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Spectral detection of stress-related pigments in salt-lake succulent halophytic shrubs

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ABSTRACT

The spectral detection of vegetation pigment concentrations has a high potential value, but it is still underdeveloped, especially for pigments other than chlorophylls. In this study, the seasonal pigment dynamics of two *Tecticornia* species (samphires; halophytic shrubs) from north-western Australia were correlated with spectral indices that best document the pigment changes over time. Pigment dynamics were assessed by analysing betacyanin, chlorophyll and carotenoid concentrations at plant level and by measuring reflectance at contrasting seasonal dates. Plant reflectance was used to define a new reflectance index that was most sensitive to the seasonal shifts in *Tecticornia* pigment concentrations. The two *Tecticornia* species turned from green to red-pinkish for the period March–August 2012 when betacyanins increased almost nine times in both species. Chlorophyll levels showed the opposite pattern to that of betacyanins, whereas carotenoid levels were relatively stable. Normalised difference indices correlated well with betacyanin ($r = 0.805$, using bands at 600 and 620 nm) and chlorophyll ($r = 0.809$, using bands at 737 and 726 nm). Using knowledge of chlorophyll concentrations slightly improved the ability of the spectral index to predict betacyanin concentration ($r = 0.822$ at bands 606 and 620 nm, in the case of chemically determined chlorophyll, $r = 0.809$ when using remotely sensed chlorophyll). Our results suggest that this new spectral index can reliably detect changes in betacyanin concentrations in vegetation, with potential applications in ecological studies and environmental impact monitoring.

1. Introduction

Plant pigments and variations in their concentrations can be correlated to the state of vegetation during seasonal changes, such as the decline of chlorophylls and the increase of carotenoids and anthocyanins during autumn (Archetti, 2009). Pigments in plants can be measured directly by chemical and instrumental analyses or indirectly by spectrometry, since the reflectance in the visible region of the spectrum varies according to the composition and the concentration of many components (Ustin et al., 2004; Ustin et al., 2009; Ollinger, 2011). Research on the spectral detection of plant pigments has led to the development of several spectral indices, but most investigations have focussed on chlorophylls due to their importance for photosynthesis (Gamon et al., 1992; Gamon and Surfus, 1999; Gitelson et al., 2009; Peñuelas et al., 2011). There have been fewer spectral studies of other plant pigments, perhaps because their overlapping absorbance with chlorophylls makes it difficult to define indices that are able to provide accurate estimates of non-chlorophyll pigments (Sims and Gamon, 2002).

An advantage of using spectrometry to detect pigments is that vegetation condition can be evaluated at large scales (e.g. by satellites), and it is relatively fast compared to traditional methods (Kerr and Ostrovsky, 2003). Additionally, spectrometry via remote sensing can be especially useful in areas where human intervention is difficult due to their fragility and/or inaccessibility, such as deserts or flooded areas (Shuman and Ambrose, 2003). Wetlands and salt marshes are complex ecosystems that show particular interactions between structure (soil and vegetation cover, species composition), and function (nutrient cycling, primary productivity) (Kelly et al., 2011). In seasonal wetlands, the occurrence of flooding events alternating with drought periods controls plant distribution, with

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environmental gradients resulting in unique microhabitats with highly specialised species. Due to difficult accessibility and complex bio-physical dynamics, wetlands and marshes pose big challenges for monitoring using traditional techniques such as vegetation surveys and other sampling methods (Zomer et al., 2009).

In Western Australia, the halophytic endemic *Tecticornia* genus (formerly *Halosarcia*) of the Amaranthaceae family (formerly Chenopodiaceae) is dominant in saline inland areas (Wilson, 1980; Shepherd and van Leeuwen, 2010). Australia is a centre of diversity for this particular group (Shepherd and Wilson, 2007). Many species grow in ephemeral inland salt lakes that are subject to periodic changes in salinity, waterlogging and drought (Shepherd and van Leeuwen, 2010; English and Colmer, 2011). It has been observed that *Tecticornia* species, especially those occurring in salt marshes, change colouration under unfavourable conditions (e.g. drought or waterlogging) from green to red-pinkish tones (Datson, 2002). Changes in colouration seem to be the result of chlorophyll degradation, as well as increases in other pigments like betalains, which are recognized for their role as photoprotective pigments (Stintzing and Carle, 2004; Brockington et al., 2011). Betalains are a group of pigments commonly found in the Amaranthaceae family (Strack et al., 2003). Two types of betalains are known: the yellow and orange betaxanthins and the red-violet betacyanins, with absorption maxima at 460–480 and 540 nm, respectively (Stintzing and Carle, 2004). Although the biological functions of betalains are not well understood, their accumulation has been associated with protection against UV radiation (Ibdah et al., 2002) and can be induced by abiotic stresses such as salinity (Hayakawa and Agarie, 2010; Zhao et al., 2010; Rabhi et al., 2012).

Despite the importance of *Tecticornia* species as keystone species dominating in some areas of Australia (Wilson, 1980; Shepherd and Wilson, 2007), the dynamics and the nature of their pigments have not been spectrally evaluated, and the potential application of spectral signals for the assessment of the condition of these ecosystems is novel. In addition, large areas occupied by *Tecticornia* have experienced intensive land use changes in the last past decades, such as groundwater extraction and flow diversion for mining, and secondary salinity in agricultural landscapes (Timms, 2005). Thus, the evaluation of plant condition and the changes associated with seasons and stress is of importance for conservation and land management.

In this study we assessed the seasonal changes in colouration and the spectral response to these changes for two *Tecticornia* species from the Fortescue Marsh, the largest inland marsh of the Pilbara region in Western Australia. After elucidating the yet unknown *Tecticornia* betacyanin pattern by HPLC-DAD-MS^n, our main goal was to identify a spectral index that best reflects differences in pigment concentrations. Our hypothesis was that due to seasonal changes, pigment concentration would vary during the year and that the changes in light reflectance could be spectrally detected. To test this hypothesis, we quantified plant pigment dynamics by sampling and analysing plant tissue pigment concentrations (betalains, chlorophylls and carotenoids) and by measuring light reflectance at plant level across seasons. Plant reflectance was subsequently used to define a new reflectance index that was most sensitive to the seasonal shifts in *Tecticornia* pigment concentrations.

2. Methods

2.1. Study area

Field work was performed in an area on the northern shore of Fortescue Marsh, a basin in the Fortescue River catchment located 120 km north of Newman, Pilbara region, Western Australia (22° 22.9′ S, 119° 20.1′ E). The Fortescue Marsh has been recognized for its conservation value and ecosystem services including habitat for several endemic species (Shepherd and van Leeuwen, 2010). The marsh is subjected to episodic monsoonal summer floods alternating with prolonged drought during winter, when soils dry and salinity increases considerably (Skrzypek et al., 2013). Rains mainly occur from October to March with an average of approximately 400 mm per year. A strong seasonality is also observed in air temperature and solar radiation (Fig. 1). Prior to the study period, heavy rain fell in January and March; during the study period, rain was negligible and conditions became progressively drier but with lower temperature and lower radiation load. Two *Tecticornia* species were studied: *Tecticornia indica* subsp. *bidens* (Nees) K. A. Sheph. and Paul G. Wilson, a C4 species occurring towards the outer margins of the marsh, and the C3 species *T. auriculata* (K. A. Sheph. and Paul G. Wilson) distributed from the *T. indica* habitat to several hundred meters into the saline marsh at slightly lower elevations (for photographs see Fig. S1). *Tecticornia* species are the dominant perennial shrubs in the area representing almost 90% of the total plant cover (Moir-Barnetson, 2015)

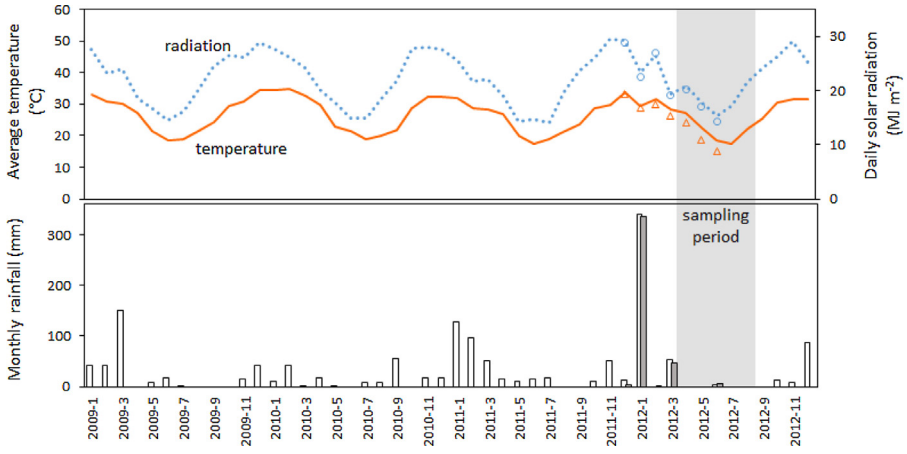

Fig. 1. Weather conditions in the study area. The top panel shows monthly average air temperature (°C; solid line for nearby permanent weather station, triangles for measurements at study site) and daily total solar radiation (MJ m^{-2}; dotted line for nearby permanent weather station, circles for measurements at study site). The bottom panel shows monthly total rainfall (mm; white bars for nearby permanent weather station, grey bars for measurements at study site). The shaded area indicates the period during which spectral and chemical sampling was carried out. The 2009–2011 weather data are from stations Marillana (radiation and rainfall; 30 km S of study site) and Wittenoom (temperature; 100 km W of study site), which were obtained from the Bureau of Meteorology database (bom.gov.au/climate/data).

2.2. Experimental design

Plant sampling for pigment analysis and spectral measurements was done in three plots per species, with four plants per plot. The size of each plot was 25 m^2. Plots were chosen based on a dominance of 90% of the target species and there was a minimal distance of 100 m between each plot. *Tecticornia* plants sampled within each species were similar in plant height and crown diameter. Sampling was done in (i) early March 2012 (middle of 'wet' season), (ii) early July 2012 (middle of dry season), and (iii) late August 2012 (late dry season).

2.3. Pigment analysis

Representative parts of the succulent plant 'stems' (i.e., reduced and fused leaves) were sampled for pigment analysis and tissue water content. Two large branches (from lowest succulent article to tip) were kept on ice in the dark for up to 6 h during transport to the laboratory and then snap-frozen in liquid N_2 and subsequently freeze-dried and stored at −20 °C to preserve the samples. Betacyanin and chlorophyll were analysed for plants sampled at three strategic times: March, July and August 2012. The reason for choosing these sampling periods was that they represent a transition from hot and moist to cooler but drier and stressed conditions, when the seasonal changes in colouration occur.

Prior to quantitative analyses, the water-soluble pigments present in the *Tecticornia* spp. were identified by HPLC-DAD-MS^n. For this purpose, freeze-dried *Tecticornia* stems or *Amaranthus tricolor* leaves (generously provided by Sanjay Nene of the National Chemical Laboratory, Pune, India) were extracted with McIlvaine's buffer (disodium hydrogenphosphate-citric acid buffer, pH 6.5) additionally containing 50 mM L-ascorbate. The aqueous extract was filtered (0.45 μm) into amber glass vials and stored at −20 °C until HPLC analyses. HPLC-DAD-MS^n analyses were conducted as described previously at Schweiggert et al. (2009), except for the different column temperature (25 °C) and a different elution gradient, being based on 1% v/v formic acid in water (solvent A) and methanol (solvent B). The used elution gradient was: from 100 to 90% A in 7 min, from 90 to 80% A in 20 min, from 80 to 60% A in 8 min, from 60 to 35% A in 10 min, isocratic at 35% A for 5 min, from 35 to 0% A in 2 min, isocratic at 0% A for 3 min, and then from 0% to 100% A in 5 min. Total run time was 60 min at a flow rate of 1 ml/min. All further parameters of the HPLC-DAD-MS^n system were set as previously described at Schweiggert et al. (2009).

For quantitative betacyanin analyses, extraction and quantification were done following von Elbe (2001): freeze-dried tissue was pulverised in a ball-mill to a fine powder and then extracted in water using 100 mM MES buffer (2-N-morpholino-ethanesulfonic acid) with pH adjusted to 5.5 using NaOH and ascorbic acid at 50 mM (Strack et al., 2003). 1.8 ml of extracting solvent was added to 60 mg of tissue powder and first mixed in a vortex for 1 min and then in a mechanical shaker for 3 h at 4 °C in the dark. Samples were then centrifuged for 20 min at 15,000 rpm (4 °C). The supernatants were filtered (0.45 μm) and 1.2 ml was transferred to a disposable cuvette for spectral absorbance readings. Blanks of 1.2 ml of extracting solvent were recorded every 10 samples to ensure no drift in the calibration of the instrument. Betacyanin absorbance was read at 538 nm (Strack et al., 2003) on a Cary 3 UV/Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA, spectral bandwidth range 0.2–4 nm; wavelength accuracy ± 0.2 nm). Betacyanin concentrations were calculated using 727 g mol^{-1} as the molecular weight and 60 l mol cm^{-1} as the molar extinction coefficient (von Elbe, 2001) and were expressed on a dry weight basis. Chlorophylls and carotenoids were extracted using cold methanol according to Sims and Gamon (2002). 60 mg of tissue powder was added to 1.8 ml 100% cold methanol and mixed thoroughly in a shaker for 3 h in darkness at 4 °C. Samples were then centrifuged for 15 min at 1500 rpm at 4 °C. The supernatant was filtered (0.45 μm) and absorbance was measured in the same spectrophotometer as described above, at 470, 652.4 and 665 nm. Chlorophylls and carotenoids were estimated according to equations in Wellburn (1994).

2.4. Plant spectral measurements

Plant spectral measurements were performed using a handheld spectrometer (JAZ, Ocean Optics Dunedin, FL, USA) to measure reflectance between 400 and 1200 nm (visible and near-infrared wavelengths) but due to high noise at the end of the spectrum, we only used the bands between 400 and 800 nm. The spectrometer has a spectral resolution of 0.35 nm, giving a total of 1139 bands. Reflectance was estimated as a percentage between a maximum, a pure white reference surface, and a minimum reference value obtained by blocking all light from the fiber optic. Measurements were performed between 12:00 and 13:00 on two consecutive cloud-free days. Plant spectral measurements were taken from the north at two angles, 45° and 90° as determined by a clinometer. Distance between the sensor and the target was 10 cm.

In order to find an index which best estimated pigment concentration across seasons and species, the Pearson correlation coefficient (r) was calculated between pigment concentration (for betacyanin and chlorophyll) and all possible two-band normalised difference indices of the form:

$$I_{ij} = (\lambda_i - \lambda_j)/(\lambda_i + \lambda_j) \quad (1)$$

where λ is the reflectance in the wavelengths *i* and *j*. As we had spectral measurements with 1139 bands the number of all possible two-band combinations was 1295043 (noting that inverting the *i* and *j* in the equation above yields the same correlation with inverted sign). By taking this approach, we are also effectively including some indices which have already been used by researchers for different purposes, for example, the Photochemical Reflectance Index (PRI_{570}) (Peñuelas et al., 2011) which has been shown to be sensitive to carotenoid pigments, and which corresponds with λ_i and λ_j of 531 and 570 nm. The widely used Normalised Difference Vegetation Index (NDVI) (Gamon and Surfus, 1999) uses the reflectance in the near-infrared (NIR) and red bands, but different authors have used different wavelengths for NIR and red and it has also been used with broadband sensors (Table S1).

In order to assess the possible influence of chlorophyll on the correlation between betacyanin and spectral indices, with the aim of identifying the bands most sensitive to betacyanins *per se*, we fitted the following model from Guerschman et al. (2009):

$$C_{betacyanin} = a_0 + a_1 \times C_{chlorophyll} + a_2 \times I_{ij} \quad (2)$$

where $C_{betacyanin}$ and $C_{chlorophyll}$ refer to the concentration of both pigments in mg g^{-1} dry matter, a_0, a_1 and a_2 are the parameters associated to the linear model and I_{ij} corresponds, as in Eq. (1) to all possible two-band indices of the form $I_{ij} = (\lambda_i - \lambda_j)/(\lambda_i + \lambda_j)$. By finding the values of *i* and *j* which maximise the correlation index of the multiple linear model we are effectively identifying a normalised index that best predicts $C_{betacyanin}$, once $C_{chlorophyll}$ is taken into account. This analysis was carried out using the chemically determined chlorophyll concentrations, as well as the spectrally estimated chlorophyll concentrations.

2.5. Statistical analysis

Pigment concentrations and spectral values were compared between dates and species using a repeated measures analysis (Littell et al., 1996) which was performed using the Pro-Mixed pro-

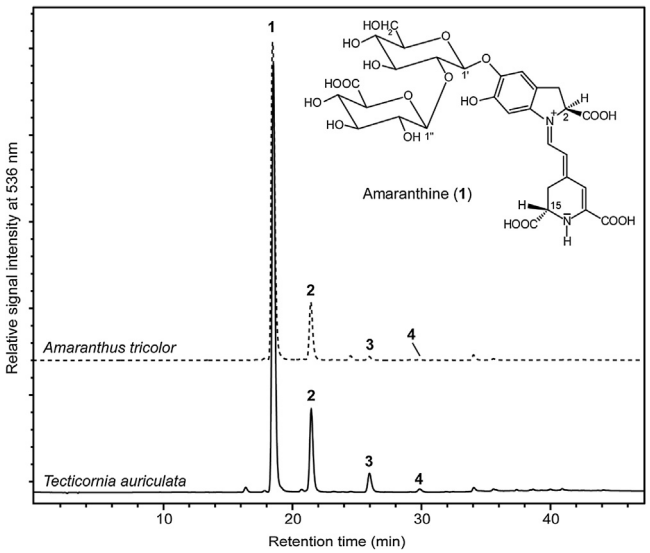

Fig. 2. HPLC separation of betacyanins from *T. auriculata* and *A. tricolor* leaves. Pigment profiles of *T. indica* and *T. auriculata* were identical (*T. indica* is not shown). Peak assignment, 1: amaranthine, 2: isoamaranthine, 3: betanin, 4: isobetanin (see Table 1).

gram from SAS (1999). In all cases we used a significance level of $P < 0.05$.

3. Results

3.1. *Identification of* Tecticornia *spp. betacyanins by HPLC-DAD-MS^n*

In both *T. indica* and *T. auriculata*, an identical set of compounds with betacyanin-specific absorption properties was detected by HPLC-DAD-MS^n (Fig. 2). Identification of amaranthine (**1**), isoamaranthine (**2**), betanine (**3**), and isobetanine (**4**) in *Tecticornia* samples was accomplished by comparing retention times, UV/Vis absorption and mass spectra (Table 1) to data obtained with an authentic *Amaranthus tricolor* sample. For the latter, the betalain profile including UV/Vis absorption and mass data was previously reported by Cai et al. (2005). After verifying the previously reported identity of amaranthine (**1**), isoamaranthine (**2**), betanin (**3**), and isobetanin (**4**) in the *Amaranthus* sample by HPLC-DAD-MS^n (Table 1), the respective compounds **1-4** were identified in both *Tecticornia* samples by co-chromatography and a corresponding comparison of the analytical data reported in Table 1. The profile of water-soluble pigments was highly similar in both *Tecticornia* sp., containing the betacyanins amaranthine (**1**) and isomaranthine (**2**) as major as well as betanin (**3**) and isobetanin (**4**) as minor constituents (Fig. 2). In addition, the betacyanins detected in *Tecticornia* were strikingly similar to those found in the *Amaranthus* sample (Fig. 2), being characteristic for several members of the Amaranthaceae (Cai et al., 2005).

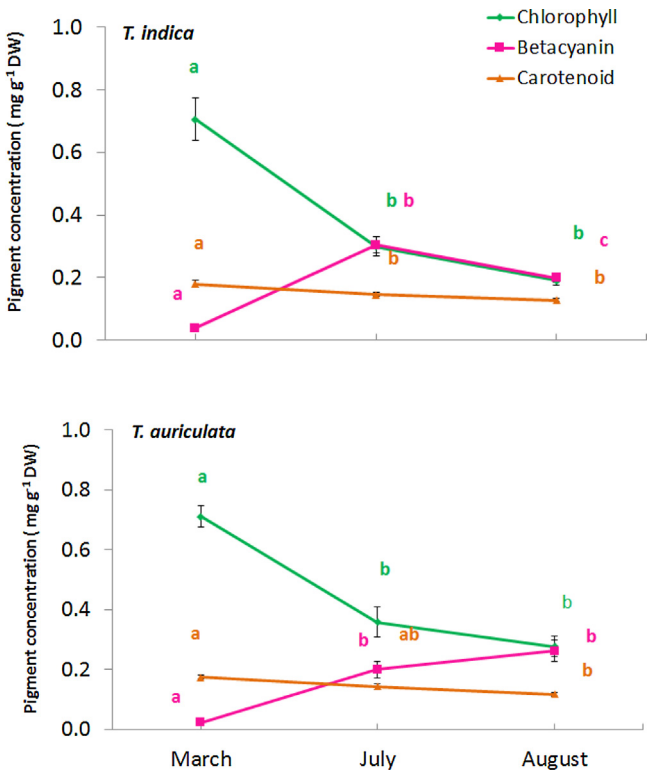

Fig. 3. Betacyanin, chlorophyll and carotenoid concentrations for *T. auriculata* and *T. indica* at Fortescue Marsh for March, July and August 2012. Different letters indicate significant differences with time for each individual pigment at $P = 0.05$. Precipitation in the month preceding the sampling was 40.6, 5.0 and 0.0 mm for the March, July and August samplings, respectively.

3.2. *Pigment dynamics*

Betacyanin and chlorophyll concentrations showed strong seasonal dynamics, with opposing trends, whereas carotenoid concentrations were relatively stable. Betacyanin concentrations increased almost nine times in both species (Fig. 3, $P < 0.001$) from the end of the wet summer (March) to the end of the dry winter (August), while plant colour changed from green to red-pinkish (Fig. S2). No significant differences in betacyanin concentrations were detected between species ($P = 0.53$, Fig. 3). For *T. indica*, betacyanin concentration was higher during July and then declined significantly towards the end of August. In *T. auriculata* betacyanin concentration continued to increase until the end of the dry season, although the differences between July and August were not statistically significant ($P = 0.22$, Fig. 3). Contrary to betacyanin concentrations, chlorophyll concentrations were significantly higher during March and significantly ($P < 0.05$) lower during July-August. Carotenoid concentrations declined slowly, showing significantly lower values at the end of the dry season ($P < 0.05$, Fig. 3).

Table 1
HPLC retention times, UV/Vis absorption maxima and mass spectral data of betalains from *Tecticornia* spp. and *Amaranthus tricolor*.

PeakNo.	Compound identity	HPLC retention time [min]	UV/Vis absorption maximum [nm]	$[M+H]^+$ *m/z*	HPLC-ESI-(+)-MS^n *m/z* (rel. intensity)
1	Amaranthine	18.3	535	727	[727]: 389 (100), 551 (37)
2	Isoamaranthine	21.4	535	727	[727]: 389 (100), 551 (21)
3	Betanin	26.0	535	551	[551]: 389 (100)
4	Isobetanin	29.8	535	551	[551]: 389 (100)

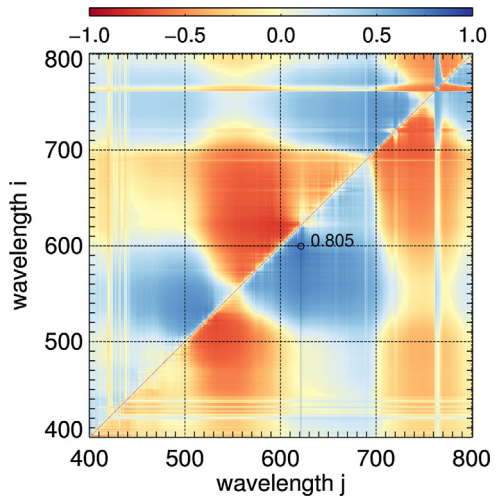

Fig. 4. Correlation coefficients between betacyanin concentration (mg g^{-1} DW) and different normalised wavelength ratios for *T. auriculata* and *T. indica*. The correlations are based on observations for both species at three different dates in 2012. The difference normalised ratios using 600 and 620 nm showed the highest correlation with betacyanin concentration (r = 0.805). Note that the correlation array is symmetrical (with inverted sign) along the diagonal axis.

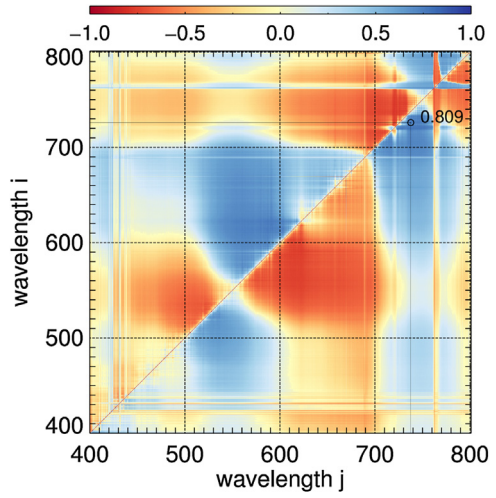

Fig. 5. Correlation coefficients between chlorophyll (a + b) concentration (mg g^{-1} DW) and different normalised wavelength ratios for *T. auriculata* and *T. indica*. The correlations are based on observations for both species at three different dates in 2012. The normalised ratio for 726 and 737 nm showed the highest correlation with chlorophyll concentration (r = 0.809).

3.3. Spectral reflectance

Tecticornia reflectance spectra changed seasonally along with the pigments dynamics. As trends were quantitatively and qualitatively similar for the two species, correlations between pigment composition and reflectance spectra were analysed for the combined dataset comprising both species. The best correlation between betacyanin concentration and spectral reflectance was found for the normalised ratio using the bands at 600 and 620 nm (r = 0.805, Fig. 4), but high correlations were also found for bands in the range 510–550 nm, which indicates a possible influence of the peak absorption of betacyanins at near 540 nm (Fig. 4). Among the indices most established in literature, the Structure Insensitive Pigment Index (SIPI) (Peñuelas et al., 1995) was the one that best correlated (r = 0.73) with betacyanin. In contrast, other indices showed weak or no correlation with betacyanin concentration (Table S1). Regarding chlorophylls, the pattern was very similar to

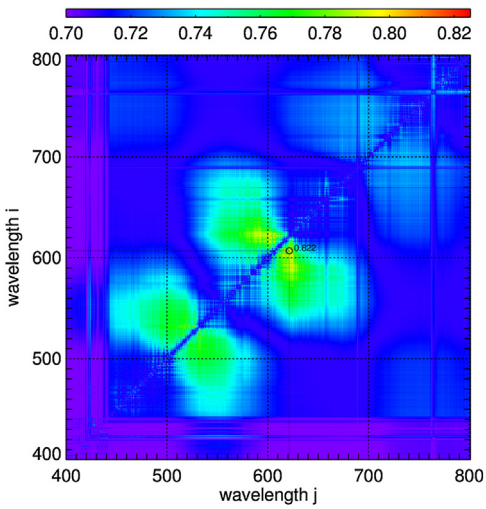

Fig. 6. Correlation coefficients for the multiple correlation of betacyanin concentration (mg g^{-1} DW) with different normalised wavelength ratios (nm) and measured chlorophyll concentrations. The analysis is based on combined data for *T. auriculata* and *T. indica* for three dates in 2012.

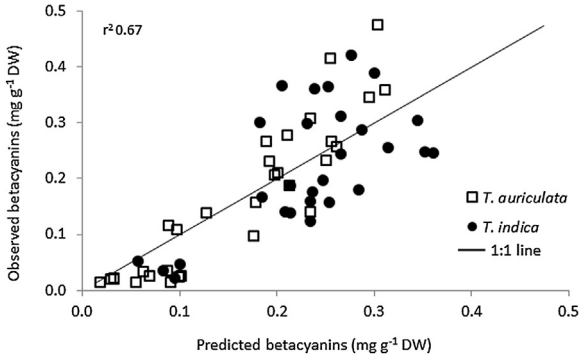

Fig. 7. Observed versus predicted betacyanin concentration for *T. indica* and *T. auriculata* for March, July and August 2012. Predicted values obtained according the model in Eq. (2) using the normalised index of wavelengths 606 and 621 nm.

that observed for betacyanins but with opposite sign (Fig. 5). The highest correlation (r = 0.809) with chlorophyll was found for a normalised index using the bands at 737 and 726 nm, which detects the 'red edge effect' (Sims and Gamon, 2002). Also, high correlation values were found between chlorophyll and indices calculated using the bands in the range of 600–620 and 580–600 nm (Fig. 5). The clear opposing patterns of Figs. 4 and 5 show that concentrations of betacyanin and chlorophyll were strongly and negatively correlated with each other.

Given the strong negative correlation between betacyanin and chlorophyll concentrations, it is important to examine if consideration of the chlorophyll concentration can improve the ability of the spectral index to predict betacyanin concentration. Fig. 6 shows correlation coefficients for multiple correlations based on Eq. (2), using measured chlorophyll concentration and normalised indices of all possible wavelengths. The normalised index using the wavelengths 606 and 621 nm allowed for a better prediction of betacyanin concentration (r = 0.822) than the index that did not consider chlorophyll concentration (Fig. 4). The parameters a_0, a_1 and a_2 for this model were 0.309, −0.160 and −2.373 respectively.

The regression between the observed and the predicted betacyanin concentration using the normalised ratio 606/621 and measured chlorophyll concentration showed a good fit (r^2 0.67, Fig. 7), suggesting a potential and reliable use of this method to estimate this pigment via remote sensing.

Finally, having established that betacyanin concentrations could be estimated with the new normalised difference index, and that taking into account the (chemically) measured chlorophyll concentrations improved the accuracy of the betacyanin estimates, we then performed a final test to determine if the same improved accuracy could be achieved using spectrally estimated chlorophyll concentrations. Chlorophyll was estimated using the wavelengths 726 and 737 nm (Fig. 5) and then used to fit Eq. (2). The parameters a_0, a_1 and a_2 were 0.309, −0.160 and −2.373, and the r^2 for the regression was 0.64 (Fig. S2), almost as good as in the model using measured chlorophyll concentrations. Thus, both pigments, chlorophyll and betacyanin, can be simultaneously spectrally detected using the models and the wavelengths presented here.

4. Discussion

Variations in pigmentation in leaves and other tissues have been widely studied in relation with plant phenological and physiological changes (Demmig-Adams, 1990; Chalker-Scott, 1999; Lee, 2002). However, despite its high potential value, the detection of these changes by spectrometry is still underdeveloped, especially for pigments other than chlorophylls. In this study, we showed that seasonal pigment changes of two stem-succulent halophytic shrubs (*Tecticornia* species), specifically the red-pinkish betacyanins, can be detected using remote sensing.

Seasonal changes in betacyanin concentrations were observed in the two *Tecticornia* species, with levels increasing from the wet season to the dry season. Changes in betacyanins have also been described for other species in the Amaranthaceae with an increase in levels under stress. As examples, in *Suaeda salsa* (Zhao et al., 2010), *Suaeda japonica* (Hayakawa and Agarie, 2010) and *Sarcocornia fructicosa* (Duarte et al., 2013) betacyanin synthesis increased after increased light intensity, lower temperatures and during drought. In these species betacyanins appear to be the major stress-related pigment, more than anthocyanins or carotenoids. Chlorophylls in the two *Tecticornia* species, in contrast with the betacyanins, were highest during the wet-season and decreased during the dry season. However, carotenoids did not show substantial changes over time. Given the potential for a protective role of betacyanins under stress conditions (Stintzing and Carle, 2004), a role that also carotenoids have (Young, 1991), it is likely that betacyanins play an important function in *Tecticornia* species.

The changes observed in the two *Tecticornia* species due to betacyanins colouration was accurately detected by using a linear model considering the total chlorophyll concentration (which was also spectrally estimated using the normalised ratio of reflectance at wavelengths 726/737 nm proposed in this study), and a normalised ratio for betacyanins using the reflectance at wavelengths 606/621 nm as also proposed here. Gamon and Surfus (1999), who have explored many spectral indices related to chlorophylls and anthocyanins, have concluded that compared to other indices, the two-bands reflectance ratio approach has the advantage of reducing the multidimensional spectrum to a few values. However, although indices such as PRI or ARI have demonstrated strong correlation with changes in carotenoids and anthocyanins, respectively, in other species (Stylinski et al., 2002; Gitelson et al., 2006), these indices showed a weak correlation with betacyanins in the two *Tecticornia* species (Table S1). Due to the prominence of betacyanins in *Tecticornia* shoot tissues, the new proposed index detects seasonal pigment changes more accurately than those other two existing indices.

Although our results relate to measurements at plant scale, the dominance of *Tecticornia* species in the outer areas of Fortescue Marsh (>90%) plus the strong seasonality observed in their pigments makes detecting these changes at larger scales plausible. A preliminary study done at plot level and using the same technique (and device) confirmed the significant increase of the normalised index using 606/621 nm wavelengths for this vegetation towards the end of the dry season, corresponding with observed changes in pigments in these *Tecticornia* plants (V. Marchesini, unpublished data). We recommend further studies at larger scales, including the evaluation of the normalised index 606/621 nm that we propose for assessment of betacyanins, using airborne or satellite hyperspectral remote sensors.

Tecticornia species cover a large proportion of saline ecosystems of Australia (Wilson, 1980) and might be suitable for revegetation/stabilisation of degraded mining or saline areas (Thompson and Thompson, 2004). The spectral detection of vegetation condition by observing changes in betacyanins, as well as chlorophylls, is a promising method to monitor seasonal changes and might also enable detection of other, non-seasonal changes including from possible impacts on *Tecticornia* communities. The new spectral index presented in this study offers an alternative for other indices in vegetation dominated by species with betacyanin pigments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jag.2016.07.002.

References

Archetti, M., 2009. Classification of hypotheses on the evolution of autumn colours. Oikos 118, 328–333.
Brockington, S.F., Walker, R.H., Glover, B.J., Soltis, P.S., Soltis, D.E., 2011. Complex pigment evolution in the Caryophyllales. New Phytol. 190, 854–864.
Cai, Y., Sun, M., Corke, H., 2005. HPLC characterization of betalains from plants in the Amaranthaceae. J. Chromatogr. Sci. 43, 454–460.
Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70, 1–9.
Datson, B., 2002. Samphires in Western Australia: a field guide to Chenopodiaceae tribe Salicornieae, Perth, Australia.
Demmig-Adams, B., 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim. Biophys. Acta—Bioenergetics 1020, 1–24.
Duarte, B., Santos, D., Marques, J.C., Caçador, I., 2013. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback—Implications for resilience in climate change. Plant Physiol. Biochem. 67, 178–188.
English, J.P., Colmer, T.D., 2011. Salinity and waterlogging tolerances in three stem-succulent halophytes (*Tecticornia* species) from the margins of ephemeral salt lakes. Plant and Soil 348, 379–396.
Gamon, J.A., Surfus, J.S., 1999. Assessing leaf pigment content and activity with a reflectometer. New Phytol. 143, 105–117.
Gamon, J.A., Peñuelas, J., Filed, C.B., 1992. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sens. Environ. 41, 35–44.
Gitelson, A.A., Keydan, G.P., Merzlyak, M.N., 2006. Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophys. Res. Lett. 33, L11402.
Gitelson, A.A., Chivkunova, O.B., Merzlyak, M.N., 2009. Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. Am. J. Bot. 96, 1861–1868.
Guerschman, J.P., Hill, M.J., Renzullo, L.J., Barrett, D.J., Marks, A.S., Botha, E.J., 2009. Estimating fractional cover of photosynthetic vegetation, non-photosynthetic vegetation and bare soil in the Australian tropical savanna region upscaling the EO-1 Hyperion and MODIS sensors. Remote Sens. Environ. 113, 18–18.

Hayakawa, K., Agarie, S., 2010. Physiological roles of betacyanin in a halophyte, *Suaeda japonica* Makino. Plant Prod. Sci. 13, 351–359.
Ibdah, M., Krins, A., Seidlitz, H.K., Heller, W., Strack, D., Vogt, T., 2002. Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ. 25, 1145–1154.
Kelly, M., Tuxen, K.A., Stralberg, D., 2011. Mapping changes to vegetation pattern in a restoring wetland: finding pattern metrics that are consistent across spatial scale and time. Ecol. Indic. 11, 263–273.
Kerr, J.T., Ostrovsky, M., 2003. From space to species: ecological applications for remote sensing. Trends Ecol. Evol. 18, 299–305.
Lee, D.W., 2002. Anthocyanins in autumn leaf senescence. Adv. Bot. Res. 37, 147–165. Academic Press.
Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC.
Moir-Barnetson, L., 2015. Ecophysiological responses to changes in salinity and water availability in stem-succulent halophytes (*Tecticornia spp.*) from an ephemeral salt lake. In: PhD Thesis. Plant Biology. The University of Western Australia.
Ollinger, S.V., 2011. Sources of variability in canopy reflectance and the convergent properties of plants. New Phytol. 189, 375–394.
Peñuelas, J., Baret, F., Filella, I., 1995. Semi-empirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. Photosynthetica 31, 221–230.
Peñuelas, J., Garbulsky, M.F., Filella, I., 2011. Photochemical reflectance index (PRI) and remote sensing of plant CO_2 uptake. New Phytol. 191, 596–599.
Rabhi, M., Castagna, A., Remorini, D., Scattino, C., Smaoui, A., Ranieri, A., Abdelly, C., 2012. Photosynthetic responses to salinity in two obligate halophytes: *Sesuvium portulacastrum* and *Tecticornia indica*. S. Afr. J. Bot. 79, 39–47.
SAS, 1999. In: Wolfinger, R., Chang, M. (Eds.), Comparing the SAS GLM and MIXED Procedures for Repeated Measures. SAS Institute Inc, Cary, NC.
Schweiggert, R.M., Villalobos-Gutierrez, M., Esquivel, P., Carle, R., 2009. Development and optimization of low temperature enzyme-assisted liquefaction for the production of colouring foodstuff from purple pitaya (*Hylocereus sp*. [Weber] Britton & Rose). Eur. Food Res. Technol. 230, 269–280.
Shepherd, K.A., van Leeuwen, S.J., 2010. *Tecticornia globulifera* and *T. medusa* (subfamily Salicornioideae: Chenopodiaceae), two new priority samphires from the Fortescue Marsh in the Pilbara region of Western Australia. Telopea 13, 349–358.
Shepherd, K.A., Wilson, P.G., 2007. Incorporation of the Australian genera *Halosarcia*, *Pachycornia*, *Sclerostegia* and *Tegicornia* into *Tecticornia* (Salicornioideae, Chenopodiaceae). Aust. Syst. Bot. 20, 319–331.
Shuman, C.S., Ambrose, R.F., 2003. A comparison of remote sensing and ground-based methods for monitoring wetland restoration success. Restor. Ecol. 11, 325–333.
Sims, D.A., Gamon, J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81, 337–354.
Skrzypek, G., Dogramaci, S., Grierson, P.F., 2013. Geochemical and hydrological processes controlling groundwater salinity of a large inland wetland of northwest Australia. Chem. Geol. 357, 164–177.
Stintzing, F.C., Carle, R., 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci. Technol. 15, 19–38.
Strack, D., Vogt, T., Schliemann, W., 2003. Recent advances in betalain research. Phytochemistry 62, 247–269.
Stylinski, C., Gamon, J., Oechel, W., 2002. Seasonal patterns of reflectance indices, carotenoid pigments and photosynthesis of evergreen chaparral species. Oecologia 131, 366–374.
Thompson, S.A., Thompson, G.G., 2004. Adequacy of rehabilitation monitoring practices in the Western Australian mining industry. Ecol. Manage. Restor. 5, 30–33.
Timms, B., 2005. Salt lakes in Australia: present problems and prognosis for the future. Hydrobiologia 552, 1–15.
Ustin, S.L., Roberts, D.A., Gamon, J.A., Asner, G.P., Green, R.O., 2004. Using imaging spectroscopy to study ecosystem processes and properties. Bioscience 54, 523–543.
Ustin, S.L., Gitelson, A.A., Jacquemoud, S., Schaepman, M., Asner, G.P., Gamon, J.A., Zarco-Tejada, P., 2009. Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. Remote Sens. Environ. 113, 67–77.
von Elbe, J.H., 2001. Betalains. In: Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc.
Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313.
Wilson, P.G., 1980. A revision of the Australian species of Salicornieae (Chenopodiaceae). Nuytsia 3, 3–154.
Young, A.J., 1991. The photoprotective role of carotenoids in higher plants. Physiol. Plant. 83, 702–708.
Zhao, S.-Z., Sun, H.-Z., Chen, M., Wang, B.-S., 2010. Light-regulated betacyanin accumulation in euhalophyte *Suaeda salsa calli*. Plant Cell Tissue Organ Cult. (PCTOC) 102, 99–107.
Zomer, R.J., Trabucco, A., Ustin, S.L., 2009. Building spectral libraries for wetlands land cover classification and hyperspectral remote sensing. J. Environ. Manage. 90, 2170–2177.