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Biochemical and Morphological Characteristics in Maturing Achenes from Purple-Hulled and Oilseed Sunflower Cultivars

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The blackbird feeding preference for sunflower oilseed variety Jacques Discovery (JD) over purple-hulled Neagra de Cluj (NdC) has been attributed to anthocyanin. To test this hypothesis, we compared biochemical and morphological properties in the achene over the maturation period. Comparisons included total phenols, anthocyanin, tannin, fat/oil, protein, nonstructural sugars, and phytomelanin contents, as well as mass properties and moisture content. Only three differences were observed: (1) Hull mass was significantly higher for NdC than JD. (2) Anthocyanins were synthesized by NdC only. (3) JD was higher in oil content. Each of these factors may play a role in reduced preferences exhibited by birds for NdC.

Bird-resistant traits in ripening agricultural crops usually involve a combination of morphological and biochemical

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characteristics (Bullard and York, 1984). In developing bird-tolerant cultivars the two modes of protection are usually pursued simultaneously, resulting in cultivars that contain traits of both. This has been a productive approach in sorghum (Voight, 1966) and corn (Dolbeer et al., 1982) and is the one being followed by plant geneticists working on bird-resistant sunflower varieties (Fox and Linz, 1983; Foley and Hanzel, 1986). Morphological features thought to increase resistance include concave heads, long bracts, heads that face the ground, head-to-stem distances exceeding 15 cm, and seeds with tough fibrous hulls (Parfitt, 1984). Biochemical features thought to increase resistance include chlorogenic acid (Harada, 1977) and anthocyanins in purple-hulled varieties (Dolbeer et al., 1986; Fox and Linz, 1983; Mason et al., 1986). Chlo-

rogenic acid has since been rejected (Fox and Parfitt, 1982).

Anthocyanin in purple-hulled varieties is entirely concentrated into a highly accessible thin layer in the pericarp hypodermal cells (Knowles, 1978) among hairs protruding from the surface. Thus, the chemical is readily accessible to removal through contact or by extraction. Anthocyanin pigments are pH sensitive: Below pH 3 solutions display their most intense red coloration while in the range pH 4–5 they become almost colorless. Further increases in pH give rise to purples or blues similar to that observed *in vivo* in purple-hulled sunflower varieties (Jackman and Yada, 1987). Anthocyanins are particularly recognized among the phenolics for their instability, which makes it difficult to relate laboratory extractions and analyses with the natural state found in the plant source. The extraction of NdC hulls with water produces an extract that turns brown on standing, and the often-used 1% HCl in methanol extraction medium will hydrolyze acylated anthocyanins at room temperature (Holm, E. T., 1987, personal communication).

In one series of laboratory taste preference tests of achenes from purple-hulled Neagra de Cluj (NdC) and three other sunflower varieties on individually caged red-winged blackbirds (rwbb; *Agelaius phoeniceus*), there were some indications of anthocyanin avoidance (Mason et al., 1986). However, a companion series of aviary and field tests of feeding preference among heads from the standing crops (Dolbeer et al., 1986) indicated that initially birds avoided NdC but that the resistance "began to break down under severe feeding pressure when a convenient source of preferred sunflower seed was depleted".

When the anthocyanin question was pursued further, the present assessment of maturing achene characteristics was compared with feeding preference behavior (Mason et al., 1989) in field, outdoor aviary, and laboratory tests. The objectives were to measure several biochemical and morphological properties of NdC and Jacques Discovery (JD, a commercial oilseed variety) achenes at each of six maturational stages, to correlate these data with those from the behavioral tests at each stage, and to attempt to relate behavioral responses to differences in the biochemical and morphological properties.

MATERIALS AND METHODS

Sampling. Sunflower heads of synchronous maturity from JD and NdC varieties (planted 14 June 1986; Erie County, OH; Mason et al., 1989) were collected randomly throughout the field on six dates from 19 Aug to 29 Sept 1986 (5, 10, 15, 25, 35, and 45 days after bloom (DAB)). To minimize biochemical changes that could influence chemical analytical test data, each head was quick-frozen immediately after cutting by immersion in liquid nitrogen. Subsequently, in a cold room an achene sample for each test category was separated from debris, lyophilized, and held at -5°C . For tests requiring dehulling, hulls were removed from the respective sample aliquot by hand with forceps. All grinding of whole achenes, meats, or hulls was to 20 mesh with a Wiley mill.

Achene, Meat, and Hull Weights: Moisture. Hulls were separated from the meats of three randomly selected 50 achene sample aliquots and weighed. Whole achene mass was determined on another set of three randomly selected sample aliquots. For moisture determinations, achene samples collected fresh were held in a 30°C drying room for 3 weeks.

Phytomelanin (Armored Layer) Detection. Six randomly selected achene sample aliquots from each sunflower sample were placed in a 3:1 saturated potassium dichromate-concentrated sulfuric acid solution and held for 30 min (Seiler et al., 1984). After each immersion, a black coating on the hull indicates the presence of an armored layer while an achene without the layer appears white.

Phenolic Compounds. For examination of total phenolics, 1-g sample aliquots of ground whole achenes were extracted with

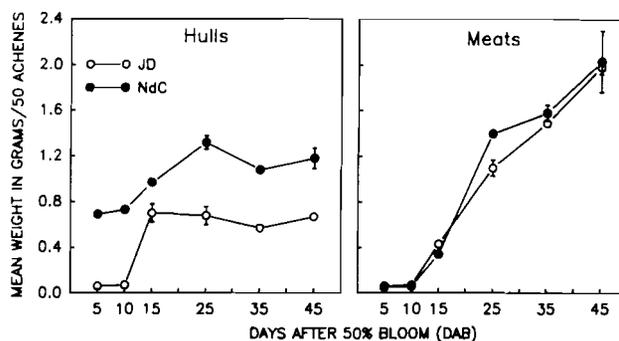


Figure 1. Mean weight (g) of hulls and meats from 50 achenes in each maturity class (i.e., 5, 10, 15, 25, 35, and 45 DAB) in JD and NdC.

5 mL of methanol for 1 h on a platform shaker and centrifuged at 4500 rpm for 10 min. A 50- μL sample of the supernatant was combined with 2 mL of water and 0.2 mL of Folin-Ciocalteu reagent (Sigma, St. Louis, MO). The solution was vortexed and allowed to stand for 1 min. Next, 5 mL of 1 M ethanolic ammonia was added and the solution vortexed and timed for 10 min to determine A_{750} of the phosphomolybdate blue color on a Beckman DK2A spectrophotometer. Catechin was used for the standard curve, and data are reported in terms of catechin equivalents.

Tannin analyses were conducted by determining the protein-binding activity of methanol extracts from ground achenes in a bovine serum albumin solution, using quantities identical with specifications for analysis of sorghum grain (Bullard et al., 1981).

A new method was developed for determining anthocyanin concentrations in achene hulls. A 250-mg ground hull sample aliquot was extracted with 10 mL of 0.5% HCl in methanol by shaking for 15 min on a platform shaker, the mixture immediately centrifuged at 6500 rpm for 10 min, and the supernatant stored in a freezer until analysis. In 280–800-nm spectra conducted on a Beckman DK2A recording spectrophotometer, the absorbance maximum for NdC anthocyanin was found to be at 528 nm. Since cyanidins are reported to be one of the important structural components involved in anthocyanin formation in sunflowers (Holm, E. T., personal communication), and its absorption maximum was almost identical with that of ND anthocyanin, cyanidin-Cl (K&K Laboratories, Plainview, NY) served as the reference standard for quantitative analysis purposes. Anthocyanin A_{528} values were determined on all NdC and JD samples and are reported as cyanidin equivalents.

Total Nonstructural Carbohydrates (Sugars). Ground meats were analyzed by standard methods (Smith et al., 1964) for total nonstructural carbohydrates (TNC) by the Range Science Nutritional Analysis Laboratory, Colorado State University, Fort Collins, CO. Briefly, ground meats were digested in H_2SO_4 , and an iodometric technique was used to analyze the resulting extract.

Nitrogen and Protein. Ground meats were analyzed for nitrogen and protein content by the Range Science Nutritional Analysis Laboratory, Colorado State University, Fort Collins, CO, using a standard micro-Kjeldahl method (Cole and Parks, 1946).

Lipids. Ground meats were analyzed for fat and oil content by Agricultural Consultants, Inc., Brighton, CO. Two-gram samples were extracted for 4 h on a Goldfish fat/oil extractor, according to AOAC methods (1975).

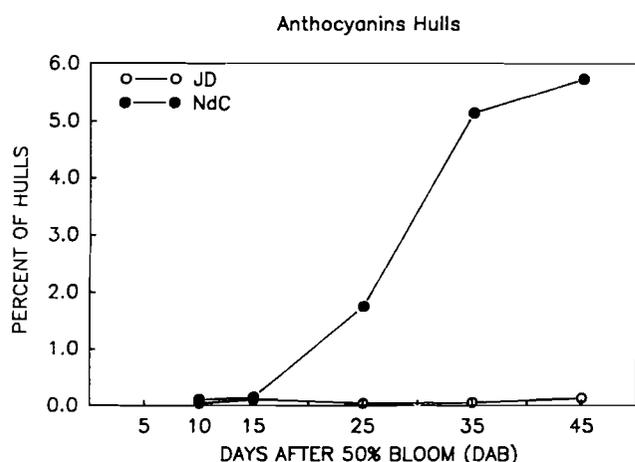
Data Analyses. Each sample (i.e., achenes from either NdC or JD collected at one of the six sampling dates) was analyzed by each of the above methods for the respective value. Mean values, collapsed across DAB, were computed for NdC and JD in each assessment. These means were then evaluated in a Wilcoxon signed rank two-tailed test with $P = 0.05$ level of significance.

RESULTS

Achene, Meat, and Hull Weights. NdC had significantly higher achene and hull mean weight values than JD (Figure 1). There were no overall differences in meat weights or in moisture content between NdC and JD. Developmentally, biosynthesis of hull structures predominated until 10 DAB, with little change in achene mass.

Table I. Biochemical Properties of Ripening Achenes from Neagra de Cluj (NdC) and Jacques Discovery (JD) Varieties

variety	DAB ^a	achene			meat		hull:
		% moisture	% fat/oil	% phenolics	% protein	% sugars	% anthocyanin
JD	5	77	5.0	0.0			0.00
JD	10	81	4.8	0.13	17.89	38.65	0.04
JD	15	74	14.1	0.17	22.45	17.71	0.11
JD	25	58	40.1	0.10	21.82	3.37	0.04
JD	35	48	49.8	0.12	18.11	1.55	0.06
JD	45	22	48.7	0.63	20.35	3.91	0.13
NdC	5	78	2.6	0.05			0.11
NdC	10	82	3.2	0.11	17.81	36.75	0.11
NdC	15	75	5.8	0.12	23.04	25.17	0.15
NdC	25	57	27.0	0.15	31.12	2.53	1.75
NdC	35	42	30.6	0.35	31.42	2.61	5.14
NdC	45	21	33.3	0.46	30.07	4.58	5.73

^a Days after bloom.**Figure 2.** Percent of anthocyanin in hulls from JD and NdC for each maturity class (i.e., 5, 10, 15, 25, 35, and 45 DAB).

By 15 DAB hull development had plateaued, and meat weights began to increase with a burst of development between 15 and 25 DAB and then a slower, steadier growth until 45 DAB.

Phytomelanin Detection. There was no armored layer detected on the hulls of either variety.

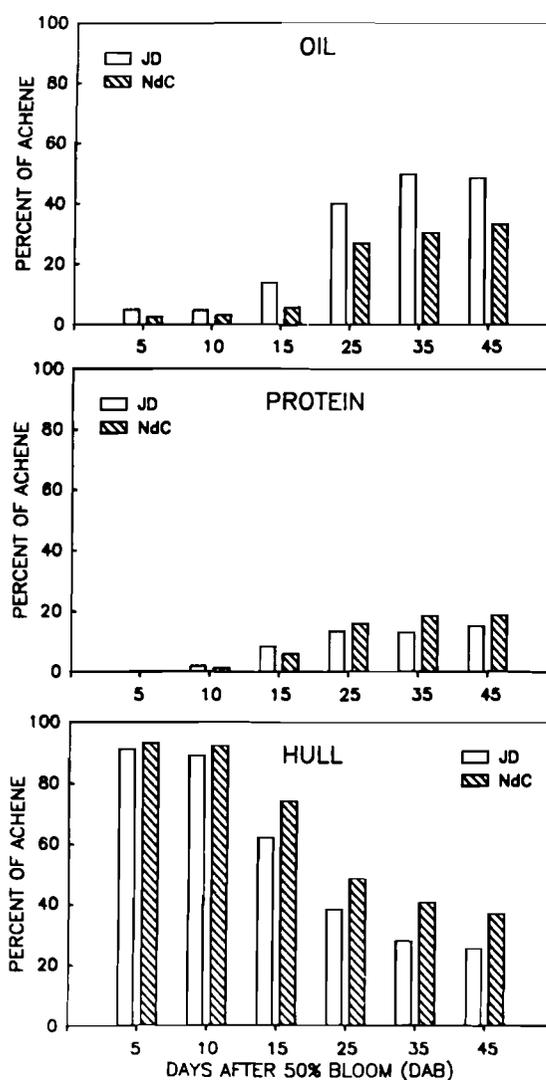
Phenolic Compounds. Similar levels of total phenolics (Table I) were detected in both NdC and JD. Tannins were not detected in either variety, but obviously other compounds such as chlorogenic acid (Harada, 1977) were present.

Anthocyanin concentrations were significantly higher in NdC than JD. Table I and Figure 2 indicate that up to 0.13% of the anthocyanin values was attributed to trace quantities of other compounds detectable by the analytical method. Biosynthesis of anthocyanin began between 15 and 25 DAB and accelerated over later sampling dates. By 45 DAB, anthocyanins comprised nearly 6% of NdC hull mass.

Total Nonstructural Carbohydrates. There were no differences in TNC for the two varieties. Table I suggests that sugars were a major component in the meats of both varieties until 15 DAB. However, little biosynthesis occurred subsequently, and at later sampling dates, sugars were a minor component of meat masses.

Protein. There were no overall differences between JD and NdC in nitrogen content or percent protein. However, the data do suggest greater protein values in NdC at 25, 35, and 45 DAB (Table I; Figure 3).

Lipids. Meats from JD samples were significantly higher in oil than meats from NdC samples. Examination of Table I and Figure 2 indicates that oil biosynthesis

**Figure 3.** Percent of oil, protein, and hull in achenes from each maturity class (i.e., 5, 10, 15, 25, 35, and 45 DAB) of JD and NdC.

began between 10 and 15 DAB, with the bulk of biosynthesis occurring between 15 and 25 DAB.

DISCUSSION AND MANAGEMENT IMPLICATIONS

NdC and JD appear to differ most in hull weight (NdC is greater), anthocyanin concentration (NdC is higher), and oil concentration (NdC is lower). Any one of these features may influence consumption by birds.

Regarding anthocyanin as a taste repellent to *rwbb*, the chronology of biosynthesis in this study raises some questions concerning the influence of anthocyanin con-

centration on feeding behavior. JD was highly preferred at each test date in one-choice aviary tests (Mason et al., 1989), yet at 15 DAB there are only 0.15% anthocyanin and 1.75% at 25 DAB in NdC hulls. It was between 25 and 35 DAB where the big increase (nearly 3-fold) in anthocyanin synthesis occurred. The heaviest feeding for NdC in the no-choice test and for both varieties in the one-choice test occurred at 10 and 15 DAB. Our results coincide with an oilseed sunflower damage chronology study conducted between 1979 and 1982 by Cummings et al. (1989). In their studies (adjusted to our DAB scale), over 75% of the total bird damage occurred within the first 28 DAB, with the peak occurring between 16 and 25 DAB.

However, total hull anthocyanin on a dry-weight basis may be somewhat misleading. Factors other than quantity could influence chemoreception in rwbb. For example, moisture content in the hypodermis cellular structure could increase the release of anthocyanin into the moist mouth of a bird during the brief manipulation required for dehulling. We did not observe an appreciable drop in moisture content (Table I) of the samples until after 15 DAB, and then it decreased as anthocyanin increased. This hypothesis is not consistent, however, with the results obtained in recent studies; two experiments involving visual examination of rwbb feeding behavior on immature NdC indicated that taste was not involved in rwbb avoidance (Mah, 1988).

Regarding oil content, some consider that birds prefer foods containing greater amounts of lipid (Besser, 1978). If rwbb seed consumption is related to oil content, the higher levels in JD (Table I; Figure 2), especially at 25, 35, and 45 DAB, could influence the feeding behavior of rwbb in tests involving a feeding choice between the two. In a study similar to this one, Samanci (1987) compared bird damage to oilseed variety hybrid 894, NdC, and candidate morphological resistant hybrids BRS-1 and ND 80116-15-1 and concluded that "genotypes with higher kernel and oil contents suffer heavier damage".

Differences in achene and hull mass may also have influenced rwbb feeding patterns in our studies. The difference in hull mass was especially noticeable in the early stages (Table I; Figures 1 and 2). At 15 DAB hull formation was nearly complete, very little oil formation had taken place, and there were no significant differences in protein or sugars, yet JD was highly preferred in field and aviary studies (Mason et al., 1989). Thus, until 15 DAB hull mass is the best explanation we have of preference differences in this study. The pattern may have continued throughout the remaining test dates considering the differences in hull mass between the two varieties.

The optimal feeding theory predicts that birds should choose the food item maximizing the net energy intake (Pyke, 1984). If this applies to the feeding choices in our study and a thinner hull makes dehulling an easier task, birds would tend to favor JD as a food item. Oil is a rich source of energy and dehulling activity by birds consumes feeding energy, meaning birds would benefit by (1) expending less energy in dehulling JD (2) to reach a meat of higher energy value.

Across the industry, sunflower breeders generally recognize that hull mass and seed size are inversely correlated with oil content (Fick, 1978; Fox and Linz, 1983). Perhaps, this is the reason that confectionary varieties are less desirable than oilseed varieties (Besser, 1978; Fick, 1978; Fox and Linz, 1983). Lofgren (1978) published data of 45.8% and 28.2%, respectively, for the average hull and oil content in four typical confectionary varieties while Dorell (1978), in the same book, gave values for five oilseed

cultivars averaging 24.4 and 50.5%, respectively. These data are very similar to ours for NdC and JD (Figure 2), indicating that NdC is more like a confectionary variety than an oilseed variety. Samanci (1987) went so far as to agronomically categorize it with two candidate morphological genotypes having thick hulls and low oil content.

Thus, evidence is mounting that factors other than anthocyanin could be involved in reduced preference for NdC when it is accompanied by the presence of an oilseed variety. Further studies are being planned to explore the importance of hull mass and oil content in rwbb feeding behavior and to conduct a specific taste repellent study on extracted sunflower anthocyanin.

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