TABLE I

Physical and Chemical Characteristics of Oils and Their Molecularly Distilled Fractions

Fraction No.	Temperature range (°C.)	Weight % of original oil	Nature and color of the fraction	Acid value	Iodine value (Wijs)	Saponification value	Refractive index	Unsaponifiable matter (%)
1	2	3	4	5	6	7	8	9
Karanja oil (*Pongamia glabra*, Vent)								
1	Below 110	4.6	Semi-solid; lemon yellow	74.1	74.1	.....[g]	.....	46.1[e]
2	110–170	5.8	Solid; orange-yellow	36.7	56.6	.....[g]	.....	59.7[e]
3	170–220	4.9	Viscous;reddish orange	5.5	84.9	.....[g]	1.4943[b]	9.8[e]
4	220–240	28.7	Liquid; yellow	0.5	90.9	193.6	1.4688[b]	1.9[e]
5	240–250	19.8	Liquid; pale yellow	0.3	93.3	194.6	1.4678[b]	0.4[e]
6[d]	.....	36.2	Liquid; dark orange	0.3	90.6	191.5	1.4678[b]	0.3[e]
Karanja oil	.....	.....	Liquid; brownish orange	5.4	87.2	188.0	1.4803[b]	6.2
Malkanguni oil (*Celastrus paniculatus*, Willd)								
1	Below 110	14.0	Solid; whitish yellow	189.1	75.0	228.6	1.4482[c]	12.6[f]
2	110–140	9.2	Semi-solid; yellow	117.1	79.8	257.6	1.4582[c]	4.3[f]
3	140–185	10.7	Viscous liquid; orange-yellow	25.2	71.8	305.7	1.4728[c]	3.2[f]
4	185–210	23.2	Liquid; orange-yellow	3.9	105.9	264.6	1.4610[c]	2.4[f]
5[d]	.....	42.9	Liquid; dark brown	3.9	100.8	227.8	1.4600[c]	1.9[f]
Malkaguni oil	.....	.....	Liquid; reddish brown	49.3	92.6	242.8	1.4601[c]	3.9[f]
Undi oil (*Calophyllum inophyllum*, Linn)								
1	Below 130	3.9	Solid mass, greenish yellow	183.2	91.6	199.2	1.4570[c]	1.0[f]
2	130–180	11.5	Jelly-like viscous; yellow	136.1	100.9	192.8	.....	2.4[f]
3	180–220	4.9	Viscous liquid; orange-yellow	76.1	107.7	195.2	1.4947	2.6[f]
4	220–240	11.0	Liquid; dark yellow	7.3	86.1	194.6	1.4605[c]	1.3[f]
5	240–250	17.8	Liquid; pale yellow	1.3	86.4	193.2	1.4559[c]	0.2[f]
6[d]	.....	50.9	Liquid; brownish black	0.6	86.6	191.5	1.4550[c]	1.4[f]
Undi oil	.....	.....	Liquid; dark green	27.6	92.3	197.1	1.4640[c]	1.3[f]
Sesame oil (*Sesamum indicum*, L.)								
1[a1]	Below 180	1.7	Semi-solid; orange-yellow	74.2	94.3	107.8	.....	49.2[e]
2[a1]	180–200	1.8	Semi-solid; orange-yellow	10.3	104.2	142.3	.....	30.2[e]
3[a1]	200–220	7.1	Liquid; pale yellow	0.6	107.4	193.5	1.4689[b]	2.3[e]
4[a1]	220–240	31.7	Liquid; pale yellow	0.2	109.8	200.7	1.4679[b]	1.4[e]
5[a2]	240–260	49.0	Liquid; pale yellow	0.1	111.2	200.4	1.4680[b]	0.2[e]
6[d, a2]	.....	8.7	Liquid; blackish yellow	0.1	110.8	201.0	1.4699[b]	0.1[e]
Sesame oil	.....	.....	Liquid; yellow	1.6	109.4	193.7	1.4694[b]	1.2[e]

[a1] Baudouin test, positive. [a2] Baudouin test, negative. [b] At 30°C. [c] At 60°C. [d] Residual oil. [e] Ethyl ether. [f] Petroleum Ether. [g] Titration end-point could not be judged.

3. Sesamin could be isolated from the first fraction of molecularly distilled sesame oil by crystallization.
4. Karanjin and pongamol were similarly separated from the first fraction of molecularly distilled karanja oil.
5. With malkanguni oil there was some fractionation of the glycerides.
6. Elimination curves of karanja, malkanguni, undi, and sesame oils are given.

REFERENCES

1. Bailey, A. E., Oliver, G. D., Singleton, W. S., and Fisher, G. S., Oil and Soap, *20*, 251 (1943).
2. Bhat, S. G., Kane, J. G., and Sreenivasan, A., J. Am. Pharm. Assoc. (Sci. ed.), *43*, 543 (1954).
3. Detwiler, S. B. Jr., Bull, W. C., and Wheeler, D. H., Oil and Soap, *20*, 108 (1943).
4. Embree, N. D., Chem. Revs., *29*, 317 (1941).
5. Gangadharan, P. R. J., Natarajan, S., Wadhwani, T. K., Giri, K. V., Narayanamurty, N. L., and Iyer, B. H., J. Ind. Inst. Sci., *35*, 69 (1953).
6. Haller, H. L., McGovran, E. R., Goodhue, L. D., and Sullivan, W. N., J. Org. Chem., *7*, 183 (1942).
7. Haller, H. L., La Forge, F. B., and Sullivan, W. N., J. Econ. Entomol., *35*, 247 (1942).
8. Heftmann, E., J. Am. Oil Chemists' Soc., *24*, 404 (1947).
9. Hickman, K. C. D., Ind. Eng. Chem., *29*, 1107 (1937).
10. Komori, S., Kikuchi, S., Hachiya, K., Shinsugi, A., and Agawa, T., J. Chem. Soc., Japan, Ind. Chem. Sect., *54*, 225 (1951).
11. Patel, S. M., and Sreenivasan, A., J. Sci. & Ind. Res. (India), *9B*, 31 (1950).
12. Rangaswami, S., and Seshadri, T. R., Ind. J. Pharmacy, *3*, 3 (1941).
13. Rawlings, H. W., Oil and Soap, *16*, 231 (1939).
14. Riemenschneider, R. W., Swift, C. E., and Sando, C. E., Oil and Soap, *17*, 145 (1940).
15. Sabashi, K., Nakayama, A., and Hirao, S., Bull. Japan Soc. Sci. Fisheries, *16*, 46 (1950).
16. Subbarao, N. V., Veerabhadrarao, J., and Seshadri, T. R., Proc. Ind. Acad. Sci. (India), *10A*, 65 (1939).
17. Tetsujiro Obara and Keijiro Oka., J. Japan. Soc. Food Nutrition, *3*, 138 (1951).
18. Toshio Agawa, Suburo Komori, and Yoichi Nishimura, J. Oil Chemists' Soc., Japan, *1*, No. 1, 12 (1952).
19. Ueno, S., Komori, S., and Agawa, T., J. Chem. Soc., Japan, Ind. Chem. Sect., *52*, 67 (1949).
20. Villavecchia, V., and Fabris, G., Z. Angew. Chem., *17*, 505 (1893).
21. Wachs, W., Z. Lebensm-Untersuch. U. -Forsch, *96*, 168 (1953).
22. Yamakita, I., Araki, Y., and Shimamoto, S., Bull. Inst. Chem. Res., *24*, 81 (1951).

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Effect of Maleic Hydrazide Applied to the Cotton Plant on the Development of Free Fatty Acids in the Seed

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MALEIC HYDRAZIDE, a remarkably consistent plant growth inhibitor among many species, has been applied to cotton plants in concentrations of 0.5 to 1.0% (3) and 0.48% (8) as an inhibitor of secondary growth. It has been applied to cotton plants by many other investigators (5, 6, 7, 9, 10, 15, 16, 17), but in none of these studies have the effects of maleic hydrazide on the storage properties of the seed been reported. In a field experiment on cotton plants, considerably higher concentrations of maleic hydrazide than those hitherto reported were applied prior to and at defoliation to determine their effects on secondary growth. Seed harvested from these plants was made available to investigators at

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the Southern Regional Research Laboratory. The samples were used to determine the effect of the inhibitor on the rate of deterioration of the seed during storage as measured by the development of free fatty acids. Results reported are for one season. Successful inhibition of deterioration in cottonseed during storage for three months or more is of marked interest in the Cotton Belt and of particular significance to the cottonseed crushing industry.

This paper represents a preliminary report, the purpose of which is to serve as a basis or starting-point for others who might be interested in pursuing this work further. In all probability the effects noted will also be of interest to investigators who are applying maleic hydrazide in other fields.

Materials and Methods

It has been shown that the rate of formation of free fatty acids as well as other manifestations of deterioration in naturally moist cottonseed is dependent on the moisture and free fatty acids contents of the seed at the time of harvest (12); also, that if the moisture content of naturally moist seed is reduced artificially by aeration, the rate of formation of free fatty acids likewise decreases (12). Conversely the rate of formation of free fatty acids by cottonseed can be increased by artificially increasing the moisture content (14). When the relative humidity of the atmosphere surrounding the seed exceeds 84%, increases in moisture content are accompanied by growth of microorganisms which, in turn, contribute their share to increasing the production of free fatty acids and liberating more moisture (4).

The various seed lots used in this experiment were exposed to relative humidities of 75, 91, and 100%, as a method of artificially increasing the moisture content and thereby promoting the formation of free fatty acids. Desiccators were used with suitable connections, manifolds, and saturated salt solutions (11) to insure exposure of the seed, a Stoneville 2B variety (1950), to a uniform atmosphere of the desired relative humidity.

In all, six lots of seed were received. Two were from untreated control plants, labeled 1A (9.6% moisture and 3.15% free fatty acids) and 1B (9.7% moisture and 2.5% free fatty acids). Two were from plants treated with 3% by volume of a 30% maleic hydrazide formulation; maleic hydrazide was present as the diethanol amine salt dissolved in water (spray was applied at the rate of 15 gal. per acre). One treated at defoliation was labeled 2A (11.3, 9.7% moisture and 1.71% free fatty acids); the other treated 16 days prior to defoliation was labeled 2B (10.9, 9.4% moisture and 1.80% free fatty acids). The remaining two samples were from plants treated with 15% by volume of a 30% maleic hydrazide formulation at defoliation, 3A (10.6% moisture and 1.04% free fatty acids), and 16 days prior to defoliation, 3B (9.7% moisture and 1.5% free fatty acids).

According to Tharp (18), the usual rule is to make the application of defoliants to cotton plants at the time when approximately 50% of the bolls are open. It is to be expected therefore that only a few bolls would be open 16 days prior to defoliation. In applying maleic hydrazide according to the schedule outlined in this investigation, it is visualized that at least three different situations were created: a) maleic hydrazide came in contact with open bolls; b) mature but unopened bolls were "contaminated" by contact with bolls of group one during harvest and ginning; and c) seed from immature bolls may have picked up maleic hydrazide by translocation as well as by later "contamination" as with group two. At this time one can only note that these possibilities exist. It would be premature to suggest how the effects measured were brought about.

Results

In spite of thorough mixing of the individual samples on their arrival at the Southern Regional Research Laboratory, moisture contents varied considerably, ranging from 9.4% to 11.3% (Table I). At 91% R. H. and at 100% R. H. all samples showed increases in moisture content, ranging from 1.0 to 3.0% after six weeks of storage (Table I). For the longer intervals of storage, namely, 16, 20, and 23 weeks at 100% R. H. and 16, 20, and 28 weeks at 91% R. H., all lots except 3A and 3B showed significant increases in moisture content. After 23 weeks of storage at 100% R. H. lots 3A and 3B increased in moisture content by 1.5% and 2.0%, respectively. The moisture contents of all lots at 91% R. H. and 100% R. H. were considerably lower than might have been expected under these relative humidities at 78°F. (13) even though all six samples at each relative humidity were exposed to the same air supply, channelled through the same manifold. Little change occurred in the moisture contents of the various samples at 75% R. H. during the storage interval of 28 weeks; the range was 9.9% to 11.5%.

Table II shows that free fatty acids contents at the time of harvest of the untreated lots, 1A and 1B, were 3.15% and 2.57%, respectively. These values are in excess of that for prime seed, 1.8%, and indicate that this seed was damaged (sometimes called "weathered") (1). A check of the weather conditions after defoliation indicated that sufficient rain had fallen to cause the seed to become field-damaged

TABLE I

The Effect of Maleic Hydrazide Applied to the Cotton Plant on the Moisture[a] Uptake of Seed Harvested from the Plant When the Seed[b] Is Stored at 78°F. and Various Relative Humidities

Storage Weeks	Percentage Moisture Content					
	1A	1B	2A	2B	3A	3B
0	9.6%	9.7%	11.3, 9.7%	10.9, 9.4%	10.7%	9.7%
	100% Relative Humidity					
6	12.6	12.5	12.2	11.5	11.9	11.3
16	15.7	16.0	15.2	15.2	12.2	12.0
20	18.1	18.7	16.8	16.6	.....	.....
23	.....	.....	.....	.....	12.7	12.2
28	.....	.....	.....	.....	14.2	14.5
	91% Relative Humidity					
6	12.1	12.1	12.5	12.4	11.7	11.3
16	12.8	12.8	13.3	13.1	11.9	11.6
20	13.5	13.6	14.1	14.1	.....	.....
28	15.6	16.0	16.0	16.9	13.1	12.8
	75% Relative Humidity					
6	11.3	11.1	9.9	10.5	11.3	11.2
16	11.6	11.1	10.4	10.9	11.5	11.2
20	11.3, 20.6[c]	11.2	10.6	10.5, 9.9	.....	.....
28	11.2	11.2	10.0		11.5	11.1

[a] Each figure represents an average of duplicate values. Method of the American Oil Chemists' Society was used (2).

[b] Samples 1A and 1B = from different untreated control lots.
Samples 2A and 2B = sprayed with 3% of a 30% maleic hydrazide formulation, A–at defoliation and B–16 days prior to defoliation.
Samples 3A and 3B = sprayed with 15% of a 30% maleic hydrazide formulation, A–at defoliation and B–16 days prior to defoliation.

[c] No explanation is available for this high moisture content.

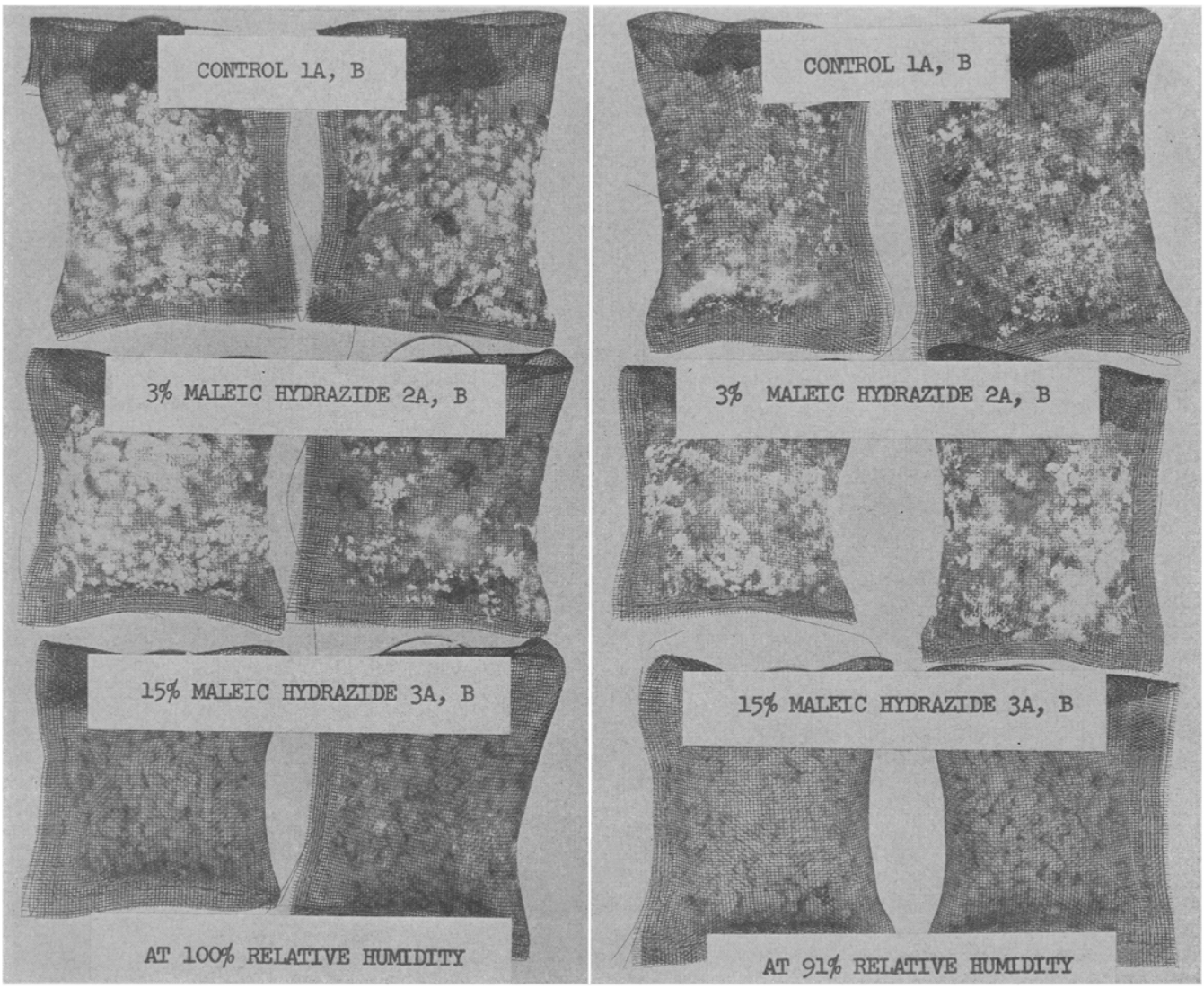

FIG. 1. Effect of maleic hydrazide sprayed on cotton plants at defoliation (A) and 16 days prior to defoliation (B) on the growth of mold on the harvested seed stored for 7 months at 78°F. and 100% R. H. (left) and 91% R. H. (right).

(1). At 100% R. H. these lots of seed began forming free fatty acids rapidly after the sixth week of storage. At 91% R. H. free fatty acids did not form rapidly until after the 20th week of storage. No real "break" (a sudden rapid rise in free fatty acids content) was observed for seed lots stored at 75% R. H. The free fatty acids contents of samples 2A and 2B at the time of harvest were 1.71% and 1.80%, respectively (Table II). These values indicate that the seed was in prime condition as far as the free fatty acids content was concerned. These seeds were exposed to the same unfavorable conditions prior to harvest as the untreated seed, yet the free fatty acids content was that of prime seed. It is possible that the maleic hydrazide treatment had protected this seed in the field. Free fatty acids formed rapidly in these samples after the 16th week of storage at 100% R. H. and after the 20th week at 91% R. H. At 75% R. H. the rise in free fatty acids content was very slow (Table II). At harvest time the free fatty acids content of samples 3A and 3B were 1.04% and 1.50%, respectively (Table II). The low values indicate clearly that the seed was in prime condition with respect to free fatty acids and that the maleic hydrazide treatment had similarly protected this seed from field damage. At 100% R. H. the free fatty acids content began to rise rapidly only after the 23rd week. No rapid rise in free fatty acids occurred in these lots at either 91% R. H. or 75% R. H. (Table II).

Although mold counts were not obtained for the various samples and other investigators have pointed out misconceptions that may occur if much weight is placed on gross visual observations, such observations have been recorded here because they appear to be directly correlated with the development of free fatty acids. It is of interest therefore to compare the following observations on the appearance and odor of the whole seeds, kernels, and ground meats with the rapid development of free fatty acids in the samples. After six weeks of storage at 100% R. H. lots 1A and 1B had a musty odor, noticeable in the whole seeds, kernels, and ground meats, although spores were not visible on the seeds or kernels. After 16 weeks however many spores were visible, and an odor of putre-

TABLE II

The Effect of Maleic Hydrazide Applied to the Cotton Plant on the Development of Free Fatty Acids[a] in Seed Harvested from the Plants When the Seed[b] Is Stored at 78°F. and Various Relative Humidities

Storage Weeks	Percentage Free Fatty Acids					
	1A	1B	2A	2B	3A	3B
0	3.15%	2.57%	1.71%	1.80%	1.04%	1.50%
100% Relative Humidity						
2	2.74	3.00	1.89	2.05	1.20	1.85
6	4.22	3.78	2.13	2.13	0.87	1.52
16	14.49	10.39	5.75	5.69	1.95	2.30
20	40.18	34.31	19.39	18.15	.....	.....
23	.....	.....	.....	.....	2.44	1.91
28	.....	.....	.....	.....	5.15	8.88
91% Relative Humidity						
2	3.13	2.80	2.08	1.95	1.30	1.16
6	4.48	3.71	1.92	1.96	1.66	2.42
16	5.83	3.23	4.71	3.04	1.28	2.62
20	6.70	5.56	3.52	4.47	.....	.....
28	28.40	24.09	28.96	26.05	1.97	3.06
75% Relative Humidity						
2	3.03	2.48	1.61	1.85	1.18	1.90
6	4.35	3.80	0.60	2.09	1.90	1.84
16	4.09	4.10	2.62	2.67	1.75	2.06
20	6.00	4.89	3.04	3.24	.....	.....
28	6.06	4.44	2.37	4.50	1.53	2.37, 3.12

[a] Each figure represents an average of duplicate values. Method of the American Oil Chemists' Society was used (2).
[b] Samples 1A and 1B = from different untreated control lots.
Samples 2A and 2B = sprayed with 3% of a 30% maleic hydrazide formulation, A–at defoliation and B–16 days prior to defoliation.
Samples 3A and 3B = sprayed with 15% of a 30% maleic hydrazide formulation, A–at defoliation and B–16 days prior to defoliation.

faction was noted in the kernels. On examining the data in Table II, it is seen that at 100% R. H. lots 1A and 1B exhibited a rapid rise in the formation of free fatty acids between the sixth and sixteenth weeks. This rapid rise is termed a "break" in the development of free fatty acids, occurring during the same interval when the musty odor was first noted and the seeds eventually became covered with mold. Similar "breaks" occurred in lots 2A and 2B between 16 and 20 weeks of storage, in lot 3B between 23 and 28 weeks of storage at 100% R. H., and in lots 1A, 1B, 2A, and 2B between 20 and 28 weeks at 91% R. H. In every case where a "break" occurred, the same sequence of observations for the appearance and odor of the whole seeds, kernels, and ground meats was noted. (See Figure 1 for gross appearance of samples at 28 weeks of storage.) Since molds were beginning to appear only at the 28th week of storage in lots 3A and 3B at 100% and 91% R. H. and a "break" in the development of free fatty acids occurred in only one out of four samples, it is possible that the higher concentration of maleic hydrazide was exerting a fungistatic effect and that the apparent inhibition of the formation of free fatty acids may have arisen from this activity.

Throughout the 28 weeks of storage under 75% R. H. however all lots of seed were clean in appearance and no odor of mold could be discerned. When the kernels were examined, all were found to be clean and the odor of mold could not be detected in the whole kernels or the ground meats. The ground meats were a pale yellow color and had the odor of fresh cottonseed. It is assumed that a "break" in the development of free fatty acids was not imminent. Also, moisture contents remained uniform and similar so that at any particular sampling date moisture contents of all samples were generally within experimental error of each other. Nevertheless marked differences in free fatty acids contents developed by the 28th week of storage. Here, then, it would appear that inhibition of the formation of free fatty acids, particularly in sample 3A (Table II, Col. 6), where the treatment was applied at defoliation and the free fatty acids content was still that of prime seed, resulted from the activity of maleic hydrazide on the seed itself. In this connection it is reassuring to note that Christensen did not observe growth of molds in cottonseed stored at relative humidities under 84% (4).

A duplicate set of experiments were conducted during the following season (1952) by one of the authors (Carns), and again seed was made available to investigators at the Southern Regional Research Laboratory. Because of the drought that year, results from similar storage experiments with the seed were not as clear-cut as those obtained in the 1951 study. For this reason data obtained in 1952 are not reported.

Summary

The available evidence suggests that maleic hydrazide may have contributed to inhibition in the formation of free fatty acids in the seed during field exposure and the presampling period; to inhibiting the proliferation of mold on the seed at high relative humidities during storage and to reducing the rate of formation of free fatty acids; and to inhibiting the slow rate of formation of free fatty acids by the seed itself apparently in the absence of mold growth. The seed appeared to be protected for approximately 16, 20, and 20–28 weeks at 100%, 91%, and 75% R. H., respectively. The evidence seems to favor only slightly the higher concentration of treatment. However its application prior to or at defoliation seemed to have no measurable effect on the development of free fatty acids.

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REFERENCES

1. Altschul, A. M., "Cottonseed and Cottonseed Products." ed., Bailey, A. E., p. 200, Interscience Publishers Inc., New York, 1948.
2. American Oil Chemists' Society, Official and Tentative Methods, 2nd ed., Chicago, Am. Oil Chemists' Soc., 1946, with 1950 revisions.
3. Burleson, C. A., and Hubbard, J. L., "Cotton Defoliation in the Lower Rio Grande Valley, Texas Agr. Exp. Sta. Prog. Rpt. *1397*, 5 p., 1951.
4. Christensen, C. M., Olafson, J. H., and Geddes, W. F., Cereal Chem., *26*, (2), 109–128 (1949).
5. Crafts, A. S., Currier, H. B., and Day, B. E., Hilgardia, *20*, 57–80 (1950).
6. Currier, H. B., and Crafts, A. S., Science, *111*, 152–153 (1950).
7. Ergle, D. R., and McIlrath, W. J., Bot. Gaz., *114*, 114–122 (1952).
8. Hall, W. C., "The Effect of Sucrose and Maleic Hydrazide on the Chemical Defoliation of Second Growth of Cotton," Tex. Agr. Exp. Sta., Prog. Rpt., *1356*, 3 p., 1951.
9. Hall, W. C., Truchelut, G. B., and Lane, H. C., Assoc. of South. Agr. Workers Proc., *50*, 175–176 (1953).
10. Holtsun, J. T. Jr., and Porter, W. K. Jr., South. Weed Conf. Proc., *4*, 47–52 (1951).
11. International Critical Tables, vol. 1, 67–68, McGraw-Hill Book Co. Inc., New York, 1926.
12. Jensen, E. A., Lambou, M. G., Andrews, F. R., Mayne, R. Y., Karon, M. L., Curet, M. E., Wilcox, F. B., Altschul, A. M., Newby, Wales, and Bollens, W. F., J. Am. Oil Chemists' Soc., *28*, 241–245 (1951).
13. Karon, M. L., and Adams, M. E., J. Am. Oil Chemists' Soc., *25*, 21–22 (1948).
14. Karon, M. L., and Altschul, A. M., Plant Physiol., *19*, 310–325 (1944).
15. McIlrath, W. J., Amer. J. of Bot., *37*, 816–819 (1950).
16. McMurray, S. F., South. Weed Conf. Proc., *4*, 87 (1951).
17. Naylor, A. W., and Davis, E. A., Bot. Gaz., *112*, 112–126 (1950).
18. Tharp, W. H., private communication.

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