

## Research Note

***Vitis* seed longevity after prolonged cold storage**

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**Key words:** seed, germination, *Vitis berlandieri*, *Vitis cinerea*.

**Introduction:** Propagation of grapevine by seed is generally used only in breeding programs to develop new varieties for commercial use. The long generation time of grapevines necessitates breeding programs to sow their seeds as soon as possible. Few reports exist regarding germination of *Vitis* seed after prolonged storage. Storage life of *Vitis* seeds is generally limited to 2–3 years (WENDEL 1981, ZANKOV 1981). Seed longevity of native North American *Vitis* species is of interest as wildlife habitat (WENDEL 1981) and forestry weeds (TRIMBLE and TRYON 1979, HAYWOOD 1994).

This report describes seed viability and seedling emergence of inter- and intraspecific crosses of *Vitis* from root-stock breeding experiments after 16 and 33 years storage.

**Material and Methods:** Seeds were collected in 1968 and 1985 and stored at 4 °C in sealed containers. Seeds from intraspecific crosses of *V. berlandieri* and of *V. cinerea* obtained from the University of Illinois were planted at Merbein in 1968. Three mature vines from the 1968 crosses were used for *V. berlandieri* x *V. vinifera* Sultana crosses in 1984. Sufficient seeds (> 60) from 8 crosses were available for sowing (Table).

Seed viability was tested to determine whether embryos had potential to germinate. Seeds were surface-sterilized in 2 % v/v sodium hypochlorite for 2 min and rinsed in tap water. Seeds that floated during rinsing were considered unlikely to germinate (WINKLER *et al.* 1974) and were discarded. At least 25 potentially viable seeds of each cross were placed on filter paper soaked in distilled water in closed petri dishes for 72 h. Seeds were then cut transversely and placed cut side down on fresh filter paper soaked in 1 % w/v 2,3,5 triphenyltetrazolium chloride (MACKEY 1972). Seeds that developed red formazan deposits after 48 h were considered viable.

The remaining potentially viable seeds were sown approximately 5 mm deep into a 20 mm layer of steam-sterilized, washed quartz sand over a sterilized seed-raising mix (1:1:1 sand:peat moss:composted sawdust) on 31 Oct. 2001 (*i.e.* storage times of 16 or 33 years). Each cross was sown into a separate 14 l polystyrene crate (42 x 29 x 12 cm) and watered thoroughly. Crates were kept at 4 °C for 42 d before they were transferred to a glasshouse (average maximum temperature  $\pm$  SE: 25  $\pm$  5 °C). Crates were watered twice a day and glasshouse humidity maintained above 80 %. Successful seedling emergence was recorded once the seedling hook was free of the sand. Observations continued until 182 d after sowing.

**Results and Discussion:** Tetrazolium staining indicated no 33-year-old seeds were viable, while 97 % of 16-year-old seeds in each cross were viable (data not shown). Seed viability after storage depends upon seed maturity at harvest, seed moisture content, drying conditions and storage temperature (IBPGR 1985). Seeds of *V. vinifera* stored for 2 years in sealed glass jars at 10 °C or 20 °C had greater viability than when stored at 30 °C (MAEDA *et al.* 1985). *V. aestivalis* seed viability decreased when stored in fibreglass mesh envelopes in soil for 11 years (WENDEL 1981).

Table

Seed viability and seedling emergence of *Vitis* species crosses collected in 1968 and 1985 planted in October 2001

Cross	Seed age (y)	Sinking (%) <sup>1</sup>	Emergence (%) <sup>2</sup>
<i>V. berlandieri</i> 7652 x <i>V. berlandieri</i> 7651	33	83	0(67)
<i>V. cinerea</i> 882-6 x <i>V. cinerea</i> 9	33	86	0(66)
<i>V. cinerea</i> 55 x <i>V. cinerea</i> 194-1	33	51	0(66)
<i>V. cinerea</i> 58 x <i>V. cinerea</i> 194-1	33	47	0(66)
<i>V. cinerea</i> Indian Creek #8 x <i>V. berlandieri</i> 7651	33	95	0(66)
<i>V. berlandieri</i> 54-72 x <i>V. vinifera</i> Sultana	16	-	8.3(60)
<i>V. berlandieri</i> 56-100 x <i>V. vinifera</i> Sultana	16	100	1.5(66)
<i>V. berlandieri</i> 56-12 x <i>V. vinifera</i> Sultana	16	97	1.5(66)

<sup>1</sup> Calculated from number of seeds required to obtain at least 25 seeds for viability testing. No seeds of *V. berlandieri* 54-72 x *V. vinifera* Sultana were tested for viability.

<sup>2</sup> Total number of seeds planted shown in brackets.

No seedlings from the 1968 crosses emerged (Table). At least one seedling emerged from each of the 1984 crosses, including 5 seedlings of *V. berlandieri* 54-72 x *V. vinifera* Sultana. Successful seedlings emerged within 126 d of sowing. Storage in refrigerated, sealed containers for 33 years was detrimental to all seeds, while some seeds were able to survive for 16 years. Fungal contamination of inviable seeds may have overestimated their germination potential (MACKAY 1972). Improved storage methods, e.g. rapid drying to low humidity (IBPGR 1985) may increase emergence of 16-year-old seeds or extend longevity.

These results extend the known maximum survival times of *Vitis* seeds by approximately 5 years as *V. aestivalis* seeds germinated successfully in a laboratory incubator after 11 years *in situ* storage (WENDEL 1981). *V. vinifera* seeds stored for 3 years had low germination rates (<5 %) and seeds stored for 6 and 15 years failed to germinate (ZANKOV 1981). Germination rates of seeds from interspecific crosses using *V. vinifera* females sown immediately after berries were harvested ranged from 1.2 to 45.5 % (PATIL and PATIL 1993). The emergence rate of 8.3 % for *V. berlandieri* 54-72 x *V. vinifera* Sultana seeds after 16 years storage was presumably less than if sown immediately. Original emergence data were not recorded; seeds of similar crosses made in 2000 sown in 2001 had much higher emergence rates (*V. cinerea* x *V. vinifera* Cabernet Sauvignon: 28 %, *V. cinerea* x *V. vinifera* Riesling: 56 %, *V. cinerea* x *V. berlandieri*: 52 %; unpubl. data).

**Conclusion:** Under our storage conditions, 16-year-old seeds of *V. berlandieri* x *V. vinifera* crosses were still capable of successful emergence, while 33 years appeared to

exceed the maximum storage time for successful emergence of *V. berlandieri* or *V. cinerea* seedlings. The proportion of emerging seedlings was relatively low for the particular interspecies crosses, suggesting long-term storage of *Vitis* seeds is an inefficient practice for either breeding or conservation purposes. Seed storage appears less reliable for long-term conservation of grapevine germplasm than the resource-intensive cultivation of seedlings as individual vines, but may provide useful material for other purposes. Using established seed storage guidelines may extend longevity of grapevine seeds.

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