# COMPOSITIONAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH THE CHEMICAL DEFOLIATION OF COTTON[1]

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(WITH THREE FIGURES)

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In a previous publication (**13**) evidence was presented in support of the auxin–ethylene balance hypothesis as an explanation for natural leaf abscission. It was found that the two commercial defoliants, Shed-A-Leaf (sodium chlorate–pentaborate) and Endothal (disodium-3,6-endoxohexahydrophalate + ammonium sulphate), accelerated ethylene production in cotton leaves. This observation has since been confirmed for other defoliants by another investigator (**16**).

The present study was designed to investigate the role of ethylene in natural and chemically induced leaf abscission. It has also become of interest to re-investigate the role of the carbohydrate and nitrogen fractions of the leaf in relation to defoliation as well as to determine any physiological or compositional changes in the cotton leaf blade and plant during chemically induced defoliation and, if possible, to assess their importance to the basic mechanism of abscission. The first problem has been the subject of two recent papers (**5, 9**). Evidence has been presented by V. L. HALL (**9**) that the susceptibility of the cotton plant to defoliation varies inversely with reducing sugars, starch, and dextrin in the leaves, whereas his data showed a direct relation between total nitrogen in the leaf and per cent. leaf drop. On the other hand, EATON and ERGLE (**5**) have provided extensive data showing that the percentage of bolls shed by cotton plants is not directly related to the carbohydrate and nitrogen status of the plant.

DENNY (**6**) has reviewed the literature up to 1933 concerning compositional changes in leaves prior to leaf fall. Although the bulk of the earlier workers reported an autumnal shift in leaf composition, he (**6**) concluded from his own work with *Viburnum dentatum* and *Syringa vulgaris* that the dry weight of leaves was nearly constant for the period of sampling (41 days prior to frost); no important changes were observed in sugars or polysaccharides, while nitrogen losses were confirmed only for Viburnum. Abscission records were not kept, and therefore it is not known whether any correlation actually existed between leaf drop and leaf composition.

BROWN and ADDICOTT (**3**) have reported upon the anatomy of leaflet abscission in *Phaseolus vulgaris* and have noted that starch disappeared rapidly from all tissues except those in the abscission zone, and that there was a polar movement of sucrose when applied to the pulvinal region. The sucrose polymerized to starch in the stem. To the authors' knowledge, how-

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ever, a quantitative study of compositional changes occurring during chemical defoliation has not been reported heretofore.

## Methods

### 1950 Experiments

Four experiments were conducted during the spring, summer, and fall of 1950, with the experiment conducted during the fall continuing into late winter and early spring of 1951. The experiments of spring, summer, fall, and late winter–early spring will be referred to as experiments 1, 2, 3, and 4. Stoneville 2B, Deltapine, Acala, and Rowden 41B varieties were used in the first three experiments, whereas only the Stoneville 2B variety was used in the fourth experiment. All plants were started in the greenhouse and grown in three-gallon jars. Experiment 1 was started in early spring and grown in Lufkin fine sandy loam which is a poor soil low in nitrogen. The defoliants were applied on August 15. The plants of experiment 2 were grown simultaneously with those of experiment 1 and differed only in that they were cultured in manured Houston clay with two applications of ammonium nitrate. They were sprayed on August 23. The plants of experiment 3 were identical to those of experiment 2 except floral buds were removed daily. Experiment 4 was started in the greenhouse during the winter of 1950 and was terminated in the spring of 1951. The cultural conditions for the experiment were the same as for experiment 2. Experiment 4 was designed to study the influence of maleic hydrazide and sucrose, singly and in combination with Endothal and Shed-A-Leaf.

Eighty plants were used in both experiments 1 and 2 (20 of each variety); 24 plants were used in experiment 3 (six of each variety); and 80 plants were used in experiment 4. All plants were defoliated when approximately 50% of the bolls had opened. A commercial wetting agent, Nonic 218, was added to all defoliants which were freshly prepared just prior to application. The defoliant sprays were applied with a power-driven atomizer sprayer until the foliage was wetted. A high defoliant concentration of 5% was utilized in all experiments except experiment 4 where a 2% concentration was employed. Sucrose was applied at 3.5 and 5% and maleic hydrazide at 4800 p.p.m. (table I).

In all experiments, the number of leaves per plant was determined prior to defoliant application and seven to nine days after application. From the difference the percentage defoliation was determined. This figure included both the leaves that abscised naturally and those that fell when gentle pressure was applied to simulate field conditions. All data presented are the averages of the individual treatments and, in the case of the first three experiments, represent a composite of the four varieties used.

### 1951 Experiments

Two lots of Stoneville 2B plants were grown in manured Houston Clay in the greenhouse at College Station. The first lot involved 10 plants which

were defoliated on June 15 with Shed-A-Leaf at a rate equivalent to six pounds per acre. The second lot of 24 plants provided the plant material for both the ethylene experiment (tables II and III) and the respiration measurements (table IV). The plants defoliated with 100 p.p.m. ethylene were cut off at the ground line and placed in beakers of water under large bell jars with the gas for 72 hours. Leaf samples were taken at the beginning, at 48 hours, and at 72 hours. Comparable controls were also placed under bell jars for 48 hours to see what effect the bell jar environment had upon defoliation and leaf composition. The plants used for the respiration measurements were sprayed with 2% Shed-A-Leaf, and leaf samples were taken three hours later and at 24 hour intervals up to 72 hours.

Replicated field plot experiments were conducted at the Weslaco, Temple, and Lubbock stations during the summer of 1951. These stations were selected because they represent diverse farming practices, climates, and growing conditions. Therefore it is believed that some significance can be attached to similarities in plant reaction to chemically induced defoliation when grown under such greatly different conditions, whereas marked deviations from the norm are of questionable significance. A uniform defoliant (Shed-A-Leaf), at the rate of seven to eight pounds per acre, was applied by means of tractor-mounted spray equipment. The mode of action of Shed-A-Leaf was compared to that of ethylene used at College Station (tables II and III).

Leaf samples were collected at the time of defoliant application at all locations. At College Station, samples were also taken 24, 48, and 72 hours after application. In the Lower Rio Grande Valley and Temple experiments, leaf samples were collected 24 and 48 hours after application, while at Lubbock, samples were collected 48 and 72 hours after application (table III). Daily observations were made of plants at all localities throughout most of the defoliation process. Defoliation counts were made as soon as the abscission process was completed, and the per cent. of leaf fall for the plots was averaged. With the exception of the plants at Lubbock, the per cent. of leaf fall was determined about eight days after the defoliant was applied.

The experiment in Lower Rio Grande Valley was performed near Donna, Texas, under irrigated conditions of ample moisture. The Deltapine variety of cotton was used and the defoliant was applied in early July. The Temple experiments utilized the Empire variety of cotton grown under extremely unfavorable conditions. At the time of defoliant application (August 20), the plants were under very serious drought stress. The plants at Lubbock were grown under what may be called semi-dry land conditions. Approximately 11 inches of rain fell during the season, which was supplemented by a four acre-inch irrigation in April and a two acre-inch irrigation in late July. Application of the defoliant to the Storm Proof cotton variety normally grown in the High Plains was made in early October.

## Sampling, analytical and chemical methods

In 1950, the leaves on the main stalk of the control plants of the four experiments were sampled at the time of defoliant application, those of the plants of experiment 4 sprayed with 5% sucrose were sampled at 3 and 55 hours after application, and those of the plants sprayed with maleic hydrazide were sampled one week after application. In 1951 the plants were sampled as outlined in the previous section and summarized in tables II and III and figures 1 and 2. The plants at College Station were separated into leaf blades, petioles, and stems (figs. 1, 2). Only leaf blades were sampled at the other stations. The stem portion included the upper three fourths of the main stalk; the woody basal portion was discarded. In all

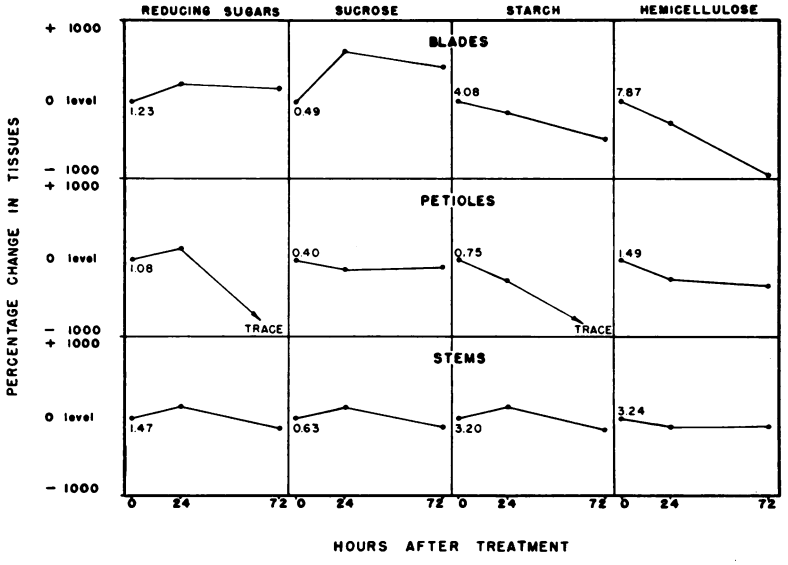


FIG. 1. Changes in carbohydrate fractions of blades, petioles, and stems during defoliation of cotton plants grown at College Station in 1951. All values are expressed as per cent. of dry weight.

cases the samples were oven-dried as soon as possible at 80° C and ground to pass an 80-mesh screen. Carbohydrates were determined by methods described in detail elsewhere (**4, 11**). Total nitrogen, including nitrates and nitrites, was determined by the modified Kjeldahl method using salicylic acid and sodium thiosulphate. Soluble nitrogen, extracted with 80% ethanol from oven-dry material, was determined by the Kjeldahl method after preliminary reduction with reduced iron powder.

The respiratory rates of control leaves and leaves sprayed with Shed-A-Leaf were measured in a standard Warburg respirometer by the leaf-disk method previously described (**11**). Oxygen uptake was measured with 0.2 ml. of 20% KOH in the center well to absorb $CO_2$. Carbon dioxide was determined by the direct method of Warburg. All measurements were made

in very diffuse light at 30° C, and at a stroker rate of 120 strokes per minute. Fifteen minutes of equilibration were allowed before closing the manometer stopcocks. Gas exchange was measured for one hour in all cases. Several flasks of the same treatment were used, and the two results in closest agreement were averaged for the data of table IV.

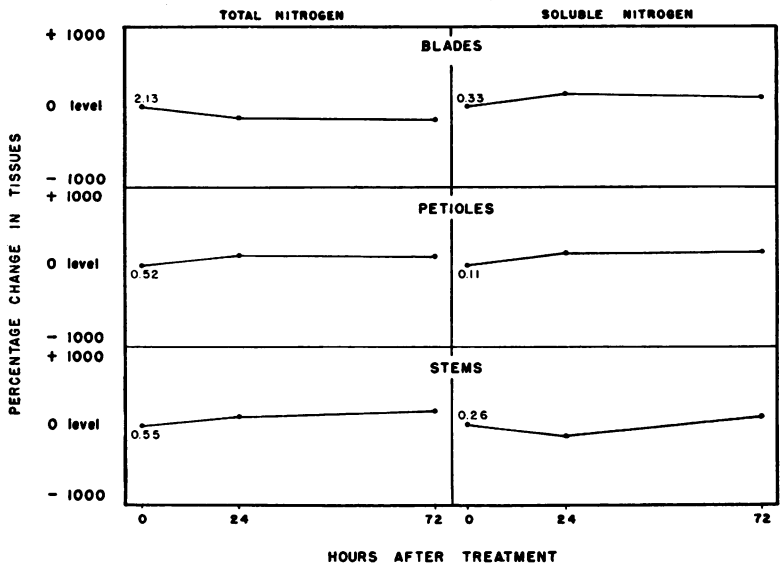


FIG. 2. Changes in nitrogen fractions of blades, petioles, and stems during defoliation of cotton plants grown at College Station in 1951. All values are expressed as per cent. of dry weight.

## Results

### 1950 EXPERIMENTS

Five different techniques were utilized in an attempt to alter the carbohydrate and nitrogen composition of the cotton plant: (a) The plants were grown in a low nitrogen supply, a procedure which has been shown to cause carbohydrate accumulation as well as decreased nitrogen content (**4**); (b) The plants were grown on a high nitrogen supply causing an increase in nitrogen content and a decrease in carbohydrate content; (c) Sucrose was applied as a spray to induce a build up in carbohydrate content; (d) The flower buds were removed which caused an increase in carbohydrate content as has been shown not only for cotton (**4**) but for other plants as well (**1, 10**); (e) Maleic hydrazide, which has been reported to cause an accumulation of carbohydrates in the cotton leaf (**18**), was applied as a spray.

The effects of these treatments on the carbohydrate composition of the main stalk leaves and their influence upon the per cent. of defoliation are summarized in table I. It was found that the low nitrogen treatment in experiment 1 increased the carbohydrate content when compared to the plants supplied with high nitrogen in experiment 2. The debudding and

## TABLE I

THE RELATIONSHIP OF CARBOHYDRATE COMPOSITION (AS PER CENT. DRY WEIGHT OF MAIN STALK LEAVES) AND AVERAGE PERCENTAGE DEFOLIATION AS SHOWN IN EXPERIMENTS OF 1950.

| Experiment and treatment | Average per cent. defoliation | Carbohydrates of main stalk leaves at time of spraying | | | | |
|---|---|---|---|---|---|---|
| | | Reducing sugars | Sucrose | Starch | Hydrolyzable residue | Total |
| Experiment 1, low N supply | | | | | | |
| Control | 00.0 | 1.03 | 1.03 | 2.90 | 7.25 | 12.48 |
| 5% Endothal | 12.7 | | | | | |
| 5% Endothal + 3.5% sucrose | 76.7 | | | | | |
| 5% Shed-A-Leaf | 14.5 | | | | | |
| 5% Shed-A-Leaf + 3.5% sucrose | 60.5 | | | | | |
| Experiment 2, high N supply | | | | | | |
| Control | 00.0 | 0.70 | 0.91 | 2.30 | 6.20 | 10.11 |
| 5% Endothal | 26.5 | | | | | |
| 5% Endothal + 3.5% sucrose | 32.3 | | | | | |
| 5% Shed-A-Leaf | 26.5 | | | | | |
| 5% Shed-A-Leaf + 3.5% sucrose | 57.5 | | | | | |
| Experiment 3, debudded | | | | | | |
| Control | 00.0 | 0.93 | 1.32 | 2.95 | 7.80 | 13.00 |
| 5% Endothal | 85.5 | | | | | |
| 5% Shed-A-Leaf | 82.5 | | | | | |
| Experiment 4, maleic hydrazide, sucrose | | | | | | |
| Control | 00.0 | 0.86 | 2.02 | 2.35 | 7.40 | 12.63 |
| 5% sucrose (3 hrs. after application) | 00.0 | 1.19 | 2.62 | 2.07 | 7.10 | 12.98 |
| 5% sucrose (55 hrs. after application) | 00.0 | 0.86 | 2.05 | 3.32 | 7.20 | 13.43 |
| 4800 p.p.m. maleic hydrazide (1 week after application) | 00.0 | 1.88 | 1.20 | 4.80 | 7.90 | 15.60 |
| 2% Endothal | 42.5 | | | | | |
| 2% Endothal + 5% sucrose | 94.3 | | | | | |
| 2% Endothal + 4800 p.p.m. maleic hydrazide | 66.8 | | | | | |
| 2% Shed-A-Leaf | 83.6 | | | | | |
| 2% Shed-A-Leaf + 5% sucrose | 94.4 | | | | | |
| 2% Shed-A-Leaf + 4800 p.p.m. maleic hydrazide | 89.2 | | | | | |

maleic hydrazide treatments caused the greatest increase in leaf carbohydrates; whereas some increase in leaf carbohydrates resulted from the use of the sucrose sprays.

It is recognized that the defoliant concentration employed in the first three experiments was too high for optimum results, but this concentration was employed to ascertain the possible tonic or protective effect of sucrose in alleviating defoliant toxicity. The poor defoliation obtained with Endothal or Shed-A-Leaf alone in experiments 1 and 2 was at least partially attributable to the high defoliant concentration. Little evidence exists in

these data (table I) to support the report of V. L. HALL (9) that a correlation exists between leaf carbohydrates and the percentage defoliation effected. Rather, the correlation in the present data appears to be more an interaction between defoliant treatment and leaf composition during the defoliation process.

## 1951 EXPERIMENTS

A few notations of general interest are presented on plants defoliated with Shed-A-Leaf at the four locations in 1951 and others not included in this report. The growing conditions modify greatly the time and nature of the plant response to chemical defoliants as reported by V. L. HALL (9).

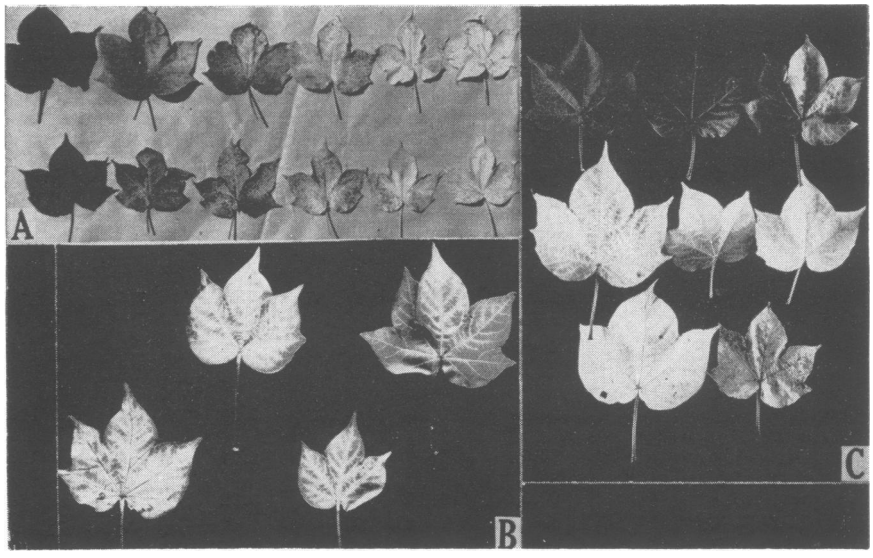


FIG. 3. Representative cotton leaves photographed during the abscission process showing the similarity in the pattern of degreening (loss of chlorophyll). A. Leaves from Lower Rio Grande Valley plants defoliated with Shed-A-Leaf. Left to right, progressive stages from defoliant application until leaf fall. B. Leaves enclosed with 100 p.p.m ethylene for 72 hours. C. Leaves from plants grown at College Station showing degreening stages during natural abscission.

Plants grown in the greenhouse in general have responded very quickly to the defoliant and exhibit signs of defoliant action a few hours after application, and defoliation is complete within five to seven days. Conversely, the plants grown in the High Plains area of Texas always respond very slowly to any type of defoliant. It generally takes several days before any visible symptoms of defoliant action on the plants can be observed, and in many cases three weeks are required before defoliation is complete. Some differences in response can be attributed to poor spray coverage, but for greenhouse plants or the small type of plant usually grown on the High Plains, complete coverage is not difficult to achieve. However in the Lower Rio Grande Valley, the rank type of growth, with 40 to 100 leaves per plant not uncommon, makes good coverage extremely difficult.

The usual leaf symptoms appearing 24 hours or longer after defoliant application are chlorotic spotting and localized dehydration of the leaf. The size of the spots is regulated by the size of the defoliant droplet and the rapidity of action of the defoliant. The spotting is probably caused by localized absorption and partial tissue destruction in the immediate area of contact with the droplet. Occasionally the leaves become red. Of particular interest was the degreening (loss of chlorophyll) that first appeared in some plants in the early stages more or less concomitantly with the spotting effect and which increased until leaf drop. In most cases, however, this chlorosis is masked in the field by the rapidity of defoliant action resulting

TABLE II

THE RELATIONSHIP OF CARBOHYDRATE AND NITROGEN COMPOSITION (AS PER CENT. DRY WEIGHT OF LEAF BLADES) AND AVERAGE PERCENTAGE DEFOLIATION AS SHOWN IN EXPERIMENTS OF 1951.

| Sample | Average per cent. defoliation | Carbohydrates | | | | | Nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | Reducing sugars | Sucrose | Starch | Hydrolyzable residue | Total | Total | Soluble |
| College Station Grown in 3-gal. jars outside. Defoliated with SAL. 6 lbs./acre | 87 | 1.23 | 0.49 | 4.08 | 7.85 | 13.67 | 2.13 | 0.33 |
| Lower Rio Grande Valley Irrigated, field grown. Defoliated with SAL. 8 lbs./acre | 65 | 1.23 | 0.30 | 4.05 | 8.20 | 13.78 | 2.00 | 0.26 |
| Temple Dry land, field grown. Defoliated with SAL. 8 lbs./acre | 32 | 0.63 | 1.95 | 3.02 | 6.00 | 11.60 | 2.27 | 0.40 |
| Lubbock Semi-dry land, field grown. Defoliated with SAL. 7 lbs./acre | 79 | 1.05 | 0.56 | 0.39 | 2.18 | 4.18 | 3.35 | 0.49 |
| College Station Grown in 2-gal. jars outside. Defoliated with 100 p.p.m. Ethylene gas | 96 | 2.50 | 1.80 | 4.75 | 8.50 | 17.75 | 2.56 | 0.44 |

in quick drying and browning of the foliage, which soon obscures the degreening that occurs. In this connection a series of photographs of representative cotton leaves from the Lower Rio Grande Valley plants were photographed showing the progressive loss of chlorophyll from the time of defoliant application until leaf drop (fig. 3 A). These leaves are to be compared with leaves induced to abscise by treatment with ethylene gas (fig. 3 B) and leaves abscising naturally (fig. 3 C). The similarity in the pattern of degreening in all three cases is striking.

In general agreement with the 1950 results, no definite trends or correlations are apparent between the carbohydrate and nitrogen fractions of the leaf blade and the average percentage defoliation obtained (table II). The

Lubbock samples were the lowest in total carbohydrates and highest in total and soluble nitrogen but had a higher percentage of defoliation than the Temple or Lower Rio Grande Valley plants. On the other hand the College Station plants which were defoliated with 100 p.p.m. ethylene were highest in starch, reducing sugars, and total carbohydrates, intermediate in nitrogen, and gave the highest percentage defoliation. The extremely low reserve carbohydrate content of the Lubbock plants is noteworthy. It should

TABLE III

CHANGES IN CARBOHYDRATE AND NITROGEN COMPOSITION OF LEAF BLADES DURING DEFOLIATION AS SHOWN IN EXPERIMENTS OF 1951. ALL VALUES AS PER CENT. OF DRY WEIGHT.

| Treatment | Time after application | Carbohydrates | | | | | Nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | | Reducing sugars | Sucrose | Starch | Hydrolyzable residue | Total | Total | Soluble |
| | *hrs.* | | | | | | | |
| | | | | College Station | | | | |
| Control | 0 | 1.23 | 0.49 | 4.08 | 7.87 | 13.67 | 2.13 | 0.33 |
| 6 lbs. SAL. | 24 | 2.60 | 3.01 | 3.15 | 2.88 | 11.64 | 1.64 | 0.58 |
| 6 lbs. SAL. | 72 | 2.07 | 2.03 | 0.83 | 0.85 | 5.78 | 1.46 | 0.35 |
| | | | | Lower Rio Grande Valley | | | | |
| Control | 0 | 1.23 | 0.30 | 4.05 | 8.20 | 13.78 | 2.00 | 0.26 |
| 8 lbs. SAL. | 24 | 0.75 | 1.01 | 3.41 | 6.15 | 13.32 | 2.41 | 0.30 |
| 8 lbs. SAL. | 48 | 1.20 | 0.78 | 3.15 | 6.00 | 11.13 | 2.01 | 0.27 |
| | | | | Temple | | | | |
| Control | 0 | 0.63 | 1.95 | 3.02 | 6.00 | 11.60 | 2.27 | 0.40 |
| 8 lbs. SAL. | 24 | 0.13 | 0.44 | 2.75 | 6.10 | 9.42 | 2.24 | 0.57 |
| 8 lbs. SAL. | 48 | 0.75 | 1.29 | 1.34 | 6.20 | 9.58 | 2.22 | 0.44 |
| | | | | Lubbock | | | | |
| Control | 0 | 1.05 | 0.56 | 0.39 | 2.18 | 4.18 | 3.35 | 0.49 |
| 7 lbs. SAL. | 48 | 0.83 | 0.49 | 0.20 | 2.53 | 4.06 | 3.07 | 0.56 |
| 7 lbs. SAL. | 72 | 0.99 | 0.99 | None | 1.16 | 2.64 | 2.56 | 0.50 |
| | | | | College Station | | | | |
| Control | 0 | 2.50 | 1.80 | 4.75 | 8.50 | 17.75 | 2.56 | 0.44 |
| Control | 48 | 1.23 | 1.82 | 3.64 | 7.75 | 14.44 | 2.51 | 0.45 |
| 100 p.p.m. $CH_2=CH_2$ | 48 | 0.60 | 0.68 | 0.51 | 7.11 | 8.90 | 2.54 | 0.56 |
| 100 p.p.m. $CH_2=CH_2$ | 72 | 0.10 | 0.15 | None | 2.63 | 2.88 | 1.96 | 0.48 |

be pointed out also that the relatively low amount of defoliation of the Lower Rio Grande plants was in part due to inadequate coverage of the foliage.

When changes in the carbohydrate and nitrogen composition of leaf blades were followed during the early stages of defoliation (up to 72 hours) of plants grown at the different locations, several trends in common were noted. The highest percentages of defoliation (table II) were associated with a rapid depletion of carbohydrates, particularly the reserve and struc-

tural carbohydrates (starch, hemicellulose, etc.) (table III). These fractions underwent rapid hydrolysis as shown by the general increase in their equivalent in soluble sugars at 24 and 48 hours. Eventually, however, considerable translocation or metabolic transformation occurred as evidenced by a net decrease in total carbohydrates of the blades. Changes in total nitrogen and soluble nitrogen followed much the same trends. The plants showing the most defoliation were those showing the greatest change in total nitrogen during the 72 hour period.

The control plants placed under bell jars for 48 hours showed some decrease in leaf blade carbohydrates and nitrogen. However, the change in these constituents in the plants enclosed with ethylene was much greater suggesting that ethylene accelerates enzyme activity, an effect observed in other plant organs (**12, 15**). The results with ethylene, although more pro-

TABLE IV

EFFECT OF DEFOLIANT (SAL.) ON GAS EXCHANGE IN COTTON LEAF DISKS. MEASUREMENT WAS MADE THREE HOURS AFTER SPRAY APPLICATION AND AT 24 HOUR INTERVALS THEREAFTER.

| Treatment | Time after application | $Q_{O_2}$ | Per cent. of control | $Q_{CO_2}$ | Per cent. of control | R. Q. |
|---|---|---|---|---|---|---|
| | *hrs.* | | | | | |
| Control | 3 | 1.34 | .... | 1.20 | .... | 0.89 |
| Defoliant | 3 | 2.89 | 215.6 | 2.71 | 225.8 | 0.93 |
| Control | 27 | 1.65 | .... | 1.63 | .... | 0.99 |
| Defoliant | 27 | 3.12 | 189.0 | 2.82 | 173.00 | 0.90 |
| Control | 51 | 1.76 | .... | 1.93 | .... | 1.09 |
| Defoliant | 51 | 3.80 | 215.9 | 3.73 | 193.2 | 0.98 |
| Control | 75 | 1.70 | .... | 1.84 | .... | 1.08 |
| Defoliant | 75 | 3.45 | 202.8 | 3.56 | 193.4 | 1.03 |

nounced, are comparable to those obtained with the defoliant at the other stations, and suggest a common mode of action.

Analyses of leaf blades, petioles, and stems of the College Station plants (fig. 1) indicated the nature of the changes observed in the leaf blades which are summarized in table III. Chemical analyses of the samples taken at 24 hours show that the reserve fractions of the petioles and the blades were undergoing simultaneous hydrolysis. A comparison of the values for soluble carbohydrate and nitrogen fractions at 24 hours with the initial values indicates that these products were also being translocated out of the blade and petiole into the stem. The higher starch value at 24 hours in the stem compared to the initial content in this organ indicates polymerization. By 72 hours, both starch and reducing sugars in the petioles had fallen to trace amounts, although both fractions were still present in the blades. Total nitrogen in the petioles at 72 hours, however, had not changed appreciably, although soluble nitrogen had steadily increased. These changes in the

petiole probably have special significance in abscission. The decrease in total carbohydrates of the stem taking place between 24 and 72 hours suggests that translocation was occurring from the stem to the roots. Unfortunately, roots were not sampled to substantiate this interpretation.

A comparison of the various carbohydrate fractions in the leaf blades, petioles, and stems (figs. 1, 2) before and up to 72 hours after defoliant application shows a deficit when the fractions are totaled. It appeared that the difference was more than could be accounted for by translocation to the roots and therefore an accelerated respiration was indicated. This interpretation was confirmed by respiratory determination employing the Warburg apparatus (table IV). The respiratory rate was approximately doubled in the plants sprayed with defoliant during the period of 72 hours as measured either by oxygen uptake or carbon dioxide production. The respiratory quotient suggests that carbohydrates were the primary substrates being respired under aerobic conditions.

## Discussion

By considering the observations and results of this study as a whole, several events assume a paramount position in the chemically induced type of abscission process which have much in common with changes observed in the natural type and ethylene-induced type of abscission. The first of these events is the degreening phenomenon that occurred in all three types of abscission. GAWADI and AVERY (**8**) noted that in the abscission process of leaf fall separation of the leaf from the stem occurs when the leaves begin to lose their green color. BROWN and ADDICOTT (**3**) listed the events leading to abscission in the bean leaf and described the pulvinus as changing in color from deep green through pallid green and yellow-green to yellow. They noted a positive correlation between the color of the pulvinus and abscission. Typically, if the pulvinus is green, leaf fall will not take place; if it is yellow, only a light touch is necessary to bring about separation. OLMSTEAD (**19**) studied the effect of photoperiod on abscission in the sugar maple and stated that abscission may involve a loss of green color but this loss may be a side effect of the aging process related to photoperiod and may not in itself lead directly to abscission. In studying the degreening of oranges, ROPER and MILLER (**20**) noted that the synthetic auxins offered no promise as degreening agents and none had a major effect upon respiration. On the other hand, ethylene greatly stimulated the respiratory rate and gave excellent degreening. They concluded from their work that reactions which cause degreening are natural ones and the addition of ethylene is the most effective means known to make these processes occur at the maximum rate possible. Cotton petioles were observed to redden slightly, lose green color, and have total loss of color in the blade and petiole when the plants were enclosed in an ethylene atmosphere where a high rate of abscission occurred (**13**). However, when the cotton plants were pretreated with naphthaleneacetic acid, degreening of leaves or petioles did not take place

nor did they abscise to any extent. In the present study, the similarity in the pattern of degreening noted during chemically induced abscission, natural abscission, and ethylene-induced abscission, indicates rather clearly the possibility that ethylene is the causal agent in all three cases.

Other important events, such as the rapid hydrolysis of reserve compounds to soluble constituents, their polar translocation in the plant, and the greatly stimulated respiratory rate, all seem to occupy key roles in the defoliation process. The results of this study, as well as other published (**3, 13, 17, 21**) and unpublished observations (**14**) lead us to postulate that the physiological course of events during chemically induced defoliation proceeds, more or less, along the following pathway. As the defoliant strikes the leaf, it is absorbed and brings about a temporary inhibition of the metabolic processes of the leaf; respiration is slowed for an hour or two (**13**), leaf processes are partially inactivated, and some localized tissue destruction occurs. It is believed that these reactions lower the auxin content (**13, 17**) and are followed by a rapid stimulation of both hydrolytic and respiratory enzymes. The reserve constituents of the leaf, such as starch, the hemicelluloses, and other structural carbohydrates, and the proteins and insoluble nitrogen fractions are rapidly hydrolyzed as evidenced by their decrease and by the concurrent increases in the soluble fractions, such as sugars and soluble nitrogen. Respiratory enzymes become greatly stimulated as shown by accelerated oxygen uptake and increased carbon dioxide output as soon as three hours after defoliant application. The respiratory quotient indicates that the soluble carbohydrates are the primary substrates being oxidized under aerobic conditions. In some cases it has been shown that increased aerobic respiration is favorable to increased ethylene production (**7, 12, 13, 15**). The initial evolution of ethylene may further lower the auxin level or inactivate auxin, shifting the auxin-ethylene ratio in the favor of ethylene; initiating the events leading to degreening and chlorophyll destruction (**20**) and further stimulating both hydrolase and respiratory enzymes. BENNETT (**2**) has shown that the hemicelluloses of the corn stalk are made up largely of pentosans which yield pentoses upon hydrolysis. In other work (**12**) it has been demonstrated that the pentoses, particularly arabinose, served as excellent substrates for ethylene production in *in vitro* experiments. With the high rate of hydrolysis of the reserve components of the leaf blade, a rapid accumulation of soluble compounds occurs resulting in their translocation out of the blade into the petiole, and eventually on into the stem. The foregoing events undoubtedly accelerate respiration in the petiole, production of ethylene, a loss in the auxin gradient (**21**) across the petiole, digestion of the cell and cell wall materials, and cell division in the so-called abscission layer.

Finally, the results of the present paper do not support entirely the conclusions of V. L. HALL (**9**) that the starch or nitrogen content *per se* governs the susceptibility of the cotton plant to chemical defoliation. Rather it appears that the events that take place during the abscission process which

involve the rate of depletion of leaf carbohydrates and nitrogen, the accelerated respiratory rate, and the relative ratio between auxin and ethylene are as important, or more so, in regulating the course of abscission. In all fairness to V. L. HALL (**9**) it should be pointed out that he suggested that carbohydrate depletion is an important factor in the effectiveness of chemical defoliation and that there are other factors which determine the susceptibility of the cotton plant to chemical defoliation. It appears logical that the significance of a low starch content in defoliation is that it indicates the labile carbohydrates are exhausted and the reserve structural carbohydrates of the leaf are more subject to serve as enzymatic substrates under the influence of chemical defoliants. The writers' findings on the absence of a direct relation between carbohydrates and nitrogen and defoliation are in closer agreement with the final conclusions of EATON and ERGLE (**5**) although their work involved shedding of bolls in cotton. At the present time, there is no reason to believe that shedding and defoliation are basically different.

## Summary

Results of experiments conducted in 1950 and 1951 which involve studies of compositional and physiological changes associated with the chemical defoliation of cotton are reported. Five techniques were employed in 1950 to alter the carbohydrate content of cotton leaves and study the effect upon the percentage defoliation obtained. In 1951 experiments were performed at four locations, and the mode of action of a uniform defoliant applied to plants grown under widely different cultural and environmental conditions was compared with plants defoliated with 100 p.p.m. of ethylene. Chemical changes in the carbohydrate and nitrogen fractions were followed by analyses at the time of defoliant application and thereafter at 24 hour intervals up to 72 hours during defoliation. Warburg respirometer measurements were made during abscission.

Little evidence was found for any correlation between leaf blade carbohydrates and the percentage defoliation effected in the 1950 work. There appeared to be more of an interaction between defoliant treatment and leaf composition during the defoliation process.

A similarity in the pattern of degreening (chlorophyll loss) of the leaf during natural abscission, defoliant-induced abscission and ethylene-induced abscission is illustrated and discussed.

No definite trends or correlations were found between the carbohydrate and nitrogen fractions of the leaf blade and the percentage defoliation obtained in 1951.

A rapid hydrolysis of the reserve constituents of the leaf blade and the polar transport of their soluble products occurred under the influence of the defoliant. The results with ethylene, although more pronounced, were comparable with those obtained with the defoliant.

The respiratory rate of the leaves sprayed with defoliant, when measured either by oxygen uptake or carbon dioxide production, approximately

doubled that of unsprayed leaves. The respiratory quotient indicated that carbohydrates were the primary substrates being oxidized under aerobic conditions.

The rapid hydrolysis and the rate of depletion of reserve compounds in the leaf blade and petiole brought about by the polar translocation of the mobile compounds as well as by oxidation in respiration, are believed to play a prominent role in chemically induced defoliation.

Based on the evidence of the present and other studies, a physiological course of events during chemical induced defoliation is proposed and discussed.

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