

2

Evolutionary Ecology of Secondary Compounds in Ripe Fruit: Case Studies with Capsaicin and Emodin

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Introduction

Vertebrate-consumed fruits should be characterized by features that facilitate seed dispersal – features that increase attractiveness to any organism capable of ingesting and defecating viable seeds. Yet, as any inquisitive child with outdoor experience can certainly attest, most wild fruits appear delicious and taste bad. Astringency is the rule, not an exception (Herrera, 1982). It is equally puzzling that most species of birds and mammals rely only partially on fruits or not at all. Because wild fruits are ‘designed’ to be eaten, they should embody the proverbial free lunch. Why do they so often remain seemingly untouched for long periods (McCarty *et al.*, 2002; Tang *et al.*, 2005)?

A potential explanation emerges when one adopts a broad view of fruits and frugivores (Janzen, 1977; Cipollini and Levey, 1997a; Levey and Martínez del Río, 2001). Fruits are more than packages of seeds and nutritious pulp, and frugivores are more diverse than those that can be easily seen. In particular, secondary compounds are a universal constituent of fruits, and microbes are ubiquitous frugivores that are generally detrimental to plant fitness (because they do not disperse seeds). To understand the ecology of fruits and vertebrate frugivores, one must view fruit–frugivore interactions as an evolutionary triad between fruiting plants, their mutualists (vertebrate frugivores), and their predators and parasites, including seed predators, insects and microbial frugivores (Herrera, 1982; 2002; Cipollini, 2000; Jordano, 2000; Tewksbury, 2002; Levey, 2004). Secondary compounds are thought to mediate many of these interactions (Cipollini, 2000; Jordano, 2000; Herrera, 2002).

We believe that the key to understanding the functional significance of any secondary compound in fruits is to examine its role in an integrative manner. This requires knowledge of its biosynthetic pathway, the costs and

benefits of its production, and the impact it has on the complete spectrum of organisms that interact with the plant, including mutualists and antagonists. Effects should not be viewed in isolation. Ideally, studies of the evolutionary ecology of secondary compounds should be intraspecific, because individual variance in the trait of interest can be more easily separated from variation in other, unrelated, traits and because natural selection acts on individuals.

These points are not new (Berenbaum, 1995a; Wink and Schimmer, 1999; Cipollini, 2000). We raise them because progress in the field of fruit secondary metabolites continues to be slow and the potential for significant discoveries seems high – we know of no ecological study that has rigorously explored fruit secondary compounds and *not* found an impact of some kind. These impacts, however, are bewilderingly broad in scope, including effects on microbes, arthropods, vertebrate seed dispersers and vertebrate seed predators (Cipollini, 2000). In addition, the literature is largely a hodgepodge of studies about the effects of a particular compound on a particular organism, often without any ecological or evolutionary context.

In this chapter, we synthesize ongoing and past work on two prominent secondary metabolites in ripe fruit, emodin and capsaicinoids. Our goal is to illustrate the importance of adopting an integrative approach in deciphering the ecological role and evolutionary context of these compounds. To place our review into a broad context, we start with a short critique of how secondary compounds have been viewed by plant ecologists. We then describe the biosynthetic pathways of emodin and capsaicinoids, where they occur in plant tissue, and how their occurrence is linked to abiotic conditions. Next, we shift our attention to biotic interactions, organizing our discussion around three sequential phases of plant life-history: pre-dispersal, dispersal, and post-dispersal. Finally, we draw attention to the similarities and differences between secondary compounds in fruits and in other plant tissues. Our unifying theme is that an unusually wide array of selection pressures on fruits and seeds has led to an unusually diverse set of functions for secondary compounds in ripe fruits.

Terminology and function of plant secondary compounds

Traditionally plant metabolites have been divided into those with a direct role in primary metabolism (i.e. growth and development) and those without such a role (i.e. 'secondary'; Whittaker and Feeny, 1971; Stamp, 2003a). In this framework, the classification of secondary compounds is simple: if a metabolite is not in a major and essential pathway – photosynthesis, respiration or uptake – it is a secondary compound. Most of the theory that developed around this dichotomy assumed a defensive role for secondary compounds. This assumption persists, despite much controversy about the evolutionary ecology of secondary compounds (Berenbaum, 1995a; Hamilton *et al.*, 2001; Koricheva, 2002; Lerdau and Coley, 2002; Stamp, 2003a,b).

For the purpose of this chapter, we focus attention on the tenuous distinction between what is 'primary' and what is 'secondary' in plant metabolism. It is now widely accepted that production processes of primary and secondary metabolites are often linked via biosynthetic pathways; they are not as independent as their classification implies. This linkage may lead to trade-offs between growth, defence and reproduction. For example, there can be a negative correlation between growth and production of some tannins, suggesting that tannins are sometimes produced at the expense of growth (Coley, 1986; Sagers and Coley, 1995). More fundamentally, primary compounds can function as secondary compounds by playing a role in plant defence (e.g. citric acid; Cipollini and Stiles, 1992) and secondary compounds can have non-defensive functions (e.g. protection from UV; Dixon and Paiva, 1995).

The important point is that many plant compounds can perform 'primary' and 'secondary' functions, depending on when and where they are produced (Herms and Mattson, 1992; Berenbaum, 1995b). In fruits, their production is often tightly controlled, especially during the ripening process (Lund and Bohlman, 2006). This view of secondary compounds in fruits has important implications for the evolution of fruits, as we will explain towards the end of this chapter.

Biosynthesis of emodin and capsaicin

Emodin

Emodin belongs to the anthraquinones, a group of more than 170 natural phenolic compounds that comprise the largest group of natural quinones (Thomson, 1987, 1997; Harborne *et al.*, 1999). There are two distinct biosynthetic pathways leading to anthraquinones in higher plants: the acetate-malonate pathway in Polygonaceae, Leguminosae and Rhamnaceae, and the O-succinylbenzoic acid pathway in the Bignoniaceae and Verbenaceae (Evans, 1996; Dewick, 1998; Harborne *et al.*, 1999). The basic chemical structure of anthraquinone is an anthracene ring (tricyclic aromatic) with two ketone groups in positions C9 and C10. In plants, anthraquinones are mostly present as sugar derivatives (glycosides) but the free form (aglycones) are widely distributed as well (Thomson, 1987, 1997; Harborne *et al.*, 1999). Among the commonest naturally occurring anthraquinone aglycones in higher plants are emodin, rhein, chrysophanol, aloe-emodin and physcion (Evans, 1996; Harborne *et al.*, 1999). Several biochemical pathways that transform one anthraquinone to another have been discovered. For example, chrysophanol is synthesized in plants by dehydroxylation of emodin, an enzymatic conversion that is mediated by NADPH (Anderson *et al.*, 1988). It also appears that physcion is derived from emodin (Thomson, 1997). The anthraquinone glycosides are formed when one or more sugar molecules, mostly glucose or rhamnose, are bound to the aglycone by a β -glycoside linkage to the hydroxyl group at position C8

(in the case of glucose) or the one at C6 (in the case of rhamnose) (Dewick, 1998). Among the most common emodin-related glycosides are emodin-8-glucose, frangulin and glucofrangulin (Harborne *et al.*, 1999).

Within-species variation among the chemical and morphological traits of ripe fruits has been studied in *Rhamnus alaternus* (Rhamnaceae; Izhaki *et al.*, 2002). Variation in chemical traits of its fruit (emodin, macronutrients and minerals) was typically much higher than variation in morphological traits (e.g. fruit size). This discrepancy may be due to differences in environmental conditions between microsites that imposed greater variability on fruit nutrient composition than on fruit morphological traits, and by lower selective pressure by birds on fruit chemical traits than on morphological traits. Alternatively, it may be explained by differences in measurement error between chemical and physical traits.

Izhaki (2002a) also found that emodin concentration in fruits was positively correlated with concentrations of non-structural carbohydrates and negatively correlated with lipid concentration. Such interrelationships between chemical constituents in fruit may indicate synergistic and antagonistic interactions between these constituents, some of which might also be explained by plant–environment relations. Emodin levels were also found to depend on season and light intensity, perhaps reflecting a trade-off between plant development and defence (Paneitz and Westendorf, 1999). However, no study has explored the effect of abiotic conditions on emodin concentration in fruit.

Capsaicinoids

The secondary metabolite capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), along with a series of homologous alkyl vanillylamides called capsaicinoids, are responsible for the pungent ('hot') flavour of chilli fruit (Iwai *et al.*, 1979; Kawada *et al.*, 1985; Govindarajan, 1986; Cordell and Araujo, 1993). Capsaicinoids are unique to the genus *Capsicum* (Solanaceae; Govindarajan *et al.*, 1987; Govindarajan and Sathyanarayana, 1991; Bosland, 1994), and they are produced only in the fruit (Fugiwake *et al.*, 1982; Suzuki and Iwai, 1984). Thus, their adaptive function cannot be ascribed to interactions in other plant tissues. In addition, because capsaicinoids are both odourless and colourless (Iwai *et al.*, 1979; Kawada *et al.*, 1985; Cordell and Araujo, 1993), they are unlikely to function as signalling compounds, and thus any adaptive function should be tightly linked to direct defence of the developing embryos. Capsaicinoids are produced during fruit maturation, after elongation is complete (Estrada *et al.*, 1999), and are the result of a condensation reaction between the end products of two distichous pathways, the phenylpropanoid pathway and the fatty-acid biosynthesis pathway. The phenylpropanoid pathway contributes the aromatic head-group, while the fatty acid pathway provides the acyl moiety (Sukrasno and Yeoman, 1993). The phenylpropanoid pathway produces compounds encompassing a large range of structural and secondary bioactive plant

chemicals, all of which are unique to plants and bacteria (Kessler and Baldwin, 2002). The initial conversions of phenylalanine to phenolic compounds such as cinnamates, coumarates, tannins, saponifiable cell-wall phenolics, monolignols, flavonoids and caffeic acids is a part of the phenylpropanoid pathway common to all angiosperms. However, the conversion from ferulic acid to capsaicin is known to occur only in the genus *Capsicum* (Sukrasno and Yeoman, 1993; Dixon and Paiva, 1995). Starting with labelled phenylalanine, Hall and Yeoman (1991) found that a mere 1% of precursor was incorporated into capsaicin, while nearly 50% was incorporated into 'lignin-like' substances. One of the most important products of phenylpropanoid metabolism is a class of compounds, monolignols, which polymerize to form lignin, a structural component of plant cell walls. Importantly, lignin and capsaicin share common precursors in developing fruit; thus it appears that carbon can be allocated either to lignin production or capsaicin production but not both. It remains unclear whether or when these precursors may be limiting. If they are in short supply, a trade-off in allocation is set up between investment towards physical defence (lignin) and chemical defence (capsaicin). An important implication is that one type of defence cannot be understood without considering the other type of defence, and that the genetic and physiological mechanisms controlling production of capsaicin hold the key to revealing where and when capsaicin is produced.

Recent investigations support the hypothesis that capsaicinoid production is a monophyletic, derived trait. *Capsicum lanceolatum*, *C. rhomboideum*, *C. lycianthoides* and *C. geminifolium* are non-pungent and appear basal to all pungent taxa (Walsh and Hoot, 2001; L. Bohs, Utah, 2006, personal communication). Furthermore, a single quantitative trait locus, *cap*, accounts for 34–38% of the phenotypic variation in capsaicinoid content (Blum *et al.*, 2002). The only comprehensive theory advanced to explain the origin of pungency in *Capsicum* centres on an initial radiation from the arid, high-elevation, interior valleys of Bolivia (McLeod *et al.*, 1982). The proposed ancestral species in this radiation was a form of *C. chacoense*. More recent work using nuclear and chloroplast DNA supports a radiation of pungent taxa from dry mountainous regions, either in Bolivia or Peru (Walsh and Hoot, 2001). This is based on the earliest-branching pungent species (*C. eximium* and *C. cardinasii*) and inconclusive bootstrap values separating the baccatum clade (*C. chacoense* and *C. baccatum*). Finally, the discovery of a natural polymorphism for fruit pungency in an accession of *C. chacoense* from south-eastern Bolivia (1959 collection by Paul Smith, USDA #PI260433, polymorphism discovered by P. Bosland, New Mexico), along with the discovery of wild polymorphic populations in two additional species, *C. baccatum* and *C. eximium* (Tewksbury *et al.*, 2006) demonstrates that this genus may not always have pungent fruit. While early collectors have noted rare encounters with non-pungent plants (D'Arcy and Eshbaugh, 1974), details of this polymorphism have never been examined. The discovery and description of zones of polymorphism for capsaicinoid production may be a first step in understanding the origin and adaptive

significance of capsaicinoids in wild fruit, and their role in the radiation of the genus.

We conclude this section by summarizing three important differences in the occurrence of emodin and capsaicin. Taken together, they strongly suggest that emodin is a more primitive (derived early) compound with generalized effects, and capsaicin is more recently derived with more restricted effects.

1. Emodin is found throughout the plants that contain it, whereas capsaicin occurs only in fruit and on seeds (Iwai *et al.*, 1979; Izhaki, 2002a). This difference suggests that the ecological functions of capsaicin may be more tightly linked to the activity of frugivores and seed predators than those of emodin.
2. Emodin is produced in a wide variety of plant families and even by some fungi, whereas capsaicin appears restricted to a single genus of Solanaceae, *Capsicum* (Bosland, 1998; Izhaki, 2002a), again suggesting a more specialized role of capsaicin.
3. Although concentrations of both emodin and capsaicin can vary widely among nearby plants, this variation is much more extreme for capsaicin: to our knowledge, polymorphism for emodin production does not occur in any species, whereas it has been found in at least three wild species of *Capsicum* (Tewksbury *et al.*, 2006).

Because these three differences between capsaicin and emodin will be reflected throughout the following sections on ecological functions, they can be viewed as an organizing framework. We caution, however, that our discussion is restricted to only capsaicin and emodin, and that they may or may not be representative of other secondary compounds with similar characteristics.

Pre-dispersal effects

Without chemical protection prior to dispersal, fruits and seeds are especially vulnerable to pathogens and predators because they occur in large numbers for a relatively long time in a predictable place, and are both nutritious and moist. This pre-dispersal phase includes both ripe and unripe fruit. Removal or damage to unripe fruit is obviously detrimental to plant fitness. Removal of ripe fruit, on the other hand, may be either detrimental or beneficial, depending upon whether the seed is killed or dispersed after removal. In this section, we consider effects of emodin and capsaicin on all organisms that reduce consumption of fruit by seed dispersers.

Concentrations of emodin are highest in unripe fruits (Tsahar *et al.*, 2002), while concentrations of capsaicinoids are highest in ripe fruits (Estrada *et al.*, 2000). The reduction in emodin concentration with fruit ripening is typical of fruit secondary metabolites, suggesting a generalized defensive role and potential conflicts with beneficial consumers. By

contrast, the increase in capsaicinoid concentration with fruit ripening seen in *Capsicum* suggests a more specialized role for these chemicals – one that is focused explicitly on fruit consumption. If emodin and capsaicinoids were simply a by-product of a plant's general metabolic processes (Ehrlén and Eriksson, 1993), one would not expect their concentrations to change markedly during fruit maturation, nor that these changes would be so common among other secondary compounds and species.

Protection of fruit and seeds prior to seed dispersal is critical before and after the fruit ripens, but trade-offs and selective pressures shift during fruit ripening. For example, early in fruit development, herbivores will often consume fruit, creating significant fitness costs (Hulme and Benkman, 2002), and chemicals that deter herbivory in other plant parts are often found in unripe fruits (Ehrlén and Eriksson, 1993). During fruit maturation, chemicals that deter beneficial seed dispersers are detrimental, and most plant secondary metabolites rapidly decrease in concentration as fruits ripen (Cipollini and Levey, 1997a). However, in almost all fruits there is a period of vulnerability after fruit maturation but before dispersal (Howe, 1977; Thompson and Willson, 1979), during which pre-dispersal seed predation by invertebrates and microbes can destroy fruit (Hulme and Benkman, 2002). Protecting ripe fruit may be more difficult for a plant than protecting unripe fruit, as chemicals involved in the protection of ripe fruit may also harm legitimate dispersers, necessitating more selective fruit chemistry (Herrera, 1982; Cipollini and Levey, 1997a; Tsahar *et al.*, 2002; Tewksbury, 2002; Schaefer *et al.*, 2003). Chemical protection of unripe fruit might thus be directed primarily at herbivores, while chemical protection of ripe fruit may be most often directed against microbial and invertebrate attack, as these organisms thrive on the same nutritional qualities that attract legitimate dispersers – sugars, lipids, carbohydrates and proteins (Herrera, 1982). However, considerable overlap is expected, as consumers and fruit chemistry often cross the boundary between ripe and unripe fruit. Indeed, in the two systems we examine here, both emodin and capsaicin appear to deter a broad spectrum of organisms, including herbivores and microbes.

Emodin

Emodin has larvicidal activity against dipterans (Yang *et al.*, 2003) and inhibits growth of some protozoa (Wang, 1993), bacteria (Wang and Chung, 1997) and fungi (Kim *et al.*, 2004). Among vertebrates, emodin strongly deters food consumption by yellow-vented bulbuls (*Pycnonotus xanthopygus*; Pycnonotidae), house sparrows (*Passer domesticus*; Passeridae), and white-footed mice (*Peromyscus leucopus*; Cricetidae) (Sherburne, 1972; Tsahar *et al.*, 2002) and can be lethal when force-fed to redwing blackbirds (*Agelaius phoeniceus*; Icteridae), European starlings (*Sturnus vulgaris*; Sturnidae) and one-day-old cockerels (*Gallus domesticus*; Phasianidae) (Wells *et al.*, 1975; Schaefer *et al.*, 1983). Unfortunately, many of these studies are

fundamentally limited by a lack of ecological context. Most were not done in the field and effects were tested using unusually high concentrations of emodin on organisms that rarely or never encounter emodin in nature. A notable exception is a study that examined natural variations in emodin concentration in *Rhamnus alaternus* fruit and reported that plants with more emodin suffered lower pre-dispersal seed damage by insects (Tsahar *et al.*, 2002). However, it is unclear whether variation in emodin or some other factor generated the differences in seed damage. Thus, the most we can conclude about emodin's function during the pre-dispersal phase is that it has great potential for thwarting attacks on unripe fruit and that this potential is general across a wide range of taxa.

Capsaicinoids

The functional role of capsaicinoids in unripe and ripening fruits is clearer than that of emodin. Like emodin, capsaicin inhibits growth of many types of microbes and invertebrates *in vitro* and under laboratory conditions (Debkiyaniya *et al.*, 1980; Cichewicz and Thorpe, 1996; Oh *et al.*, 2004; Zeyrek, 2005). However, the polymorphism for pungency in some species of wild chilli allows one to move beyond this type of study and examine the effects of capsaicin in an ecologically relevant setting. In Bolivia, ripe fruits on wild pungent and non-pungent *Capsicum chacoense* plants were monitored for fungal infection (J.J. Tewksbury *et al.*, 2006, unpublished results). After 45 days, no fruits on pungent plants showed signs of fungal attack, whereas 12% of fruits on non-pungent plants did so. This experiment does not rule out effects of other compounds which might vary between pungent and non-pungent plants, but it strongly suggests an anti-fungal role for capsaicinoids. A similar experiment using pungent and non-pungent varieties of *C. chacoense* also demonstrated that capsaicin deters consumption of chilli fruits and seeds by small rodents in the field and in the laboratory (Tewksbury *et al.*, 1999; Tewksbury and Nabhan, 2001). This is noteworthy because rodents are generally seed predators. Interestingly, capsaicin does not strongly or consistently deter consumption of foliage by large herbivorous mammals (Andelt *et al.*, 1994; Wagner and Nolte, 2000; Santilli *et al.*, 2004), which are more likely than small rodents to defecate ingested seeds in a viable condition (Janzen, 1971).

Dispersal effects

For the purposes of this review, we define dispersal as the phase between the time when a fruit is removed from a plant by a seed disperser and when its seeds are eventually defecated, dropped or regurgitated. We include fruit selection by seed dispersers, as it ultimately determines which fruits are consumed.

Emodin

Birds and mammals appear universally sensitive to variations in emodin concentration, although this sensitivity is not equal among taxa (Izhaki, 2002a). For example, yellow-vented bulbuls (*Pycnonotus xanthopygos*) in feeding trials distinguished between diets containing 0%, 0.001% and 0.002% emodin, always preferring the diet with the lowest concentration (Tsahar *et al.*, 2002). House sparrows (*Passer domesticus*) were less sensitive than bulbuls. They did not distinguish between 0.001% and 0.002% diets but did between 0.005% and 0.01%. Functionally, this means that bulbuls are sensitive to the natural range of emodin variation in ripe fruits (0.001–0.002%), whereas house sparrows are only sensitive to the range of variation between ripe and unripe fruits (0.001–0.01%) (Tsahar *et al.*, 2002). Sherburne (1972) also reported sensitivity of vertebrates to emodin: American robins (*Turdus migratorius*) and white-footed mice (*Peromyscus leucopus*) tended to avoid ripe fruits coated with emodin but readily consumed uncoated (control) fruits and very rarely consumed unripe fruits, which contain the highest concentrations of emodin.

Despite the widespread detrimental effect of emodin on fruit consumption by vertebrates, ripe fruits that contain emodin are frequently consumed by a wide variety of seed-dispersers (Herrera, 1984; Izhaki and Safriel, 1985; Izhaki, 2002b; Tsahar *et al.*, 2002), suggesting that the net benefits of fruit consumption somehow outweigh the negative effects of emodin. In particular, the nutritional reward of carbohydrates, lipids and proteins in fruit pulp can be viewed as more important to consumers than the presence of co-occurring secondary compounds (Cipollini and Levey, 1997b). This does not mean that consumers are blind to natural variation in compounds such as emodin. Tsahar *et al.* (2002) found that removal of *Rhamnus alaternus* fruits was significantly higher on plants with lower concentrations of emodin in one of two years of study.

Consumption of emodin has physiological effects on frugivores, which probably influence seed fate and hence plant fitness. Domestic chicks force-fed 3.7–37 mg/kg and American robins force-fed approximately 0.07–70 mg/kg developed severe diarrhoea (Sherburne, 1972; Wells *et al.*, 1975). In contrast, yellow-vented bulbuls feeding voluntarily on a fruit-based diet containing 0.01% (wet mass) of emodin consumed as much of the diet and in a similar pattern to birds on a control diet but had longer intervals between defecations, strongly suggesting a costive effect of emodin (Tsahar *et al.*, 2003). At concentrations of 0.001%, 0.005% and 0.01%, emodin generally increased digestive efficiency of dry matter, nitrogen, fat and organic remains (mostly carbohydrates and protein) by approximately 5–10%. Because this increase could not be attributed to differences in consumption rates, it was likely to have been due to the slowing of transit time through the gut, allowing for more complete assimilation of digesta (Afik and Karasov, 1995). From a bird's perspective, higher digestive efficiencies are presumably beneficial. From a plant's perspective, the costive effects of emodin are probably beneficial because

they increase the time between defecations and potentially the distance that seeds are dispersed away from the parent plant and the distance between where seeds are deposited in sequential defecations. Longer gut retention times may also increase the probability that seeds will be thoroughly cleaned of pulp, which is important in species whose seeds require removal of pulp in order to germinate (Barnea *et al.*, 1991; Traveset *et al.*, Chapter 4, this volume).

Capsaicinoids

Birds are the primary consumers and dispersers of wild chillies (Tewksbury and Nabhan, 2001; Levey *et al.*, 2006). Capsaicin has no effect on the feeding behaviour of captive birds (Norman *et al.*, 1992; Tewksbury and Nabhan, 2001). To determine whether this lack of discrimination also occurs in natural settings, we trimmed all fruit from five pungent and eight non-pungent *Capsicum chacoense* plants and wired in their place cut branches with 10–11 ripe fruit from either pungent or non-pungent plants. We monitored fruit removal on branches from pungent and non-pungent plants, and replaced branches where removal had occurred with new branches of the other pungency treatment (i.e. a branch bearing pungent fruit would follow one bearing non-pungent fruit). We found no difference in removal rates of pungent and non-pungent fruits, suggesting that the presence of capsaicin in ripe chilli fruits has no cost to the plant in terms of fruit preference by seed-dispersing birds (Fig. 2.1).

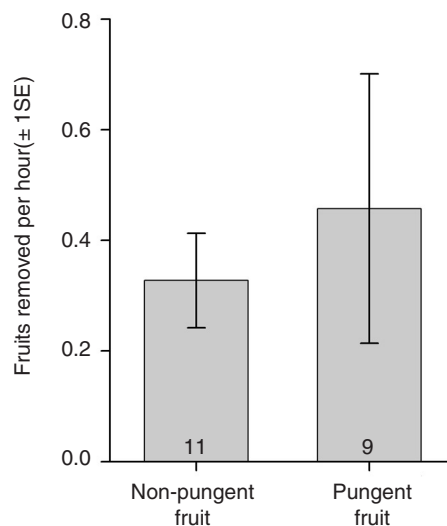


Fig. 2.1. Rate of fruit removal (per hour) for non-pungent and pungent *C. chacoense* fruit in Bolivia. Branches with 10 fruits per branch were wired onto chilli bushes where all natural fruit had been removed, and these branches were observed to determine when fruits were removed. Sample size, provided at the base of each bar, is the number of branches monitored.

Once ingested, capsaicin affects the gut processing of seeds. To determine the nature of this effect we studied the most common dispersers of chillies in Bolivia (small-billed elaenia, *Elaenia parvirostris*; Tyrannidae) and in Arizona (curve-billed thrasher, *Toxostoma curvirostre*; Mimidae). Individuals of each species were fed non-pungent chillies injected with a solution containing capsaicin or a control solution, and the time of all defecations were recorded until the last seeds were voided (J. J. Tewksbury *et al.*, 2006, unpublished results). For both species, capsaicin increased seed retention times by an average of 15–20% compared with controls.

Longer retention times induced by capsaicin probably mean that *Capsicum* seeds are dispersed more widely by birds, which would be beneficial to parent plants. Such a benefit may be countered by post-dispersal impacts to the seeds as a result of longer exposure to digestive enzymes and physical processing in the gut (see below). Longer retention times may benefit avian dispersers if they increase assimilation of nutrients in fruit pulp, as happens with emodin; but we did not explore that possibility.

Post-dispersal effects

Although the post-dispersal phase technically includes all processes from the time of seed deposition to the time of fruit production by the next generation of plants, we restrict the scope of our review to what happens to seeds before germination.

Emodin

The effects of emodin on post-dispersal seed fate are unexplored in species that naturally produce emodin. All that is known comes from experiments on sunflowers (*Helianthus annuus*; Asteraceae) and corn (*Zea mays*; Poaceae), in which application of emodin inhibits germination by 98% and 55–76%, respectively (Hasan, 1998). Seeds of two species of *Rhamnus* will not germinate unless freed of pulp (Gourley and Howell, 1984; Barnea *et al.*, 1991). Emodin is a common constituent of *Rhamnus* fruit pulp but whether it accounts for inhibition of germination is unclear. Likewise, nothing is known about emodin effects on ecologically relevant species of post-dispersal seed predators and pathogens.

Capsaicin

Pungency is most likely to affect seed fate through changes in germination and in vulnerability to seed predators and pathogens. Effects on germination are tied to the differences, described above, in gut retention time. In *C. chacoense* there is no difference between pungent and non-pungent fruits in total percentage of germinated seeds; seeds with and

without a coating of capsaicin have equal probabilities of germinating (J.J. Tewksbury *et al.*, 2006, unpublished results). However, seeds from pungent and non-pungent fruits differ substantially in how time in the gut affects seed viability. Seeds from non-pungent plants are not affected by long periods in the gut, whereas seeds from pungent plants decrease from 80% viability if defecated within 30 min to 45% when defecated after 3 h (J.J. Tewksbury *et al.*, 2006, unpublished results). We are currently exploring the reason for the greater susceptibility of pungent seeds to long gut-transit times. Given that capsaicinoids and lignin share a biosynthetic pathway, we hypothesize that pungent plants gain chemical protection from capsaicinoids at the cost of reduced physical protection by lignin. Less lignin in seed coats may mean a greater susceptibility of seeds to physical and chemical breakdown during gut passage.

Capsaicinoids appear to have little influence on post-dispersal predation of *Capsicum* seeds. In Bolivia we coated naturally non-pungent *C. chacoense* seeds with capsaicin and compared their removal to control seeds (naturally non-pungent *C. chacoense* seeds coated with ethanol, the carrier we used for capsaicin). We found no difference in removal rates (J.J. Tewksbury *et al.*, 2006, unpublished results). However, the presence of capsaicin appears to reduce the extent of fungal (*Fusarium* spp.) infection of seeds; infection levels are higher in non-pungent than in pungent *C. chacoense* seeds. This effect is complicated by the fact that passage of seeds through a bird's gut reduces both the amount of capsaicin on seeds and the level of fungal infection, at least temporarily (J.J. Tewksbury *et al.*, 2006, unpublished results).

Discussion

Our take-home message is that fruit secondary compounds have multiple functions on multiple taxa over multiple time scales (Table 2.1). We restricted our review to emodin and capsaicin because no other fruit secondary compounds have been as thoroughly studied in a wide variety of ecological contexts, except perhaps glycoalkaloids (Cipollini and Levey, 1997b,c; Levey and Cipollini, 1998; Wahaj *et al.*, 1998). Despite our fairly narrow scope, the broad spectrum of effects we have described is likely to be typical of fruit secondary metabolites (Wink, 1998; Wink and Schimmer, 1999; Cipollini, 2000). Essentially, all secondary compounds examined in an ecologically relevant way have been reported to have at least some effects on fruit or seed consumers (Cipollini and Stiles, 1991, 1992, 1993; Murray *et al.*, 1994; Bairlein, 1996; Cipollini and Levey 1997a,b,c; Levey and Cipollini, 1998; Wahaj *et al.*, 1998; Stanley and Lill, 2001; Tewksbury and Nabhan, 2001; Witmer, 2001; Tsahar *et al.*, 2002; Schaefer *et al.*, 2003; Tsahar *et al.*, 2003; Tsang and Corlett, 2005). Studies that report no effects are rare; we are aware of only one: Struempf and Martínez del Rio (1999) found no effect of amygdalin on food consumption by cedar waxwings (*Bombycilla cedrorum*; Bombycillidae).

Table 2.1. Properties and functions of emodin and capsaicin in fruits before, during, and after seed dispersal.

Plant life-history phase	Property or possible function	Emodin	Capsaicin
Pre-dispersal	Higher concentration in unripe fruits	+	+
	Protect fruits and seeds by deterring consumption by: vertebrates	+	+
	invertebrates	+	0
	fungi	+	+
Dispersal	Detectable by dispersing birds	+	0
	increase gut retention time	+	+
Post-dispersal		+	+
		+	+
		– (Chicken and American robin)	+
	Seed viability after bird ingestion	Not studied	2 pungent (<i>C. chacoense</i>) 0 non-pungent (<i>C. chacoense</i>) + pungent (<i>C. annuum</i>)
	Protect seeds from predation by:		
	rodents	Not studied	+
	fungi	Not studied	+
	ants	Not studied	0

+ = positive effect; – = negative effect; 0 = no effect. Study species are listed in parentheses.

An important corollary is that a narrow exploration of the role of a secondary compound is practically guaranteed to yield an equally narrow and probably misleading interpretation. For example, a study on capsaicin's effect on retention time of seeds in bird guts would lead one to conclude that slower passage through the gut would benefit *Capsicum* plants by ensuring wider dispersal. An examination of seed predation, however, would no doubt reveal that seeds held longer in the gut are stripped of capsaicin and more prone to seed predation than seeds not passed through the gut or those passed more rapidly. In addition, seeds held for >3 h in the gut suffer a reduction in the percentage germinating, presumably due to damage by digestive processes. From the plant's perspective, the *net effect* of a secondary compound is the relevant currency of natural selection. The important point is that this net effect is impossible to gauge without a multifaceted examination of potential effects.

The example with capsaicin, gut passage and germination raises another issue that is often overlooked – metabolic trade-offs. Most studies on secondary metabolites focus on potential benefits, with little recognition of costs. Capsaicin is produced via the phenylpropanoid pathway, which is significant because lignin is also produced by it, and therefore capsaicin and lignin compete for precursors (Sukrasno and Yeoman, 1993). This results in a potential trade-off between physical protection (lignin) and chemical protection (capsaicin), and suggests a mechanistic explanation of the apparently fragile nature of pungent seeds reflected by their lack of germination success after exposure to long gut-transit times. Similarly, emodin concentration in *Rhamnus alaternus* fruits is negatively correlated with amounts of neutral detergent fibre and acid detergent fibre, both of which contain lignin, and other types of fibre (Izhaki *et al.*, 2002). Unfortunately, we cannot understand such trade-offs without a better knowledge of how fruit secondary compounds are created and changed during ripening; fruits and their constituents are highly dynamic. Thus, research on fruit secondary metabolites needs to acknowledge the complex interplay between biosynthetic pathways, genetic regulatory mechanisms, and environmental cues during ripening (Berenbaum, 1995; Lund and Bohlman, 2006).

Although not universal (Cipollini *et al.*, 2004), the kinds of trade-offs described above may be common, because the phenylpropanoid pathway is responsible for the production of practically all fruit secondary metabolites (Dixon and Paiva, 1995). The extent to which metabolic trade-offs determine concentrations of secondary metabolites in plants is under debate, with the outcome largely depending on how limiting metabolic precursors are under different conditions and the relative costs of producing different metabolites (Stamp, 2003a). To our knowledge, only two studies have experimentally examined the relationship between the availability of a potentially limiting precursor and the concentration of secondary compounds in wild fruits. Based on a greenhouse experiment with a desert plant, *Ochradenus baccatus* (Rubiaceae), glucosinolate concentration in fruit pulp was positively affected by the sulphur content

in soil and was negatively related to water conditions (Lotan, 2005). On the other hand, *Solanum carolinense* (Solanaceae) fruits on plants grown under two nitrogen and two water treatments showed essentially no variation in concentrations of secondary compounds (Cipollini *et al.*, 2004), suggesting that the types of trade-offs we propose for products of the phenylpropanoid pathway are not universal.

Why do fruit secondary metabolites so often have multiple functions?

Multifunctionality of fruit secondary metabolites should not come as a surprise or be viewed as extraordinary. The reason extends beyond previous explanations of multifunctionality, which are based on the simple premise that a compound with more than one benefit will be favoured over a compound with a single benefit (Wink, 1998; Wink and Schimmer, 1999; Cipollini, 2000). That premise begs the question of *how* such variation in function may arise in the first place. What unique characteristics of fruit underlie multifunctionality?

We suggest that fruits are an unusually effective generator of bioactive chemicals. Through their seeds, they link one generation to another and therefore play a central role in determining the fitness of fruiting plants. More fundamentally, because fruit secondary metabolites occur in both pulp and seeds, their impacts span the period from fruit inception through seed dispersal and germination. In species with seed dormancy, this period can be longer than the lifespan of the parent plant. In all species, it includes exposure to practically all types of organisms that typically interact with plants. These organisms include microbes, arthropods and vertebrates. Furthermore, they also encompass mutualists, predators and pathogens. Secondary metabolites mediate interactions with all of them. This enormous variation in type of interaction is essentially an incubator for compounds with multiple functions across diverse taxa of fruit and seed consumers.

In contrast to fruits, leaves generally experience a single type of biotic interaction: herbivory. Any organism that consumes leaf tissue is typically detrimental to plant fitness, whereas organisms that consume fruits may be either beneficial or detrimental. Thus, secondary compounds in leaves have a much narrower scope of selection pressure than those in fruits. In flowers, secondary compounds have the potential to mediate interactions with mutualists (pollinators) and antagonists (nectar robbers, herbivores) in a way similar to what we propose for fruits. Some secondary compounds that occur in nectar are known to deter at least one species of nectar robber, while stimulating nectar consumption by pollinators (Irwin *et al.*, 2004; Tadmor-Melamed *et al.*, 2004; Singaravelan *et al.*, 2005, 2006).

This perspective of fruit secondary metabolites aligns with an unconventional view of fruit pulp. Mack (2000) proposed that fruit pulp originated as a medium in which protective compounds and structures could encase seeds, essentially adding a layer of chemical and physical

protection over whatever physical protection was already provided by the seed coat. The evolution of frugivory resulted from vertebrates that specialized in consuming these types of seeds, and gradually developed a more gentle treatment of seeds as plants developed other types of pulp constituents that were more accessible and nutritious to those vertebrates. In much the same way, insect pollination is thought to have derived from insects that initially preyed upon pollen and gradually switched to feeding on nectar as it became available. This view should force a re-evaluation of the distinction between secondary compounds and nutritional rewards in fruit pulp. Carbohydrates, lipids and proteins are typically considered ecologically and evolutionarily distinct from secondary metabolites, yet they occur only because other secondary metabolites created the opportunity in what we view as an evolutionary incubator for such compounds. Furthermore, they fit the classical definition of a secondary metabolite as a compound with no primary (i.e. physiological) function.

More fundamentally, this confusion over categorization requires one to re-examine the usefulness of the term 'secondary metabolite'. Categorizing plant metabolites as primary and secondary can be useful in whole-plant physiological studies where growth is defined as primary and everything else is considered secondary (most notably reproduction, where scents and colours are mediated by secondary metabolites). These categories become conflicted and even irrelevant when a broader ecological or evolutionary view is taken. Major adaptive shifts can be mediated by chemicals for which no 'primary' purpose is known (Bradshaw and Schemske, 2003), but the fitness advantage conferred through the production of the chemical is no less important. Thus, as our understanding of the role of compounds associated with increasing fitness becomes more complete, the false construct of categorizing these compounds as primary or secondary may fall from favour. Indeed, some investigators have begun using the term 'specialized' metabolite, rather than secondary metabolite, to describe compounds that appear to have a relatively narrow function within plant tissues.

If one accepts the premise that fruit secondary metabolites have diverse and multiple functions, the question of how to characterize compounds such as lipids and capsaicinoids has an easy answer: lipids and capsaicinoids are mechanistically different but share some functional similarity as mediators of interactions between plants and their biotic environment. Thus, the primary importance of secondary compounds in fruit pulp may be that essentially *all* compounds in ripe fruit should be given equal billing and regarded, collectively, as primary determinants of fruit fate, seed fate, and ultimately the fitness of fruiting plants.

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