Environmental and Experimental Botany 99 (2014) 180–188

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Patterns of night-time water use are interrelated with leaf nitrogen concentration in shoots of 16 deciduous woody species

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ARTICLE INFO

Article history:
Received 20 July 2013
Received in revised form
19 November 2013
Accepted 25 November 2013

Keywords:
Night-time gas exchange
Nitrogen availability
Nocturnal transpiration
Predawn stomatal opening
Water-use strategies

ABSTRACT

Although the impact of nitrogen availability on the night-time water relations of plants has received a lot of attention during the last decade, knowledge of how these two traits are interrelated is contradictory and still limited. The aim of the current study was to investigate the impact of leaf nitrogen concentration on night-time (E_{nap}) and daytime (E_d) transpiration rate, nightly water-use percentage of daytime water use (NWU), and increase in night-time transpiration rate (INT) in artificial predawn hours in the cut shoots of 16 woody species measured in the controlled conditions of a growth chamber. Two distinct patterns of night-time water use associated with leaf nitrogen concentration ([N]) were observed: shoots with high NWU were characterised by significantly ($P < 0.05$) lower [N], whereas shoots with highest INT had greater [N]. The forward stepwise regression analysis revealed that variability in E_d, NWU and INT depended on [N], whereas the impact of other predictor variables (leaf phosphorus concentration, habitat soil water content and light availability) was insignificant ($P > 0.05$). Our results suggest that high NWU could potentially compensate limited nitrogen uptake in species able to grow in nutrient-poor habitats. Furthermore, night-time stomatal regulation mechanisms may differ between species according to their [N] and this may explain the contradictory results between previous studies.

1. Introduction

The impact of plant mineral nutrition on night-time transpiration has been discussed in several studies during the last decade (Snyder et al., 2003, 2008; Daley and Phillips, 2006; Caird et al., 2007; Dawson et al., 2007; Howard and Donovan, 2007, 2010; Marks and Lechowicz, 2007; Scholz et al., 2007; Christman et al., 2009; Kupper et al., 2012). Although the uptake of nitrogen may depend on overall transpiration rate of trees (McDonald et al., 2002; Cramer et al., 2008; Tullus et al., 2012; Sellin et al., 2013), the causality between night-time transpiration and nitrogen acquisition has remained ambiguous. Snyder et al. (2003) suggested that night-time transpiration may enhance the uptake of mineral nutrients like nitrate from the soil. Scholz et al. (2007) provided evidences that N-fertilised savannah trees had lower nocturnal stomatal conductance and water use than trees growing in less fertile control conditions. Recently, Zeppel et al. (2012) demonstrated substantial increase in nocturnal stomatal conductance under rising CO_2 concentration in *Eucalyptus sideroxylon* saplings and they suggested that increased nutrient demand with rising [CO_2], as a result of an increased leaf area of trees, could potentially explain the increase in night-time stomatal conductance. Nevertheless, Caird et al. (2007) pointed out that the experimental design of Scholz et al. (2007) did not allow the separation of direct effects due to reduced plant demand for nutrients from the indirect effect of plant size or water status. Moreover, other experiments in several plant species do not confirm increased night-time transpiration in nitrogen deficiency (Caird et al., 2007; Christman et al., 2009; Howard and Donovan, 2007, 2010). Also hybrid aspen saplings grown at extremely low soil nitrogen concentration did not show water loss at night, although the air vapour pressure deficit (VPD) was sufficient for transpiration induction (Kupper et al., 2012). At the same time, fast-growing shade intolerant tree species characterised by high [N] have shown greater night-time transpiration and stomatal conductance (Daley and Phillips, 2006; Marks and Lechowicz, 2007) as compared to shade-tolerant species or species with lower [N].

For many species the night-time stomatal conductance is not stable throughout the night. An endogenous, gradual increase in stomatal opening during predawn hours has been reported in species from different environments (Lasceve et al., 1997; Donovan et al., 2003; Bucci et al., 2004; Dodd et al., 2005; Caird et al., 2007; Howard and Donovan, 2007; Kupper et al., 2012). Recently, Resco de Dios et al. (2013) concluded that 23–56% of nocturnal sap flow variation associated with endogenous circadian regulation in *Eucalyptus globulus*. Although the mechanism of endogenous stomatal opening in the dark is unclear, it is possible that a by-product of starch metabolism may affect guard-cell osmoregulation at night (Lasceve et al., 1997), causing greater stomatal opening in plants,

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http://dx.doi.org/10.1016/j.envexpbot.2013.11.017

which starch levels are high (Caird et al., 2007). However, the endogenous increase in night-time water flux was suppressed by the low soil/leaf nitrogen concentration in hybrid aspen saplings (Kupper et al., 2012). This leads to the idea that the increase in stomatal opening before dawn may depend on [N], which determines plant photosynthetic capacity and affects accumulation of soluble sugars (Lewis et al., 2011). Such endogenous stomatal opening might be adaptive for plants, as it provides full assimilation capacity in humid morning hours, when transpiratory water loss associated with CO_2 uptake is comparatively small (Kaiser and Kappen, 1997; Oren et al., 1999).

The aim of the current study was to investigate how leaf nitrogen concentration of woody species is related to night- and daytime transpiration rates, nightly water-use percentage of daytime water use (NWU) and increase in night-time transpiration rate (INT) in artificial predawn hours. We suppose that NWU is a species-specific characteristic of plant water use, which allows the estimation of a plant's relative ability to transpire at night (Kupper et al., 2012). Although several studies (Snyder et al., 2003; Scholz et al., 2007; Kupper and Sellin, 2009; Sellin and Lubenets, 2010; Ogle et al., 2012) have investigated the variability of nightly transpiration in natural conditions, NWU has not been measured simultaneously in several species in controlled conditions until now. We suggest that the NWU and INT may provide new insights into the nocturnal patterns of species-specific water use in relation to plant nitrogen acquisition. We set up two hypotheses: (1) species characterised by low [N] demonstrate high shoot night-time water-use percentage of daytime water use (NWU), since this may compensate limited N acquisition in habitats where nitrogen availability is low; (2) species with high [N] exhibit endogenous increase in shoot night-time transpiration rate in predawn hours. This may enhance the assimilation of CO_2 in the early morning and support intensive growth in habitats where nitrogen availability and light competition are high.

2. Materials and methods

2.1. Sample shoots and experimental conditions

The experiment was carried out on shoots of 16 deciduous tree and shrub species (Table 1), collected from different habitats in Rõka experimental site (Kupper et al., 2011), Järvselja Experimental Forest District, and Tartu County in south-eastern Estonia in July and August 2011 and 2012 (Table 2). A number of habitats were sampled to get plant material from various soil conditions and to ensure variability in leaf nitrogen concentration. Because some species were less available for shoot collection a different number of habitats were covered for different species. The average diameter of sampling sites ranged 10–30 m, whereas the distance between different sites/habitats was 50–50 km. We were careful in collecting samples of species with clonal growth (*Salix caprea*) to ensure that they are not genetically identical: the sample shoots were taken as far as possible from each other. Some of the species with possible clonal nature (*Padus avium*, *Populus tremula*) were individually planted in habitat 10 to be sure that they were genetically heterogeneous. Hybrid aspen (*Populus* × *wettsteinii*) was the only micropropagated species and its sample trees belong to clone C05-99-34 (according to the Finnish Plant Production Inspection Centre).

The sample shoots were measured in controlled conditions of a Percival AR-95 HIL growth chamber (Percival Scientific Inc., Perry, IA), while responses in shoot water relations and leaf gas exchange were observed. Four and seven cycles of measurements were conducted in 2011 and 2012, respectively, where each cycle lasted ~22 h (Table 3) and comprised 8–16 shoots collected from 2 to 4 species per cycle. Altogether, 127 shoots were taken from the upper canopy position of small (up to 6 m tall) trees/shrubs, whereas each shoot was taken from separate individuals (Table 2). First a big branch was cut off a tree with secateurs and then the sample shoot was cut off the branch with a razor blade under deionised (Direct-Q3 UV water purification system; Millipore SAS, Molsheim, France) and freshly degassed water (T-04-125 ultrasonic-vacuum degasser; Terriss Consolidated Industries, NJ), and inserted into water-filled plastic container. The shoots were transported to the laboratory, where they were re-cut under water. In the laboratory, the submerged leaves of the cut shoots, demonstrating clear signs of water infiltration, were removed during re-cutting of the shoots. The distance between the previous and last cut was at least three internodes. The re-cut shoots (20–40 cm tall; bearing 3–66 leaves depending on leaf size and species) were inserted into 100 ml plastic flasks filled with deionised and degassed water and sealed with adhesive mass to prevent water evaporation out of the flask. The flasks with sample shoots were weighed and placed into the growth chamber at 21:30 h. A timetable of the experiment and the growth chamber parameters are given in Table 3.

The photosynthetic photon flux density (PPFD) inside the chamber at the level of the shoots was held 450–550 μmol m^{-2} s^{-1} during daytime. The relative air humidity (RH) and the air temperature (T_A) were measured with two HMP45A sensors (Vaisala, Helsinki, Finland) located at shoot height. Measurements of leaf temperature (T_L) were conducted on 31 leaves (one leaf per measurement cycle per species) with 4 fast-response temperature sensors (MT2; Delta-T Devices, Burwell, UK). T_L averaged 22.9 ± 0.2 and 22.0 ± 0.1 °C during the day and night, respectively. The higher T_L in the daytime as compared to the night hours resulted in similar values in leaf-to-air vapour pressure difference (VPD_L) – 1.11 and 1.14 kPa for day and night, respectively – despite higher RH during the day (Table 3). The readings of the HMP45A probes and leaf temperature sensors were recorded with a DL2e data logger (Delta-T Devices) and stored as 10-min averages.

2.2. Environmental conditions in plant habitats

Volumetric soil water content (SWC; %) of the habitat was measured in the uppermost soil layer with ThetaProbe type ML2x (Delta-T Devices, Burwell, UK) in 10–12 replications per species in every site. The soil water content varied from 2.3 to 42.2%.

Light availability in shoot position was estimated by the hemispherical photographic technique (Hale and Edwards, 2002). Photographs were taken with a Coolpix digital camera (Nikon Coolpix 950, Tokyo, Japan) equipped with a FC-E8 fisheye lens (Nikon, Tokyo, Japan) and the hemispherical images were analysed by applying WinSCANOPY software (Regent Instruments Inc., Quebec, Canada). The light conditions of the shoot were characterised by indirect site factor (ISF), i.e. the proportion of diffuse solar radiation reaching a given location. The ISF varied from 0.19 to 0.99.

2.3. Measurements of water relations and gas exchange

To estimate shoot water loss, the flasks with sample shoots were weighed regularly at 21:30, 05:30, 09:30 and 14:30 h. The weight loss (mg) of flasks was assumed to be equal to shoot water loss. The average shoot water loss was calculated for four different time periods (local time): 'night' (from 21:30 to 05:30 h), 'artificial predawn' (from 05:30 to 09:30 h), 'night and artificial predawn' (from 21:30 to 09:30 h) and 'daytime' (from 09:30 to 14:30 h). The time periods are presented according to daylight saving time (DST): UTC + 3 h (for Estonia). The sunrise varied mainly from 5:00 to 5:20 h and the sunset varied from 21:16 to 21:38 h during the experiment (except on 18.07.12 when sun rose at 4:36 and fell down at 22:06 h). Hence, the beginning and the end of the 'night' was coincidental