Environmental and Experimental Botany 72 (2011) 358–367

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Quantitative and qualitative changes in carbohydrates associated with spring deacclimation in contrasting *Hydrangea* species

Majken Pagter[a,*], Isabelle Lefèvre[c], Rajeev Arora[b], Jean-Francois Hausman[c]

[a] Department of Horticulture, Aarhus University, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark
[b] Department of Horticulture, Iowa State University, Ames, IA 50011, USA
[c] Department EVA Environment and Agrobiotechnologies, Centre de Recherche Public-Gabriel Lippmann, 41, rue du Brill, 4422 Belvaux, Luxembourg

ARTICLE INFO

Article history:
Received 2 October 2010
Received in revised form 21 February 2011
Accepted 22 February 2011

Keywords:
Climate change
Freezing tolerance
1-Kestose
Soluble sugars
Water status

ABSTRACT

Cold deacclimation and associated changes in soluble carbohydrates and water status of two *Hydrangea* species differing in susceptibility to frost injuries was followed under natural conditions. In fully cold hardy plants of *H. macrophylla* stem freezing tolerance fluctuated in parallel with changes in air temperature, while in a seasonal perspective increased temperatures caused a sigmoid deacclimation pattern in both *H. macrophylla* and *H. paniculata*. Timing of deacclimation was approximately synchronized in the two species, but *H. paniculata*, the hardier species based on mid-winter hardiness, deacclimated faster than *H. macrophylla*, indicating that deacclimation kinetics were not correlated with mid-winter hardiness. In both species concentrations of soluble sugars decreased during deacclimation and were highly correlated with stem cold hardiness and air temperatures. This suggests that sugar hydrolysis may be an important temperature-driven mechanism of deacclimation in *Hydrangea*. Accumulation patterns of specific carbohydrates differed between the two species, suggesting that they utilize different strategies to overcome cold. In *H. paniculata*, deacclimation was associated with an increase in stem water content, which occurred shortly before bud burst and hence may be a prerequisite for leafing out.

1. Introduction

In temperate perennials cold hardiness is a seasonal process synchronized with the external seasonal changes in temperature and photoperiod. In autumn plants cold acclimate, whereby they become increasingly tolerant to subzero temperatures. Maximum hardiness is reached midwinter, and in spring plants loose acclimated cold hardiness by deacclimation (Weiser, 1970). Successful over wintering therefore not only requires sufficient maximum freezing tolerance, but also proper timing and rates of acclimation and deacclimation (Suojala and Lindén, 1997). Seasonal timing of acclimation and deacclimation may be an important trait affecting mortality, growth and quality of introduced crops cultivated far from their origin. Studies of the kinetics (timing and rates) of cold acclimation and deacclimation in response to the critical environmental stimuli are additionally much needed, given current predictions of climate change, where growing conditions may favour an altitudinal and poleward shifts in vegetation (Hughes, 2000; Parmesan, 2006).

Parallel to cold acclimation in autumn, temperate-zone woody perennials form terminal buds and develop dormancy (Rohde and Bhalerao, 2007). Despite favourable growth conditions dormancy often inhibits or prevents growth and deacclimation (Kalberer et al., 2006); risk of untimely deacclimation is, therefore, a concern for plants that are no longer dormant. Cold deacclimation is strongly dependent on temperature and can occur much faster than cold acclimation (Leionen et al., 1997; Taulavuori et al., 1997; Kalberer et al., 2007). Previous studies indicate that the rate of deacclimation is not a linear response but may change as deacclimation progresses. In addition, the rate and/or timing of deacclimation may vary between species, cultivars, ecotypes etc., demonstrating genetic variability for deacclimation kinetics and genetic adaptation to the local climate (Leinonen et al., 1997; Suojala and Lindén, 1997; Kalberer et al., 2007).

Regulation of cold acclimation in the autumn and the underlying physiological, biochemical and molecular responses have been extensively studied (Benedict et al., 2006; Welling and Palva, 2006). Less is known about the process of deacclimation (Kalberer et al., 2006). A close association has been established between accumulation of soluble carbohydrates and acquisition of cold tolerance in the autumn (Wanner and Junttila, 1999; Cox and Stushnoff, 2001), whereas the importance of alterations in carbohydrate metabolism in deacclimation is less clear. Some studies have found a correlation between decreasing sugar concentrations and the loss of cold hardiness, and suggested a mechanistic role of carbohydrate catabolism in deacclimation (Svenning et al., 1997; Tinus et al., 2000). In contrast, others have noted a decline in soluble carbohy-

* Corresponding author. Tel.: +45 89993388; fax: +45 89993496.
E-mail addresses: majken.pagter@agrsci.dk (M. Pagter), rarora@iastate.edu (R. Arora), hausman@lippmann.lu (J.-F. Hausman).

0098-8472/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2011.02.019

drates preceding a loss of cold hardiness and, therefore, suggested no unequivocal relationship to spring-deacclimation (Sauter et al., 1996; Lennartsson and Ögren, 2004). To withstand subfreezing temperatures perennials employ two major strategies; supercooling (freeze avoidance) and extracellular freezing (freeze tolerance). Both freezing resistance mechanisms have been associated with accumulation of carbohydrates. In freezing tolerant tissues the protective function of sugars has been ascribed to their ability to stabilize membranes and proteins during freeze-induced dehydration (Crowe et al., 1998; Minorsky, 2003). In deep supercooling cells sugars are believed to aid in depressing the nucleation temperature (Kasuga et al., 2007). In stems of woody plants cortical tissues are strictly non-supercooling, while xylem ray parenchyma cells may exhibit either strategy (Quamme et al., 1972; Karlson et al., 2004). Seasonal changes in soluble carbohydrates in woody perennials have been analyzed in many studies. However, most of these studies have focused on intact stems (Cox and Stushnoff, 2001; Pagter et al., 2008), xylem tissue (Sauter and van Cleve, 1994; Sauter et al., 1996) or more seldom bark tissue (Thomas et al., 2004). Little is known about seasonal changes in soluble carbohydrates in both tissue types, including whether the type and/or concentration of specific sugars differ depending on how the tissues are adapted to freezing (Kasuga et al., 2007).

Hydrangeas are popular flowering shrubs, widely used and commercially important in landscape horticulture. *Hydrangea macrophylla* is native to Japan (McClintock, 1957) and thrives in maritime regions, but grows and flowers in most temperate regions where it is not damaged by cold temperatures. However, even in the relatively mild climate of Denmark frost-injury/winter-kill of current year shoots is a common problem in its cultivars. In contrast, *H. paniculata* is much less susceptible to frost (Suojala and Lindén, 1997; Pagter et al., 2008). Insufficient mid-winter hardiness may account for some of the frost injuries encountered in *H. macrophylla*, but likely late acclimation in fall and/or premature deacclimation in spring also limit successful cultivation of *H. macrophylla* (Adkins et al., 2003). Seasonal changes in cold hardiness of *H. macrophylla* and *H. paniculata*, determined on a rough time-scale, have previously been correlated with variation in a limited number of soluble carbohydrates in intact stems (Pagter et al., 2008). Recently we additionally examined the timing and rate of deacclimation of *H. macrophylla* and *H. paniculata* under simulated warm spell conditions (22 °C/17 °C day/night) (Pagter et al., 2011). Constant warm temperatures, however, may not adequately reflect the deacclimation conditions experienced under natural conditions, where continuously varying temperatures exist and where deacclimation presumably is much slower. Hence, the present study was conducted to address the following two main questions: (1) Which characteristics (if any) of deacclimation kinetics are important for *Hydrangea* to avoid frost injuries in spring? (2) Are soluble carbohydrates involved in controlling the rate and degree of deacclimation in *Hydrangea* stems and does the accumulation of soluble carbohydrates differ between bark- and xylem tissues? The hypotheses are: Re (1) susceptibility of *Hydrangea* to frost injuries during spring-deacclimation depends on the timing and rate of deacclimation in response to increasing temperatures. Re (2) genotype-specific differences in cold hardiness during deacclimation are due to variability in alterations in carbohydrate metabolism.

2. Materials and methods

2.1. Location, climate and plant material

The experiment was carried out using three-year-old vegetatively propagated and commercially produced *Hydrangea macrophylla* ssp. *macrophylla* (Thunb.) Ser. cv. Alma and *Hydrangea paniculata* Sieb. cv. Vanille Fraise plants grown in 5-L pots containing sphagnum peat. For each species 45 plants were purchased (early January 2009), at which time the plants had been maintained outside (Gunnar Christensen's Nursery, Denmark, latitude 55°26′N) since spring 2008. Hence, the plants had undergone cold hardening under natural conditions. Following delivery the plants were maintained outside (Department of Horticulture, Aarhus University, Denmark, latitude 55°18′N) where the pots were buried in the soil to avoid root frost injuries and to facilitate plants' removal and moving when needed. Local air temperature and day length data were obtained from the department's climate station, which is operated by the Danish Meteorological Institute. The winter and spring months of 2009 were relatively warm, with the lowest air temperature of −7.9 °C being recorded in the beginning of January (Fig. 1). Daily minimum air temperatures were below zero on 39 days, with the last freezing event occurring in the beginning of April. The daily maximum air temperature dropped below the freezing point on two occasions, implying that the plants did not experience extended periods of freezing temperatures. Rather the temperatures fluctuated around ca. 0 °C in the months of January and February, around ca. 5 °C in March and around ca. 10 °C in April and May. In January and February small differences between day and night temperatures (i.e. daily maximum and minimum temperatures) were observed, whereas in March and particularly April and May this difference increased.

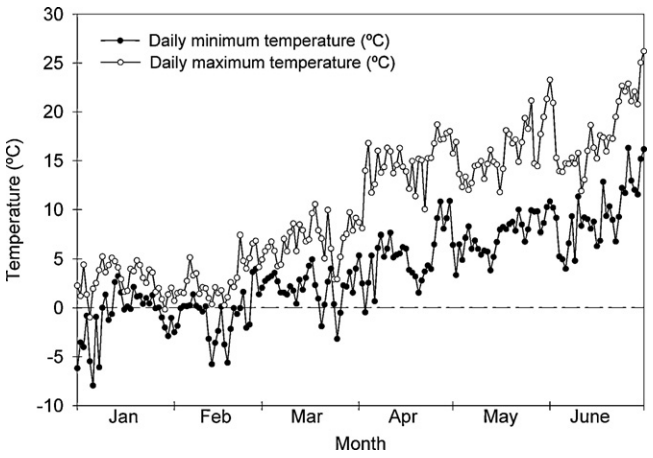

Fig. 1. Minimum and maximum daily air temperatures (°C) at the experimental site from January to July 2009.

Before initiation of the experiment it was verified that both species had emerged from dormancy. Dormant status (or lack thereof) was estimated by moving potted plants into a greenhouse and inducing bud-break at 20 °C day/night, 18-h photoperiod and 100-300 μmol m^{-2} s^{-1}. The majority of buds broke within two and three weeks in *H. macrophylla* and *H. paniculata*, respectively, implying that dormancy had been broken in both species. Measurements and sampling of current year shoots were carried out ca. every two weeks from January 2009 to June 2009. Samples were randomly collected from five plants per species.

2.2. Determination of cold hardiness

Freezing tolerance was determined on one control (4 °C) and six subfreezing temperatures using the electrolyte leakage method. One (*H. macrophylla*) or two (*H. paniculata*) 3-cm-long pieces of internodal stem tissue were rinsed under cold running tap water for 15 s and then under cold running demineralised water for 15 s. After rinsing the samples were placed in 70-mL test tubes containing 100 μL of demineralised water (to initiate ice formation), and samples were placed in a temperature-controlled