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Effects of a Phosphate Defoliant on Cotton Leaves: Water Relations and Free Amino Acids[1]

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ABSTRACT

The phosphate defoliant DEF (*S,S,S*-Tributylphosphorothrithioate) was sprayed on presquaring greenhouse-grown cotton (*Gossypium hirsutum* L.) plants. Plant water status and leaf area data were taken at 3-hour intervals during the 1st day and daily thereafter. Stomatal diffusion resistance was measured during the 1st day only. Leaves were analyzed for total N, soluble N, and distribution of free amino acids. Little change in any of the parameters measured was noted the 1st day. On subsequent days the DEF-treated leaves had lower leaf water potential recovery values (predawn measurement) and lower specific weights (fresh weight basis). DEF-treated leaves, however, had a higher specific leaf weight on a dry weight basis. Leaf N percent did not change as a result of the DEF treatment. Soluble N increased up to twice that of the controls by the 3rd day. The distribution of free amino acids in DEF treated leaves was altered. The concentrations of all amino acids except aspartic acid, glutamic acid + glutamine, proline and valine were significantly increased in the DEF treated leaves. The exceptions remained stable or tended to decrease. The results indicated that DEF-treated leaves may be mobilizing the leaf protein prior to abscission, but that transport out of the leaf is impeded. Further experiments are needed to substantiate this notion.

Additional index words: Gossypium hirsutum L., DEF, Asparagine, Soluble N.

THE chemicals *S,S,S*-Tributylphosphorotrithioite and *S,S,S*-Tributylphosphorotrithioate (DEF) were first reported to have defoliant activity in 1956 (Woofter, 1956; Goyette, 1956; and Hall, 1957) and were introduced commercially in 1957 (Anonymous, 1960). Since their introduction they have become the standard defoliants in the cotton (*Gossypium hirsutum* L.) industry (Walhood and Addicott, 1968; Cooper et al., 1968; and Weaver, 1972).

Reports on the biochemical, physiological, and compositional changes coincident with defoliant treatment in general are numerous (e.g., Hall, 1951; Hall and Lane, 1952). Respiration of leaf tissue treated with defoliants other than DEF was observed to increase (Leinweber and Hall, 1959a; Felton and Downing, 1958). Other compositional features have been examined and observed to change in defoliant-treated leaves. Starch content decreased 24 to 72 hours after treatment (Hall and Lane, 1952) but high starch content accompanied resistance to defoliation, (Hall, 1951). In a later study Leinweber and Hall (1959b) looked at the starch content of defoliant-treated leaves as a function of leaf age. They found that in general the starch content of treated leaves increased during the first 10 hours, although different defoliants had different effects in this aspect. Endothal had more pronounced effects than did amino triazole. Younger leaves showed more pronounced increases than did older ones. They did not continue their study beyond 10 hours.

Soluble N increased in one study (Hall and Lane, 1952) after 24 hours, which would result if leaf proteins in defoliant-treated leaves were being hydrolyzed into free amino acids. Leinweber and Hall (1959b) also looked at changes in soluble N. During the first 10 hours soluble N increased with more pronounced effects on older leaves, again an indication of induced senescence.

DEF effects on cotton leaves are not as well documented, however. The reports of Katterman and Hall (1961) and Hall et al. (1961) are the most recent avail-

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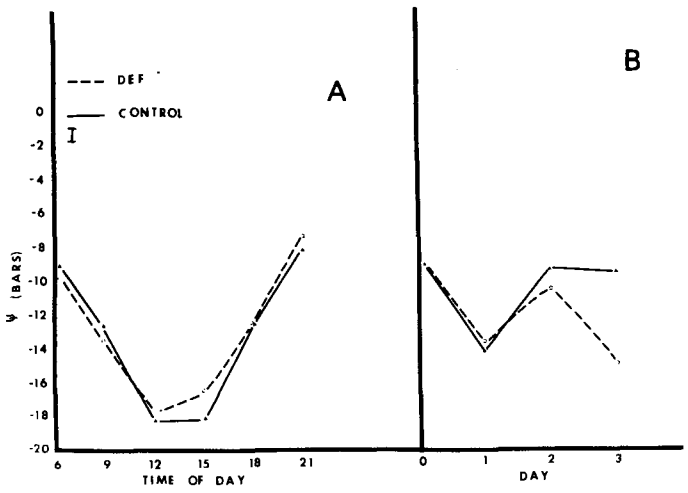

Fig. 1. The effects of a DEF application on diurnal changes in leaf water potentials (A) and daily changes in water potential recovery values (ψ_R) (B). SE bar for the experiment as determined by ANOV is shown.

able. They showed that respiration of DEF-treated leaves increased, that the sulfhydryl group content decreased to values similar to older more senescent leaves, and that free amino acids increased. The free amino acids were checked by paper chromatography and individual amino acids were not identified nor quantitated.

Because data on changes in leaf composition the day of and 1 day after defoliant treatment are contradictory and incomplete and because data on effects of the phosphate defoliants are scarce, we felt a fresh look at the whole area was needed. We treated leaves with a prominent defoliant, DEF, and measured physiological and compositional changes which occurred in leaves during the treatment and subsequent days. We had techniques and equipment not available to previous researchers, which enabled us to chart the compositional changes, especially of the amino acids and water status, more completely.

MATERIALS AND METHODS

Cotton plants of the 'Stoneville 213' cultivar were grown in the greenhouse in 20-cm clay pots filled with 1/3 Jiffy Mix[a], 1/3 masonry sand, and 1/3 top soil (v/v/v). The root medium was kept moist by frequent watering and biweekly additions of a complete nutrient solution.

Two weeks after emergence the plants were thinned to one per pot. Treatments were imposed at the early square stage of development (6 to 7 weeks after emergence). Treatments were replicated three times in a randomized block design and consisted of an untreated control and spray application of *S,S,S*-Tributylphosphorotrithioate (DEF[a]). The chemical was diluted with water and applied to the plants with a three-nozzle boom that contained T X-8 conejet tips spaced 0.5 m apart. Pressure was maintained at 2.25 kg/cm^2 with CO_2, and the boom was passed over the plants at 4 km/hour. Total spray volume was 187 liters/ha to give a rate of application of 1.26 kg ai/ha. The chemical was applied at 0900 hours on the day of treatment. Sampling of leaves (leaves at the first three nodes) began at 0600 hours and continued at 3-hour intervals until 2100 hours.

[a] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

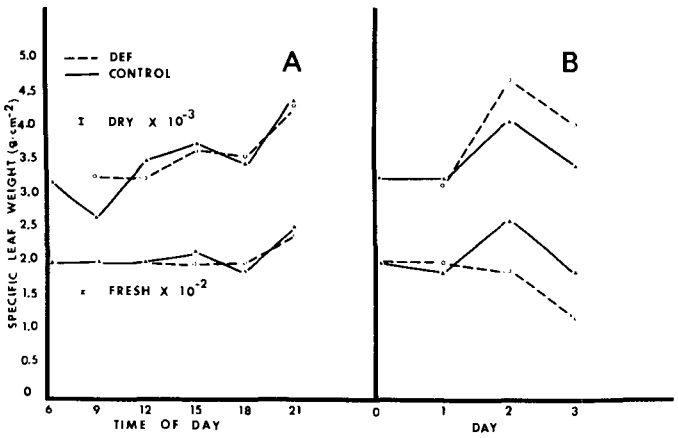

Fig. 2. The effects of a DEF application on diurnal (A) and daily (B) changes in specific leaf weights on a fresh and dry weight basis. SE bars for the experiments as determined by ANOVS are shown.

Then leaves were sampled daily only at 0600 hours through the 3rd day following treatment. Leaf water potentials and leaf water potential recovery values were determined at each sampling time with a Scholander pressure bomb (Scholander et al., 1965). The 0600 hour samples only should be considered leaf water potential recovery values since 0600 hours was before dawn and these values represent the highest water potential the plants will attain during the day. Leaf areas of harvested leaves were determined with a Lambda portable area meter[a]. Leaf fresh weights and stomatal diffusion resistances were also determined, except that no stomatal diffusion resistance determinations were made for the 0600 hour samples. The leaves were then frozen, lyophilized, weighed, and finely ground using a Wiley Mill[a]. Leaf N content was determined with a Coleman N analyzer[a]. Free amino acids were determined by the ninhydrin assay (Spies; 1957) on an alcoholic extract of the lyophilized leaves. Two replications of the 0600 hour samples only were analyzed for distribution of free amino acids using a Beckman 121 amino acid analyzer[a].

RESULTS AND DISCUSSION

During the day of the DEF treatment, no effects on leaf water potential values (Fig. 1), specific leaf weights (Fig. 2), or stomatal diffusion resistance (data not shown) were observed. There were, however, observable effects later. The leaf water potential recovery values for DEF-treated leaves diverged significantly from control leaves and on the 2nd and the 3rd days averaged 6 bars lower than the control. By the 3rd day the DEF-treated leaves were visibly affected and had large desiccated spots and necrotic areas. The abscission zone had also formed by this time. The lower water potential of these leaves probably reflects both the presence of the desiccated spots and an increased resistance to water flow. Changing leaf water content also is reflected in the specific leaf weight effects which were noticeable beginning with the 2nd day. The desiccated spots in the DEF-treated leaves contributed to the lower specific leaf weight values for fresh weight basis.

DEF-treated leaves had a higher specific leaf weight on a dry weight basis. The reason is not readily apparent from the data at hand. It could be that the DEF treatment inhibits translocation out of the leaf, yet does not inhibit photosynthesis. This would result in assimilate accumulation by the leaf, and thereby in denser leaves with higher specific leaf weights.

Table 1. Leaf composition of N and free amino acids as affected by DEF treatment.

Leaf component	Treatment	Date and time after treatment: Day 0 0600	Day 0 0900	Day 0 1200	Day 0 1500	Day 0 1800	Day 0 2100	Day 1 0600	Day 2 0600	Day 3 0600	SE†
% N	Control	4.13	4.15	4.10	3.76	3.90	4.05	4.20	4.07	3.81	0.11 ns
	DEF	–	4.13	4.03	3.74	3.74	3.90	4.21	4.17	4.08	
Free amino acids‡	Control	100	105	107	108	120	106	83	82	70	6.2**
% of control	DEF	–	102	102	112	115	95	99	137	143	

** is listed whenever the ANOV for treatment differences was significant at the 0.01 level of probability. † SE is presented for the experiment.
‡ Day 0 0600 taken as 100%.

Table 2. Free amino acid composition of DEF treated cotton leaves compared with controls.

Amino acid	Days after treatment: 0 Control	0 DEF	1 Control	1 DEF	2 Control	2 DEF	3 Control	3 DEF	SE†
	μmoles/g dry wt								
Aspartic acid	11.3	10.5	10.6	13.4	11.5	7.1	9.3	6.2	0.90 ns
Threonine	1.0	1.0	0.6	1.4	0.8	5.2	0.8	4.0	0.26**
Serine	3.2	3.3	1.2	1.6	1.4	3.5	0.9	3.6	0.35*
Asparagine	2.0	2.2	1.5	3.0	1.8	18.2	1.2	28.7	1.92**
Glutamic acid + glutamine	12.2	12.9	9.5	15.5	13.1	10.0	9.6	9.7	1.12 ns
Proline	0.4	0.4	Tr	0.3	1.1	1.2	0.9	0.9	0.12 ns
Glycine	0.4	0.3	0.1	0.2	0.2	0.4	0.1	0.4	0.02**
Alanine	8.1	10.6	2.3	4.4	3.3	8.9	2.2	6.0	0.22**
Valine	0.1	0.3	0.6	0.4	0.3	0.3	0.3	1.3	0.18 ns
1/2 cystine	0.7	0.8	0.6	1.1	0.8	0.9	0.5	1.1	0.06**
Methionine	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	–
Isoleucine	Tr	Tr	0.1	0.2	0.1	0.5	0.2	0.8	0.03**
Leucine	Tr	Tr	0.2	0.2	0.1	0.3	0.2	0.4	0.03*
Tyrosine	Tr	Tr	Tr	Tr	Tr	Tr	0.1	Tr	0.004*
Phenylalanine	0.2	0.2	0.1	0.2	0.1	0.3	0.2	0.4	0.01*
γ-aminobutyric acid	5.2	5.2	4.6	4.8	5.8	23.8	5.0	20.0	0.96*
Ethanolamine	1.5	1.4	1.5	2.3	2.0	2.6	1.7	3.1	0.13**
Ammonia	8.0	11.5	3.1	7.0	4.3	5.8	3.0	6.7	0.89*
Lysine	0.1	0.1	0.2	0.3	0.3	0.7	0.5	1.8	0.09**
Histidine	Tr	Tr	Tr	0.1	Tr	0.4	0.1	0.6	0.01**
Arginine	Tr	Tr	Tr	Tr	Tr	0.1	Tr	Tr	0.01*

* and ** are listed whenever the ANOV treatment differences were significant at the 0.05 or 0.01 level of probability, respectively.
† SE is presented for the experiment.

Leaf N percent was not affected by the DEF treatment (Table 1). Free amino acids or soluble N, however, did increase and soluble N in DEF-treated leaves was double that in the controls by day 3. This was true even though the soluble N content of the controls decreased significantly during the experiment. Hall and Lane (1952) showed a decrease in total N in treated cotton leaves and Hall (1951) suggested that the positive relationship he observed between leaf N content and sensitivity to defoliation was probably casual (the result of starch content changes rather than N per se). Hall and Lane (1952) also reported an increase in soluble N in leaves treated with other defoliants, but not as great as in our results. The soluble N was probably generated from leaf proteins by protein hydrolysis.

Since leaf percent N did not change significantly and specific leaf weight (dry weight basis) increased, then leaf N content must have increased in the DEF-treated leaves. This was not verified statistically, but the rationale for this is the same as for the specific leaf weight increase, with the proviso that additional N entered via the transpiration stream. If phloem transport is indeed hindered by formation of the abscission zone, then an increase in leaf N content would be expected.

Measurement of soluble N or free amino acids by the ninhydrin assay is only an approximation because each amino acid present has a slightly different extinction coefficient. To learn more about the composition and content of the soluble N fraction the individual amino acids must be known. They were determined and the results are shown in Table 2. Most amino acids were affected by the DEF treatment. Glutamic acid and aspartic acid, the most abundant amino acids in cotton leaf protein (McMichael and Elmore, 1977), with percentage values of 14 and 11%, respectively, were not significantly affected. With protein hydrolysis of leaf proteins, the two amino acids that should have increased most in the soluble N fraction are aspartic acid and glutamic acid. Asparagine, the aminated version of aspartic acid did increase quite dramatically; but glutamine, the corresponding aminated version of glutamic acid, was not present in large enough concentrations to differentiate it from glutamic acid in our system. Glutamic acid was probably being released to the soluble N fraction by protein hydrolysis but was being metabolized rapidly to other amino compounds including asparagine.

It is known that N is transported as one of the amides (usually asparagine or glutamine) in plants (Atkins et al. 1975). Many plants specifically transport

N in one amide form or the other. The accumulation pattern of amino acids in this experiment indicate asparagine as the transport amide in cotton.

The data from this experiment support the notions that asparagine is the transport amide and that translocation of metabolites out of the leaf is inhibited by DEF. Although not yet proved, data from other sources support asparagine as the transport amide in cotton (Radin, 1977; Capdevila and Dure, 1977; Elmore and Leffler, 1976). Additional experiments are needed to test both hypotheses.

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