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RELATIONSHIP OF CHLOROGENIC ACID CONCENTRATION IN SUNFLOWER ACHENES TO BIRD PREDATION OF SUNFLOWER

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Blackbird feeding on sunflower is a significant problem in parts of the Northern Great Plains. Therefore, experiments were initiated to determine the relationship of chlorogenic acid (CA) in sunflower achenes to bird predation and the amount of variability for CA in sunflower genotypes. Eight inbred sunflower (*Helianthus annuus* L.) genotypes with variable levels of resistance to blackbird (*Agelaius phoeniceus* L. and *Xanthocephalus xanthocephalus* Bonaparte) and house sparrow (*Passer domesticus* L.) predation were evaluated at two locations in 1979 and 1980 for seed and hull CA. Differences were observed among inbred sunflower genotypes for CA concentration in both hull and seed. However, no significant correlation between percent CA in seed and mean percent bird damage over the four tested environments was observed. An unexpected positive correlation for hull CA and bird damage was found, however. Achene samples were taken from three positions within the sunflower head (outside, middle and inside) from seven inbred lines at three sampling dates. Genotype influenced CA level in both achene components while sampling date influenced CA level in the seed only. Position of achenes within the sunflower head did not influence CA concentration of either hull or seed. Thus, it is unlikely that the CA concentration of physiologically mature sunflower achenes (hull or seed) causes the observed bird predation pattern (edge to center) on sunflower heads. CA concentration is not apparently related to bird damage.

Key words: Sunflower, bird feeding, chlorogenic acid, *Helianthus annus* L., blackbird

Les rapports entre la teneur en acide chlorogénique des akènes du tournesol et leur destruction par les oiseaux.

Titre abrégé: Acide chlorogénique et destruction des akènes chez le tournesol.

Les ravages faits par les carouges dans les cultures de tournesol entraînent des pertes importantes dans certaines régions du nord des Grandes Plaines. Nous avons donc mené des expériences visant à déterminer les rapports entre le teneur en acide chlorogénique des akènes et le degré de destruction par les oiseaux ainsi que la variabilité de la teneur en acide chlorogénique d'un génotype de tournesol à l'autre. Nous avons mesuré la teneur en acide chlorogénique (CA) du grain et des enveloppes de huit génotypes autofécondés de tournesol (*Helianthus annuus* L.) présentant divers degrés de résistance aux attaques du carouge à épaulettes (*Agelaius phoeniceus* L.), du carouge à tête jaune (*Xanthocepahlus xanthocephalus* Bonaparte) et du moineau domestique (*Passer domesticus* L.) à deux emplacements, en 1979 et en 1980. Nous avons observé des différences dans la teneur en CA des génotypes, dans le grain et dans l'enveloppe. Toutefois, nous n'avons déterminé aucune corrélation significative entre la teneur en CA du grain et le taux de destruction moyen. Une corré-

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lation positive inattendue a cependant été observée entre la teneur en CA de l'enveloppe et les dommages dus aux oiseaux. Nous avons prélevé des éhcantillons d'akènes de trois positioins différentes dans le capitule des tournesols (à l'extérieur, au centre et à mi-chemin entre ces deux positions) chez sept lignées autofécondées et à trois dates différentes. La génotype semble influer sur la teneur en CA du grain et de l'enveloppe tandis que la date d'échantillonnage n'influe que sur la teneur en CA du grain. La position de l'akène dans le capitule ne semble pas influer sur la teneur en CA du grain ni sur cells de l'enveloppe. Ainsi, il semble peu vraisemblable que l'habitude qu'ont les oiseaux de s'attaquer d'abord aux akènes situés à l'extérieur soit attribuable à la teneur en CA des akènes matures (grain et enveloppe). Par ailleurs, il ne semble pas y avoir de rapport entre le teneur en CA et les ravages causés par les oiseaux.

Mots clés: Tournesol, ravages par les oiseaux, acide chlorogénique , *Helainthus annuus* L., carouge.

Redwinged blackbirds (*Agelaius phoeniceus* L.) and yellow-headed blackbirds (*Xanthocephalus xanthocephalus* Bonaparte) often cause significant damage to the sunflower crop near wetland areas in the Northern Great Plains of Canada and the United States (Besser 1978). Modification of sunflower plant morphology produced some reduction of damage due to bird feeding (Parfitt 1984). However, additional sources of resistance to bird predation are needed to develop highly resistant sunflower genotypes. The present study was initiated to determine whether phenolic acids in sunflower achenes would reduce bird feeding and whether significant variability for phenolic acids in sunflower achenes or seeds was available for use in sunflower breeding programs.

Sorghum (*Sorghum bicolor* L. Moench.) germplasm with known resistance to bird predation has been in use since the mid 1960s. McMillan et al. (1972) reported a highly significant negative correlation ($r = 0.622$) between bird damage and tannin (a phenolic acid) in the grain pericarp. Tipton et al. (1970) reported that the tannic acid concentration of bird-resistant sorghum was approximately eight times that of bird-susceptible sorghum hybrids. Green (1974) related in vitro organic matter digestion and bird resistance to tannin content of sorghum, and found a highly significant negative correlation ($r = 0.91$) between digestion and tannin content.

Sunflower achenes do not contain tannins but do contain other types of phenolic acids. Chlorogenic acid (CA) is the major phenolic acid in sunflower (Dorrell 1978). Genetic differences for levels of CA in sunflower have been reported by various researchers. Dorrell (1976) reported levels of 1.42–4.0% CA for 167 accessions from the world collection grown at Morden, Manitoba. Some of the highest CA concentrations were observed for improved oil varieties (Peredovik and Krasnodarets). Dorrell (1976) examined 42 wild sunflower seed samples and found CA concentration to be low (1.58–2.70%). Commercial oilseed hybrids probably contain higher levels of CA since seed CA content is highly correlated with high oil concentration and low hull percentage of achenes (Dorrell 1976). Sosulski et al. (1972) reported a CA range of 1.5–2.0% for seed from four sunflower genotypes, while Pomenta and Burns (1971) listed the CA range of seeds from 10 sunflower genotypes grown commercially in various parts of the U.S. as 1.14–2.22%. CA concentration is also strongly influenced by environmental factors. Short growing seasons and cool temperatures are associated with low levels of CA (Dorrell 1978).

Pomenta and Burns (1971) reported that seeds located within 5 cm of the center of the head contained up to twice as much CA as seeds located toward the periphery, indicating the need for careful sampling due to position effect. They suggested that differences in physiological maturity between less mature achenes at the center of the head and more mature achenes toward the margin may affect CA levels. Conversely, Dorrell (1978) has reported that seed CA concentration increases steadily during seed development. Thus, high CA achenes would be located at the periphery of the heads. If location of the achenes within the head affects CA percentage of hull or seed at physiological maturity (20–35% moisture), the observed bird feeding patterns in sunflower (from the outside of the head) could be correlated with the location of high CA achenes in the sunflower head.

Two experiments were performed to elucidate the relationship between CA concentration and predation by redwinged or yellowheaded blackbirds and house sparrows (*Passer domesticus* L.).

MATERIALS AND METHODS

A method described by Sosulski (pers. commun.) was used for CA extraction. Achenes were dehulled and separated into hull and seed. Each sample was dried for 2 h at 75°C. Hulls were ground with a Wylie mill to pass a 20-mesh screen; seeds were finely ground with a mortar and pestle. Samples (50 mg) were placed in 70% methanol solution and shaken for 2 h. After filtration, the absorbance of each sample was measured at 328 nm (Milic et al. 1968). The concentration of CA was determined by reference to a standard curve prepared with pure CA.

Date × Genotype × Head Position

Two heads from each of seven inbred sunflower genotypes were harvested on three dates in 1980. Heads were divided into three concentric rings of achenes approximately 2.5 cm wide. Achenes from each ring were collected and stored in plastic bags at 0°C prior to CA determinations. The experiment was arranged as a 3 × 7 × 3 factorial with two replications. Sampling date, genotype, and radial position of achene to the head were considered to be fixed factors, while heads sampled (replications) was a random factor. Analyses of variance were computed for percent CA in the hull and seed using the Statistical Analysis System's general linear models procedure. After the data were analyzed, means were compared using Tukey's multiple range test.

Inbred × Environment

Eight inbred genotypes varying in susceptibility for resistance were evaluated at two locations in 1979 and 1980, one incurring blackbird damage (Alice or Chaffee) and the other sparrow damage (Fargo). At each location inbreds were grown in two-row plots, 7.62 m long, in a randomized complete block design with two replicates. Achenes were taken from sunflower plants that had reached physiological maturity to ensure that maximum CA content had been established (Dorrell 1978). Samples were taken prior to significant bird predation. Achene samples were consistently taken from an area 4–5 cm in diameter, 2 cm from the periphery of the sunflower head. Samples were sealed in plastic bags and stored at 0°C before analysis for CA. Bird damage was estimated as a percentage of the total seeds removed from the head.

Analyses of variance were computed for hull CA percentage, seed CA percentage, and percent bird damage. Genotype was considered to be a fixed factor while environments (location × year) and replications/environment were random. The Statistical Analysis System's general linear models procedure was used to calculate the analyses of variance. After the data were analyzed, genotypic means were compared using Fisher's protected LSD procedure. Correlations between percentage hull CA vs. percent bird damage and percentage seed CA vs. percent bird damage were computed on an individual plant basis for each of the four environments tested. Correlations were also calculated using the genotypic means averaged over environments.

The eight inbreds used in the inbred × environment experiment and the 41 additional inbred genotypes were grown at Fargo in 1980, harvested in bulk, and tested for CA percentage to determine if greater variability could be detected among the additional inbreds.

RESULTS AND DISCUSSION

Date × Genotype × Head Position

Hull CA and seed CA concentrations were influenced by genotype (Table 1). HA291 had significantly higher CA in the hull than the other inbreds (Table 2). Date of sam-

Table 1. Analysis of variance for hull CA and seed CA of seven inbred sunflower genotypes sampled by head position on three dates

Source of variation	df	Mean square Hull CA	Seed CA
Date (D)	2	0.010	0.592**
Inbred (I)	6	0.492**	1.065**
I × D	12	0.023*	0.105*
Position (P)	2	0.022	0.007
D × P	4	0.003	0.002
I × P	12	0.006	0.010
I × D × P	21	0.004	0.016
Error	56	0.012	0.048

*,** Significant at the 0.05 and 0.01 levels, respectively.

Table 2. Means for hull CA and seed CA for seven inbred sunflower lines sampled by head position on three dates

Source	Hull CA	Seed CA
Inbred		
Peredovik†	0.15*ab*	1.80*cd*
Impira INTA†	0.09*a*	1.53*b*
Advance 946†	0.11*a*	1.53*b*
HA 291	0.60*c*	1.24*a*
HA 234	0.17*ab*	1.75*c*
Chernianka†	0.16*ab*	1.93*cd*
HA 138	0.24*b*	1.99*d*
Date		
9-22-80	0.20*a*	1.55*a*
10-3-80	0.25*a*	1.75*b*
10-13-80	0.22*a*	1.76*b*
Head position		
1 (outside ring)	0.20*a*	1.67*a*
2 (middle ring)	0.22*a*	1.68*a*
3 (inside ring)	0.25*a*	1.72*a*

† Inbred selection derived from open-pollinated variety.
a-d Means within source with the same letter are not significantly different at the 1% level by Tukey's multiple range test.

pling affected seed CA levels, but not hull CA. CA increased as the seeds matured. A significant I × D interaction was observed for hull and seed CA content. All other interaction effects were nonsignificant. The I × D interaction may be attributable to differences among the inbreds with respect to maturity or rate of dry-down.

While differences among inbreds and among dates were significant, differences among rings sampled within the head (head position) were nonsignificant for hull or seed CA. Thus, head position has no significant influence on CA concentration of sunflower achenes (hull or seed) either directly or through two-factor or three-factor interactions.

Bird feeding was observed to proceed from the edge toward the center of the heads for all seven of the sunflower lines. Birds feed first on the achenes at the periphery of the head of both physiologically immature and mature sunflower heads. Dorrell's results (1978) indicated that the more mature achenes at the periphery of an immature sunflower head would have higher levels of CA than achenes positioned toward the center. Our study indicated that position of achenes within the head did not significantly affect the levels of CA in the seed or hull when the head had reached physiological maturity. Although significant, there was not a strong relationship between sampling date and CA (Table 2). Thus, bird feeding patterns are probably a response to other factors such as achene succulence and accessibility, not differential amounts of CA throughout the sunflower head.

Inbred × Environment

Inbreds had a significant influence on hull CA percentage and the inbred × environment (I × E) interaction was highly significant (Table 3). For seed CA levels, the influence of inbreds and the I × E interaction were highly significant. These results indicate that CA concentration of hulls and kernels is strongly influenced by the genotypes that were tested. The highly significant influence of replication/environment on percent damage is a reflection of uneven predation levels often observed within natural bird predation environments. The highly significant I × E interaction observed for all response variables indicates that genotypic influence on these traits is strongly conditioned by the environment.

Means for hull CA percentage, seed CA percentage, and percent bird damage for the

Table 3. Analysis of variance for hull CA percentage, seed CA percentage, and bird damage percentage of eight inbred sunflower genotypes grown at four environments

		Mean squares			
Source	df	Hull CA	Seed CA	df	Bird damage
Environment (E)	3	0.253	0.067	3	1465
Replication/E	4	0.066	0.021	4	6400**
Inbred (I)†	7	1.867*	0.936**	7	5151
E × I	20	0.555**	0.134**	18	2574**
Experimental error	18	0.055	0.036	17	693
Sampling error	206	0.070	0.042	180	827

† The *F* test for inbreds was I/(E×I).
*,** Significant at the 0.05 and 0.01 level, respectively.

eight inbreds are listed in Table 4. HA291 had a hull CA content significantly higher than any of the other inbreds. It also had the highest hull CA level in the date × genotype × head position experiment.

Seed CA variation was continuous with significant differences between genotypes with relatively high, medium, or low CA contents. Since the *F* test was not significant, no comparisons were made between genotypes for percent bird damage. However, it was interesting to note that the genotypes Advance 946, Sputnik, and Impira INTA, evaluated as somewhat resistant to bird predation in a preliminary 1978 test, had the least damage in 1979 and 1980. Thus, it is quite likely that differences did exist between genotypes (unpubl. data). Unfortunately, this experiment did not provide the necessary precision to detect statistically significant differences.

Blackbirds and sparrows remove the achene from the sunflower head, remove the hull, and eat the seed. Thus, if CA is to deter bird predation on the basis of taste aversion, CA concentration within the seed is probably the most important factor conditioning resistance. Since the hull is dry and the CA is in the hull itself, we believed that birds would not be exposed to a strong aversive stimulus (high CA) when extracting the seed. Bird predation was not appreciably deterred (43.47% damage) in HA291, the only genotype with high levels of CA in the hull (Table 4). The CA level within the seed is about 10 times the level observed in the hull for most genotypes with significant differences between genotypes

Table 4. Mean percent hull CA, seed CA, and bird damage for eight inbred sunflower genotypes grown at four environments

Inbred	Plants evaluated for CA	Hull CA	Seed CA	Plants evaluated for bird damage	% damage
Impira INTA (R†)‡	35	0.12*a*	1.51*b*	32	29.8
Advance 946 (R)‡	25	0.16*a*	1.52*b*	20	12.5
Chernianka (S†)‡	43	0.18*a*	1.87*c*	40	64.5
HA 234 (S)	46	0.19*a*	1.55*b*	44	35.2
Sputnik (R)‡	15	0.22*a*	1.54*ab*	11	14.3
Peredovik (S)‡	29	0.24*a*	1.91*c*	25	34.2
HA 138 (S)	16	0.30*a*	1.64*b*	14	45.7
HA 291 (S)	50	1.05*b*	1.28*a*	44	43.5

† R = resistant; S = susceptible.
‡ Inbred selections from named open-pollinated variety.
a-c Means with same letter were not significantly different at the 1% level by Fisher's protected LSD test.

across the observed range of concentrations (1.28–1.91%). However, genotypes with the highest CA concentration, Peredovik and Chernianka, also have high levels of bird predation. The more resistant genotypes appear to have low to medium CA percentages. Correlations between bird damage percentages and hull CA percentage or seed CA percentage were calculated using genotypic means averaged across environments. Neither hull or seed CA was significantly associated with bird predation ($P<0.05$, 7 df) and all correlation coefficients were positive — 0.24 and 0.36, respectively.

Since appreciable plant-to-plant variability within genotypes was observed for hull and seed CA percentage and percent bird damage, correlations between percent bird damage and CA percentage in hull or seed were calculated on a per-plant basis for each of the four environments (Table 5). Significant positive correlations ($P<0.05$) for hull CA were observed for the Fargo location (sparrow feeding). Correlation coefficients for seed CA were generally positive but non-significant ($P<0.05$). The absence of significant correlations between seed CA percentage and percent bird damage suggests that high CA levels in the seed do not deter bird feeding. This study was initiated with the hypothesis that the opposite relationship would occur, as was found for tannins in grain sorghum. A number of seed characteristics (oil percentage and seed/hull ratio) that were positively correlated with CA concentration of seed might make high CA sunflower achenes more attractive as a food source for birds (Dorrell 1978). However, there is no obvious explanation for the observed positive correlations between hull CA percentage and percent bird damage.

Table 5. Correlation coefficients for hull and seed CA vs. estimated bird damage (per plant)

Location-year	Plants evaluated	Hull CA	Seed CA
Fargo, 1979	37	0.29*	0.10
Alice, 1979	29	0.25	0.02
Fargo, 1980	61	0.27*	0.15
Chaffee, 1980	103	−0.06	0.17†

†,* Significant at the 0.10 and 0.05 levels, respectively.

The range of CA concentration in the seed was 0.89–2.51%, with an overall mean of 1.55% for the 49 inbreds used in these experiments. CA concentrations of 0.11–1.04% with a mean of 0.27 were observed for hulls of the inbreds. This does not represent a substantial increase in variability for CA content in the hull or seed.

The experimental data presented here indicate that CA concentrations from 0.10 to 1.0% in the hull and from 1.2 to 2.1% in the seed do not deter bird predation. The results from the date × genotype × head position experiment support this conclusion. There was no indication that substantially more variability exists for this trait in the world collection of domestic sunflower or in wild sunflower genotypes (Dorrell 1976). Therefore, breeding for increased CA in hull or seed to deter bird predation is probably not a useful breeding strategy for the development of bird-resistant sunflower.

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