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## Induced frost tolerance in leafy spurge roots: Changes in carbohydrate metabolism

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The resistance of leafy spurge (*Euphorbia esula* L.) to conventional weed control practices is due primarily to an extensive perennial root system that is well adapted to survive many different chemical, biological and environmental stress situations, including the harsh winters of the Northern Great Plains.

If the biochemical and physiological mechanisms that enable leafy spurge roots to acquire and maintain tolerance to freezing were understood, alternative control strategies, in addition to herbicide treatments and/or the use of biological control agents, might be developed that would reduce the ability of the root system to overwinter and effectively limit the plant's aggressive pattern of vegetative regrowth and infestation. Laboratory studies with 2-month-old greenhouse-grown leafy spurge plants showed that the tolerance of the roots to freezing at -7°C for 1 week was induced over a period of 3 or 4 weeks of acclimation at 4°C. Cold-induced frost tolerance in roots was marked by a rapid reduction in reserve starch levels and a corresponding increase in soluble carbohydrates. After a cold treatment for 4 weeks, starch levels were reduced over 50% and soluble carbohydrate levels increased over 4-fold.

Analysis of the soluble carbohydrates showed that sucrose levels increased more than 6-fold after 4 weeks of cold acclimation and represented over 84% of the total soluble carbohydrates in the roots. In contrast to sucrose, glucose levels were less than 7% of the total soluble carbohydrates and increased to only a limited extent after 4 weeks of cold acclimation. Even though endogenous sucrose concentrations increased dramatically during the induction of frost tolerance, concentrations were not high enough by themselves to provide effective cryogenic protection against freezing injury at -7°C.

The rapid loss of starch reserves in roots during the induction of frost tolerance was associated with a 3-fold increase in  $\alpha$ -amylase activity and a 2-fold increase in phosphorylase activity. Important enzymes responsible for sucrose synthesis were UDPglucose pyrophosphorylase and sucrose synthase. The activity of these two enzymes together with differential rates of starch hydrolysis and the absence of measurable invertase activity in both control and cold-induced roots may account for the observed accumulation of sucrose during the development of frost tolerance.

Even though a direct relationship between frost tolerance and carbohydrate metabolism was not established in these studies, key enzymes responsible for starch hydrolysis and sucrose synthesis may indirectly affect the establishment of frost tolerance and serve as possible early targets for biochemical regulation and reduced winter survival of the plant.