

Pigments in flowers are not only attractive: Anthocyanins as a sunscreen for the pollen grains

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are responsible for most of the red, pink, purple and blue colours of flowers and fruits, where they mostly play a signalling role. In the flowers, plants synthesise anthocyanins to selectively attract pollinators, which is crucial for the sexual reproduction of animal-pollinated plants. The pigmentation pattern of the petals is the main guide for pollinators, although other parts of the flowers, such as the anthers, can also be engaged in this function. Apart from animals, the transfer of the pollen grains between plants can be performed either by the wind or by water. In these types of plants, flowers are usually inconspicuous and gathered in catkins. As they do not need animals to perform the pollination and consequently, they do not need to attract them, one might expect the absence of anthocyanins in these types of flowers, above all if we bear in mind that the synthesis of these secondary metabolites represents a metabolic cost for the plant. However, it is quite interesting to observe in nature some examples of wind-pollinated plants that show red or purple colourations in their flowers. This is the case of the three main species of the genus *Populus* L. in the Iberian Peninsula: *P. nigra* L., *P. alba* L. and *P. tremula* L., that all show purple-red anthers in their male catkins.

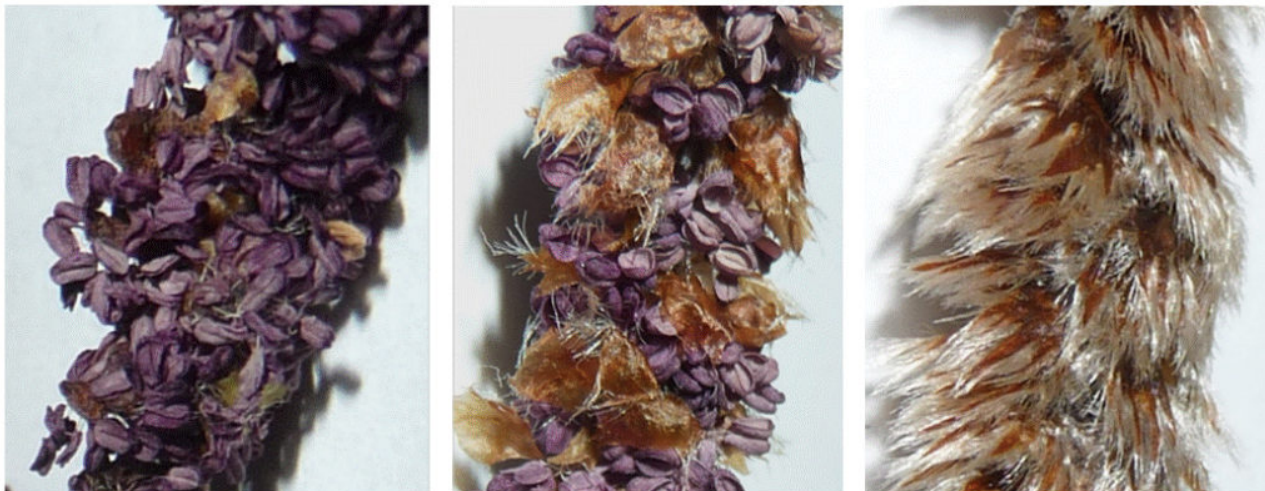


Fig. 1. Detail of the male catkins (lyophilised) of *Populus nigra* (left), *P. alba* (middle) and *P. tremula* (right), showing the coloured anthers and the degree of hairiness of the bracts

The first objective of this work was to figure out the possible role of this type of pigments in the anthers. Apart from flowers and fruits, anthocyanins can be present in vegetative parts of the plants such as leaves, stems or even roots, where their presence has been related to protective roles. In the case of autumn leaves, it has been proposed that anthocyanins can shield photosynthetic

tissues from excess light and, consequently, they are protecting foliar nutrient resorption during senescence. In the case of the anthers of the three *Populus* species studied in this work, we have hypothesised that the role of the anthocyanins may also be photoprotective. In these three species of poplars, flowering takes place before foliar emergence and, consequently, anthers and pollen grains are exposed to sunlight. Thus, the synthesis of anthocyanins might be aimed to avoid the damage of the male gametes by excessive sunlight.

The HPLC-DAD-MS analyses of the extracts of the anthers of these three *Populus* species revealed the presence of several anthocyanins (up to 17 different pigments), but in all cases cyanidin 3-O-glucoside was the major one with percentages higher than 60%. Light can induce several enzymes of the biosynthetic pathway of anthocyanins and cyanidin is one of the primary anthocyanins in that pathway. For this reason, cyanidin derivatives are commonly the first anthocyanins synthesised in response to light. For comparative purposes, the red autumn leaves of one of the *P. tremula* specimens were also analysed in the present study. At this location, where anthocyanins are performing a photoprotective role, cyanidin 3-O-glucoside was almost the only compound detected (more than 92%).

Catkins of these three species possess hairy bracts that can provide partial physical shading to the anthers. However, there are differences in the degree of pilosity of the bracts among the different species (Fig. 1). It has been very interesting to observe a reverse relationship between the total anthocyanin content of the anthers and the degree of hairiness of the catkins: the highest total anthocyanin content was observed in *P. nigra* samples, where the bracts hardly supply physical shading. On the contrary, the lowest total anthocyanin content was observed in *P. tremula* samples, where the hairs of the bracts were long and dense and cover all the anthers. Thus, as the physical shading increases, the need of anthocyanins to perform their photoprotective role is lessened and, consequently, their syntheses seem to be reduced.

Furthermore, the study of the differences in the anthocyanin profile of the anthers of these three species has resulted in other interesting results. Whereas most of the compounds are present (but in different percentages) in all the studied species, some of them are exclusive of only one species. These single compounds that are species-indicative can, therefore, be used as chemotaxonomic markers. In addition, the employment of statistical techniques in the data matrix constituted by the total anthocyanin content and by the percentages of the different anthocyanins in each sample has allowed a classification of the samples which is in accordance with the initial classification of the specimens carried out on the basis of their morphological characters.

All these results reveal the usefulness of the anthocyanin composition of the anthers of these three species of *Populus* for chemotaxonomic purposes.

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