RELATIONSHIP OF SEASONAL TRENDS IN CARBOHYDRATE AND NITROGEN LEVELS AND EFFECTS OF GIRDLING AND SPRAYING WITH SUCROSE AND UREA TO THE NUTRITIONAL INTERPRETATION OF BOLL SHEDDING IN COTTON

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Received January 2, 1952

The shedding of young cotton bolls has for many years been widely attributed to nutritional relations; insufficient carbohydrate and nitrogen supplies have most often been thought of in this connection. This view of the cause of boll shedding is possibly quite old, but the first reference with which the writers are familiar is that of MASON (**20**) in 1922 in which he said that both the cessation of growth of the main axis and the augmented susceptibility to shedding could be attributed to a correlation factor which tended to deflect the supply of elaborated food from the apical part of the plant to the fruit developing on the basal fruiting branches. In 1934 MASON and PHILLIS (**21**) wrote that the bolls drain the vegetative plant of its food materials, the leaves yellow and fall off as nitrogen is drained away, and as the strain increases the flower-buds and bolls themselves are starved and shed. In 1931, one of the present writers (**2**) stated that the nutritional dominance of bolls over the vegetative growth of the cotton plant had frequently been observed to inhibit boll setting and terminal bud and branch development. Eaton, in a section of a Field Station report by KING and LOOMIS (**17**) stated that a very close relation exists between the rate at which organic products are made available by photosynthesis and the number of bolls that the cotton plant retains. None of the foregoing conclusions was supported by original analytical data but it was known from earlier work that the nitrogen content of vegetative parts of the cotton plant declines with the advance of the summer. MASON (**20**) had found that the removal of all leaves caused a prompt shedding of nearly all young bolls carried by Sea Island cotton plants, and EATON (**2**) had found that when previously set bolls were removed from Upland plants these were replaced within a matter of 10 days with new bolls. HAWKINS *et al.* (**15**) measured the trends in carbohydrate levels in cotton plants throughout a summer and concluded in 1933 that the percentage of shedding is regulated by the amount of plant food available for the development of the young bolls. The conclusions drawn by WADLEIGH (**23**) in 1944 were that when developing ovules in cotton bolls already set have sufficiently depleted nitrogenous or carbohydrate reserves to a level of inadequacy for further ovule development, the additional young bolls abscise because of ovule abortion; after a plant had set a number of bolls sufficient to deplete its nitrogen reserves, all subsequently formed young bolls abscised and, very soon thereafter, young squares abscised and the terminal buds of the fruiting branches aborted; there was little relation between percentage of abscission and treatment.

The present paper raises a question on the adequacy of the nutritional interpretation. Chemical and shedding data are presented on the effects of girdling and of spraying cotton plants with solutions of sucrose and urea and of combinations of these treatments and, also, on the seasonal march of carbohydrate and nitrogen levels in the cotton plant and its bolls through the fruiting period. The thought that spraying cotton plants with sucrose might supply a new approach to this old problem was suggested by remarks of F. W. Went in June 1947 when he reported outstanding growth responses with tomatoes sprayed with sucrose solutions. WENT and CARTER (**25**) have reviewed the literature on the uptake of sugars from solutions. In one of their experiments in a dark room, unsprayed tomato plants grew 15.8 mm. in six days; plants sprayed on the upper sides of the leaves with 10% sucrose grew 27.5 mm. and those sprayed on the underside grew 37.0 mm. Another of their experiments showed that the entry of sucrose was not dependent upon open stomata; the stomata in the tomato, as in cotton, are more numerous in the lower than in the upper epidermis. The authors pointed out that the upper cuticle is thicker than the lower and, also, that the concentration of the sucrose spray should make little difference since the concentration always increases as evaporation progresses.

Urea sprays have been tested for a longer period than sucrose sprays. In general (**6, 11, 12, 16, 20**) plants on soils that respond to soil applications of nitrogen also respond to urea sprays and sometimes more promptly and with certain advantages. EMMERT and KLINKER (**6**) have reported that the tendency for urea sprays to cause leaf burning is avoided when sufficient sucrose is added to the solution. Because of the current interest in both sucrose and urea sprays, the results of the experiments involving these are given more attention in this paper than they would otherwise have received.

Methods

The sucrose and urea spray experiment of 1947 at Shafter, California, was with Missdel × Acala $F_8$10-13 plants in six-foot row segments for each plot in five randomized blocks. There was an average of 6.2 plants per plot, extra plants were treated at the end of each plot to provide sample material. Previously set bolls (an average of one per plant) were removed on July 3. All subsequent flowers and shed bolls were counted daily. The plants were sprayed six days per week, usually in the late afternoon, starting on July 3 and continuing until August 26. Samples for analyses consisted of four leaf blades from the middle third of the main stem of each of 12 plants, the petioles of these leaves, the middle third of the main stems of the same 12 plants and 13-day-old bolls (10 to 14 per sample). These samples were collected on July 16, July 31, and August 14. On the collection dates, half of the samples were taken in the late morning and half in the early afternoon. Plant heights were measured weekly. Before weighing and drying, the samples were agitated in two changes of water to remove surface materials. In spraying, a spreader was used and the upper surfaces of all leaves were thoroughly wetted. The four treatments were 20% sucrose spray, 1% nitrogen (Nugreen) spray, 20% sucrose plus 1% nitrogen, and water spray only. The plots were irrigated weekly.

The 1948 experiment was also with a Missdel × Acala strain and as in 1947 the plots were irrigated weekly. The treatments were started on August 14 and continued for 11 days. Plants were treated in four ways: (a) sprayed each second day on their upper leaf surfaces with 2% nitrogen (six times), (b) girdled above a large fruiting branch, (c) both girdled and sprayed as above, and (d) untreated. There were 10 consecutive plants for each treatment in each of four blocks with extra treated plants in each plot for samples which were collected on August 21. By August 21, few of the girdles had been bridged by new tissue. Duplicate samples from eight plants from each treatment were collected, one sample being from the upper two blocks and one from the lower two. The root samples were composed of the tap and larger secondary roots as lifted with a shovel. The leaf and stem samples were from the middle third of the main stalks. Each day's flowers (August 14 to 24 inclusive) were counted and marked with a different colored tag. On August 29 the tags from the healthy bolls remaining on the plants were collected for the boll-shedding record.

The carbohydrate and nitrogen analyses were made by methods previously described (**5, 7**). The organic acids in the leaves were determined by methods described by PUCHER *et al.* cited elsewhere (**8**).

Results

EFFECTS OF SUCROSE AND UREA SPRAYS IN 1947

The carbohydrate and nitrogen concentrations found in the plants of the spray experiment in 1947 are reported in table I as the averages of the three collections. The effects of the treatments on plant growth, flowering and boll retention are shown in table III. Starting during the second week of spraying, there was evidence of marginal burning of the leaf blades sprayed with urea alone. This burning progressed and by late August the plants sprayed with urea alone had about half the leaf area of the other treatments. When the same amount of urea was used with 20% sucrose there was no burning; the latter plants, like the sucrose-sprayed plants, had the same appearance as the control (water spray) plants. Table I shows that far less nitrogen was accumulated in the leaves when the urea was combined with sucrose than when the urea was used alone. This depression in urea absorption would account for the absence of burning.

When the experiment was started, a gummy coating of sucrose (both sucrose alone and sucrose plus urea) remained on the surface of the leaves from one day to the next. But early in the second week, it became difficult to distinguish the leaves that had been sprayed the evening before with sucrose from those that were sprayed only with water. The samples were collected in moist cotton bags and kept in the shade until they could be taken to the laboratory for washing and weighing. On one of the sample dates, the plants had been sprayed in the early morning; the leaves when collected were coated with sucrose. When taken from the bags for washing those sprayed with sucrose were found to be very wet. The solution on the surface of these leaves (not found on the leaves treated with urea or water)

TABLE I

CARBOHYDRATE AND NITROGEN LEVELS IN VARIOUS PARTS OF COTTON PLANTS SPRAYED WITH WATER, SUCROSE AND UREA. PERCENTAGES ON FRESH WEIGHTS. MEANS OF COLLECTIONS OF JULY 16, JULY 31, AND AUGUST 14.

Plant part	Spray treatment	Moisture	Carbohydrates					Nitrogen	
			Hexose	Sucrose	Starch	Sum	Hemicellulose	Soluble	Insoluble
Leaf blades	Water only	80.7	0.05	0.13	0.28	0.46	0.76	0.16	0.54
	Sucrose	80.7	.07	.15	.31	.53	.83	.14	.58
	Urea	79.6	.01	.02	.20	.23	.83	.32	.73
	Sucrose and urea	80.1	.07	.09	.22	.39	.84	.21	.60
	L.S.D. 5%	.09	.08	.10	.19	.20	.06	.08	.23
Petioles	Water only	82.9	.09	.15	.26	.50	....	.20	.11
	Sucrose	83.8	.08	.24	.26	.58	....	.20	.08
	Urea	83.4	.06	.12	.17	.36	....	.24	.08
	Sucrose and urea	83.9	.10	.10	.24	.44	....	.17	.08
	L.S.D. 5%	1.0	.03	.14	.06	.18	....	.03	.05
Stems	Water only	75.4	.22	.56	.56	1.34	....	.10	.12
	Sucrose	77.9	.14	.45	.47	1.04	....	.09	.12
	Urea	77.3	.11	.38	.43	.93	....	.12	.13
	Sucrose and urea	76.7	.15	.44	.48	1.15	....	.11	.12
	L.S.D. 5%	3.9	.08	.19	.15	.35	....	.02	.05
13-Day Bolls	Water only	87.1	1.24	.04	.88	2.16	....	.12	.18
	Sucrose	87.6	1.26	.02	.79	2.08	....	.11	.18
	Urea	86.7	1.06	.04	.90	1.97	....	.15	.21
	Sucrose and urea	87.1	1.17	.02	1.01	2.21	....	.13	.20
	L.S.D. 5%	.85	.52	.04	.19	.58	....	.026	.026

was apparently the result of leaf water withdrawn by osmosis from inside the leaves.

The dew-point was reached during about half of the nights of the experiment and it was approached on the remaining nights. With this high nightly humidity it seems evident that there was enough moisture withdrawal by osmosis from leaves to let the sucrose solution drip to the ground. This runoff caused by osmosis apparently did not occur until after the surface cuticle had been altered during the first week of spraying. The analytical data of table I show little evidence that sucrose was absorbed. The observations are pertinent, nevertheless, that between about 4 P.M. and 4 A.M.

there was an opportunity for epidermal uptake of sucrose and that after the first week the epidermis was permeable, at least to water. Computations made at several stages of the experiment showed that enough sucrose was being applied to have supported double the number of bolls being developed by the plants.

Sucrose when used as a spray alone (table I) gave slight nonsignificant increases in the carbohydrates of the leaves and petioles. These increases, however, were balanced by non-significant losses in the stems and bolls 13 days old. There was no significant effect of sucrose spray on either of the soluble or insoluble nitrogen fractions in any of the tissues.

Urea used alone reduced the sugar and starch in leaves, petioles and stems and these reductions were for the most part significant. There was a small loss of carbohydrate in the bolls as well. With only one exception (insoluble nitrogen in petioles), the urea spray increased both soluble and insoluble nitrogen in all tissues; these nitrogen gains were usually significant. Some of the carbohydrate reduction associated with the urea spray is reflected in gains in the carbon content of the nitrogen compounds. Using for purposes of comparison, the factor 6.25 to convert the total nitrogen to protein, there was in the leaves a gain of 2.18% in terms of protein equivalent against the reduction of 0.23% found for carbohydrate. In the stems the protein gain failed to compensate for the carbohydrate loss. In the bolls, the loss of carbohydrate was 0.19% and the protein gain was 0.38%. The spray of sucrose and urea resulted in slight non-significant losses in total carbohydrate (a small gain in the bolls) and slight gains (petioles excepted) in total nitrogen. All of the sprays caused significant increases in hemicellulose concentrations in the leaves.

In general, it would seem that the effect of the sucrose spray and the sucrose and urea spray on the carbohydrate concentrations in the plants were too small to merit consideration. Some interest can be attached to the increased nitrogen but lowered carbohydrate concentrations in all tissues resulting from the use of the urea spray alone. There are yet to be considered some analyses (table II) of the concentrations of various organic acids in the leaves. It has been recurrently suggested that the organic acids may be involved in the initiation of reproductive processes.

All of the sprays reduced the concentrations of citric and malic acids (table II). The effects of the urea spray in reducing malic acid and of the sucrose spray in increasing the unidentified acids were especially marked. The reduction in malic acid by the urea spray was not compensated by gains in the other acids and the loss shows up significantly in the total organic acids. The low total organic acids found in the leaves sprayed with urea are associated most logically with reduced concentrations of bases which have been found in other plants as well as in cotton (**8**) when fertilized with ammonium salts. Any association between changes in the organic acids and the retention of bolls, if such existed, might be the significant loss in citric acid and gain in the unidentified acids that resulted from the sucrose spray.

TABLE II

ORGANIC ACIDS OF LEAVES. DATA EXPRESSED AS MILLIGRAM EQUIVALENTS PER 100 GRAMS DRY WEIGHT.

Treatment	Citric	Malic	Oxalic	Unidentified	Total
Water only	77.2	99.5	11.7	24.1	202.4
Sucrose	58.6	87.0	12.3	37.3	195.3
Urea	62.0	70.9	11.7	26.3	170.1
Sucrose and urea	65.8	89.3	11.5	28.9	196.0
L.S.D. 5%	16.7	11.6	0.6	4.7	28.4

As shown in table III, there was an apparent disadvantage from all the sucrose and urea spray treatments on plant height, but these differences lacked statistical significance. There were no significant effects of the various sprays on the number of flowers produced. Each of the three spray treatments reduced the number of bolls retained; in each case, these reductions were significant. The trends of the four treatments in flower production and boll shedding were noted to follow similar courses, *i.e.*, the three sets of sprayed plants remained like the controls in flowering rates but gradually fell below in boll retention. It seems evident that if any of the sucrose sprayed on the leaves was taken up by the plants it was offset by reduced photosynthesis.

This experiment like the one that followed in 1948 was conducted under conditions of high nitrate supply. The irrigation water at Shafter, California, adds 25 pounds of nitrate nitrogen per acre foot of application. About four acre feet of water, or 100 pounds of nitrogen, are applied annually and cotton at the Shafter Station has shown no response to additional applications.

Effects of girdling and urea sprays in 1948

The girdling and spraying with urea in this experiment were done at a time when the cotton plants were shedding over half of their bolls. The girdle was cut above one of the lower fruiting branches that had four or five leaves; special care was taken to avoid injuring the xylem as much as possible. A pair of razor blades were set 5 mm. apart in a block of wood

TABLE III

GAIN IN PLANT HEIGHT (JULY 4 TO AUGUST 25), AND NUMBER PER PLOT (ABOUT SIX PLANTS) OF FLOWERS (JULY 3 TO AUGUST 21) AND OF BOLLS RETAINED (JULY 3 TO AUGUST 27).

Spray treatment	Height	Flowers	Bolls retained
	cm.		
Water only	51	188	80
Sucrose	47	189	57
Urea	43	203	69
Sucrose and urea	41	204	65
L.S.D. 5%	11	34	11

with the edges protruding only far enough to cut just through the bark of most of the stems. As soon as the strip of bark was removed, the wound was wrapped with scotch tape. The girdles were cut, the first urea spray applied, and the tagging of flowers was started on the same day. The samples for chemical analyses were collected a week later. On the eleventh day, none of the plants showed any appearance of having been injured by the girdling and there was at that time only minor marginal leaf burning from the spray of 2% nitrogen applied each second day (six times in all).

The urea spray (both with and without girdling) more than tripled the soluble nitrogen of the leaves and almost doubled that in the stems (table IV). There was a substantial increase in protein (insoluble) nitrogen in the leaves but not in the stems. Urea spray, relative to the control plants, and

TABLE IV

EFFECT OF UREA SPRAY AND GIRDLING ON CARBOHYDRATE, NITROGEN, PHOSPHORUS, AND POTASSIUM CONCENTRATIONS. THE SAMPLES WERE COLLECTED ONE WEEK AFTER STARTING THE TREATMENTS. DATA ARE EXPRESSED AS PERCENTAGES ON FRESH WEIGHT.

Part of plant	Treatment	Moisture	Carbohydrates				Nitrogen		Phosphorus	Potassium
			Hexose	Sucrose	Starch	Sum	Soluble	Insoluble		
Leaves	Control	77.6	0.12	0.19	0.40	0.71	0.14	0.60	0.056	0.047
	Urea	75.4	.02	.04	.36	.42	.51	.72	.060	.044
	Girdle	77.6	.12	.20	.58	.90	.15	.54	.048	.038
	Urea and girdle	74.5	.06	.06	.49	.61	.48	.69	.052	.042
Stems	Control	74.0	.16	.70	.31	1.17	.06	.11	.032	.48
	Urea	72.6	.08	.44	.28	.80	.11	.11	.039	.54
	Girdle	71.0	.28	1.46	.77	2.51	.05	.11	.020	.41
	Urea and girdle	70.0	.21	.80	.96	1.97	.12	.13	.024	.41
Roots	Control	71.2	.20	1.26	1.08	2.54	.04	.10	.046	.38
	Urea	71.2	.14	.98	1.10	2.22	.06	.10	.050	.40
	Girdle	71.9	.14	.74	.60	1.48	.06	.09	.038	.34
	Urea and girdle	71.2	.14	.64	.66	1.44	.05	.09	.042	35

the urea and girdling treatment, relative to the girdled plants, caused 25 to 40% reductions in the sums of sugars and starch in the leaves and stems. Girdling alone increased the concentrations of sugars plus starch by 27% in the leaves and by 100% in the stems. In the large roots there was a 60% reduction in carbohydrate. Treatment with urea and girdling, relative to the control plants, reduced the carbohydrate in the leaves a little, but increased that in the stems from 1.17 to 1.97%; this increase was associated with an increase from 0.17 to 0.25% in total nitrogen. During the period of the observations, flowering rates (table V) were not influenced by any of the treatments. Relative to the control plants the urea spray reduced the number of bolls retained to one half. Girdling reduced the number of bolls retained to one third. Urea and girdling together reduced the number to one fifth. Relative to the control plants all of these reductions were statistically significant. Neither the large increase in nitrogen from urea treatment and the carbohydrate gain from girdling, nor the increases in both from urea and girdling increased boll retention *per se;* instead there were large decreases.

SEASONAL TRENDS IN CARBOHYDRATES LEVELS

As a further basis for evaluating possible effects of carbohydrate and nitrogen levels on boll shedding, there have been assembled in table VI, from other experiments, results showing the seasonal march of carbohydrate concentrations in the middle third of the main stems of five varieties (12 plants per sample) at College Station, Texas, in 1945 and in three varieties at Shafter, California, in 1947. At Shafter, data were obtained on nitrogen, as well as carbohydrate concentrations, in leaves (four leaf blades from the middle third of each of the 12 stems) and in 13-day-old bolls (12 bolls usually from the above plants). In both years, the plants were sampled about July 15, August 1, and August 15. At College Station the plants were sampled from randomized plantings in mid-afternoon. At Shafter, half of

TABLE V

FLOWERS PER 10 PLANTS DURING PERIOD OF SPRAYING, AUGUST 14 TO 24 INCLUSIVE, AND NUMBER OF HEALTHY BOLLS FROM THESE THAT REMAINED ON PLANTS ON AUGUST 29.

Treatment	Flowers	Bolls retained
Control	78	30
Urea	69	15
Girdle	71	9
Urea and girdle	77	6
L.S.D. 5%	....	7

the samples were collected in mid-morning and half in mid-afternoon. In the instance of the Stoneville and Acala varieties, the sampling was divided between two consecutive days reversing the order of collection in the two morning and two afternoon samplings. During both years, the plants had started to flower in early July and were fruiting rapidly and retaining nearly all of their bolls on July 15. By August 1, there was a little shedding but most of the bolls were being retained. By August 15 over half of the bolls were being shed. All plants were grown in plots irrigated weekly.

The middle third of the main stem provides a creditable index tissue for carbohydrate levels in the cotton plant. As shown in table VI, carbohydrates accumulate in the stems in concentrations three times those of the leaves. Carbohydrates being moved downward to the roots and much of those interchanged between the fruiting branches pass through this portion of the plant. Bolls 13 days from flowering were used because at this age they are well beyond the stage that they might be failing to develop prior to shedding; also they are five to eight days ahead of carbohydrate utilization in oil synthesis in the seed and in cellulose deposition in the secondary thickening of the walls of the lint cells.

The large decrease in the carbohydrates of the Missdel x Acala strain in late July (table VI) is perhaps of some general interest in that the strain had been selected by G. J. Harrison for its high resistance to Verticillium wilt; but Stoneville and Acala plants with bolls removed to increase their carbohydrates also appeared to have some extra resistance. In another paper (**3**) in which some of these data were used, attention has been called

TABLE VI

CARBOHYDRATE AND NITROGEN LEVELS THROUGH THE PERIOD OF BOLL SETTING. THE FIRST SAMPLES WERE TAKEN ABOUT JULY 15 (EARLY FRUITING), AUGUST 1 (HEAVY BOLL SETTING), AND AUGUST 15 (HEAVY BOLL SHEDDING). DATA EXPRESSED AS PERCENTAGES ON FRESH WEIGHT.

Location, tissue, and variety	Total sugars			Sum of sugars and starch			Total nitrogen		
Sampling:	1	2	3	1	2	3	1	2	3
College Station, 1945									
Stems, middle									
Arkansas Rowden B 4	2.05	1.83	1.44	2.52	2.97	3.77	....	....	....
D & Pl 14	2.03	1.97	1.95	2.97	3.03	3.25	....	....	....
Hi-Bred	1.10	2.04	2.09	1.83	4.02	3.97	....	....	....
Stoneville 2 B	2.13	2.35	2.30	2.83	3.73	3.69	....	....	....
Oklahoma Triumph 92	2.03	2.36	1.58	2.75	4.02	3.80	....	....	....
Shafter, 1947									
Stoneville 2 B									
Leaves	.58	.49	.46	1.09	.87	.78	0.84	0.75	0.78
Stems, middle	1.03	1.51	1.36	1.38	2.25	2.31	.25	.24	.20
Bolls, 13 days	1.85	1.51	1.31	3.33	2.73	2.46	.28	.26	.25
Acala p 18									
Leaves	.36	.51	.23	.99	.97	.69	.86	.77	.72
Stems, middle	1.46	1.37	.94	1.79	1.96	1.63	.25	.18	.13
Bolls, 13 days	2.04	1.61	1.02	3.50	2.69	1.79	.29	.18	.13
Missdel x Acala									
Leaves	.25	.13	.15	.74	.23	.42	.81	.61	.66
Stems, middle	1.22	.50	.41	2.10	1.06	.88	.29	.21	.18
Bolls, 13 days	1.49	.93	1.40	2.47	1.51	2.50	.32	.33	.19
Means:									
College Station									
Stems	1.87	2.11	1.87	2.58	3.55	3.69	....	....	....
Shafter									
Leaves	.40	.38	.28	.94	.69	.63	.84	.71	.72
Stems	1.25	1.13	.90	1.76	1.76	1.60	.26	.21	.17
Bolls	1.79	1.35	1.24	3.10	2.31	2.25	.30	.26	.19

to significant differences in carbohydrates between the five varieties at College Station and, also, to the fact that the Stoneville at Shafter had only 60% as much sugar plus starch as at College Station.

Directing attention to the levels of carbohydrates in the stems of the Stoneville and Acala varieties at Shafter, there was a substantial gain in the sugars of Stoneville (1.03 to 1.36%) between mid-July and mid-August and during the same period there was a somewhat larger loss (1.46 to 0.94%) in Acala. Between these dates starch increased in Stoneville from 0.35 to

TABLE VII

CARBOHYDRATE AND NITROGEN CONCENTRATIONS IN THE MIDDLE THIRD OF COTTON STEMS AND IN BOLLS 13 DAYS OLD IN SAMPLES FROM FIRST, SECOND AND THIRD PLANTINGS. DATA EXPRESSED AS PERCENTAGES ON FRESH WEIGHT.

Variety and planting	Sucrose			Total Sugars			Sum sugars and starch			Total nitrogen		
Sample dates:	July 14–15	July 29–30	August 11–12	July 12–15	July 29–30	August 11–12	July 14–15	July 29–30	August 11–12	July 14–15	July 29–30	August 11–12
Wet												
Stems												
Stoneville 2 B												
First planting	0.89	1.25	1.02	1.03	1.51	1.36	1.38	2.25	2.31	0.25	0.24	0.20
Second planting		.81	.92		1.27	1.29		2.11	2.14		.22	.21
Third planting			.73			1.35			2.28			.19
Acala p18												
First planting	.24	1.06	.69	1.46	1.37	.94	1.79	1.96	1.63	.25	.18	.13
Second planting		.76	.98		1.06	1.27		1.53	1.74		.18	.16
Third planting			.81			1.17			1.62			.18
Bolls												
Stoneville 2 B												
First planting	.08	.00	.07	1.85	1.51	1.31	3.33	2.73	2.46	.28	.26	.26
Second planting		.04	.01		1.10	1.41		2.51	2.74		.24	.27
Third planting			.05			1.32			2.95			.26
Acala p18												
First planting	.00	.00	.02	2.06	1.61	1.02	3.50	2.69	1.79	.29	.29	.13
Second planting		.04	.07		1.75	1.69		3.00	2.01		.26	.10
Third planting			.00			1.18			2.22			.30
Means—3 moistures												
Stems												
Stoneville 2B												
First planting	.66	.76	.79	1.20	1.06	1.18	2.07	2.13	1.86	.24	.18	.20
Second planting		.85	.89		1.37	1.31		2.27	2.44		.22	.20
Third planting			.88			1.37			2.11			.19
Acala p18												
First planting	.84	1.01	0.72	1.41	1.37	1.01	2.26	2.96	1.90	.26	.20	.19
Second planting		.84	1.15		1.28	1.47		1.94	2.31		.18	.17
Third planting			.78			1.13			1.59			.17
Bolls												
Stoneville 2B												
First planting	.03	.02	.05	2.02	2.42	1.46	4.17	3.12	2.70	.29	.26	.25
Second planting		.01	.12		1.42	1.79		2.71	3.32		.25	.26
Third planting			.03			1.26			2.57			.25
Acala p18												
First planting	.05	.02	.01	2.08	1.87	1.14	3.80	3.04	2.23	.30	.29	.23
Second Planting		.05	.05		1.31	1.44		2.50	2.36		.29	.28
Third planting			.00			1.09			2.27			.28

0.95% and in Acala from 0.33 to 0.69%. Both varieties, it will be recalled, were retaining most of their bolls in mid-July and shedding most of them in mid-August. At College Station, with a similar shedding performance, there was no mid-July to mid-August change in the five-variety sugar average but there was an average gain in starch from 0.71 to 1.82%. These data fail to provide a carbohydrate explanation for the boll shedding in August. The carbohydrate data for the bolls 13 days old are yet to be considered.

Nitrogen concentrations were found to follow a downward course in all tissues in California and, in keeping with earlier data, this was probably the case at College Station also. The possible significance of the declining nitrogen levels in connection with boll shedding should be considered with the fact that in the short experiment of 1948, the urea spray increased nitrogen and caused more rather than less shedding. Neither at College Station nor at Shafter were the cotton yields increased by nitrogen fertilization.

Step-by-step decreases occurred in the young bolls in the concentrations of both sugars and starch in going from mid-July to mid-August (table VI). In mid-July there is evidence of rather strong polar transport from the leaves to the stems and from the stems to the bolls. In both varieties the carbohydrate differentials between the stems and bolls had almost disappeared by mid-August. This failure of the 13-day-old bolls to maintain favorable sugar differentials relative to the stems might appear on the basis of the evidence of table VI to provide an explanation of the increased shedding of young bolls as the cotton plant becomes older. The data of table VII provide an opportunity for a further examination at this point.

In 1947, there were two plantings of both the Stoneville and Acala varieties that were made later than the one that supplied data for table VI. Each of the three plantings was grown at three moisture levels: wet, irrigated on each side of the rows each week; intermediate, irrigated on one side each week; and dry, irrigated on one side each second week.

The three plantings were three weeks apart. The second planting started flowering 15 days after the first and the third 15 days after the second. Three sets of samples (table VII) were collected from the first planting, two from the second planting and one from the third; all three plantings were sampled at the last date and the first two at the intermediate date. For the four-day periods preceding and including July 14, July 29, and August 11 the average maximum temperatures were 93, 95, and 95° F respectively, the minimum temperatures were 60, 62, and 65°, the relative humidities 34, 34, and 32%, and the day length 14.4, 14.1 and 13.7 hours. The respective mean values for the actual sampling days were maximum 96, 96, and 98, minimum 62, 63, and 64, and humidity 33, 35, and 32. As computed by T. H. MacDonald of the U. S. Weather Bureau, the mean clear-day horizontal-plane total radiation for the period June 30 to July 19 was 770 gram calories per sq. cm. per day and 700 gram calories for the period July 26 to August 14.

The carbohydrate and nitrogen data are presented both on the basis of the results in the wet plots and on the basis of the means of wet, intermediate, and dry treatments. Each of the three plantings of both varieties of cotton retained nearly all bolls during the first weeks of their flowering. By mid-August, the first plantings of both varieties were shedding most of their new bolls whereas the last plantings were retaining their bolls.

In the first planting, the concentration of total sugars in both the Stoneville and Acala bolls was higher in mid-July than at later dates and higher also than any levels reached by the later plantings. For the means of the three levels of moisture supply in mid-August, the concentrations of sugar and starch, and under the wet treatment the concentrations of sugars, were equally low in the stems and in the 13-day-old bolls of the first and last plantings. Also in the means, only minor differences were found in mid-August in the concentrations of nitrogen in the two plantings. This lack of difference between the young and old plants in the level of carbohydrate and nitrogen supply in the stems and in the operational levels within their 13-day-old bolls affords no insight as to why the older plants should have been shedding their bolls profusely at the same time the young plants were retaining their bolls.

The interpretation of the data for the bolls might have been aided had bolls during the first days of their development been analyzed; but during this early period there is no certain basis for distinguishing between bolls that are going to shed (these presumably would be low in carbohydrate) and the bolls that will be retained. The general observation is accordingly appropriate that whether or not carbohydrate levels within very young bolls are causally involved in shedding, antecedent differences are not shown in the stems of the shedding and non-shedding plants nor are differences reflected in the 13-day-old bolls of such plants.

Discussion

Some of the literature on the shedding of cotton bolls is pertinent to the present discussion. Similar percentages of boll shedding have been found with variations in manuring and fertilization. EWING (**10**) obtained widely different growths and numbers of flowers on poor soil and on rich bottom soils, but the percentages of bolls that shed were much the same. In a less striking field experiment (**22**) similar percentages of shed bolls were found when the proportions and amounts of N, P, and K were varied.

Data on the carbohydrate and nitrogen accumulations within the plant in relation to NO_3 supply are available. WADLEIGH (**23**) using four nitrate levels in sand cultures in a whitewashed greenhouse in the summer, pointed out that regardless of treatment 60 to 70% of all blossoms produced were shed and gave thought to the possible bearing of nitrogen and carbohydrate deficiencies on shedding. Calculations from Wadleigh's data have shown that for his four NO_3 levels there were respectively 3.6, 3.5, 2.8, and 3.5 bolls per 100 grams of fresh stems and leaves. EATON and RIGLER (**5**) also used four NO_3 levels in sand cultures—the highest one being four times as great as Wadleigh's highest. The experiment was repeated under muslin shades in a greenhouse in the winter and fully exposed outdoors in the summer. The four NO_3 treatments gave an average of 12% of bolls shed under low light and of 40% under high light. At low light, the root bark contained an average of 1.16% of sugars plus starch and the plants had an average relative fruitfulness of 4.0 bolls per 100 grams of fresh stems and leaves; under high light the root bark contained 2.5% of sugars plus starch and the relative fruitfulness was 6.8 bolls per 100 grams of stems and leaves. Under low light there was a heavy shedding of floral buds and under high light there was very little. Both the high and low nitrate levels reduced sharply the sizes of the plants but at corresponding nitrate levels the stems and leaves weighed almost the same under low and high light. The light intensity thus altered relative fruitfulness but neither it nor the associated changes in carbohydrate influenced vegetative weight. In both of the experiments, the NO_3 treatments that resulted in larger plants also resulted in proportionately more bolls. In the interpretation of this experiment, it seemed pertinent to ask why, if carbohydrate supply limited boll setting under low light, the high-light plants did not reduce their carbohydrate supply down to the low-light level before these plants started to shed.

From the foregoing experiments, the conclusion seems to be supported that neither the carbohydrate or nitrogen levels in the plants influence relative fruitfulness very much, *i.e.*, the partition of growth between vegetative and reproductive activities. Within given light intensities, the partition of carbohydrate utilization remained quite uniform but between intensites there were marked differences. Neither carbohydrate nor nitrogen levels in the plants nor the level of NO_3 supply seem in the above experiments to have constituted critical factors in boll shedding but this evidently does not apply to the shedding of floral buds.

Something comparable to the foregoing was found (**11**) when the sulphate supply for cotton plants in greenhouse sand cultures was increased from a low level, that resulted in little or no sugars in the leaves and stems and very little growth, to a high level that gave large plants with normal sugars and many bolls. Over the SO_4 range there were successively 3.8, 4.0, 3.8, and 4.0 bolls per 100 grams of fresh stems and leaves.

The present experiments with urea and sucrose sprays, used alone and together, and with girdling and urea sprays, alone and together, were found to have had consistently adverse effect on boll retention. The sucrose spray failed to increase carbohydrate levels in the plant but the urea spray when used alone decreased carbohydrate and increased nitrogen. Girdling increased carbohydrate levels in the plant stems materially and when combined with urea spray increased both carbohydrate and nitrogen. The organic acids of the cotton leaves were altered by the sucrose and urea sprays but if these changes were related to boll shedding the effect was adverse, or at least masked by other effects of the treatments. Had the foregoing treatments produced neutral effects on boll retention, they could be more readily comprehended; the strongly adverse effects are difficult to understand.

During the course of the investigations, it has been felt that a great weakness in the nutritional theory of boll shedding, as regards carbohydrate and nitrogen levels in plants, rested in the fact, as illustrated in table VI, that the seasonal march in shedding rates is not correlated with the seasonal march in stem carbohydrates. The results in the short-period experiment of 1948 with urea spray, which greatly increased nitrogen in the tissues but caused only a trace of leaf injury, have tended to make it seem unlikely that the seasonal downward trend in nitrogen levels in the cotton plant were responsible for late season shedding. As nitrogen becomes deficient, both vegetative growth and flowering are slowed down together.

Examinations of very young bolls for carbohydrate and nitrogen contents during the period when they might or might not be going to shed is impracticable because changes prior to abscission may take place before the slowing of growth can be noted. The use of 13-day-old bolls for analysis, it was believed, might avoid the weakness attached to analyzing very young bolls and thereby provide a basis for judging whether important differences exist in the carbohydrate relations of the bolls themselves during the early season when nearly all bolls are being retained and the late season when nearly all are shedding.

When 13-day-old bolls from plants in their early, medium and late fruiting period were analyzed, step-by-step decreases in carbohydrate levels were found as the plants became older. The least carbohydrate was found in these bolls at the time when the plants were shedding most heavily. Early in the fruiting period, the bolls had much higher concentrations than the stems, but by mid-August the concentrations of the stems and bolls were nearly equal; this suggested that plant-age factor was involved in polar transport and the possibility that transport of carbohydrate to the bolls might be a critical factor in boll retention. An extension of the same experiment, however, included later plantings of the same two varieties of cotton and these data did not support this suggestion. These later plantings provided stems and bolls for collection from old plants that were shedding and from young plants that were not, all on the same days of sample collections in mid-August. The results of these measurements were rather surprising in that nearly the same concentrations of carbohydrate and nitrogen were found in mid-August in the bolls 13 days old from the old and from the young plants. Time or season of sampling rather than plant age, or shedding performance, seemed to determine the carbohydrate levels in the bolls. There was very little difference in maximum and minimum temperatures or humidity on and preceding the three sampling dates but there was a reduction in day length. If day length was the critical factor in the carbohydrate accumulation in the stems and bolls, the reason, or mode of action, remains obscure. American Upland cottons are day-length neutral. The photoperiodic cottons express their day-length reactions primarily in the retardation until short days of the development of their first fruiting branches. The first fruiting branches of the short-day cottons often shed many of the early floral buds.

Recognizing that the carbohydrate level found in a plant tissue is only the resultant between the diverse processes of photosynthesis, respiration, translocation, and conversions to proteins and other materials, the low level of carbohydrate found in the young bolls in mid-August might thus be considered as being the result of more rapid utilization. This explanation seemingly will not hold in the present instance; bolls set in mid-August require longer to mature than those set in mid-July and they are smaller when mature.

The early observation (**2**) that the removal of all bolls from cotton plants was followed promptly by stimulated vegetative growth and a rapid replacement of the bolls has seemed to support the nutritional interpretation of boll shedding. Since, as shown in the present paper, the period of heavy boll shedding in cotton is not accompanied by any diminution in the carbohydrate levels in the plant stems, it seems appropriate to give brief consideration to some of the points in boll shedding that would need to be reconciled in any alternate interpretation. Small floral buds die in place rather extensively when cotton plants are grown under low light intensities. This death of buds (as distinct from the usual boll shedding) has been attributed to the associated low levels of carbohydrates in the plants. Also, there is a special characteristic of boll shedding which is always of interest; namely, that it is only during the first week or so following anthesis that bolls customarily absciss. It is extremely rare to find large floral buds shedding prior to anthesis. The uniformity found in the relative fruitfulness measurements under given environmental conditions, but over wide ranges of nutrient supply, leads to the idea of a balance between the number of leaves on cotton plants and the number of bolls that are set or, as a possible corollary, a balance between some mobile anti-auxin type of material developed in growing cotton bolls and the auxin produced in cotton leaves. A number of investigators have studied the effects of organic chemicals on boll abscission. In one experiment (**3**) it was found that weekly spraying with 4-chlorophenoxyacetate decreased both relative fruitfulness and the number of bolls per plant; beta-naphthoxyacetate increased significantly the number of bolls set but, like naphthaleneacetate, was without significant effect on relative fruitfulness. In the abscission of bean leaves in has been found (**1**) that ethylene treatment leads to rapid disintegration in the abscission zone, whereas 2,4-dichlorophenoxyacetic acid delays all anatomical changes. Going further, several investigators (**13, 14, 18**) have developed the idea that the ethylene-auxin balance is the critical relation in leaf abscission.

It is not easy to conclude when a subject such as the foregoing has been adequately investigated or when conclusions are sufficiently well supported. In the light of the literature, as here briefly reviewed, and the interpretations provided by the experimental data presented, it would seem, nevertheless, that the view often held that the cotton plant sets only as many bolls as it can nourish (or more specifically in the present paper, as many as it can supply with carbohydrate and nitrogen) requires new or other evidence for its support. It does not follow, however, if many more bolls were set (perhaps by auxin application) that the plant would then be able to maintain the necessary carbohydrate and nitrogen supply so that all could be brought to full development without a reduction in their size. As pointed out elsewhere it is common to find that the bolls constitute over 60% of the above ground dry weight of the cotton plant at the end of the summer; it would seem doubtful that this order of reproductive efficiency could be pushed a great deal higher. But between locations, years, and varieties substantial differences in relative fruitfulness are often indicated. Although the relative fruitfulness of small and large plants may be the same under a given environment, it still follows that the highest yields result under the nutritional conditions that give the largest plants. Plants that have lost their floral buds through insect injury are commonly found to be rank-growing and unproductive; they may set a top crop after insect control but still remain relatively unfruitful due to the self-shading of so much of the foliage.

Summary

The adequacy of the nutritional interpretation of boll shedding in cotton has been re-examined with special reference to carbohydrate and nitrogen relations. The investigation included chemical analyses of plants that were girdled and those sprayed with sucrose and urea in various combinations. The seasonal march of carbohydrates and nitrogen in leaves, stems and bolls 13 days old and the shedding of bolls were followed.

Spraying the upper leaf surface of plants daily through the fruiting period with 20% sucrose, 1% nitrogen (urea) and the two in combination failed to improve growth and resulted in large significant decreases in the number of bolls that were set. Flowering rates were not affected. Sucrose was not accumulated. Nitrogen was absorbed and resulted in much lower sugar and starch concentrations in all tissues. The urea when used alone burned the margins of the leaves severely but not when it was combined with sucrose; the sucrose when combined with urea greatly reduced nitrogen accumulation.

In an experiment in August when control plants were shedding over half of their bolls, girdling, 2% urea spray (six times) and the two together caused large increases in boll shedding. The girdling increased carbohydrate levels, and the urea spray increased nitrogen and reduced carbohydrate; the two in combination increased both.

The carbohydrate levels in the middle third of cotton stems increased from mid-July, when nearly all bolls were being retained to mid-August when most of them were being shed. In mid-July, the carbohydrate concentrations in bolls 13 days old were much higher than in the stems but by mid-August the two were much alike. This evidence of failure of polar transport to the bolls as the plants become older did not provide an explanation of boll shedding; bolls of first, second and third plantings (the first plantings shedding most of their bolls and the latter retaining their bolls) when sampled in mid-August all had similar concentrations of carbohydrates and nitrogen.

It is concluded that the nutritional theory of boll shedding is poorly supported at least as regards carbohydrate and nitrogen relations. Even though the relative number of bolls carried by a cotton plant, or the percentage of bolls shed, is apparently not influenced by the level of carbohydrate or nitrogen supply, the absolute number it may develop is so affected. Within varieties and environments, the number of bolls per 100 grams of fresh stems and leaves remains rather constant even though nutritional factors may cause marked differences in plant growth. This relationship has suggested that boll shedding is controlled by the balance between auxin produced in the leaves and an anti-auxin or inhibitory material from developing bolls that is moved out into the fruiting branches.

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