

Role of Anthocyanins in Plant Defence

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Abstract. In addition to their well-documented beneficial effects on plant physiological processes, anthocyanins have also been proposed to function in a diverse array of plant/animal interactions. These include the attraction of pollinators and frugivores, as well as the repellence of herbivores and parasites. The optical properties of anthocyanins may serve as visual signals to potential herbivores, indicating a strong metabolic investment in toxic or unpalatable chemicals. Anthocyanins have also been implicated in the camouflage of plant parts against their backgrounds, in the undermining of insect crypsis, and in the mimicry of defensive structures. These hypotheses have in recent years attracted strong theoretical support and increasing experimental evidence. We emphasize that both the defensive and the physiological functions of anthocyanins may operate in plants simultaneously.

2.1 Introduction

In their natural environments, plants run the risk of multiple attacks by many different species of herbivores and pathogens. Phytophagous species feature in all major groups of vertebrates; mammals and birds are undoubtedly the most injurious to plants, but reptiles, amphibians, and fish also inflict damage (Schulze et al. 2002). Invertebrates, too, have the potential to devastate plant communities. In a collection of German woodlands, for example, Reichelt and Wilmanns (1973) identified numerous species of leaf chewers, excavators, leaf miners, bark borers, wood borers, sap suckers, bud and shoot eaters, root eaters, and seedling eaters. Some herbivores feed preferentially at the canopy, others focus on lower branches or seedlings; some parasitize the sugars and nitrogen flowing through leaf veins, others will eat the lamina tissues surrounding the veins; some cause the plants to develop galls in which the animals live and feed, yet others employ chemical signals to redirect the flow of plant nutrients in their direction (Karban and Baldwin 1997). Little wonder, therefore, that plants have evolved elaborate strategies of avoidance and/or a sophisticated armoury of morphological devices to counteract herbivore attacks. Chemical weaponry, too, is known to play a significant role in plant defence. A vast

assortment of secondary metabolites has been demonstrated to act as antifeedants, toxins, warning signals, or precursors to physical defence systems (Bennett and Wallsgrove 1994; Harborne 1997). Among them are the phenolics, a large group of structurally diverse compounds that includes terpenoids, cinnamic acids, catechols, coumarins, tannins, as well as certain flavonoids such as the anthocyanins, the subject of this chapter.

There are several different ways anthocyanins might assist plants in their defence against other organisms. These include both direct roles as chemical repellents and more indirect roles as visual signals. In common with other flavonoids, certain anthocyanins have demonstrable antiviral, antibacterial, and fungicidal activities (Konczak and Zhang 2004; Wrolstad 2004, and references therein). They have the potential, therefore, to protect plants from infections by pathogenic microorganisms. In general, however, the antimicrobial activities of anthocyanins are appreciably less effective than those of other phenolic compounds, such as key flavonols and hydroxycinnamic acids that are also likely to be present in the shoot (Padmavati et al. 1997; Werlein et al. 2005). Moreover, anthocyanins have not been found to be toxic to any higher animal species (Lee et al. 1987). Aphid survival rates, for example, are unaffected by anthocyanins in their diet (Costa-Arbulú et al. 2001). Thus, direct chemical defence is unlikely to be a major function of these pigments in plants. There is, in contrast, strong theoretical support and growing empirical evidence for a role of anthocyanins in the defence from “visually oriented” herbivores. This discussion focuses largely on the defensive roles of anthocyanins as visual cues, though some of these mechanisms also involve associated chemical or mechanical components such as poisons and thorns.

Although it is generally accepted that the colours of flowers and fruits enhance reproductive success by facilitating communication between plants, their pollinators, and seed-dispersers (Ridley 1930; Faegri and van der Pijl 1979; Willson and Whelan 1990; Weiss 1995; Schaefer et al. 2004), there is no *a priori* reason to assume that flower, fruit and leaf colours cannot also serve in defence from herbivory. This is achievable if the colours (i) undermine an herbivorous invertebrate’s camouflage, (ii) are aposematic, (iii) mimic an unpalatable plant or animal, or (iv) serve in plant camouflage (see Hinton 1973; Givnish 1990; Cole and Cole 2005; Lev-Yadun 2006). Of course, anthocyanins are not the only class of pigments that might contribute to defence in these ways. In several species, leaf variegation caused by pigments unrelated to anthocyanins has been shown to correlate to reduced herbivory (Cahn and Harper 1976; Smith 1986). Such examples may, however, help to understand the principles that operate when anthocyanins serve in defence. Moreover, it has long been recognised that the non-photosynthetic plant pigments have the potential to serve more than one function concurrently (Gould et al. 2002; Lev-Yadun et al. 2002, 2004; Schaefer and Wilkinson 2004; Lev-Yadun 2006). The UV-absorbing dearomatized isoprenylated phloroglucinols, for example, serve a defensive role in the stamens and ovaries of *Hypericum calycinum*, but an attractive role in the petals of the same species (Gronquist et al. 2001). Thus, the various functional hypotheses concerning pigmentation in leaves and other plant parts need not contrast or exclude any other functional explanation for specific types of plant colouration, and those traits, such as colouration, that might have more than one type of benefit, may be

selected for by several agents. Indeed, Armbruster (2002) suggested that plants for which anthocyanins are deployed in the defence of vegetative organs would be the *more likely* also to use anthocyanins to attract pollinators and seed dispersers. Combinations of traits that simultaneously enhance both pollination and defence would likely confer a disproportionate fitness advantage (Herrera et al. 2002). Such synergistic gains may act for a quicker and more common evolution of the red plant organ colour trait.

2.2 Hypotheses

Hypotheses for an anti-herbivory function of anthocyanic plant organs include:

- (i) aposematism (conspicuous colouration serving to parry predators) in poisonous fruits and seeds (Cook et al. 1971; Harborne 1982; Williamson 1982), flowers (Hinton 1973), and thorns (Lev-Yadun 2001, 2003a, 2003b, 2006);
- (ii) mimicry of dead or senescing foliage (Stone 1979; Juniper 1994), of thorns and spines (Lev-Yadun 2003a), and of ants, aphids, and poisonous caterpillars (Lev-Yadun and Inbar 2002);
- (iii) camouflage of seeds against the background of the soil substrate (Saracino et al. 1997, 2004), and of variegated foliage in forest understory herbs (Givnish 1990);
- (iv) the undermining of herbivorous insect crypsis by leaf variegation (Lev-Yadun et al. 2004; Lev-Yadun 2006);
- (v) attraction of herbivores to young, colourful leaves, diverting them from the more costly older leaves (Lüttge 1997); and
- (vi) signalling to insects by red autumn leaves that the trees are well defended (Archetti 2000; Hamilton and Brown 2001; Schaefer and Rolshausen 2006).

Each of these hypotheses is discussed in detail below.

2.3 Reluctance to Accept Hypotheses on Defensive Colouration

Prior to the year 2000, much of the published information on defensive plant colouration, including anthocyanin-based ones, had been largely anecdotal. As Harper (1977) commented in his seminal book about the possibility of defensive colouration operating in plants, botanists have been surprisingly reluctant to accept ideas that are commonplace for zoologists. The relative scarcity of papers on defensive colouration in botany as compared to those in zoology was highlighted in the annotated bibliography on mimicry and aposematism by Komárek (1998). It should be appreciated, however, that that it has taken zoologists more than a century to understand the defensive role and the genetic mechanisms of pigmentation in animals (Hoekstra 2006); the effort needed to achieve the same progress in botany

would surely not be smaller. Thus, our explanations for the role of pigments in plant defences remain imperfect. Notwithstanding the difficulties involved in providing concrete evidence for plant defensive colouration, it has therefore been extremely encouraging to note a recent wave of interest in this area, particularly in relation to foliar anthocyanins.

2.4 Colour Vision in Animals

A frequent criticism of the anti-herbivory hypotheses for foliar anthocyanins is that herbivorous insects may lack ocular receptors for red light. Insects have up to five kinds of photoreceptors sensitive to different regions of the visible and UV spectrum (Kelber 2001; Kelber et al. 2003). Butterflies of the genera *Papilio* and *Pieris* have arguably the most sophisticated colour vision system of the insects studied so far, including a red receptor maximally sensitive around 610 nm (Arikawa et al. 1987; Shimohigashi and Tominaga 1991). However, most of the insects that have been examined to date – including the phytophagous aphid *Myzus persicae* – possess only three types of photoreceptors, maximally sensitive to green, blue, and ultraviolet light, respectively (Briscoe and Chittka 2001; Kirchner et al. 2005). In the absence of a red light receptor, it could be argued that insects would be unable to perceive the visual cue presented by anthocyanins.

There is nevertheless good evidence that red is recognised by insects. Döring and Chittka (2007) recently summarised the results from 38 studies in which the behavioural responses to red or green stimuli were compared in aphid species. In 28 of those studies, the aphids had been observed to move preferentially towards the green stimulus, and in only one of the reports had aphids not demonstrated a colour preference. In the remaining studies, the experimenters had varied the shade of the green stimulus, and found that insects moved preferentially towards whichever stimulus was the brighter, red or green. It is likely, therefore, that both chromatic (wavelength related) and achromatic (intensity related) information is involved, as is known also to be used by frugivorous birds (Schaefer et al. 2006). There are insufficient data to state with confidence whether aphids tend to avoid red light or, instead, are simply more attracted to green than to red light. Only one publication has addressed this issue, albeit indirectly: in a “no-choice” experiment, Nottingham et al. (1991) recorded positive phototaxis towards red targets by the bird cherry aphid *Rhopalosiphum padi*. Although response rates were very low, that experiment suggested that the insects were not innately repelled by red objects.

Detail of the mechanism by which insects lacking a red photoreceptor perceive red colours remains to be resolved. A colour opponency mechanism has been proposed, which may explain colour discrimination in certain aphid species (Döring and Chittka 2007). This requires negative excitation in the blue and UV, and positive excitation in the green waveband. It is unclear, however, how such a mechanism might facilitate perception of anthocyanic leaves which, compared to green leaves, typically reflect smaller quantities of both green light (Neill and Gould 1999) and UV radiation (Lee and Lowry 1980). It may simply be that red leaves are less attractive to insect herbivores because the excitation of their green receptor is

lower than when excited by green leaves (Thomas Döring, personal communication). Contrasts in colour and/or brightness between red leaves and the visual background are likely also to be important (Dafni et al. 1997).

2.5 Anthocyanins and Other Red Pigments

Anthocyanins usually appear red in leaf cells, but depending on their chemical nature and concentration, the vacuolar pH, and interactions with other pigments, they can result in pink, purple, blue, orange, brown, and even black leaf colours (Schwinn and Davies 2004; Andersen and Jordheim 2006; Hatier and Gould 2007). Many of the published articles on plant defensive colouration have assumed red foliage to be the outcome of the production of anthocyanins, this despite the fact that other pigments – carotenoids, apocarotenoids, betalains, condensed tannins, quinones and phytomelanins – can also contribute to plant vermilion (Davies 2004). There is, moreover, a dearth of systematic information on the full complement of pigments in all plant organs at all developmental stages. This lack of data precludes detailed taxon-wide comparisons of the involvement of anthocyanin, or indeed any pigment, in plant defence. Clearly, if only visible cues (hue, lightness, and colour saturation) are involved in defence, the chemical nature of a pigment would be unimportant to a herbivore; red warnings would be similarly effective irrespective of whether they were generated by anthocyanins, carotenoids, or betalains. If, on the other hand, the efficacy of the warning relied on a combination of attributes, for example the reflection of red light *plus* the presence of a toxic or olfactory phenolic derived from an offshoot in the anthocyanin biosynthetic pathway, then the pigment type could be critical.

2.6 Olfactory Signals

An important, if not critical issue is whether or not olfactory signals are involved along with the visual ones. From studies of deceptive pollination, wherein insects are lured to flowers but receive no sugar reward, we know that signalling to animals can involve a combination of both visual and olfactory components (Dafni 1984; Ayasse et al. 2000; Schiestl et al. 2000); there are good reasons to think that the same may be true in the defence of vegetative organs (Pichersky and Gershenson 2002). The identification of olfactory volatiles is achievable using modern laboratory equipment (e.g., Jürgens 2004; Jürgens et al. 2002, 2003; Pichersky and Dudareva 2007), but such procedures are difficult to accomplish in the field. It is, in addition, very difficult to identify the specific molecules that deter specific herbivores from among the many volatile molecules that plants omit, and there is the possibility that deterrence operates only if several molecules are sensed simultaneously. The fact that not all animals respond similarly to any chemical signal or cue, should also be considered.

2.7 Aposematic Colouration

Aposematic colouration, a well-known phenomenon in animals, has until recently been given little attention in plants. Often, a brightly-coloured animal (red, orange, yellow, white with black markings, or combinations of these colours) is dangerous or unpalatable to predators – a trait that confers a selective advantage because predators learn to associate the colouration with unpleasant qualities (Cott 1940; Edmunds 1974; Gittleman and Harvey 1980; Harvey and Paxton 1981; Wiklund and Järvi 1982; Ruxton et al. 2004). Although several authors had noted a similar association between conspicuous colouration and toxicity in plants (Cook et al. 1971; Hinton 1973; Harper 1977; Wiens 1978; Rothschild 1980; Harborne 1982; Williamson 1982; Knight and Siegfried 1983; Smith 1986; Lee et al. 1987; Coley and Aide 1989; Givnish 1990; Tuomi and Augner 1993), only in the past decade have the scope and significance of this phenomenon been appreciated. Indeed, the possibility of aposematic colouration was discounted in some of these earlier studies (Knight and Siegfried 1983; Smith 1986; Lee et al. 1987; Coley and Aide 1989). A related phenomenon, olfactory aposematism in poisonous plants, has also been proposed (e.g., Eisner and Grant 1981; Harborne 1982; Launchbaugh and Provenza 1993; Provenza et al. 2000) although this has received scant attention.

2.7.1 Poisonous Plants

The first detailed hypothesis for a possible defence from herbivory attributable to red colouration (and other colours) was published by Hinton (1973), who proposed that colourful poisonous flowers should be considered aposematic, and that they probably have mimics. His review about deception in nature was published in a book about illusion; this was not a biological book, but rather dealt with art. His hypothesis was briefly referred to by Rothschild (1980) in her discussion on the roles of carotenoids, but otherwise did not stir botanists or ecologists to pursue this issue. Indeed, Harper (1977), who had written the comment about botanists being reluctant to accept things that were commonplace for zoologists, omitted to explain why zoologists who dealt with animal aposematism, and who were also involved in research on plant-animal interactions, had not recognized how common are these phenomena in plants. Harborne (1982) proposed that the brightly coloured, purple-black berries of the deadly *Atropa belladonna* warn grazing mammals of the danger to consume them. Williamson (1982) also proposed that brightly coloured (red, or red and black) seeds lacking an arillate or fleshy reward (e.g. *Erythrina*, *Ormosia*, and *Abrus*) might be aposematically coloured to warn seed eaters of their toxicity. These hypotheses were written only as short paragraphs within long reviews, however, and there has been no further effort to study the function of their colouration.

2.7.2 Thorny Plants

In English there are three terms for pointed plant organs: spines (modified leaves), thorns (modified branches), and prickles (comprising cortical tissues, e.g. in roses). For the purposes of this discussion, we refer to plants as “thorny” if they produce any

of the three types of sharp appendages. Thorny appendages provide mechanical protection against herbivory (Janzen and Martin 1982; Janzen 1986; Myers and Bazely 1991; Grubb 1992; Rebollo et al. 2002) because they can wound mouths, digestive systems (Janzen and Martin 1982; Janzen 1986), and other body parts of herbivores. They might also inject pathogenic bacteria into herbivores (Halpern et al. 2007). Thus, once herbivores learn to identify thorns – and their bright colours and associated markings should help in their recognition – they can avoid harmful plants displaying them.

The flora of countries such as Israel, which has a millennia-long history of large-scale grazing, clearly and “sharply” indicates the ecological benefit of being thorny when grazing pressure is high. A continuous blanket of spiny shrubs such as *Sarcopoterium spinosum*, as well as many types of thistles, covers large tracts of the land. The thorns effectively impede the rate at which an herbivore feeds within the canopy of the individual plant, and this presents an overall considerable advantage to such plants over non-defended ones. Spiny plants, such as *Echinops* sp. (Asteraceae), which normally grow as individuals or in small groups, sometimes become the most common perennial plant over many acres in heavily grazed lands. The same is true for many other taxa.

Thousands of thorny species have colourful or otherwise conspicuous markings (e.g. Fig. 2.1A), many of which can be considered to be aposematic (Lev-Yadun 2001). We will not discuss this common phenomenon in the thorniest taxon – the Cactaceae – since they lack anthocyanins, and use betalains instead (Stafford 1994). Lev-Yadun (2001) categorised two types of thorn ornamentation, which are typical of many thorny plant species: (i) colourful thorns, and (ii) white spots, or white and colourful stripes, in leaves and stems associated with the thorns. Both types have been recorded for approximately 2,000 species originating from several continents in both the Old and New World (Lev-Yadun 2001, 2003a, 2003b, 2006; Rubino and McCarthy 2004; Halpern et al. 2007; Lev-Yadun unpublished). It has been proposed

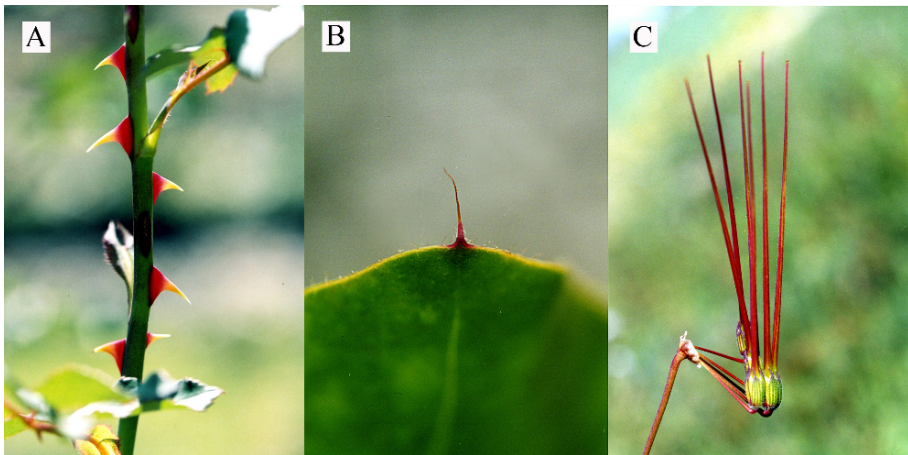


Fig. 2.1 Thorns and their mimics. (A) Anthocyanic thorns on a rose stem. (B) Red mucron at the apex of a leaf of *Limonium angustifolium*. (C) Red fruit of *Erodium laciniatum*. See Plate 1 for colour version of these photographs

that the pigmentation in thorns and associated organs (in many cases resulting from anthocyanins) are cases of vegetal aposematic colouration, analogous to such colouration of poisonous animals, that serves to communicate between plants and herbivores (Lev-Yadun 2001, 2003a, 2003b, 2006; Lev-Yadun and Ne'eman 2004; Rubino and McCarthy 2004; Ruxton et al. 2004; Speed and Ruxton 2005; Halpern et al. 2007). Interestingly, spiny animals also show the same phenomenon (Inbar and Lev-Yadun 2005; Speed and Ruxton 2005).

Colourful thorns are especially common in the genera *Agave*, *Aloe* and *Euphorbia*. *Agave* species can have two types of thorns in their leaves: spines at the leaf apex, and/or teeth along the leaf margins (Lev-Yadun 2001). In addition, many *Agave* species also have colourful stripes running along the margins that enhance spine and tooth visibility. The spines and the teeth along the margins of the leaves are brown, reddish, gray, black, white, or yellow; these colours are known to serve in aposematism. The same phenomenon is true for *Aloe* species for which the colourful thorns can be white, red, black, or yellow, and for thorny African *Euphorbia* species, many of which have colourful thorns or colourful markings associated with thorns along the ribs of the stems (Lev-Yadun 2001). Anthocyanins are known to contribute to the markings in *Agave* and *Euphorbia*, though the carotenoid rhodoxanthin is involved in leaf colouration in *Aloe*. Colourful thorns advertise their defensive quality directly, unlike poisonous aposematic organisms in which the poison is advertised indirectly (Ruxton et al. 2004; Speed and Ruxton 2005).

Since the colouration and markings of thorns in plants are so widespread, they are probably neither a neutral nor a random phenomenon. Lev-Yadun (2001) proposed that, like aposematic colouration in animals, the conspicuousness of thorns would be of adaptive value since herbivorous animals would remember the signal, and subsequently tend to avoid tasting such plants. Annual and perennial plants usually survive damage caused by herbivores (Williamson 1982; Crawley 1983; Ohgushi 2005), so an herbivore reacting to aposematic colouration would be of direct benefit to the individual plant, which would probably suffer fewer repeated attacks. Hence, as with animals (Sillén-Tullberg and Bryant 1983), there is no need to propose kin/group selection, or altruism, as the evolutionary drive for the spread of this character. Indeed, there are several probable reasons for the quick and easy route to aposematism in plants in general. Thorny or poisonous plants are already well-defended, even without aposematic colouration. Plants recover much better than most animals from herbivore (predator) damage (Crawley 1983; Ohgushi 2005). Thus, the original thorny, aposematic mutant would have had a good chance of survival and producing offspring, even despite the risks associated with being conspicuous to herbivores and being partly eaten. Furthermore, an herbivore might pass over an aposematic individual and eat its non-aposematic neighbour, thereby reducing the competition between the aposematic and neighbouring plants. A recent hypothesis proposed that since thorns harbour many types of pathogenic microbes, their aposematism serves to signal both about their biological risks as well as the mechanical ones (Halpern et al. 2007). It is possible, therefore, that the evolution and spread of aposematism progressed even more swiftly in plants than in animals.

2.8 Defensive Mimicry

Defensive mimicry is said to occur if a plant gains protection against herbivory by resembling a noxious or unpalatable model species. Williamson (1982) proposed that because plants are sessile organisms, mimicry is less likely to be successful in plants than in animals; plant mimics are less likely to be mistaken for their models than are the mobile animal mimics. Moreover, because plants that have been partially eaten by herbivores can often regenerate new organs, defensive mimicry would in theory provide less advantage to plants than animals. In contrast, Augner and Bernays (1998) studied the theoretical conditions for an evolutionary stable equilibrium of defended, signalling plants and of plants mimicking these signals. Their model showed that mimicry of plant defence signals could well be a common occurrence; even imperfect mimics had the potential to invade a population of defended model plants. Theoretically, natural selection would allow the success of even imperfect mimics (Edmunds 2000). Wiens (1978) estimated that about 5% of the land plants are mimetic, listing various types of protective plant mimicry. Several types of anthocyanin-related defensive mimicry have been proposed, especially in recent years, and these are discussed below.

2.8.1 Mimicry of Dead Leaves

An interesting hypothesis in relation to anthocyanins was published in a brief note by Stone (1979), who proposed that young leaves of understory palms mimic old or dead leaves. He observed that the combination of chlorophylls and anthocyanins in the developing leaves of several species produced a dull brown colour which “strikingly mimics the drab color of dying or withered dead leaves”. The leaves, moreover, showed no evidence of browsing damage, possibly because they appeared unpalatable to potential herbivores.

Juniper (1994) suggested a similar explanation for the abundant red, brown and even blue flushes of young leaves in tropical trees and shrubs from the families Annonaceae, Fabaceae subfam. Caesalpinioideae, Guttiferae, Lauraceae, Meliaceae, Rutaceae, and Sapindaceae. It was postulated that to a phytophagous insect, such leaves would appear dull, like advanced senescent foliage. “If you do not look like a normal leaf ... if you do not feel like a leaf to a palpating insect, because you are soggy; if you do not have the posture of a proper leaf, i.e. you are hanging down like wet facial tissue; if you do not smell like a leaf because you have no photosynthesis and, if on the first suck or bite, you do not taste like a leaf because you have no sugars ... you might escape being eaten” (Juniper 1994).

Young foliage that acquires a protective advantage by resembling older, non-green leaves would be an example of cryptic mimicry (Pasteur 1982). The idea seems to have been generally accepted (Juniper 1994; Dominy et al. 2002; Gould 2004; Karageorgou and Manetas 2006; Lev-Yadun 2006; Manetas 2006) although there is little in the way of supporting experimental data. As is the case for many of these anthocyanin defence hypotheses, the chemical nature of the pigments in many such red leaves has not been tested, the anatomical location of the pigmentation is not always known, and possible physiological roles of the colouration are unclear.

2.8.2 Defensive Thorn Automimicry

Since aposematic thorns are so common, it is not surprising that mimics of this type of defence exist. Lev-Yadun (2003b) described two variations of thorn mimicry: (i) thorn-like imprints on lamina margins (a type of weapon automimicry, until recently believed to be exclusive to animals), and (ii) mimicry of aposematic colourful thorns by colourful, elongated, and pointed plant organs (buds, leaves and fruit) which, despite their appearances, are not sharp (Fig. 2.1B).

Weapon automimicry (in which the mimic has part of its structure resembling some other part) of horns and canine teeth has been observed in several mammalian species (Guthrie and Petocz 1970) and has been proposed to be of greatest value in intraspecific defence. This intriguing idea has now been suggested to occur in plants. More than 40 *Agave* species show what Lev-Yadun (2003b) described as “thorn automimicry”. In those *Agave*, the developing leaves press strongly against one another as they grow. Teeth along the leaf margins press against the lamina surfaces of other leaves, leaving an impression of the teeth along the non-spiny parts. This phenomenon is particularly conspicuous in *A. impressa*, for which the teeth impressions are white. However, because many of the real thorns in other *Agave* species are red, brown and black, probably as the outcome of expression of anthocyanins, some of the imprints contain reddish colouration. Similar impressions of colourful teeth are obvious in fronds of the palm *Washingtonia filifera*, a common ornamental and a feral tree in Israel, as well as in certain *Aloe* species. It remains to be demonstrated that such leaf patterns effectively contribute to herbivore deterrence. If they do, then the imprints, which give the visual impression of a more extensive system of thorns than actually exists, should be considered a transitional type between Batesian and Müllerian mimicry; the mimicking of real thorns by non-thorny plants would be Batesian, and the illusion of more thorns than occurs in reality would be a Müllerian-Batesian intermediate (Lev-Yadun 2003b).

Colourful, thorn-like appendages have been observed in several species that grow wild in Israel (Lev-Yadun 2003b). They are especially prominent in the fruit of various *Erodium* species, an annual in the family Geraniaceae. *E. laciniatum* subsp. *laciniatum*, *E. crassifolium*, and *E. arborescens* all have elongated, beak-like fruits with pointed apices (Fig. 2.1C). These self-dispersing structures are usually pigmented red from anthocyanins, and although they look like thorns, their tissues are soft. In *Limonium angustifolium*, a wild and domesticated perennial of the Plumbaginaceae, the apical portions of its large leaves are red; again, this resembles a spine though its tissues are soft (Lev-Yadun 2003b). Such non-thorny plants that mimic thorny ones are cases of Batesian mimicry. There are two possible evolutionary routes toward mimicry of colourful thorns. In the first, an aposematic thorny plant may have lost its thorny characteristics but retained the shape and aposematic signal. In the second, a non-aposematic and non-thorny plant may acquire the signal, becoming a primary mimic. Alternatively, the structure and colouration may have a different, unknown function. The mode of evolution of these mimics has not been studied.

2.8.3 Defensive Animal Mimicry by Plants

Some plants utilise anthocyanins (and other pigments) to mimic insects, the presence of which would likely discourage visits by potential herbivores. It is widely accepted that many animals visually masquerade as parts of plants, thereby gaining protection from predators, or crypsis from their prey (Cott 1940; Wickler 1968; Edmunds 1974; Ruxton et al. 2004). Classic examples listed in Cott (1940) include: fish and crabs that resemble algae; geckos and moths that look like lichens; many insects, amphibians and reptiles that resemble leaves; spiders, caterpillars, moths, beetles, amphibians, lizards and birds that mimic tree bark; stick-insects that resemble branches; and Thomisid spiders that are disguised as flowers. Flowers, too, are known to mimic animals to attract pollinators (Dafni 1984). However, the possibility of animal mimicry as a defence mechanism of plants against herbivores has been largely overlooked. Evidence for the involvement of anthocyanins in plant mimicry of ants, aphids and caterpillars is discussed below.

2.8.4 Ant Mimicry

Ants are well known to defend plants from invertebrate herbivory. Indeed, in certain cases, the relationship between ants and their plant hosts has been recognized as mutualistic (Madden and Young 1992; Jolivet 1998). The potential benefit from ant-attendance, and therefore from mimicry of ant presence, is obvious. Ants bite, sting and are aggressive, and most insectivorous animals and herbivores will avoid them. Thus, ants have become models for a variety of arthropods, which have evolved to mimic them (Wickler 1968; Edmunds 1974). The importance of ants in defending plants was demonstrated in a field experiment in which the removal of ants and aphids resulted in a 76% increase in the abundance of other herbivores on narrow-leaf cottonwoods (Wimp and Whitham 2001). Not surprisingly, therefore, many plant species invest resources in attracting ants, providing them with shelter, food bodies and extrafloral nectaries (Huxley and Cutler 1991). Certain plants even tolerate aphid infestation to gain anti-herbivore protection from aphid-attending ants (Bristow 1991; Dixon 1998).

Ant mimicry has been observed in several plant families. The mimicry takes the form of conspicuous, darkly-coloured spots and flecks, usually 2–10 mm long, on the plant's epidermis. These markings resemble ants in size, shape and in the direction of their spatial patterns, which look like columns of ants. Dots predominate in some individual plants, flecks in others (Lev-Yadun and Inbar 2002). This phenomenon has so far been found on the stems and petioles of *Xanthium trumarium* (Asteraceae) and *Arisarum vulgare* (Araceae) growing in Israel, and in several other plant species growing in Eastern USA and Northern Greece (Lev-Yadun unpublished). Real ant swarms, as seen from a distance, comprise many moving dark flecks, each varying in size from several mm to over 1 cm. The swaying of leaves, stems and branches in the wind, in combination with the dark spots and flecks, many of which are arranged in lines, give the illusion that the ant mimics are moving. Olfactory components may also be involved, though this has not been tested. The ant-mimicking colouration has not been analysed for pigment composition, but it is likely that anthocyanins are

involved since they are abundant in the flowers and/or foliage of these species. In any case, it seems evident that ant mimicry would be highly beneficial to plants, since they could acquire herbivore deterrence without paying the cost of feeding or housing real ants (Lev-Yadun and Inbar 2002).

2.8.5 Aphid Mimicry

A phenomenon similar to ant mimicry is aphid mimicry. Lev-Yadun and Inbar (2002) described aphid mimicry in *Paspalum paspaloides* (= *P. distichum*), for which the dark-pigmented anthers are the size, shape and colour of aphids. The stems of *Alcea setosa* are also covered with dark flecks that look like aphids, and similar morphological features have been found in several wild grasses growing in North Carolina (Lev-Yadun, unpublished). Plants which look infested would likely be left untouched both by grazers and insects (Lev-Yadun and Inbar 2002). Several studies have shown that early infestation by aphids and other homopterans has a negative impact on host plant preferences and larval performance of other insect herbivores. Finch and Jones (1989) reported that large colonies of the cabbage aphid *Brevicoryne brassicae* and of the peach aphid *Myzus persicae* deterred ovipositioning by the root fly *Delia radicum*. Inbar et al. (1999) demonstrated that the presence of Homopterans (whiteflies) not only altered adult cabbage looper (*Trichoplusia ni*) host selection, but also actually reduced the feeding efficiency of their offspring. Aphids respond to crowding by enhanced dispersal (Dixon 1998), and it is probable, therefore, that they avoid previously infested or infestation-mimicked hosts. The clear zoological data are consistent with a potential defensive value in aphid mimicry by plants, but the hypothesis requires testing experimentally.

2.8.6 Mimicry of Aposematic Poisonous Caterpillars

The final example of red colouration from anthocyanins mimicking insects for defence is that of immature legume pods, which often resemble aposematic poisonous caterpillars. Lev-Yadun and Inbar (2002) described pods of several wild annual legumes (*Lathyrus ochrus*, *Pisum elatius*, *Pisum humile*, and *Vicia peregrina*) that were comparable in general shape, size and colour to those of Lepidopteran caterpillars. The pods were ornamented with apparent “spiracles” or other conspicuous spots in various shades of red and purple. In one of the species (*V. peregrina*), two different phenotypes were observed. The first had red spots along the length of the pods, similar to those of *L. ochrus*, *P. elatius*, and *P. humile*. The second phenotype was characterized by red circles with green centres along the pods. Lev-Yadun and Inbar (2002) proposed that these morphological traits may serve as herbivore-repellence cues, and are part of the defence system of the plants. Caterpillars employ a large array of defences that reduce predation. Unpalatable caterpillars armed with stinging and irritating hairs, functional osmeteria (scent glands) or body-fluid toxins often advertise their presence by aposematic colouration and aggregation (Cott 1940; Bowers 1993; Eisner et al. 2005). The usual warning colours are yellow, orange, red, black, and white, often in stripes along the body and/or spots, especially around the abdominal spiracles. By mimicking aposematic

caterpillars with red “spiracle spots”, wild legumes may reduce immature pod predation (Lev-Yadun and Inbar 2002). It has been shown that ungulates actively select leaves in the field by shape and colour, and avoid eating spotted ones (e.g., Cahn and Harper 1976) but there seems to be no published data on the response of vertebrate herbivores to aposematic (or cryptic) caterpillars. Again, the possible involvement of olfactory deterrence has not been studied.

The examples of ant, aphid and caterpillar mimicry may signal unpalatability to more than one group of animals in two ways (Lev-Yadun and Inbar 2002). First, insect mimicry would reduce attacks by insect herbivores, which refrain from colonizing or feeding on infested plants because of perceived competition and/or induced plant defences. Second, where the insect mimicked is aposematic, this could deter larger herbivores from eating the plants. None of these hypotheses concerning the various types of defensive insect mimicry has been tested directly, though there is good indirect evidence from studies on insects, and the hypotheses appear reasonable.

2.9 Camouflage

2.9.1 Whole Plants and Seeds

There is a remarkable dearth of scientific information on the use and effectiveness of plant pigments to camouflage plant organs from potential herbivores. Given that plants are largely sessile and often produce seeds that are dispersed on bare ground or on plant litter, an ability to blend in with their general surroundings (eucrypsis) would present them with an obvious advantage. To our knowledge, however, not a single monograph has been published on this topic, though several workers have alluded to the possibility of camouflage. In the New Zealand tree *Pseudopanax crassifolius*, for example, the combination of chlorophylls and anthocyanins produces a brown colour in seedling leaf laminae (Gould 1993), effectively concealing them (at least to human eyes) amongst the background leaf litter. *Lithops*, too, appears to be well camouflaged amidst the rocks and gravel in the desert (Cole 1970; Cole and Cole 2005), although betalains, rather than anthocyanins, are involved in that taxon.

Camouflage has been postulated to act as defence in seeds of various *Pinus* species (Saracino et al. 1997, 2004; Lanner 1998); in a field experiment involving various combinations of soil types and seed colours, light grey seeds, which were rich in polyphenols, were observed to be predated less if the seeds were on a similar coloured substrate, suggesting that eucrypsis functioned as a protective strategy against predation by granivorous birds. The predation of black seeds, in contrast, did not vary consistently with substrate colour.

From years of field work it has become evident to one of the authors (Lev-Yadun) that anthocyanins play a significant role in plant camouflage, yet there has been no systematic study on this phenomenon. Since seed survival in particular is critical for plants, and camouflage of seeds is so obvious, it is therefore difficult to understand why such studies, which are commonplace in zoology have not featured similarly in botany.

2.9.2 Variegation in Understory Herbs

The leaves of many understory herbs in certain temperate and tropical floras are variegated. Their colours are diverse; many of these plants are variegated green and white, others are mottled with red, purple or even black, often the result of anthocyanin accumulation. Givnish (1990) proposed that variegation in evergreen understory herbs in the forests of New England serves as camouflage. He argued that in that dappled light environment, variegation would disrupt the leaf outlines as perceived by colour-blind vertebrate herbivores such as deer. Unfortunately, this appealing hypothesis lacks empirical data. As stated by Allen and Knill (1991), “Givnish’s hypothesis, elegant in its simplicity, demands to be tested experimentally.”

2.10 Undermining Crypsis of Invertebrate Herbivores

Another recent hypothesis holds that red and yellow pigments in both vegetative and reproductive shoots can undermine the camouflage of invertebrate herbivores (Lev-Yadun et al. 2004; Lev-Yadun 2006). Thus exposed, the invertebrates would be vulnerable to predation. Moreover, potential herbivores would likely avoid settling on plant organs with unsuitable colouration in the first place, thereby compounding the benefit to the plants.

Plants provide the habitat and food for many animals. Intuitively, the common optimal camouflage for herbivorous insects would be green, since many (e.g., aphids, caterpillars, grasshoppers) have evolved green colouration (Cott 1940; Purser 2003). It has been claimed that the considerable variation in the colours of leaves and stems, as well as those of flowers and fruits, could serve to undermine the camouflage of invertebrate herbivores, especially insects (Lev-Yadun et al. 2004; Lev-Yadun 2006). For example, colour differences between the upper and lower surfaces of leaves, or between the petiole, veins and leaf lamina, are common across diverse plant taxonomic groups. They occur across a range of plant forms, from short annuals to tall trees, and in habitats ranging from deserts to rain forests, and from the tropics to temperate regions. When a given leaf has two different colours, such as green on its upper (adaxial) surface and blue, brown, pink, red, white, yellow or simply a different shade of green on its lower (abaxial) surface, a green insect (or otherwise coloured one) that is camouflaged on one of the leaf surfaces will not be camouflaged on the other. The same is true for vein, petiole, branch, stem, flower, or fruit colouration. Moreover, when a green herbivore moves from one green region to another, passing through a non-green region, it would immediately become more conspicuous to its predators (Lev-Yadun et al. 2004). The foliage of many plants is simply too colourful to allow the universal camouflage of folivorous insects, and it compels small animals to cross “killing zones” of colours that do not match their camouflage (Fig. 2.2). This is a special case of “the enemy of my enemy is my friend”, and a visual parallel of the chemical signals that are emitted by plants to signal wasps when attacked by caterpillars (Kessler and Baldwin 2001). It is also a natural parallel to the well-known phenomenon of industrial melanism (e.g., Kettlewell 1973; Majerus 1998), which illustrated the importance of plant-based camouflage for herbivorous insect survival.



Fig. 2.2 Variegated red autumn leaf of *Acer* may undermine the crypsis of herbivorous insects. See Plate 1 for a colour version of this photograph

Interestingly, the antithesis of this, that background chromatic heterogeneity *promotes* herbivore crypsis, has also been argued by some workers (Merilaita 2003; Merilaita et al. 1999; Schaefer and Rolshausen 2006). Using a mathematical model to simulate the evolution of cryptic colouration against different backgrounds, Merilaita (2003) concluded that the risk of prey being detected was appreciably lower in the more visually complex (more colourful) habitats. Similarly, Schaefer and Rolshausen (2006) showed that a variegated leaf comprising red primary veins and pink or white secondary veins on a green lamina could effectively camouflage a diverse assortment of computer-generated insects, many more than could be accommodated by a green-only leaf.

This dichotomy of opinion is, perhaps, resolvable if the ratio between the size of the herbivore and that of the colour patch on the plant be taken into consideration. Green insects that are smaller than, say, the average red patch on a leaf would run the greater risk of being exposed to their predators. Conversely, those types of variegation that consist of small-scale mosaics, wherein each patch is smaller than an insect, are unlikely to facilitate the undermining of the animal's camouflage.

There is a rich literature on the effects of background matching on crypsis in animals (e.g., Cott 1940; Endler 1984; Ruxton et al. 2004). However, the role of anthocyanins in undermining crypsis on plant surfaces awaits experimental confirmation.

2.11 Red Young Leaves Divert Herbivores from More Costly Old Ones

The young, expanding leaves of many woody plant species in the tropics are red (Stone 1979; Lee et al. 1987; Juniper 1994; Richards 1996; Dominy et al. 2002). Lüttge (1997) proposed that the colourful young leaves may attract herbivores such as primates, diverting them from the metabolically more expensive and photosynthetically active older leaves. The hypothesis, which was not elaborated even by Lüttge (1997), remains to be tested in the field. However, young leaves are

usually chemically and physiologically less defended than old ones (Coley and Barone 1996). This might cause difficulties in interpreting data from field observations.

2.12 Signalling by Red Autumn Leaves

2.12.1 General

The spectacular phenomenon of red autumn foliage of deciduous shrubs and trees, especially in eastern USA and Canada (Matile 2000; Hoch et al. 2001) has received broad scientific attention in recent years, especially in relation to the potential for defensive colouration by anthocyanins. For decades, many scientists believed that these colours simply appeared after the degradation of chlorophyll that masked these pigments, and that they served no function. Recently, however, it has been shown that for many plants, anthocyanins are not simply unmasked, but rather are synthesized from precursors by leaves in mid senescence (Hoch et al. 2001; Lee 2002; Lee et al. 2003; Ougham et al. 2005). Thus, the question of the possible physiological and ecological benefits of this colouration has attracted considerable scientific attention. There is very good evidence for physiological benefits of autumn leaf colouration, such as an enhanced recovery of foliar nitrogen owing to the protection of degrading chloroplasts by anthocyanins from the effects of photooxidation (Hoch et al. 2001; Yamasaki 1997; Chalker-Scott 1999; Matile 2000; Hoch et al. 2003; Feild et al. 2001; Lee and Gould 2002a, 2002b; Close and Beadle 2003; Schaefer and Wilkinson 2004; Gould 2004). These physiological advantages notwithstanding, certain hypotheses for non-physiological functions of autumn leaf colouration also merit consideration.

An early hypothesis held that autumn leaves function as a fruit flag (Stiles 1982). The reddening of leaves adjacent to ripe fruits was postulated to attract frugivorous birds, thereby enhancing the chances of seed dispersal. The hypothesis was tested experimentally by Facelli (1993), who tied plastic, leaf-shaped “flags” of different colours and sizes to the infructescences of *Rhus glabra*; birds removed the most fruit from the shrub when the flags were large and red. Other experiments, however, failed to confirm the hypothesis (e.g. Willson and Hoppes 1986), and this may have stimulated the search for alternative ecological explanations. Since the year 2000, several variations on possible signalling roles for anthocyanins in defence have been proposed in relation to autumn foliage.

2.12.2 Signalling of Defensive Potential

It has been proposed that the red and yellow colours of autumn leaves signal to potential herbivores that the plants are chemically well defended (Archetti 2000, 2007a, 2007b; Hamilton and Brown 2001; Hagen et al. 2003, 2004; Archetti and Brown 2004; Archetti and Leather 2005; Brown 2005). The hypothesis is a classic example of Zahavi’s handicap principle (Zahavi 1975), which states that because signalling is metabolically expensive, it is therefore likely to be reliable. Organisms that operate under the handicap principle send *honest* signals for the receiver to

evaluate (Zahavi 1975, 1977, 1987; Grafen 1990; Zahavi and Zahavi 1997). Accordingly, Hamilton and Brown (2001) argued that trees which face the more intense pressure from herbivory would likely present the more ostentatious displays of colourful foliage; those displays would correlate to, and be perceived by insects as, a greater metabolic investment by the plant into active defence compounds. Archetti (2000) theorised that the production of red foliage as a warning signal would benefit both parties, the tree and the parasitic insects; the tree gains from a reduction in browsing, and the insects gain information on where not to lay their eggs. Thus, far from being “a kind of extravagancy without a vital function” (Matile 2000), autumn foliage was considered to be an adaptive phenomenon resulting from the coevolution between insects and trees.

There is evidence, experimental as well as theoretical, both for and against this signalling hypothesis (Archetti 2000; Hamilton and Brown 2001; Holopainen and Peltonen 2002; Wilkinson et al. 2002; Hagen et al. 2003, 2004; Archetti and Brown 2004; Schaefer and Wilkinson 2004; Archetti and Leather 2005; Brown 2005; Ougham et al. 2005; Sinkkonen 2006a, 2006b; Schaefer and Rolshausen 2006, 2007; Archetti 2007a, 2007b; Rolshausen and Schaefer 2007; Lev-Yadun and Gould 2007). Among those papers consistent with the hypothesis, Hamilton and Brown (2001) noted that tree species for which autumn foliage was especially rich in red or yellow pigmentation were among those that potentially risked colonisation by the greatest diversity of specialist aphid species. Hagen et al. (2003) found that in mountain birch, the first trees to develop autumn colouration were the least likely to show insect damage in the following season. Similarly, Archetti and Leather (2005) found a negative correlation between aphid counts and the proportions of red or yellow leaves in a population of bird cherry (*Prunus padus*).

Arguing against the signalling hypothesis are compelling data recently published by Schaefer and Rolshausen (2007). These authors experimentally manipulated the colour of leaves in mountain ash, then monitored visits to the leaves by winged aphids. Contrary to the predictions of Archetti’s (2000) coevolutionary hypothesis, aphid counts were similar for both green and red leaves. Noting a positive correlation between aphid numbers and fruit production, however, Schaefer and Rolshausen (2007) concluded that aphids probably do select their hosts non-randomly, but they do not use leaf colour as a cue.

Other workers have argued that rather than serving as a signal to potential herbivores, the synthesis of anthocyanins in autumn shoots might better be explained in terms of the possible benefits to senescent leaf physiology (e.g. Wilkinson et al. 2002; Schaefer and Wilkinson 2004; Ougham et al. 2005). This leads to an important point: as far as we are aware, none of the proponents of the leaf signalling hypothesis have adequately addressed the question of the timing of autumn leaf colouration. Why would a plant preferentially protect from herbivores a dying leaf soon to detach from the branch, yet leave its younger, productive, acyanic leaves vulnerable to herbivory? There are possible explanations for this, of course, though none has been tested experimentally. For example, in autumn the phloem sap is particularly rich with the nutrients remobilized from senescing leaves, and signalling by anthocyanins could well benefit the tree by deterring phloem sap feeders, such as aphids. (Incidentally, Holopainen and Peltonen (2002) argued the opposite of this –

that autumn foliage *attracts* aphids because they associate the bright colours with the availability of a nitrogen feast! The authors' reasoning was based largely on the well-known attractive effects of yellow colours found in carotenoids, however, rather than of the red anthocyanins). Other possibilities are that the timing of autumn red leaves coincides with visits of a particularly aggressive herbivore, or else a key stage in the animal's lifecycle; again, these require empirical testing. Plant physiologists, on the other hand, have demonstrated a function of anthocyanins that is unique to the senescing foliage: by reducing levels of photooxidative stress on the degenerating chloroplasts, anthocyanins improve rates of nitrogen recovery from autumn leaves (Hoch et al. 2001, 2003). In terms of explaining the phenology of anthocyanin production, therefore, evidence for physiological benefits of autumn leaf colouration currently appears to outweigh that for defensive signalling.

2.12.3 The “Defence Indication Hypothesis”

Schaefer and Rolshausen (2006) proposed a variation on the signalling hypothesis which goes some way towards bridging the gap between the putative physiological and defensive functions of anthocyanins in autumn leaves. Elaborating on previous ideas by Willson and Whelan (1990) on the evolution of fruits colours, and by Fineblum and Rausher (1997) concerning the common biochemical pathways for fruit and flower colour and defensive molecules, Schaefer and Rolshausen (2006) formulated the “defence indication hypothesis”. The hypothesis holds that foliar anthocyanins evolved in response to abiotic stressors such as drought, cold, and strong light. However, because anthocyanins share the same phenylpropanoid biosynthetic pathway with many defensive phenolic compounds (such as condensed tannins), the upregulation of anthocyanin biosynthesis inevitably leads to elevated defensive strengths. Fewer herbivorous insects would feed on plants that have strong anthocyanic pigmentation because it correlates with the strength of a chemical defence. Thus, anthocyanins in leaves at once serve to mitigate the effects of environmental stress and to indicate to potential herbivores their level of investment in defensive compounds. The hypothesis is an attractive alternative to Archetti's (2000) coevolutionary model, and warrants experimental testing.

2.12.4 Aposematism of Red Autumn Leaves

If the “defence indication hypothesis” is accepted, it follows that plant parts rich in anthocyanins might serve as aposematic (warning) colouration for chemical-based unpalatability. Even if red autumn leaves are not chemically well-defended, but have a low nutritive value (another case of unpalatability) they could still be considered aposematic. The possibility of aposematism in chemically defended plants has been appraised in previous studies (Cook et al. 1971; Hinton 1973; Harborne 1982; Lev-Yadun and Ne'eman 2004; Lev-Yadun 2006); red autumn leaves are simply another case of such phenomena. We emphasize that aposematism does not exclude the possible simultaneous operation of any other types of visual or non-visual defence.

As in other cases of aposematism (Cott 1940; Wickler 1968; Lev-Yadun 2003b), it seems likely that mimics of aposematic autumn leaves also exist. Indeed, the

widespread phenomenon of red autumn leaves in some areas may partly be the result of Müllerian and/or Batesian mimicry. When toxic or unpalatable red leaves of different species mimic each other they should be considered Müllerian mimics, and when non-toxic and palatable leaves mimic toxic ones they should be considered Batesian mimics. The possible role of mimicry in the evolution of red (or yellow) autumn colouration has not been explored (Lev-Yadun and Gould 2007).

2.13 Conclusions and Suggestions for Further Research

It is evident that our understanding of the possible defensive functions of anthocyanic colouration is far from complete. Evidence for most of the hypotheses remains largely theoretical or anecdotal, and even the signalling hypothesis, for which there is the most supportive experimental data, has not been appraised systematically across more than a few taxonomic groups. Few studies have measured levels of both the anthocyanins and the herbivore antifeedants in a plant organ so that we might assess whether red leaves really do serve as an honest signal of defence commitment. Similarly, information on possible associated roles of olfactory signals, on the effectiveness of anthocyanins in mimicry and camouflage, and on the genetics of these phenomena, are lacking in most cases. Perhaps most surprisingly, there is a paucity of feeding trials involving red foliage and herbivores other than aphids. We hope that in the coming years we will see considerable progress in the experimental study of these fascinating and complicated issues.

As a caveat, it should be noted that spatial and temporal variation in anthocyanin function have the potential to confound experiments which aim to test for the involvement of anthocyanins in defence. For example, the functions of anthocyanins might well change over the course of the plant's ontogeny from, say, a defensive role in the vegetative shoot to that of an insect attractant in the reproductive shoot. Similarly, anthocyanin function could vary from organ to organ; in a leaf the anthocyanins might function as visual cues, but in an underground root they would be more likely to operate as a toxin. The relative importance of physiological versus defensive roles of anthocyanins could also change over time, or following exposure to biotic and/or abiotic stressors. The fact that many tissues are simultaneously pigmented by several pigments rather than only by anthocyanins further adds to the level of complication. Such complicated systems are not going to yield their secrets easily!

2.13.1 Leaf Colouration and the Level of Risk

There are also inherent difficulties associated with testing plants in the field for defensive colouration. Well-defended organisms are attacked less. Thus, a low abundance of herbivores on a plant bearing anthocyanic leaves could either mean that the plant faces no risk, or else that there is potentially a very high risk that is invisible because of effective deterrence. An illustrative example of this, though involving green leaves rather than red ones, is provided by various summer plants from the Negev desert in Israel. Several abundant alkaloid-rich, poisonous or thorny plants tend to form lush, green islands in the dry summer when all surrounding plants have turned yellow and have been in many cases grazed to their roots. Even under

such extreme grazing pressure those green plants are ignored by the flocks of sheep, goats, donkeys and camels that pass them daily (Lev-Yadun and Ne'eman 2004). The lack of attacks on these green plants is a clear indication for their very good defensive qualities rather than of a low level of risk. When a good defence operates, and there are only a small number of attacks on the defended organism, experimental noise may be more evident than the signals themselves.

2.13.2 No Defence is Perfect

It would be naive to assume that any defence mechanism is perfect. All types of defence incur a cost, and therefore require an organism to make physiological and developmental decisions about the level of contribution that can be achieved at any point in time. Risks of herbivory vary according to whether or not there are neighbouring plants, the plant's vigour and relative dominance within a stand, the abiotic conditions, seasonality, interactions with viral, bacterial, and fungal pathogens, the nature and strengths of herbivores, and the presence of parasites and predators of those herbivores. Thus, the requirements for defences have no constant value, but rather, change continuously according to the prevailing biotic and abiotic conditions.

The potential for herbivory pressure to outweigh the level of defence commitment poses a further problem for scientific investigations into anthocyanin function. An anthocyanic leaf that has been infested with phytophagous insects, such as the young, red, rose leaf bearing aphids shown in Fig. 2.3, could indicate one of two things: either the level of protection afforded by anthocyanins was inadequate in this instance, or anthocyanins are not involved in the defence of this species from aphids. Clearly, insect counts alone are insufficient evidence to argue either for or against the involvement of anthocyanins in defence. The evaluation of a defensive role for anthocyanins requires controlled laboratory and field experiments in which the levels of both herbivore pressure and chemical protectants can be monitored independently.

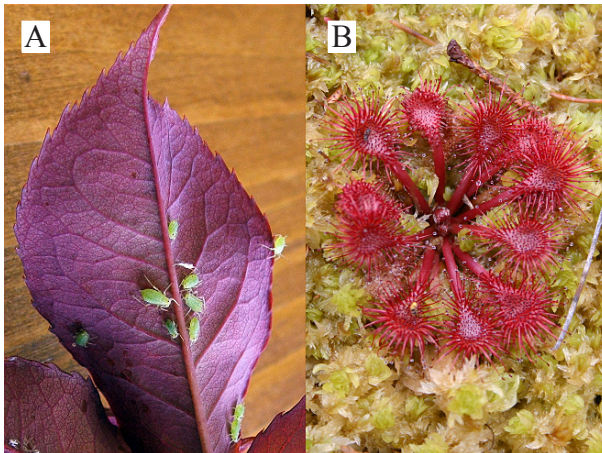


Fig. 2.3 Imperfect defence, and the exceptions. (A) Aphids feeding from the midrib of the red underside of a rose leaf. (B) Anthocyanic leaves of the New Zealand sundew *Drosera spathulata* (photo: Dean O'Connell). See Plate 1 for colour version of these photographs

2.13.3 Exceptions

There are also plants for which foliar anthocyanins are obviously not involved in anti-herbivory. One example is *Drosera spathulata*, a common sundew found in bogs throughout Australasia and parts of Asia, which typically has burgundy coloured leaves clad in stalked, glandular hairs (Fig. 2.3B). Sticky exudates from those hairs attract, ensnare, suffocate, and eventually digest small insects. The red pigments, a mix of anthocyanins (Ichiishi et al. 1999), clearly did not evolve in this species to repel insects; they may function to attract prey, but a physiological role is just as likely, as has been postulated for anthocyanins in other carnivorous plants (Moran and Moran 1998; Mendez et al. 1999).

The existence of exceptions does not, of course, vitiate hypotheses for defensive functions of anthocyanins in other red-leafed species. This is an important point because some workers have used a similar fallacious argument to dismiss certain physiological explanations in favour of defensive hypotheses for foliar anthocyanins (Dominy et al. 2002). We emphasize that regardless of the selective pressure(s) that led to the evolution of anthocyanin biosynthesis in vegetative organs, both the defensive and the physiological functions of these pigments may operate in plants simultaneously.

Armed with contemporary knowledge of anthocyanin chemistry biosynthesis and induction, as well as improved technology for monitoring plant performance in the field, and a library of information on defence mechanisms in animals, the time is ripe for enthusiastic good scientists to pursue the study of anthocyanins in plant defence. We hope that this review will prompt such studies.

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