CONTROL OF GUT RETENTION TIME BY SECONDARY METABOLITES IN RIPE SOLANUM FRUITS

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Abstract. We tested whether compounds in ripe Solanum americanum fruits affect gut retention time of S. americanum seeds in Cedar Waxwings (Bombycilla cedrorum). Glycoalkaloids were of special interest because they commonly occur in ripe Solanum fruits and are associated with diarrhea in humans. Also, we determined the influence of gut retention time and the presence/absence of two glycoalkaloids, α-solasonine and α-solamargine, on germination of S. americanum seeds. In one trial, we measured seed retention times of 10 waxwings fed three types of artificial fruits, Control (containing no secondary metabolites), Low Concentration (containing low concentrations of α-solasonine and α-solamargine, matching those in ripe S. americanum fruit), and Extract (containing an ethanol extract of ripe S. americanum fruits, with many unidentified secondary metabolites). Seeds in Low Concentration fruits were not defecated more quickly than those in Control fruits, but seeds in Extract fruits were. Thus, ripe S. americanum fruits contain a chemical or chemicals with a laxative effect. Also, seeds from Control fruits were deposited in more defecations and at lower densities in each defecation than those from Extract fruits. In a second trial, we compared retention times of seeds in control fruits and in fruits with high concentrations of glycoalkaloids, typical in ripe fruits of other Solanum species. These high concentrations had a significant constipative effect on seed passage. Percentage germination and mean germination time of defecated seeds were not influenced by mean retention time in waxwing guts. However, proportionately fewer seeds germinated from Low Concentration fruits than from Control fruits. These results suggest that plants have more control over seed processing by frugivores than generally acknowledged. Secondary metabolites in ripe fruits can increase or decrease retention time and thereby influence seed deposition patterns (e.g., number of defecations with seeds, number of seeds per defecation, and presumably, dispersal distance).

Key words: Bombycilla; frugivory; fruit; glycoalkaloids; laxative; retention time; secondary metabolites; seed dispersal; seed germination; solamargine; Solanum; solasonine.

INTRODUCTION

Despite the importance of fruiting plants to frugivores (Foster 1982, Terborgh 1986, Levey 1988) and frugivores to fruiting plants (Howe et al. 1985, Schupp 1988, Chapman and Chapman 1995) reciprocal selection pressures have not resulted in tight coevolutionary relationships between these groups. Morphological adaptations for frugivory in animals appear rare or inconsistent among taxa and/or studies (Herrera 1984, Jordano 1987, Karasov and Levey 1990, Levey and Duke 1992, Witmer 1994, Ricklefs 1996) and, likewise, frugivores have not strongly influenced many morphological and nutritional traits of fruits (Herrera 1989, 1992, Ficker and Chapman 1993, Jordano 1995, Tamboia et al. 1996). One reason for the apparent lack of evolutionary responses of plants to seed dispersers may be their inability to influence what happens to seeds after they are ingested (Wheelwright and Orians 1982, Howe 1984). In support of this hypothesis, plant–frugivore coevolution is perhaps most apparent in the one group of plants, mistletoes, that can control where their seeds are defecated (Walsberg 1975, Restrepo 1987, Reid 1991). Sticky viscin in their fruits can influence quality of dispersal by causing birds to wipe defecated seeds on branches of a diameter appropriate for germination and establishment (Reid 1989, 1991, Sargent 1995).

Recently, Murray et al. (1994) proposed another, more general, mechanism by which plants may influence quality of seed dispersal. They provided experimental evidence that secondary metabolites in fruit pulp influence retention time of seeds in frugivore guts, mediating a tradeoff between dispersal distance (retention time is positively correlated with dispersal distance) and seed viability (retention time is negatively correlated with viability). This hypothesis is appealing because it provides an evolutionary explanation for secondary metabolites in ripe fruit (Janzen 1977, Herrera 1982, Cipollini and Levey 1997) and suggests that plants have more control over seed dispersal than previously thought.

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Murray et al.’s (1994) results are difficult to interpret for two reasons. First, the method of adding secondary chemicals to artificial fruits by soaking them in aqueous fruit extracts could have resulted in oxidation and breakdown of metabolites, production of by-products by microbes, and chemical conversions (cf. Cilliers and Singleton 1990). As such, the concentrations of secondary metabolites and fruit pulp nutrients—which also affect germination and gut passage rates (Witmer 1996a)—could not be controlled or standardized. Second, Murray et al. (1994) did not directly compare germination success of seeds that had passed through the birds’ guts with and without the fruit extract. Thus, they could not distinguish between direct effects of pulp secondary metabolites on germination vs. indirect effects due to variation in retention time caused by the same or other compounds.

Here we test Murray et al.’s (1994) hypothesis on the role of secondary metabolites in ripe fruit, addressing the confounding factors described above. Murray et al. (1994) used fruits of the tropical solanaceous shrub Witheringia solanacea (which is now thought to be W. meiantha; K. Murray, personal communication). We focused our work on a related temperate species, Solanum americanum Mill., because this species contains glycoalkaloids, which we suspected could produce laxative effects. Glycoalkaloids are common in ripe Solanum fruits and when ingested in large amounts are known to cause diarrhea in mammals (Zitnak 1979). Some species, like S. americanum, exhibit low concentrations in ripe fruits, whereas others, like S. carolinense L., have levels so high that their ripe fruits are considered toxic (Zitnak 1979, Van Gelder 1990, Cipollini and Levey 1997b).

We conducted three experiments. In the first, we tested whether S. americanum seeds in artificial fruits containing a low concentration of glycoalkaloids are defecated more quickly than seeds in fruits without glycoalkaloids. In the same set of trials, we also tested for the presence of other secondary metabolites with laxative effects by measuring retention times of seeds in fruits made with an extract from ripe S. americanum fruits. At low concentrations, we found no effect of glycoalkaloid on retention time and so, in the second experiment, we conducted similar tests using an increased glycoalkaloid concentration in the artificial fruits. The third experiment tested whether gut retention time and presence of glycoalkaloids influence germination of S. americanum seeds.

**METHODS**

**Study organisms**

Cedar Waxwings are seed dispersers that feed on a large variety of fleshy fruits (Witmer 1996b). We captured waxwings in blueberry fields near Gainesville, Florida. They were housed separately in cages (dimensions 0.5 × 0.5 × 0.5 m) behind one-way mirrors and maintained on a banana-based diet (Denslow et al. 1987) and water, both provided ad libitum. Birds were held on a 14 h:10 h light:dark cycle at 23°C.

A roll of plastic sheeting suspended behind each cage provided a single layer of plastic that was pulled through slots in the back and front of the cage, then under the one-way mirror. This setup allowed us to observe birds and collect defecations with minimal disturbance to the birds. To control for diurnal rhythm of gastrointestinal motility (Duke 1982), all trials were conducted ~3 h after lights came on in the morning.

Solanum americanum, a native species in the southeastern United States, is common in disturbed areas. In our area it is an annual or short-lived perennial, producing fruits from July to October. Ripe fruit are often abundant (>100/plant) on larger plants during this time. Fruits are dark purple and ~0.5 cm in diameter when ripe. They contain 71 ± 2 seeds (n = 26; Tamboia et al. 1996), each ~1.5 mm in diameter.

We have no records of waxwings consuming S. americanum fruits. Nonetheless, we feel justified using this system because (1) captive waxwings readily consumed S. americanum fruits and, in fact, preferred them to their maintenance diet (D. Levey, unpublished data); (2) wild waxwings consume fruits that are essentially identical to S. americanum in size, color, and nutrient content (Martin et al. 1951, Witmer 1996b, Cipollini and Levey 1997a); (3) a close relative of waxwings, Phainopepla nitens, has been reported to consume Solanum fruits (Martin et al. 1951); (4) lack of feeding records does not mean waxwings avoid S. americanum; and (5) waxwings and S. americanum were the closest phylogenetic matches we had available to the species studied by Murray et al. (1994).

Glycoalkaloids are virtually restricted to the Solanaceae and are composed of C27 steroid aglycones to which various branched carbohydrate moieties are attached (Ripperger and Schriever 1981). The best known compounds are the potato glycoalkaloids, α-solane and α-chaconine (Gregory 1984, Van Gelder 1990). The structurally related compounds α-solasonine and α-solamargine occur more broadly among Solanum species (Ripperger and Schriever 1981) and are the focus of our study. They occur in ripe S. americanum fruit in relatively low concentrations (<1 mg/g), but can be found in much higher concentrations in ripe fruits of other Solanum species (e.g., S. carolinense; 10–30 mg/g; Cipollini and Levey 1997a).

**Extraction of glycoalkaloids and formulation of agar fruits**

**Extraction and purification of α-solasonine and α-solamargine.** We isolated α-solasonine and α-solamargine by column chromatography of crude glycoalkaloid extracts from oven-dried samples of S. aculeatissimum (provided by M. Weissenberg, Volcani Center, Bet Dagan, Israel). S. aculeatissimum was used because of the ease with which pure α-solamargine and α-so-
lasonine can be extracted and purified from this species. We extracted oven-dried fruit pulp with hot ethanol, rotoevaporated the filtrate to dryness, and redissolved it in 1% acetic acid. We then shifted the pH to 11.5 using 30% ammonia and held the extract at 70°C for 20 min. After the extract had cooled overnight at room temperature, we extracted the crude glycoalkaloid precipitate using chloroform. After removal of the chloroform by rotoevaporation, the crude glycoalkaloid was taken up with water-saturated n-butanol and chromatographed on a neutral alumina column using water-saturated n-butanol as the solvent. Pure fractions were authenticated by co-chromatographing them on thin layer silica gel with authentic $\alpha$-solanine and $\alpha$-solamargine (authenticated using thin-layer chromatography, high-performance liquid chromatography, and fast atom bombardment mass spectroscopy by M. Weissenberg, Volcani Center, Bet Dagan, Israel). They were then collected, rotoevaporated to dryness, and dissolved in a minimal amount of 80% ethanol for addition to agar fruits.

Preparation of crude $S.\ americanum$ extracts.—We obtained a crude extract of $S.\ americanum$ fruits by heating 20 g of freeze-dried seedless pulp in 200 mL of 70°C ethanol (95%) for 30 min. The extract was filtered through a glass fiber filter, rotoevaporated to near dryness, and kept frozen until time of use. This broad-spectrum extraction procedure resulted in the extraction of a wide range of primary (nutritional) and secondary metabolites, including $\alpha$-solanine and $\alpha$-solamargine (Leven et al. 1979, Dey and Harborne 1989).

Preparation of agar fruits.—Artificial agar fruits mimicked pulp chemistry of ripe $S.\ americanum$ fruits. They were made by dissolving the following in 92.7 mL of a hot 2% agar solution: sugars (5.37%: glucose: fructose: sucrose; 2:2:1), lipids (0.7%: corn oil: peanut oil; 1:1), complex carbohydrates (0.7%: starch: pectin; 3:1), and protein (0.6%: soy protein extract). Concentrations of these constituents were determined by prior chemical analysis of ripe $S.\ americanum$ fruits (Cirollini and Levey 1997a). We used citric acid to adjust the pH of agar fruits to 5.7, which is the average pH of ripe $S.\ americanum$ fruits (M. Cirollini, unpublished data).

We prepared four types of agar fruits: “Control” fruits had only the components listed above; they lacked glycoalkaloids. “Low Concentration” fruits also contained glycoalkaloid at 0.008% ($\alpha$-solamargine: $\alpha$-solanine; 5:3), which mimicked the naturally low alkaid concentration of ripe $S.\ americanum$ fruits. “High Concentration” fruits were identical to Low Concentration fruits but contained glycoalkaloids at 0.600% ($\alpha$-solamargine: $\alpha$-solanine; 5:3), a concentration approaching that of “toxic” fruits such as $S.\ carolinense$ (Cirollini and Levey 1997a). “Extract” fruits were prepared using 7 mL of $S.\ americanum$ crude extract in 93 mL of 2% agar. Extract fruits did not require any added nutrients because the 7 mL of crude extract included nutrients and secondary metabolites at levels equal to those of real $S.\ americanum$ fruit. Agar solutions were cooled to 70°C prior to the addition of glycoalkaloid or extract. After secondary metabolites were added, the agar solutions were further cooled to 50°C for the addition of $S.\ americanum$ seeds. Approximately 125 seeds, freshly extracted from fruits of at least 10 $S.\ americanum$ plants (to control for variation in seed quality among fruits and plants; see Germination Experiment) were placed into each well of a plastic tissue culture plate (Falcon #3077; 0.5-mL wells; each well produced one artificial fruit) and covered with agar. Fruits were then allowed to gel at room temperature. We made new fruits each morning and used them within 1–2 h.

Low glycoalkaloid concentration and $Solanum$ extract experiment

We used this experiment to test whether mean retention time of seeds was affected by (1) $\alpha$-solanine and $\alpha$-solamargine at the low concentrations occurring in ripe $S.\ americanum$ fruits and (2) unknown secondary metabolites in ripe $S.\ americanum$ fruits.

On the day of a trial, birds were allowed to feed on seedless fruits of one of the three agar types (Low Concentration, Extract, or Control) for ~2 h. This assured that their guts would be full of the appropriate fruit type at the start of the trial, thereby eliminating possible effects of the maintenance diet on retention time. Also, we found that birds eating the test fruits at the start of a trial were more likely to continue eating them after the trial started. Fruit type on the first two days of trials was determined randomly; all birds were given the same fruit type on each of those days. Trials were often unsuccessful and had to be repeated. After the second day of trials, fruit type was determined randomly for each bird, unless the bird needed only a single fruit type to complete the experiment.

At time zero (3 h after lights came on), birds were force-fed two fruits with seeds. (Meal size was typically two fruits when birds were feeding normally.) They were then allowed to resume feeding on seedless fruits, ad libitum. We collected defecations every 5 min for 90 min, which a pilot study ($n=8$ birds) confirmed was usually long enough for all seeds to pass.

We force-fed because some birds refused to eat seedless fruits until their guts were nearly empty, whereas others ate fruits readily. This variation in feeding behavior concerned us because waxwings normally maintain a full gut (Levey and Grajal 1991, Witmer 1994), and their consumption rate highly influences retention time (D. Levey, unpublished data). Thus, variation in feeding behavior could override any effects of secondary metabolites on retention time. To validate that force-feeding did not itself affect retention time, we compared retention times of Control fruits between eight force-fed birds and the same birds after they had
eaten an identical fruit voluntarily and quickly (within 20 min after the start of a trial). We found no difference in mean retention time between force-fed and non-force-fed trials (32.9 ± 9.6 min vs. 30.1 ± 6.7 min, respectively; paired t = 0.997, df = 7, P = 0.352).

We also observed large temporal variation in consumption rate within birds, even within single trials. We thought it prudent to control for this variation, but could not measure consumption rate within trials without disturbing the birds. Instead, we used analysis of defecation rate to decide when birds were consuming seedless fruits at a normal and consistent rate after force-feeding. This approach is justified by a strong positive correlation between consumption rate and defecation rate ($r^2 = 0.81$, $P = 0.006$, $n = 10$ trials; pilot study). Based on pilot trials, we knew that continuously feeding waxwings averaged at least one defecation every 5 min ($\bar{X} = 0.6\pm 0.6$ defecations/5 min, $n = 10$ trials) and rarely had two or more consecutive 5-min periods without defecations ($\bar{X} = 3.7\pm 0.7\%$ of consecutive 5-min intervals without defecations, $n = 16$ trials). Also, continuously feeding birds defecated most seeds between 15 and 35 min, so this time period was most critical for normal defecation behavior. We used these data to define a priori criteria for determining when a bird was not eating and defecating at a normal rate, in which case the trial was rejected. Trials were repeated for each bird until a trial (1) averaged 1 defecation/5-min interval between 15 and 60 min, (2) did not have any consecutive 5-min periods without defecations between 15 and 35 min, and (3) did not have more than two consecutive periods without defecations between 15 and 60 min.

Seeds were carefully removed from defecations and counted. In calculations, the midpoint of each 5-min interval was used to represent the passage time for all seeds defecated within the interval. Mean retention time is the integrated average time between ingestion and excretion of seeds (Warner 1981, Robbins 1993):

$$\text{Mean retention time} = \sum f_i t_i,$$

where $f_i$ is the fraction of total ingested seeds excreted at time $t_i$.

**High glycoalkaloid concentration experiment**

We used this experiment to test whether high concentrations of (α-solamnine and α-solamargine, typical in some ripe Solanum fruits, affected mean retention time of seeds. Because birds would not voluntarily consume any High Concentration fruits, we force-fed them three times: 15 min before the trial, at time zero, and 15 min later. Two fruits were fed each time because this feeding rate approximately matched the waxwings’ natural consumption rate of 6 fruits/h (2.15 ± 0.59 g/h, $n = 10$ birds; one fruit = 0.36 g). Fruits fed at time zero contained seeds; all others did not. We did not force-feed fruits after 15 min for fear it would cause birds to defecate seeds in their colon prematurely. To eliminate confounding effects of other food types in the gut, birds were not given any other food during these trials, although they had water ad libitum. Control fruits were force-fed in the same manner.

**Germination experiment**

We used this experiment to test whether gut retention time affected percentage germination and mean germination time of seeds collected in the Low Concentration and Control trials. Each defecation was rinsed in a 0.5-mm sieve to separate seeds from agar. Care was taken to minimize physical scarification. Once counted, seeds were placed on top of a mixture of potting soil, topsoil, and sand (4:2:1). Seeds from each retention period were evenly distributed in separate cells (approximately $3 \times 5 \times 7$ cm) and were planted the same day they were collected from the plants and fed to the birds. Trays of seeds were placed in a greenhouse and watered daily for 30 min at ~14:00 h. Trays were rotated 180° daily to ensure an even distribution of water to all cells. Germination was monitored daily, and all seedlings were removed. We define germination as the appearance of cotyledons, although, technically, germination occurs before this time. We monitored germination for at least 62 d; the exact length of time varied because we sowed different trays on different dates and stopped monitoring all trays on the same date, after germination had nearly ceased (<2% of all cells had newly germinated seeds during the final 4 d of monitoring). Because the order of Low Concentration and Control trials was randomized, seeds from both types of fruits were allowed essentially equal time for germination. Mean germination time is the integrated average time between sowing and germination of seeds:

$$\text{Mean germination time} = \sum f_i t_i,$$

where $f_i$ is the fraction of total seeds germinating at time $t_i$.

**Statistical analysis**

**Retention times.**—We used a multivariate, repeated-measures ANOVA, with fruit type (Low Concentration, Extract, and Control) as a “within” variable and mean retention time as the dependent variable (Abacus Concepts 1989). We used the same model to test for differences among fruit types in two other descriptors of gut retention time, transit time (i.e., time of first appearance of seeds) and the time at which the last seed was defecated. All $P$ values were adjusted by Greenhouse-Geisser epsilon.

We performed two planned contrasts on fruit type. The first tested whether mean retention times differed between Low Concentration and Control fruits. The second compared mean retention times of Extract and Control fruits. Again, $P$ values were adjusted by Greenhouse-Geisser epsilon.

Mean retention times of seeds from High Concentration and Control fruits were compared with a paired
**TABLE 1.** Mean retention time of *Solanum americanum* seeds from three types of artificial fruits (low concentration of glycoalkaloid, high concentration of glycoalkaloid, extract of fruit pulp) in Cedar Waxwings.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ss</th>
<th>ms</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>9</td>
<td>1079.5</td>
<td>120.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit type</td>
<td>2</td>
<td>677.9</td>
<td>338.9</td>
<td>6.28</td>
<td>0.009</td>
</tr>
<tr>
<td>Fruit type × subject</td>
<td>18</td>
<td>54.2</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In all tests, alpha was 0.05, and we report means ± 1 SD, unless otherwise noted.

Germination.—Although we originally intended to analyze germination data from each 5-min interval separately, it was necessary to combine intervals for two reasons. First, sample sizes were often low (<5 seeds), especially in the early and late intervals of each trial. Second, we felt it important to use a multivariate, repeated-measures ANOVA because such a model controls for correlation among the repeated measures. Because there were often several time intervals for each bird in which no seeds were defecated and because the multivariate approach cannot accommodate such missing values (Abacus Concepts 1989), we combined all 5-min intervals so that there were no missing values for any bird. The combined time intervals were 0–27.5 min, 32.5–42.5 min, 47.5–57.5 min, and 62.5–87.5 min. Percentage germination within each interval was arcsine-transformed to reduce positive mean-variance relationships. We used a multivariate, repeated-measures ANOVA with fruit type (Low Concentration, Control) and time interval as “within” factors and percentage germination as the dependent variable. The same ANOVA model was then used with mean germination time as the dependent variable.

**RESULTS**

*Low glycoalkaloid concentration and Solanum extract experiment*

Fruit type significantly affected mean retention time of seeds (*F*_{2,18} = 6.28, *P* = 0.009; Table 1). Mean retention time of seeds from Low Concentration and Control fruits were essentially identical (*P* = 0.77; planned comparison), but seed retention differed significantly between Control and Extract fruits (*P* = 0.009; planned comparison; Fig. 1A). Mean retention time of seeds from Extract fruits was ~75% that for control fruits. Also, the pattern of seed deposition differed between Control and Extract fruits; seeds in Extract fruits were deposited in fewer defecations than seeds in Control fruits (X ± 1 so = 12.6 ± 3.5 and 16.1 ± 4.8, respectively, paired *t* = 2.9, df = 9, *P* = 0.02), which means seed density was higher in these defecations than in those from control fruits.

Cumulative frequency curves for percentage of seeds defecated vs. time were essentially identical until 20 min (Fig. 2A). Transit time (i.e., time of first appearance of seeds) did not differ for the three fruit types (*F*_{2,18} = 2.95, *P* = 0.08; repeated-measures ANOVA). After 20 min, seeds from Extract fruits started to appear more quickly in defecations than seeds from Control or Low Concentration fruits. Between 20 and 40 min, seeds from Low Concentration fruits were defecated less frequently than seeds from Control fruits, although this difference did not persist and was not large enough to create a significant difference in retention times of seeds from Control and Low Concentration fruits. However, this hint of longer retention times of Low Concentration fruit foreshadowed a greater and significant effect on mean retention time with higher levels of glycoalkaloids. The difference in seed retention between Extract and Control fruits persisted until the last seed out (*F*_{2,18} = 4.10, *P* = 0.03; repeated-measures ANOVA). In summary, effects of fruit pulp constitu-
ents were not reflected in transit time; they became evident only after ~15% of seeds were defecated. The difference from Control fruits persisted and was significant for Extract fruits, but not for Low Concentration fruits.

**High glycoalkaloid concentration experiment**

High Concentration fruits significantly increased mean retention time (paired $t = 2.3$, df = 9, $P = 0.025$). Seeds from High Concentration fruits took 36% longer to pass than seeds from Control fruits (Fig. 1B).

As with Low Concentration and Extract fruits, the time of first seed defecation did not differ from Control fruits ($t = 1.94$, df = 9, $P = 0.08$; Fig. 2B). Between 30 and 85 min, however, seeds from Control fruits appeared much more quickly than those from High Concentration fruits. There was no difference between the two types of fruit in time of appearance of the last seed ($t = 1.52$, df = 9, $P = 0.16$).

**Germination experiment**

Seeds started to germinate 7–10 d after sowing. Sowing density (number of seeds/cell) was negatively correlated with percentage germination ($F_{1,276} = 4.96$, $P = 0.03$). However, the variation in germination explained by sowing density was extremely low ($r^2 = 0.01$) and we therefore decided that inclusion of sowing density in the ANOVA model was not worth the cost in lost degrees of freedom. Regardless, the effect of sowing density would be the same on seeds defecated early and late because both sets of seeds were defecated and sown with few other seeds.

Percentage germination was not affected by gut retention time ($F_{3,24} = 2.37$, $P = 0.096$; Table 2). Despite the relatively low $P$ value, there was no trend in percentage germination with increasing time. In particular, seeds that were defecated earlier did not have a higher percentage germination than seeds defecated later (Fig. 2).

![Cumulative frequency distribution of seeds from artificial fruits eaten and defecated by Cedar Waxwings. Fruit types are as in Fig. 1A and 1B. Error bars are not shown because they obscure differences among the lines.](image)

**TABLE 2.** Repeated-measures ANOVAs of (a) percentage germination and (b) mean germination time of *Solanum americanum* seeds from two types of artificial fruits (with and without glycoalkaloids) retained in waxwing guts for four nonoverlapping time periods.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ss</th>
<th>ms</th>
<th>$F$</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>a) Percentage germination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject</td>
<td>8</td>
<td>1.78</td>
<td>0.22</td>
<td>5.63</td>
<td>0.045</td>
</tr>
<tr>
<td>Fruit type</td>
<td>1</td>
<td>1.11</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit type × subject</td>
<td>8</td>
<td>1.57</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>0.71</td>
<td>0.24</td>
<td>2.37</td>
<td>0.01</td>
</tr>
<tr>
<td>Time × subject</td>
<td>24</td>
<td>2.41</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit type × time</td>
<td>3</td>
<td>0.10</td>
<td>0.03</td>
<td>0.63</td>
<td>0.60</td>
</tr>
<tr>
<td>Fruit type × time × subject</td>
<td>24</td>
<td>1.31</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Mean germination time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject</td>
<td>8</td>
<td>473.7</td>
<td>59.21</td>
<td>0.15</td>
<td>0.71</td>
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<tr>
<td>Fruit type</td>
<td>1</td>
<td>28.5</td>
<td>28.5</td>
<td>0.54</td>
<td>0.66</td>
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<tr>
<td>Fruit type × subject</td>
<td>8</td>
<td>1542.1</td>
<td>192.8</td>
<td></td>
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</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>35.9</td>
<td>12.0</td>
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<tr>
<td>Time × subject</td>
<td>24</td>
<td>530.1</td>
<td>22.1</td>
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<tr>
<td>Fruit type × time</td>
<td>3</td>
<td>43.9</td>
<td>14.6</td>
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<td>0.62</td>
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<tr>
<td>Fruit type × time × subject</td>
<td>24</td>
<td>579.1</td>
<td>24.1</td>
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</table>
3A). Seeds from fruits containing glycoalkaloids had significantly lower percentage germination than seeds from control fruits ($F_{1,8} = 5.63, P = 0.045$; Fig. 3A; Table 2). Percentage germination from Control fruits was approximately 50% higher than that of seeds from Low Concentration fruits. Rate of germination did not differ between seeds from Low Concentration and Control fruits (Fig. 4). Although both curves reached an asymptote, which suggests seed germination was essentially complete, it is possible that ungerminated seeds had prolonged dormancy; we stress that our experiment tells us little about seed viability. Thus, while our results could suggest that glycoalkaloids inhibit germination permanently, they confirm only that glycoalkaloids slow germination rate.

Mean germination time was not affected by gut retention time ($F_{3,24} = 0.54, P = 0.657$; Table 2). Seeds defecated within the first time interval did not germinate sooner than seeds from the last time interval (Fig. 3B). Also, fruit type did not affect mean germination time ($F_{1,8} = 0.15, P = 0.71$; Fig. 3B; Table 2).

**DISCUSSION**

**Secondary metabolites and gut retention time**

Glycoalkaloids at concentrations typical of ripe *Solanum americanum* fruit did not decrease gut retention time of seeds in Cedar Waxwings. In fact, at high concentrations glycoalkaloids increased retention time by 36%. However, ripe *S. americanum* fruits do appear to contain a laxative chemical or chemicals (for ease of discussion, we will henceforth assume a single chemical), because seeds in artificial fruits with a pulp extract were defecated more quickly than seeds in control fruits.

Compounds in fruit pulp are widely known to have laxative effects in humans (Willimott 1933, McMillan and Thompson 1979). For example, sorbitol in Rosaceous fruits (e.g., prunes) and glucosides of emodin in Buckthorn fruits (*Rhamnus* spp.) are often used by humans as natural laxatives (Meyers 1983). At issue is whether such compounds have similar effects on natural seed dispersers. Our results suggest they do and therefore support Murray et al.’s (1994) conclusion that a compound in *Witheringia solanacea* pulp decreases retention time of seeds in another frugivorous bird, *Myiodes melanoce*. Murray et al.’s (1994) evidence for such a compound was questioned, since effect of dietary sugar concentration on retention time was not controlled (Witmer 1996a). Because our Control and Extract fruits were essentially identical except for presence of secondary metabolites in extract fruits, our study addressed this concern.

We hypothesized that glycoalkaloids could produce laxative effects when consumed by frugivores. Our data do not support this hypothesis, as glycoalkaloids at low concentrations did not affect retention time and at high concentrations, increased it. Nonetheless, for two rea-
sons we feel it premature to reject our hypothesis. First, we tested only a single species of seed-disperser—a bird. Mammalian seed dispersers could respond as predicted. Indeed, organisms differ in their response to glycoalkaloids, depending upon the binding affinity of the glycoalkaloid for sterols and upon the quantity and type of sterol within the cell membrane (Roddick et al. 1990, Keukens et al. 1992, Cipollini and Levey 1997c). Second, we only tested the two most important glycoalkaloids in ripe S. americanum and S. carolinense fruits. Other glycoalkaloids in these species might contribute to a laxative effect but their levels in fruit tissues are so low—virtually undetectable by HPLC—that we think it unlikely that they affect gut retention time. The structurally similar potato glycoalkaloids (α-solanine and α-chaconine), however, commonly occur in other Solanum spp. and their saponin-like properties can cause diarrhea in mammals (Willimott 1933, McMillan and Thompson 1979, Dalvi and Bowie 1983, Hopkins 1995).

Retention time and germination

Seeds are often altered by passage through vertebrate guts. In particular, treatment in the gut often increases or decreases germination rate or success (Izhaki and Safriel 1990, Barnea et al. 1991, Ellison et al. 1993, Levey 1986, Lieberman and Lieberman 1986). Thus, it is reasonable to expect that the degree of treatment (i.e., retention time) will influence germination. Indeed, Murray et al. (1994) found a negative correlation between gut retention time of seeds in Witheringia fruits and proportion of seeds germinating. Their interpretation of this result and their retention time experiment was that a compound in Witheringia fruit pulp reduced gut retention time, which in turn increased seed viability. Note that Murray et al. (1994) did not consider direct effects of secondary metabolites on seed viability; they inferred indirect effects via retention time. They could not test for direct effects because they lacked data on germination of seeds passed in the absence of secondary metabolites.

In contrast to Murray et al.’s (1994) results, we found no effect of retention time on seed germination, but did find a direct inhibitory effect of glycoalkaloids. In particular, α-solamargine and α-solasonine delayed seed germination, even at the low concentrations typical of ripe S. americanum fruits. It is unclear from our results whether the glycoalkaloids decreased viability or simply caused a delay in germination. A reduction in viability would be puzzling, given that the glycoalkaloids are naturally present in ripe fruits at the concentrations we used. A delay in germination would likewise be surprising because the Low Concentration seeds had such a short exposure time (<2 h) to glycoalkaloids in the fruit. This short exposure time was the only difference between Control and Low Concentration fruits.

Implications for seed dispersal

A central implication of our results is that plants appear to have more control than previously thought over how quickly seeds are passed and, presumably, how they are dispersed. Our results suggest that both laxative and constipative compounds occur in S. americanum, albeit not simultaneously present in high enough concentrations to have opposing effects. In hindsight, presence of both types of compounds is not surprising; reports in the medical literature clearly document that fruit consumption can increase or decrease defecation rate (Birnberg 1933, Willimott 1933, Joslin et al. 1938, Fries et al. 1950, McMillan and Thompson 1979). Secondary metabolites in fruit may have many other functions, as well (reviewed in Cipollini and Levey 1997b). Nonetheless, by altering retention time of seeds, some of them almost certainly affect patterns of seed deposition and may thus have consequences for seed dispersal and plant fitness (Levey 1986, Murray 1988, Jordano 1992). We now discuss possible advantages and disadvantages to plants of variation in seed retention time within frugivores and then discuss how our results relate to the natural history of Solanum.

Retention time.—Probably the most widely recognized consequence of longer retention times is increased dispersal distance (Murray 1988, Clench and Mathias 1992, Jordano 1992). Yet the link between retention time and dispersal distance is not clear, especially if frugivores return frequently to a fruiting tree (Lambert 1996). Furthermore, longer dispersal distances are not necessarily beneficial. For example, in plants that live in patchy environments, long retention times may increase the risk of seeds being dispersed into adjacent areas of unsuitable habitat, because in a patchy environment the probability of encountering a similar habitat decreases with distance from the starting point (Grace 1991, Lechowicz and Bell 1991, Bell 1992). Also, once a seed is carried beyond the vicinity of high mortality near its parent, dispersal distance likely becomes less important (Clark and Clark 1984, Howe et al. 1985, Augspurger 1988, Merg 1994).

Consequences of variation in seed retention time may also depend upon the type of seed disperser. Many seed predators, for example, also disperse some seeds (Janzen 1981, Levey 1986, Lambert 1989). In these species, shorter retention times are likely to increase the probability that seeds will survive gut passage (Janzen 1984, Lambert 1989, Jordano 1992, Gardener et al. 1993). Movement rates of frugivores may likewise determine whether longer or shorter retention times are beneficial to the fruiting plant. For instance, it has been hypothesized (H. Horn, personal communication) that because birds are generally more mobile than mammals of equivalent size, shorter retention times are beneficial for bird dispersal (to avoid dispersal outside the proper habitat) and longer retention times are beneficial for mammal dispersal (to ensure adequate dispersal dis-
stance within the habitat). Perhaps not coincidentally, mammal-dispersed *Solanum* fruits have higher concentrations of glycoalkaloids, which are constipative, than bird-dispersed *Solanum* fruits, at least one of which contains a compound that significantly speeds seed passage in a frugivorous bird (Cipollini and Levey 1997b, this study).

The picture that emerges is that variation in seed retention time will likely affect dispersal distance, but that advantages and disadvantages of this variation will differ widely, depending on plant habitat requirements, seed germination, behavior of dispersers, and the internal treatment of seeds by frugivores. Variation in seed retention time is also likely to influence the number of defecations with seeds and the average number of seeds per defecation, from a given meal of fruit. Indeed, we found that a 26% increase in gut passage time resulted in 28% more defecations containing seeds. Because the number of seeds per fruit and number of fruits ingested were controlled in our study, slower gut passage also resulted in fewer seeds per defecation. A similar relationship between retention time and defecation pattern has been observed in other frugivorous birds (Levey 1986). In this context, increased retention time is likely to be beneficial for three reasons. First, it increases the number of dispersal events (i.e., sites to which seeds are dispersed; e.g., Janzen 1981). Second, because defecations with fewer seeds produce fewer seedlings, it reduces competition among seedlings, which can be severe (Howe 1989, Loiselle 1990). Third, because seed predators are more likely attracted to high than low densities of seeds in defecations (Janzen 1982, Kaspari 1993, Merg 1994, Moegenburg 1994), it may decrease postdispersal seed predation.

Glycoalkaloids and the natural history of *Solanum*.—Our results relate to the natural history of two groups of *Solanum* spp. One group retains high levels of glycoalkaloids in ripe fruit, is dispersed by mammals, exhibits delayed germination, and has ripe fruits that persist on the plant for long periods. The other group has low levels of glycoalkaloids in ripe fruit, is dispersed primarily by birds, exhibits rapid germination, and has fruits that are consumed quickly after ripening. We report elsewhere on these patterns and describe in detail two species, one belonging to each group—*S. carolinense* and *S. americanum*, respectively (Cipollini and Levey 1997a, 1997b).

A proposed function of secondary metabolites in ripe fruit is to prevent seed germination until after dispersal (Cipollini and Levey 1997b). Indeed, not only do glycoalkaloids reduce percentage germination of *Solanum* seeds (this study), but their concentrations are highest by far in *Solanum* spp. of the first type. Presumably, species of the first group (i.e., those with slow removal rates) have a greater need to inhibit germination of seeds within fruits than species whose fruits are removed rapidly. High glycoalkaloid concentration in these species may also tie into their dispersal ecology and the constipative effects we observed on gut retention times of seeds. As discussed in the previous section, slowed seed passage could positively affect seed shadows produced by mammals, which are much less mobile than birds. Rapid seed passage, on the other hand, is more likely to be beneficial to bird-dispersed *Solanum* seeds. Indeed, *Solanum* spp. of the first type have higher concentrations of glycoalkaloids than bird-dispersed *Solanum* fruits, at least one of which contains a compound that significantly speeds seed passage in a frugivorous bird (Cipollini and Levey 1997b, this study).

**Conclusion**

Our experiments support the work of Murray et al. (1994) by showing that *Solanum americanum* fruits contain a compound or compounds that enhance gut passage rates of seeds in a frugivorous bird. Contrary to our expectations, glycoalkaloids are not responsible for these effects, but may act instead to delay seed passage and perhaps to inhibit seed germination. Unlike Murray et al. (1994), we found no evidence that gut passage rates have any influence on germination. It is possible, however, that under conditions less ideal than those encountered by our seeds, we might have discovered a positive effect of rapid seed passage on percentage germination of defecated seeds.

Our results provide an evolutionary explanation for secondary metabolites in ripe fruit. They also suggest that plants might exert some control over seed shadows produced by frugivores by affecting gut passage rates with specific laxative and/or constipative chemicals. We believe continued study of the ecological roles of secondary metabolites of ripe fleshy fruits will uncover mechanisms by which plant–frugivore interactions are mediated. This underemphasized area deserves increased attention by ecologists interested in understanding and explaining the evolution of plant–frugivore interactions.

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