From Forest Nursery Notes, Summer 2008

134. Using sequential digital images to study seed germination and dormancy.
Geneve, R. L. and Dutt, M. Propagation of Ornamental Plants 8(1):13-16. 2008.

Propagation of Ornamental Plants
Vol. 8, № 1, 2008: 13-16

USING SEQUENTIAL DIGITAL IMAGES TO STUDY SEED GERMINATION AND DORMANCY

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Abstract

A sequential imaging system was developed to study seed germination and dormancy. Images were collected automatically at set time intervals as frequent as 30 min for up to 5 days using a flat-bed scanner interfaced with a computer. The system permitted individual seeds to be followed throughout germination and early seedling growth. Sequential still images were made into simple movie clips that exposed features of germination not easily seen in traditional germination studies. Using this system, we were able to followed imbibition patterns after scarification treatments in honeylocust (*Gleditsia triacanthos* L.) seeds with impermeable seed coats (physical dormancy). Imbibition movies clearly showed different patterns of water entry into the seed coat following acid vs. physical scarification. We also used the system to measure seedling growth in redbud (*Cercis canadensis* L.) embryos following chilling stratification to relieve endogenous physiological dormancy. The major difference between embryos isolated from non-chilled and chilled seeds was the time to initiate radicle growth. After growth was initiated, growth rates were similar for non-chilled and chilled seeds.

Key words: *Cercis*, Computer-aided imaging, *Gleditsia*, honeylocust, physical dormancy, physiological dormancy, redbud

INTRODUCTION

Several methods have been developed for capturing digital images during seed germination. These include charged coupled device (CCD) cameras (Howarth and Stanwood 1993, Dell'Aquila et al. 2000) and flatbed scanners (Geneve and Kester 2001, Sako et al. 2001). Video and still camera usage is relatively expensive and requires very consistent lighting for optimal performance. Flatbed scanners are an inexpensive alternative that provides consistent lighting and the ability to capture usable images from very small seeds (Geneve and Kester 2001). Flatbed scanner germination systems have been very useful in providing individual digital images to study aspects of seed quality such as seedling size as an indicator of seed vigor (Dutt and Geneve 2007, Geneve 2005). Recently, we developed a non-destructive system for capturing sequential digital images over time that provides additional precision and insight concerning aspects of seed germination (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. The objective was to show how this computer-aided system could document initial water uptake in seeds following physical or acid scarification. 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°C for four weeks. Non-stratified seeds were acid scarified, 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. This was performed immediately following stratification or after 48 h for non-stratified seeds.

Honeylocust seeds or redbud embryos were placed in 6-cm diameter plastic Petri dishes containing one piece of transparent cellulose film (Celorey-PUT, Cydsa

Received: October 11, 2007

Accepted: January 10, 2008

Monterrey, Mexico) with sterile water (2 ml for honeylocust and 1 ml for eastern redbud). Petri dishes were sealed with Parafilm and placed on a flat bed scanner (HP Scanjet 5370 C with transparency adapter). The scanner was controlled using a SigmaScan Pro 5.0 for Windows (SPPC Science, Chicago, IL) macro written in Visual Basic that allowed capture of timed interval scans. Scans were taken at hourly intervals. Gray scale images (stored as 200 or 300 dpi, tiff files) were analyzed by creating an overlay that matched the seed or seedling size and measured using SigmaScan Pro's area function. Data was recorded as the increase in seed size until radicle emergence for honeylocust and seedling size (mm^2) for excised redbud embryos.

The visual basic macro program designed to drive the HP PrecisionScan Pro flatbed scanner and collect images every sixty minutes includes the following codes:

```
Sub Main
Dim App As Object
Dim Name As String
    Name = InputBox("enter experiment name",
"expt name", "file name")
    Duration = InputBox("enter number of scans",
"scan number", "120")
    Interval = InputBox("enter seconds between
scans", "seconds", "3000")
AppActivate ("HP PrecisionScan Pro")
For I = 1 to Duration
    Imagename = Name + I + ".tif"
    SendKeys "%T"
    SendKeys "U"
    SendKeys "^S"
    SendKeys Imagename
    SendKeys "~"
    Wait 600
    SendKeys "%T"
    SendKeys "U"
    Wait Interval
    Next I
End Sub
```

RESULTS AND DISCUSSION

Physical dormancy in honeylocust

Honeylocust seeds have a typical palisade epidermal layer with thick walled macroscleried cells responsible for maintaining physical dormancy and restricting imbibition (Baskin and Baskin 1998). Only a few seeds imbibed when treated for 30 min with concentrated H_2SO_4 (data not included). In acid scarified legume seeds, Liu et al. (1981) showed a general reduction in the materials covering macroscleried throughout the seed. Therefore, between 30 and 60 min was required to begin the process of removing these surface materials in honeylocust seeds. 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 acid. However, if this were the case it would be 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. 2). 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.

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. This suggests that initial penetration of water to inner regions of the seed did not compensate

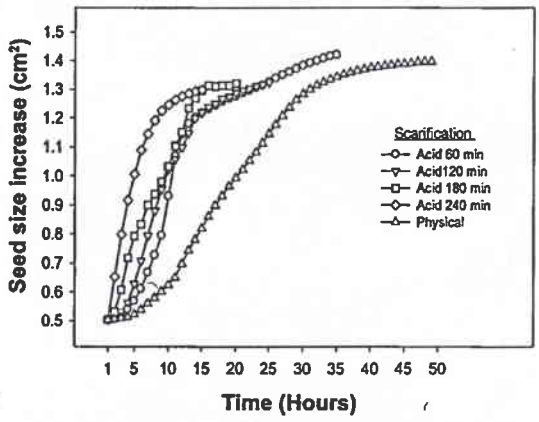

Fig. 1. Imbibition in honeylocust seeds treated with concentrated sulphuric acid or physically scarified. Data was fit to a three parameter sigmoidal curve with R^2 between 0.98 and 0.99 and each curve was significant at $p \leq 0.01$.

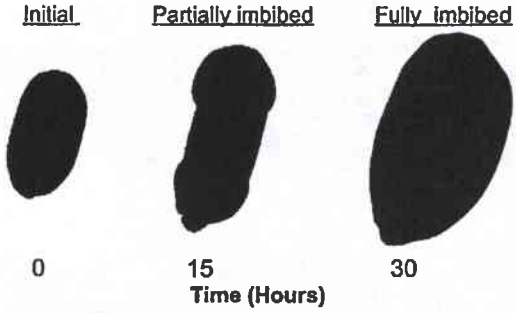

Fig. 2. Water entry in honeylocust seeds treated with acid showing the "dumbbell" shape in partially imbibed seeds.

for the single water entry point in physically scarified seeds.

Scarified honey locust seeds showed typical phases in seed size increase that were indicative of water uptake during imbibition (Fig. 1). However, the lag phase of germination was reduced with increasing acid duration leading to faster germination. Seeds treated with acid for 60, 120, 180 and 240 min reached 50% of their final germination at 35, 24, 19 and 15 h after imbibition, respectively. Physically scarified seeds required 49 h to reach 50% germination. Seeds that imbibed more slowly (i.e. 60 min acid and physical scarification) germinated at a larger seed size suggesting that embryo hydration might be more important for determining germination time rather than total seed hydration.

Dormancy release in eastern redbud seeds

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 chilling stratification (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 in isolated embryos. The current results clearly show that isolated redbud embryos from stratified seeds grew into larger seedlings than untreated embryos after six days (Fig. 3). 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. 3 A).

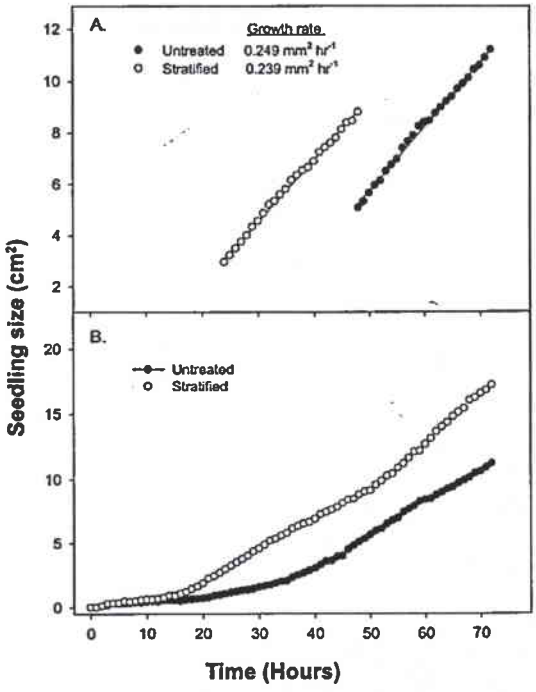

Fig. 3. Seedling size in isolated eastern redbud embryos from untreated or stratified (4 weeks at 5°C) seeds. A) Linear regression for the 24 h period following radicle emergence. Untreated seedling size = 0.248 x h – 6.71; R^2 = 0.996. Stratified seedling size = 0.239 x h – 2.62; R^2 = 0.997. B) Seedling size following embryo excision in untreated and stratified seeds over 72 h.

CONCLUSIONS

The two experiments described in this paper demonstrated that sequential digital images captured with the flat bed scanner can be used for a variety of growth related aspects of seed germination. Analysis of sequential digital images offers an alternative research tool to study time sensitive processes related to seed germination. It allows easy identification and analysis of changes in seed and seedling morphology during development. 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.

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