Structural Adaptation in Bird-dispersed Whitebark Pine and Siberian Stone Pine Seeds

Tillman-Sutela, Eila [1]; Kauppi, Anneli [2]; and Tomback, Diana F.[3]

[1]Finnish Forest Research Institute, Muhos Research Unit, 91500 Muhos, Finland, e-mail eila.tillman@metla.fi
[2]University of Oulu, Department of Biology, P.O. Box 3000, 90014 Oulun yliopisto, Finland, e-mail anneli.kauppi@oulu.fi,
[3]University of Colorado at Denver and Health Sciences Center, Department of Biology, P.O. Box 173364, Denver, CO 80217-3364,USA, e-mail Diana.Tomback@cudenver.edu

The large and wingless seeds of *Cembrae* pines are dispersed by nutcrackers (Genus *Nucifraga*; Family *Corvidae*), which cache seeds in the soil during late summer and autumn. A high percentage of undeveloped seeds, irregularity of large cone crops, and lack of detailed studies on the anatomy of stone pine seeds hamper effective seedling production. We studied basic structures, maturity, and water imbibition by whitebark pine (*Pinus albicaulis* Engelm.) and Siberian stone pine (*Pinus sibirica* Du Tour) seeds, as well as structural changes during a 90-day multi-step treatment of whitebark pine seeds, using scanning electron (FESEM), transmission electron (TEM) and light microscopy (LM) and histological stainings. The thick and hard seed coat was permeable to water. However, the very small pits of the sclerotesta of the seed coat together with phenol compounds, particularly in both the pigmented sarcotesta and in the multilayered nucellar tissue inside the seed coat, hindered the entrance of stain molecules. These structures apparently constitute an essential protection against drying, if seeds are left uncovered, or against microbes and predators when seeds are cached in the soil. They also partly explain the existence of a *Cembrae* pine soil seed bank, which is a very unusual phenomenon among conifers. Other striking differences compared to many other conifer seeds were clustering of the large, thin-walled megagametophyte cells, and great accumulation of starch in both the megagametophyte and the embryo of the untreated and pre-treated seeds. Protein bodies of the embryo were in early development stages, whereas in the megagametophyte their stages varied. Lipids were in easily soluble form and therefore difficult to observe with LM. Lipid bodies were less abundant and smaller in the embryo than in the megagametophyte. Ultrastructurally, they formed a layer in the periphery of most embryo cells. The megagametophyte cells were packed with lipid bodies of variable form and sizes. Abundance of starch grains and soluble lipid bodies may facilitate removal of reserves needed for the physiological maturation and the structural differentiation of the embryos during the time when seeds are buried in the soil. Microscopy revealed enlargement of the moistened embryo at the beginning of the pre-treatment, and early structural differentiation of the embryo rather than actual growth through cell division and increasing cell numbers until seedling emergence. Although the seed coat was mature, our results indicated that the embryo and the megagametophyte were still immature. The morphological differences and variant chemical maturity of the seed structures may be an advanced

adaptation to bird-dispersal, and well-controlled pre-treatment simulating natural conditions should result in improved germination.