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What Parts of Fleshy Fruits Contain Secondary Compounds Toxic to Birds and Why?

ANAT BARNEA,*† JEFFREY B. HARBORNE‡ and C. PANNELL*
*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK;
‡Department of Botany, School of Plant Sciences, University of Reading, Whiteknights,
Reading RG6 2AS, U.K.

Key Word Index—Ivy; holly; yew; hawthorn; fruits; secondary metabolites; frugivorous birds.

Abstract—Saponins, flavonoids and cyanogenic glycosides were surveyed in pulps and seeds of wild, bird-dispersed fleshy fruits of hawthorn (*Crataegus monogyna*), ivy (*Hedera helix*), holly (*Ilex aquifolium*), and yew (*Taxus baccata*). Interactions between three of the four species (hawthorn, holly, and yew) and their avian seed dispersers were studied in the field. The results indicate that when different bird species foraged on the same fruit they spend similar periods of time on the tree and eat a similar number of fruits at each feeding bout. Frugivorous birds stayed on all plant species for short periods of time (1.3–5.3 min) and consumed only a few fruits in each feeding bout (4.3–6.5 min). There is a differential occurrence of secondary compounds in fruit parts: in most cases allelochemicals were found in pulps but not seeds. These findings confirm the hypothesis that mild toxicity in the pulp can prevent consumption of too many fruits in one foraging bout and regulate seed retention time. This, combined with the short visits ensures better seed dispersal, as only few seeds will be deposited in one site at one time. Yew presents a special case, since cyanogenic glycosides were identified only in seed-coats, while both the fleshy aril and seed content are free of this toxin. A possible ecological explanation is suggested for this finding. The concentrations of some compounds may change during fruit ripening.

Introduction

In fleshy fruits dispersed by animals, it is generally accepted that chemical defense allocated to the immature fruit should be neutralized upon maturation so that the pulp becomes attractive to dispersal agents (Janzen, 1983; McKey, 1975). The seeds must be protected from disperser's guts and seed predators. This can be done either mechanically, e.g. by a hard seed coat, or chemically, by toxic compounds (Janzen, 1983).

However, it has also been suggested that frugivores avoid fruits of some species because they are toxic (Janzen, 1978). Toxins in certain fruit species may have evolved to prevent consumption of fruits by birds or mammals that disperse seeds into unsuitable habitats (Sorensen, 1983). It is also possible that some fruit species have evolved mild toxins to prevent too many fruits from being eaten per foraging bout. This may have important consequences to seed dispersal by preventing too many seeds from being deposited in one site (Sorensen, 1982).

Relatively little is known about secondary compounds in wild fleshy fruits and their ecological role. Most of the existing information is either very general, referring to "plant extract", or to other plant parts (i.e. leaves, stems, etc.). Even in cases where identification was done in fruits, usually the whole fruit was tested, not separating the pulp (which is digested by dispersers) from the seed(s) (which are usually excreted intact). Similarly, in only few cases have workers tested and compared secondary compounds in unripe and ripe fruits of the same species.

Therefore, the aim of this work was to examine the secondary compounds in pulps and seeds of ripe (and, when possible, of unripe) wild fleshy fruits that are bird-dispersed. Four species, each from a different family, were included in the analysis:

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†Present address: The Rockefeller University, Box 137, 1230 York Avenue, New York, NY 10021–6390, U.S.A.

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hawthorn (*Crataegus monogyna*, Rosaceae), ivy (*Hedera helix*, Araliaceae), holly (*Ilex aquifolium*, Aquifoliaceae), and yew (*Taxus baccata*, Taxaceae). All these species are common native plants in England and bear fleshy edible dispersal structures that enclose the seeds and are consumed by birds. For convenience, diaspores of all species (drupes in the case of hawthorn and holly, berries in ivy, and arillates in yew) will be termed hereafter as "fruits" and dispersal structures as "pulps" in an analogous sense.

Interactions between three out of the four species (hawthorn, holly and yew) and their avian dispersers were studied in the field. Direct observations on fruiting individuals determined which bird species dispersed the fruits; number of fruits that were consumed at each feeding bout; and duration of visits to the fruiting plants. Fruit samples were collected from the observed individual trees and kept for biochemical analysis.

Saponins, flavonoids and cyanogenic glycosides have variously been detected in the four plant species (Table 1). They are known to have various toxic effects and some evidence is available about their possible ecological role in interactions with animals. Cyanogenic glycosides are toxic only through their release of hydrocyanic acid (HCN), which occurs either when the tissues in which they are present have been broken after ingestion, or as a result of plant cell damage before ingestion (Cooper and Johnson, 1984). This production of the poisonous HCN can affect a wide range of organisms, since its site of action is inhibition of the cytochromes of the electron transport system. In general the highest concentrations of cyanogenic glycosides in plants are found in leaves, but they also occur in seeds, roots and other plant tissues (Cooper and Johnson, 1984).

Flavonoids are, in many plants, a significant barrier against herbivore feeding, although we know little at present of the detailed chemistry of the substances causing avoidance (Harborne, 1988). Related phenolic compounds of various types have been implicated as feeding deterrents also in birds but the taste and toxicity of natural flavonoids have rarely been studied to any degree in an ecological context (Rosenthal

TABLE 1. SAPONINS, FLAVONOIDS AND CYANOGENIC GLYCOSIDES PREVIOUSLY IDENTIFIED IN HAWTHORN, IVY, HOLLY AND YEW

Species	Compound	Part of plant	Reference
Crataegus monogyna	Saponins	Fruit	Rossiiskaya *et al.*, 1989 Gibbs, 1974
	Flavonoids: Vitexin and Vitexin-4′-rhamnoside	Leaf	Pietta *et al.*, 1986
	Cyanogenic glycosides	Seeds	Gibbs, 1974
Hedera helix	Saponins	Leaves	Facino *et al.*, 1987 Hubrecht, 1988
		All parts	Frohne and Pfander, 1984
		Berries	Hostettmann, 1980
	Cyanogenic glycosides	Leaves	Gibbs, 1974
Ilex aquifolium	Saponins	Leaves	Balansard and Flandrin, 1946
	Cyanogenic glycosides	Leaves	Gibbs, 1974
		Ripe fruits	Willems, 1988 and 1989
		Stems	
		Leaves	
	Flavonoids: Quercetin glycosides (Rutin)	Leaves	van Gelderen, 1987
Taxus baccata	Cyanogenic glycosides	Leaves	Frohne and Pfander, 1984
		All parts except fruit	Cooper and Johnson, 1984

and Berenbaum, 1991, 1992). Present evidence indicates that common flavonoids are harmless to human beings; however, this can be related to the presence of an efficient detoxifying system. As with insects, toxicity, reduction of appetite and antifeedancy that is caused by common flavonoids (including rutin and quercetin), have been demonstrated (Shaver and Lukefahr, 1969; Ohem and Holzl, 1988).

Saponins are widespread in numerous plant species, but their main function is still not clear. It has been suggested that they may serve as a chemical defense mechanism in plants, imparting resistance to pests of diseases (Birk, 1969). Saponins are distinguished by their bitter taste, which deters animals from feeding on plants in which saponins are present (Cheeke, 1976). These compounds are generally harmless to mammals when ingested, although large quantities can be irritant and cause vomiting and diarrhoea. However, saponins are highly toxic to fish and snails (Cooper and Johnson, 1984), and some of them are responsible for growth depression of chicks and for decrease in food consumption (Draper, 1948; Cooney *et al.*, 1948).

Materials and Methods

Study area

Field Observations. Observations of hawthorns, hollies and yews were made during October 1990–January 1991 at two localities in Oxfordshire, southern England: Brasenose Wood (400 acres of mixed deciduous wood) and University Parks in Oxford. At least three trees of each species was observed. Observations were made for at least two hours each, between 0900–1500 h. Hawthorns were observed for a total of 8 h, hollies for 9.5 h and yew for 4 h. Each visit by an individual bird to any of the trees of each species under observation was defined as a feeding visit if the bird was seen to eat or try to eat at least one fruit. As far as possible we recorded the following for each feeding visit: bird species, duration of visit, and number of fruits taken (meal size).

Plant material. Ripe haws were collected at Brasenose Wood, from three trees, at three different times during the fruiting season: in November and December 1990 and January 1991. Fruits of ivy were collected from the same location twice, from three individuals: unripe fruits in November 1990, and ripe fruits which were observed to be eaten by birds in February 1991. Ripe fruits of holly (from two trees) and yew (three trees) were collected in Oxford, in November 1990. Seed coats of yew that had been stripped off and thrown away by greenfinches were collected from the soil beneath these trees. All fruits were dissected to separate the dispersal structure i.e. the flesh or aril from the seed(s). Different collection were kept separately at −28°C until use.

Detection of cyanogenic glycosides. The presence of cyanogenic glycosides was determined separately for pulps and seeds (in the case of yew, seed coats were tested separately from seed content). We used a qualitative test, based on the work of Feigl and Anger (1966). Filter paper strips were prepared by dipping them for 2 min in a 1:1 mixture of two freshly prepared solutions of 1% copper ethyl acetoacetate and 4,4′-tetramethyldiaminodiphenylmethane in chloroform. Pieces of the fruit tissue to be tested (1.0–1.5 g of pulp or seeds) were placed in a test tube, a few drops of water and toluene were added and the material was crushed with a glass rod. The tube was then firmly corked, with the paper strips suspended inside from the cork so as not to touch the macerated tissue and left to incubate at 40°C for 2 h. The cellular disruption of the tissue presumably brings the glycoside into contact with the glycosidase usually also present in the plant. The enzymatic release of HCN changes the almost colourless reagent on the filter strips to a blue colour. If the reaction was negative the tube was left at room temperature for a further 24–48 h and re-examined (Pusey, 1963). The limit of detection is 1 µg HCN.

Identification of flavonoids. Seeds and pulps of unripe and/or ripe fruits of each tested species were extracted separately for 5–10 min with boiling 95% ethanol and the extracts subsequently concentrated on a watchglass in an air draught. The aqueous concentrates were collected with capillaries and applied directly to chromatoplates (Harborne, 1988). TLC (thin layer chromatography) was used to test the presence of flavonoids in plant extracts. Authentic samples, used as markers were chromatographed alongside the alcoholic extracts. Unless otherwise noted, we used 20×20 cm cellulose plates coated with fluorescent indicator. After drying the plates were examined in UV light, then sprayed with ammonia and examined again.

Vitexin. Authentic samples was purchased from Apin Chemicals Ltd., England. Hawthorn extracts of ripe seeds and pulps and authentic samples were chromatographed two-dimensionally using the solvent BAW (BuOH–HOAC–H_2O, 4:1:5) in the first direction and 50% HOAc in the second.

Rutin. An authentic sample was available in our collection (J.B.H). Extracts of unripe and ripe ivy seeds and pulps and authentic sample were chromatographed two-dimensionally using BAW in the first direction and 10% HOAc in $CHCl_3$ in the second (Harborne, 1988; Stahl, 1969). Seeds and pulps of holly fruits were chromatographed as follows: (i) one dimensionally on 10×10 cm plates of silica gel, using BAW as solvent, (ii) one-dimensionally on same plates and HOAc–Me_2CO–MeOH–toluene, 5:5:20:70 as solvent (Stahl, 1969), (iii) two-dimensionally using BAW in the first direction and 5% HOAc in the second (Harborne, 1988).

Identification of saponins. Seeds and pulps of unripe and ripe ivy fruits were extracted separately with methanol. After concentration in a rotary evaporator, extracts were partitioned between butanol and water (Hostettmann, 1980). TLC was used to test the presence of saponins in butanol layers of the extracts. Authentic samples of α-hederin (hederagenin-3-*O*-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranoside) and hederagenin 3-*O*-α-L-arabinopyranoside were obtained from Professor K. Hostettmann, Switzerland. These samples were used as markers and chromatographed alongside the pulp and seed extracts on 20×20 cm silica gel plates in three solvents: (1) $CHCl_3$-MeOH-H_2O,. 13:7:2 (Hostettmann, 1980), (2) BAW (BuOH-HOAc-H_2O, 4:1:5; Stahl, 1969), (3) BuOH-H_2O, 1:1 (Harborne, 1988). After drying all chromatograms were sprayed with $SbCl_3$, heated for 10 min at 100°C and inspected in UV light.

Results

Field observations

The results obtained of all individuals for each of the four tree species were combined. Hawthorns were observed to be mostly eaten by blackbirds (*Turdus merula*, Turdidae) and redwings (*Turdus iliacus*, Turdidae); yew fruits by blackbirds and starlings (*Sturnus vulgaris*, Sturnidae) and holly fruits by blackbirds. In the case of fruits being eaten by two species of birds, the tested variables (duration of visit and meal size) were compared between these species. Table 2 presents the data for hawthorn (eaten by blackbirds and redwings) and yew (eaten by blackbirds and starlings). In all cases the differences were found to be insignificant: when different species of birds foraged on the same fruits they spent similar periods of time on the tree and ate similar numbers of fruits at each feeding visit. Therefore we considered the birds as a group and the data for the tested parameters in each case were calculated together. The results show that birds stayed on all three fruiting tree species for short periods of time (range of 1.3–5.3 min), and consumed only a few fruits in each feeding bout (Table 3).

TABLE 2. DURATION OF VISIT (min), AND MEAL SIZE (no. of fruits) OF BLACKBIRDS, REDWINGS AND STARLINGS FEEDING ON FRUITS OF HAWTHORN (*C. Monogyna*) AND/OR YEW (*T. baccata*) AND THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE BIRD SPECIES

<table>
<tr><th></th><th colspan="4">Plants and Dispersers</th></tr>
<tr><th>Variable</th><th>Hawthorn
Blackbirds</th><th>Redwings</th><th>Yew
Blackbirds</th><th>Starlings</th></tr>
<tr><td>Duration of visit*</td><td>3.1±2.6</td><td>3.1±1.6</td><td>1.8±0.2</td><td>1.6±0.8</td></tr>
<tr><td></td><td colspan="2">ns</td><td colspan="2">ns</td></tr>
<tr><td></td><td>(19)</td><td>(7)</td><td>(3)</td><td>(9)</td></tr>
<tr><td>Meal size†</td><td>6.0±4.7</td><td>4.7±3.3</td><td>5.0±2.7</td><td>4.1±2.9</td></tr>
<tr><td></td><td colspan="2">ns</td><td colspan="2">ns</td></tr>
<tr><td></td><td>(25)</td><td>(9)</td><td>(4)</td><td>(11)</td></tr>
</table>

ns, not significant at $P<0.05$

(*n*), no. of observations (all data presented are X±S.E.).

*Wilcoxon two sample test (Us values are 80.5 and 16.5 respectively, ns).

†One way analysis of variance, ANOVA (after square root transformation: $F_{(1,32)}=0.50$ and $F_{(1,13)}=0.43$ respectively).

TABLE 3. DURATION OF VISIT (min), AND MEAL SIZE (no. of fruits) OF FRUGIVORES FEEDING ON FRUITS OF HAWTHORN (*C. monogyna*), YEW (*T. baccata*) AND HOLLY (*I. aquifolium*)

Variable	Plant Hawthorn	Yew	Holly
Duration of visit	3.0±2.4	1.3±0.6	5.3±4.4
	(26)	(12)	(10)
Meal size	5.7±4.4	4.3±2.8	6.5±4.3
	(34)	(15)	(24)

(n), no. of observations (all data presented are X±S.E.).

Biochemical analysis

Cyanogenic glycosides. Table 4 presents results of the Feigl and Anger tests for the presence of cyanogenic glycosides in different fruit parts. Cyanogenic glycosides are present in pulp of haw, ivy and holly fruits, but not in their seeds. In ripe haws, pulp contains more cyanogenic glycosides in November–December than later (January), as the reaction in the first case is stronger than the second. There is a similar tendency in unripe and ripe ivy pulps; the reaction in unripe pulps is stronger than in ripe pulps. In the case of yew fruits, cyanogenic glycosides are found only in seed-coats, while both the pulp and seed are toxin-free.

TABLE 4. RESULTS OF FEIGL AND ANGER TEST FOR THE DETECTION OF CYANOGENIC GLYCOSIDES IN DIFFERENT PARTS OF FRUITS OF HAWTHORN, IVY, HOLLY AND YEW

	Plant species						
	C. monogyna			*H. helix*		*I. aquifolium*	*T. baccata*
	Ripe fruits			Fruits		Ripe fruits	
Tested tissue	Nov.	Dec.	Jan.	Unripe	Ripe	Nov.	Nov.
Seed							
Intact seed	— (2)	— (2)	— (2)	— (2)	— (2)	— (2)	
Only seed content							— (4)
Only seed coat							+ (3)
Pulp	+ (3)	+ (2)	+ weak (2)	+ (3)	+ weak (2)	+ (2)	— (6)

+, positive reaction, occurred within 2–5 hours.
—, no reaction even after 48 hours.
(n), no. of replicates.

Flavonoids and saponins. Table 5 shows that vitexin occurs in haw pulp but not in seeds. Rutin is present in pulp but not seed of both unripe and ripe ivy fruits. Similar results were found in ripe pulp and seed of holly. Table 6 shows that the saponin α-hederin is not present in seeds and pulps of unripe ivy fruits. However, in ripe fruits this saponin is probably present in both seed and pulp. Hederagenin 3-*O*-α-L-arabinopyranoside is detectable in unripe seeds in only one solvent system, but is definitely present in unripe pulp (detectable in all three solvent systems). In ripe seed, this saponin is absent whereas it is present in ripe pulp.

TABLE 5. RESULTS OF THIN LAYER CHROMATOGRAPHY TESTS FOR THE IDENTIFICATION OF FLAVONOIDS IN PULP AND SEED EXTRACTS OF HAWTHORN, IVY, HOLLY AND YEW FRUITS

		Plant species			
		C. monogyna	*H. helix*		*I. aquifolium*
		Ripe fruits	Fruits		Ripe fruits
Compound	Tested tissue	Nov.	Unripe	Ripe	Nov.
Vitexin*	Seed	—			
	Pulp	+			
Rutin†	Seed		—	—	—
	Pulp		+	+	+

*R_f (×100) 57 in BAW, 68 in 50% HOAc; dull brown to bright yellow (UV and UV+NH_3)
†R_f (×100) 46 in BAW, 29 in 5% HOAc; dull brown to bright orange (UV and UV+NH_3)

TABLE 6. PRESENCE/ABSENCE OF SAPONINS IN FRUITS OF IVY, *HEDERA HELIX*

	α-Hederin	Hederagenin 3-α-arabinoside
Unripe fruit		
Seed	—	(+)
Pulp	—	+
Ripe fruit		
Seed	(+)	—
Pulp	(+)	+

(+), detectable by chromatography in only 1 of 3 systems used (see Experimental).

+, detectable in 2 or 3 systems; —, not detectable.

Discussion

The four plant species investigated (hawthorn, ivy, holly and yew) are known to be toxic to many animals and probably contain further secondary compounds, other than those which are discussed here. Thus yew is known to contain taxines, a complex mixture of alkaloids (Frohne and Pfander, 1984; Miller, 1980). Many cases of yew poisoning have been recorded involving humans (Burke *et al.*, 1979; Feldman *et al.*, 1987) and other mammals (Knowles, 1949; Ogden, 1988). Holly is often described as one of the more important poisonous plants (Frohne and Pfander, 1984). Ingestion of the red berries produces vomiting, diarrhoea, and mild narcosis (Willems, 1988). The ivy saponins cause allergic and irritant contact dermatitis (Gafner *et al.*, 1988; Hausen *et al.*, 1987). These compounds also show molluscicidal activity (Hostettmann, 1980), and act as antifeedants in caterpillars, which cause death in high concentrations (Hubrecht, 1988). Saponins as well as flavonoids (Pietta *et al.*, 1986) were also identified in haw fruits (Rossiiskaya *et al.*, 1989).

However, information is limited on the distribution of these toxins within the fruit parts, since most analyses have been carried out on whole fruits. Such is the case for example with saponin content in hawthorn and ivy fruits (Rossiiskaya *et al.*, 1988; Hostettmann, 1980) as well as cyanogenic glycosides in holly fruits (Willems, 1988). We believe that in ecological studies, it is important to know where in the fruit the toxins occur: a compound can have an effect on a frugivore, pest, or predator only if it is present in that fruit part which is consumed. This study, which tested for the presence of allelochemicals in pulp and seed separately, shows indeed that in most cases they do not occur in both fruit parts. Thus cyanogenic glycosides are found in pulp, but not in seed of three of the four investigated species (haw, ivy and holly; Table 4). Some of the flavonoids show a similar pattern: vitexin (previously identified in extract of hawthorn plants (Pietta *et al.*, 1986)) is present in pulp but not seed. Rutin is found in pulp but not seed of unripe ivy fruits and ripe holly (Table 5). Rutin, known to cause reduction of appetite (Ohem and Holzl, 1988), was previously identified in holly only in leaves (van Gelderen, 1987), and to the best of our knowledge has not been recorded before in ivy. The same tendency of differential occurrence seem to exist also in the ivy saponin hederagenin-3-*O*-α-L-arabinopyranoside. This compound is not found, or found significantly in seeds but is common in pulp.

Our findings do not support the general assumption that secondary compounds are likely to be present in seed, to protect them from predators, but not in pulp which should be attractive to dispersal agents (Janzen, 1983; McKey, 1975). However, they confirm another hypothesis, previously suggested by Sorensen (1982), which argues that mild toxicity in the pulp can prevent consumption of too many fruits in one foraging bout, and ensures better seed dispersal, as only few seeds will be deposited in one site at one time. Field observations support this idea, as average meal size of birds feeding on different plant species was always less than 10 fruits and sometimes even much smaller (Snow and Snow, 1988; Sorensen, 1984 and Table 3 in this study). There are obviously other factors that regulate meal size, such as predation risk, volume of digestive system, or the amount of food which the bird already has in its stomach. Nevertheless, meal size for the different bird and plant species was found to be quite similar (Tables 2 and 3), and therefore it seems that small quantities of certain secondary metabolites regulate the number of fruits which are consumed at each feeding bout. This, combined with the rather short period of visit of birds on the trees (mean of 3.0, 1.3 and 5.3 minutes for haw, yew and holly respectively, Table 3), which might be explained by the great risk of predation that temperate-zone birds seem to face (Snow and Snow, 1988) ensures that seeds will be dispersed away from the parent plant, and in different sites.

The existence of mild toxicity in the pulp might also affect seed retention time in the frugivore's digestive system. This could be the case especially with the saponins which are known to cause diarrhoea (Cooper and Johnson, 1984). And indeed, seed retention times are relatively short, mostly ranging from a few minutes to less than an hour (Barnea *et al.*, 1991; Herrera, 1984; Sorensen, 1984). The shorter time the seeds stay in the digestive systems the less they are exposed to chemical activity which might harm their viability. Although there is evidence that in some cases modification of seed coat structure due to ingestion might enhance germination (Barnea *et al.*, 1990, 1991), too long retention times might cause a stronger reduction of seed coat that could reduce germination success.

Yew is a special case since cyanogenic glycosides are found only in seed coats, while both the pulp and seed are toxic free in this respect (Table 4). Beside frugivorous bird species which ingest the fleshy aril and defecate the seed intact, yew also has an important seed predator, the greenfinch (*Carduelis chloris*). Greenfinches discard the pulp, strip off the seed coat and eat only the seed contents (Snow and Snow, 1988 and personal observations). Therefore it was suggested by these authors that toxic compounds might be concentrated in the seed coat and protect the seeds from predation: when the whole fruit is consumed by frugivorous birds, only the pulp, which is free from cyanogenic glycosides, is digested and the seeds are defecated intact. Therefore, in this case, the toxic compunds have no effect on the birds, and seed dispersal is achieved. However, if seed predators attempt to eat the seeds they might be exposed to poisoning. And indeed, yew fruits are avoided by seed predators like the wood pigeon (*Columba palumbus*) and some of the larger crows (Snow and Snow, 1988). These birds swallow fruits whole and normally grind up the seed coat with the seed in their stomach. Only predators like the greenfinches, that are capable of separating the toxic seed coat from the endosperm can forage on yew seeds. Our results strongly confirm this idea, and are the first to provide evidence of the influence of cyanogenic glycosides on the foraging behaviour of birds.

The results of this study also indicate that there may well be changes in concentrations of toxins during fruit ripening. Both in ripe haws (when comparing pulps from November and December with those which were collected in January), as well as in unripe and ripe ivy fruits, pulp seems to contain more cyanogenic glycosides earlier in the season than later (Table 4). This reduction of concentration might ensure and promote fruit consumption when seeds are mature and ready to be dispersed.

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