ADVENTITIOUS SHOOT FORMATION ON EMBRYOS OF *PINUS PALUSTRIS* MILL., P. *ELLIOTTII* ENGELM. AND P. *PALUSTRIS* X *ELLIOTTII*.

Michael Stine and Harry E. Sommer
School of Forest Resources, UGA, Athens

Abstract. Adventitious shoot formation on the embryos of longleaf pine (P. *palustris* Mill.), slash pine (P. *elliottii* Engelm.) and their hybrid (P. *palustris* x *elliottii*) were compared. Using modified procedures of Reilly and Brown (1976), entire mature embryos were placed in a liquid media of Blaydes major salts, iron as Fe-EDTA, minor salts of Brown (Sommer and Brown, 1980), Doy's vitamins and 2% sucrose. The media was supplemented with napthaleneacetic acid at concentrations of 0.1, 0.01 and 0.001 mg./1. and 6-benzylaminopurine at concentrations of 1, 5, 10, 15 and 20 mg./1. The experimental design was a 3x5 factorial, with 20 explants of each species established for each hormone combination. The embryos were kept in the liquid media for ten days and then were transferred to a solid media of the following composition: major salts of Risser and White at half-strength, iron as Fe-EDTA, minor salts of Brown, Doy's vitamins, 1% sucrose and 0.6% Phytagar.

Adventitious shoots first appear on raised areas of the epidermis of the cotyledons of longleaf pine and the hybrid after 3-4 weeks in culture. Shoots form along the entire length of the cotyledon, with the fastest elongation of needles occurring on shoots located on the most distal portion of the cotyledon. With slash pine, most of the adventitious shoots form near the tips of the cotyledons, with only occasional formation elsewhere. A few of the slash pine embryos formed adventitious shoots on their hypocotyls, while longleaf pine and the hybrid did not. The experiment is still ongoing and at present it is not possible to identify the optimal concentrations of hormones for adventitious shoot initiation.

Scanning electron microscopy (SEM) was used to examine the organization and surface morphology of adventitious shoots. Samples were fixed in 2% glutaraldehyde/0.1 M cacodylate buffer, dehydrated with an ethanol series, critical point dried with CO_2 and coated with gold/palladium prior to viewing with a Cambridge Stereoscan Mark 2A. SEM showed the adventitious shoots of longleaf pine and the hybrid, to form on raised areas of the epidermis of the cotyledons, while in slash pine, shoot formation was not limited to raised areas. Stoma were found on the needles of adventitious shoots of longleaf and slash pine (the hybrids yet examined are younger, and stomata have not yet been found after 5-7 weeks in culture) after 11-12 weeks in culture. The stoma form in rows and appear to be occluded with epicuticular wax.

Literature Cited

Reilly, K. J. and C. L. Brown. 1976. In vitro studies of bud and shoot bud and shoot formation in Pinus radiata and Psuedotsuga menziesii. Georgia Forest Research Paper No. 86.

Sommer, H. E. and C. L. Brown. 1980. Embryogenesis in tissue cultures of sweetgum. For. Sci. 26: 257-260.