SEED VIABILITY DETECTION USING COMPUTERIZED FALSE-COLOR RADIOGRAPHIC IMAGE ENHANCEMENT

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ABSTRACT

Seed radiographs are divided into density zones which are related to seed germination. The seeds which germinate have densities relating to false-color red. In turn, a seed sorter may be designed which rejects those seeds not having sufficient red to activate a gate along a moving belt containing the seed source. This results in separating only seeds with the preselected densities representing biological viability lending to germination. These selected seeds demand a higher market value. Actual false-coloring isn't required for a computer to distinguish the significant gray-zone range. This range can be predetermined and screened without the necessity of red imaging. Applying false-color enhancement is a means of emphasizing differences in densities of gray within any subject from photographic, radiographic or video imaging. Within the 0-255 range of gray levels, colors can be assigned to any single level or group of gray levels. Densitometric values then become easily recognized colors which relate to the image density. Choosing a color to identify any given density allows separation by morphology or composition (form or function). Additionally, relative areas of each color are readily available for determining distribution of that density by comparison with other densities within the image.

INTRODUCTION

Forestry is severely challenged by abusive cutting and global environmental change while its value increases as a revenue source for wood products. These social, environmental and economic strains are further reflected by biological ones. The seeds of economically important indigenous tree species are difficult, expensive and labor-intensive to collect, store and germinate. Considering that natural germination rates range from less than 50 percent to more than 90 percent, it then becomes significant to recognize good or viable seeds from poor or nonviable ones. An example shows that in 1991, 1.2 billion pine seedlings were planted in the southeastern United States. Assuming an average of 70 percent germination, at least 2 billion seed were required to meet the demand. If an average of 10,000 seeds per pound is assumed, then this required about 200,000 pounds of pine seeds. At an average of $40 per pound, this is an $8 million dollar investment per year in the southeastern U.S. only. Nurserymen are willing to pay an additional $5 per pound for enhanced viability seeds.

Using the same figures as 1991, but with enhanced germination at 95% and increasing the average price per pound to $45, would result in a savings of $2,375,000 per year. If you consider the expenses of intensive labor to cull out the multiple seedlings planted to reach the goal of only 1.2 billion seedlings then there is an additional manpower savings in time and energy. The consistency of germination would

further allow planting to be timed with the 5-day weather forecast as to when to actually plant to account for local weather optimal for germination.

Radiographs of pine seeds clearly show empty and full seeds but will not distinguish which of the full seed will germinate. Densitometric values indicate variable but distinct density patterns within the seed seemingly unrelated to seed anatomy. Computerized imaging and densitometry assigns a color value to each of several density ranges, resulting in four of five different colored patterns which are more obvious than the original shades of gray density. Comparing the color distribution of seed which germinate to those that do not germinate leads to predicting individual seed viability. The significance of separation potential is within the gray level of densities physiologically active in the seeds. Actual false-coloring is not requisite to distinguishing densities so that a computer could detect predetermined gray ranges aside from false-coloring. For practical applications this would eliminate expense and software, as the computer would select by reading only the gray ranges of interest.

MATERIALS AND METHODS

We used a high-resolution TV digitizing system for scanning the images because it was important to be able to accurately compare different radiographs. The camera gain was adjusted so that the area of each film which had been covered by the lead letter label had a gray level of 250. This procedure reduces errors due to differing fog levels or developer activity for different individual films.

The radiographs each consisted of an array of several seeds, all of the same species, but with different germination potential, as confirmed by laboratory germination tests. A separate digital image of each seed in the array was created under identical illumination and recording conditions. The digitized image size in pixels was either 512 x 512, 256 x 256, or 512 x 64, depending on the size and shape of the species of seed. The contrast and brightness controls on the camera were adjusted for each array of seeds so that the full 0-255 range of gray levels was utilized. The density was not inverted, so the highest density values corresponded to the highest x-ray scattering (in our case, primarily due to water). We used a Dage model 81 high resolution video camera (Dage-MTI, Inc., Michigan City, IN) with an Androx ICS-400 image-processing board (Imaging Solutions Corp., Natick, MA) in a Sun 3/260 workstation (Sun Microsystems, Mountain View, CA). Our image-capture system is described in Hindus (1).

A montage of digital images of selected seeds (one image each of several categories of germination potential or x-ray appearance) was created for each species. This montage was then saved as a single image. A color lookup table was created for each montage by dividing the 0 to 255 density range into four or five sub-ranges colored in the sequence, green, yellow, blue, red, cyan, from lowest to highest density.

These limits were determined by moving a cursor around the image. At each point selected by the cursor, all of the pixels in the image with that gray-level blue flash on and off rapidly. In this way, both the optical density distribution and the morphology of the seeds could be taken into account in making decisions about color assignment. Colors were chosen to provide good contrast between areas of adjacent density ranges, while following a general plan of bright or warm colors for dense areas and dark or cool colors for light (empty) areas. The percent of the total pixel area of each seed representated by each color was found by counting the number of pixels of each color using a PIXAR II image computer.

The boundaries of the colors were fairly evenly-spaced, but adjusted to emphasize a difference in the seed which germinated. We chose to use the color red to indicate the density range which best correlated visually with germination potential. The background density was sufficiently low to be left uncolored, while all of the seed structure could be colored. The percentage of each color of each seed was obtained from pixel counts, and quantitatively related to germination potential. The image manipulation was done on a Raster Model One/80 graphics terminal (GS Computer Services, Inc., Nashua, NH), using the Sun 3/260 as a host. The software used was a portion of the Sterecon system described by Marko et al (2).

The pattern distribution is confirmed as lipid or free-water zones by magnetic resonance imaging after nuclear magnetic resonance analyses. Seed radiography is described with application examples by Vozzo et al (3, 4, 5, 6, 7, 8, 9, 10).

DISCUSSION

Water and air are natural radiopaque agents. That is, both are x-ray absorbent and therefore provide density on a radiograph. Even though each is requisite for seed germination, they are non-selective for viable and non-viable tissue. Additionally, seeds have numerous natural cavities which will accumulate both water and air. These are important factors because neither water nor air are then indicators of seed viability when imaged alone. Figures 1 and 2 show that significant amounts of water are imbibed by both viable and non-viable seeds. Fig. 1 is of Juglans nigra L. (Black Walnut) freshly collected and dry. Fig. 2 is of the same individuals radiographed after complete imbibition. Note that many walnuts appear full in Fig. 2 that do not in Fig. 1. The difference is water uptake. How then can good seeds be separated from nonviable seeds? An early false-coloring, density selective radiographic technique was xeroradiography. Using selenium activated aluminum, a positive or negative mode gave seeds another image perspective (Fig. 3). However, xeroradiography did not separate the nonviable seeds even though it did offer image advantages for morphology. False-color densities do separate seeds according to their viability. The walnuts in Fig. 4 represent an empty, a viable, and a nonviable image. Images A and D. are of the same individual. It is empty seed and will not germinate. However, images B and C appear full in black and white radiography and are expected to germinate. Actually, seed B germinated and seed C did not. There is a difference in their images after false-coloring (Figs. E and F respectively). Before false-coloring, the seeds can not be separated by radiography. Walnut is a practical specimen to illustrate image enhancement because it is relatively large and has an easily distinguished anatomy, i.e. embryo, cotyledon (stored food), and protective layerings.

Similar imaging for seven other tree seed species gave comparative results, viz false-coloring enhanced shades of gray density to separate seed which germinated from those which did not: Cornus florida L. (flowering dogwood), Fraxinus spp L. (ash), Liquidambar styraciflua L. (sweetgum), Liriodendron tulipifera L. (yellow-poplar), Magnolia grandiflora L. (southern magnolia), Pinus elliottii Engelm (slash pine), and Pinus taeda L. (loblolly pine). Each is seen as Figs. 5 - 11.

As represented in all species, the empty seeds have no red and are almost all green and yellow. The full seeds which germinated are predominanty red but may have also small areas of blue, green and yellow. Considering that even normal, healthy, viable seeds have tissue of low density, it is not unexpected to see the cool colors present. The absence of red in empty and poor seeds indicates a lack of heavier, denser tissue, requisite for initiating biochemical processes. Intermediate seeds are also evident as those individual within each Fig. 4 - 11 with an insignificant red present. These seeds may either germinate very slowly (as evidenced by germination rate), or not at all.

Germination trials showed that those individuals selected as being good seed were not only viable but vigorous, i.e. they had an extended primary root emerged from the seedcoat. Their germination rate was expressed simply by observing the number of seeds germinated every seventh day from their placement inside an incubating germination control box. The germination trial lasted 28 days.

The walnuts in Fig. 4, for example, show that images B and E represent viability while images C and F exhibit insufficient red to germinate. But what is the red representing?

Using magnetic resonance imaging (MRI) we can superimpose radiographs (conventional and false-colored) with MRI film and associate radiographic density and false-color red with proton presence in MRI. The proton presence is a combined imaging of free-water molecules and lipids. We think then, that the red false-color densities represent the distribution and percent of free-water and lipids necessary for germination. This is a valid biological assumption.

These findings have commercial application for separating substances by their inherent densities. Any matter with a radiopaque signature could be sorted by selecting an ejection device triggered by a color representing that signature. Theoretically, seeds or other matter could be introduced to a moving belt which passes under a radiographic beam. The beam projects sufficient energy to penetrate the subject and excite an image processor. A computer translates the subject's registered density from the radiographic receptor into spectral wavelengths of visible light. Pre-selected ranges of gray levels between 0 and 255 are assigned a visible light energy. Signals emitted from the color translators activate an ejection/selection gate along the moving belt which then channels each subject into its collector.

Commercial value is related to grading standards and quality control. A color-sorting system is currently available which can be mated to a radiographic survey unit. After determining the applicable density-color translations, this technique will sort and grade transparent, translucent, or opaque subjects unaffected by radiographic energies.

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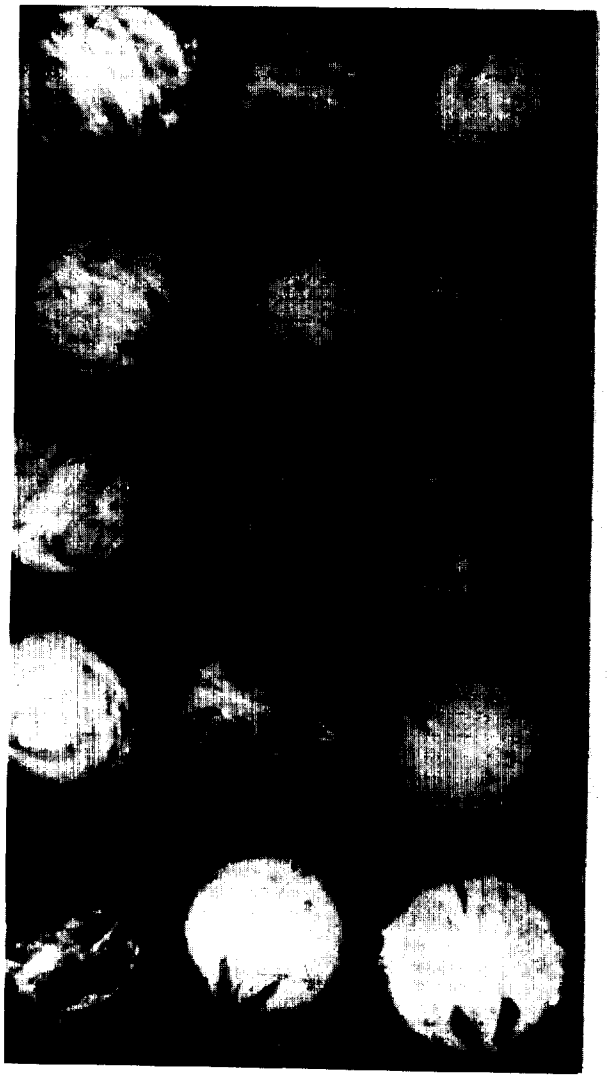

Figure 1. Conventional radiography of Juglans nigra seeds freshly collected. Note empty and full seeds.

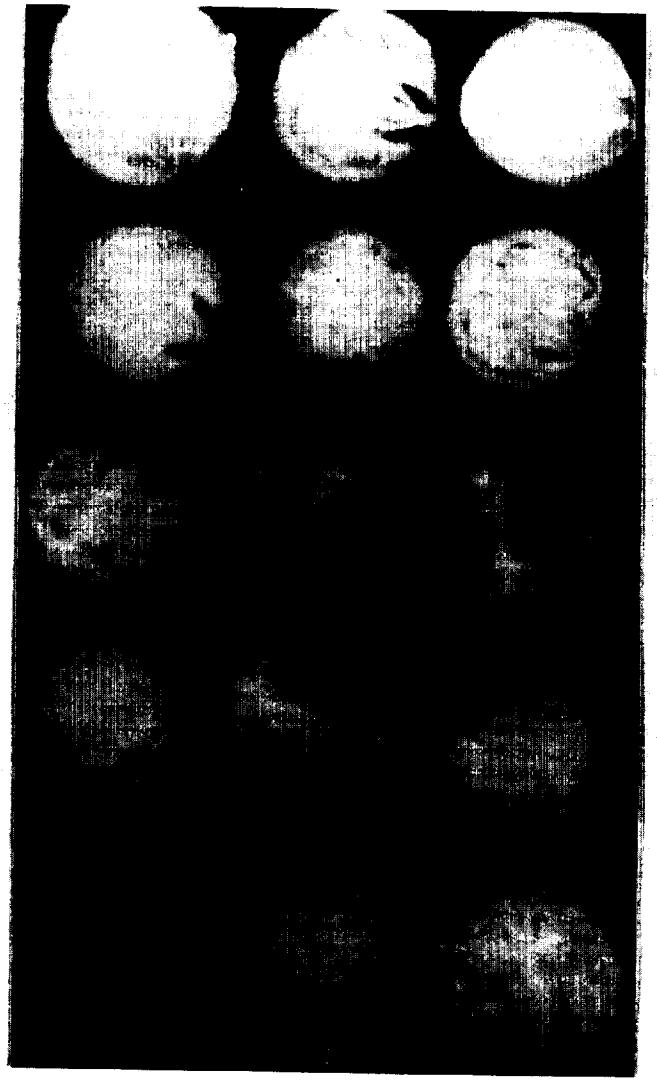

Figure 2. Same individuals as in Fig. 1, but after imbibition and water uptake. Note now that seeds appear full due to water density.

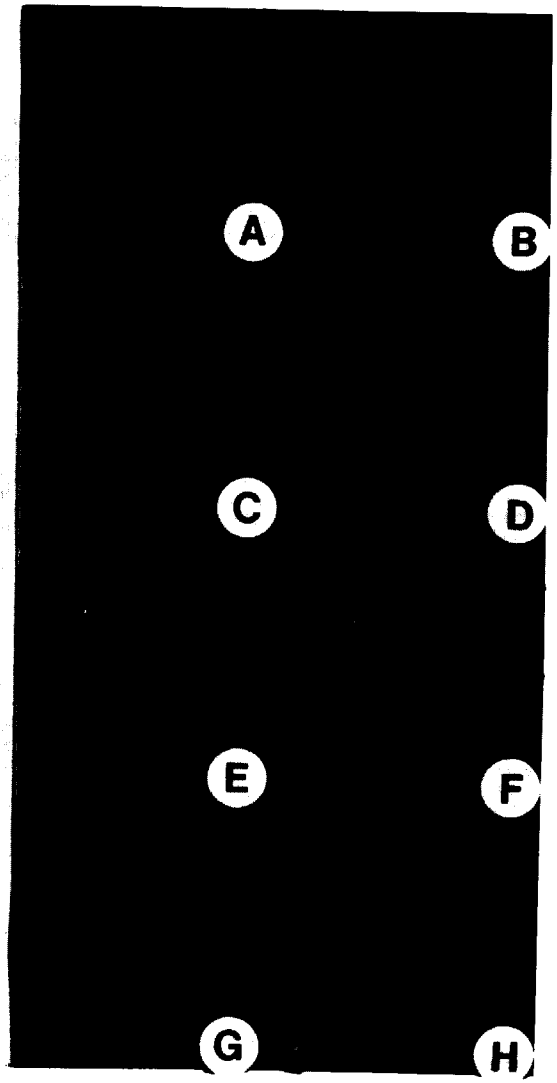

Figure 3. Xeroradiography of Juglans nigra seeds (C, D, E, F) as compared with conventional radiography (A, B, G, H). Note empty seeds in left column and full seeds in right column.

Figure 4. Juglans nigra seeds. Compare images A and D, B and E, and C and F of individual seeds. Only seed B, E germinated.

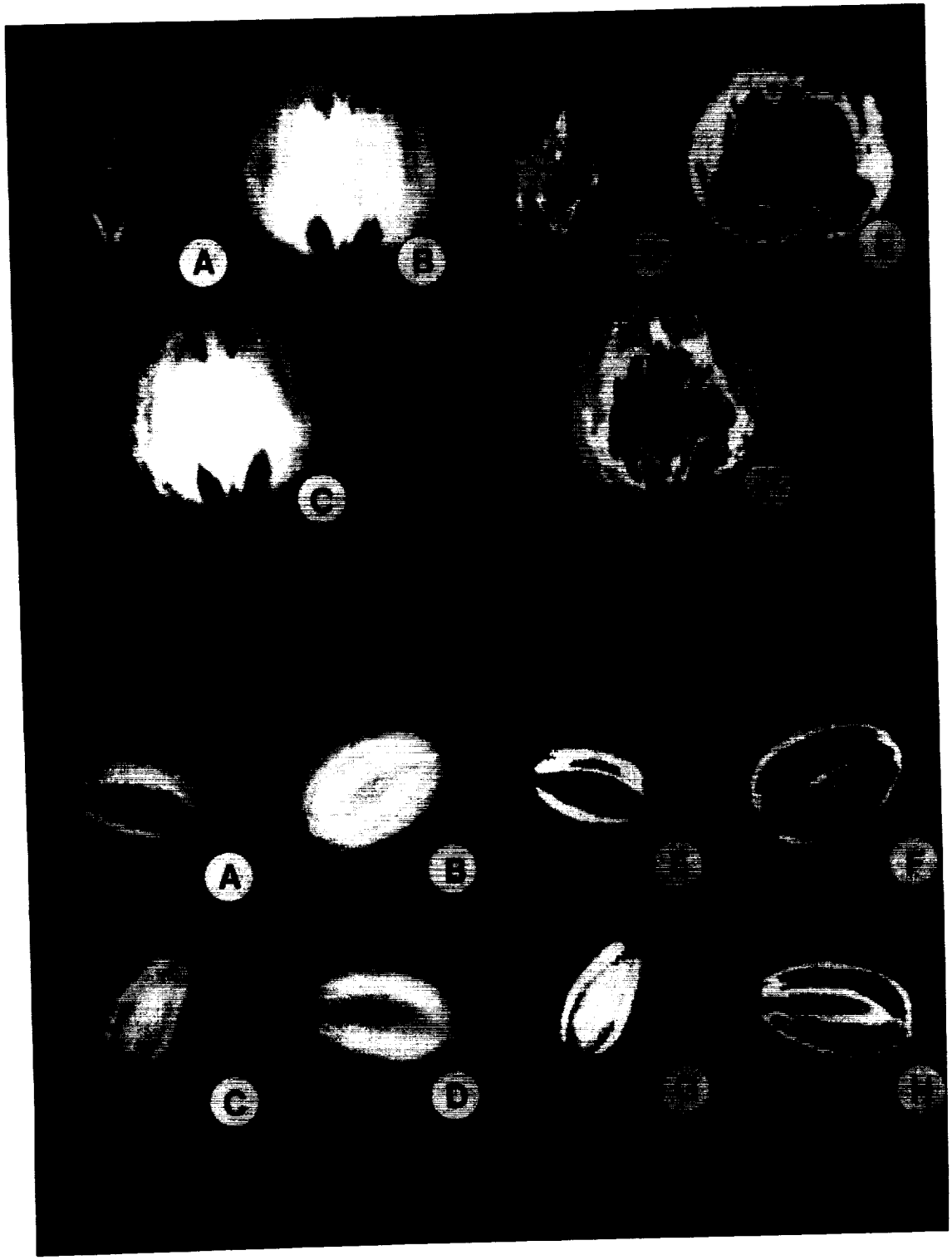

Figure 5. Cornus florida seeds. Individual B, F germinated.

Figure 6. Fraxinus spp seeds. Individuals C, I and E, K germinated.

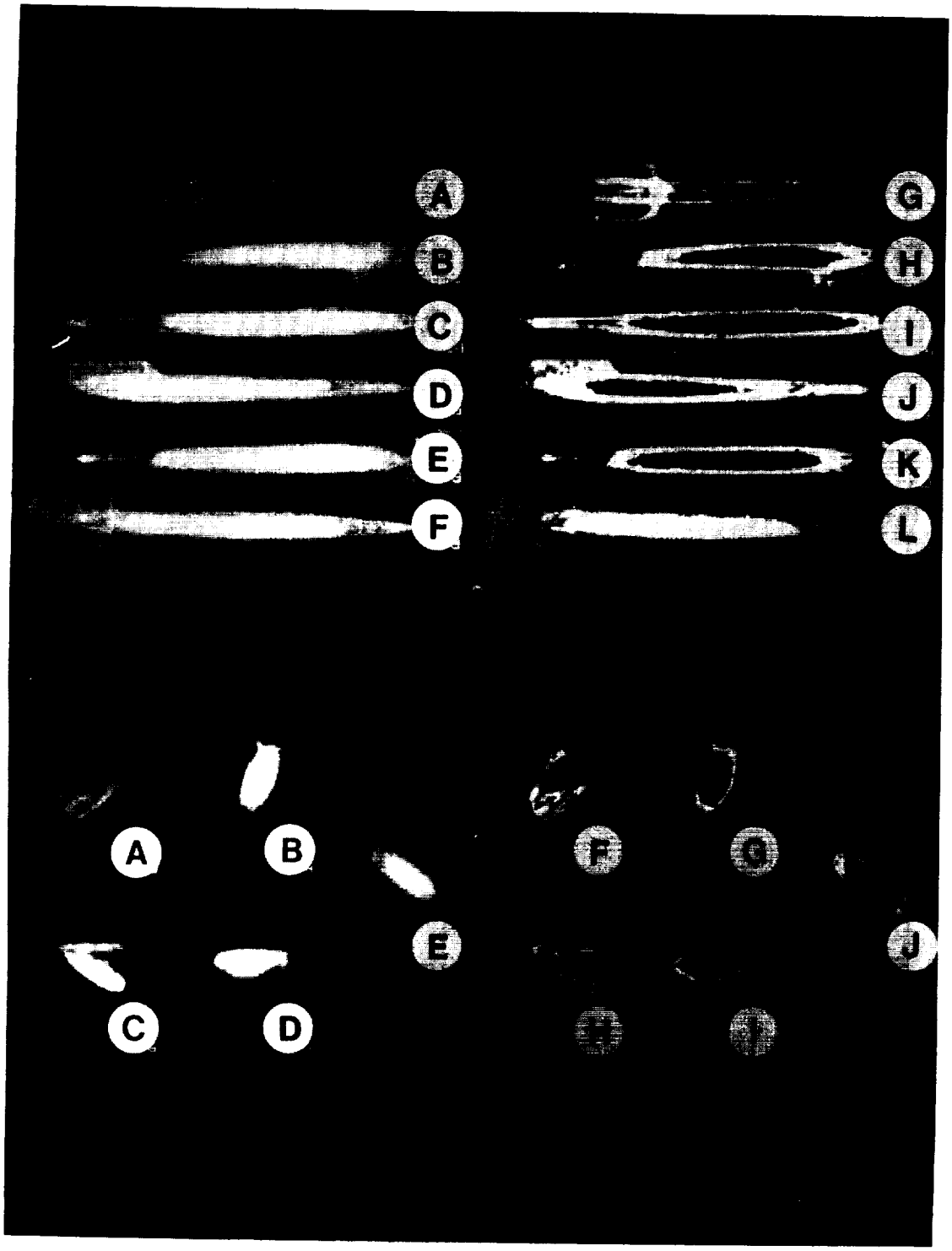

Figure 7. Liquidambar styraciflua seeds. Individuals D, I and E, J germinated.

Figure 8. Liriodendron tulipifera seeds. Individual D, H germinated.

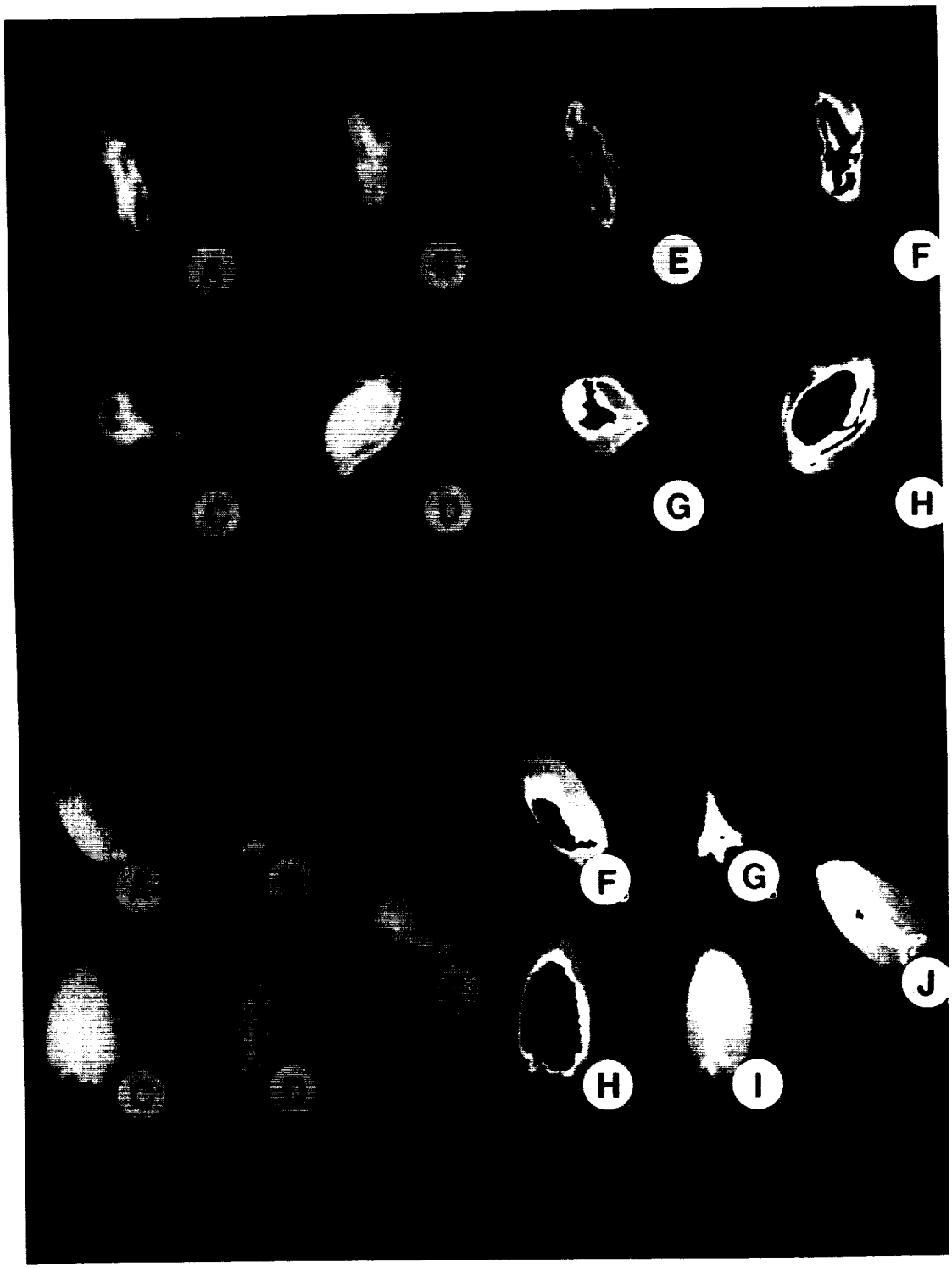

Figure 9. Magnolia gandiflora seeds. Individual C, H germinated.

Figure 10. Pinus elliottii seeds. Individual B, F germinated.

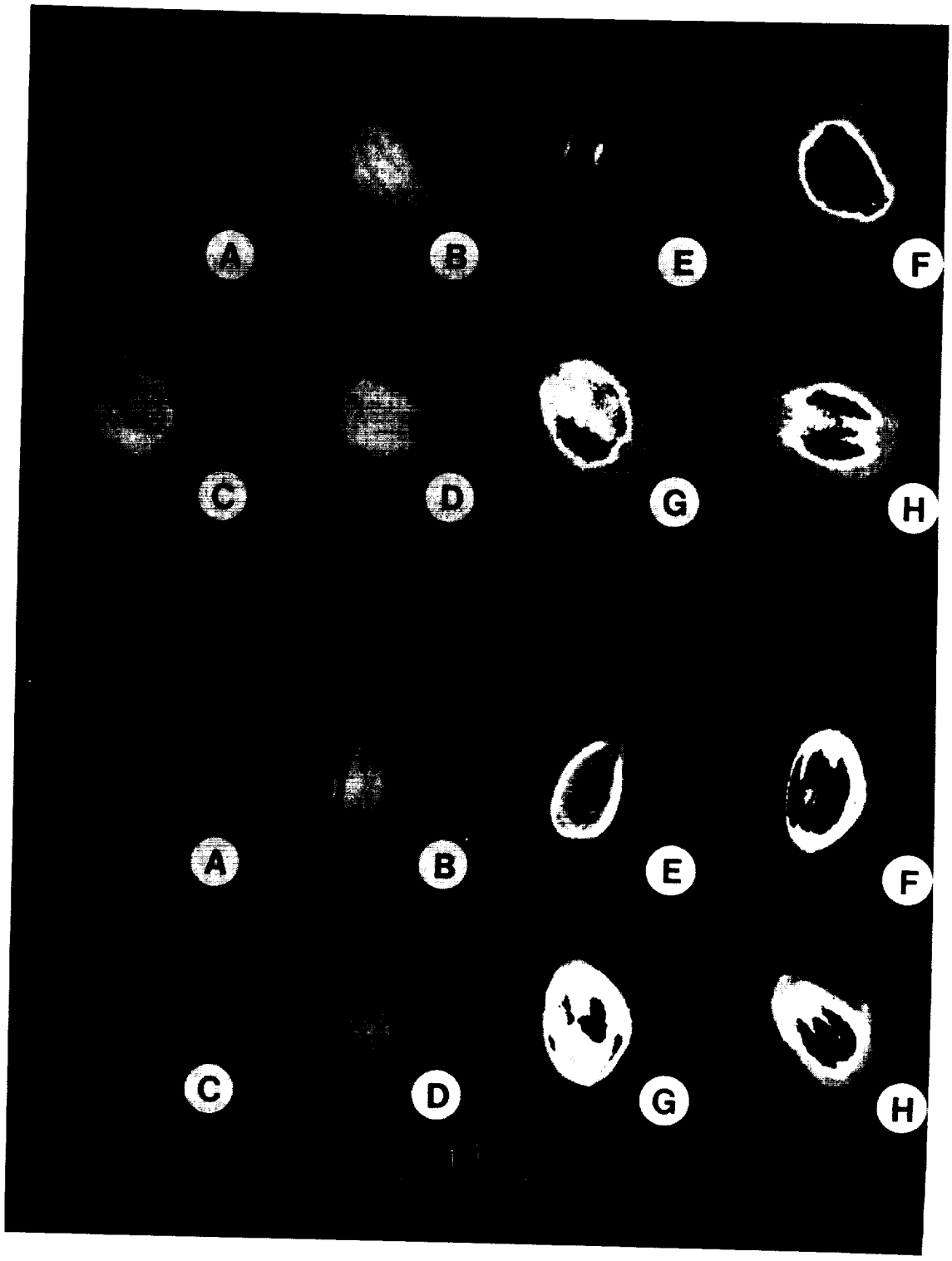

Figure 11. Pinus taeda seeds. Individual B, F germinated.