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Short Communication

Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*?

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Abstract

The biological function of juvenile leaves pigmented with anthocyanin is poorly understood. The role anthocyanins play in UV protection was assessed in juvenile leaves of two *Syzygium* species (*S. luehmannii* and *S. wilsonii*) which contain high anthocyanin concentrations. HPLC was used to separate UV-absorbing anthocyanins from other soluble UV-absorbing phenolic compounds. The isolated anthocyanins (predominantly malvidin-3,5-diglucoside) contributed little to the total absorbance of UV-A and UV-B radiation. This was because the non-acylated anthocyanins only effectively absorbed shortwave UV-B radiation and the strong absorbance by other compounds. These results suggest that the UV protection hypothesis is not valid for anthocyanins in juvenile *Syzygium* leaves.

Key words: Anthocyanin, UV protection.

Introduction

Juvenile leaves pigmented with anthocyanin are a common and visually striking feature of many tropical and subtropical woody species from a range of higher plant families (Richards, 1952; Lee and Lowry, 1980; Lee *et al*., 1987; Tuohy and Choinski, 1990). Anthocyanins also have a wide distribution in mature (Hrazdina, 1992; Harborne and Grayer, 1988) and senescent (Chang *et al*., 1989; Ji *et al*., 1992) leaves. They are located in a range of leaf tissues including the upper epidermis (Burger and Edwards, 1996) and the lower epidermis (Lee *et al*., 1979) and they also occur in the upper (Woodall *et al*., 1998) and lower mesophyll tissues (Lee *et al*., 1987). The biological function of juvenile leaf anthocyanins is poorly understood.

Anthocyanins belong to the flavonoid group of plant phenolic compounds. The C_6-C_3-C_6 flavonoid base absorbs strongly in the UV spectrum (Markham, 1982). Epidermal flavonoids strongly attenuate UV radiation in mature evergreen leaves (Day *et al*., 1994). Mesophyll flavonoids are also important to the absorption of UV radiation, particularly during leaf expansion when UV radiation may not be fully attenuated by the developing cuticular and epidermal layers (DeLucia *et al*., 1992). Anthocyanins generally have two absorption maxima, one between 270–290 nm and the other in the visible spectrum at 500–550 nm (Markham, 1982). Acylation of anthocyanins with aromatic organic acids increases their UV absorbance by addition of another absorption maximum in the 310–320 nm range. The strong UV absorption of anthocyanins has led to the hypothesis that they may protect expanding leaves from the harmful effects of UV radiation (Lee and Lowry, 1980; Coley and Kursar, 1996).

Consistent with the UV protection hypothesis are observations that mature leaves of red Coleus varieties with epidermal anthocyanins were damaged less by UV-B and UV-C radiation than non-anthocyanin accumulating varieties (Burger and Edwards, 1996). Stapleton and Walbot (1994) demonstrated that the DNA in *Zea mays* plants that contain flavonoids (primarily anthocyanins) was protected from damage caused by UV radiation relative to the DNA in plants that were genetically deficient in these compounds. Anthocyanins were also shown to protect cell suspension cultures of *Centaurea cyanus* from the damaging effects of UV radiation (Takahashi *et al*., 1991).

A UV protective function for anthocyanin in juvenile leaves of shade-tolerant evergreen trees has not been shown. In juvenile mango (*Mangifera indica*) leaves, anthocyanin localization (in cell layers just above the lower epidermis) and concentration (a small proportion

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of the total phenols) argues against a role in UV protection (Lee *et al*., 1987). However, the presence of high anthocyanin concentrations in the palisade mesophyll of juvenile *Syzygium* leaves (Woodall *et al*., 1998) may mean that anthocyanins are important UV absorbing compounds, especially if the cuticle and epidermis do not fully attenuate incident UV radiation. Therefore the possible involvement of mesophyll anthocyanins as UV-absorbing compounds in red juvenile *Syzygium* leaves was examined.

Materials and methods

Leaf samples for HPLC analysis were collected from well-illuminated trees growing within the grounds of The University of Queensland campus, Brisbane, Australia. Leaves less than 30% fully expanded were used because this leaf class contains the highest anthocyanin levels (Woodall *et al*., 1998).

Material for HPLC analysis (1 g FW) was ground in liquid nitrogen and extracted with 10 cm^3 of methanol (70%), H_2O (25%) and formic acid (5%). Extraction in 1% HCl/methanol gave identical chromatograms, with no indication of anthocyanin breakdown in 1% HCl. There was no evidence of artefact formation of anthocyanins extracted in the presence of formic acid. After centrifugation, 100 μl of supernatant was used for HPLC analysis, using a Beckman System Gold HPLC, with a Beckman 128 pump system, Beckman 507 autosampler and a Beckman 168 diode array detector. Soluble phenolic compounds were separated on a C18 Beckman ultrasphere 25 cm column. The initial conditions were 100% solution A switching between 10 and 30 min to a linear gradient from 0% solution B to 70% solution B at a flow rate of 1.5 cm^3 min^{-1}. Between 40–45 min the percentage of solution B was increased linearly to 100%. Solution A comprised 5% formic acid/10% methanol/85% H_2O (by vol.) and solution B, 50% methanol/10% formic acid/40% H_2O (by vol.). Fractions of 1.0 cm^3 were collected and assayed for total phenols. The Folin-Ciocalteu (Sigma chemicals Folin concentrate) reaction was used to determine phenol content, following the methodology described by Booker *et al*. (1996). Partial and total acid hydrolysis was used to identify hydrolysis intermediates and aglycones respectively (Strack and Wray, 1989). Extracts from tissues containing known anthocyanins were used as standards (Harborne and Grayer, 1988).

Results and discussion

The retention time for the main anthocyanin peak in both *Syzygium luehmannii* and *S. wilsonii* was at 23 min (Fig. 1a, b) and co-eluted with malvidin-3,5-diglucoside (Mv-3,5-diglucoside) an anthocyanin common in Myrtaceous flowers (Lowry, 1976). In 1% HCl/methanol the principal *Syzygium* anthocyanin had absorption maxima at 275 nm and 535 nm (Fig. 2a). These spectral characteristics are similar to those reported for Mv-3,5-diglucoside by Francis (1982). Partial and total acid hydrolysis of the main peak confirmed that the anthocyanin was malvidin-3,5-diglucoside (data not shown). Malvidin-3,5-diglucoside was always the main anthocyanin isolated from *S. luehmannii* and *S. wilsonii*, contributing to over 70% of the total peak area at 520–540 nm (Fig. 1a, b). A second, unidentified anthocyanin was present as a shoulder on the major Mv-3,5-diglucoside (Fig. 1a, b). The aglycone of this anthocyanin was identified as peonidin. Several other small anthocyanin peaks were recorded (Fig. 1a, b). Hydrolysis of crude anthocyanin extracts confirmed that malvidin was the abundant aglycone (>70% of total peak area at 520–540 nm) with smaller amounts of peonidin and traces of pelargonidin, cyanidin, delphinidin, and petunidin. None of the isolated anthocyanins (both major and minor peaks) displayed spectral properties characteristic of anthocyanins acylated with aromatic acids.

Anthocyanins isolated from *S. luehmannii* and *S. wilsonii* leaves, less than 30% expanded, accounted for only 4.5% and 6.3% of the total mean absorbance 270–280 nm respectively (Fig. 1c, d). Anthocyanins from these leaves also contributed less than 5% to the total UV-B absorbance (Fig. 1e, f) and were of negligible importance to the absorption of UV-A radiation (Fig. 1g, h). Additionally anthocyanins were only 3.4% and 5.5% of the total phenols in *S. luehmannii* and *S. wilsonii*, respectively (Fig. 1i, j). Some absorbance attributed to anthocyanins, particularly in the UV-B, may have been due to other co-eluting compounds. Thus, these percentages probably overestimate the contribution of anthocyanin absorbance. Unfortunately it was not possible to express the results in terms of the percentage of total mesophyll UV absorbance because it was not possible to separate the epidermal layer from the mesophyll tissue in the expanding leaves of *Syzygium*.

The anthocyanins isolated from juvenile *Syzygium* leaves effectively absorb a narrow range of shortwave UV-B radiation (Fig. 2a). In fact, many of the compounds that eluted before 25 min absorbed strongly in the UV-B (in particular, radiation less than 300 nm) and less strongly in the UV-A region. Generally compounds that eluted after 25 min absorbed strongly in both the UV-A and UV-B regions. The UV absorption spectra of two such compounds are shown in Fig. 2b. These spectra are similar to those of flavonoids (Markham, 1982).

A key feature likely to make anthocyanins more effective for UV-B protection is the presence of acylated aromatic acids (phenolic acids), due to the superimposition of the aromatic acid absorption upon that of the pigment absorption (Harborne, 1957). Certainly, a study that supported the UV protection hypothesis for anthocyanins used a species which contained an anthocyanin that was acylated with cinnamic acid (*Coleus* in Burger and Edwards, 1996). Similarly, the absorption spectrum for extracted flavonoids of *Zea mays* had a peak at 310–320 nm which may indicate the presence of anthocyanins acylated with aromatic acids (Stapleton and Walbot, 1994).

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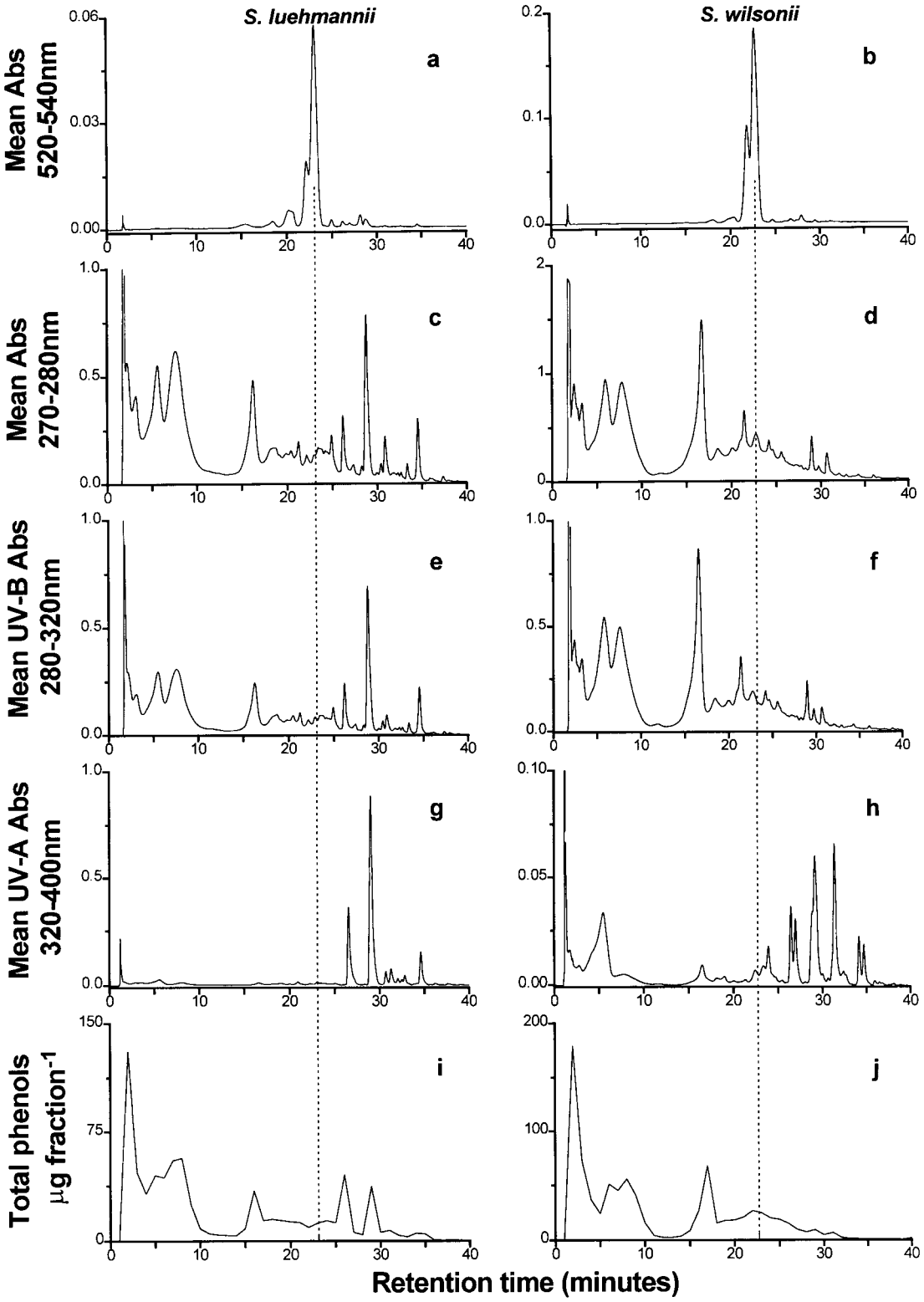

Fig. 1. Separation of anthocyanin pigments by HPLC from other soluble phenolic compounds extracted from field-grown *S. luehmannii* and *S. wilsonii* leaves less than 30% expanded. Mean absorbance (Abs) 520–540 nm for detection of anthocyanin (a), *S. luehmannii* (b), *S. wilsonii*. Mean absorbance 270–280 nm (c), *S. luehmannii* (d), *S. wilsonii*. Mean UV-B absorbance (280–320 nm) (e), *S. luehmannii* (f), *S. wilsonii*. Mean UV-A absorbance (320–400 nm) (g), *S. luehmannii* (h), *S. wilsonii*. Total phenols are expressed as μg of catechin equivalents per fraction (i), *S. luehmannii* (j), *S. wilsonii*. Similar chromatograms were obtained from two other extractions and separations.

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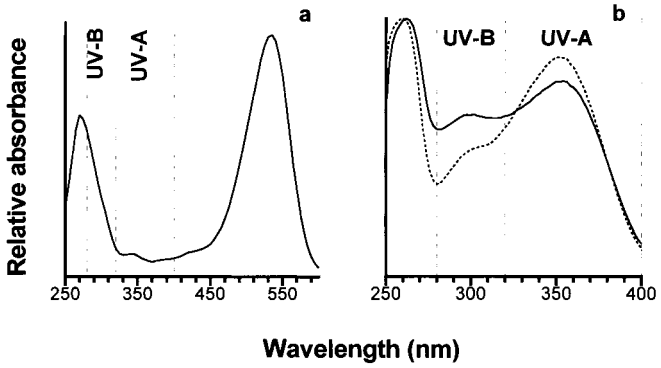

Fig. 2. Relative absorbance spectra of: (a), isolated *S. luehmannii* anthocyanin in 0.1% HCl/methanol (retention time 23 min see Fig. 1a); (b), *S. luehmannii* flavonoids in 0.1% HCl/methanol (Fig.1e, g) with retention times 26.5 min (·····) and 28.5–29 min (——). Spectra are relative to the maximum absorbance.

Although the chemical structure of anthocyanins is important in determining their potential role, so too is their abundance in relation to other UV-absorbing compounds. In leaves of *Coleus* (Burger and Edwards, 1996), *Zea mays* (Stapleton and Walbot, 1994) and cell cultures of *Centaurea cyanus* (Takahashi *et al*., 1991) anthocyanins were the major UV-absorbing compounds. This is not the case in *Syzygium* juvenile leaves (Fig. 1c–h), where anthocyanins comprise a small proportion of the total UV absorbance.

In conclusion, the absence of anthocyanins with aromatic acyl groups and the abundance of other compounds which strongly absorb UV radiation both suggest that the UV protection hypothesis (Lee and Lowry, 1980; Coley and Kursar, 1996) is not valid for the anthocyanins in developing *Syzygium* leaves.

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