TABLE 1

Effect of Reaction Conditions on Aflatoxin Content of Undelinted Whole Cottonseed[a]

Ammoniation conditions[b]		
Peak ammonia pressure kg/cm²	Peak temperature °C	Total aflatoxins[c] μg/kg
1.4	65	110
1.4	71	56
1.4	74	59
1.4-1.8	77	41
1.4-1.8	82	4

[a]4% Ammonia concentration; 30 min reaction time at peak conditions.

[b]15% Moisture content.

[c]Values reported are means of 4 replicated runs.

Experience with detoxification of aflatoxin-contaminated cottonseed meals indicates that the presence of moisture promotes destruction of aflatoxin with ammonia gas. The influence of higher moisture levels, with a limit of approximately 15%, probably will enhance the destruction of aflatoxins in undelinted whole cottonseed.

This study supports the feasibility of ammoniating undelinted whole cottonseed as an effective means of reclaiming contaminated cottonseed for use as a feed for ruminant animals.

ACKNOWLEDGMENTS

A. O. Franz Jr. provided technical assistance.

REFERENCES

1. Lough, O.G., *Hoard's Dairyman 124*:586 (1979).
2. Fowler, R.G., *Prog. Agric. Ariz. 30*(2):3 (Summer 1979).
3. Dollear, F.G., G.E. Mann, L.P. Codifer Jr., H.K. Gardner Jr., S.P Koltun and H.L.E. Vix, *J. Am. Oil Chem. Soc. 45*:862 (1968).
4. Masri, M.S., H.L.E. Vix and L.A. Goldblatt, U.S. Patent 3,429,709 (1969).
5. Gardner, H.K. Jr., S.P. Koltun, F.G. Dollear and E.T. Rayner, *J. Am. Oil Chem. Soc. 48*:70 (1971).
6. Prevot, A., and M. Jemmali, *Zesz. Probl. Postepow Nauk Roln. 189*:125 (1977).
7. Cavanagh, G.C., and M.E. Ensminger, *Livestock Bus. 1*:1 (1968).
8. McKinney, J.D., G.C. Cavanagh, J.T. Bell, A.S. Hoversland, D.M. Nelson, J. Pearson and R.J. Selkirk, *J. Am. Oil Chem. Soc. 50*:79 (1973).
9. Price, R.L., O.G. Lough and W.H. Brown, *J. Food Prot. 45*:341 (1982).
10. Koltun, S.P., E.T. Rayner, J.I. Wadsworth and H.K. Gardner Jr., *J. Am. Oil Chem. Soc. 56*:803 (1979).
11. Jorgensen, K.V., and R.L. Price, *J. Ag. Food Chem. 29*:555 (1981).
12. Pons, W.A. Jr., and A.O. Franz Jr., *J. Assoc. Off. Anal. Chem. 60*:89 (1977).

[Received November 7, 1985]

Chemical and Microscopic Studies of the Matrix Substance in Pigment Glands of Cotton (*Gossypium hirsutum* L.) Seeds

Lawrence Yatsu[a,*], Thomas J. Jacks[a], Henry W. Kircher[b,1] and Mary An Godshall[c]

[a]Southern Regional Research Center, ARS, USDA, P.O. Box 19687, New Orleans, LA 70179; [b]Department of Agricultural Chemistry, University of Arizona, Tucson, AZ, and [c]Sugar Processing Research Inc., New Orleans, LA

Cottonseeds contain gossypol, a toxic substance, that renders the otherwise nutritious seeds inedible. However, because the gossypol is concentrated in small, intercellular glands, it is possible to separate gossypol from other seed constituents by pulverizing the seeds and removing the glands. This procedure is practicable because gossypol remains with the glands even during seed pulverization and manipulation in hexane. Many believe that the gossypol remains within the glands because the glands are virtually indestructible, protected by tough, resilient "plates." However, we show that most of the isolated glands are broken after comminution. The gossypol is held in a water-soluble matrix within the lumen of the glands. Analysis of aqueous extracts of isolated glands showed that the bulk of the extract is a non-dialyzable arabinogalactan. We suggest that the matrix substance is an arabinogalactan.

Over 33 million tons of cottonseed was produced worldwide last year, an increase of 50% over the last decade (1). The seed, a by-product of fiber production, contains an excellent vegetable oil and high grade proteins (2). However, the protein and sometimes even the oil, are rendered unfit for human consumption because of the presence of gossypol (a toxic substance) in the seeds (3-5).

Because virtually all of the gossypol in cottonseed is concentrated in glands (6,7), it is possible to separate the gossypol from the rest of the seed constituents by pulverizing the seeds and removing the pigment glands (8-10). This procedure is practicable because gossypol remains with the glands during comminution and suspension in hexane. Many believed, and still believe, that the glands retain the gossypol because they are virtually indestructible, protected by tough, resilient "plates" (6,8,10,11).

We showed, however, that many (perhaps most) of the isolated glands were broken in pulverized cottonseed meal and concluded that intact glands really were not necessary for procedures such as the Liquid Cyclone Process (LCP) to work (7). In this communication, we show that the gossypol is enmeshed in a water-soluble matrix within the lumen of the glands. We show that

[1]Deceased.

*To whom correspondence should be addressed.

matrix material remains with the glands, even after the glands have been broken and suspended in hexane. We present chemical and physical data which suggests that the matrix substance is an arabinogalactan.

MATERIALS AND METHODS

A pigment gland-enriched fraction from an LCP was suspended in a mixture of Freon 113 and hexane (sp. g. about 1.37 to 1.38) and the bottom fraction discarded. This process was repeated several times. The enriched pigment gland preparation was then dried. This was the purified pigment gland preparation used for our studies. An aqueous extract of pigment glands was obtained by extracting purified glands with water for an hour and then centrifuging. The supernatant was saved and the pellet resuspended in fresh water and extracted once more. The combined extract was filtered through a 0.45 μ Millipore filter and lyophilized.

Samples of the aqueous extract were chromatographed on silica gel plates with the solvent system n-butanol: ethanol:H_2O:acetic acid (50:30:15:5, v/v/v/v) (12). Visualization of the sugars was accomplished by spraying the plates with 2N H_2SO_4 and heating at 95 C for 10 min. Trimethylsilyl (TMS) derivatives of the extract were

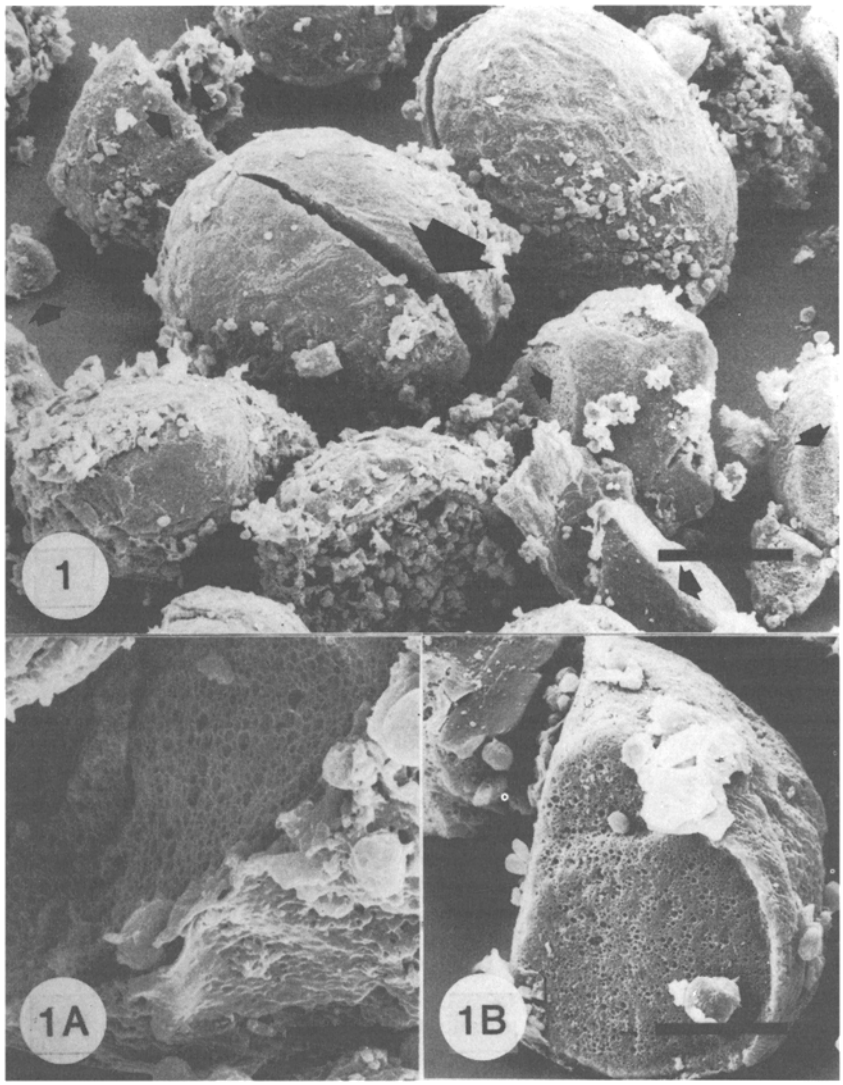

FIG. 1. An SEM micrograph of purified pigment glands from an LCP. There are many broken glands and gland fragments (small arrows); even the two intact glands in the center have large cracks in them (large arrow). The fact that the glands did not lose their contents after breaking and soaking in hexane indicates the matrix substance is neither a fluid nor soluble in hexane. The matrix material can be coated with gold-palladium for SEM. Bar indicates 40 μm. (1A) A higher magnification micrograph taken at the crack (pointed out by the large arrow) in the gland shown in Fig. 1. Notice the sponge-like matrix that fills the lumen of the gland; spherules containing gossypol and related pigments are held by this matrix. Bar indicates 4 μm. (1B) A micrograph of a gland fragment, similar to the ones designated by small arrows in Fig. 1, showing more clearly the nature of the material associated with the broken glands. The material on the outside of the fragment is identical to the matrix substance inside the gland lumens (visible within the cracks of the split glands in Fig. 1A). Bar indicates 40 μm.

chromatographed in a gas liquid chromatograph (GLC) using an OV101-coated fused silica capillary column and an HP 5880 gas chromatograph equipped with a flame ionization detector (FID). The samples were held at the injection temperature of 175 C for 2 min and then programmed to run at 4 C/min for 10 min. A sample of the aqueous extract was dialyzed in a regenerated cellulose bag (3500 MWt cut-off) over toluene-saturated deionized H_2O for 100 hr and lyophilized. A portion of the retentate was hydrolyzed in 2N H_2SO_4 by refluxing for 4 hr. The H_2SO_4 was neutralized with $Ba(OH)_2$; then the excess barium was removed with CO_2. A portion of the hydrolysate was equilibrated in pyridine for 24 hr and the TMS derivatives analyzed as described above.

For scanning electron microscopy (SEM) pigment glands were placed onto adhesive-coated aluminum stubs and coated in a Technics Hummer II D.C. sputtering device with 30–40 nm of gold-palladium. Glands, extracted with distilled water and lyophilized, were prepared in the same manner. The coated samples were examined in a Cambridge Steroscan 250 Scanning Electron Microscope.

RESULTS AND DISCUSSION

Figure 1 is an SEM micrograph of a purified pigment gland preparation. Contrary to popular belief, pigment glands are not unduly tough, nor do they necessarily re-

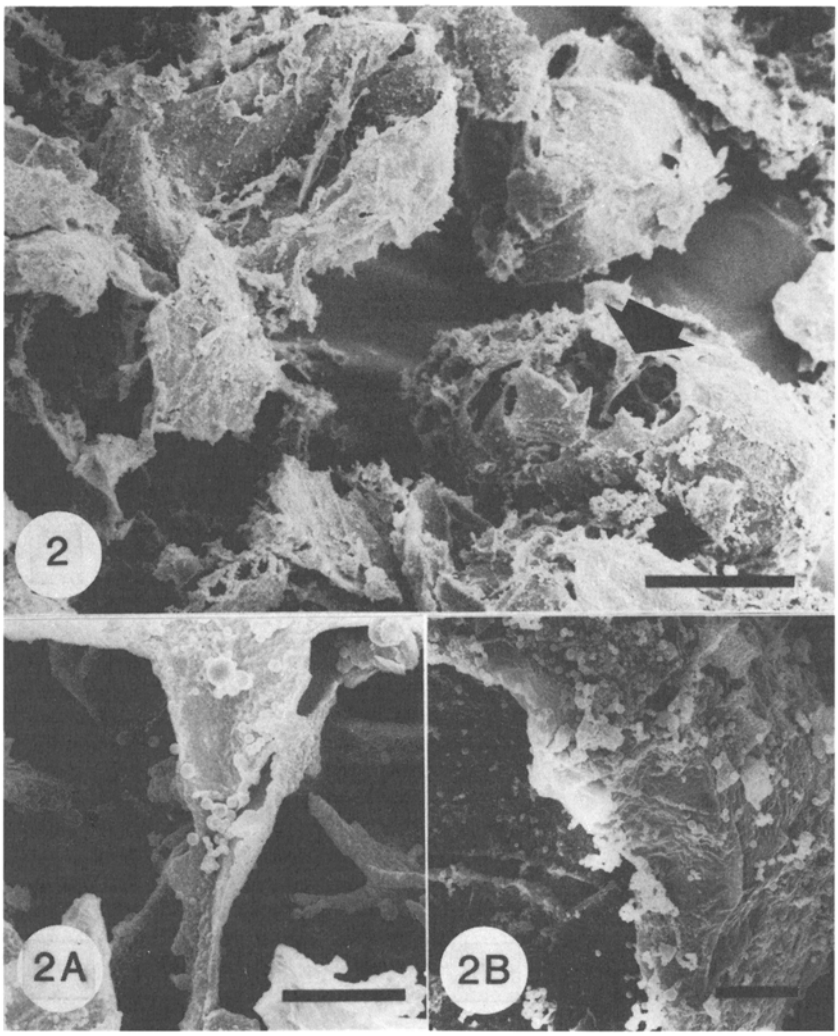

FIG. 2. An SEM micrograph of pigment glands similar to those shown in Fig. 1, but after water-extraction. The outstanding difference between glands shown in this micrograph and those in Fig. 1 is the lack of spongy matrix material. None of the glands or gland fragments possessed spongy matrix substance after water-extraction; consequently, it was possible to look into the interiors of gland lumens and see cell walls, gossypol-containing spherules, etc. In Fig. 1, these entities were hidden under the matrix material. Bar indicates 200 μm. (2A) A higher magnification micrograph of a break in the glandular cell wall shown in Fig. 1 (arrow). The water-extraction has washed away the matrix substances, and it is possible to see the inside of the gland. Note the cell walls and spherules. Bar indicates 30 μm. (2B) A micrograph, similar to Fig. 2A but of a mature gland, showing the interior. Notice that the cell walls seen in Fig. 2A are absent; the cell walls lyse away during maturation and are absent in mature glands. Bar indicates 10 μm.

main intact after pulverization. Many of the profiles shown in the micrograph are fragments of broken glands, and the two intact glands in the center of the micrograph have cracks in them. Figure 1A is a higher magnification view of the crack, showing the interior of the gland. Note that the lumen (interior) of the cracked gland is filled with a spongy appearing substance. Remnants of spherules that contained gossypol and gossypol-related pigments can be seen in the fractured matrix. The contents of the broken gland did not leak out when the gland was cracked or wash away in the hexane and Freon used in the isolation of the glands; rather, it remained a solid, substantive entity that can be coated with gold-palladium. Figure 1B is a micrograph showing a fragment of a broken gland, similar to the ones at the small arrows in Figure 1. Note that the sponge-like material on the outside of broken gland fragments is identical to the matrix material inside the cracked glands.

Figure 2 is an SEM micrograph of water-extracted pigment glands. Water-insoluble constituents, such as pigment gland walls, fragments of gland associated cells, cell contents and other debris, can be seen. Figure 2A is a higher magnification micrograph taken at the fissure in the gland shown in Figure 2. This gland was rather immature, as evidenced by the presence of cell walls in the lumen of the gland. Figure 2B is a micrograph showing the interior of a mature gland. The obvious difference between Figures 1 and 2 is the lack of matrix material associated with pigment glands in the latter. The matrix material, which obscures the interior of the glands, was solubilized away by water. Note the spherules in Figures 2A and 2B that are visible after aqueous extraction. These spherules, which contain gossypol and related pigments, can be seen in situ with transmission electron microscopy (7), but are hidden from view in scanning electron microscopy by the matrix material.

Water extracts contained about 25% of the weight of purified pigment glands. Chemical analysis (13) of the aqueous extract showed that it was 81% carbohydrate. TLC revealed that the extract contained sucrose, raffinose, stachyose and an unidentified trisaccharide and pentasaccharide. A large spot, probably oligo- or polysaccharides, and peptides remained at the origin. GLC showed a trace of sucrose, raffinose and possibly another trisaccharide but no monosaccharides.

Dialysis of the aqueous extract showed that 67% was non-dialyzable. The hydrolysate of the retentate contained arabinose, galactose and glucose in a 1:3.3:0.07 ratio (22.8% arabinose, 75.6% galactose, 1.5% glucose). It is interesting to note that Reeves et al. (14,15) claimed that the "resin glands" contained pentosans a half century ago. These findings are consistent with our previous suggestion that "aggregates" of gossypol-containing spherules were held together by a hexane-insoluble, water-soluble bonding substance. Because non-dialyzable, arabinogalactan polymers made up the largest portion of the aqueous extracts of isolated glands and because arabinogalactans are hexane-insoluble, we suggest that the matrix substance in pigment glands that enmeshes the gossypol-containing spherules into aggregates is an arabinogalactan. This would not only resolve how broken glands can retain gossypol, but also explain why the glands are so "sensitive" to water (6,11).

As we noted in the introduction, there has been an enormous surge in cottonseed production in the world during the last few years. There are probably many reasons for this increase, not the least of which is the fact that cotton is still one of the finest textile fibers known (from both the producer's and consumer's point of view). Unlike fibers derived from fossil fuel, cotton is a renewable resource. We feel the demand for cotton will continue for the foreseeable future, and if the production of cotton is to continue, it behooves us to study ways to salvage something from cottonseeds.

ACKNOWLEDGMENTS

Bruce Ingber assisted on the scanning electron microscope.

REFERENCES

1. U.S.D.A. Foreign Agricultural Service, Foreign Agric. Circ., *Oilseeds and Products,* FOP 1-85 (Jan. 1984/85).
2. Olson, R.L., *Oil Mill Gaz. 77*(9):7 (1973).
3. Withers, W.A., and J.F. Brewster, *J. Biol. Chem. 15:*161 (1913).
4. Pons, W.A. Jr., J.C. Kuck and V.L. Frampton, *J. Am Oil Chem. Soc. 38:*104 (1961).
5. Damaty, S., and B.J.F. Hudson, *J. Sci. Food Agric. 26:*1667 (1975).
6. Moore, A.T., and M.L. Rollins, *J. Am. Oil Chem. Soc. 38:*156 (1961).
7. Yatsu, L.Y., T.P. Hensarling and T.J. Jacks, *J. Am. Oil Chem. Soc. 51:*548 (1974).
8. Spadaro, J.J., R.M. Persell, C.G. Reuther Jr., H.L.E. Vix, E.J. Laborde, J.W. Latham, R.L. Jaeger, E.F. Pollard and E.A. Gastrock, *J. Am. Oil Chem. Soc. 27:*336 (1950).
9. Vix, H.L.E., P.H. Eaves, H.K. Gardner Jr. and M.G. Lambou, *J. Am. Oil Chem. Soc. 48:*611 (1971).
10. Gardner, H.K. Jr., R.J. Hron Sr. and H.L.E. Vix, *Oil Mill Gaz. 78*(6):12 (1973).
11. Boatner, C.H., C.M. Hall, M.L. Rollins and L.E. Castillon, *Bot. Gaz. 108:*484 (1947).
12. Roberts, E.J., M.A. Clarke and M.A. Godshall, *Short Report No. 2,* Sugar Processing Research Inc., New Orleans, LA (1984).
13. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem. 28:*350 (1956).
14. Reeves, R.G., and C.C. Valle, *Bot. Gaz. 93:*259 (1932).
15. Reeves, R.G., and J.O. Beasley, *J. Agric. Res. 54:*711 (1937).

[Received May 15, 1985]