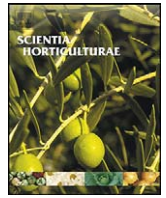


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Optimized scarification protocols improve germination of diverse *Rubus* germplasm

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ABSTRACT

Seed collections of the wild relatives of cultivated blackberry and raspberry (*Rubus* species) are maintained at the National Clonal Germplasm Repository, Corvallis, OR. Information on wild species germination requirements is rarely available, and germination may be poor or slow, making it difficult for scientists to use them for breeding improved cultivars. Eight diverse *Rubus* species in 6 of the 12 *Rubus* subgenera from seed stored at -20°C for 1–23 years were studied. Seed weight, seed-coat thickness and hardness varied widely. Scarification with sulfuric acid (98% H_2SO_4) or sodium hypochlorite (14% NaOCl) was followed by germination treatments of deionized water (DI), smoke gas or a combination of gibberellic acid (2.03 mg/L GA_3) and potassium nitrate (34 mg/L KNO_3) during stratification. The commonly used scarification protocols were not effective for many species; but effective scarification exposure was established based on the amount of embryo damage seen with 2,3,5 triphenyl tetrazolium chloride (TZ) viability testing. H_2SO_4 scarification followed by a treatment with KNO_3 and GA_3 during stratification was highly effective for the most species. Two species in subgenus *Anoplobatus* had a hilar-end hole that allowed rapid germination of unscarified seed. Some species with extremely hard seed coats had little or no germination, and longer scarification times are suggested based on seed size, seed-coat thickness and hardness and viability testing.

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1. Introduction

Germplasm collections are valuable to plant breeders as a source of new genes or traits to improve cultivated crops. The National Clonal Germplasm Repository (NCGR) in Corvallis, OR preserves more than 1300 wild-collected *Rubus* seed accessions at -20°C . These seeds are available to plant breeders for blackberry and raspberry crop improvement, however little is known about the germination requirements of most of the species. Often they do not germinate using the commonly used protocols found in the literature. In addition the seed collections were made by many plant collectors over many years and information on the seed maturity and initial viability is often not known. The genus *Rubus* is very diverse, includes over 750 species in 12 subgenera, and is found on all continents except Antarctica (Finn, 2008). The deep dormancy of many *Rubus* species seeds makes it difficult to use wild germplasm for blackberry and raspberry breeding programs (Clark et al., 2007; Daubney, 1996). Seed germination of the resulting hybrids is one

of the largest challenges in blackberry breeding as there is large variation in germination based on genotype (Clark et al., 2007).

Rubus seed germination is constrained by both physical and physiological dormancy. An impermeable seed coat imposes mechanical resistance to growth and may contain chemical inhibitors; slow germination may also be due to the slow maturation of the dormant embryo (Zasada and Tappeiner, 2003). The endosperm and testa of dormant blackberry seeds contain growth inhibitors that degrade or are leached out during stratification under moist conditions, resulting in breaking dormancy and germination after about 5 months (Lasheen and Blackhurst, 1956). Polyphenols in the seed coat may also be linked with seed dormancy and longevity (Werker et al., 1979). Heit (1967b) noted that a hard seed coat or the combination of a hard seed coat and a dormant embryo will inhibit germination.

Sulfuric acid (98% H_2SO_4) scarification and a long stratification period, first warm and then cold, are required for satisfactory germination of hard-seeded species (Heit, 1967a). A scarification procedure using concentrated H_2SO_4 neutralized with calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) described by Jennings and Tulloch (1965) is a widely used protocol for *Rubus* seed germination. H_2SO_4 scarification of 30 min is recommended for the small seeded raspberries (*Rubus idaeus* L., *Rubus occidentalis* L.) and up to 3 h for the larger seeded

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