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ORIGINAL ARTICLE

Frost hardiness, carbohydrates and bud morphology of *Picea abies* seedlings after different lengths of freezer storage

JAANA LUORANEN[1], JOHANNA RIIKONEN[1], RISTO RIKALA[1] & SIRKKA SUTINEN[2]

[1]*Finnish Forest Research Institute, Suonenjoki Research Unit, Suonenjoki, Finland, and* [2]*Finnish Forest Research Institute, Joensuu Research Unit, Joensuu, Finland*

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Abstract
In Nordic conditions, conifer seedlings are frozen stored up to eight months. However, there is only little information on the extended freezer storage (more than six months) on frost hardiness (FH) and carbohydrate concentrations in the shoots of Norway spruce seedlings. FH of two-year-old Norway spruce [*Picea abies* (L.) Karst.] container seedlings were placed in storage on 23 October and kept in storage for 172, 193, 228 or 256 days. Bud structure and carbohydrate concentrations of the needles were analysed. DT_{50} (temperature causing 50% increase in proportion of needles, stem and buds after freezing exposure) of the measured parts of the seedlings decreased, and changes in DT_{50} of buds and stems were small between the 172nd and the 193rd day of freezer storage. The concentration of starch increased and the concentration of soluble sugars decreased during the first 172 days of freezer storage. Longer duration of freezer storage, however, did not cause any further changes. The first phase of bud development (pointed lower needle primordial) was already seen in the autumn. The bud development did not change during freezer storage but both the length of the primordial shoot and that of the whole bud increased from October through mid-July. This study suggests that Norway spruce seedlings can be frozen-stored safely for longer periods (eight to nine months) than they are generally stored for currently (six to seven months).

Keywords: *Bud development, carbohydrates, frost hardiness, freezer storage, Norway spruce,* Picea abies.

Introduction

It has been estimated that in North America and the Nordic countries, over half of all conifer seedlings produced are frozen stored (Colombo, 1996; Rikala & Konttinen, 2008). In Nordic conditions, seedlings are generally placed in freezer storage in October or November. Planting of these seedlings is possible not until May and June next year, indicating that seedlings are frozen stored for several months, up to eight months.

In autumn, the seedlings are usually placed in freezer storage after a benchmark frost hardiness (FH) level has been exceeded (O'Reilly et al., 2000). This benchmark FH level varies from one area to another. In Finland, freezer storage generally starts around mid-October, when FH of the seedlings is well below -20°C (Luoranen et al., 2009). Long-term freezer storage may have some adverse effects on seedling physiology such as drought stress and consumption of carbohydrate reserves for maintenance respiration (Camm et al. 1994; Jiang et al., 1994; Ögren, 1997; Ritchie, 1982;). These changes may cause reduced photosynthetic recovery (Jiang et al., 1995) and reductions in growth and stress resistance of the seedlings after planting (Jiang et al., 1994; L'Hirondelle et al. 2007; Ritchie, 1982; Wang & Zwiazek, 1999). In some experiments, FH of conifer species has been gradually lost during freezer storage (Jacobs et al., 2008b; Omi et al., 1991; Ritchie, 1982; Ritchie et al., 1985;). Soluble sugars contribute to FH by acting as osmolytes, compatible solutes and energy reserves (Guy et al., 2008). Consequently, it is often assumed that the loss of FH is related to the reduction in the non-structural carbohydrate concentrations during freezer storage (Chomba et al., 1993; Jiang et al., 1994; Ritchie, 1982). However, the experimental evidence on this matter is scarce.

The aim of this study was to examine how freezer storage up to nine months affects FH, morphology,

Correspondence: Jaana Luoranen, Finnish Forest Research Institute, Suonenjoki Research Unit, Juntintie 154, FI-77600, Suonenjoki, Finland.
E-mail: jaana.luoranen@metla.fi

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