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Rediscovering leaf optical properties: New insights into plant acclimation to solar UV radiation

Paul W. Barnes [a, *], Stephan D. Flint [b], Ronald J. Ryel [c], Mark A. Tobler [a], Anne E. Barkley [a], Jason J. Wargent [d]

[a] Department of Biological Sciences and Environment Program, Loyola University New Orleans, 6363 St. Charles Avenue, New Orleans, LA 70118, USA
[b] Department of Forest, Rangeland and Fire Sciences, UIPO 441135, University of Idaho, Moscow, ID 83844-1135, USA
[c] Department of Wildland Resources, Utah State University, 5230 Old Main Hill, Logan, UT 84322-5230, USA
[d] Institute of Agriculture & Environment, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand

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ABSTRACT

The accumulation of UV-absorbing compounds (flavonoids and other phenylpropanoid derivatives) and resultant decrease in the UV transmittance of the epidermis in leaves (T_{UV}), is a primary protective mechanism against the potentially deleterious effects of UV radiation and is a critical component of the overall acclimation response of plants to changing UV environments. Traditional measurements of T_{UV} were laborious, time-consuming and destructive or invasive, thus limiting their ability to efficiently make multiple measurements of the optical properties of plants in the field. The development of rapid, nondestructive optical methods of determining T_{UV} has permitted the examination of UV optical properties of leaves with increased replication, on a finer time scale, and enabled repeated sampling of the same leaf over time. This technology has therefore allowed for studies examining acclimation responses to UV in plants in ways not previously possible. Here we provide a brief review of these earlier studies examining leaf UV optical properties and some of their important contributions, describe the principles by which the newer non-invasive measurements of epidermal UV transmittance are made, and highlight several case studies that reveal how this technique is providing new insights into this UV acclimation response in plants, which is far more plastic and dynamic than previously thought.

1. Introduction

Ultraviolet radiation (UV; 280–400 nm) represents a relatively small, but important part of solar spectrum for higher plants. Exposure to UV, especially the shorter wavelengths in the UV-B region (280–315 nm), has the potential to result in a number of deleterious effects in plants, including disruption of the integrity and function of biological macromolecules (DNA, proteins and lipids), oxidative damage, the partial inhibition of photosynthesis, and ultimately reduction in growth and productivity (Ballaré et al., 2011; Jordan, 2002). Because of these effects, plants have developed mechanisms to detect UV and then protect and repair sensitive targets from direct and indirect UV-induced damage (Jenkins, 2009; Rizzini et al., 2011; Jansen and Bornman, 2012). One of the most commonly reported protective responses of plants to UV is the induction of flavonoids and other phenylpropanoids (Searles et al., 2001; Li et al., 2010). These compounds appear to have multiple functions in photoprotection in plants (Agati et al., 2013). Some flavonoids accumulate within mesophyll tissue and are thought to function as antioxidants, thereby reducing oxidative damage that can be induced by UV-B and high PAR (Olsson et al., 1998; Tattini et al., 2005). Other compounds, including certain flavonols and hydroxycinnamic acids (HCAs), are known to function as UV "sunscreens" and accumulate in epidermal tissue where they effectively reduce the penetration of UV to the underlying photosynthetic mesophyll (Mazza et al., 2000; Bidel et al., 2007; Li et al., 1993). Perhaps because of the efficiency of these and other protective measures, negative effects of ambient or realistically-

Abbreviations: F_{BL}, chlorophyll fluorescence induced by blue light; F_o, initial fluorescence yield; F_{UV}, chlorophyll fluorescence induced by UV radiation; F_v/F_m, ratio of variable to maximum fluorescence; PAR, photosynthetically active radiation (400–700 nm); PFD, photon flux density (400–700 nm); T_{UV}, epidermal UV transmittance; UV, ultraviolet radiation (280–400 nm); UV-A, ultraviolet-A radiation (315–400 nm); UV-B, ultraviolet-B radiation (280–315 nm).

* Corresponding author.
E-mail address: pwbarnes@loyno.edu (P.W. Barnes).

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enhanced solar UV-B on plant growth, photosynthesis and productivity are generally small or difficult to detect in field-grown plants (Searles et al., 2001; Newsham and Robinson, 2009).

The ability of plants to accumulate these protective UV-absorbing compounds and change their optical properties (primarily epidermal transmittance) in response to days or weeks of exposure to UV-B radiation has long been known (Caldwell et al., 1983). Indeed, a meta-analysis of 62 studies has shown that the increased production of UV-B-absorbing compounds is one of the most commonly observed responses of field-grown plants to UV-B-supplementation simulating ozone depletion (Searles et al., 2001). This acclimation response entails a measurable energetic/carbon cost (Snell et al., 2009; Guidi et al., 2011), varies with species and genotype [e.g., (Day et al., 1992; Randriamanana et al., 2015)], is influenced by other environmental factors (e.g., UV-A (315–400 nm), visible light (400–700 nm) and temperature (Guidi et al., 2011; Bilger et al., 2007; Flint et al., 2004; Gotz et al., 2010; Siipola et al., 2015)) and is linked with cross-tolerance to other abiotic and biotic stresses (e.g., drought and herbivory (Mazza et al., 2013; Bandurska et al., 2013)).

Until recently, the determination of epidermal UV transmittance (T_{UV}), the optical property of primary interest, has involved measuring the transmittance of epidermal peels using an integrating sphere [e.g., (Robberecht and Caldwell, 1978)] or measuring UV penetration into leaves with microprobes [e.g., (Vogelmann, 1989)]. However, these techniques are laborious, time-consuming and destructive or at least invasive. The development of rapid, nondestructive optical methods of determining T_{UV} (UVA-PAM, (Kolb et al., 2005); Dualex/Multiplex, (Goulas et al., 2004)) has permitted the examination of UV optical properties of leaves with increased replication, on a finer time scale, and enabled repeated sampling of the same leaf over time. This technology has thus allowed for studies examining acclimation responses to UV in plants in ways not previously possible. Here we provide a brief overview of the earlier studies examining leaf UV optical properties and point to some of their major contributions, describe the principles by which the newer non-invasive measurements of epidermal UV transmittance are made, including some of the necessary precautions, and highlight several case studies that reveal how this technique is providing new insights into the plastic and dynamic nature of this UV acclimation response in plants.

2. Early approaches and findings

Many of the initial studies on leaf UV optical properties examined the natural variation in T_{UV} that exists among species or ecotypes and the anatomical factors that influence this filtering mechanism. The comprehensive companion studies by Caldwell, Robberecht and Billings (Caldwell et al., 1980) and Robberecht, Caldwell and Billings (Robberecht et al., 1980) showed that the UV transmittance of epidermal peels from plants originating from low UV-B environments (i.e., low elevation sites in the Arctic) was generally higher and more variable than those of plants from high UV-B environments (i.e., high elevation equatorial alpine habitats). Results from these, and follow-up comparative UV-sensitivity studies of Arctic-alpine plants (Caldwell et al., 1982; Barnes et al., 1987), provided strong circumstantial evidence that solar UV was indeed an important ecological and selective factor along this latitudinal gradient. Species surveys were also conducted by Day, Vogelmann and DeLucia (Day et al., 1992) and others [e.g., (Qi et al., 2010)] using microprobes, and these studies documented considerable variation in the UV screening capabilities among major plant growth forms (grasses, broad-leaved herbaceous species and needle-leaved conifers). These differences were related to variation in the levels of UV-absorbing compounds as well as leaf anatomical structures (Day, 1993). Other studies, again with microprobes, showed that the UV penetration into leaves could be influenced not only by the epidermis but also by surface features, such as waxes and trichomes (Karabourniotis et al., 1999; Karabourniotis and Bornman, 1999).

The use of microprobes further allowed for the three-dimensional examination of internal UV-filtering in leaves (Alenius et al., 1995) and these studies revealed that the epidermis was non-uniform in its UV filtering effect (Day et al., 1993). This heterogeneity in UV penetration into leaves resulted from differences in UV transmittance among cellular components (e.g., vacuoles vs. cell walls), the spatial patterns of epidermal tissue (e.g., the distribution and arrangement of stomatal guard cells vs. other epidermal cells), and the optical focusing and reflective properties of different cells and tissues (Karabourniotis et al., 2000; Vogelmann et al., 1996).

Both integrating sphere [e.g., (Robberecht and Caldwell, 1983)] and microprobe techniques [e.g., (Sullivan et al., 1996; Olsson et al., 1999; Cen and Bornman, 1993)] were used to demonstrate that T_{UV} decreased (and UV-absorbing compounds increased) in response to UV-B exposure when plants were grown under elevated UV-B, indicating that many plants possessed the potential to acclimate to a range of UV exposures including enhanced UV-B levels associated with ozone depletion.

3. Chlorophyll fluorescence as a non-invasive probe of leaf optical properties

3.1. Principles and tests of the technique's validity

In 1997 Bilger, Veit, Schreiber and Schreiber (Bilger et al., 1997) introduced a non-invasive technique to measure T_{UV} based on chlorophyll fluorescence. This approach provided indirect estimates of T_{UV} by measuring the fluorescence yield of chlorophyll (F_o, $\lambda > 650$ nm) induced by UV and blue-green (BG) radiation (Fig. 1). The technique is based on the premise that both UV and BG

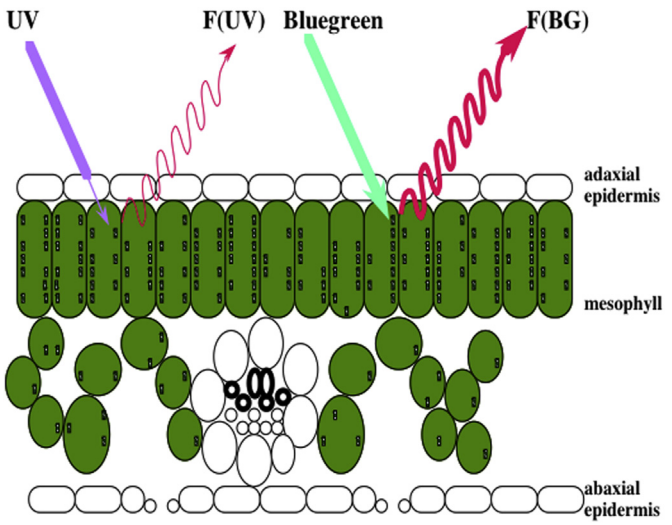

Fig. 1. Principle of measurement of epidermal UV transmittance using chlorophyll fluorescence. The technique involves the near simultaneous measurement of chlorophyll fluorescence induced by both UV (F_{UV}) and visible (e.g., bluegreen (BG); F_{BG}) radiation. For typical green leaves, F_{UV} is influenced by the amount of UV-absorbing compounds in the epidermis whereas F_{BG} is not. Fluorescence induced by BG (F_{BG}) thus serves as a reference to account for variation in chlorophyll content and chloroplast distribution in the underlying mesophyll. Provided certain precautions and standards are utilized, the ratio of F_{UV} to F_{BG} provides a robust estimate of T_{UV} or concentrations of epidermal UV-absorbing compounds.

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