# Red-winged blackbird (*Agelaius phoeniceus* L). feeding response to oil and anthocyanin levels in sunflower meal

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ABSTRACT. Bird damage to sunflowers might be reduced by the development of resistant cultivars. Neagra de Cluj may be one such cultivar, with high levels of anthocyanin (a possibly aversive flavour) in achene hulls, but low oil yield. Four experiments were designed to assess the importance of oil content and anthocyanin concentration in feeding preferences expressed by red-winged blackbirds (*Agelaius phoeniceus* L.). Differences in oil concentration of 15% (w/w) were reliably discriminated in two-choice tests: higher concentrations were preferred. Conversely, all anthocyanin concentrations (0·5, 1·0, 2·5 and 5·0% [w/w]) were avoided, and two-choice tests suggested that higher concentrations (2·5 and 5·0%) were relatively more aversive. Both anthocyanin concentration and oil content could influence Neagra de Cluj resistance to bird depredation. Of these two characteristics, oil may be relatively more important.

KEYWORDS: Anthocyanin; blackbird; *Agelaius phoeniceus*; flavour; oil content; sunflower; resistant variety; bird damage

## Introduction

Damage by passerine bird species, including red-winged blackbirds (*Agelaius phoeniceus* L.), house sparrows (*Passer domesticus* L.), and goldfinches (*Carduelis tristis* L.), to oilseed sunflower can be severe (Besser, 1978), particularly in fields near marsh roost sites of blackbirds. In part, this reflects the long period of time that sunflowers are susceptible to depredation—from early seed development (about 5 days after bloom) until harvest (Cummings *et al.*, 1987).

One promising means of reducing bird damage may be the development of bird-resistant varieties of sunflower. As has been the case with other crops, such as maize, sorghum, and pears (e.g. Bullard, York and Kilburn, 1981; Greig-Smith *et al.*, 1983; Summers and Huson, 1984; Dolbeer, Woronecki and Mason, 1988), efforts to develop resistant sunflower varieties have emphasized morphological and chemical traits. Morphological features thought to increase resistance include concave heads, long bracts, heads that face the ground, head-to-stem distances in excess of 15 cm, and seeds with tough, fibrous hulls held tightly in the head (Parfitt, 1984). Presumably, these characteristics make extraction of seeds from heads more difficult for birds. Chemical features thought to increase resistance (via taste repellency or adverse postingestional effects) include anthocyanins (purple pigments) present in the seed hulls of some varieties (Dolbeer *et al.*, 1986; Mason *et al.*, 1986). Chlorogenic acid also was once considered a potential taste repellent present in sunflower (Harada, 1977), but the effectiveness of this compound is now doubted (Fox and Parfitt, 1982).

In a series of recent behavioural experiments (Mason *et al.*, 1989), the preferences of birds between two sunflower varieties, Jacques Discovery (a commercial oilseed cultivar) and Neagra de Cluj (NdC; a purple-hulled experimental bird-resistant cultivar) were assessed in the field, outdoor aviary, and laboratory at six dates during the course of seed development. Concurrently, both varieties were studied biochemically (Bullard *et al.*, 1989). Field results showed that Jacques was preferred to NdC, although the latter was damaged when the former became unavailable. Aviary and laboratory findings were consistent with these data. In terms of biochemistry, there were differences between the two varieties in anthocyanin concentration (NdC was higher), oil content (NdC was lower), and seed coat mass (NdC was higher). Any of these differences could have influenced consumption by birds, but oil content and anthocyanin concentrations could be parametrically evaluated in the laboratory. For this reason, the present experiments were designed to assess the importance of anthocyanin and oil as determinants of red-winged blackbird feeding preference.

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## Materials and methods

### *Subjects*

Male red-winged blackbirds were mist-netted (Sandusky, Ohio, $n = 60$) or decoy-trapped (Gainesville, Florida, $n = 60$) and shipped to the Monell Center, Philadelphia. Upon arrival, the birds were group-housed ($n = 15$/group) in cages (2 × 1 × 1·8 m). (After 4 weeks of adaptation to laboratory conditions, birds were randomly selected, and individually caged (61 × 36 × 41 cm) under a 6:18 h light/dark cycle that maximized feeding rates without reducing the total quantity consumed relative to *ad libitum* 24 h consumption (Rogers, 1974).

### *Sunflower meal*

Sunflower meal (25 kg) from which all oil had been extracted was obtained from National Sun Enterprises (Enderlin, North Dakota). Upon delivery, this meal was sieved to remove hull slivers and other contaminants. All particles ⩾12-mesh (1·4 mm diameter) in size were discarded. The prepared meal was used to formulate test samples, as described below.

### *Preperation of oil samples*

Sunflower oil (obtained from National Sun Enterprises), prepared meal, 1·0% w/w xanthate-gum granulating agent (Kelfo®), and water were mixed in a high-speed blender to produce six uniform samples that differed only in oil content (0, 10, 20, 30, 40 or 45% w/w, respectively). These oil levels were chosen to bracket both the oil content normally found in mature NdC achenes (30%) and the oil content normally present in mature commercially available sunflower varieties (40–45%). After preparation, each sample was spread on aluminum foil to dry. After drying, meal samples were crumbled by hand, sieved, and 9–14 mesh (1·2–2·0 mm diameter) particles were retained for use in behavioural tests.

### *Preparation of anthocyanin samples*

Whole NdC achenes were submerged in a 0·04% hydrochloric acid/methanol solution that extracted anthocyanin (Bullard *et al.*, 1989). After 48 h, the solvent solution was decanted into a rectangular glass dish (30 × 38 × 6·3 cm) and the dish was placed in a fume hood. Air drawn over the dish evaporated the solvent until only a thin layer of anthocyanin remained. This layer was scraped from the glass and transferred to a 2 litre flask. The flask contents were extracted overnight, first with cyclohexanone and then with diethyl ether, to remove non-polar contaminants (i.e. waxes and oil). Anthocyanin residues were mixed in a high-speed blender with sunflower meal (containing 30% oil [w/w], to ensure measurable consumption), 1·0% w/w Kelfo and water to produce uniform meal samples containing anthocyanin concentrations of 0, 0·5, 1·0, 2·5 and 5·0% (w/w). These levels were chosen to bracket the anthocyanin concentration (2–5%) normally present in NdC achene hulls. After drying as previously described, meal samples were crumbled by hand, and 9–14 mesh particles were retained for use in behavioural tests. Because the anthocyanin caused treated meal samples to differ in colour from control samples (0% anthocyanin), McCormick purple food colouring was added to the latter to minimize the influence of colour cues on feeding by birds. In our experience, this dye has no apparent taste qualities detectable by red-winged blackbirds.

### *Experiment 1: one-choice oil tests*

During a 3-day pretreatment period, 30 individually housed birds were presented with 20 g Purina Flight Bird Conditioner in a 7·5 cm diameter metal cup positioned in the centre of the front of each cage. Food was presented for 6 h, after which cups were removed from the cages and consumption was measured. No feed was present in the cages during the 18 h dark period.

On day 4, mean consumption by each bird during the pretreatment period was computed, and birds were assigned to six groups on the basis of consumption. The bird with the highest consumption was assigned to the first group, that with the second highest to the second group, and so forth. This ensured that the groups were balanced with respect to consumption before treatment.

On treatment days 5–8, each bird was presented with one cup containing 20 g prepared sunflower meal at the start of the 6 h light period. The oil contents of meal samples presented to the six groups were 0, 10, 20, 30, 40 and 45% (w/w), respectively. Consumption of meal was assessed at 2, 4 and 6 h daily.

### *Experiment 2: two-choice oil tests*

Two-choice tests are more sensitive than one-choice tests in detecting food preference and avoidance (e.g. Dragoin, McCleary and McCleary, 1971). Experiment 2 provided a measure of the birds' ability to discriminate between oil levels in a choice situation.

Thirty experimentally naive birds were individually housed and assigned to six groups on the basis of pretreatment consumption, as described in experiment 1. Treatment trials were similar to those described for one-choice tests, except that two cups were presented at the front of each cage. During each trial, one cup contained a 20 g sample of 30% oiled sunflower meal (So) while the other cup contained 0, 10, 20, 30, 40 or 45% oiled meal (S+). The 30% oil level in control meal samples was chosen because mature NdC achenes contain approximately 30%

oil. Cup positions were randomly assigned on a daily basis, and consumption was measured at 2, 4 and 6 h.

*Experiment 3: one-choice anthocyanin tests*

Thirty experimentally naive birds were individually housed, and assigned to five groups on the basis of consumption, as previously described. During the treatment period, each bird received a single cup holding 20 g of prepared sunflower meal containing 30% oil and one of the five levels of anthocyanin (0, 0·5, 1·0, 2·5 or 5·0% [w/w] for groups 1–5, respectively). Otherwise, procedures were identical to those described in experiment 1.

*Experiment 4: two-choice anthocyanin tests*

Thirty experimentally naive birds were individually housed, and assigned to five groups on the basis of pretreatment consumption, as previously described. The procedures followed during the treatment period were identical to those described in experiment 2. On each day, one cup contained sunflower meal with 30% oil only (So) while the other cup contained meal with 30% oil and one of the five levels of anthocyanin (S+).

*Analysis*

For one-choice tests (experiments 1, 3), consumption during the treatment period was assessed in three-factor analyses of variance (ANOVAs: oil or anthocyanin concentrations, days, intervals), with repeated measures over days and intervals. For two-choice tests (experiments 2, 4), preference ratios for S+ samples were calculated by dividing S+ consumption by total (S+ and So) consumption for each bird at each measurement interval on each treatment day. Ratios varied from 1·0 (consumption of S+ only) to 0·0 (consumption of So only), and were assessed in three-way ANOVAs (i.e. oil or anthocyanin concentrations, days, intervals), with repeated measures over days and intervals. Because there were no significant interval effects in these ANOVAs, actual consumption (g) after 6 hours was assessed in three-way ANOVAs (i.e. oil or anthocyanin concentrations, days, cups). For all experiments, Tukey HSD *post hoc* tests were used to isolate significant differences among means (Linton and Gallo, 1975).

## Results

*Experiment 1: one-choice oil tests*

There was an interaction between concentrations and measurement intervals ($F = 2{\cdot}4$; 10, 48 d.f.; $P < 0{\cdot}2$). Meal containing 10% oil was consumed in greater quantities than other meals at 4–6 h (*Figure 1*); at 2 h, no significant differences in consumption among meals were observed.

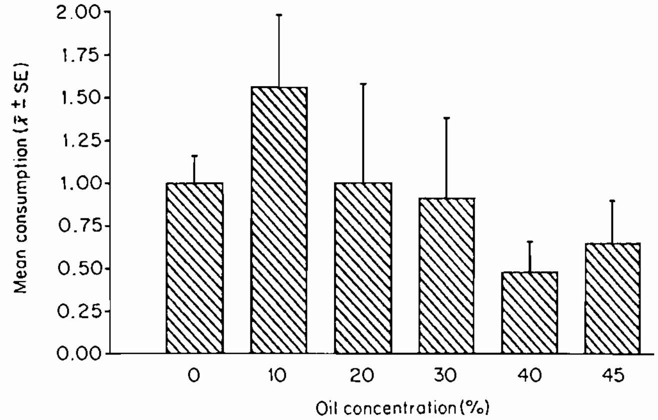


FIGURE 1. Mean consumption (g) of oiled sunflower meal (0, 10, 20, 30, 40, 45% [w/w]) by red-winged blackbirds after 6 h in one-choice oil tests (experiment 1). Capped vertical bars represent standard errors of the means (SE)

*Experiment 2: two-choice oil tests*

When preference ratios were examined, a significant difference among oil concentrations was obtained ($F = 4{\cdot}0$; 5, 24 d.f.; $P < 0{\cdot}009$). Tukey tests ($P < 0{\cdot}05$) indicated that preference ratios for 45% oil were significantly higher than preference ratios for 0% or 10% oil (*Figure 2a*). Otherwise, there were no significant effects.

When actual consumption after 6 h was examined, there was a significant interaction between concentrations and cups ($F = 5{\cdot}3$; 5, 24 d.f.; $P < 0{\cdot}002$). Tukey tests ($P < 0{\cdot}05$) revealed that (a) consumption of 0% or 10% (S+) oil was < 30% (So) oil, (b) there were no differences in consumption between 20% or 30% (S+) oil and 30% (So) oil, and (c) consumption of 40% or 45% (S+) oil was greater than consumption of 30% (So) oil (*Figure 2b*).

*Experiment 3: one-choice anthocyanin tests*

There were significant differences among concentrations ($F = 12{\cdot}05$; 4, 25 d.f.; $P < 0{\cdot}0001$). *Post hoc* tests revealed only that consumption of 0·0% (control) meal was greater than consumption of meal containing any anthocyanin concentration (*Figure 3*).

In addition to the main effect for concentrations, there was an interaction between concentrations and measurement intervals ($F = 7{\cdot}11$; 8, 50 d.f.; $P < 0{\cdot}00001$). Examination of this effect showed that while consumption of control meal was always greater than consumption of anthocyanin-treated meal, the difference in consumption became less pronounced across measurement intervals.

*Experiment 4: two-choice anthocyanin tests*

There were significant differences among concentrations ($F = 4{\cdot}2$; 4, 25 d.f.; $P < 0{\cdot}01$) and days

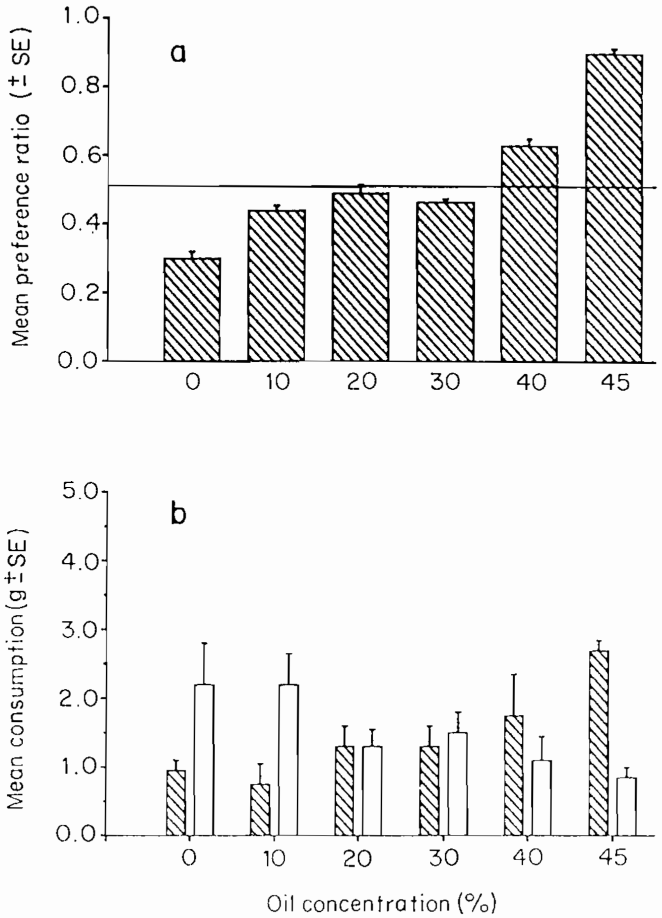


FIGURE 2. (a) Mean preference ratios (S+ consumption/total consumption) expressed by red-winged blackbirds in two-choice oil tests (experiment 2). A ratio of 1·0 reflects consumption of S+ only, while a ratio of 0·0 reflects consumption of So only. Indifference is indicated by a ratio of 0·5. (b) Mean consumption in two-choice oil tests after 6 h. Capped vertical bars represent SE. ▧, S+, 0–45% oiled meal; □, So 30% oiled sunflower meal

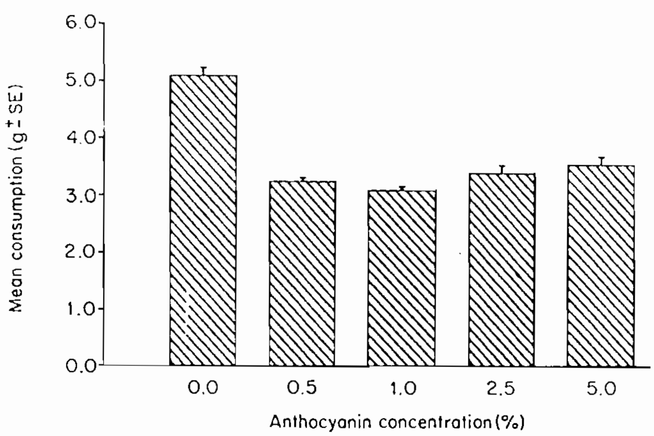


FIGURE 3. Mean consumption (g) of 30% [w/w] oiled sunflower meal containing 0·0, 0·5, 1·0, 2·5 or 5·0% [w/w] anthocyanin by red-winged blackbirds in one-choice anthocyanin tests (experiment 3). Capped vertical bars represent SE

($F = 3{\cdot}3$; 3, 75 d.f.; $P < 0{\cdot}02$). Preference ratios for 0·0% anthocyanin were greater than preference ratios for 2·5% or 5·0% anthocyanin ($P < 0{\cdot}05$), but not 0·5% or 1·0% anthocyanin (*Figure 4a*). Examination of the days effect showed that preference ratios for day 1 were higher than those for day 2 ($P < 0{\cdot}05$).

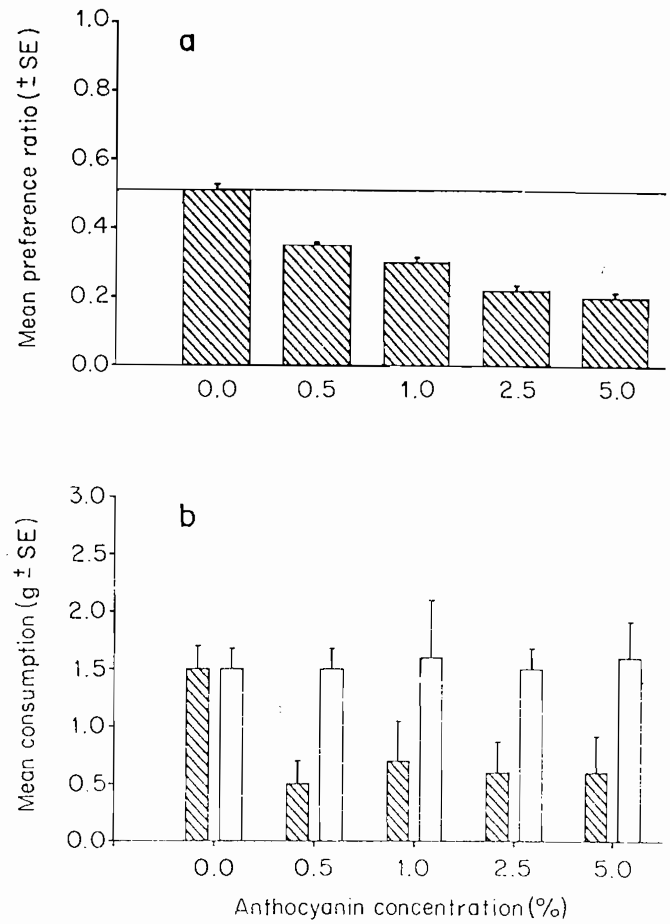


FIGURE 4. (a) Mean preference ratios (S+ consumption/total consumption) expressed by red-winged blackbirds in two-choice anthocyanin tests (experiment 4). A ratio of 1·0 reflects consumption of S+ only, while a ratio of 0·0 reflects consumption of So only. Indifference is indicated by a ratio of 0·5. (b) Mean consumption in two-choice anthocyanin tests after 6 h. Capped vertical bars represent SE. Key as in *Figure 2*

When actual consumption after 6 h was examined, there were significant differences among days ($F = 42{\cdot}5$; 3, 75 d.f.; $P < 0{\cdot}00001$). Tukey tests ($P < 0{\cdot}05$) showed that consumption was lowest on day 1. Consumption was higher on day 2 than on day 1, but lower than on days 3 and 4. Consumption on day 3 did not differ from that on day 4. Furthermore, there was a significant difference in consumption between cups ($F = 90{\cdot}1$; 1, 25 d.f.; $P < 0{\cdot}00001$); S+ consumption ($1{\cdot}1 \pm 0{\cdot}1$) was lower than So consumption ($2{\cdot}4 \pm 0{\cdot}2$). Finally, there were significant interactions between concentrations and cups ($F = 6{\cdot}1$; 4, 25 d.f.; $P < 0.002$) and between days and cups ($F = 3{\cdot}1$; 3, 75 d.f.; $P < 0{\cdot}03$). *Post hoc* examination of the former effect showed that there were significant differences in consumption between S+ and So when S+ was 0·5%, 1·0%, 2·5% or 5·0% anthocyanin. In each of these cases, S+ consumption was significantly lower than So consumption ($P < 0{\cdot}01$; *Figure 4b*). *Post hoc* examination of the interaction between days and cups showed that S+ consumption on days 1 and 2 was significantly less than that on days 3 and 4

($P < 0{\cdot}01$). Consumption of So was less on day 1 than on days 2–4 ($P < 0{\cdot}05$).

## Discussion

The results of one-choice oil tests indicated greater consumption of meal containing 10% oil than of meal containing higher or lower concentrations. It may be that birds were responding to the caloric density of test diets (i.e. consuming more of the low-oil than of the high-oil meal to achieve the same caloric intake). This explanation, however, does not account for the observation that more of the 10% diet was eaten than of the 0% diet. Nevertheless, the fact that greater consumption of 10% was evident only after 4 h suggests that differential consumption was caused by factor(s) other than taste (e.g. postingestional feedback).

The results of two-choice tests reveal that red-wings can discriminate between foods with oil levels that differ by 15% and that they may be able to discriminate between samples that differ by smaller amounts. Where discrimination occurred, the meal sample with the higher oil was preferred. Whatever the factors mediating consumption, the results of two-choice tests provide evidence that red-wings possess a fine capability for discriminating among the oil concentrations present in foods. The sensisitivity of the birds in the present experiment was at least equivalent to human (Mela and Christensen, 1987) and rat (Naim, Brand and Kare, 1987) sensitivity to oil concentrations.

The results of one-choice anthocyanin tests suggest that any anthocyanin concentration was repellent relative to control meal, and that 0·5% anthocyanin was as aversive as 5% anthocyanin. However, the fact that consumption increased over measurement intervals suggests that the birds could habituate to the aversive character of anthocyanin if no alternative foods were available. This is consistent with the results of both field and aviary tests (Dolbeer *et al.*, 1986; Mason *et al.*, 1989). While commercial oilseed varieties are preferred to NdC, when the former are not available, the latter is damaged.

The results of two-choice tests confirm the results of one-choice tests in terms of anthocyanin repellency, but provide greater resolution in terms of concentration effects. Specifically, 5·0% or 2·5% anthocyanin preference ratios were significantly lower than 1·0%, 0·5% or 0·0% anthocyanin. While 0·5% and 1·0% anthocyanin were rejected relative to 0·0% anthocyanin (actual consumption after 6 h), the data support the possibility that increases in anthocyanin concentration were at least weakly associated with increases in repellency when alternative, untreated food was available. That no habituation (i.e. no increase in consumption among daily measurement intervals) was observed, probably reflects the availability of untreated meal and is consistent with the field and aviary evidence alluded to above. In this regard, it is perhaps important to note that consumption increased over treatment days. While not specific to anthocyanin-treated meal samples, this general increase may reflect habituation to the testing context (i.e. an increase in the willingness of birds to approach and sample foods).

## Management implications

Both low oil content and high anthocyanin levels could contribute to reduced damage of NdC sunflowers by depredating birds. Changes in either characteristic may affect the performance of this variety in the field. For example, significant increases in oil content (necessary to make NdC commercially viable as an oilseed cultivar) without concomitant increases in anthocyanin may result in greater damage to this variety by birds. Even high levels of anthocyanin may not protect purple-hulled sunflowers with high oil contents. In the present experiments, no anthocyanin concentration eliminated consumption, and consumption of oil anthocyanin concentrations increased over days, suggesting habituation. Finally, it is interesting to note that the ability of red-wings to discriminate between samples with different oil contents is between 10 and 15%, approximating to the difference in oil between NdC and commercial oilseed varieties. It may be that the reduced damage to NdC by birds reflects the fact that this variety is merely a lower-quality food (relative to other sunflower varieties) and not a resource with strongly aversive qualities, *per se*.

In addition to anthocyanin and oil content, it remains to be determined whether NdC seed coat mass contributes to the bird tolerance of this variety. Coat mass clearly affects the tolerance of other sunflower varieties (Parfitt, 1984). Further experimental work on the importance of this variable is planned.

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