THE FUNCTIONAL ROLE OF ANTHOCYANINS IN LEAVES

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FRONTISPIECE

Anthocyanic and acyanic leaves of *Elatostema rugosum* (Urticaceae) photographed in the Waitakere Ranges, New Zealand (Source: K. S. Gould).

ABSTRACT

The anthocyanins, a relatively small group of pigments in the diverse flavonoid family, are largely responsible for the red-blue colouration in a large number plant species worldwide. Their occurrence in fruits and flowers seems to offer clear benefits in attracting pollinators and aiding seed dispersal, however their presence in the vacuoles of leaves remains obscure.

The accumulation of anthocyanin pigments in leaves can be induced by a host of disparate environmental and anthropogenic stressors, such as UV-exposure, wounding, pathogen infection, high light, chilling, pollution, osmotic stress, and nutrient deficiency. Anthocyanins are in some species expressed throughout leaf development; in others the pigments are found exclusively in the young, rapidly expanding leaves, or at later stages of leaf senescence. Many researchers have explored the possible functional role(s) of anthocyanins within leaves, although none of the hypotheses provides a unified explanation for the diverse range of environmental triggers, or for the variability in the pigment's location and expression at particular stages of development.

Differences in the cellular location of cyanic pigments had little effect on the optical properties of leaves from several native New Zealand species. Instead, the absorptance of green-yellow light was strongly proportional to the concentration of anthocyanin. Red leaves absorbed up to 17 % more PAR (400-700 nm) than green leaves of same species. The reflectance of red light was independent of leaf anthocyanin content.

In *Lactuca sativa*, the absorption of light by anthocyanic cell vacuoles in the upper epidermis led to a reduction in the light incident on subjacent chlorenchyma. Under high irradiances, the dissipation of excess energy through non-photochemical processes (qNP) was lower in the red regions of the leaves than in the green regions. Red *L. sativa*, maintained higher photochemical efficiencies (ΦPSII), and had greater rates of photochemical quenching (qP) than the green tissues. Moreover, the cyanic areas were photoinhibited significantly less (7 %), as measured by levels of maximum PSII efficiency (Fv/Fm), than green areas during a high light treatment of 1300 μmol m^{-2} s^{-1}. Chloroplast suspensions from shade adapted *L. sativa* generated less superoxide ($O_2^{\cdot -}$) through the Mehler reaction and had reduced rates of

chlorophyll bleaching, when irradiated with 300 μmol m^{-2} s^{-1} of red light rather than white light. These data demonstrated the impact of the light-filtering effects of anthocyanins on the photophysiology of the leaves.

Complementary to their light-shielding functions, anthocyanins also demonstrated potent antioxidant capabilities at pH values typical of both the cytoplasm and the vacuole. The addition of cyanidin-3-malonylglucoside, which was colourless at the cytoplasmic pH, to a chloroplast suspension receiving high irradiance resulted in the significant scavenging of $O_2^{\cdot -}$. The red, flavylium form of anthocyanin was also oxidised by $O_2^{\cdot -}$. After 15 minutes, this oxidation equated to a 40 % reduction in antioxidant potential of the anthocyanin, as measured by cyclic voltammetry. The data suggested that anthocyanins could provide widespread cellular protection to cellular membranes, organelles, and DNA.

Analysis of the overall antioxidant defence in red and green leaves of *Elatostema rugosum*, a shade-adapted herb native to New Zealand, and *Quintinia serrata*, a native tree found on exposed ridges, provided evidence for a photoprotective role of anthocyanins. Red leaves of *E. rugosum* were on average five-times more effective at scavenging DPPH radicals than were green leaves. The anthocyanins constituted the most active phenolic component, providing a greater relative contribution to the antioxidant pool than the flavones, flavonols, and hydroxycinnamic acids. In contrast, red and green leaves of *Q. serrata* exhibited comparable ranges in antioxidant activities. The data suggest that for some species, anthocyanins can supplement the pool of low molecular weight antioxidants but are not a prerequisite for protection from oxidative stress. It is likely that the localised accumulation of anthocyanins in the leaves of *Q. serrata* serves to shield photosynthetic tissues that are the more susceptible to photodamage.

In conclusion, the accumulation of anthocyanins represents a multifunctional mechanism to; i) directly reduce ROS through scavenging and possibly metal chelation, acting in conjunction with other antioxidants, and ii) to shield photosynthetic processes experiencing excessive irradiances, thereby reducing the extent of photooxidation, photoinhibition, and wasteful dissipatory systems. Such protection may be vital for leaves experiencing stressful environments.

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PREFACE

The chapters in this thesis have been written in the format of individual papers that either have been recently submitted for publication or have already been published. As such, each chapter introduces its particular topic separately, describes the methods used, and discusses the results in detail as independent entities. In contrast, the general introduction, background sections, and conclusions refer to the thesis as a whole.