A Closer Look at Seed Germination and Dormancy©

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INTRODUCTION

Several methods have been developed for capturing digital images during seed germination (Dell'Aquila et al., 2000; Geneve and Kester, 2001; Sako et al., 2001). Flatbed scanners are an inexpensive alternative to video and still cameras that provides consistent lighting and the ability to capture usable images from very small seeds (Geneve and Kester, 2001). Recently, we developed a nondestructive system for capturing sequential digital images over time that provides additional precision and insight concerning aspects of seed germination and dormancy (Geneve et al., 2006).

In this study, captured sequential digital images were used to evaluate seed dormancy release in two woody legume species with different dormancy types. Honeylocust (*Gleditsia triacanthos* L.) seeds have physical dormancy and require scarification to allow imbibition. Eastern redbud (*Cercis canadensis* L.) seeds have physiological dormancy and require chilling stratification. In this case, seedling growth over time in excised embryos was used as an indicator of release from dormancy following chilling.

MATERIALS AND METHODS

Seeds of honeylocust were acid scarified for 30 to 240 min in concentrated H_2SO_4 or physically scarified by nicking the center of the seed using a file. Seeds of redbud were treated with concentrated H_2SO_4 for 30 min and stratified at 4 ºC for 4 weeks. Nonstratified seeds were acid scarified and imbibed, but did not receive chilling. Embryos were surgically removed from redbud seeds by slitting the seed coat at the radicle end to expose the embryo.

Honeylocust seeds or redbud embryos were imaged as described by Geneve et al. (2006). Seeds were placed in 6-cm diameter plastic Petri dishes containing one piece of transparent cellulose film with sterile water. Petri dishes were sealed with Parafilm and placed on an Hewlett-Packard Scanjet flat bed scanner with transparency adapter. Scans were taken at hourly intervals and saved as gray scale TIFF files.

RESULTS AND DISCUSSION

Honeylocust Physical Dormancy. Honeylocust seeds have a typical palisade epidermal layer with thick-walled macroscleried cells responsible for maintaining physical dormancy and restricting imbibition. Only a few seeds imbibed when treated for 30 min with concentrated H_2SO_4 (data not included). As seeds received increasing durations of acid treatment from 60 to 240 min, they showed faster imbibition and quicker germination (Fig. 1). This suggests that additional entry points for water were being exposed across the seed surface with increasing exposure to

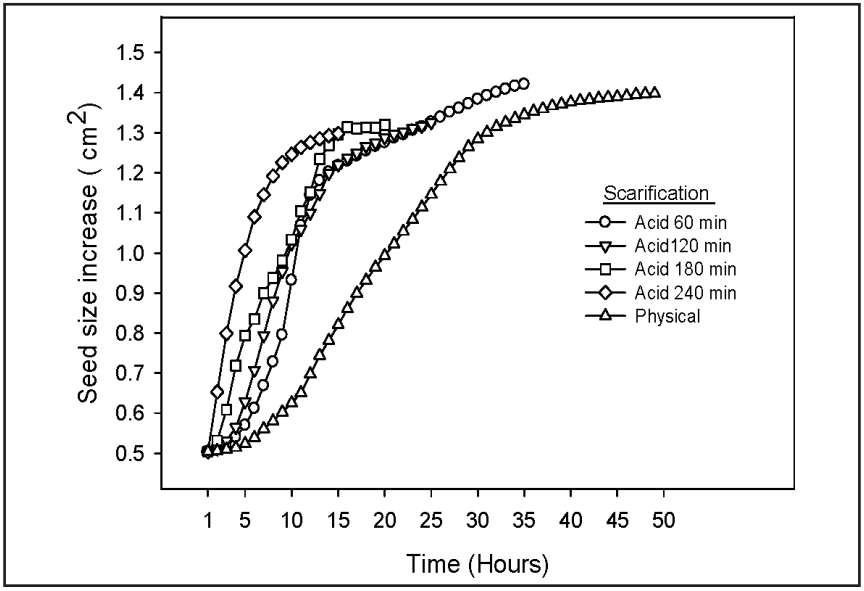

Figure 1. Imbibition in honeylocust seeds treated with concentrated sulphuric acid or physically scarified. Data was fit to a three parameter sigmoidal curve with R_2 between 0.98 and 0.99 and each curve was significant at $P \leq 0.01$.

acid (Fig. 2). It was anticipated that acid-treated seeds would show uniform water uptake over the entire seed surface. However, when seed size was followed on an hourly basis, acid-treated honeylocust seeds showed more water initially entering at the polar ends of the seeds (Fig. 3). This suggests that the cells in these regions were more susceptible to acid scarification than cells in the middle of the seed or that they are more adept at allowing water entry.

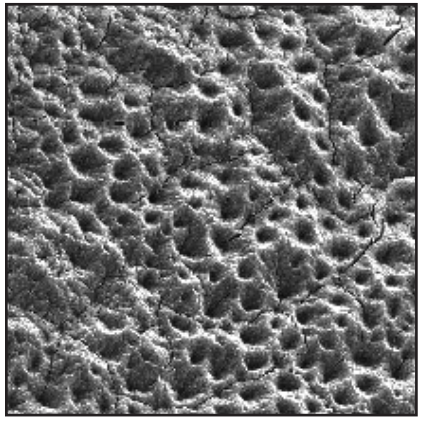

Figure 2. Electron micrograph of acid scarified honeylocust seed coat.

Seeds physically scarified at one location on the seed imbibed slower than acid-treated seeds and took the longest to germinate. A single entry point for water restricted water uptake compared to the greater surface area exposed to water in acid-treated seeds. Physical scarification provided a deeper intrusion through the seed coat compared to the surface etching from acid scarification (Fig. 3). This suggests that initial penetration of water to inner regions of the seed did not compensate for the single water entry point in physically scarified seeds.

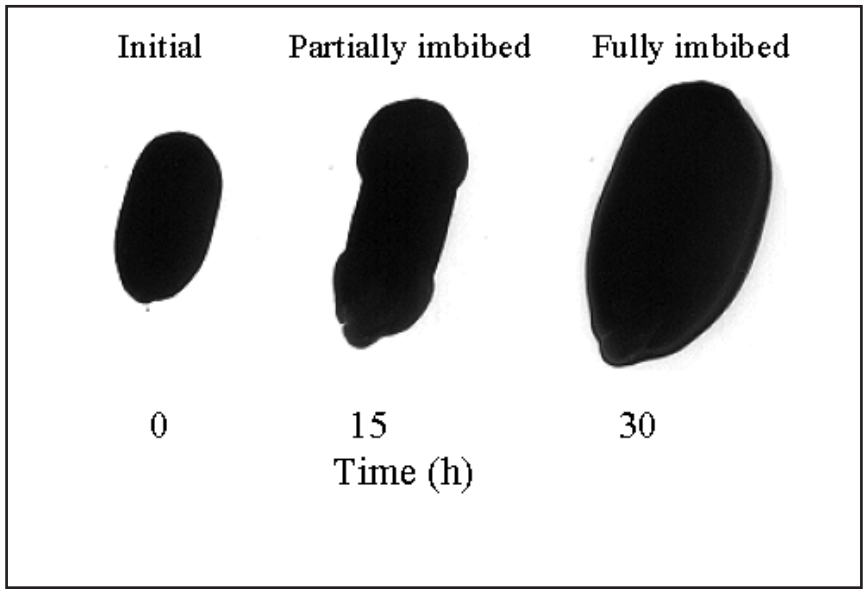

Figure 3. Water entry in honeylocust seeds treated with acid showing the "dumbbell" shape in partially imbibed seeds.

Dormancy Release in Eastern Redbud. Eastern redbud seeds have intermediate physiological dormancy (Geneve, 1991). One of the characteristics of seeds with non-deep or intermediate physiological dormancy is that the embryo shows increased growth potential following moist chilling (Hartmann et al., 2002). Growth potential is the relative ability of the radicle to penetrate the seed covering and permit germination to proceed. One measure of growth potential is seedling size following germination. The current results clearly show that isolated redbud embryos from stratified seeds grew into larger seedlings than untreated embryos after 6 days (Fig. 4). However, these results also show that the major impact of stratification was the reduction in the time to initiate radicle growth rather than overall growth rate of the seedlings following radicle emergence. Untreated embryos began germination approximately 27 h later than stratified embryos. Following initiation of growth the growth rates of the seedlings were identical (Fig. 4A).

These two experimental examples demonstrate that sequential digital images can be used for a range of growth-related aspects of seed germination. Analysis of sequential digital images allows easy identification and analysis of changes in seed and seedling morphology during germination. This technique would allow the researcher to more precisely identify key stages of development during seed germination for physiological or biochemical analyses and track seeds or seedlings on an individual basis.

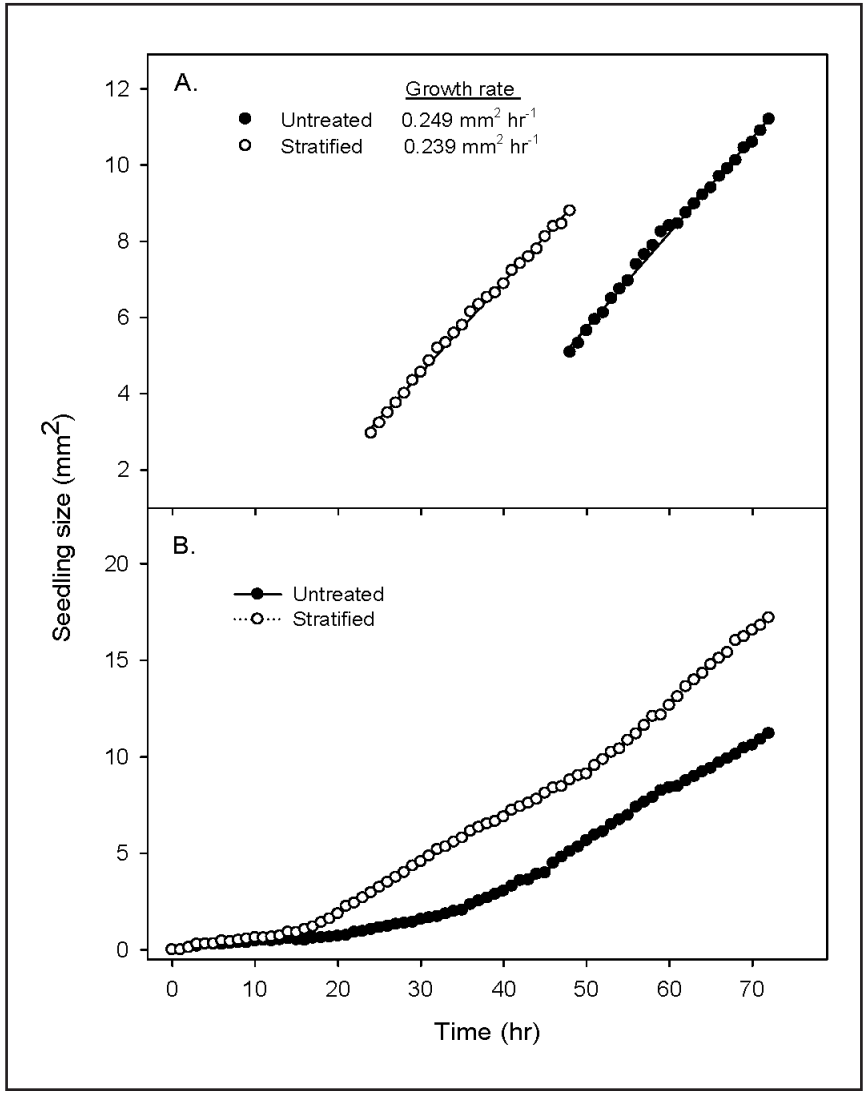

Figure 4. Seedling size in isolated eastern redbud embryos from untreated or stratified (4 weeks at 5 °C) seeds. (A) Linear regression for the 24-h period following radicle emergence. (B) Seedling size following embryo excision in untreated and stratified seeds over 72 h.

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