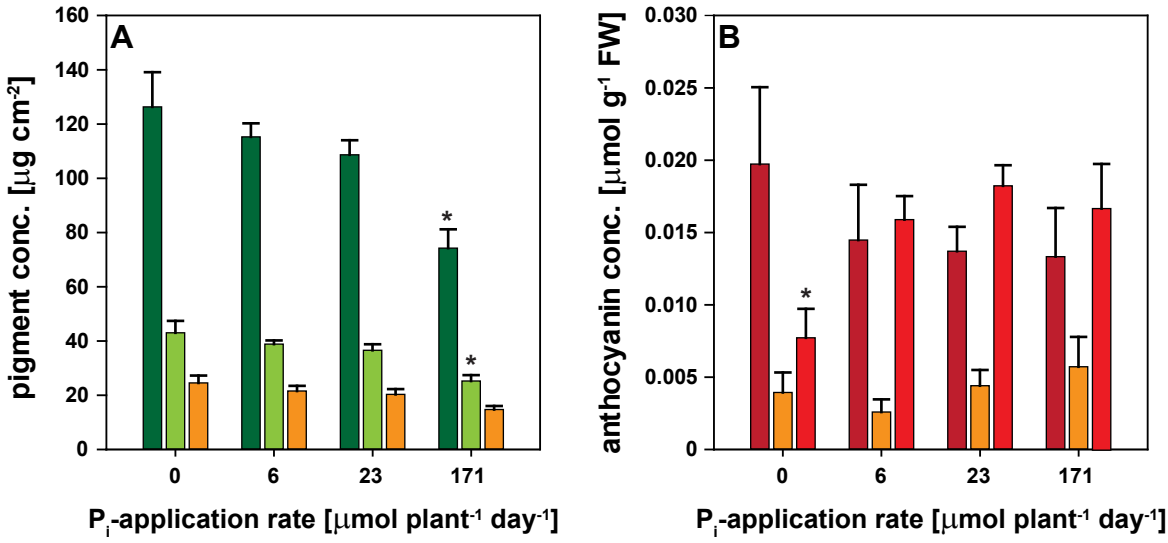
Supplemental Figure S1



Supplemental Figure S1: Accumulation of photosynthetic pigments and anthocyanins in leaves of *Hakea prostrata* plants grown at a range of inorganic phosphate (P_i) supplies. At the highest P_i supply, mature leaves became chlorotic as a sign of P_i toxicity. A, Chlorophyll a (dark-green bars), chlorophyll b (light-green bars) and carotenoid (orange bars) concentrations in mature leaves. B, anthocyanin concentration in young (dark red bars), mature (orange bars), and senescing (red bars) leaves. Four-month old nursery seedlings were grown in nutrient solution for 12 weeks before being exposed to the indicated P_i supplies in nutrient solution (experiment A). After three weeks of treatment, a destructive harvest was undertaken. Values are means ± SE, n = 3 to 6. Treatment effects were analyzed using a One-Way Repeated Measures Analysis of Variance (ANOVA) for A or a Two-Way ANOVA for B. Significant differences across treatments according to Tukey's honestly significant difference (HSD) test at $P < 0.05$ are indicated by (*).

Methods

Analysis of photosynthetic pigments

Photosynthetic pigments were extracted from two freshly-harvested mature leaf discs of *Hakea prostrata* (0.07 cm^2 each) using 100% methanol (Wellburn, 1994). Aliquots of 385 μL extract were transferred to a 96-well plate resulting in a 1-cm path length and the absorbance of carotenes, xanthophylls, and chlorophylls (a and b) was determined spectrophotometrically at 470, 653 and 666 nm, respectively, using the Multiskan Spectrum Plate Reader (Thermo Scientific, Scoresby, Australia).

Analysis of anthocyanins

The amount of anthocyanin in leaf samples was determined using a modified pH-differential method (Wrolstad et. al., 2005). The extracts used for P_i analysis were also used for anthocyanin assays. Aliquots of 60 μL of the extracts were combined with 240 μL of either 25 mM potassium chloride buffer (pH 1.0) or 400 mM sodium acetate buffer (pH 4.5). The samples were incubated in the dark for 15 minutes at room temperature before their absorbance was measured at 520 nm and 700 nm (Multiskan Spectrum Plate Reader, Thermo Scientific, Scoresby, Australia). Concentrations were calculated using the molar absorptivity of the predominant anthocyanin in the sample (cyanidin-3-glucoside, ε = 26,900 L mol^{-1} cm^{-1}).

References

Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology **144:** 307-313

Wrolstad RE, Durst RW, Lee J (2005) Tracking color and pigment changes in anthocyanin products. Trends in Food Science & Technology **16:** 423-428.