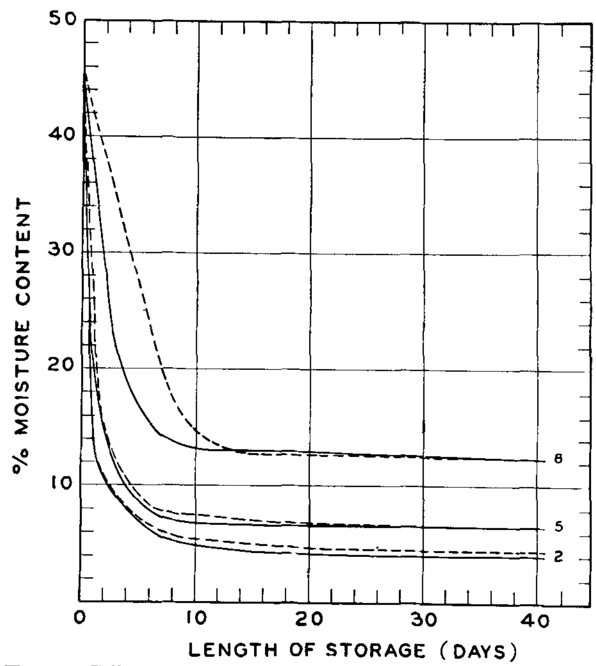


FIG. 7. Effect of length of storage at constant relative humidity and temperature on the moisture content of freshly dug Spanish peanuts.

| | No. 2 | No. 5 | No. 8 |
|---|---|---|---|
| --- 25°C. | 22.5% R.H. | 53.3% R.H. | 86.4% R.H. |
| —— 35°C. | 21 % R.H. | 50.7% R.H. | 85.8% R.H. |

rium moisture content at 35° is lower than that at 25°. The slight differences are probably the result of the slightly lower relative humidity at 35°C.

## Summary and Conclusions

The hygroscopic equilibria and rates of attainment of hygroscopic equilibrium of freshly dug, naturally cured, and artificially cured peanuts have been determined over the range of 11-93% relative humidity at 25°C. The hygroscopic equilibrium does not appear to be dependent on the method of curing. At a constant relative humidity, the moisture content is distributed so that the moisture content of the skins is twice that of the meats and in general the moisture content is lowest in the meats, and is increasingly greater in hearts, shells, and skins, respectively.

At 25° and 35°C. the relative humidity is of greater importance than the absolute humidity. Circulation of the air over the samples greatly increases the rate of attainment of hygroscopic equilibrium. At the higher temperature, the rate of attainment of hygroscopic equilibrium is further increased because of the increased rate of diffusion of moisture in the seed.

Inasmuch as the hygroscopic equilibrium is the same at both 25° and 35°C., it is more advantageous when speed of drying is important to use the higher temperature because equilibrium is attained more rapidly.

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# Gossypol and Gossypurpurin in Cottonseed of Different Varieties of G. barbadense and G. hirsutum, and Variation of the Pigments During Storage of the Seed*

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## Summary

Selected varieties of cottonseed comprising three Sea Island cottons, four Egyptian cottons, and 10 upland cottons providing two or more varieties having the following characteristics: short, intermediate, and long staple; low, intermediate, and high content of lipids; and low, intermediate, and high content of protein, were planted and grown under as nearly identical conditions as possible.

The initial contents of moisture, lipids, nitrogen, gossypol, and gossypurpurin of the harvested seed were determined, after which samples of all of the seed were stored for a year under identical conditions and analyzed periodically for gossypol and gossypurpurin.

A definite relation was found between the species of the seed and their content of gossypol and gossypurpurin. Sea Island and Egyptian seed of the species *G. barbadense* contained more gossypol and very much more gossypurpurin than seed of the species *G. hirsutum*. Within the species *G. barbadense* Sea Island seed contained more gossypol and less gossypurpurin than Egyptian seed.

Gossypurpurin increased during storage of all of the seed whereas gossypol varied in a number of different ways, increasing in some, decreasing in others, and remaining relatively constant in a few samples of stored seed.

A discussion is presented of the theoretical and practical implications of these observations.

## Introduction

BECAUSE of the economic importance of cotton and cottonseed, the effect of genetic and environmental factors on the properties of the lint and the composition of the seed has been the subject of extensive investigations (28) although relatively few have dealt with the content of pigments in cottonseed from different varieties of cotton grown in different

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environments. Most investigations of cottonseed pigments have been limited to the determination of gossypol, long recognized as the principal pigment occurring in cottonseed, but now known to be accompanied by other pigments of closely related structure (1, 2, 4, 6, 7, 9) as well as small amount of carotenoids (23, 26).

Investigations (6, 33) of the colors of hydraulic-pressed cottonseed oils produced under similar processing conditions from two commercial samples of prime cottonseed before and after storage of the seed revealed the fact that the colors of the oils produced from one lot of seed were initially greater and increased more rapidly during storage of the seed than did that produced from the other seed. It is evident from this work that the content of pigments in different cottonseeds and their variation during storage are of practical importance as well as theoretical interest.

The first investigation (30) of the gossypol content of different varieties of cottonseed was carried out with a large number of samples of commercial seed collected from oil mills throughout the cotton belt of the United States. The gossypol content of the individual samples of seed varied as much as 300% and that of seed of the same variety grown in the same location varied as much as 200% in two consecutive years. A general correlation was noted between the contents of gossypol and oil in the seed and the area of production; seeds from the Southwest had low contents of oil and gossypol; seed produced in the Southeast section had contents of oil and gossypol of intermediate value; and seed produced in the Pacific Coast region were highest in contents of both oil and gossypol. No indication was found of the influence of variety on the contents of either gossypol or oil.

Gallup (15) determined the gossypol and oil contents of seed of the same variety, Oklahoma Triumph 44, obtained from plants grown during successive seasons on experimental plots in several adjacent counties. The effect of various fertilizers was also investigated. Approximately parallel variations in the contents of oil and gossypol were noted. Gallup concluded that the contents of gossypol and oil are influenced only slightly by increased supplies of plant nutrients but that they are influenced markedly by climatic conditions prevailing during growth. High rainfall during the growing season appeared to cause an increase in the amount of gossypol and oil in the seed.

More recently, an investigation (31) was made of the contents of moisture, lipids, nitrogen, and gossypol in a large number of samples of cottonseed obtained from plants grown successive years in experimental plantings at two places in Russia where it was stated that growing conditions differed widely from each other. It was found that the gossypol content of a given variety of seed was affected by both location and year of growth, but no data were given concerning the climatic conditions which might have caused the observed variations in composition of the seed. It was also observed that the average gossypol content of all varieties of seed of a given species was characteristic of the species even though seed of different varieties of the same species contained widely varying amounts of gossypol. The average content of gossypol was least in the case of different varieties of *G. herbaceum*, intermediate in varieties of *G. hirsutum*, and highest in varieties of *G. barbadense*. These results indicated to Smirnova (31) that both environmental and genetic factors independently affect the gossypol content of cottonseed.

Podolskaya (26) reported that the concentration of the carotenoid pigment which she had previously detected in cottonseed and cottonseed oil (23) was a characteristic of the species. The content of the pigment in seed of three varieties of *G. hirsutum*, 114, Navrotski, and 8517, was 0.096, 0.093, and 0.077%, respectively, whereas that of seed of two varieties of *G. barbadense* was 0.114 and 0.193%, respectively. The seed which were examined were of different agronomic origin, and no investigation has been reported of the effect of conditions of growth on the content of the carotenoid pigment in cottonseed.

Podolskaya (24, 25) also made several qualitative observations concerning the occurrence of "red gossypol" (21, 22) and of the yellow and orange pigments of immature cottonseed (24, 25) in different kinds of cottonseed. She noted that the relative concentrations of all of these pigments were initially higher and increased more rapidly during storage of Egyptian cottonseed, *G. barbadense*, than of American seed, *G. hirsutum*. Since "red gossypol" has been shown (1, 2) to be a mixture of gossypol and gossypurpurin, and the relative concentrations of "red gossypol" were estimated on the basis of the absorption band at 570 m$\mu$ which is the location of the principal absorption band of gossypurpurin (1, 6), observations concerning "red gossypol" can be interpreted directly in terms of gossypurpurin.

No systematic investigations have been reported of the variation of pigments during storage of cottonseed. As noted above, Podolskaya reported an increase in the amount of gossypurpurin during storage of several samples of cottonseed. She also reported (25) that during storage of a sample of variety 114 of *G. hirsutum* the gossypol content decreased from an initial value of 1.15% of the dry weight of the kernels to 0.75% after storage of the seed for four months.

In the present investigation there were examined different varieties of *G. barbadense* and *G. hirsutum* which were grown during one season under as nearly identical conditions as possible in order to determine the influence of genetic factors on the content of pigments in the kernels. The seed examined were representative of the types of cotton most widely grown in the United States. They comprised a number of varieties of upland seed (*G. hirsutum*) having different varietal characteristics with respect to length of fiber and contents of lipids and nitrogen as well as several varieties of Egyptian and Sea Island seed. The pigmentation of the seed was determined shortly after harvesting after which all of the samples of seed were stored at an average temperature of 80°F. and subsequent periodic determinations of content of pigments were made.

## Materials

*Selection of Varieties.* The following series of seed was selected for the pigment investigation reported here. Three varieties of Sea Island, $12B_2$, Seaberry, and TZRV, from Florence, South Carolina; four varieties of Egyptian, Pima, Earlipima, SXP, and Amsak, from Sacaton, Arizona; and 10 varieties of

*G. hirsutum,* Delfos 651, Bobdel, Deltapine 14 (833), Stoneville 2B (8275), Coker 100-7, Coker 4 in 1 Strain 7, Wilds 17, Half and Half, McNamara Naked seed, and Cook Naked seed, from planting stock assembled at Stoneville, Mississippi, for yield trials and genetics studies (20). A planting of Hopi, an Arizona Indian cotton (sample No. 23), failed to germinate. The first six varieties of *G. hirsutum* were selected for their characteristic low or high contents of oil and nitrogen. Wilds 17 and Half and Half were selected because of their long and short staples, respectively. The two varieties of Naked seed were selected because of the possible correlation between the absence of fuzz fibers and the presence and variation of the pigments in these varieties.

*Conditions of Growth and Storage.* All of the varieties were grown during the same season (1945) in neighboring experimental plots at the U. S. Cotton Field Station at Stoneville, Mississippi (20). The soil is a Sarpy sandy loam which was treated with 200 lbs. of sodium nitrate per acre prior to planting.

Growing conditions were not identical for all of the seed because of the long season required for maturing the Egyptian and Sea Island varieties. Two plantings were made of the Sea Island and Egyptian varieties, a normal early planting on May 14 and a second later planting on May 31 corresponding to the planting of the upland varieties.

The upland varieties were harvested on November 7, and the Sea Island and Egyptian varieties on November 18. All of the seed from the upland varieties and that from the early (normal) plantings of Sea Island and Egyptian varieties were mature when harvested, but only about 75% of the bolls from the late plantings were open. Only the seed from mature bolls was analyzed.

All plants were grown under extremely wet conditions (20), and the open bolls were exposed to considerable rainfall. Infestations of weevils were held down on the upland and early plantings of the Sea Island and Egyptian varieties, but considerable damage occurred in the late plantings of Sea Island and Egyptian varieties (16).

The seed was shipped to New Orleans, Louisiana, immediately after harvesting. Samples of the seed were stored at 38°F. in sealed containers and determinations of the composition and initial contents of pigments were carried out as soon as possible after receipt of the seed. For determination of the variation of pigments during storage, samples of the seed were stored in closed glass containers protected from direct light and at an average temperature of 80°F. which occasionally varied $\pm$ 5°F. from the average.

## Methods

All samples were analyzed for moisture, lipids, and nitrogen using the Official Methods of the American Oil Chemists' Society (19).

The pigments were determined by spectrophotometric analysis of chloroform extracts of the seed kernels. For this purpose extracts were prepared by equilibrating weighed samples of kernels (0.5 to 1.0 g.), ground to pass a U. S. No. 50 sieve, with U.S.P. chloroform (25 ml.) in low actinic flasks for 24 hours. This procedure has been shown (3, 6) to yield complete extraction of gossypol, gossypurpurin, and gossyfulvin from cottonseed. The cold chloroform extracts were filtered through folded Whatman No. 2 filter paper into pre-cooled low-actinic flasks. The filtered extracts were maintained at 38°F. and were analyzed within a period of 48 hours.

The absorption spectra measurements were made with a Coleman monochromator spectrophotometer. Examination of the absorption spectra of the chloroform extracts throughout the region from 360 to 650 m$\mu$ indicated the presence of significant amounts of gossypol and gossypurpurin and no detectable amounts of gossyfulvin or the carotenoid pigment.

The concentration of gossypurpurin in the seed was calculated on the basis of the specific extinction coefficients at 570 m$\mu$ of the chloroform extracts compared with that of pure gossypurpurin at the same wavelength (1, 6). The antimony trichloride-spectrophotometric method (3) was applied to the chloroform extracts for the determination of the concentration of gossypol in the kernels.

The specific extinction coefficients at 360 to 370 m$\mu$ for gossypol in the extracts calculated on the basis of the antimony trichloride method were compared with the specific extinction coefficients of the untreated chloroform extracts in the same region. This procedure failed to reveal the presence of any appreciable amounts of the yellow extraglandular pigment (7) recently detected in cottonseed. The chloroform extracts were shaken with a few drops of concentrated hydrochloric acid prior to treatment with antimony trichloride for comparison with the antimony trichloride reaction product of the nonacidified extracts (1, 2, 4, 9). but no appreciable amounts of gossyfulvin could be detected in any of the seed by this procedure.

## Results

*Composition and Initial Content of Pigments.* Table 1 shows the contents of lipids, nitrogen, moisture, gossypol, and gossypurpurin of different varieties of Sea Island and Egyptian seed, *G. barbadense,* including those obtained from seed planted at the same time as the faster maturing seed of *G. hirsutum.* The seed in each group are arranged in the order of decreasing content of lipids. The contents of lipids and nitrogen appear to be roughly inversely proportional to each other. There does not appear to be any general relation between the content of either gossypol or gossypurpurin and lipids, nitrogen, or moisture. The composition of Sea Island and Egyptian seed obtained from the late planting differs in many respects from that of the corresponding seed obtained from the normal planting, but the late planting does not appear to have exerted any consistent influence on the composition of the seed except for a somewhat reduced content of moisture in all but one sample of the seed obtained from the late planting as compared with the corresponding seed obtained from the normal planting.

The composition of seed of different varieties of *G. hirsutum* is shown in Table 2 in which the seed are arranged in order of decreasing content of lipids. A general tendency can be noted toward increase in nitrogen with decrease in lipids. However, the actual contents of lipids and nitrogen of the different varieties of seed are not consistent with the reported varietal characteristics of the seed with respect to these two components. With the exception of a very rough inverse relationship between the contents of lipids and nitrogen, no correlation can be observed between the relative amounts of any of the seed com-

TABLE 1

Composition and Initial Content of Gossypol and Gossypurpurin in Cottonseed of Different Varieties of *G. barbadense*

| Sample No. | Sub-species and variety | Composition of seed | | | | Initial pigment content | |
|---|---|---|---|---|---|---|---|
| | | Kernels | | | Delinted seed | | |
| | | Lipids [1] | Nitrogen [1] | $H_2O$ | $H_2O$ | Gossypol [2] | Gossypurpurin [2] |
| Normal Planting [3] | | % | % | % | % | % | % |
| 3 | Sea Island $12B_2$ | 41.35 | 4.78 | 6.60 | 10.79 | 2.54 | 0.0184 |
| 1 | Sea Island Seaberry | 40.75 | 4.86 | 6.67 | 10.88 | 3.35 | 0.0254 |
| 2 | Sea Island TZRV | 39.41 | 5.18 | 6.30 | 11.18 | 2.80 | 0.0244 |
| 5 | Egyptian Earlipima | 41.56 | 5.09 | 7.08 | 9.99 | 2.30 | 0.0360 |
| 7 | Egyptian SXP | 40.62 | 5.53 | 6.90 | 9.94 | 1.94 | 0.0299 |
| 4 | Egyptian Amsak | 40.23 | 5.32 | 7.09 | 9.93 | 1.92 | 0.0370 |
| 6 | Egyptian Pima | 38.64 | 5.18 | 7.56 | 9.90 | 2.56 | 0.0485 |
| Late Planting [4] | | | | | | | |
| 8 | Sea Island Seaberry | 40.32 | 4.85 | 6.77 | 10.33 | 3.04 | 0.0189 |
| 9 | Sea Island TZRV | 39.33 | 5.30 | 6.74 | 10.72 | 2.75 | 0.0538 |
| 10 | Sea Island $12B_2$ | 35.14 | 5.46 | 3.12 | 10.04 | 1.94 | 0.0270 |
| 11 | Egyptian Amsak | 40.57 | 4.85 | 6.74 | 10.57 | 2.78 | 0.0173 |
| 14 | Egyptian SXP | 38.88 | 5.36 | 6.52 | 9.48 | 2.02 | 0.0245 |
| 12 | Egyptian Earlipima | 37.07 | 5.23 | 3.84 | 9.83 | 2.26 | 0.0205 |
| 13 | Egyptian Pima | 33.35 | 5.35 | 5.30 | 9.51 | 2.54 | 0.0262 |

[1] Calculated on a moisture-free basis.
[2] Calculated on basis of weight of kernels.
[3] Planted May 14 and harvested November 18; mature seed.
[4] Planted May 31 and harvested November 18; about 75 per cent of bolls open.

ponents in these varieties of upland cottonseed. The relationship between content of lipids and gossypol observed in previous investigations (15, 30) was not detected in these seed. Neither does there appear to be any relation between the reported characteristic staple length of the lint of the different varieties and the content of either gossypol or gossypurpurin in the kernels.

In Table 3 are shown the average and the lowest and highest initial concentrations of gossypol and gossypurpurin found in different varieties of the species, *G. hirsutum*, and different varieties of Egyptian and Sea Island seed of the species, *G. barbadense*. As shown by these values, the average content of gossypol is less, and the average content of gossypurpurin is markedly less in seed of different varieties of *G. hirsutum* than in Egyptian or Sea Island seed. Egyptian seed obtained from the normal planting contained less gossypol and more gossypurpurin than Sea Island seed grown under the same conditions.

The differences in conditions of growth resulting from the normal and late planting of seed of different varieties of *G. barbadense* caused changes in their content of gossypol and gossypurpurin, but the changes in the Sea Island seed were the opposite of those in the Egyptian seed. All varieties of Sea Island seed obtained from the late planting contained less gossypol and two varieties contained more gossypurpurin than the corresponding varieties obtained from the normal planting whereas the Egyptian seed obtained from the late planting contained about the same or somewhat more gossypol and less gossypurpurin than the corresponding varieties obtained from the normal planting.

*Variations in Content of Gossypol and Gossypurpurin During Storage of Cottonseed.* The curves in Figure 1 show the variation of gossypol during storage of the seed. The numbers of the curves correspond to the sample numbers listed in Table 1 and 2, and the curves are grouped as follows: curves 1 through 7 on the left are for Sea Island and Egyptian seed obtained from the normal planting, curves 8 through 14 are for the corresponding seed obtained from the late planting, and curves 15 through 22, 24, and 25 are for seed of different varieties of *G. hirsutum*. The time of storage was calculated as the number of days elapsed between harvesting of a given sample of seed and the determination of its content of gossypol.

The concentration of gossypol in the three varieties of Sea Island seed obtained from the normal planting (Figure 1, Curves 1 to 3) was initially high and

TABLE 2

Composition of Seed of Different Varieties of *G. hirsutum* [1]

| Sample | Variety | Varietal characteristics | Staple length | Composition of seed | | | | Initial pigment content | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Kernels | | | Delinted seed | | |
| | | | | Lipids [2] | Nitrogen [2] | $H_2O$ | $H_2O$ | Gossypol [3] | Gossypurpurin [3] |
| | | | | % | % | % | % | % | % |
| 15 | Delfos 651 | High oil, high nitrogen | Long | 42.12 | 4.61 | 7.01 | 9.66 | 1.89 | 0.00462 |
| 16 | Bobdel | High oil, high nitrogen | Long | 40.71 | 4.87 | 6.78 | 9.28 | 1.38 | 0.00592 |
| 25 | Cook Naked Seed | Naked | Short | 40.71 | 4.84 | 5.86 | 9.78 | 1.57 | 0.00665 |
| 20 | Coker 4 in 1 (7) | High oil, low nitrogen | Long | 40.53 | 4.84 | 6.50 | 9.02 | 1.89 | 0.00622 |
| 21 | Wilds 17 | ............... | Long | 40.30 | 5.08 | 6.12 | 9.59 | 1.67 | 0.00547 |
| 19 | Coker 100-7 | Low oil, high nitrogen | Long | 39.91 | 5.15 | 7.14 | 10.07 | 2.09 | 0.00778 |
| 18 | Stoneville 2B | High oil, low nitrogen | Intermed. | 39.87 | 4.72 | 6.76 | 9.28 | 1.94 | 0.00671 |
| 24 | McNamara | Naked | Short | 39.10 | 5.42 | 6.10 | 9.95 | 2.12 | 0.00826 |
| 17 | Deltapine 14 (833) | Low oil, high nitrogen | Intermed. | 38.57 | 4.97 | 6.99 | 9.34 | 1.87 | 0.00670 |
| 22 | Half and Half | ............... | Short | 38.18 | 5.39 | 6.58 | 9.66 | 1.98 | 0.00810 |

[1] Planted May 31 and harvested November 7; mature seed.
[2] Calculated on moisture-free basis.
[3] Calculated on basis of weight of kernels.

TABLE 3

Initial Content of Gossypol and Gossypurpurin in Seed of the Species, *G. hirsutum* and *G. barbadense*

| Species | Sub-species | Number of varieties examined | Time of planting | Average initial content of pigments in kernels [1] | |
|---|---|---|---|---|---|
| | | | | Gossypol | Gossypurpurin |
| | | | | % | % |
| *G. hirsutum* | ........... | Ten | Normal [2] | 1.84 (1.38-2.12) | 0.00664 (0.00462-0.00826) |
| *G. barbadense* | Egyptian | Four | Normal [3] | 2.18 (1.92-2.56) | 0.0378 (0.0299-0.0485) |
| *G. barbadense* | Sea Island | Three | Normal [3] | 2.90 (2.54-3.35) | 0.0227 (0.0184-0.0254) |
| *G. barbadense* | Egyptian | Four | Late [4] | 2.40 (2.02-2.78) | 0.0221 (0.0173-0.0262) |
| *G. barbadense* | Sea Island | Three | Late [4] | 2.56 (1.94-3.04) | 0.0332 (0.0189-0.0538) |

[1] Figures in parentheses give maximum and minimum values for varieties within each group.
[2] Planted May 31 and harvested November 7; maturity complete.
[3] Planted May 14 and harvested November 18; maturity complete.
[4] Planted May 31 and harvested November 18; about 75 per cent of bolls open.

increased rapidly during the first two months of storage, after which it remained unchanged or decreased slightly during subsequent storage of the seed. In the case of the Egyptian seed obtained from the normal planting, the amount of gossypol in Amsak, Pima, and SXP cottonseed (Figure 1, Curves 4, 6, and 7) changed very little during storage whereas the amount of gossypol in Earlipima cottonseed (Figure 1, Curve 5) increased rapidly throughout the period of storage.

The variation of gossypol during storage of Sea Island cottonseed obtained from the late planting followed a different course in each variety. Gossypol increased rapidly over a period of seven months in TZRV (Curve 9); it increased rapidly during the first two months and less rapidly thereafter in Seaberry (Curve 8); and it increased slightly and then decreased to its initial value in $12B_2$ (Curve 10). The concentration of gossypol in three varieties of Egyptian seed obtained from the late planting increased rapidly during the first two months of storage, after which it increased less rapidly in Earlipima and SXP (Curves 12 and 14), and decreased in Amsak (Curve 11). The concentration of gossypol changed very little during storage of Pima seed (Curve 13), increasing slightly during the initial storage period and then decreasing slightly.

There does not appear to be any relation between the variation of gossypol during storage of the seed obtained from the late planting and that of the corresponding seed obtained from the normal planting.

The variation of gossypol during storage of seed of different varieties of *G. hirsutum* (Figure 1, Curves 15 through 25) does not appear to follow any consistent pattern. There was relatively little change in the content of gossypol during storage of Delfos, Stoneville 2B, and Cook Naked seed (Curves 15, 18, and 25); the concentration of gossypol increased during storage of Bobdel, Deltapine, and McNamara seed (Curves 16, 17, and 24); and it decreased during storage of Coker 100-7, Coker 4 in 1, Wilds 17, and Half and Half seed (Curves 19, 20, 21, and 22). No relation is apparent between the initial content

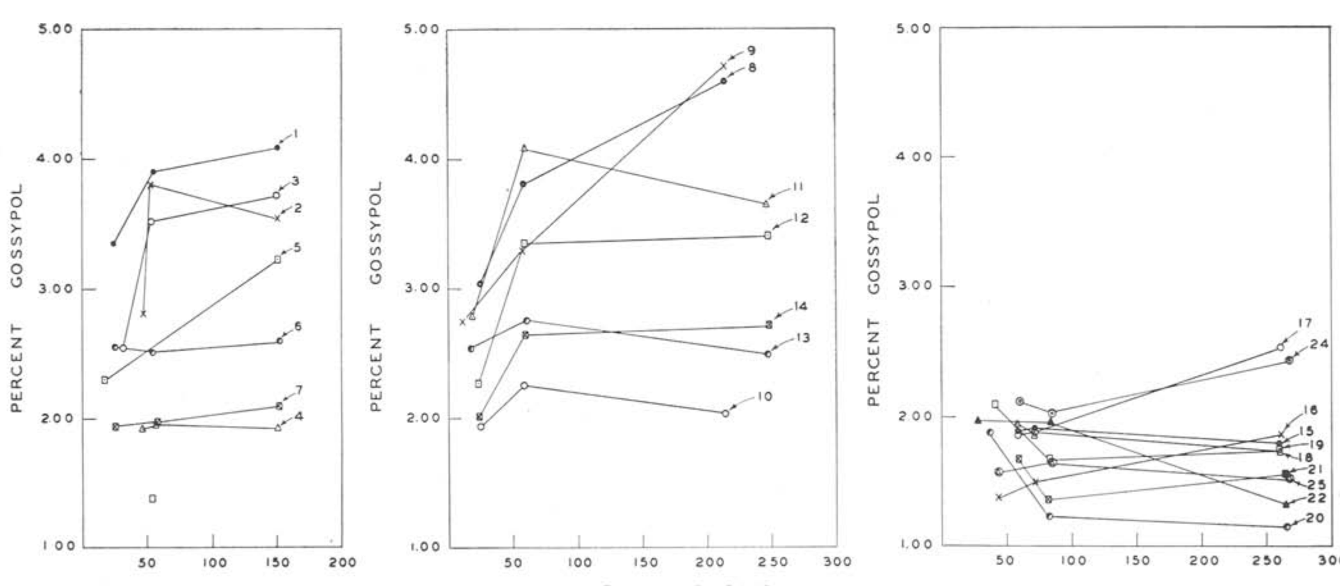


FIG. 1. Variation in content of gossypol during storage of cottonseed of different varieties of *G. barbadense* and *G. hirsutum*. Curves 1 through 7 are for different varieties of Sea Island and Egyptian seed obtained from a normal planting; curves 8 through 14 are for the corresponding seed obtained from a later planting; and curves 15 through 22, 24, and 25 are for seed of different varieties of *G. hirsutum*.

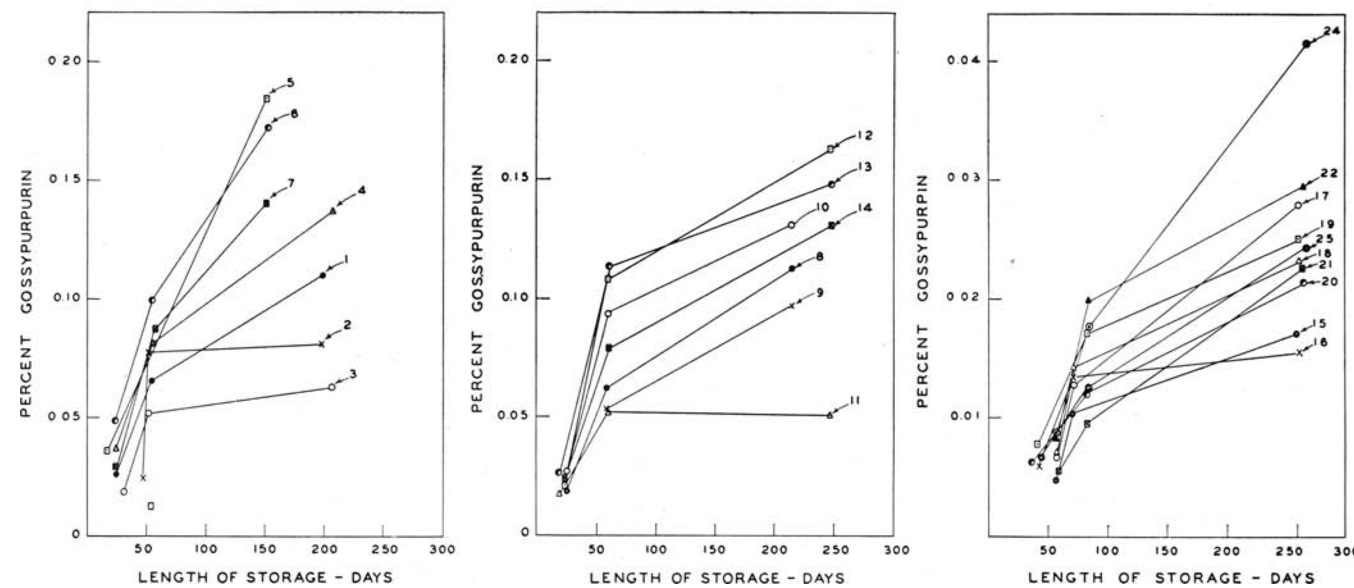


FIG. 2. Variation in the content of gossypurpurin during storage of cottonseed of different varieties of *G. barbadense* and *G. hirsutum*. Curves 1 through 7 are for different varieties of Sea Island and Egyptian seed obtained from a normal planting; curves 8 through 14 are for the corresponding seed obtained from a late planting; and curves 15 through 22, 24, and 25 are for seed of different varieties of *G. hirsutum*.

of gossypol and the rate of its formation or disappearance during storage of the seed.

The curves in Figure 2 show the variation of gossypurpurin during storage of the seed. The numbers of the curves correspond to the sample numbers listed in Tables 1 and 2, and the curves are arranged as in Figure 1. The time of storage is taken as the number of days between harvesting of a given sample of seed and determination of its content of gossypurpurin.

In all of the samples of seed examined the content of gossypurpurin increased during storage. The increase was very rapid during the first two months of storage, but during more prolonged storage gossypurpurin formed very much more slowly in most cases and remained relatively unchanged in the case of Sea Island TZRV from the normal planting, Egyptian Amsak from the late planting, and Bobdel (Figure 2, Curves 2, 11, and 16, respectively).

The content of gossypurpurin was initially much higher and increased more rapidly during storage of Sea Island and Egyptian seed (Figure 2, Curves 1 through 14) than that of seed of different varieties of *G. hirsutum*. However, for different varieties of the same species there does not appear to be any relation between the initial content of gossypurpurin and its formation during storage of the seed.

## Discussion

Seed of different varieties of Sea Island and Egyptian cotton of the species *G. barbadense* were found to contain more gossypol and very much more gossypurpurin than seed of different varieties of *G. hirsutum*. These observations are consistent with those reported for gossypol (31) and gossypurpurin (25) in cottonseed of Russian production. The conclusions of Smirnova (31) that the content of gossypol is a species characteristic has been confirmed and the generalization can be extended to include gossypurpurin, which has been shown (7) to constitute the other intraglandular pigment of cottonseed.

Within the species *G. barbadense* Sea Island seed obtained from the normal planting contained more gossypol and less gossypurpurin than Egyptian seed obtained from the same planting, thus indicating that the content of these pigments might be a sub-species characteristic. However, the relatively large and inconsistent changes in content of these pigments in seed obtained from the late planting demonstrates that gossypol and gossypurpurin are independently affected by conditions of growth and maturity. These observations stress the necessity for further investigations to determine the relative importance of the influence of environmental and genetic factors on the content of these pigments.

No pigments other than gossypol and gossypurpurin were detected in the seed. However, the methods used were not sufficiently sensitive for detecting traces of pigments other than gossypol and gossypurpurin so that the possibility of the occurrence of small amounts of other pigments is not precluded.

Podolskaya (25) has suggested that gossypol, which has been found (11, 13, 14, 29) to form during the early stages of development of the seed, is the precursor of red gossypol, which she detected (24, 25) in mature and stored cottonseed but failed to recognize as a mixture of gossypol and gossypurpurin. Gossypurpurin has been shown (1, 6) to be a derivative of gossypol which can be prepared *in vitro* by reaction of gossypol with ammonium hydroxide, and it increases continuously during storage of cottonseed, but this increase is not accompanied by a parallel decrease in gossypol, and the concentration of gossypol is always very much greater than that of gossypurpurin. Thus it appears that although the variation of gossypol and gossypurpurin during storage of cottonseed furnishes evidence in support of the hypothesis (10) that the intraglandular pigments participate in the metabolism of the seed, further investigations are needed in order to determine the nature of their interaction.

The observation that gossypurpurin occurs in highest concentration in Sea Island and Egyptian seed and increases during storage of all kinds of cottonseed suggests that gossypurpurin, or a decomposition product of this pigment, is responsible for the dark colors of oils produced from Sea Island and Egyptian seed as well as those produced from stored cottonseed.

The failure to detect any direct relation between the content of gossypol and gossypurpurin and other varietal and species characteristics of cottonseed suggests the possibility of breeding cottonseed for optimum content of these pigments without adversely affecting other desirable characteristics of the seed such as content of lipids and protein and quality of lint. Whether seed should be chosen for high or low content of the intraglandular pigments will depend upon the method chosen for processing cottonseed. In processing cottonseed by the conventional hydraulic and screw-press methods, as well as by recently introduced solvent extraction methods, the pigments have only a nuisance value, and control of color constitutes an important processing problem (2, 6, 8, 17, 18, 33). Consequently, if cottonseed are to be processed by any of the aforementioned methods, efforts of geneticists should be directed toward the development of varieties having a low content of pigments. On the other hand, by application of the recently developed gland flotation process for the mechanical fractionation of cottonseed (5, 32), intact pigment glands are obtained as a by-product of lightly colored oil and meal. The pigment glands have been shown (7) to constitute from three to five per cent of the weight of the kernel, and to contain all of the gossypol and gossypurpurin of the seed. Gossypol, the principal component of the glands, constitutes from 35 to 50 per cent, and gossypurpurin from one to three per cent of the weight of the separated glands. It has been found (12) that gossypol can readily be prepared from the glands in good yields. Thus, if practical uses can be developed for gossypol and its derived products, it would be desirable to use the gland flotation process for cottonseed, and to develop strains of cottonseed having a high content of pigments.

## Summary

A selection of cottonseed for planting was made which comprised seed of three varieties of Sea Island cotton, four varieties of Egyptian cotton, and 10 varieties of upland cotton providing two or more varieties having the following characteristics: short, intermediate, and long staple; low, intermediate and high content of nitrogen. The seed analyzed were obtained from plants grown under identical conditions.

The initial contents of moisture, lipids, nitrogen, gossypol, and gossypurpurin were determined, after which the samples of seed were stored under identical conditions and analyzed for gossypol and gossypurpurin at fixed intervals over a period of a year. Seed of the species *G. barbadense* contained more gossypol and very much more gossypurpurin than seed of the species *G. hirsutum.* Within the species *G. barbadense* Sea Island seed contained more gossypol and less gossypurpurin than Egyptian seed.

The content of gossypurpurin increased during storage of all of the seed, whereas that of gossypol varied in a number of different ways, increasing in some, decreasing in others, and remaining relatively constant in a few samples.

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