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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)

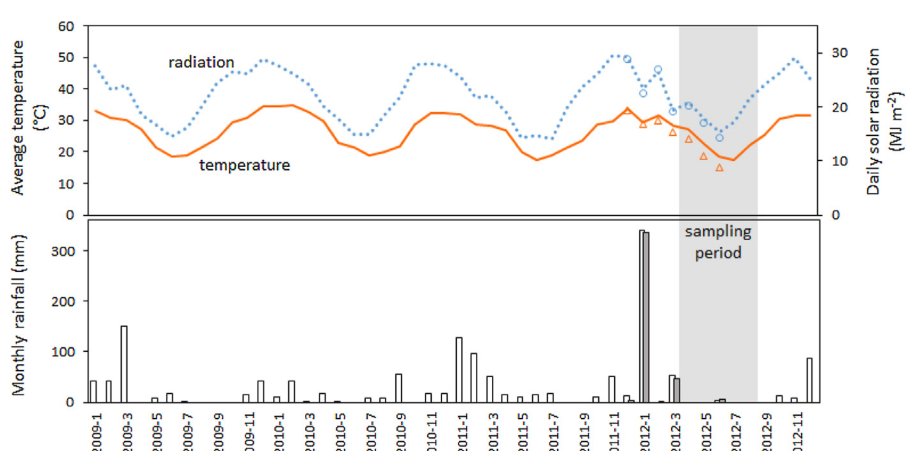

Fig. 1. Weather conditions in the study area. The top panel shows monthly average air temperature (°C; solid line for nearby permanent weather station, triangles for measurements at study site) and daily total solar radiation (MJ m^{-2}; dotted line for nearby permanent weather station, circles for measurements 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).