

Use of Computer Imaging to Evaluate the Initial Stages of Germination in Woody Tree Seeds[©]

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INTRODUCTION

Seed germination begins with the initiation of water uptake by the dry seed and ends with the protrusion of the radicle from the fully imbibed seed. Measurement of initial water uptake is usually by measuring fresh weight gain, which is laborious and requires physical handling of each seed. Such techniques require pooling of seeds to make different samples to estimate average values and submitting to statistical analysis. These methods do not record growth performance and variation on an individual seed basis. Dell Aquilla et al. (2000) and McCormac and Keefe (1990) have described image analysis systems to monitor the imbibition in cabbage and cauliflower seeds. Such techniques, though useful, require the setup of sophisticated and expensive equipment. The computer imaging system developed by Geneve and Kester (2001) uses a simple Petri dish germination system that is inexpensive and amenable to automated capture of sequential digital images in real time.

In this study, the techniques developed by Geneve and Kester (2001) 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 a physical dormancy and a physiological dormancy requiring a chilling stratification. In this case, radicle growth in excised embryos is an indicator of release from dormancy following chilling. Therefore, radicle length was measured on an hourly basis in nonchilled and chilled seeds to determine specific growth rates.

MATERIAL AND METHODS

Seeds of honeylocust were acid scarified for 30 or 60 min in concentrated H₂SO₄ or physically scarified by nicking the center of the seed using a file. Seeds of redbud were treated with concentrated H₂SO₄ for 30 min and stratified at 4°C for 4 weeks. Nonstratified seeds were acid scarified, but did not receive chilling. After 4 weeks, embryos were surgically removed from redbud seeds.

Two honeylocust seeds or four redbud embryos were placed in 6-cm-diameter plastic petri dishes containing one piece of transparent cellulose film (Celorey-PUT, Cydsa Monterrey, Mexico). Honeylocust seeds were surface sterilized in 10% Clorox[®] solution for 10 min and washed in distilled water before being placed in a Petri dish containing 2 ml of distilled water. Petri dishes were sealed with Parafilm[®] and placed on a flat bed scanner (HP Scanjet 5370C with transparency adapter). The scanner was controlled using a SigmaScan[®] Pro 5.0 for Windows (SPPC Science, Chicago, Illinois) macro written in Visual Basic that allowed for timed interval scans. For this experiment, scans were taken at hourly intervals. Gray scale images (stored as 200 dpi, tiff files) were analyzed using another SigmaScan macro that allowed for batch processing of the various images in a short period of time.

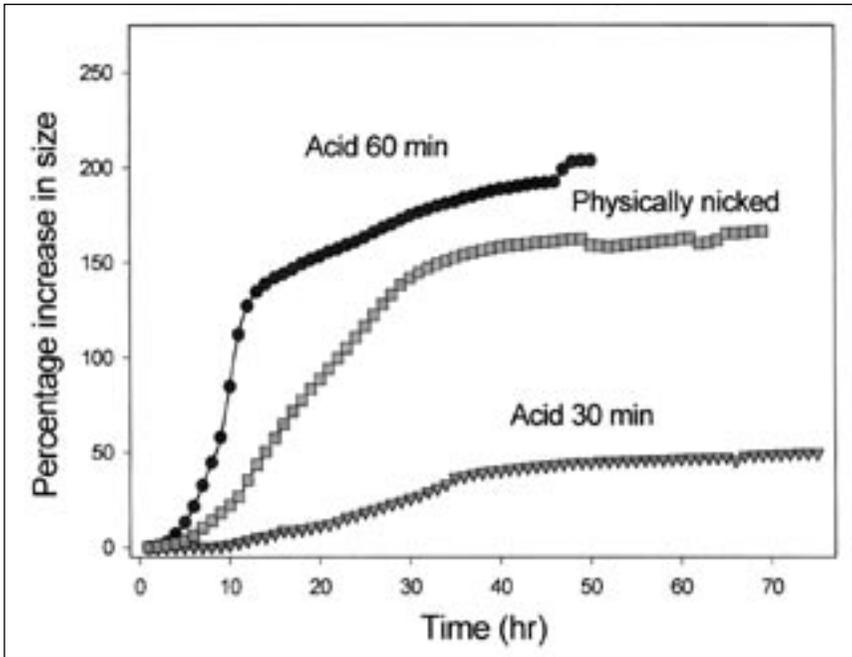


Figure 1. Increase in seed size for acid vs. physically scarified honeylocust seeds.

Data was recorded for percentage increase in seed size until radicle emergence for honeylocust and radicle length (mm) for 3 days in excised redbud embryos.

RESULTS AND DISCUSSION

Honeylocust seeds treated with concentrated H_2SO_4 for 60 min had a faster imbibition rate compared with seeds that were acid scarified for 30 min or physically scarified (Fig. 1). Seeds treated with concentrated H_2SO_4 (60 min) reached 50% of their final imbibed size within 11 h after imbibition compared to 20 h for physically scarified seeds (significant at $P < 0.01$). Seeds treated with concentrated H_2SO_4 (30 min) remained small and did not imbibe much water or germinate for the study period.

According to Woodstock (1988) hardseededness may be due to a compact arrangement of cellulose microfibrils in the cell wall, involving an irreversible change in micellar structure during maturation and dehydration of the seed. Honeylocust seeds have a palisade epidermal layer with thick walled malpighian cells. Subsequently, 30-min acid scarification was not enough to adequately scarify the epidermal layer leading to reduced imbibition.

There was less variation in the rate of water uptake between seeds treated with concentrated H_2SO_4 (60 min) compared to physically scarified seeds (Fig. 2). This may be due to a larger and more uniform disruption of surface area cells in acid scarified seeds compared to a single wound site on the seed coat for nicking or may be due to the nonprecise nature of physically nicking the seeds. However, Fig. 2 does show how the imaging system can easily compute water uptake on a single-seed basis for such an analysis.

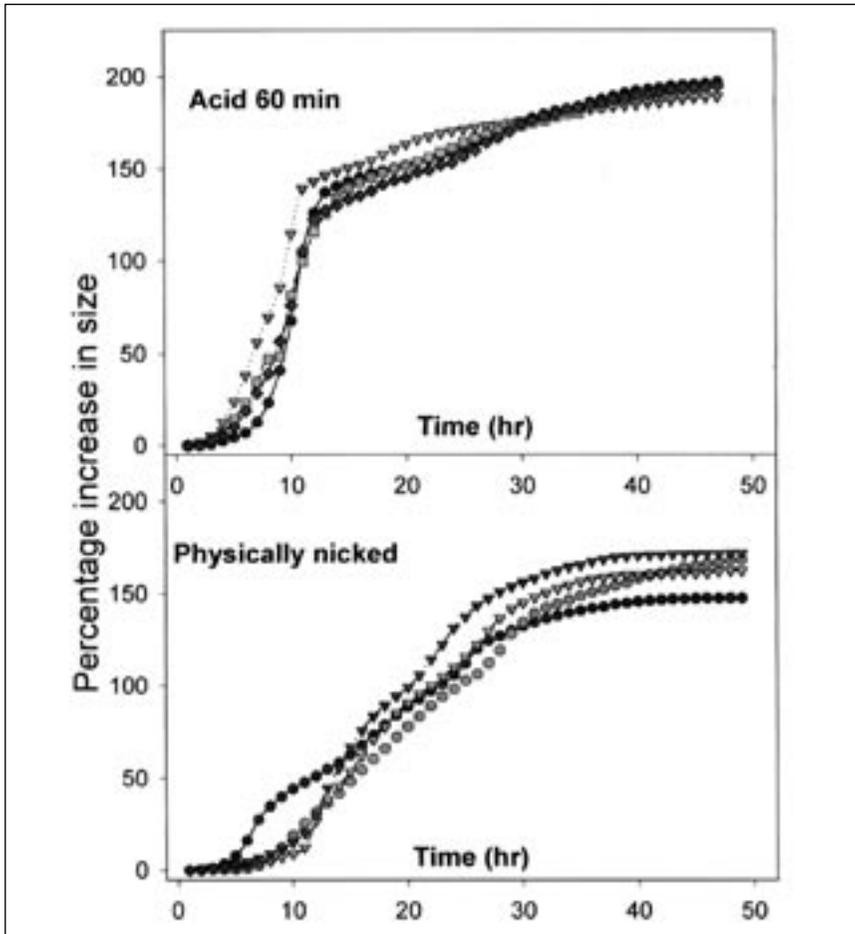


Figure 2. Impact of type of scarification on uniformity of water uptake in individual honeylocust seeds.

Baskin et. al. (2000) suggested that in legume seeds, the lens (strophiole) is the first place on the seed coat for water entry when hard seeds become permeable under natural conditions. In contrast, for acid scarified legume seeds, Liu et al. (1981) showed a general reduction in the materials covering macrosclerids throughout the seed. Therefore, rather than a single entry point for water, it would be anticipated that acid-treated seeds would show uniform water uptake over the entire seed surface. However, when water entry was followed on an hourly basis, acid-treated honeylocust seeds showed asymmetric water uptake across the seed with more water initially entering at the chalazal and micropylar ends that produced a “dumbbell” shaped appearance (Fig. 3). This suggests that the cells in the polar regions of the seed were more susceptible to acid scarification than cells in the middle of the seed.

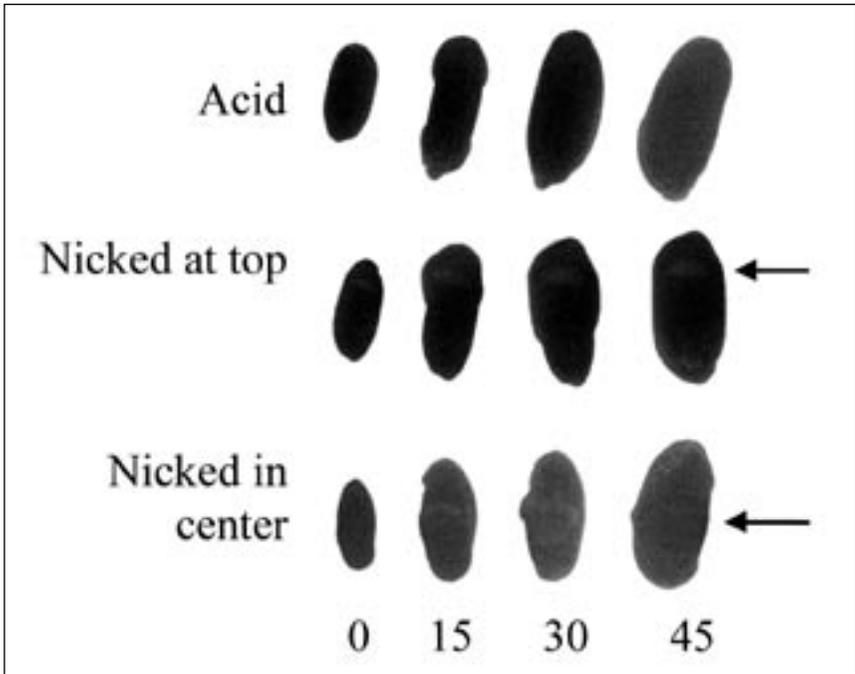


Figure 3. Water entry over the first 45 h in seeds treated with acid or physically scarified by nicking the seeds at the top or center of the seed. Arrows indicate location of physical nicking.

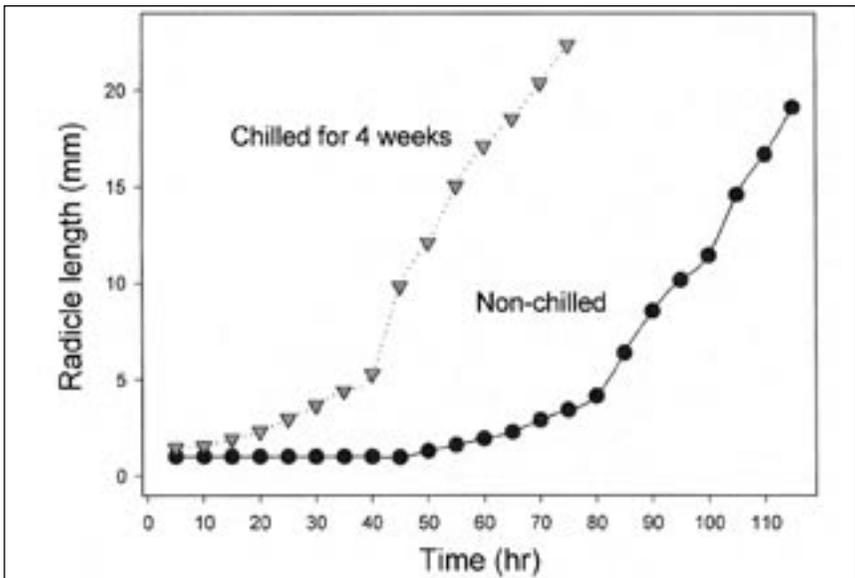


Figure 4. Increase in radicle length in dormant and nondormant redbud embryos isolated from the seed coat.

Physically scarified seeds showed initial water uptake at the point of nicking with water spreading from the center of the seed to the opposite ends of the seed or from one end to the other end of the seed depending on the initial nicking point (Fig. 3). The sequential images captured by the flat bed scanner allowed us to document the water uptake at hourly intervals and enabled us to see the position of water uptake in the honeylocust seeds as would not be possible by former techniques.

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). Geneve (1991) showed that isolated redbud embryos from chilled seeds grew faster than nonchilled embryos. However, these measurements were performed by hand and done every 24 h. In contrast, using the computer-aided imaging system, radicle length could be measured every hour and a precise growth rate calculated with little researcher investment in time (Fig. 4). As predicted, nonchilled redbud embryos took 48 h to initiate growth and required 90 h for radicles to reach 10 mm in length, while embryos chilled for 4 weeks initiated growth immediately and reached a radicle length of 10 mm in only 45 h (Fig. 4; Significant at $P \leq 0.01$).

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. It enabled easy identification and analysis of water entry into seeds. This technique revealed changes in seed morphology that were previously undocumented for seeds with physical dormancy. This technique can also be used for assessing seeds with other types of dormancy. Also the use of sequential imaging holds promise for an automated system to assess seed quality in seed lots.

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