SEPARATING LIVE FROM DEAD LONGLEAF PINE SEEDS: GOOD AND BAD NEWS

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Abstract—Of all southern pine seeds, longleaf pine (*Pinus palutris* Mill.) are the most difficult to collect, process, treat, and store while maintaining good seed quality. As a result, interest in techniques for separating filled dead from live longleaf pine seeds has developed. The good news is that new technologies are becoming available to evaluate seed quality, but the bad news is that they seem to have limited application to longleaf pine. Tests suggest that incubating, drying, and separating, chlorophyll fluorescence, and near infrared techniques do not help improve longleaf pine seed quality. The incubating-drying-separating method is inefficacious because variability in the seed coat wing stub affects seed flotation. The chlorophyll fluorescence method measures changes in chlorophyll content as seeds mature or are damaged, but such changes do not seem to occur in pine seeds. The near infrared method seems to offer the best potential. The use of near infrared scanning technologies can determine changes in seed constituents, but we have not been able to determine which measurable seed constituents may change as viability declines.

INTRODUCTION

Techniques to improve seed quality, such as winnowing (commonly used for millennia), have as an ultimate goal the capability to separate filled dead from live seeds. Generally, those of us interested in improving the performance of tree seeds have followed the lead of scientists working with agricultural and horticultural seeds. We are working to identify methodologies for improving seed quality, which may address forestry needs.

For decades, the most effective means of improving southern pine seed quality has been to remove all unfilled seeds and then separate damaged or poorly developed seeds from filled seeds. Technology to accomplish these tasks has been based on seed flotation or the use of mechanical equipment. Aspirator and specific gravity table techniques work well for many tree species, but the most effective quality improvements have been achieved by density separation processing. Typically, poor viability is common in some lots of longleaf (*Pinus palustris* Mill.) and other southern pine species, so newer technologies are being sought to improve seed-lot performance. The increased demand for longleaf pine seeds over the last decade has reinforced the need for better separation technology. The primary use of containers for longleaf pine seedling production—and the resulting economic benefits of sowing only one filled seed per container cavity—necessitate techniques to cull filled but dead seeds. Of course, the ultimate goal is to achieve 100 percent germination. But is it possible to determine which of two seemingly identical filled seeds is dead, and which is alive?

This paper reports on a series of evaluations of longleaf pine seeds using newer technologies that potentially could improve seed quality. We considered three different approaches. The first is incubating-drying-separating (IDS) fluid separation. Certain IDS procedures have been used for a number of years, and IDS can help separate nonviable from viable seed lots of a number of coniferous species (Bergsten 1987, Downie and Wang 1992, Simak 1984). This methodology, however, has not been critically evaluated with longleaf pine seeds.

The second approach, chlorophyll fluorescence (CF), is used to measure plant photosynthetic health. Studies have found that CF scans can be used as a noninvasive method for determining seed maturity and quality. Jalink and others (1998) reported that CF scanning may be a new sorting technique to improve the quality of some species of agricultural seeds. This method has yet to be evaluated for sorting southern pine seeds.

Near infrared (NIR) spectroscopy is the third approach to evaluating seed quality. Williams and others (1985) found NIR a suitable, noninvasive technique to measure protein content of whole grains. Other applications of NIR technology continue to be developed for seed, plant, and forest products use. Portable equipment now available can scan seeds in milliseconds, making NIR attractive as a potential commercial technology.

Research on all three technologies is good news, because they provide possible ways to improve the quality of southern pine seeds. We present the results of our evaluations of each technology using longleaf pine seeds.

INCUBATING-DRYING-SEPARATING

The IDS process is based on the principle that water imbibed by live seeds is lost at a slower rate than water imbibed by dead filled seeds when both are subjected to uniform drying conditions. Ideally, seeds can then be separated in a liquid medium into a nonviable floating fraction and a viable sinking fraction based on the resulting density differences between the two fractions.

Previous studies of IDS technology have shown limited success with southern pine seeds. Karrfalt (1996) reported that his attempts using IDS to remove fungus-damaged seeds from slash pine (*P. ellliottii* Engelm.) failed completely. Donald (1985) achieved positive results, but with slash pine seeds in South Africa. His results indicated that the IDS technique is of little value for low-viability lots, but that separation of a better quality seed lot will improve its germination capacity.

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McRae and others (1994) studied IDS treatment of loblolly (*P. taeda* L.) and slash pine seeds and its effect on seed cost and efficiency in the nursery. They reported the IDS treatment was used successfully to separate filled-dead from filled-live seeds, but information was inadequate to determine if an economic advantage could be expected from the treatment. McRae and others (1994) wrote that the wing stub and seed size of longleaf made it difficult to evaluate seeds using IDS technology.

Our objective is to determine the optimal IDS and related technology to sort filled-dead from filled-live seeds of longleaf pine. We conducted a series of tests with three lots of longleaf pine seeds: South Carolina1992, Mississippi 1987, and Mississippi 2000. The application of IDS to the lots failed because of excessive and erratic flotation patterns caused by wing and seed coat characteristics (Creasey 2002). Further evaluations used a combination of Prevac (pressure vacuum system) and imbibitional density separation process (DSP).

Prevac is a treatment used to improve the quality of seeds from many tree species. It mechanically separates damaged seeds and debris in a lot by means of a partial vacuum. The vacuum forces water into damaged seeds, causing them to be heavier and sink when the vacuum is released. The DSP method is an alternative to IDS, but generally it is less reliable. It is a method of separating filled-dead seeds from filled-live ones by monitoring moisture uptake and establishing a sink/float relationship over time. Filled-live seeds sink, while weak, dead, or empty seeds remain floating at a predetermined cut-off time.

Incubation-drying-separating, Prevac, and imbibitional DSP treatments were not successful with longleaf pine seeds. Neither Prevac treatment for 30 or 60 seconds of vacuum at 27 inches mercury nor imbibitional DSP for 24, 43, or 60 hours provided any consistent improvement in seed performance (Creasey 2003) (fig. 1). As McRae and others (1994) found for IDS, the variability of the seed coat and its attached wing stub created flotation problems that prevented the successful application of these techniques.

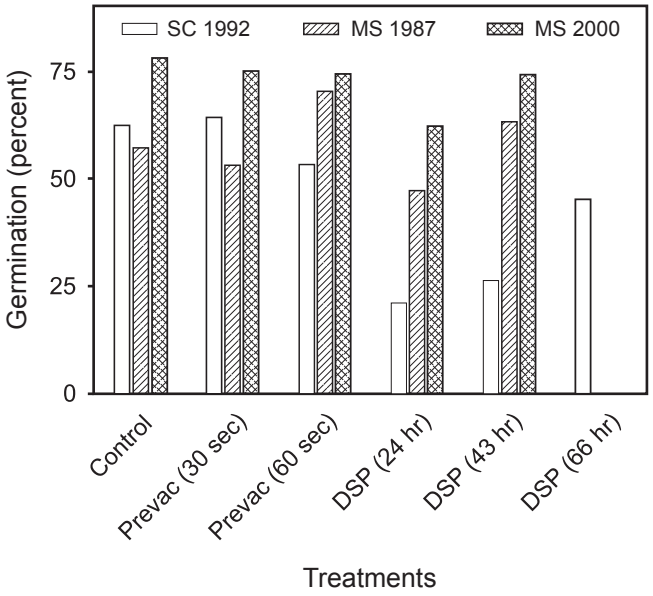

Figure 1—Germination of three lots of longleaf pine seeds subjected to no treatment, Prevac treatment for 30 seconds, and imbibitional density separation processing (DSP) for 24, 43, and 66 hours.

CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence is a nondestructive and instantaneous method to measure differences in plant function by assessing the magnitude of chlorophyll fluorescence signals. When chlorophyll molecules absorb light during photosynthesis, a small portion of that light is re-emitted, or fluoresced. Numerous studies have used CF to estimate photosynthetic efficiency, which is an indirect measure of plant stress (Adams and others 1990, Gentry and others 1989).

More recently, Ward and others (1995) and Steckel and others (1989) have used CF to estimate seed maturation. For many species, the amount of chlorophyll in the seed coat decreases during maturation, and for carrot (*Daucus carota* L.) and cabbage (*Brassica oleracea* L.) seeds, the change has been related to germination (Jalink and others 1998, Steckel and others 1989).

Because longleaf pine seeds have large embryos with considerable amounts of chlorophyll, we decided to evaluate CF as a method of sorting for viability improvement. Chlorophyll fluorescence was evaluated using Satake Corporation's SeedScan™ technology (Satake Corporation 2002). The SeedScan™ is a tabletop seed-by-seed maturity sorter that is designed to separate seeds based on their germination potential. The unit separates a seed lot into six fractions based on levels of chlorophyll fluorescence. We scanned individual longleaf pine seeds and tracked them through germination tests, enabling us to determine the relationship between the scanning spectra and germination. We evaluated several replications of 100 seeds each. Although CF is related to germination in some species, we could demonstrate no such relationship when scanning longleaf pine seeds.

NEAR INFRARED SPECTROSCOPY

Near infrared radiation is in the wavelength range of 780 to 2,500 nm, where 400 to 7,800 nm is visible light and above 2,500 nm is infrared. A commercial breakthrough for NIR spectroscopy came when it was shown that this technology could be used to determine the protein content in whole grains (Williams and others 1985).

Today, NIR technology is widely used not only in chemical, pharmaceutical, and food industries, but also in agriculture and wood technology (Downy 1985). The reason for the popularity of NIR spectroscopy is that little or no sample preparation is needed, saving both time and the cost of chemicals (Lestander 2003).

The main use of NIR spectroscopy within the field of seed science is quantifying seed moisture content and chemical constituents like proteins and oils (McClure 1994, Norris 1988). It is now being used as a quantitative tool that relies on chemometrics to develop calibrations relating reference analysis of the seed or plant material to that of the NIR optical spectrum. In other words, germination data have to be correlated to the measured spectrum on the same seeds.

Lestander (2003) has demonstrated the potential of using multivariate NIR spectroscopy for conifer seed classification.

He found that filled viable and nonviable Scots pine (*P. sylvestris* L.) seeds could be separated with an accuracy of < 95 percent.

We have evaluated NIR technology both in informal tests with U.S. Department of Agriculture Forest Service Forest Products scientists and more formally with Brimrose Corporation's Seed Meister™ system. Brimrose's Seed Meister™ AOTF-NIR spectrometer is specially designed for high-speed discrimination, quantification, and sorting of hybrid agricultural seeds (Brimrose Corporation 2002a). The spectrometer has a six-port sorter controlled and selectable by software and can evaluate 16,000 wavelengths (30 scans) per second. The NIR scanning technology can determine oleic and linoleic acid content in sunflower (*Helianthus* spp.) and protein and oil content of soybean (*Glycine* spp.) seeds (Brimrose Corporation 2002a, 2002b).

If the chemical composition of dead seeds were different from live ones, it would be feasible to use NIR methodology to sort them based on viability. We scanned individual seeds from several lots of 100 using NIR systems and had them germinate. We could not discern relationships among scanning spectra and germination potential for longleaf pine seeds.

SUMMARY

Three different technologies were evaluated to determine the feasibility of separating filled-dead from filled-live longleaf pine seeds. The results of the IDS tests were negative primarily because of the nature of the seeds. Portions of the seed coat wings are retained after seed processing, and the wing stubs vary markedly in both size and shape, making separation using flotation problematic. Although IDS is effective in sorting seeds of some tree species, the technology has shown little potential with longleaf pine. Even with other southern pine species, IDS is a complicated procedure that will be restricted to few commercial operations and may be hard to justify economically.

Chlorophyll fluorescence and NIR techniques are new approaches to evaluating seed quality. Both techniques are nondestructive and instantaneous and offer excellent opportunities for commercial application. Chlorophyll fluorescence depends upon changes in chlorophyll content within the seed coat. In some agricultural species, the technique effectively separates dead from live seeds, because chlorophyll content changes as the seed mature—normally declining as maturity is reached. Conifer seed physiology is such that these changes in chlorophyll content do not occur, so the content is similar in both dead and live mature seeds.

Near infrared technology would seem to offer the best potential for a fast, effective means of sorting seed. We think it logical that as viability in a seed is lost, the composition of some major biochemical component would change, although we have found no chemical change that relates to loss of viability. So, even though NIR has some potential for rapid sorting of seeds on a commercial scale, we have yet to find any relationship between scanning spectra and viability for longleaf pine.

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