

A GLYCOALKALOID IN RIPE FRUIT DETERS CONSUMPTION BY CEDAR WAXWINGS

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ABSTRACT.—Many ripe fruits contain toxic chemicals that presumably protect them against attacks by frugivores that do not disperse seeds. These secondary metabolites may be generally toxic, representing an evolutionary compromise between defense from pathogens and attraction of seed dispersers (the General Toxicity hypothesis). Alternatively, they may be nontoxic to seed dispersers and simultaneously toxic to vertebrate frugivores that do not disperse seeds (the Directed Toxicity hypothesis). To tease apart these hypotheses, we tested whether consumption of artificial fruit agar by captive Cedar Waxwings (*Bombycilla cedrorum*) was reduced by the presence of α -solanargine, a glycoalkaloid common in solanaceous fruits. We recorded consumption of three artificial fruit types differing in α -solanargine concentration and a control fruit that lacked α -solanargine. Waxwings were strongly and equally deterred by all concentrations of α -solanargine. These concentrations, 0.1, 0.2, and 0.3% wet mass, are commonly found in some *Solanum* fruits. In a second trial, we addressed another hypothesis, the Nutrient-Toxin Titration hypothesis, which predicts that the deterrent effects of α -solanargine can be overridden by highly nutritious fruit pulp. We offered waxwings three types of artificial fruits that varied in nutrient concentration but not in α -solanargine concentration. Nutrient content had no effect on consumption when α -solanargine was present. In summary, our results are inconsistent with both the Directed Toxicity and the Nutrient-Toxin Titration hypotheses. Received 24 June 1996, accepted 27 August 1997.

BIRDS ARE PRIMARY CONSUMERS of fruits and dispersers of seeds. At one interface of this mutualistic interaction is the match between the nutritional requirements of birds and the chemical content of fruits (Martínez del Río and Restrepo 1993). Nutrients of particular interest to researchers have been lipids, proteins, and carbohydrates (Martínez del Río et al. 1989, Worthington 1989, Karasov and Levey 1990, Levey and Grajal 1991, Place and Stiles 1991, Witmer 1994, Afik and Karasov 1995). Largely overlooked are secondary metabolites, which promise to yield new insights for studies of fruit-frugivore interactions, as they have for studies of plant-herbivore interactions (Cipollini and Levey 1997b). For example, some secondary metabolites in ripe fruit pulp are paradoxical because they appear to deter fruit consumption—and hence seed dispersal—by seed-dispersing birds (Herrera 1982, Cipollini and Levey 1997c). How can their presence be explained in the context of mutualism? Here, we use Cedar Waxwings (*Bombycilla cedrorum*) to test two

sets of hypotheses, formally proposed by Cipollini and Levey (1997c), that address the evolutionary significance of fruit secondary metabolites.

The Directed Toxicity hypothesis states that secondary metabolites in ripe fruit are toxic to vertebrate frugivores that do not disperse seeds but are not toxic to those that disperse seeds (Janzen 1975). This hypothesis gains credence from observations that seed-dispersing birds consume a wide range of “toxic” fruit (Martin et al. 1951, Kear 1968, Heiser 1969, Herrera 1982, Jordano 1987) and appear to be broadly tolerant of naturally occurring toxins (Herrera 1985). Alternatively, the General Toxicity hypothesis posits that secondary metabolites are repellent to all frugivores, regardless of how they treat seeds (Cipollini and Levey 1997c). This hypothesis is bolstered by studies concluding that variation in behavior and morphology among frugivores cannot account for variation in fruit traits (Herrera 1992, Jordano 1995, Tamboia et al. 1996).

The Nutrient-Toxin Titration and Removal Rate hypotheses focus on interspecific variation among plants and the possible interactions of nutrient content, secondary chemistry, mi-

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crobial attack, and fruit removal by seed dispersers (Cipollini and Stiles 1992a, b, 1993, Cipollini and Levey 1997a, b, c). At issue is whether fruits that are high in nutrient content require more chemical protection because they are especially attractive to microbes (which rot fruits and are detrimental to plant fitness), or require less protection because they are removed especially quickly by frugivores.

The Nutrient-Toxin Titration hypothesis posits a positive correlation between nutritional quality and concentration of secondary metabolites. Fruits with high levels of nutrients are predicted to be highly protected. Their highly rewarding pulp both requires protection from microbes and allows it, because seed-dispersing frugivores are well compensated for tolerating secondary metabolites when they consume such fruits. This hypothesis fits with the recent demonstration that the detoxification of plant secondary metabolites by birds may have a high energetic cost (e.g. Guglielmo et al. 1996). Thus, the high energy content of fruit pulp may help compensate for the high costs of detoxifying secondary metabolites. Alternatively, the Removal Rate hypothesis predicts that fruits with high levels of nutrients will be the least protected by secondary metabolites because rapid removal of these highly preferred fruits will preempt microbial colonization and growth. One can tease apart these hypotheses by testing a central assumption of the Nutrient-Toxin Titration hypothesis: i.e. fruit choice is influenced by an interaction of nutrient content and secondary metabolite content. More specifically, secondary metabolites should deter the consumption of fruits that are low in nutrients but not of fruits that are high in nutrients.

We report two sets of experiments. In the first, which tested the Directed Toxicity hypothesis versus the General Toxicity hypothesis, we measured consumption by Cedar Waxwings of three artificial fruit-pulp media that contained different amounts of glycoalkaloid versus consumption of a control artificial fruit medium that lacked glycoalkaloid. Because Cedar Waxwings consume a wide variety of fruits and disperse seeds in those fruits (Martin et al. 1951, Witmer 1996a), the Directed Toxicity hypothesis predicts that the presence of glycoalkaloids in fruits should not deter waxwings from feeding on those fruits. The General Tox-

icity hypothesis predicts the opposite. The second experiment tested the assumption of the Nutrient-Toxin Titration hypothesis that consumption will be influenced by an interaction between fruit nutrient content and secondary metabolite concentration. We held glycoalkaloid content constant and varied nutrient content of artificial fruits to determine whether glycoalkaloid deterrence would be reduced if nutritional rewards were high.

METHODS

Study system.—A wide diversity of glycoalkaloids is found in solanaceous plants (Ripperger and Schrieber 1981). Concentrations are highest in unripe fruit (Zitnak 1979), but some species retain levels in ripe fruit sufficient to kill a 1- to 2-kg vertebrate after consumption of less than 10 fruits (see Cipollini and Levey 1997c). Although such extreme toxicity is uncommon, many species are considered toxic (Heiser 1969).

Alpha-solanine and α -chaconine are the best known of the glycoalkaloids in *Solanum*. However, α -solasonine and α -solamargine are structurally similar and are found in more species (Ripperger and Schrieber 1981). They are the dominant glycoalkaloids in *Solanum americanum* and *S. carolinense*, both common plants in the eastern United States. Their combined concentrations in ripe fruits range from 0.008% wet mass in *S. americanum* to 0.26% in *S. carolinense* (Cipollini and Levey 1997c). Alpha-solamargine and α -solasonine are similar in structure and equally repellent to at least four species of frugivorous vertebrates (Cipollini and Levey 1997c). Although some studies have suggested that these two compounds have interactive effects on antifungal activity and membrane disruption (Roddick 1987, Roddick et al. 1990), our studies have shown that they are not synergistic (Cipollini and Levey 1997a, c). Because of the similar and non-interactive effects of α -solamargine and α -solasonine on frugivores, and because we could more easily extract and purify α -solamargine, we used it to model total concentration of α -solamargine and α -solasonine.

We used concentrations of α -solamargine ranging from 0.1 to 0.3% wet mass. These concentrations, which span a three-fold range along the upper limits of naturally occurring concentrations, were selected for two reasons. First, we wanted to match concentrations used by Cipollini and Levey (1997c) so that the results of the two studies would be directly comparable. Second, we were looking for a high level of tolerance toward glycoalkaloids, as predicted by the Directed Toxicity hypothesis, and as found for Cedar Waxwings toward capsaicin, another apparently noxious compound occurring in some ripe solanaceous fruits (Mason et al. 1991, Norman et al. 1992).

TABLE 1. Composition (in grams) of artificial fruit used in experiments. Ingredients were added to a 2% agar solution at 70°C and adjusted to pH 5.7 with citric acid.

Fruit type	Sugar ^a	Carbo ^b	Lipid ^c	Protein ^d	Fiber ^e	α -Solamargine	Water
Alkaloid concentration experiment							
Control	5.39	0.70	0.035	0.56	0.00	0.00	93
0.1%	5.39	0.70	0.035	0.56	0.00	0.10	93
0.2%	5.39	0.70	0.035	0.56	0.00	0.20	93
0.3%	5.39	0.70	0.035	0.56	0.00	0.30	93
Nutrient-toxic titration experiment							
Control	4.00	1.00	0.58	0.50	4.00	0.00	90
Low nutrient	1.20	0.30	0.15	0.15	8.00	0.20	90
Avg. nutrient	4.00	1.00	0.58	0.50	4.00	0.20	90
High nutrient	7.20	3.20	0.85	0.82	0.00	0.20	90

^a Glucose : fructose : sucrose (1:1:1 ratio).

^b Complex carbohydrates; starch : pectin (3:1).

^c Peanut oil : corn oil (1:1).

^d Soy protein isolate.

^e Milled pure cellulose fiber.

Cedar Waxwings feed on a wide diversity of fruits, especially small, sugary, low-lipid fruits (Witmer 1996b), similar to many species of *Solanum*. We have no records of Cedar Waxwings eating *S. americanum* or *S. carolinense* fruits in the wild. However, captive waxwings readily consume *S. americanum* (D. Levey unpubl. data), and fruits of other *Solanum* species are taken by many species of birds, including a relative of waxwings, the Phainopepla (*Phainopepla nitens*; Martin et al. 1951).

Waxwings were captured near Gainesville, Florida, in May 1995 and held individually in cages (ca. 0.5 × 0.5 × 0.5 m) behind one-way mirrors. They were maintained on a banana-based diet (Denslow et al. 1987) provided *ad libitum* and kept on a 12h : 12h light : dark cycle at a constant temperature of 23°C. A slit at the bottom of the mirrors allowed us to place food dishes into cages with little disturbance to the birds. All birds maintained a constant body mass and appeared to be in good health. We tested preferences using artificial fruit-pulp media that contained concentrations of nutrients typical of solanaceous fruits and matching those used in a previous study (Cipollini and Levey 1997c). We did not couple our use of artificial fruits with trials using real *Solanum* fruits because consumption patterns of natural fruits by other animals relate to the secondary metabolite content of those fruits, and consumption patterns of real and mimetic fruits match well (Cipollini and Levey 1997b, c).

Extraction of α -solamargine.—We extracted α -solamargine from dried pulp of *Solanum khasianum*, which has a high content of solamargine. Pulp was soaked in hot ethanol that was then roto-evaporated to dryness. The residue was dissolved in 1% acetic acid, and 30% ammonia was added to shift the pH to approximately 11.5. The solution was heated to 70°C for 15 min and then cooled overnight at 4°C.

The crude glycoalkaloid precipitate was extracted with chloroform, which was roto-evaporated to dryness and taken up in water-saturated 1-butanol. The solution was fractionated on a neutral alumina column, and purity of α -solamargine fractions was verified using thin-layer chromatography. Additional details on this methodology are provided in Cipollini and Levey (1997c).

Alkaloid concentration experiment.—This experiment tested whether consumption of artificial fruits containing α -solamargine was lower than that of fruits not containing α -solamargine. Also, we tested for an effect of α -solamargine concentration on consumption.

Artificial fruits were made from four types of agar (Table 1). Control agar lacked glycoalkaloid; 0.1% agar had 0.1 g α -solamargine added to 100 g warm agar solution, 0.2% agar had 0.2 g α -solamargine added, and 0.3% agar had 0.3 g added. Because taste perception of glycoalkaloids is pH-dependent, at least in humans (Zitnak 1979), we adjusted the pH of all agars to 5.7 (i.e. the average pH of *S. americanum* and *S. carolinense* fruits; Cipollini and Levey 1997c). Once ingredients had been added to the agar solution, we poured the solution into shallow pans. After cooling, we cut it into small cubes (ca. 3 × 3 × 3 mm; hereafter, "fruits"), which were then presented to the birds. Fruits were stored at 20°C and used within four days.

Each of eight birds was presented with a weighed petri dish containing one of the four fruit types. After 15 min, we removed the dishes, weighed them, waited 15 min, re-weighed them, and placed them in the cage of another bird. Fifteen-minute periods of feeding and fasting were chosen because waxwings typically eat every 5 to 15 min throughout the day (Holthuijzen and Adkisson 1984, Witmer 1994, D. Levey unpubl. data). Thus, 15 min was sufficient

time for a hungry bird to feed and for a satiated bird to become hungry.

The order of presentation was randomized, with the restriction that a bird was not given the same dish twice. This cycle was repeated eight times until each bird had been exposed to each dish once and each of the four fruit types twice. We repeated this entire process twice a day for three days, over a five-day period. We define a "trial" as a set of four presentations, each containing two 15-min periods with each fruit type (i.e. for each bird, six trials \times four presentations per trial = 24 presentations). We did not reuse fruits from one trial to the next, and within each trial, far more fruits were offered on each dish than could be consumed. Fruits did not change appearance over the course of a trial (4 h), and fruits that were picked up and dropped back into the dish did not appear to be different from others in the dish; we did not attempt to remove them. Fruits dropped outside the dish were put back into the dish before weighing. We used change in mass of the fruits during the 15-min periods between presentations to correct for water loss during the previous 15 min.

Nutrient-toxin titration experiment.—Four types of fruits were made (Table 1). Control fruits contained no glycoalkaloids and had nutrient levels typical of ripe *Solanum* fruits (Cipollini and Levey 1997c). The other three fruits had 0.2 g α -solamargine added per 100 g agar, the average concentration used in the alkaloid concentration experiment. "Average" nutrient fruits were identical to control fruits, except for the presence of α -solamargine. "Low" nutrient fruits had a 70% decrease in overall nutrient content, and "high" nutrient fruits had a 70% increase. Specific nutrients were increased or decreased within the limits of natural variation in fleshy fruits (Stiles 1980, Johnson et al. 1985, White 1989, Witmer 1994). Cellulose fiber was added to keep dry matter approximately constant across diets as nutrient levels were changed.

Presentation of fruits and data collection were as described for the alkaloid concentration experiment with one exception: at the end of each 15-min presentation, we collected and weighed fruits that were picked up and dropped outside the dish by the birds. We noticed but failed to record the number of dropped fruits in the alkaloid concentration experiment and thought it prudent to record them in this experiment. We hoped that such information would provide insight about whether waxwings can sense glycoalkaloid or nutrient content of fruits before ingestion.

Experimental design considerations and statistical analysis.—Our short-term, single-choice feeding trials are a compromise between long-term, single-choice trials and multiple-choice trials. Long-term, single-choice trials may yield results that are difficult to interpret because animals may eventually eat a

less-preferred diet as they become hungry. Also, when nutrient levels vary among food types, high consumption rates may be a result of compensation for lower-quality food rather than a result of preference for that food. Furthermore, long-term, single-choice trials may poorly mimic biologically relevant conditions, because birds rarely are forced to feed upon one food type for long periods of time. Indeed, fruit-eating birds often have several types of fruits in their digestive tracts simultaneously (Loiselle 1990, White and Stiles 1990).

Multiple-choice tests, on the other hand, often exaggerate preferences and may suffer from lack of independence (Peterson and Renaud 1989, Roa 1992). In addition, lack of biological relevance may be a problem because birds rarely encounter many equally accessible food items simultaneously. This shortcoming is highly relevant to fruit-eating birds because their preference patterns are tightly dependent on how fruits are presented in time and space (Moermond and Denslow 1983, Levey et al. 1984).

Repeated, short-term presentations and randomization reduced problems of nonindependence and gave the birds frequent opportunity to consume preferred fruits, minimizing starvation effects. Also, although our birds were not starving, they were sufficiently hungry to always show interest in consuming fruits when dishes were first placed in the cages. Thus, if we found a deterrent effect of α -solamargine, it was strong enough to repel hungry birds. In addition, short-term, single-choice experiments, where animals repeatedly encounter varying types of foods and quickly decide whether to eat, closely mimic how waxwings feed in the wild.

In both experiments, we used $\log(1 + g \text{ agar consumed in each 15-min presentation})$ as the dependent variable. Log transformation was necessary because means and variances were positively correlated (Sokal and Rohlf 1981). Preferences were analyzed by multivariate repeated-measures ANOVA (SuperANOVA; Abacus Concepts 1989) in which fruit type and trial were independent variables ("within" factors). When fruit type was a significant factor, we proceeded with planned comparisons using sequential Bonferroni tests to control for group-wide type I error (Rice 1989).

Because birds were more likely to drop fruits the more they ate of a given fruit type, we analyzed number of dropped fruits by ANCOVA, with amount of fruit consumed as the covariate. We used average consumption and average number of dropped fruits for each bird for each agar type ($n = 32$).

RESULTS

Alkaloid concentration experiment.—Fruit type significantly influenced consumption ($F = 15.2$, $df = 3$ and 21 , $P < 0.0001$). No interaction terms

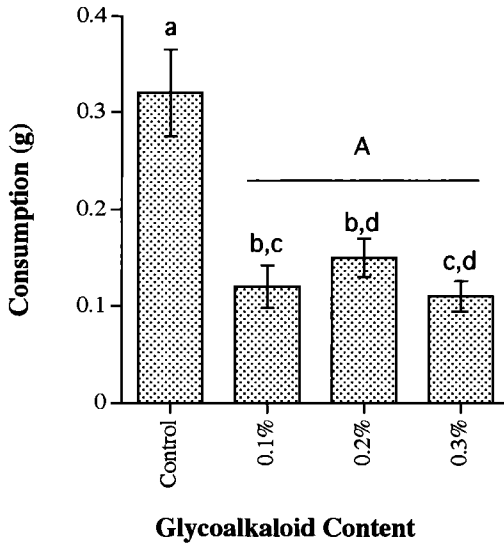


FIG. 1. Consumption (g wet mass) by Cedar Waxwings of control fruits and three fruits containing different amounts of α -solamargine. Values are $\bar{x} \pm$ SE. Letter pairs indicate planned comparisons; non-significant differences have two lowercase letters, and significant differences have one uppercase and one lowercase letter. The horizontal line indicates a comparison between the average consumption of the set of α -solamargine-containing fruits and the control fruit.

were significant or close to significant. Birds did not distinguish among different concentrations of α -solamargine; 0.1, 0.2, and 0.3% fruits were eaten with equal frequency (planned contrasts, $P_s > 0.15$). Furthermore, there was no trend of decreasing consumption with increasing α -solamargine content (Fig. 1). The significant effect of fruit type resulted from higher consumption of control fruits relative to fruits containing α -solamargine (i.e. average of 0.1%, 0.2%, and 0.3% vs. control; planned comparison, $P < 0.0001$). Control fruits were eaten at approximately twice the rate of fruits containing α -solamargine. Also, control fruits were consumed at a rate typical of both wild and captive waxwings feeding on similar diets (Holthuijzen and Adkisson 1984, Martínez del Rio et al. 1989, Levey and Grajal 1991, Witmer 1994).

Nutrient-toxin titration experiment.—Birds distinguished among fruit types ($F = 8.3$, $df = 3$ and 24 , $P = 0.008$). Trial and fruit type \times trial terms also were significant ($F = 2.7$, $df = 5$ and 35 , $P = 0.025$ and $F = 2.5$, $df = 15$ and 105 , P

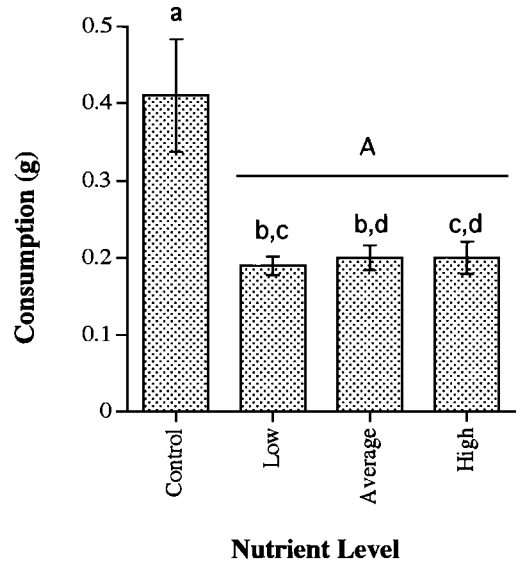


FIG. 2. Consumption (g wet mass) by Cedar Waxwings of fruits containing equal amounts of α -solamargine and three levels of nutrients versus control fruit (which lacked α -solamargine). Notation as in Figure 1.

$= 0.004$, respectively). The trial effect resulted from low consumption during the second trials on the second and third days of the experiment; consumption was approximately 20% lower in these trials compared with all others. The fruit type \times trial term was significant largely because consumption of low- and high-nutrient fruits fell substantially on the second trial of the second day and because consumption of control fruits fell over the course of the last three trials. All other rates of consumption stayed fairly constant over the course of the trials. Thus, the following pattern of preference did not change as a result of the interaction between fruit type and trial. Nutrient content did not influence preference; all fruits containing α -solamargine and varying levels of nutrients were consumed in equal amounts (planned contrasts, $P_s > 0.82$), and all were consumed at approximately half the rate of control fruits (planned contrast, $P < 0.0001$; Fig. 2).

Controlling for amount consumed, fruit type was marginally significant in explaining variation in the number of dropped fruits ($F = 2.51$, $df = 3$ and 24 , $P = 0.08$; Fig. 3). Low-nutrient fruits were dropped 61% more frequently than average-nutrient fruits, which were dropped 189% more frequently than high-nutrient

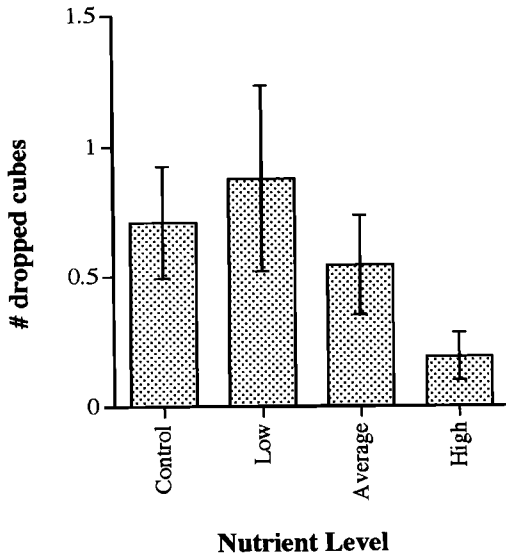


FIG. 3. Average number of fruits dropped by Cedar Waxwings during 15-min presentations during the nutrient-toxin titration experiment. Values are $\bar{x} \pm SE$.

fruits. Control fruits were dropped at an intermediate rate.

DISCUSSION

We found no support for the Directed Toxicity hypothesis; Cedar Waxwings avoided fruits that had concentrations of α -solamargine typically found in fruits of many species of *Solanum*. Our results confirm those of Cipollini and Levey (1997c), who found that two other seed-dispersing frugivores (American Robin [*Turdus migratorius*] and Virginia opossum [*Didelphis marsupialis*]) strongly avoided artificial fruits with naturally occurring concentrations of glycoalkaloids.

Despite these results, we think that it would be premature to reject the Directed Toxicity hypothesis for two reasons. First, it may apply to some secondary metabolites, but not others. Glycoalkaloids such as α -solamargine appear to be generally toxic. Yet, tropane alkaloids in ripe *Atropa belladonna* (Solanaceae) fruits are lethal to mammals but are eaten with apparent impunity by seed-dispersing birds (Heiser 1969). Capsaicin, a phenolic amide found in wild peppers (*Capsicum annuum*, Solanaceae), provides a similar although less-dramatic example (Mason et al. 1991, Norman et al. 1992).

The key issue—which compounds are deterrent to which frugivores—currently is difficult to resolve because so few compounds have been tested in controlled feeding trials. Furthermore, even if differences in deterrence among organisms are documented, it must be shown that the compound is less active toward effective seed dispersers than toward less-effective seed dispersers, a distinction that we did not test.

Second, directed toxicity may apply on a different level than we discussed; e.g. not to birds versus mammals or to seed-dispersing vertebrates versus non-seed-dispersing vertebrates, but to microbes versus vertebrates. Certainly, the potential exists for plants to evolve compounds directed solely against frugivorous microbes because such microbes are universally detrimental to plant fitness. In contrast, the distinction between seed-dispersing and non-seed-dispersing vertebrates is less clear-cut (e.g. Levey 1986). In support of directed toxicity toward microbes, α -solamargine strongly inhibits growth of fruit-rot fungi on media that mimic fruit pulp (Cipollini and Levey 1997a). Similar inhibitory effects of fruit secondary metabolites on microbes appear to be common (McKee 1959, Herrera 1982, Roddick 1987, Cipollini and Stiles 1992b, 1993). In at least some cases, these secondary metabolites are not strongly deterrent to seed-dispersing frugivores (Norman et al. 1992, Cipollini and Stiles 1993).

Nutrient-toxin tradeoff and removal rate hypotheses.—The Nutrient-Toxin Titration hypothesis predicts a preference for high-nutrient fruits over low-nutrient fruits, when secondary metabolite concentration is held constant. We did not find this pattern; waxwings strongly reduced their consumption of all fruit types containing α -solamargine, regardless of nutritional quality. Two other species of seed-dispersing frugivores show similarly strong responses to glycoalkaloids (Cipollini and Levey 1997c). Apparently, the energetic costs of detoxification (Guglielmo et al. 1996) are not offset by diets of higher nutritional quality.

Our rejection of the Nutrient-Toxin Titration hypothesis lends indirect support to its alternative, the Removal Rate hypothesis (Cipollini and Stiles 1992b, 1993). The latter predicts that species with fruits that are removed quickly will display low levels of secondary metabo-

lites. This prediction is upheld in fruits of at least three families, Solanaceae, Ericaceae, and Aquifoliaceae (Gargiullo and Stiles 1991, Cipollini and Stiles 1993, Cipollini and Levey 1997c).

Dropped fruits and detection of pulp constituents.—Although significant at $\alpha = 0.08$, the difference in the number of dropped fruits among the three fruit types containing α -solanamine suggests that waxwings could discriminate among these fruits, even though their consumption rates did not vary among fruit types. One interpretation is that the birds repeatedly sampled and rejected low-nutrient fruits in search of high-nutrient fruits. This behavior is consistent with detection of nutrients by taste in some fruit-eating birds (Levey 1987, Martínez del Rio et al. 1989). Dropping behavior probably was not learned (i.e. was not associated with post-ingestional effects) because the short duration of trials (15 min), random order of presentation, and similar appearance of fruit types made conditioned avoidance unlikely.

Can waxwings detect α -solanamine pre-ingestion by taste and/or odor? If so, they should have dropped many more fruits containing α -solanamine than control fruits, which was not the case. Yet, we have observed both waxwings and Northern Bobwhites (*Colinus virginianus*) approach and reject glycoalkaloid-containing fruits without tasting them (D. Levey and M. Cipollini pers. obs.). We suspect that rejection of fruits containing α -solanamine did not reflect conditioned avoidance for the reasons above, and because if trigeminal stimulation were involved, learning would be unlikely (see Clark 1996).

Conclusion.—A common compound in a widespread plant genus appears to be universally repellent to seed-dispersing vertebrates, including one of the most frugivorous species in North America (Cipollini and Levey 1997c, this study). The major implication of this finding is that the evolutionary interactions between frugivores and *Solanum* appear to be much more complex than generally expected. Teasing apart the possible causes and consequences of secondary metabolites in fruits remains a promising avenue of future research (Cipollini and Levey 1997b).

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