

**Bibliography for 2004 Tetrazolium Testing Workshop,  
Instructor: Annette Miller**

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At the Ransom Seed Laboratory we test a number of native seeds which have deep dormancy. Many of these species are so dormant they will not stain when testing for viability using the normal procedures for TZ (Tetrazolium chloride). Sometimes the endosperm stains rather than the embryo, and sometimes stain only develops after many days with sharp temperature alternation (5°-35°C). We have found the seeds will stain if they are pretreated prior to staining. We first attempt to germinate the seeds at the suggested A.O.S.A. temperature or a neutral temperature (20°C). We want the germination to reflect what would grow without treatment. This is reported as percent germination. Firm seed remaining after the test (or on one replicate at the first count if the seed has a mold problem) is cut into the cotyledon tissue using a single edge safety razor blade. The cut seeds are placed on a medium of 400 ppm GA3 (gibberellic acid, a plant growth hormone). If the seeds are small, planting on top of a blotter is sufficient. However, if the seeds are large, they may not come in contact with the substrate. In this case the seeds are placed in a shallow dish and covered with the GA3 solution. These are then placed at 5°C overnight. The next day the seeds are drained and placed in a 0.1% solution of TZ. The seeds then stain in a matter of hours. The seeds that stain are reported as percent dormant live seed, and a description of the method is given.

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Phlox is one of several species having embryos with chlorophyll (Acer, Kochia, Phlox). Metabolically active chlorophyll interferes with the development of the viability stain, Tetrazolium chloride (TZ). The result is a bright green or mottled embryo, rather than the expected red stain of a viable embryo. Several pretreatments and solution concentrations were tested on Phlox in an attempt to improve the stain. When the dry seeds are cut and placed directly into the TZ solution, the chlorophyll is not active and the TZ stain develops normally with viable embryos staining red. There is more surface damage when the seed is cut dry, however the interpretation is less difficult than evaluating the turgor of the metabolically active green embryos.