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Research article

Erythronium dens-canis L. (Liliaceae): An unusual case of change of leaf mottling

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ABSTRACT

Erythronium dens-canis is an early-flowering understory lily of southern Europe with two leaves and a single flower, although a number of plants have only one leaf and do not flower. The leaves are mottled with silvery flecks and brown patches, that gradually vanish turning to a lively green color. The nature and function of this striking variegation pattern were investigated in differently colored leaf parts following the springtime color change. Tissue organization was examined by light and electron microscopy; photosynthetic pigments were analyzed by spectrophotometry and HPLC; chlorophyll fluorescence parameters were evaluated by MINI-PAM. The results showed that brown patches originated in vacuolar anthocyanins in the subepidermal cell layer while air spaces between the upper epidermis and underlying chlorenchyma resulted in silvery flecks. The two leaf areas did not differ in photosynthetic pigments, chloroplast organization and photosynthetic parameters (F_v/F_m, NPQ, rETR). Greening of brown patches due to anthocyanin resorption was faster in non-flowering plants than in flowering ones, occurring only when young fruits were developing. Anthocyanin disappearance did not change the structural-functional features of photosynthetic tissues. As a whole the results suggest that the anthocyanin pigmentation of *E. dens-canis* leaves does not affect the photosynthetic light use and has no photoprotective function. It is proposed that the complex leaf color pattern may act as a camouflage to escape herbivores, while the reflective silvery spots may have a role in attracting pollinators of this early-flowering species.

1. Introduction

Erythronium dens-canis L. (dogtooth lily, Liliaceae) is an understory species of deciduous woods in northern Italy, locally common on north-exposed hillsides. The plant is perennial with a short growth season (a spring ephemeral) like all other *Erythronium* species, most of which are native to northern America (Allen et al., 2003; Mathew, 1992). Flowering specimens have a pair of elongate leaves and a single, nodding-postured purplish flower on top of a thin flower stalk, developing from the bulb at the end of the winter. However, a number of *E. dens-canis* plants has only a single, large, oval leaf and does not flower. The multicolored pattern of the leaves and the acuminated subterranean bulb, which gave the Linnean name of this small lily, has been known for centuries. Typically, the leaves exhibit a lively red-brown and silver-grey mottling, described by Hara (Hara, 1957) as silver-grey flecks surrounded by red-brown areas of leaf tissue. The red pigment present in these areas is believed to be an anthocyanin Ref. (Esteban et al., 2008). It is noteworthy that this pigment invariably disappears within a few weeks giving way to a bright-green leaf color all around the permanent silver-grey flecks. By this the leaves change from a red-brown/silver-grey mottling to a green/silver-grey appearance.

Variegated leaves are common among the understory plants. In most cases, e.g. *Pulmonaria officinalis* (Esteban et al., 2008), *Arum italicum* (La Rocca et al., 2011), or *Cyclamen* species (Konoplyova et al., 2008), the variegation depends on different shades of green. The lighter spots are persistent and are possibly related to strategies of light exploitation in the limiting woodland habitat (Lee, 1986), but deception of herbivores is another possibility (Givnish, 1990; Campitelli et al., 2008). Some peculiarities distinguish *E. dens-canis* from other variegated understory plants: (i) the

Abbreviations: A, antheraxanthin; β-car, β-carotene; Car, total carotenoids; Chl, chlorophyll; F_m, maximum fluorescence; Fo, minimum fluorescence; F_v, variable fluorescence; FW, fresh weight; L, lutein; MINI-PAM, mini-pulse amplitude modulated; N, neoxanthin; NPQ, non-photochemical quenching; PAR, photochemically active radiation; rETR, relative electron transport rate; V, violaxanthin; Z, zeaxanthin.

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uncommon color and complex patterns of leaf variegation (mottling), (ii) the transient nature of the leaf pigmentation, (iii) the early and short growth season that allows this plant to operate in the ephemeral open understory environment before tree leafing and canopy closure.

Several recent papers dealt with germination of *Erythronium* species (Kondo et al., 2002), phenology (Lambert et al., 2010), taxonomy (Allen et al., 2003) and metabolism (Gandin et al., 2011), but the peculiar change of color patterns has never been investigated. We have studied the nature and possible functions of leaf mottling in *Erythronium dens-canis* by examining the tissue and cell organization and photosynthetic pigments and parameters in different types of leaf areas, before and after the springtime color change. We find that the pigmentation of the red-brown areas is due to vacuolar anthocyanins of a subepidermal cell layer, whereas air spaces between adaxial epidermis and underlying chlorenchyma give rise to the silver-grey flecks. The two types of leaf areas do not differ in terms of chloroplast organization and photosynthetic pigments and parameters, prior to and following the loss of red-brown patches. We conclude that the changing leaf mottling of *Erythronium dens-canis* is influential on the photosynthetic properties, but anthocyanins do not seem to have a role as a photoprotective sunscreen. The unique leaf color pattern clearly provides an advantage to the plant in its specific growth environment and can best be interpreted in terms of camouflage against herbivores and/or of pollinator attraction of this early-flowering species.

2. Results

2.1. Plant types and flecking

The time-course of *E. dens-canis* growth and flowering in hilly woodlands in the surroundings of Bologna was followed during late winter and early spring 2012. Most fertile plants emerged from the soil and bloomed in a peak lasting only a few days around the middle of March, while a minority of plants flowered 1–2 weeks later, before tree leafing (see below). Flowering (fertile) specimens had two leaves, which often differed in both shape and level of mottling (Fig. 1A). However, most of the plants in all the sites were non-flowering and had only a single, oval leaf (Fig. 1B). Young, non-flowering plants with faint brown pigmentation continued to emerge throughout March. In the eight controlled wild populations, the average proportion of fertile plants was 12.2% (for 822 plants), but the percentage varied widely from 5.3% flowering as the lowest value (Farneto) to a maximum of 28% for the highland site of Monte Adone. Flowering also occurred in March 2012 in the

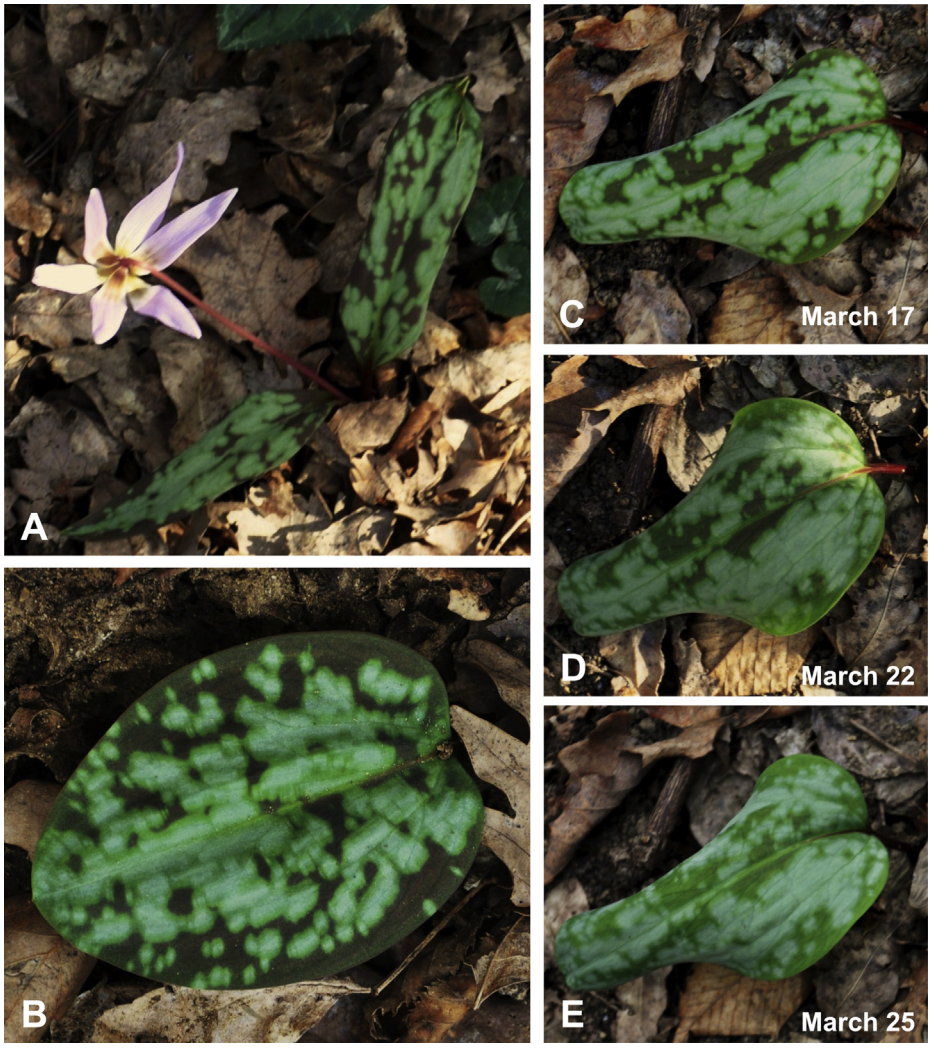

Fig. 1. (A) Flowering *Erythronium dens-canis* with two lanceolate, brown and silver-mottled leaves (Farneto site). (B) Non-flowering plant having one oval leaf with brown areas and green-brimmed silvery flecks (Monte Adone site). (C–E) A leaf of a non-flowering plant leaf undergoing color resorption in the month of March, 2012 (Farneto site). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)