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Seasonal profiles of leaf ascorbic acid content and redox state in ozone-sensitive wildflowers

Kent O. Burkey [a,*], Howard S. Neufeld [b], Lara Souza [c], Arthur H. Chappelka [d], Alan W. Davison [e]

[a] *Plant Science Research Unit, USDA-ARS and North Carolina State University, 3127 Ligon Street, Raleigh, NC 27607, USA*
[b] *Appalachian State University, Boone, NC, USA*
[c] *Department of Ecology & Evolutionary Biology, University of Tennessee, Knoxville, TN, USA*
[d] *Auburn University, Auburn, AL, USA*
[e] *University of Newcastle, Newcastle, England, UK*

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Wildflower species exhibit differences in ascorbic acid content and redox status that affect ozone sensitivity.

Abstract

Cutleaf coneflower (*Rudbeckia laciniata* L.), crown-beard (*Verbesina occidentalis* Walt.), and tall milkweed (*Asclepias exaltata* L.) are wildflower species native to Great Smoky Mountains National Park (U.S.A.). Natural populations of each species were analyzed for leaf ascorbic acid (AA) and dehydroascorbic acid (DHA) to assess the role of ascorbate in protecting the plants from ozone stress. Tall milkweed contained greater quantities of AA (7–10 μmol g^{-1} fresh weight) than crown-beard (2–4 μmol g^{-1} fresh weight) or cutleaf coneflower (0.5–2 μmol g^{-1} fresh weight). DHA was elevated in crown-beard and cutleaf coneflower relative to tall milkweed suggesting a diminished capacity for converting DHA into AA. Tall milkweed accumulated AA in the leaf apoplast (30–100 nmol g^{-1} fresh weight) with individuals expressing ozone foliar injury symptoms late in the season having less apoplast AA. In contrast, AA was not present in the leaf apoplast of either crown-beard or cutleaf coneflower. Unidentified antioxidant compounds were present in the leaf apoplast of all three species. Overall, distinct differences in antioxidant metabolism were found in the wildflower species that corresponded with differences in ozone sensitivity.
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Keywords: Antioxidant capacity; Ascorbic acid; Foliar injury; Great Smoky Mountains National Park; Native plants; Ozone

1. Introduction

Ascorbic acid (AA) is a key metabolite of antioxidant systems that protect plants from reactive oxygen species (ROS) formed as part of normal metabolism in the chloroplast and mitochondria or during periods of environmental stress associated with ozone exposure (Runeckles and Chevone, 1992; Smirnoff, 1996; Conklin and Barth, 2004). Key elements of the ascorbate mechanism include the synthesis of sufficient quantities of AA (Smirnoff et al., 2001), and adequate ascorbate–glutathione cycle capacity (Noctor and Foyer, 1998) which converts the dehydroascorbic acid (DHA) formed by antioxidant reactions into AA that can be re-utilized as an antioxidant metabolite.

While synthesized inside leaf cells, AA is present in the leaf extracellular space in many plant species (summarized in Burkey et al., 2003). The localization of AA in the leaf apoplast is thought to involve specific plasma membrane carriers that transport AA and DHA between the cytoplasm and leaf extracellular space (Horemans et al., 2000). In the case of ozone stress, AA in the leaf apoplast represents a third element of oxidative stress response (Chameides, 1989; Plöchl et al.,

* Corresponding author. Tel.: +1 919 515 1620; fax: +1 919 856 4598.
E-mail address: koburkey@unity.ncsu.edu (K.O. Burkey).

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2000; Turcsanyi et al., 2000) with the potential to scavenge ROS formed as products of ozone breakdown, protecting plasma membrane components and preventing the initiation of responses (Rao and Davis, 2001) that lead to ozone injury. Formation of ROS in the leaf cell wall following ozone exposure is a complex process. Initially, ozone decomposes in the leaf apoplast and ROS accumulate. In ozone-sensitive plants, initial ROS formation from ozone breakdown is followed by a plant-derived secondary oxidative burst in the form of hydrogen peroxide or superoxide depending on the species (Pellinen et al., 1999; Wohlgemuth et al., 2002). The secondary oxidative burst is initially localized in the leaf apoplast, and later expands into the cytoplasm and sub-cellular compartments leading to the formation of visible lesions (Pellinen et al., 1999). Potentially, ascorbic acid could serve as either a direct chemical scavenger of ozone in the apoplast, although this mechanism has been questioned (Jakob and Heber, 1998), or as a substrate for extracellular enzymes (e.g. ascorbate peroxidase) that attenuate ROS levels and thus affect the propagation of the initial ozone signal. Changes in leaf extracellular ascorbate content and redox status have been observed in ozone-treated plants (Castillo and Greppin, 1988; Luwe and Heber, 1995; Burkey, 1999), evidence that extracellular ascorbic acid is involved in detoxification of ozone and related ROS. Recent studies have found higher levels of AA in the leaf apoplast of ozone-tolerant genotypes of *Phaseolus vulgaris* (Burkey et al., 2003) and *Plantago major* (Zheng et al., 2000) compared with sensitive lines, suggesting that localization of AA in the cell wall is important.

A number of studies have examined the relationship between ozone sensitivity and the ascorbate content and redox status in leaves. The most convincing evidence that ascorbate plays a critical role in plant response to ozone stress is found in the *vtc1* mutant of Arabidopsis (Conklin et al., 1996) where a decrease in AA content is associated with increased ozone sensitivity. While it is clear that minimum levels of AA are required, the concentration of this antioxidant metabolite that naturally occurs in leaf tissue is not always well correlated with ozone tolerance (Guzy and Heath, 1993; Wellburn and Wellburn, 1996; Burkey et al., 2000). In general, these studies have focused on cultivated plants and selected tree species with less attention given to natural vegetation.

Great Smoky Mountains National Park (GRSM) encompasses an area greater than 206,000 ha in the states of Tennessee and North Carolina, and contains a wide diversity of plant and animal species characteristic of a large portion of the eastern U.S.A. There are more wildflower species in GRSM than in any other National Park (Shaver et al., 1994). Over one-half of the old-growth forest in the eastern U.S.A. is found in the Park, and greater than three-fourths of the spruce–fir ecosystem in the southern Appalachians is located in GRSM. Therefore, any detrimental effects to the vegetation are of major concern for the entire region (Shaver et al., 1994). Studies in GRSM have identified ambient ozone as an important stress factor. Ozone-sensitive, native wildflower species have been identified in the Park and species differences and seasonal trends in ozone-induced foliar injury have been documented (Neufeld et al., 1992; Chappelka et al., 1997, 2003). Given the episodic nature and unpredictability of ambient ozone episodes, plant response to ozone stress may depend on the capacity to maintain leaf antioxidant systems throughout the season. The objective of this study was to assess seasonal patterns of ascorbate pool size and redox status in leaves from natural populations of three wildflower species to determine possible relationships with ozone-induced foliar injury.

2. Materials and methods

2.1. Plant material and injury assessment

The wildflowers utilized in this study were randomly selected from natural stands in Great Smoky Mountains National Park. Species and locations included tall milkweed (*Asclepias exaltata*) at Mt. Sterling Gap, NC [Lat: 35°42′01″N, Long: 83°05′52″W, elevation 1525 m]; cutleaf coneflower (*Rudbeckia laciniata* L. var. *laciniata*) at Clingmans Dome, TN [Lat: 35°33′46″N, Long: 83°30′04″W, elevation 2015 m]; and crown-beard (*Verbesina occidentalis* Walt.) near (<2 km) the Twin Creeks Natural Resource Center in Gatlinburg, TN [Lat: 35°41′17″N, Long: 83°29′53″W, elevation 572 m]. Plants were assessed on three dates during the summer of 2001: June 12–14, July 10–12, and August 7–9.

Plants were selected and tagged during the June sampling and the same individuals assessed again in July and August. For tall milkweed, nine ozone-insensitive and 11 ozone-sensitive individuals identified the previous year (Souza et al., in press) were tagged in June when plants consisted of four to six leaf pairs. One leaf from leaf pair #2 above the base of the plant was analyzed in June and again in July with a leaf from leaf pair #3 analyzed during the August harvest. For cutleaf coneflower, 10 individuals were tagged in June within each of two populations at Clingmans Dome. The two populations were physically located either on or off the paved trail (Chappelka et al., 2003) and have been shown previously to be genetically distinct (Davison et al., 2003). The cutleaf coneflower study was initiated by analyzing the fourth leaf below the apical bud in June with leaves located one and two nodes above the original leaf analyzed during July and August, respectively. For crown-beard, six individuals were tagged and the fourth leaf pair below the apical bud was analyzed in June with leaves located one and two nodes above the original leaf analyzed during July and August, respectively.

A six point scale was used to quantify relative severity of symptoms on injured leaves (classes = 0%, 1–6%, 7–25%, 26–50%, 51–75%, and 76–100%). The mid-point of each class was used to calculate average leaf area injured.

2.2. Ambient ozone measurements

Average ozone concentrations were calculated on a biweekly basis using passive ozone samplers (Ogawa & Co., Inc., Pompano Beach, FL, U.S.A.). These passive samplers collect ozone onto a filter coated with the absorbent sodium nitrite (Krupa et al., 2001). Sampling began in May and continued through early October. Ozone was sampled at 0.5 m above the soil surface. Samplers were retrieved and mailed to the Research Triangle Institute (Research Triangle Park, NC) for analysis at the end of each sampling period.

2.3. Extracellular ascorbic acid isolation and leaf tissue harvest

Isolation of intercellular wash fluid (IWF) was conducted adjacent to remote experimental plots using an automobile battery and a DC/AC converter to provide electrical power for operating the analytical balance and clinical centrifuge required by the procedure. Leaves were excised, placed in plastic bags, and transported to the remote laboratory so that the IWF isolation began within 5 min of harvest. The mid-vein was removed from the selected leaf and the initial fresh weight determined. The leaf tissue was vacuum infiltrated with 100 mM KCl using a 60-mL polyethylene syringe as described previously