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NEW DEVELOPMENTS IN BIRD RESISTANT SORGHUMS

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In recent years there has been a major shift of emphasis from lethal to nonlethal methods of controlling bird damage to agricultural crops. In addition to being unpopular, killing large number of birds has generally been ineffective because of rapid recovery of populations. Consequently, most of the methods that are now being investigated emphasize crop protection. Since sorghums are especially vulnerable to bird damage, considerable effort has been expended in the past 20 years to develop varieties that have morphological or chemical characteristics which are resistant to bird damage (Harris, 1969; Tipton et al., 1970; McMillian et al., 1972).

Some bird resistance has been attained through genetic selection of certain morphological characteristics such as pendant heads, long awns, large glumes, and large seeds (Doggett, 1957) that physically make bird feeding difficult. However, in areas of high depredation, farmers have depended on high-tannin sorghums which more reliably resist bird damage because of their astringent "taste" properties. In areas of high rainfall farmers have also enjoyed the resistance of high-tannin sorghums to weathering (Harris and Burns, 1970; York, 1976) and preharvest sprouting in the panicle (Harris and Burns, 1970). Unfortunately, the same tannin characteristics that afford these beneficial properties are also responsible for the poor nutritional quality of many bird resistant (BR) sorghums.

In a recent review, Price and Butler (1980) attributed deleterious nutritional properties of BR sorghums to several possible tannin-related effects. In general, the suspected tannin-protein binding effects in the digestive tract can be expressed as follows: undigestible complexes with dietary protein, deactivation of digestive enzymes, and "tanning" of some areas within the digestive tract. In addition, the low palatability of high-tannin sorghums can depress feed intake and lower the weight gain. At least one investigator (Morton, 1970; Morton, 1978) has proposed that there is a link between geographical zones where a high incidence of esophageal cancer occurs and the usage of tannin-containing plants within these zones. The result is that these negative qualities in high-tannin sorghum grains cause them to have less value in the market place; and farmers that produce them are at a disadvantage.

In recent years the "detente" between proponents of crop protection and nutritional quality has resulted in an impasse to any common solution. In fact, varieties which both resist bird predation and have good nutritional quality have been recognized for at least a decade (Harris, 1969). Apparently, the barrier to progress has been in the understading of tannin biochemistry. The role of the Denver Wildlife Research Center has been to fuse the two "camps" by providing information on that most important factor. The objective of the present paper is to inform professionals involved in bird damage control of the new biochemical, agronomic, and nutritional developments in bird resistant sorghums.

Sorghum tannins: Phenols are responsible for a host of important biochemical properties in the plant kingdom that in turn elicit specific physiological and behavioral responses within the animal kingdom. In sorghums, tannins elicit the astringent effect that is primarily responsible for bird aversion to resistant sorghums. The response occurs because tannins bind with salivary and mucous epithelial proteins (Joslyn and Goldstein, 1964). Tannins from plant sources (vegetable tannins) are commonly divided into two groups: (1) hydrolyzable and (2) condensed (Ribereau-Gayon, 1972). The hydrolyzable tannins, which have a polyester structure, are readily hydrolyzed by acids or enzymes into a sugar and phenol carboxylic acid. In contrast, condensed tannins are not attached to sugars and under the action of acids undergo progressive polymerization to yield amorphous phlobaphens or tannin reds (Haslam,1966).

Tannins are usually defined as polyphenols which have molecular weights between 500 and 3000 and form stable complexes with proteins (Ribereau-Gayon, 1972). To react, the molecular size and shape must coincide with spacings within the protein matrices (Goldstein and Swain, 1963; Roux, 1972). Trimers (3-unit polymers) are the

largest size that can bind effectively with proteins. The binding activity increases as the molecules become larger, up to some maximum within the 3 to 10 monomer range, and then decrease sharply as the polymers become too large (Goldstein and Swain, 1963; Quesnel, 1968). Thus, shape and size largely determine the protein-binding characteristics of a tannin and consequently all of the other biological and biochemical properties (e.g., tanning of hides, enzyme inhibition, and nutritional effects) that have been attributed to them (Byrd et al., 1960; Haslam, 1966).

The principal tannins reported for sorghums are condensed procyanidins (Strumeyer and Malin, 1975; Fletcher et al., 1977; Haslam, 1977; Gupta and Haslam, 1978). Increasing evidence, however, suggests the presence of other proanthocyanidins (Bullard and Elias, 1980). Monomeric proanthocyanidins condense together during the development and ripening of sorghum seeds to form protein-reactive oligomers (tannins) and insoluble and unreactive polymers of very high molecular weights.

Deposition of tannins in seeds: In the past, bird resistance had been associated with "brown" sorghums, but today sharper distinctions are being made. Tannin is formed in two distinct layers of the sorghum seed, the epicarp (or outer pericarp layer) and the testa (also called nucellar layer or undercoat). The two do not contribute equally in bird resistance, and either the testa layer or pigmentation in the epicarp may be absent in a particular variety (York, 1976). Most investigators believe that the epicarp has a less important role than the presence or absence of a testa in determinimg the bird resistance of a variety (Harris, 1969). Pericarp color or pigmentation is not necessarily related to tannin content or biochemical activity (Bullard and Elias, 1980), because red pericarp color (often confused with brown) is controlled by genetic factors that are not related to those for the test. Red pericarp color involves genetic factors Y and R, whereas B_1B_2S controls the brown pericarp, the presence or absence of a pigmented testa, and tannin content (Maxson et al., 1972; Wanjari and York, 1972).

Polyphenolic changes during ripening: Several fruits and seeds are astringent when immature but lose this property during ripening (Goldstein and Swain, 1963; Singleton and Kratzer, 1969). Apparently, tannin molecules are of the appropriate size and shape for protein-binding in immature stages, but during ripening they polymerize to sizes too large to effectively bind with proteins or dissolve in an aqueous environment.

Similar changes in ripening sorghum seed have been widely referred to, until recently, as "disappearance of tannin." Results of sorghum maturation studies indicate a general increase in tannin concentrations through the immature stages and then, with few exceptions, a decrease or plateau for the fully ripened grain (Bullard and Elias, 1980). Haslam (1977) observed that as chlorophyll forms in the seed coat, procyanidins (monomers and oligomers) are rapidly synthesized to a peak level which remains approximately constant until luteolinidin pigmentation occurs. Subsequently, a decrease occurs in concentrations of oligomers as insoluble high molecular weight polymers are formed. The problem with most high-tannin sorghums is that there is not uniform polymerization in all of the tannin molecules during ripening and some retain protein-binding properties which are nutritionally deleterious in the ripened grain.

Variations among BR sorghums: The objective for genetic development would be to select genomes which provide tannins which are astringent in the immature stages and then, like fruits, become unable to bind with proteins after ripening. When we correlated the polyphenol composition of 15 mature BR sorghum grains with the results of paired preference tests on *Agelaius phoeniceus* and *Quela quela*, sorghums could be separated into two distinct groups (Bullard et al., 1980). A least preferred group had higher polyphenol values and greater uniformity in their composition. Varieties having a poor nutritional reputation fell within this group. The remaining tannin-containing varieties had generally lower polyphenol levels and much more variation among their chemical and physical properties.

Other researchers had made similar distinctions (Cummings and Axtel, 1973; Price et al., 1978), classifying sorghums into three groups on the basis of vanillin assays: Group I were low in polyphenols, Group II had large differences in polyphenol values between absolute- and acidic-methanol extracts, and Group III had about the same values for the two extracts. When vanillin assays were conducted on our varieties (Table 1), all seven of the least preferred set were Group III's, four of our most preferred set were Group II's, and two were Group I. The encouraging point is that Group II sorghums have nutritional properties equivalent to Group I, whereas the quality of III is poor.

Several possibilities could account for Group II and Group III differences, such as: (1) the polymerization process, (2) starch interactions, (3) protein interactions, (4) cell wall interactions, or (5) seed structure (Bullard and Elias, 1980). Although we do not know the specific mechanism involved in these differences, it would appear that the Group II

sorghums have the most promising characteristics for future genetic experimentation in developing bird-resistant varieties.

Our studies on eight BR varieties in the milk, light dough, firm dough, and mature stages of ripening (Bullard and Elias, 1980) offered some encouragement that a genetic solution could be found. We observed that when data were arranged according to sorghum classification (Table 2), Group II chemical and biochemical properties were similar to those for Group III varieties during the immature stages, but showed relatively larger decreases in activity for the mature grain. These are desirable trends for accomplishing our objective for genetic development. Although these particular Group II varieties had generally lower chemical and biochemical tannin activity than Group III during the immature stages, indications were that intermediate-sized oligomers with protein-binding qualities had been formed during seed development.

Current research emphasis in BR sorghum: There are several literature references to varieties which have resisted bird depredation in the field and demonstrated good nutritional quality in feeding or digestion trials (Thayer et al., 1967; Damron et al., 1968; Harris et al., 1970; Mabbayad and Tipton, 1975; Nelson et al., 1975; Oswalt, 1975; Tipton et al., 1975). One of the Group II varieties tested in our laboratory (TAM 2566) has performed well when exposed to high bird populations in Arkansas during the past 5 years (York, personal communication). Others we have tested did not discourage severe depredation during tests in Puerto Rico in early 1979. The genetic selection of BR sorghums having acceptable characteristics to both the producer and consumer is a challenging problem. I am confident, from the rapid progress of those involved and the level of interest by other plant geneticists, that we will see a pay-off in the next few years.

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Table 1. Tannin concentrations in mature BR sorghums.[a]

Classification	Chemical assay[b] (CE)	Biochemical assay[c] (TAE)
Group I (n = 2)	0.50	1.60
Group II (n = 4)	0.05	1.85
Group III (n = 9)	2.25	2.58

[a] Aliquots of combined acetone, methanol, and 90% methanol extracts from ground mature sorghum grain samples.

[b] Modified vanillin assay (Price et al., 1978) expressed in catechin equivalents (CE).

[c] Protein binding assay (Hagerman and Butler, 1978) expressed in tannic acid equivalents (TAE).

Table 2. Tannin changes in developing and ripening bird resistant (BR) sorghums overall and by group.[a]

Classification and stage	Chemical assay[b] (CE)	Biochemical assay[c] (TAE)
Overall (n = 8)		
milk	0.18	1.09
light dough	0.31	1.37
firm dough	0.43	1.47
mature	0.16	1.61
Group II (n = 3)		
milk	0.14	1.14
light dough	0.23	1.26
firm dough	0.29	1.14
mature	0.07	1.14
Group III (n = 5)		
milk	0.21	1.40
light dough	0.36	1.42
firm dough	0.51	1.68
mature	0.22	1.90

[a] Aliquots of combined acetone, methanol, and 50% methanol extracts of samples from 8 BR sorghum varieties in 4 stages of maturity (Bullard et al., 1979).

[b] Vanillin-H_2SO_4 assay (Hills and Swain, 1959) expressed in catechin equivalents (CE).

[c] Protein binding assay (Hagerman and Butler, 1978) expressed in tannic acid equivalents.