achenes at 5°C and 3.7% moisture for 27 years only decreased the germination from 92 to 82%, while storage after 49.9 years decreased germination from 92% to 42% (Walters *et al.*, 2005). Nagel and Börner (2010) reported that for cultivated sunflower seed stored at 20°C and 50% humidity, the half-viability period (P50) was only 4.3 years, decreasing to near zero by 10 years. This led them to conclude that seeds in which oil was the major storage component were more short lived at less than optimal storage conditions, while storage carbohydrates or proteins did not show an effect on seed longevity.

Seed that fails to germinate maybe dormant and dormancy can be a major problem in genebanks (IBPGR, 1982; Ellis *et al.*, 1985). Seed dormancy is mediated at least in part, by the plant hormones abscisic acid and gibberellins (Finch-Savage and Leubner-Metzger, 2006). The low germination of stored wild sunflower achenes may be the result of dormancy, and not due to seed degradation. Testing of the stored achenes using a viability test would indicate whether the achenes are alive or not.

Wild sunflower species generally have very low germination rates and high dormancy, even for freshly harvested achenes (Chandler and Jan, 1985; Seiler, 1993, 1996, 1998). Methods have been developed to overcome dormancy in wild sunflower species (Chandler and Jan, 1985; Seiler, 1993, 1996, 1998). These methods have been used on freshly harvested achenes, and achenes stored for less than five years, but have not been tested on achenes stored for longer periods and under less than optimal storage conditions.

While optimal storage conditions have predictable results, the effects of prolonged storage on germination and viability of achenes of wild *Helianthus* species stored under less than optimal conditions is not known. Knowledge of this would facilitate the long-term storage of wild sunflower achenes in genebanks and under less than ideal storage conditions. The objectives of this study were to: 1) test the germination of two wild annual sunflower species, *Helianthus annuus* L., and *H. petiolaris* Nutt. stored at room temperature for 20 years, 2) determine the viability of the achenes, and 3) determine if the germination of the achenes could be enhanced by using gibberellic acid (GA_3).

Two annual species were used in the study, wild *H. annuus* (common wild sunflower) and annual *H. petiolaris* (prairie sunflower). Wild *H. annuus* was collected near Vega (Oldham County), Texas, in a roadside ditch near railroad tracks in August, 1983. *Helianthus petiolaris* was collected near Clarendon (Donley County), Texas, in a sandy roadside ditch in August, 1983. The sites are approximately 60 km apart at an elevation of 1130 m in the panhandle of Texas. Multiple mature heads were collected from over 100 plants and bulked to form one population sample for each species. The heads were dried at 30°C for two weeks, then threshed, cleaned, and stored. Achenes contained 5 to 7% moisture when initially stored. Achenes were stored in a glass bell jar with approximately 22% relative humidity, with a surrounding air temperature of 20 to 22°C for 20 years.

Achenes were tested for germination by placing 50 achenes in a 9-cm diameter Petri dish between two pieces of filter paper saturated with 15 ml of distilled water. The Petri dishes were sealed with tape to prevent excessive water loss. Petri plates were placed in an environmentally controlled germinator (Seedburo® Equipment, Co., Chicago, IL) at

20°C with no light. Germination counts were taken at 21 days. An achene was considered germinated when the radicles reached a length of 5 mm.

In order to test the influence of a chemical treatment on germination, the same germination procedure was used as above, but with modifications. The achenes were scarified by cutting off the tip of the achene distal to the cotyledon end and pre-treated with a 1 mM gibberellic acid (GA_3) solution for one hour. Achenes were then placed in Petri plates with distilled water and the achene coat removed 24 hours later. Germination counts were taken at 21 days.

A standardized tetrazolium (TZ) test was used to assess the viability of the wild sunflower achenes (AOSA, 2002). Fifty dehulled and cut achenes were placed in a beaker with a 1% solution of 2, 3, 5-triphenyl tetrazolium chloride in reverse osmosis water and incubated at 35°C for 24 hr. Achenes that stained uniformly red were considered viable.

The study was designed as a randomized complete block (RCBD) with four replications. The experiment was repeated twice. Germination and TZ values were arcsine-transformed before statistical analyses to make percentage data closer to a normal distribution (Sokal and Rohlf, 1995). Data were analyzed using analysis of variance –ANOVA (SAS, 2009) with means separated using the least significant difference (LSD).

An analysis of variance indicated no significance difference due to replications or between the two times the experiment was conducted. Therefore, the data were combined, and means, standard errors, and LSD ($P \leq 0.05$) reflect the average of all observations. The ANOVA indicated that species and storage time were significant sources of variation.

Germination of achenes of the common wild and prairie sunflowers was low at the initial harvest in 1983, with 34.7 and 18.5%, respectively (table 1). Germination for both species decreased significantly after 20 years of storage with common sunflower decreasing by 21.7% to 13%, and prairie sunflower decreasing by 17% to only 1.5%. Testing of the viability of the species' achenes showed interesting results. Common and prairie sunflower had a TZ viability staining of 93.2% and 85.3%, respectively at harvest, and 90 and 80.2%, respectively after 20 years of storage (table 1). This would suggest that the achenes were not dead, but only dormant.

Table 1. Germination, viability, and gibberellic acid treatment of two wild sunflower (*Helianthus*) species achenes at harvest and after 20 years of storage at room temperature and moderate humidity.

Species	Harvest[a]			Storage 20 years		
	Germination	Viability (TZ)[b]	Gibberellic acid (GA_3)[c]	Germination	Viability (TZ)	Gibberellic acid (GA_3)
	%					
H. annuus (wild)	34.7±2.5[d]	93.2±1.8	77.6±2.7	13.0±2.1	90.0±4.2	88.6±1.0
H. petiolaris	18.5±1.1	85.3±1.8	62.9±2.1	1.5±0.7	80.2±1.2	85.3±1.9
LSD (0.05)	*5.9*	*5.4*	*6.6*	*4.9*	*9.4*	*NS*

[a]For comparing storage time within species, LSD ($P \leq 0.05$) = 4.3.
[b]For comparing viability within species, LSD ($P \leq 0.05$) = NS.
[c]For comparing gibberellic acid treatment within species, LSD ($P \leq 0.05$) = 5.6.
[d]Mean and standard error.

Treatment of achenes of both species at harvest with scarification and GA_3 dramatically increased germination from 34.7 to 77.6% for common sunflower, and from 18.5% to 62.9% in prairie sunflower (table 1). The scarification and gibberellic acid treatment was more effective in increasing the percentage germination of achenes stored for 20 years than for freshly harvested achenes. Germination of stored achenes of common sunflower increased from 13.0 to 88.6% with treatment, while prairie sunflower increased significantly from 1.5 to 85.3%.

Storage of wild sunflower achenes at less than ideal storage conditions did significantly reduce the percentage germination in both species, but the decrease was due to an increase in dormancy rather than a reduction of viability. Dormancy can be overcome by scarification and the exogenous application of gibberellic acid. Germination of achenes of the common wild and prairie sunflower at harvest was low, but within the range expected for these wild species (Seiler 1993, 1996, 1998). The lower germination after 20 years of storage might have been expected in wild sunflower due to their strongly imposed dormancy.

The ability of exogenously applied chemicals to overcome achene dormancy and increase germination in wild sunflower species has been previously reported (Chandler and Jan, 1985; Heiser *et al.*, 1969; Seiler, 1993, 1996, 1998). The most effective treatments are those using GA_3, which has been shown to be the primary germination-promoting hormone (Karssen *et al.*, 1989; Ellis *et al.*, 1985). Scarification and treatment of achenes of *H. annuus* and *H. petiolaris* with a 1 mM GA_3 solution significantly increased germination of the dormant achenes.

Storage of achenes at less than optimal storage conditions appeared to have some effect on the germination of wild *H. annuus* and *H. petiolaris* achenes. While the germination percentage appeared to decrease with storage time, the achenes were still viable as indicated by the TZ test and could be induced to germinate using GA_3. The low germination test results were not indicative of the actual physiological state of the achenes. Loss in dormancy during post-harvest storage and after-ripening occurs progressively more slowly the cooler the temperature (Roberts, 1973). The high dormancy in the achenes of wild sunflower was not overcome by the long storage period.

Seed moisture content and storage temperature are the major environmental factors affecting the preservation of stored seeds, with moisture content usually more critical than temperature (Bass, 1980). While the storage conditions of the current study were not ideal, the low humidity (22%) and lack of extremely high temperatures likely improved survivability. High humidity has been shown to be very detrimental for storage (Bass, 1980). In the present study, the achenes were stored at seed moisture between 5 to 7% that remained relatively constant at 6% after 20 years. According to Harrington (1970), seed moisture of 4 to 6% and storage humidity near 15% is ideal for maximum seed life. Humidity remained relatively stable in the current study because the achenes were stored in a sealed bell jar.

Temperature is equally important in influencing seed viability. The lower the storage temperature, the longer the life of the seed. According to Harrington (1970), for storage temperatures between 50°C and 5°C, for every 5°C drop in storage temperature, it doubles the life of the seed. While the storage temperature in the present study was higher

than ideal, the low seed moisture and moderate storage humidity reduced the potential detrimental effects of the high temperature.

Achenes of wild *H. annuus* and *H. petiolaris* stored for 20 years at room temperature and low humidity lost on average 21.7% and 17% germination, respectively. The tetrazolium viability test indicated that the achenes were not dead, but dormant, a trait common among the wild sunflower species. Scarification and gibberellic acid overcame dormancy in these achenes and significantly increased germination. The results of this study will be useful for genebank curation and others where wild sunflower achenes may have been stored for long periods, sometimes under less than ideal storage conditions. The achenes are dormant and should not be discarded due to their low germination. Treatment of the low-germinating dormant achenes with GA_3 should break the dormancy, allowing for the regeneration of the population. The results of the present study indicate that the problem genebanks storing achenes of wild sunflower species will have is more likely to be confounded by dormancy than by the loss in germination.

References

AOSA (Association of Official Seed Analysts). (2002). Tetrazolium testing handbook. In *Contribution No. 29*, (ed. J. Peters), Association of Official Seed Analysts Lincoln, NE, USA.

Bass, L.N. (1980). Seed viability during long-term storage. In *Horticultural Review*, (ed. J. Janick), Vol. 2, pp. 117-141, Avi Publishing Company, Westport, CN, USA.

Chandler, J.M. and Jan, C.C. (1985). Comparison of germination techniques for wild *Helianthus* seeds. *Crop Science*, **25**, 356-358.

Ellis, R.H. and Roberts, E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany*, **45**, 13-30.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1985). *Handbook of seed technology for genebanks*. 667 pp. International Board for Plant Genetic Resources, Rome.

Finch-Savage, W.F. and Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, **171**, 501-523.

Harrington, J. F. (1970). Seed and pollen storage for conservation of plant gene resources. In *Genetic resources in plants: Their exploration and conservation*, (eds. O.H. Frankel, and E. Bennet), pp. 501–521, Blackwell, Oxford.

Heiser, C.B., Smith, D.M., Clevenger, S.B. and Martin, W.C. (1969). The North American sunflower (*Helianthus*). *Memoirs of the Torrey Botanical Club*, **22**, 1-218.

IBPGR (1982). *Report of the first meeting: IBPGR ad hoc advisory group on seed storage*. International Board for Plant Genetic Resources, Rome.

Karssen, C.M., Zagorski, S., Kepcyzynski, S. and Groot, S.P.C. (1989). Key role for endogenous gibberellins in the control of seed germination. *Annals of Botany*, **63**, 71-80.

Nagel, M., and Börner, A. (2010). The longevity of crop seeds stored under ambient conditions. *Seed Science Research*, **20**, 1-12.

Pérez-García. F., Gómez-Campo, C. and Ellis, R.H. (2009). Successful long-term ultra dry storage of seed of 15 species of Brassicaceae in a genebank: Variation in ability to germinate over 40 years and dormancy. *Seed Science and Technology*, **37**, 640-649.

Pita, J.M., Martinez-Laborde, J.B., Zamnrana, E. and de la Cuadra, C. (2005). Germinability of *Vicia sativa* L. seeds after 10 years of storage in a base collection. *Genetic Resources and Crop Evolution*, **52**, 513-517.

Roberts, R.H. (1973). Predicting the storage life of seeds. *Seed Science and Technology*, **1**, 499-514.

Robertson, J.A., Morrison, W.H. and Wilson, R.L. (1979). Effects of planting location and temperature on oil content and fatty acid composition of sunflower seeds. USDA, Agricultural Research Services Results, Southern Series No.3, Washington, D.C., USA.

SAS Institute. (2009). SAS® System for Windows, Release 9.01, SAS Institute, Cary, North Carolina, USA.

Seiler, G.J. (1985). Evaluation of seeds of sunflower species for several chemical and morphological characteristics. *Crop Science*, **25**, 183-187.

Seiler, G.J. (1993). Wild sunflower species germination. *Helia*, **16**, 15-20.

Seiler, G.J. (1996). Dormancy and germination of wild *Helianthus* species. In *Compositae: Biology and Utilization*, (ed. D.J.N Hind), pp. 213-222, Kew Royal Botanic Gardens, London.

Seiler, G.J. (1998). Seed maturity, storage time, and temperature, and media treatment effects on germination of two wild sunflowers. *Agronomy Journal*, **90**, 221-226.

Sokal, R.R. and Rohlf, F.J. (1995). *Biometry: The Principles and Practices of Statistics in Biological Research*. 3rd ed. W.H. Freeman and Company, New York.

Walters, C., Wheeler, L.M. and Grotenhuis, J.M. (2005). Longevity of seeds stored in genebanks: Species characteristics. *Seed Science Research*, **15**, 1-20.