

Unripe red fruits may be aposematic

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The unripe fruits of certain species are red. Some of these species disperse their seeds by wind (*Nerium oleander*, *Anabasis articulata*), others by adhering to animals with their spines (*Emex spinosa*) or prickles (*Hedysarum spinosissimum*). Certainly neither type uses red coloration as advertisement to attract the seed dispersing agents. Fleshy-fruited species (*Rhamnus alaternus*, *Rubus sanguineus* and *Pistacia* sp.), which disperse their seeds via frugivores, change fruit color from green to red while still unripe and then to black or dark blue upon ripening. The red color does not seem to function primarily in dispersal (unless red fruits form advertisement flags when there are already black ripe fruits on the plant) because the red unripe fruits of these species are poisonous, spiny, or unpalatable. The unripe red fruits of *Nerium oleander* are very poisonous, those of *Rhamnus alaternus* and *Anabasis articulata* are moderately poisonous, those of *Rubus sanguineus* are very sour, those of *Pistacia* sp. contain unpalatable resin and those of *Emex spinosa* and *Hedysarum spinosissimum* are prickly. We propose that these unripe red fruits are aposematic, protecting them from herbivory before seed maturation.

Introduction

Advertisement is an important aspect of plant-animal relationships because it attracts pollinators to flowers¹⁻⁴ and seed-dispersing frugivores to ripe fruit.⁵⁻¹⁰ Fruit color is the typical visual signal that plants use to communicate with frugivores,^{5,6,8,11-14} although other plant parts (branches of the raceme, panicle or leaves) may also contribute to this signaling system.^{5,7}

Multicolored fruit displays, where fruits first change their color from green to a conspicuous color when they have reached full size but are still unripe and later to a second conspicuous color upon ripening, have been studied in plants of several ecosystems.^{7,8,11,15-21} Some studies indicated that bicolored fruit displays enhance seed dispersal^{7,11,15-19,21-23} but other studies did not.^{11,15,16,19,23} Thus, it seems that promotion of dispersal can only partly explain multicolored fruit displays.

Color change is a very well known and widely distributed phenomenon in flowers.^{4,24,25} In many plants, old or pollinated flowers are retained to continue their contribution to the advertisement of the whole plant or inflorescence even after they stop producing nectar and change their color. As a result, pollinators are attracted to the inflorescences but when approaching the flowers they prefer the young rewarding pre-color-change flowers to the old, unrewarding ones.^{4,24} In such a way the whole plant retains its long-range advertisement without reducing the pollination probability of its young and virgin flowers. Multicolored fruit displays seem to be a convergent phenomenon, where full size unripe colored fruits are a part of the general advertisement, but approaching frugivores prefer the more palatable and rewarding ripe fruits that have changed their color.^{7,21,22} Color changes are a broad phenomenon in young^{26,27} and senescing leaves,²⁸⁻³¹ and these color changes were proposed to serve

physiological functions (e.g., see refs. 32 and 33), anti herbivory functions (e.g., see refs. 29, 34 and 35), or both (e.g., see refs. 36–38). Color changes also characterize old aposematic spines, thorns and prickles of many taxa that lose their conspicuousness when the organ they protect matures and needs less defense.³⁹

Aposematic coloration, a well-known phenomenon in animals (e.g., see refs. 40–42), has recently been shown to be common also in thorny plants and in plants that mimic them^{39,42-57} as well as in certain poisonous plants, some of which proposed to be aposematic earlier.^{13,14,36-38,46,47,51,58-67} In animals, aposematic coloration is usually red, orange, yellow, black and white or combinations of these, which protect unpalatable, dangerous or poisonous prey species.⁴⁰⁻⁴² The similarity between aposematic colors in poisonous insects and ripe fruits has already been demonstrated,^{68,69} but their aposematic role in fruits was not considered by Herrera⁶⁸ and was not found to operate in artificial fruit-like objects introduced to domestic chicks.⁶⁹ Several types of defensive aposematic coloration have been proposed to occur in fruits that repel large herbivores: (1) brightly colored poisonous fruits,^{13,14,60,65} (2) pods of several wild annual legumes (*Lathyrus ochrus*, *Pisum humile*, *P. elatius* and *Vicia peregrina*) have conspicuous reddish spots arranged along them that appear to mimic aposematic lepidopteran caterpillars and may repel various herbivores,^{37,46,47,70} (3) colorful (yellow, red, purple or various combinations of these) aposematic thorn-like unripe soft fruits in several wild *Erodium* species and in *Sinapis alba* growing in Israel.⁴⁴

Here we report that conspicuous red but unripe fruit of several plant species are poisonous or unpalatable. We propose that in such cases fruit color may contribute to the general attraction of frugivores to the plant but at the same time serve as an aposematic signal that deters herbivores and frugivores from the dangerous or unpalatable unripe fruit. Thus, colorful aposematic unripe fruits

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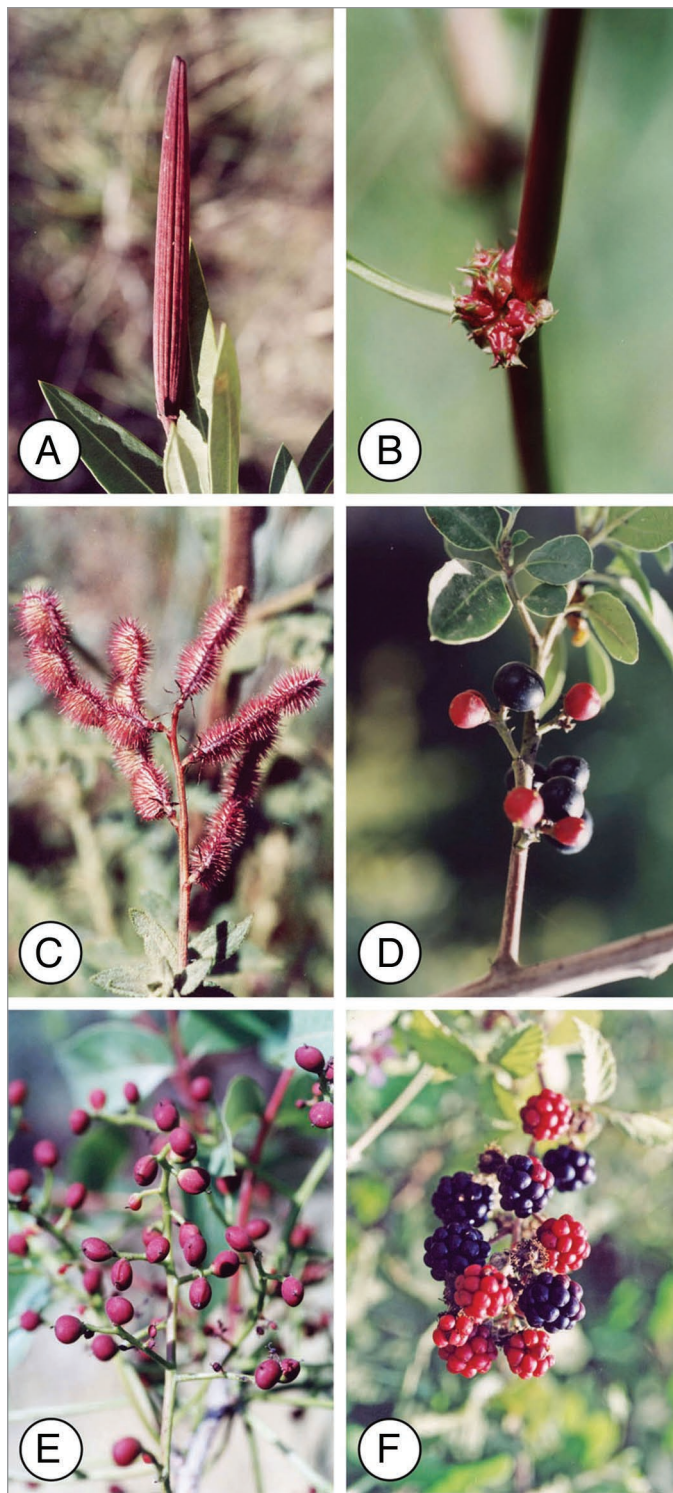


Figure 1. (A) Red unripe poisonous fruit of *Nerium oleander*. (B) The spiny unripe red fruits of *Emex spinosa*. (C) Red prickles cover the unripe pods of *Hedysarum spinosissimum*. (D) Poisonous unripe red fruits and a ripe black fruit of *Rhamnus alaternus*. (E) Red unripe fruits of *Pistacia palaestina*. (F) Red sour unripe fruits and sweet black ripe fruits of *Rubus sanguineus*.

may increase plant fitness by deterring herbivores and frugivores from consuming fruits, including their immature seeds.

Results

The species of the Israeli flora with unripe poisonous or unpalatable red fruits are described below.

Nerium oleander L., is a tall evergreen multi-stemmed shrub or small tree (Apocynaceae), common along seasonal streams, rivers and in other wet habitats. Its fruit (5–15 cm long and 6–10 mm in diameter) consists of two follicles. The young unripe small fruit is green but it turns red when fully grown but still unripe (**Fig. 1A**). Upon maturation, the fruit dries, turns brown, and its follicles twist to liberate the many small, plumed, wind-dispersed seeds.⁷¹ The whole plant, including its fruit, is extremely toxic, containing about 2% cardiac glycosides.⁷² More than ten glycosides with known chemical structure were reported from *N. oleander*. The cardiac effects of the glycosides are due to direct cardiotoxicity and an indirect effect via the vagal nerve. The direct effect is due to the inhibition of the Na-K ATP-ase pump enzyme system. This specific action increases intracellular sodium ions and serum potassium concentration.⁷³ The lethal dose of leaves reported for several animal species is about 0.5 mg per 1 kg body mass.⁷⁴

Emex spinosa (L.) Campd. is an annual plant (Polygonaceae), common in semi-arid open areas and grasslands. Its fully grown unripe fruits are red and spiny (**Fig. 1B**). When they dry, their spines turn light brown and stick to the fur of passing animals, thereby transferring the seeds over distances.

Hedysarum spinosissimum L. [= *H. pallens* (Moris) Halacsy] is an annual plant (Fabaceae), typical of sandy soils of the steppe. Red prickles cover its unripe pods (**Fig. 1C**), which turn light brown when ripe and stick to the fur of passing animals, transferring the ripe seeds.

Rhamnus alaternus L. is a tall evergreen shrub or small tree (Rhamnaceae), common in the Mediterranean maquis. Its young small fruits are green and turn red when fully grown but still unripe (**Fig. 1D**). The red fruits contain 7.8 ± 2.8 ppm of the anthraquinone emodin and are poisonous to some extent. This concentration decreases to 2.5 ± 0.9 ppm in ripe black fruits.⁷⁵ Similar fruit color change also occurs in *R. lycioides* L., in *R. punctata* Boiss., and probably also in other *Rhamnus* species.

Species of the woody genus *Pistacia* (e.g., the trees *P. atlantica* Desf. and *P. palaestina* Boiss., and the shrub *P. lentiscus* L.), grow in various habitats in the Mediterranean, steppe or in the case of *P. atlantica* even in desert districts. In *Pistacia* spp. the young green fruits turn red (or yellow in some *P. lentiscus* individuals) when fully grown but still unripe with immature seeds, and are unpalatable (**Fig. 1E**).⁷⁶ Later, upon fruit ripening and seed maturation, the fruits turn dark blue or black. Seed ripening as well as structural and chemical changes in the fruit pulp accompany this color change. The fruit softens, the concentrations of its secondary repellent compounds decrease while that of lipids and proteins increase, and it turns palatable to frugivores.⁷⁶⁻⁷⁸ The red immature fruits are less rewarding and less palatable than the mature dark ones; consequently, frugivorous birds prefer to consume dark fruits and not red ones.²³ *Pistacia* has also typical red seedless fruits that are not aborted but retained on the tree. These empty

fruits contribute to the visual attraction of fruit-bearing trees and shrubs, but are consumed by frugivores only when the dark seed containing fruits are not available.^{23,78,79}

Rubus sanguineus Friv., (= *R. sanctus* Schreb.) (blackberry) (Rosaceae) is a thorny climber, often with a shrubby form, common along streams and in other wet habitats. Compound drupes carried in groups characterize blackberries (*Rubus* spp.). The color of young small fruits is green. After an initial growth period, they reach their final size and turn bright red (Fig. 1F). However, at this stage, the fruit is still unripe and very sour. Many times, the conspicuous groups of unripe red compound drupes dominate the plants. Only later in season, when the fruits turn black and sweet (Fig. 1F) frugivorous birds consume them and disperse the seeds.^{80,81} We measured the sugar content of fruits at the three color stages. The green fruits contained 7.5%, the red 9% and the black 20% sugars. In parallel, the acidity decreased from pH 2.6–2.9 in green and red unripe fruit to pH 3.6 in black ripe fruits.

Anabasis articulata (Forssk.) Moq. is a poisonous shrub (Chenopodiaceae), common in the Negev desert. Its unripe winged fruits are purple, red or pink. After ripening, they are wind dispersed. The plant is poisonous, rich in alkaloids and is not eaten by grazers.^{51,82,83}

Discussion

In spite of the numerous studies dealing with the ecology and evolution of fruit color, the hypothesis that aposematic unripe red fruits protect their seeds has attracted only a little theoretical attention,^{37,46,47,60–62,70} a single experimental behavioral study⁶⁵ and a theoretical discussion based on detailed measurements of fruit chemistry and color and of bird visual system.¹⁴ The poisonous or otherwise unpalatable species presented here, which have unripe red fruits and the two other types of defensive aposematic coloration (mimicry of caterpillars and spines), which have recently been proposed to occur in fruits and repel large herbivores^{44,47,70} seem to represent a widespread but still almost unexplored phenomenon. The aposematic unripe fruit hypothesis is further supported by the fact that birds detect red fruits from a longer distance than black fruits.⁸⁴

Nerium oleander disperses its seeds by wind, and the red advertisement of the unripe and very poisonous fruits certainly does not promote seed dispersal. The combination of red fruits with their very poisonous latex (e.g., see refs. 71, 72 and 85) is sufficient to characterize their color as aposematic. The fact that the seeds are dispersed by wind further demonstrates the probable deterring function of the red color rather than advertisement for potential seed dispersers. Similarly, the wind-dispersed poisonous purple, red or pink fruits of *Anabasis articulata* need no advertisement to promote seed dispersal and thus may also be characterized as aposematic. The spiny unripe red fruits of *Emex spinosa* and *Hedysarum spinosissimum* are also unpalatable for large mammalian herbivores and need no advertisement to attract frugivores because they are epizoochorous, with dispersal units that adhere to animals' fur, hooves or feathers. Therefore, the red color of their fruits can also be regarded as aposematic.

The fleshy-fruited species (*Rhamnus alaternus* and other *Rhamnus* species and *Rubus sanguineus*) disperse their seeds via

frugivores only after they change color from red to black. The red fruits of *Rhamnus alaternus* are poisonous and those of *Rubus sanguineus* are very sour and low in sugar content at their red unripe stage, but sweet and not sour when black and fully ripe. It has been demonstrated for the Old World's *Rhamnus alaternus* and *R. lycioides* (= *R. palaestinus*) and for the New World's *R. cathartica* that bird species do not consume unripe red fruits.^{75,86,87} Emodin is the predominant secondary metabolite in *Rhamnus* fruits and recently it has been demonstrated that birds and small mammals are unable to detoxify high concentrations of emodin efficiently.⁸⁸ There are good indications for considerable predispersal seed predation in *R. alaternus*,⁸⁹ which may trigger the evolution of defense.

In the bird-dispersed *Rhamnus alaternus*, *Rubus sanguineus* and *Pistacia* sp. the fruits have three color stages. The first, green stage is common to all unripe small fruits. At this stage the fruit is cryptic and photosynthetically active, contributing to its own production costs and defended by both crypsis and unpalatability. Producing and bearing non-green, fully grown yet unripe fruits for substantial periods, adds to the costs of fruit production. Such a trait is supposed to be compensated for by another contribution to the fitness of the plant. Increasing the long-range advertisement of a fruit-bearing plant and its consequent attractiveness to frugivores, which also increases the probability of ripe fruit removal, can provide such compensation. Indeed, it has been demonstrated that red unripe or empty fruits of *Pistacia* contribute to the removal rate of the black ripe fruits by increasing the attraction of frugivores.⁷⁸

Our proposal that unripe red fruits are aposematic, contributes to the understanding of fruit color change and does not contradict any other explanation. Color change in fruits can be regarded as analogous to color change of flowers^{4,24} and to color changes in aposematic thorn, spine and prickle.³⁹ Pre- and post-color change organs contribute to the overall attraction of pollinators and frugivores, or repulsion of herbivores. However, when approaching the plant, pollinators visit the pre-color change rewarding flowers, while frugivores turn to the post-color-change palatable, rewarding, non-poisonous fruits. This strengthens the concept that color itself has only a limited meaning unless coupled with reward or punishment. Thus, red color can be attractive when related to rewarding fruits or aposematic when related to poisonous, sour, bitter or spiny fruits. Similarly, floral color and odor function as pollinator attractors² when coupled with reward, but there is also good evidence for their defensive function.^{90–92} Hinton⁵⁹ proposed that bright colors of poisonous flowers are not only attractive, but also aposematic. The duality of signaling systems, serving in attracting certain animals and repelling others at the same time did not get much research attention. Pollen odors in certain wind-pollinated plants that do not attract pollinators are rich with defensive molecules such as α -methyl alcohols and ketones.⁹³ The de-aromatized isoprenylated phloroglucinols may visually attract pollinators of *Hypericum calycinum* by their UV pigmentation properties, but at the same time the plant may use this pigmentation as a toxic substance against caterpillars, defending the flowers from herbivores.⁹⁴ Herrera et al.⁹⁵ proposed that plants that possess a particular combination of traits that simultaneously enhance pollination and defend from herbivores enjoy a disproportionate

