Contribution of Seed Hull Characteristics to Resistance of Sunflower to Blackbird Damage

J.R. Mason, M.A. Adams, R.A. Dolbeer, R.A. Stehn,
P.P. Woronecki, and G.J. Fox

Damage to oilseed sunflower by red-winged blackbirds (*Agelaius phoeniceus* L.) and other bird species can be severe. One promising means of reducing this damage is through development of varieties of sunflower resistant to feeding by birds (Fox and Linz, 1983 a, b,; Fox et al., 1984). A series of field and aviary trials (Dolbeer et al., 1985) showed that two experimental varieties, BRS-1 (developed by North Dakota State University) and Neagra de Cluj = NdC, had reduced levels of bird damage when compared with commercial oilseed varieties.

In conjunction with those trials, a series of five laboratory experiments were conducted with seeds from these two experimental varieties and two commercial oilseed varieties (Jacques 501 and Jacques 550). The objective was to examine the contribution of seed hull characteristics to this apparent resistance to bird feeding. We hypothesized that BRS-1 possessed morphologically resistant characteristics (i.e., seeds with tough fibrous hulls held tightly to the head -Parfitt, 1984) whereas NdC appeared repellent on the basis of flavor.

The experiments, conducted at the Monell Chemical Senses Center in Philadelphia during 1984-85, were as follows: In Experiment 1, red-winged blackbirds were presented with unhulled seeds of the four varieties to establish a preference hierarchy. In Experiment 2, rats were presented with unhulled seeds of the four varieties to determine whether resistant characteristics were general or selective for birds. In Experiment 3, red-wings were presented with hulled seeds from each of the varieties to assess whether the birds' preferences in Experiment 1 reflected hull or seed characteristics. In Experiment 4, certain solubles from NdC and J-550 seeds were extracted, and the extracts were presented to birds in aqueous solutions to investigate whether chemical characteristics of the NdC variety were responsible for its low preference rating. In Experiment 5, red-wings were presented with extracted NdC and J-550 seeds to assess whether the extraction procedure had removed the repellent characteristics.

Methods

Experiment 1.

Subjects. Thirty adult male red-winged blackbirds trapped during October 1983 were individually caged. A 9:15 hr light:dark cycle was used to maximize feeding by birds without reducing the total quantity of food consumed (Rogers, 1974). Water was always available, and before the experiment began, the birds were permitted ad libitum access to Purina Flight Bird Conditioner (PFBC) and oyster shell grit.

Stimuli. Mature sunflower heads of the four varieties (J501, J550, BRS-1, NdC) were collected from plots in Erie County, Ohio, in 1984. The seeds from each head were removed by hand and dried at 40°C. for one week.

Preference Tests. The birds were visually isolated from one another (Mason and Reidinger 1983), and adapted to 15-hrs daily food deprivation during the dark phase of each light cycle. Food-deprived (rather than satiated) birds were used to ensure that any preferences exhibited by the red-wings among the hybrids were robust.

During the first hour of light on each test day, each bird was presented with two 10-g samples of unhulled sunflower seeds. Each sample was individually presented in a metal cup (7.5-cm diameter). The cups in each pair were held together with a rubber band and placed in the center of the fronts of the cages. Different birds were presented with different pairs of varieties, so that on any day, five birds were given one of each of the six variety pairs. Across test days, presentation of varietal pairs was counterbalanced. All varieties were presented equally often on the left and right sides of the cages, and different groups of birds (n = 5/group) were presented with each of the six possible orders of variety pairs. After each test, the remaining seed samples were removed from the cages and consumption was recorded. Spillage was not assessed since pilot work indicated that it was proportional to consumption. After testing each day, the birds were given free access to PFBC until onset of darkness.

Experiment 2

Subjects. Six adult male rats (*Rattus norvegicus* L., Sprague-Dawley derived) were individually caged in a room with a 12:12 hr. light:dark cycle and an ambient temperature of 22° C. Rat chow (Wayne Lab Blox) and water were always available, except as described.

Stimuli. Seed samples from the four varieties were used.

Preference Tests. During the first hour of light on each test day, rat chow was removed from the cages, and the rats were presented with two 20-g samples of unhulled seeds as in Experiment 1. Three of the rats were presented with the

Mason and Adams are research associates, Monell Chemical Senses Center, Philadelphia, PA 19104; Dolbeer, Stehn, and Woronecki are biologists, U.S. Fish and Wildlife Service, Sandusky, Ohio 44870, and Fox is a plant breeder, Stauffer Seeds, Fargo, North Dakota (formerly research associate, Department of Agronomy).

following sequence of stimulus pairs: (a) NdC versus BRS-1; (b) NdC versus J550; (c) NdC versus J501; (d) BRS-1 versus J550; (e) BRS-1 versus J501; (f) J550 versus J501. The other three rats were presented with the stimulus pairs in the opposite order. Paper toweling was placed beneath each cage to collect spillage. The rats were not food deprived prior to test sessions so as to exaggerate any preferences that might exist among the varieties.

Experiment 3

Subjects. Twelve red-wings were randomly selected from the 30 birds used in Experiment 1.

Stimulis. Hulled J550, BRS-1, and NdC seeds were used. J501 seeds were not used, because no differences in consumption were observed between the two commercial varieties in Experiment 1.

Procedure. Preference tests were similar to those described in Experiment 1. However, only two birds were presented with each of the possible stimulus orders. Also, to keep the number of seeds presented to birds equivalent to the numbers presented in Experiment 1, 4-g (rather than 10-g) samples were used. Finally, the birds in this experiment were not food-deprived prior to testing for the reason presented in Experiment 2.

Experiment 4

Subjects. Ten red-wings were randomly selected from the remaining 18 birds used in Experiment 1.

Stimuli. Anthocyanins were extracted from NdC seed hulls by submerging the seeds either in water or in a solution of methanol (MeOH) and hydrochloric acid (1% HCl v/v). For the water extraction, the water was evaporated, leaving behind anthocyanin crystals. For the methanol extraction, the MeOH/HCl extracting solution was changed three times and extracts pooled. Dissolved anthocyanins in the samples were precipitated by adding a 1:1 mixture of hexane and diethyl ether.

Procedures as described above were used to obtain water extracts of J550 seeds, which do not contain anthocyanins. The extraction gave a tan powder of unknown composition. The yield was approximately 2.0%.

Preferences Tests. After adaptation to visual isolation for two days, the red-wings were presented with purple distilled water as their only fluid source for seven days. The shade of purple used matched the deep purple of the anthocyanin extract in aqueous solution. The colored water was presented to each bird in two calibrated 50-ml Richter tubes positioned 5-cm apart at the front of each cage. With the exception of drinking fonts which entered the cage, both tubes were concealed from the bird. Drinking during the first four hours of light was recorded, and the birds were assigned to four groups on the basis of overall consumption.

On the eighth day, and for six days therafter, all birds were given two-tube preference tests during the first four hours of light. The two experimental groups were given tests between varied concentrations of anthocyanin extract in one tube and purple distilled water in the other. Over successive days, a descending extract was presented. Presentations were then repeated in an ascending series. Control groups were given tests between two tubes containing purple distilled water. The amount of fluid consumed from each tube was measured to the nearest ml on an hourly basis during the four hour test period. Positioning of the Richter tubes was counterbalanced over days so that each bird was presented with anthocyanin solution an equal number of times on the left and right sides of the cages. At the end of testing on each day, each bird was presented with two tubes of purple distilled water.

After anthocyanin tests, birds were adapted to drinking brown water from two Richter tubes and given preference tests between concentrations of J550 extract and brown distilled water. These tests were conducted in the same fashion as that described above.

Experiment 5

Subjects. The remaining eight red-wings were used.

Stimuli. NdC and J550 seeds that had been water extracted in Experiment 4 were used after dyeing them with black food coloring (McCormick).

Preference Tests. The eight birds were randomly assigned to two groups. One group was presented twice with the following sequence of preference tests: (a) NdC versus NdC-extracted (NdC-E) seeds; (b) NdC versus J550 seeds; (c) NdC versus J550-extracted (J550-E) seeds; (d) NdC-E versus J550 seeds; (e) NdC-E versus J550-E seeds; and (f) J550 versus J550-E seeds. The other group was presented twice with the opposite order of preference tests. Other testing procedures were as previously described.

Results

Experiment 1 (Preference of birds for unhulled seeds from four varieties.)

There were no differences in consumption among groups of birds. However, there were significant differences in total consumption among variety pairs and in consumption within pairs (Table 1). Also, the interaction between con-

Table 1. Mean consumption (g) by red-winged blackbirds of NdC, BRS-1, J550 and J501 sunflower seeds in completely counterbalanced two-choice preference tests. The standard error of the mean ranged between 0.1 to 0.3 for the means presented.

	Grams of seed consumed/bird/hour[1]			
Two-choice tests	NdC	BRS-1	J550	J501
NdC vs. BRS-1	0.6a	2.1b		
NdC vs. J550	1.0a		2.1b	
NdC vs. J501	0.7a			2.4b
BRS-1 vs. J550		1.1a	2.0b	
BRS-1 vs. J501		0.6a		2.6b
J550 vs. J501			1.8a	1.7a
Overall mean consumption	0.8a	1.3a	2.0c	2.2c

[1]Means in same row with different letter are significantly ($P < 0.05$) different.

sumption among and within variety pairs was significant. Overall consumption was significantly depressed in pairs containing either BRS-1 or NdC and consumption was lowest when both of these varieties were presented in the same pair. Conversely, the highest consumption was recorded when both J501 and J550 seeds were presented in a pair.

Within pairs, there was no difference in consumption between J501 and J550. However, consumption of either of these varieties was significantly greater than consumption of NdC or BRS-1. When consumption within NdC and BRS-1 pairs was examined, consumption of BRS-1 was significantly higher than consumption of NdC.

Overall, the results reflect a clear preference hierarchy among the four varieties. J501 and J550 were preferred to either NdC or BRS-1, and BRS-1 was preferred to NdC.

Experiment 2 (Preference of rats for unhulled seeds from four varieties.)

There were no significant differences in consumption either among or within variety pairs (Table 2). Significant changes in consumption were obtained, however, across test days. Tests indicated that there was a linear increase in consumption. Such results probably reflect initial neophobia (a component of bait-shyness) toward sunflower seeds (an unfamiliar food type) that decreased over successive exposures. Spillage data revealed findings identical to those for consumption, so they are not reported here.

Table 2. Mean consumption by rats of NdC, BRS-1, J550, and J502 sunflower seeds in completely counterbalanced two-choice preference tests. The standard error of the mean ranged from 0.6 to 2.1 for the means presented.

	Grams of seed consumed/rat/hour[1]			
Two-choice tests	**NdC**	**BRS-1**	**J550**	**J501**
NdC vs. BRS-1	3.0	5.4		
NdC vs. J550	4.5		3.2	
NdC vs. J501	4.2			4.2
BRS-1 vs. J550		4.6	2.6	
BRS-1 vs. J501		4.5		2.8
J550 vs. J501			3.9	3.9
Overall mean consumption	4.2	4.2	3.6	3.6

[1]There were no significant ($P < 0.25$) differences in consumption within varietal pairs or among the four varieties in overall consumption.

The results of Experiment 2 suggest that, even under conditions designed to maximize detection of differential consumption, rats failed to exhibit preferences among the sunflower cultivars. This suggests that NdC and BRS-1 attributes that confer bird resistance do not affect consumption by rats.

Experiment 3 (Preference of birds for hulled seed from three varieties.)

There were no differences in consumption among groups of birds. However, there were significant differences in consumption among variety pairs, as well as differences in consumption within each pair (Table 3). The interaction between consumption among and within pairs was also significant. The birds consumed slightly but significantly more of either BRS-1 or NdC than J550, a result opposite to that obtained with unhulled seeds, so the bird repellent characteristics of the BRS-1 and NdC varieties were apparently concentrated or present solely in seed hulls.

Table 3. Mean consumption by red-winged blackbirds of NdC, BRS-1, and J550 seeds (with hulls removed) in completely counterbalanced two-choice tests. The standard error of the mean ranged from 0.06 to 0.14 for the means presented.

	Grams of seed consumed/bird/hour[1]		
Two-choice tests	**NdC**	**BRS-1**	**J550**
NdC vs. BRS-1	1.4a	1.4a	
NdC vs. J550	1.6a		1.3b
BRS-1 vs. J550		1.7a	1.2b
Overall mean consumption	1.5a	1.5b	1.3b

[1]Means in same row with different letters are significantly ($P < 0.05$) different.

Experiment 4 (Preference of birds for extracts from seed hulls of two varieties.)

There were differences among extraction procedures, stimulus concentrations, and within two-choice tests (Table 4). Also, there were significant two-way interactions between extraction procedures and concentrations and concentration and two-choice tests. Post-hoc tests revealed that both water and MeOH/HCl extracts of NdC and water extracts of J550 were avoided. However, avoidance of NdC extracts was relatively stronger and persisted at lower concentrations. MeOH/HCl extracts of NdC were avoided at lower concentrations than water extracts, and within two-choice tests at each concentration, less MeOH/HCl extract was consumed than water extract ($P < 0.05$).

Experiment 5 (Preference of birds for seeds with and without extracts removed from hulls.)

There were significant differences within two-choice tests and a significant interaction between days and two-choice tests (Table 5). NdC consumption was significantly lower than consumption of the available alternative in all tests. More NdC-E was consumed than NdC, but relatively less NdC-E was eaten in tests with either J550 or J550-E. When consumption in test of NdC versus J550 or J550-E was compared with that in tests of NdC-E versus J550 or J550-E, consumption of NdC-E was significantly higher than consumption of NdC. There were no significant differences in consumption between J550-E and J550.

Table 4. Mean consumption (ml) by red-winged blackbirds in two-choice drinking tests of 1) water-extracted anthocyanin from hulls of NdC seeds, 2) MeOH/HCl extracted anthocyanin from hulls of NdC seeds, and 3) water-extracted material from hulls of J550 seeds. The standard error of the mean ranged from 0.3 to 4.4 for the means presented.[1]

% Conc.	H_2O Extracted Anthocyanin (NdC)	Distilled H_2O	MeOH/HCl Extracted Anthocyanin (NdC)	Distilled H_2O	H_2O Extracted Material (J550)	Distilled H_2O
2.5	5.1a	16.1b	1.5a	10.4b	0.9a	13.4b
2.0	5.8a	17.4b	2.7a	13.6b	3.1a	11.5b
1.5	6.8a	16.3b	3.7a	12.2b	3.2a	14.0b
1.0	8.3a	14.6b	5.1a	13.5b	8.7a	9.1a
0.5	7.8a	12.1a	6.1a	13.5b	5.7a	8.2a
0.0	11.4a	8.1a	4.6a	9.5a	8.2a	4.8a

[1]Means for each pair of comparisons within a row with different letters are significantly ($P < 0.05$) different.

Table 5. Mean consumption by red-winged blackbirds of untreated and water-extracted (-E) seeds of NdC and J550 sunflower varieties in two-choice preference tests. The standard error of the mean ranged from 0.1 to 0.3 for the means presented.

	Grams of seed consumed/bird/hour[1]			
Two-choice tests	NdC	NdC-E	J550	J550-E
NdC vs. NdC-E	1.0a	2.5b		
NdC vs. J550	0.6a		1.7b	
NdC-E vs. J550		1.0a	2.0b	
NdC vs. J550-E	0.5a			1.1b
NdC-E vs. J550-E		1.3a		2.2b
J550 vs. J550-E			1.8a	1.5a
Overall mean consumption	0.7	1.6	1.8	1.6

[1]Means in same row with different letters are significantly ($P < 0.05$) different.

Discussion

The results of these experiments suggest that NdC and BRS-1 varieties possess bird repellent characteristics that do not affect consumption by rats. That unhulled NdC seed was more repellent to red-winged blackbirds than BRS-1 is consistent with two-choice outdoor aviary tests in which seeds were presented to brown-headed cowbirds (*Molothrus ater* L.) (R.A. Dolbeer, Unpubl. data). However, such preference for BRS-1 over NdC is opposite to field observations (Dolbeer et al., 1985). We speculate that BRS-1 seeds are relatively less repellent in the laboratory than in the field because the seeds by themselves do not possess all the repellent characteristics of the variety.

Before harvest, BRS-1 seeds are held tightly in concave heads, with long bracts, and the heads face the ground (Parfitt 1984). Each of these features may confer resistance to bird damage. However, the fact that hulled seeds of both resistant varieties were as preferred as hulled J501 and J550 seeds indicates that at least some repellent property (either chemical or otherwise) is inherent in the seed hulls of BRS-1 and NdC.

The bird repellency of BRS-1 seeds in our experiments was probably due to the hardness of the seed hulls, although this hypothesis remains to be tested. With regard to NdC, repellency was apparently mediated by flavor characteristic. After water extraction, NdC seeds were more readily consumed by red-wings. That the NdC cultivar remained less preferred than the J550 after water extraction suggests that at least some of the repellent flavor characteristics remained in the hulls.

We speculate that NdC repellency was due (at least in part) to anthocyanins present in the seed hulls. The extracted anthocyanin-containing fraction was repellent to red-wings in two-choice tests both at the approximate concentration present in seed hulls (2.0% w/w) and at half this concentration (1.0% w/w).

In addition to its possible value as a taste repellent for birds, anthocyanins have commercial value as natural food dyes (Vaccari et al. 1982). The anthocyanin yield from the NdC variety (approximately 2.0 percent) is higher than for any other known natural source (D. DeRovira, National Starch and Chemical Corporation, pers. commun.).

The reasons underlying the greater repellency of MeOH/HCl extract, relative to water extract, of the NdC cultivar is unclear. Possibly the difference is in the chemical compositions of the extracts themselves. There is experimental evidence that water extraction of anthocyanins from sunflower seeds yields protein-bound anthocyanin components whereas extraction using MeOH/HCl gives free anthocyanins (Pifferi and Vaccari, 1983). If this is so, then the enhanced repellency of the MeOH/HCl extract could have resulted from a material dilution effect in the water extract.

An unexpected result was that water extracts of the J550 variety were also repellent, though at relatively higher concentrations. It may be that other chemicals in sunflower hulls can exert repellent effects on birds. The possibility of additional repellent compounds in hulls is intriguing because it suggests that commercial varieties of sunflower may already possess slight bird repellent characteristics that can be enhanced.

In summary, these laboratory experiments support findings from field studies that certain varieties of sunflower possess bird-repellent properties and provide insight into the mechanisms of this repellency. We believe that varietal resistance is a viable approach for reducing the economic losses to sunflower caused by birds. Cooperative research among plant breeders, plant chemists, and vertebrate pest biologists will be needed to develop and evaluate commercially competitive lines of bird-resistant oilseed sunflower.

References

BESSER, J.F. 1978. Birds and sunflower. In Sunflower Science and Technology. (Carter, J.F., ed.). Amer. Soc. Agron., Crop Sci. Amer., Soil Sci. Soc. Amer. Inc., Madison, WI, pp. 263-278.

DOLBEER, R.A., P.P. WORONECKI, R.A. STEHN, G.J. FOX, J.J. HANZEL, AND G.M. LINZ. 1986. Field trials of sunflower resistant to bird depredations. North Dakota Farm Research 43(6):21-24.

FOX, G.J. AND G.M. LINZ. 1983a. Bird resistant sunflower. Proc. Sunflower Research Workshop, Minot, ND, 10-11.

FOX, G.J. AND G.M. LINZ. 1983b. Evaluation of red-winged blackbird resistant sunflower germ plasm. Proc. Bird Control Seminar, Bowling Green State Univ., Bowling Green, OH, 9:181-189.

FOX, G.J., G.M. LINZ, AND L. LINZ. 1984. Development of bird resistant sunflower lines. Proc. Sunflower Research Workshop, Bismarck, ND, 8.

MASON, J.R. AND R.F. REIDINGER. 1983. Importance of color for methiocarb-induced food aversions in red-winged blackbirds (*Agelaius phoeniceus*). J. Wildl. Manage. 47:383-393.

PARFITT, D.E. 1984. Relationship of morphological plant characteristics of sunflower to bird feeding. Can. J. Plant Sci. 64:37-42.

PIFFERI, P.G. AND A. VACARRI. 1983. The anthocyanins of sunflower: II. A study of the extraction process. J. Food Technol. 18:629-638.

ROGERS, J.G. 1974. Responses of caged red-winged blackbirds to two types of repellents. J. Wildl. Manage. 38:418-423.

VACARRI, A., P.G. PIFFERI, AND G. ZACCERINI. 1982. Anthocyanins of sunflower (*Helianthus annus*). J. Food Sci. 47:40-42.

WINER, B.J. 1962. Statistical principles in experimental design. McGraw-Hill Book Co., New York, NY. 907 pp.

Continued from page 11

These data indicate conditions for soil salinization exist over all the state where the saline ground waters are shallower than critical depth. In addition, conditions for salinization occur anywhere a saline water is used for irrigation, or where saline ground water is raised higher than critical depth through poor irrigation management, or through too frequent summer fallowing which promotes saline seeps.

Natural conditions of salinization in North Dakota help to identify reclamation methods and preventive measures against salinization. Reclamation includes draining high level saline groundwaters and leaching salts already concentrated in the soil root zone. Prevention includes using good quality irrigation waters and maintaining groundwater levels as deep as possible.

References

Benz, L.C., R.H. Mickelson, F.M. Sandoval and C.W. Carlson. 1961. **Ground-Water Investigations in a Saline Area of the Red River Valley, North Dakota.** Journal of Geophysical Research, 66:2435-2443.

Johnsgard, G.A. 1967. **Salt Affected Problem Soils in North Dakota.** Extension Bulletin No. 2, Cooperative Extension Service, North Dakota State University, 15 pp.

Maianu, A. 1985. **Salt Accumulation in the Rivers of North Dakota.** Journal of Environmental Quality, 14:211-217.

Maianu, A. 1985a. **Salt Accumulation in the Lakes of North Dakota.** (In revision).

Maianu, A., J.L. Richardson and P. Held. 1985. **Patterns of Salt Accumulation in the Groundwaters of North Dakota.** (In revision).

Omodt, H.W., G.A. Johnsgard, D.D. Patterson and O.P. Olson. 1968. **The Major Soils of North Dakota.** Bull. No. 472, Department of Soil Science, North Dakota State University, Fargo, 60 pp.