

Somatic Embryogenesis in Eastern and Carolina Hemlocks

P.M. Montello¹, D.G. Beleski¹, H.M. Smith¹ and S.A. Merkle¹

We have been working on a system for propagation and long-term preservation of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) via somatic embryogenesis and cryopreservation. Both species are threatened with extinction by the hemlock woolly adelgid (*Adelges tsugae*), an invasive insect that has now spread throughout the range of these two forest species. To optimize treatments for embryogenic culture initiation, we collected immature cones from four eastern and four Carolina hemlocks in Georgia and North Carolina four times during May through August, dissected the cones to obtain seeds at a range of developmental stages and cultured whole megagametophytes with embryos or embryos dissected from them on three different induction media. Over 2200 megagametophyte and zygotic embryo explants were cultured. Collection date and source tree had significant effects on induction of embryogenic callus from both species, which ranged as high as 52% for one collection of eastern hemlock. We also tested a cryopreservation protocol that we have previously applied to other embryogenic cultures for its applicability for hemlock embryogenic cultures. Three Carolina hemlock and two eastern hemlock cultures were pre-treated and cryostored using our standard procedure, testing the cryoprotectant dimethylsulfoxide (DMSO) at either 5 or 10 percent. All cryostored material of four of the five tested cultures recovered following cryostorage for over six months, regardless of DMSO treatment, but only 40 percent of samples from one eastern hemlock culture line recovered. While preliminary, our results demonstrate that cryostorage of embryogenic cultures is a feasible alternative for preservation of hemlock germplasm.

¹ Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602