Using Sequential Digital Images Captured With A Flat Bed Scanner To Evaluate Woody Plant Seeds With Different Germination Requirements

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Nature of work: Several methods have been used to capture germinating seed images including video and still cameras (1, 5) or flat bed scanners (2, 4). Video and still camera usage is relatively expensive and requires specialized lighting and camera equipment. Flat bed scanners allows for economical and high quality digitization of seed images (4). Geneve and Kester (2) developed a simple Petri dish germination system that would be amenable to automated capture of sequential digital images in real time. The objective of the current study was to demonstrate how sequential digital images could be captured during seed germination using a flat bed scanner interfaced with a computer. The power of this technology will be demonstrated by evaluating imbibition in honeylocust (Gleditsia triacanthos L.) seeds following physical or acid scarification.

Honeylocust is a large leguminous tree native to North America. Seeds have physical dormancy with a hard seed coat that is impervious to water and gases. The seed coat must become permeable to allow for moisture and gaseous uptake and consequent seed germination.

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 (6). Liu et. al. (3) noted that water impermeability in honeylocust seeds was due to the cuticle covering the macrosclereid cells at the seed coat surface. Also, the rate of imbibition of water into the seed determines the rate at which the embryo hydrates and subsequent radicle emergence.

Seeds of honeylocust were either acid scarified in concentrated H$_2$SO$_4$ for 60 minutes or physically scarified by nicking the center of the seed using a file. Two seeds were placed in 6 cm diameter plastic Petri dishes containing one piece of transparent cellulose film (Celorey-PUT, Cydsa Monterrey, Mexico). The cellulose film allows for uniform distribution of water throughout the Petri dish and being transparent allows uninterrupted image capturing by the flat bed scanner (2). Honeylocust seeds were surface sterilized in 10 % Clorox® solution for 10 minutes and
washed in distilled water before being placed in a Petri dish containing 3 ml of distilled water. Petri dishes were sealed with Parafilm™ and placed in the 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 which allowed for timed interval scans. For this experiment, scans were taken at hourly intervals. Gray scale images (stored as .tif files) were analyzed using another SigmaScan® macro which allowed for batch processing of the various images in a short period of time. Data was recorded for percentage increase in seed size till the time of radicle emergence.

**Results and Discussion:** Seeds treated with concentrated H₂SO₄ showed faster water uptake compared to physically scarified seeds (Figure 1). Acid treated seeds reached 50% of their final size within 11 hours after imbibition while physically scarified seeds required 20 hours (Figure 1). Uniform removal of the waxy coating and etching of the seed coat by acid treatment results in more rapid water uptake. This process as compared with nicking created a single point of entry of water on the seed coat. Acid treated seeds showed asymmetric water uptake across the seed with more water initially entering at the seed poles (chalazal and micropylar ends) producing a “dumbbell” shaped appearance (Figure 2). 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 (Figure 2).

The time required for radicle protrusion was about 20 hours less in acid treated seeds compared to physically scarified seeds (Figure 1). Acid treated seeds also attained a larger overall size prior to radicle emergence compared to physically scarified seeds. At the time of radicle protrusion, acid treated seeds had increased approximately 200% of their initial size, while physically scarified seeds only increased by 165%.

**Significance to the industry:** Sequential digital images captured with the flat bed scanner allowed for 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. Continued research will provide additional morphological details for seeds with other types of dormancy including physiological and morphological dormancy. The use of sequential imaging also holds promise for an automated system to assess seed quality in seed lots. This will be important for determining initial seed quality after seed harvest and for evaluating quality in stored seeds that are experiencing deterioration.
Figure 1: Imbibition following acid or physical scarification in honeylocust seeds.

Figure 2: Water entry over the first 45 hours in seeds treated with acid or physically scarified by nicking the seeds at the top or center of the seed (micropylar ends face bottom).