Environmental and Experimental Botany 119 (2015) 18–26

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Functional significance of anthocyanins in peduncles of *Sambucus nigra*

Luke J. Cooney [a,*], H. Martin Schaefer [b], Barry A. Logan [c], Bart Cox [a], Kevin S. Gould [a]

[a] *School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand*
[b] *Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstrasse D-79104, Freiburg, Germany*
[c] *Department of Biology, Bowdoin College, 6500 College Station, Brunswick, ME 04011, USA*

ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form 25 February 2015
Accepted 1 March 2015
Available online 3 March 2015

Keywords:
Anthocyanin
Photoprotection
Light screen
Plant–animal interactions
Fruit display
Peduncle

ABSTRACT

Anthocyanin accumulation in reproductive organs is normally assumed to attract animals as dispersers, yet in leaves and stems these pigments may also serve a number of protective roles. Here, we tested the requirements of both a physiological function, that anthocyanins provide photoprotection, and a communicative function, that anthocyanic reddening enhances frugivory, for *Sambucus nigra* peduncles which turn red prior to fruit ripening. We found that peduncle reddening required exposure to sunlight, and that the sub-epidermal anthocyanins significantly reduced the transmission of green light to subjacent chlorenchyma. Under saturating white light, quantum efficiencies of photosystem II were higher in red than in green peduncles. Following light stress, the decline in *Fv/Fm* was significantly greater for green portions of peduncle compared to red portions on the same organ. In contrast, we found that avian frugivory was not consistently greater for those infructescences bearing the reddest peduncles, and therefore found little evidence of a communicative function for peduncle reddening. Because peduncle senescence begins prior to fruit maturation, anthocyanins in these sterile parts of reproductive organs may confer protective benefits similar to those postulated for senescing autumn leaves.

1. Introduction

Over the past twenty years, a compelling body of evidence has accrued indicating that anthocyanins, the pigments that yield red, blue and purple colours in many plant species, serve a wide variety of important plant functions. In leaves, for example, anthocyanins appear to present a measure of protection against both abiotic and biotic stressors. They may reduce the propensity for photoinhibition, or mitigate the effects of drought, salinity, heavy metals or oxidative stress (Agati and Tattini, 2010; Chalker-Scott, 1999; Ferreyra et al., 2012; Gould, 2004; Hatier and Gould, 2009; Pollastri and Tattini, 2011). Equally, their non-green colouration can effectively camouflage seedlings against the forest litter (Fadzly et al., 2009), or deter herbivory by advertising the distribution and potency of chemical defences (Archetti and Leather, 2005; Cooney et al., 2012).

It has become clear that if we are to understand the functional significance of anthocyanin pigments in plants, we need to give equal consideration to their possible protective effects as to those in plant–animal communication. Several authors have called for research that simultaneously addresses both categories of functional hypothesis (Archetti et al., 2009; Gould, 2004; Schaefer and Wilkinson, 2004; Schaefer and Rolshausen, 2006), but such studies are rare (Karageorgou and Manetas, 2006). For the reproductive organs, for example, anthocyanin accumulation has overwhelmingly been attributed to a signalling role (Schaefer and Ruxton, 2011); anthocyanins in the peduncle, pedicel, perianth and pericarp would provide visual information to pollinators and frugivores on the availability and quality of rewards (Schaefer, 2011; Schaefer and Ruxton, 2011; Willson and Whelan, 1990). It is far from certain, however, whether this is their only or, indeed, primary function. Indeed, anthocyanins in the exocarp of apples and pears have been shown to effect a significant reduction in the severity of light stress on subjacent tissues (Steyn, 2009; Steyn et al., 2009). Thus, in the absence of empirical data, we cannot exclude the possibility that a requirement for protection from abiotic stressors, rather than that for communicating to animals, has been a key driver for the evolution of these red pigments in some floral organs.

The functional hypotheses that have been repeatedly tested on leaves provide a good starting point to investigate possible protective explanations for the presence of anthocyanins in

---

[*] Corresponding author. Tel.: +64 4 5895115; fax: +64 4 463 5331.
E-mail addresses: cooneyluke@myvuw.ac.nz (L.J. Cooney), martin.schaefer@biologie.uni-freiburg.de (H. M. Schaefer), blogan@bowdoin.edu (B.A. Logan), coxbart@myvuw.ac.nz (B. Cox), kevin.gould@vuw.ac.nz (K.S. Gould).

http://dx.doi.org/10.1016/j.envexpbot.2015.03.001
0098-8472/

reproductive organs. The light-screening hypothesis has received the most attention in recent years. Leaves that are exposed to more light than they can use for carbon assimilation show a characteristic decline in photosynthetic quantum yield (Long et al., 1994), and sustained photoinactivation can lead to the production of damaging reactive oxygen species (ROS), (Murata et al., 2007; Takahashi and Murata, 2008; Tyystjärvi, 2008). Anthocyanins absorb a proportion of the green light energy that would otherwise be intercepted by chloroplasts in the lower spongy mesophyll, and numerous studies have reported a reduction in the severity of photoinactivation for red leaves and stems, compared to green ones, when exposed to saturating light (Boldt et al., 2014; Dodd et al., 1998; Feild et al., 2001; Gould et al., 1995; Gould et al., 2010; Hughes et al., 2005; Manetas et al., 2002; Nielsen and Simonsen, 2011; Steyn et al., 2002; Tattini et al., 2014), although exceptions have been noted (Esteban et al., 2008; Júnior et al., 2012; Liakopoulos and Spanorigas, 2012; Zeliou et al., 2009). Light screening, or 'photoprotection', by anthocyanins is considered especially important during leaf senescence, a stage of enhanced susceptibility to light stress as the photosynthetic machinery disassembles for nutrient resorption (Keskitalo et al., 2005). Anthocyanic senescing leaves often display a reduction in photoinactivation, a more prolonged senescence and an improved recovery of chloroplastic nitrogen, compared to acyanic senescing leaves (Feild et al., 2001; Hoch et al., 2003; Schaberg et al., 2008; Zhang et al., 2013), although exceptions have been reported (Manetas and Buschmann, 2011; Misyura et al., 2012).

Because the peduncle (stalk of an infructescence), and the pedicels (stalks of individual fruits within an infructescence; Fig. 1A), usually abscise shortly after fruit removal, they are essentially senescing organs during fruit ripening, and like senescing leaves, may benefit from photoprotection by anthocyanins. However, no study to date has investigated whether anthocyanins play a photoprotective role in these sterile parts of reproductive organs. We have used *Sambucus nigra* (European black elder) to test this hypothesis. The species is particularly useful because both the intensity of reddening, and the proportion of peduncle that turns red, can vary substantially between individuals as well as between infructescences on the same plant (Fig. 1A), affording comparisons of the responses of red and green portions to light stress.

The peduncles of *S. nigra* also show some characteristics indicative of a role for anthocyanins in attracting seed dispersers. Its red peduncles chromatically contrast more sharply against the surrounding foliage, and their fruits are removed more quickly by birds, in comparison to its green peduncles (Schaefer and Braun, 2009). Moreover, the intensity of red colour apparently provides information on fruit quality, with the redder peduncles bearing larger fruit crops that contain more sugar (Schaefer and Braun, 2009). Nevertheless, two other features suggest that a communicative role may not be the primary function of the red pigments. First, anthocyanin accumulation precedes fruit maturation. While this may be a relatively late stage of fruit development, it does mean that peduncles appear 'unnecessarily' red while fruits are still unripe (Schaefer and Braun, 2009). Second, peduncles borne on understory *S. nigra* remain green (Schaefer and Braun, 2009). We know that anthocyanin biosynthesis per se is not constrained by low light because even in the forest understory, anthocyanin production turns ripening fruits black. This begs the question, if anthocyanins are produced primarily to attract frugivores, why do not understory plants similarly produce red peduncles? It may be that anthocyanin accumulation in *S. nigra* peduncles primarily serves a physiological function, to prevent the detrimental effects of light stress during senescence, but that the colour of the pigment has been co-opted for frugivore communication.

Here, we use two forest populations of *S. nigra* to test for communicative and light-screening roles for anthocyanins in peduncles and pedicels. To test the requirements of a communicative role, we monitored fruit removal in infructescences of varying redness, and compared peduncle colour among infructescences for which the number of berries had been experimentally manipulated. While fruit removal has previously been compared for mature infructescences bearing red and green peduncles (Schaefer and Braun, 2009), our study monitored fruit removal

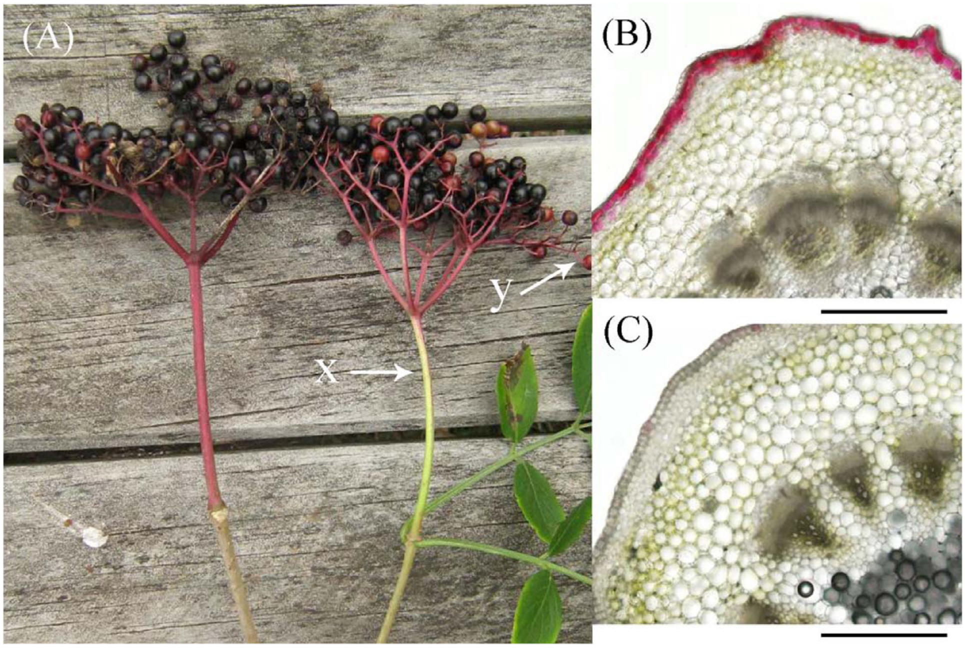

Fig. 1. Infructescences of *Sambucus nigra*. Photograph of red and green peduncles (A), and photomicrographs of transverse sections through red (B) and green (C) peduncles. Bars, 500 μm; x, peduncle; y, pedicel. (For interpretation of the references to colour in the figure legend, the reader is referred to the web version of this article.)