Urban Forestry & Urban Greening 14 (2015) 80–88

Contents lists available at ScienceDirect

Urban Forestry & Urban Greening

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

Leaf structural reddening in smoke tree and its significance☆

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ARTICLE INFO

Keywords:
Leaf reddening
Juvenile leaf
Photoprotection
Water imbalance
Smoke tree
Vein severance

ABSTRACT

The smoke tree (*Cotinus coggygria* Scop.) is not only an important ornamental tree that changes the color of its leaves in autumn but also maintains red juvenile leaves on vigorous sprouting shoots. To elucidate the process of leaf reddening in the smoke tree, we examined the structure and function of its leaf venation system, anthocyanin levels, leaf water conservation ability in red juvenile leaves and green mature leaves, leaf temperature, stomata conductance and snap strength of petiole. A combination of thermography, image analyses and photosynthetic measuring technology was used to study the facts of leaf reddening of smoke tree. We found that spatial and temporal heterogeneity in the pattern of leaf reddening in the smoke tree illustrates the interaction between internal metabolism and environmental factors. These results indicate that there are periods that show water and energy imbalances at leaf lamina and that this phenomenon is essential to leaf reddening, especially during periods of leaf expanding and leaf shedding. We consistently observed a relationship between high temperatures and leaf reddening, which indicates the latter is a photoprotective response induced by imbalances in water and energy.

Introduction

To date, many studies have proposed mechanisms for leaf reddening that are related to photoprotection (Gould et al., 1995; Feild et al., 2001; Hughes et al., 2007), anti-oxidation (Kytridis and Manetas, 2006), osmoregulation (Chalker-Scott, 1999, 2002), coevolution with insects (Archetti, 2000), photoperiod transition (Howe et al., 1995) and low temperature induction (Close et al., 2002; Pietrini and Massacci, 1998). However, because leaf reddening is a complex process that involves many factors, it has been a controversial subject for the past 100 years or more. Leaf reddening occurs in many situations, such as in juvenile leaves, old leaves, stressed leaves (Chalker-Scott, 2002) and leaves with a severed major vein (Wang, 2010). The variety of spatial and temporal conditions under which leaf reddening occurs makes it difficult to attribute the process to a single cause given that plant morphology is the result of interactions between internal metabolic processes and exogenous metamorphic actions exerted by the environment. Few studies have examined the mechanisms of heterogeneous leaf color change and even fewer have focused on the smoke tree (*Cotinus coggygria* Scop) (Chalker-Scott, 2002) or the specific variety known as 'Royal Purple' (*Cotinus coggygria* 'Royal Purple'). In this study, we measure the water conservation ability in rapidly expanding leaves, the image temperature during leaf shedding and the structural and functional venation system of smoke trees, specifically and 'Royal Purple'. We hypothesize that early leaf reddening in smoke trees is associated with the imbalances of both water and energy (Wang, 2013).

Materials and methods

Site conditions

This study was performed between spring of 2011 and autumn of 2013 at the Yinmaquan nursery and the Yanzishan forest station in Jinan City, China, which are located at E 117° 03′ 26″ and N 36° 38′ 27″ and E 117° 04′ 57″ and N 36° 43′ 13″, respectively, as well as the street tree near Shandong University. Five large smoke trees of 5 and 6 meters high and approximately 20 years old, one of which was 'Royal Purple' variety and one of which was in low vigor, were used to conduct the vein severing test and snap strength assessment in 2011. Each of 15 one-year-old seedlings without transplantation (1-0) of the common smoke tree and another 10 tree/shrub species were planted at the Yinmaquan nursery and on the hill site of the Yanzishan forest station in the spring of 2012 when support was obtained from the National Natural Science Fund of China. The tree/shrub species included the Japanese black pine (*Pinus thunbergii* Parl.), Chinese arborvitea (*Platycladus*

☆ Supported by the National Natural Science Foundation of China (ID Code: 31170671) and the Project of Science and Technology Development in Shandong, China (ID Number: 2012GNC11107).
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http://dx.doi.org/10.1016/j.ufug.2014.12.001
1618-8667/

orientalis (L.) Franco), glaucous bamboo (*Phyllostachys glauca* McClure), ginkgo (*Ginkgo biloba* L.), kousa dogwood (*Cornus kousa* Buerg.), sweetgum (*Liquidambar styraciflua* L), purple blow maple (*Acer truncatum* Bunge), Zhonghuahongye poplar (*Populus* × *euramerica* 'Zhonghong'), staghorn sumac (*Rhus typhina* Nutt) and red maple (*Acer rubrum* L.). The test is designed in random block with three replicates and five plants in each block. The soil at the Yinmaquan nursery is clay loam alluvial meadow soil, while the soil at the Yanzishan site consists of rocky cinnamon soil and the soil under the street tree near Shandong University is common cinnamon soil. The soil depth at Yinmaquan nursery and near Shandong University is more than 200 cm, while the soil depth at Yanzishan is less than 20 cm. The distance from Yanzishan and Shandong University to the major meteorological station in Jinan is less than 2 km, and Yinmaquan nursery is approximately 10 km from the Yanzishan forest station. The annual mean precipitation in Jinan city is 672.7 mm, the mean temperature is 12.7 °C, the mean temperature in July is 27.5 °C and the lowest temperature in October is 0 °C.

The number of sampled leaves and/or sampling date for the specific studies are presented in the related figure captions, e.g., the sampling number equal 11 (Sn = 11).

Leaf vein imaging and RGB image analysis

Veins were imaged at low magnification and further examined by microphotography (Nicon Eclipse-50i). Because of the protuberant and conspicuous vein structure of smoke trees, leaf veins were photographed under direct sunlight or fluorescent lighting in spring and summer. After carefully observing the structural leaf characteristics in reddening areas, we delineated the venation system manually using the magic lasso tool (Photoshop CS2, Adobe Systems Incorporated). The RGB color image analysis of venation, leaf lamina, scorched area, stressed area and leaf base area were conducted. Unless otherwise noted, RGB images were always taken at front lighting, from the up surface of the leaves and from the sunny side of the crown.

Using the magic lasso tool in Photoshop, target leaf images were manually selected, copied and pasted onto a blank image file. Each target imaging leaf was divided into tip and base sections along the lateral vein nearest to the severed location. After selecting the tip and base sections, R (red), G (green) and L (luminance) values were recorded from the color systems. To calculate the *G*/*L* and *G*/*R* values, we followed the methods as described in Wang et al. (2009). We performed a contrast analysis for the RGB values between tip and base sections of sample leaves. In addition, images taken from the hill (Yanzishan) and flat sites (Yinmaquan), as well as normal and sprouting twigs, were compared using common describing statistics. The number of sampled leaves for each study is shown in the bracket of the relative figure captions.

Measurement of leaf water conservation ability and stomatal density

Leaf water conservation ability was evaluated by using the method described by Slavik (1974). Sprouting shoots were selected from seedlings (1-0) of smoke trees that had been recently planted. Three or more samples were picked from the field and then measured under natural indoor conditions: RH 50–70% and air temperature 25–35 °C. During the transporting process from the field to the experiment site, we usually took all test materials within the same plastic bag to obtain a balance of water potential. The leaves on the sprouting shoots were classified into red juvenile or green mature leaves and then weighed with an electrical balance (Shimazhu AUY120) at specific time intervals. Water loss rate (Wl_i %) was measured and calculated using Eq. (1)

$$Wl_i\% = \frac{FW - Wawl_i}{FW} \times 100\% \quad (1)$$

where *FW* is the fresh weight at $i = 0$ and $Wawl_i$ represents the weight after persistent water loss at different time points i: $i = 1$, 2, 4, 6, 9, 12, 17, 24, 32, 40, 48,. . . hours.

Current year shoots were selected to study the stomatal density according to leaf arrangement. Leaf stomatal density was directly counted from microscope views with a magnification of 200 times and five replications. This represents the number of stomata per square mm and was used to determine the developing status of leaf transpiration capacity.

Vein severing test

Vein severance was first tested on a smoke tree with deep green leaves and a tree with thin, light green leaves near Shandong University and then on all sites mentioned in *Site conditions* section. More than 20 typical leaves located on the sunny side and lower part of the smoke trees were tested by cutting their major vein at the base, middle and top areas and then replicated at least three times at each site. Meanwhile, five or more replicates were performed for each vein severing pattern. Photo images were captured using a CCD camera (Canon IXY6.0) in 2011. The images taken on September 27, October 22, November 10, and November 19 were used to analyze their coloration.

Measurement of leaf temperature, anthocyanin and leaf stomata conductance

Leaf temperatures were measured using an infrared thermography as described (Wang and Yamamoto, 2010; Prytz et al., 2003; Chaerle and Van-Der-Straeten, 2000; Jones and Leinonen, 2003; Grant et al., 2007). Each 20 points were equally measured on 5–6 sample leaves. Leaf or leaf sections that radiated and reflected more energy than the energy they received was considered as an area with an energy imbalance. It was usually consistent with persistently abnormal high temperatures, lower stomata conductance, leaf transpiration failure, among other factors.

Anthocyanin was extracted using methanol containing 1% hydrochloric acid over a 24-hour time period and determined by optical density (OD) readings at 525 nm with an ultraviolet and visible spectrometer (UNICO UV-2102) blanked with an extracting solution. Chlorophyll was evaluated with the same solution by the optical density readings at 663 and 645 nm. The ratio between anthocyanin and chlorophyll for 15 tree/shrub species was evaluated monthly from July 2012 to Jun 2013 by using the ratio of the OD value of the extracting solution measured at 525 and 645 nm. Tree/shrub species including Japanese spindle (*Euonymus japonicus* Thunb.), fortune euonymus (*Euonymus fortunei* (Turcz.) Hand.-Maz.), red leaf photina (*Photinia glabra* (Thunb.) Maxim.), glossy privet (*Ligustrum lucidum* Ait.) and the 'Royal Purple' variety of smoke tree, along with the other 10 tree/shrub species mentioned in *Site conditions* section were studied. Leaf samples from eight deciduous tree/shrub species and three evergreen tree species were captured from the Yinmaquan nursery. The other four evergreen tree/shrub species were selected from the Shandong Forestry Research Academy, which was near the Yanzishan forest station. The relationship between water loss percent from isolated leaves in a specific time interval and leaf anthocyanin content was then regressed.

Leaf stomata conductance was measured with a portable photosynthetic system (LCi, UK) that had a chamber area of 6.25 cm^2 at the time points between 10:00 am to 12:00 am on clear days