Bulletin 789

•Amino Triazole

-- A New Abscission Chemical and Growth Inhibitor

Cotton defoliated with Amino Triazole at the rate of 1.5 pounds per acre in 26 gallons of water. Ground equipment was used. Dark spots in the background are check plots that did not receive insecticides during the growing season.

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TEXAS AGRICULTURAL EXPERIMENT STATION

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SUMMARY

Experiments were conducted with Amino Triazole, a new abscission-promoting but growth-inhibiting chemical, in the laboratory, greenhouse and in the field at College Station during 1952, 1953 and 1954.

AT caused chlorophyll destruction and impaired chlorophyll synthesis in tissues formed at the time or subsequent to the absorption of the chemical. The inhibition of chlorophyll synthesis was proportional to the concentration and the age of the tissues at the time of treatment. The possibility that the restriction of chlorophyll synthesis was due to immobilization of Mg, Fe, Mn, N, P or K by AT appeared unlikely; the inhibitory effect appeared to be prior to the protochlorophyll stage.

Translocation experiments in the greenhouse indicated that AT is readily absorbed by the roots and aerial organs of the cotton plant and is mainly translocated upward. When applied to the soil it is absorbed by the roots and apparently moves upward in the xylem, but foliar applications apparently are transported in the phloem. Although the results were seldom as clearcut or striking, field observations generally confirmed the systemic action of AT noted in the greenhouse.

Greenhouse and field tests demonstrated the effectiveness of AT as a cotton defoliant and a suppressant of secondary growth. It was compatible with other defoliants, increasing both defoliation and regrowth inhibition. When applied at the normal time of defoliant application, detrimental effects on seed or fiber properties were not observed.

Carbohydrate metabolism of AT-treated cotton plants was affected. Within 48 hours after foliage application, the aerial organs lost approximately half of the original reducing sugars and sucrose; a slight increase in starch was essentially balanced by an equivalent loss in the hemi-cellulose fraction. The result was a decrease in total carbohydrates. Fractionation of the treated plants showed that soluble sugars decreased in both the upper and basal shoot, but reserve carbohydrates increased in the upper plant and decreased in the basal parts following treatment. Preliminary experiments showed changes in nitrogen, phosphorus and potassium in AT-treated plants, but calcium values were unaffected.

Reduction in stem height and other inhibitory effects were noted for cotton sprayed with AT at two stages of growth. Elongation of oat coleoptile sections was suppressed by all concentrations above 0.84 mg/l., the inhibition increasing with increasing concentration. The stimulatory effect of indole acetic acid (IAA) on Avena section growth was further enhanced by the proper combination with AT. Relatively high concentrations of AT could relieve partially the inhibitory action of 10 mg./l. maleic hydrazide (MH) but higher concentrations of MH in combination with AT were mutually inhibitive. Below 21 mg/l., AT stimulated root growth but reduced hypocotyl growth at all concentrations when tested by means of the cucumber or cotton seedling test; in combination, IAA and AT were inhibitory to both root and hypocotyl growth. Competitive inhibition experiments showed that AT antagonized the effect of IAA in a manner qualitatively but not quantitatively expected of an anti-auxin. Its interaction with auxin appears to be of practical significance.

AT increased respiratory rates of cotton leaf blades and oat coleoptile sections. The effects of AT in combination with IAA and MH on respiration roughly paralleled their observed effects on growth.

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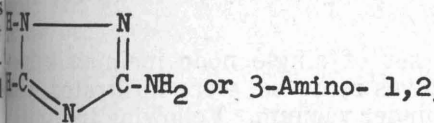
Amino Triazole -- A New Abscission Chemical and Growth Inhibitor

WAYNE C. HALL, S. P. JOHNSON and C. L. LEINWEBER*

AMINO TRIAZOLE (AT) IS A WATER SOLUBLE, heterocyclic compound composed of a five-membered ring with three nitrogen atoms. Its chemical structure is:



3-Amino- 1,2,4-Triazole

Produced under the trademark name of AMIZOL, it has a molecular weight of 84.5 and appears as transparent elongated white crystals (2). The melting point is reported between 153-159° C., depending on the investigator. Amino Triazole will react with most acids and bases to form salts; it will react with ketones and aldehydes to form many derivatives, and can be oxidized to form azotriazole although the triazole ring resists most common oxidizing agents. Preliminary toxicity studies indicate that AT is relatively nontoxic to rats (2).

AT was first discovered to have defoliating and regrowth inhibiting properties in tests with cotton conducted in 1952 by the Texas Agricultural Experiment Station (9, 10). Subsequent tests in Texas (22) and by others elsewhere (21) show that AT, alone and in combination with standard defoliants, possesses exceptional promise as a cotton defoliant and as a regrowth and general plant growth suppressant.

AT has proved effective as a pre- and post-emergence spray and as a selective herbicide (1, 3).

AT is the first defoliant reported to possess true systemic action; all defoliation chemicals used previously have been of the contact type. Although far from being the ideal defoliant, the potential impact of AT on the defoliant field may prove analgous to the discovery of DDT and its revolutionary effect on the field of insect control.

This report summarizes experiments with AT on cotton and other plant tissues conducted in the laboratory, greenhouse and in the field at College Station during 1952, 1953 and 1954.

Depending on the concentrations used, AT, singly and in combination with other compounds, possesses either stimulating or inhibiting properties on several of the basic plant processes. Because of its unique effects upon plant tissues it offers unusual possibilities for future research in both the basic and applied fields.

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METHODS AND RESULTS

The pure form of Amino Triazole, supplied under the code name ACP-981 by the American Chemical Paint Company, was used in most of the experiments reported in this study. For a few of the more critical experiments the ACP-981 compound was re-purified and re-crystalized before use.

Effects on Chlorophyll Destruction and Synthesis in Cotton

It was noted in the early work with AT that localized destruction of chlorophyll and dehydration of affected tissue often occurred when relatively high concentrations were used, particularly under conditions of high light intensity and temperature. Tissues formed at the time or subsequent to the absorption of AT were characterized by chlorosis. The degree of chlorosis varied with the concentration of AT used and the age of the plant and affected tissues at the time of treatment. When sub-lethal concentrations were used the chlorotic tissues often remained alive and eventually regained their normal color, although other manifestations of growth inhibition sometimes persisted for several months.

To check more closely the effects at AT on chlorophyll synthesis, cotton seed was planted in soil in 4-inch pots and germinated in the dark at room temperatures. When the etiolated, chlorotic seedlings had well-developed cotyledons they were thinned to four seedlings per pot and divided into six lots with five pots per lot. The six lots were sprayed in reduced light with the following concentrations of AT: 0.0 (distilled water), 8.4, 84, 210, 420, 650 and 840 mg/l. Immediately after spraying, the seedlings were placed back in the dark for an additional 24 hours to permit absorption of the AT, then left in the diffuse light of the laboratory for another day prior to being placed in the greenhouse under normal conditions of light and temperature.

The seedlings were observed daily and records kept on the number of days required for the cotyledons to obtain the relative greenness of cotyledons of seedlings germinated under normal conditions of the greenhouse. The cotyledons of all treated seedlings, except those receiving 650 and 840 mg/l. AT, eventually became normal green in color. At the two higher concentrations the cotyledons were still chlorotic along the veins when the experiments was terminated. Growth of all treated plants was severely inhibited. Growth formed subsequent to treatment was en-

tirely chlorotic. Treated plants died erratically during the experiment; by the end of 6 weeks all plants except the controls appeared to be dead. The length of time for the cotyledons to obtain normal color is roughly proportional to the concentration of AT applied (Figure 1).

To determine if AT was enhancing the light-induced destruction of chlorophyll, a concentrated acetone extract from fresh cotton-leaf powder was prepared and 15 ml. aliquots pipetted into separate test tubes. Either 15 ml. distilled water or 0.5 or 1 percent AT solution was added to the tubes. They were then placed in bright light. After 3 days the chlorophyll solution was mostly oxidized in the distilled water control tubes, but it was still bright green in the tubes containing AT. Apparently, the effect of AT on chlorophyll destruction depends on the properties of living tissue.

The possibility that the inhibition of chlorophyll synthesis was due to immobilization of Mg, Fe, Mn, N, P or K by AT appears unlikely. Single node main-stem sections from fruiting cotton plants, each containing a mature leaf, were treated by dipping the leaves momentarily into a 0.01 M AT solution. Control leaves were dipped in distilled water for comparison. The bases of the sections were immersed in a container of weak sugar solution containing sulfanilamide, being held upright in place by inserting the sections through large-meshed hardware cloth on the top of the container. The sections were placed on the laboratory bench in light. Forty-eight hours after treatment, the leaves were removed, forcing the axillary buds, which were chlorotic in the AT treated sections. As soon as the new growth

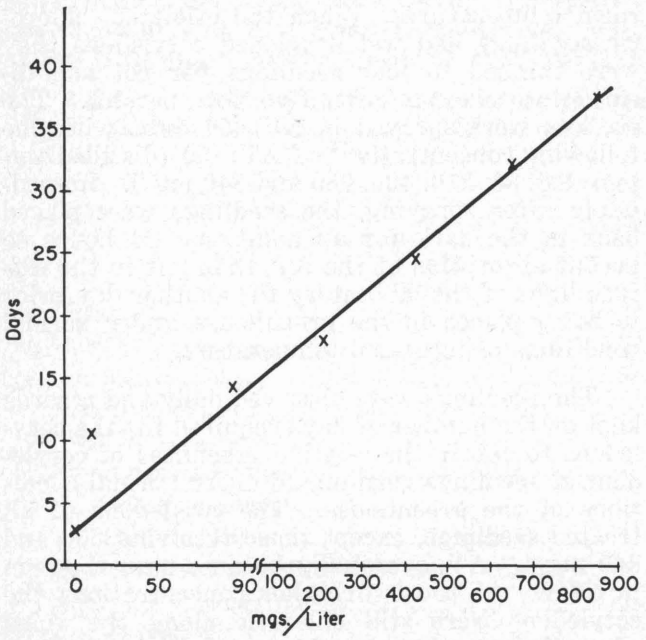

Figure 1. Days for darkgrown cotton seedling cotyledons treated with varying rates of AT to obtain normal green color.

was visible it was sprayed daily with weak sa solutions of N, Mg, Fe, Mn, P and K.

Calcium nitrate, sodium nitrate, ammoniu sulfate, urea, magnesium sulfate, magnesiu chloride, ferrous sulfate, ferric chloride, manga nese sulfate, potassium phosphate or potassiu sulfate did not prevent chlorosis, although chloro tic growth generally became green at a faster rat following applications of magnesium or potassiu salts. Regrowth from AT treated sections di not obtain the greenness or size of control sec tions.

A second set of single node main-stalk sec tions, with leaves removed, were infiltrated wit 0.0025 M AT under vacuum. Following treatmen they were sprayed daily with the various salt so lutions noted earlier. The results agreed with th first experiment involving leaf application at AT Foliage application of most of these salts to intac plants previously treated with AT did not preven chlorosis. *In vitro* tests also showed that AT does not chelate iron or magnesium.

Absorption and Translocation Experiments

Preliminary experiments on the absorption and translocation of AT were conducted in 1952 with Stoneville 2B cotton plants grown in the greenhouse and observations were made in 1953 on Deltapine cotton treated in the field. In the initial work, mature, but physiologically active plants, were treated by immersing one of the lower, fully-expanded main-stalks leaves per plant in 1 M AT for 3 hours. The previously-mentioned effect of AT in causing chlorophyll destruction and necrosis of affected tissues as well as chlorosis in newly formed tissue was used as evidence of the transport of AT to tissues remote from the point of application.

Two to 5 days following treatment the untreated leaf blades showed chlorotic and necrotic areas. At that time all main-stalk leaves were removed to force axillary growth. The new growth that developed, both terminal and lateral, showed varying degrees of chlorosis ranging from total albinoism in the upper plant to only slight chlorosis at the base.

Another preliminary translocation experiment was performed with mature cotton plants. One molar AT was applied by dipping one leaf per plant as follows: (1) a leaf blade on a vegetative lateral; (2) a fully expanded main-stalk leaf located on the middle of the stem; and (3) a fully expanded main-stalk leaf located on the second node above the cotyledonary node.

The apical meristems were excised and all plants were manually defoliated to force axillary growth 2 days after AT application.

The pattern and extent of chlorosis that appeared in the new growth indicated that: AT moved from the leaf treated on the vegetative

lateral into the main stem and mainly upward; movement occurred to tissues located above and below the treated middle stem blade as evidenced by chlorotic growth; and transport was mainly upward from the treated basal leaf.

Field observations showed that AT is readily absorbed by the aerial organs of the cotton plant. In mature but physiologically active plants the chemical is primarily translocated to the meristem regions. When relatively high concentrations were applied the pathway of transport was mainly upward to the terminal meristem and young tissues; death of the terminal meristem occurred first, followed by necrosis progressing downward along the stem.

A second greenhouse study of translocation was performed in 1953. Young vegetative plants in the four to six-leaf stage and fruiting, but actively growing plants, were cultured singly in 3-gallon jars containing fertile soil. The plants were used in the following series of experiments.

Soil Application

To one lot of four young and four mature plants, 50 ml. of 0.1 M AT were added to the soil of each jar. Prior to application, 50 percent of the plants were girdled by removing to the xylem a one-half-inch wide layer of bark just above the cotyledonary node.

Eight hours after treatment both the young and the mature plants, girdled and non-girdled, showed chlorotic and dried areas in the foliage, indicating the absorption and translocation of AT. At the end of 9 days all plants displayed marked leaf chlorosis and desiccation; new terminal and axillary growth was entirely devoid of chlorophyll; some leaves and all squares and young bolls had abscised. The AT-induced symptoms were slightly more pronounced in the girdled plants than in the ungirdled plants, and in the young plants than in the older ones. All plants eventually died.

Leaf Application

Three experiments were conducted on the absorption and movement of AT by dipping the leaf blades in a 0.01M solution. The first two experiments consisted of four young and four mature plants each. Only the four mature plants were used in the third experiment. AT was applied to either the two lower main-stalk leaves above the cotyledons or to the two first fully-expanded leaves below the apical meristem. The treatments were: (1) basal leaf treatment: young plants—girdled above the cotyledons, non-girdled; mature plants — girdled above cotyledons, non-girdled; (2) upper leaf treatment: young plants —girdled above the cotyledons, non-girdled; mature plants — girdled above the cotyledon, non-girdled; and (3) girdled at the middle of the main stem: lower leaf treatment, upper leaf treatment. The girdles were made by removing a one-half-inch wide layer of bark to the cambium.

Some of the plants showed the effects of treatment when observed 8 hours after the AT application. Nine days after treatment both young and mature plants receiving the basal leaf application showed typical AT-induced symptoms in parts above the girdle. The most marked chlorosis and necrosis occurred in the upper plant parts.

Plants receiving the top-leaf treatment showed the most accentuated effects in the apical meristem region, but some chlorosis and desiccation were apparent to the base of the plants or to the girdle. Abscission of squares, young bolls and affected leaves was noted in all plants. In the plants of the first two experiments the cotyledons abscised only in the non-girdled plants. The effects of AT generally were not apparent below the girdles. In a few of the girdled mature plants, necrotic bark tissue immediately above and below the girdle suggested a possible slight carryover across the girdle, but typical AT symptoms were not apparent below the girdle.

Defoliation and Regrowth Inhibition

Several experiments were conducted in the greenhouse with Stoneville 2B cotton and in the field with Deltapine 15 cotton to test the effects of AT, singly and in combination with commercial defoliants, on defoliation and regrowth inhibition. The cotton was mature and over 50 percent of the bolls open at the time of treatment. The sprays were applied with a hand sprayer to the greenhouse-grown cotton to wet thoroughly the foliage, but without runoff. All materials were applied in the field with a Hahn high-clearance self-propelled sprayer at the equivalent rate of 26 gallons of spray to the acre.

Table 1 summarizes the results of a representative greenhouse experiment. The percent defoliation was determined by counting leaves before and 10 days after application. All unabscised leaves, including those on unsprayed controls, were then removed and the amount of regrowth produced after 35 days was rated in terms of the control plants being 100 percent. One percent AT gave excellent defoliation and completely checked regrowth. As an additive to cyanamide sprays, AT also increased defoliation and greatly reduced regrowth.

Table 1. Effects of Amino Triazole on defoliation and regrowth inhibition of Stoneville 2B cotton grown in the greenhouse

Treatment	Concentration, %	Defoliation, %	Relative regrowth after 35 days, %
Controls	—	—	100.0
Amino Triazole	1.0	90.4	None
Monosodium cyanamide	2.0	62.8	200.0
Potassium cyanamide	2.0	95.7	120.0
Monosodium cyanamide + AT	2 + 0.25	80.7	10.0
Potassium cyanamide + AT	2 + 0.25	100.0	5.0

Results of several field experiments conducted in 1953 on the Main Station Farm and on the Brazos River Valley field laboratory are given in Table 2. The amount of defoliation was determined 8 to 9 days after spraying and the relative regrowth rated 21 days after application. AT gave acceptable defoliation over the range of 0.5 to 1.5 pounds per acre. Other field results in 1953 (22) show that 1.5 to 2.0 pounds of AT per acre gave more consistent results. The lower rates in the present experiments were used in relatively small cotton growing on the Main Station Farm. AT was found to be compatible with three chemically-different commercial defoliants. Acceptable inhibition of regrowth was obtained only with the higher rates of AT. Seedlings germinated from seed collected from AT-sprayed plants were normal unless the bolls were immature at the time of spraying.

Effects on Chemical Composition

The effects of AT on the carbohydrate composition of mature fruiting Deltapine cotton growing in the field on the Brazos River Valley Laboratory were determined as follows:

Table 2. Effects of Amino Triazole, alone and as an additive with defoliants, on defoliation and regrowth inhibition of field-grown Deltapine cotton

Treatment	Rate per acre	Av. defoliation, %	Relative regrowth after 21 days, %	Remarks
Control	——	——	100.0	.....
Amino Triazole	0.25 lb.	49.8	80	Only slight effect on regrowth, slightly chlorotic
Amino Triazole	0.5 lb.	87.6	45	Basal chlorotic regrowth
Amino Triazole	0.75 lb.	88.6	30	Regrowth chlorotic, dead terminals in some plants
Amino Triazole	1.0 lb.	90.6	20	Slight, chlorotic regrowth, mostly basal
Amino Triazole	1.5 lb.	80.0	15	Most unabscised leaves dried. Regrowth chlorotic and basal. About 50% of plants eventually died
Endothal control	5 qts.	85.1	90	Almost complete re-foliation
Endothal + AT	5 qts.+0.25 lb.	92.0	75	Checked regrowth slightly
Endothal + AT	5 qts.+0.5 lb.	93.3	60	Regrowth partly chlorotic
Endothal + AT	5 qts.+1.0 lb.	83.6	30	Regrowth mostly chlorotic
Monosodium cyanamide control	4 lbs.	36.9	150	Some regrowth initiated before application
Monosodium cyanamide + AT	4 lb.+0.25 lb.	55.0	85	Upper regrowth chlorotic
Monosodium cyanamide + AT	4 lb.+0.5 lb.	70.4	55	Upper regrowth chlorotic
Monosodium cyanamide + AT	4 lb.+0.75 lb.	72.6	40	Regrowth chlorotic
Monosodium cyanamide + AT	4 lb.+1.0 lb.	81.8	30	Regrowth mostly basal
Monosodium cyanamide + AT	4 lb.+1.5 lb.	90.3	25	Regrowth mostly basal
Shed-A-Leaf-L control	6 qts.	78.5	85	Conditions favorable for regrowth
Shed-A-Leaf-L + AT	6 qts.+0.25 lb.	97.0	70	Conditions favorable for regrowth
Shed-A-Leaf-L + AT	6 qts.+0.5 lb.	96.2	55	Conditions favorable for regrowth
Shed-A-Leaf-L + AT	6 qts.+1.0 lb.	84.6	40	Conditions favorable for regrowth

Six rows, 100 feet long, in a uniform block cotton were selected for the test. Sixteen plan sampled at random from the block as control were used to determine the original carbohydra composition of the plants at the beginning of th experiment. Immediately afterwards at 10 a.m. AT at 0.1, 0.25 and 0.5 percent concentration was applied to alternate rows with a 2-gallon hand sprayer. The sprays were applied to wet thor oughly the foliage, but without excessive runoff.

Twenty-four and 48 hours after application 16 plants were collected at random from each o the three treatments. All sampled plants wer fractionated immediately following harvest int lower, middle and upper thirds; these sample were composited into blades, petioles and stems The composite samples were dried in a force draft oven at 80° C. After drying to constan weight, the samples were ground to pass an 8 mesh screen.

The analytical methods used for determina tion of carbohydrates are given in detail elsewher (5). The sugars were extracted from oven-dry samples in a Soxhlet apparatus with 80 percen ethanol and determined by the semi-micro metho of Wildman and Hansen (23). Sucrose was de termined by inverting an aliquot of the ethano extract with concentrated HCl and computing i the usual manner. Starch contained in the Soxh let residue was determined by combined diastati and acid hydrolysis. The starch-free residue wa hydrolyzed by autoclaving with HCl and the re ducing values determined expressed as hemicell ulose.

The results are given as percentage dry weight in Table 3. The 0.25 and 0.5 percent AT sten samples collected at 24 hours were not analyzed. Results with 0.1 percent AT are generally repre sentative of the changes occurring with the high er concentrations. Figure 2 shows the percent in crease or decrease in carbohydrates from the ini tial control levels during 48 hours for this con centration only.

Within 48 hours after application of 0.1 per cent AT, the aerial organs of the cotton plant lost approximately half of the original reducing sugars and sucrose; an increase in starch content was essentially balanced by an equivalent de crease in the hemicellulose fraction. The result was a slight decrease in total carbohydrates on a whole plant basis within 48 hours after the ap plication of AT. In general, the upper plant parts (blades, petioles and stems) showed a net de crease in soluble sugars after 48 hours, whereas the reserve carbohydrates were increasing in these organs (Figure 2). On the other hand, basal plant parts showed a net decrease in both soluble and insoluble carbohydrate fractions. The only deviation from the general trend was reflected in the sucrose fraction; slight increases were shown by the upper and basal blades and lower stems. These deviations in sucrose, however, ap-

Table 3. Effects of Amino Triazole on carbohydrate composition of field-grown cotton plants as percentage dry weight after 24 and 48 hours

Fraction	Treatment, %	Blades						Petioles						Stems					
		Top		Middle		Bottom		Top		Middle		Bottom		Top		Middle		Bottom	
		24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48
Reducing sugars	Control	0.77		0.56		0.44		1.46		1.42		0.92		2.05		1.52		1.58	
	0.1 AT	0.07	0.18	0.12	1.16	0.10	0.34	0.54	0.67	0.44	0.50	0.63	0.48	1.80	1.65	0.95	0.61	0.85	0.06
	0.25 AT	0.12	0.70	0.84	0.78	0.40	0.76	0.69	0.38	0.32	0.40	0.56	0.29	—	0.23	—	0.37	—	0.50
	0.5 AT	0.34	0.54	0.76	1.11	0.52	0.82	0.20	0.48	0.59	0.44	0.42	0.38	—	0.45	—	0.32	—	0.69
Sucrose	Control	0.26		0.34		0.35		1.74		1.66		1.13		1.84		2.19		1.40	
	0.1 AT	0.57	0.46	0.49	0.00	0.59	0.53	2.21	1.12	0.43	0.43	0.21	0.08	2.00	1.00	1.36	0.75	1.50	1.55
	0.25 AT	0.44	0.22	0.83	0.42	0.21	0.36	0.52	0.19	0.36	0.58	0.76	0.44	—	0.90	—	0.81	—	0.70
	0.5 AT	1.15	0.15	0.50	0.32	1.11	0.41	3.51	0.47	1.32	0.46	0.94	0.46	—	0.58	—	0.79	—	1.50
Starch	Control	4.48		0.82		2.20		2.68		3.90		2.25		2.35		2.45		2.57	
	0.1 AT	8.04	6.28	3.09	1.48	5.44	1.54	5.78	4.75	1.50	3.00	3.12	1.68	3.40	3.07	1.95	1.15	2.21	1.99
	0.25 AT	5.07	6.67	2.38	1.98	1.80	1.52	1.32	2.70	2.79	1.71	2.57	2.69	—	0.81	—	1.01	—	1.53
	0.5 AT	1.58	5.66	2.20	2.45	1.77	2.10	3.81	3.25	3.40	3.44	2.15	3.40	—	1.68	—	1.84	—	1.68
Hemicellulose	Control	3.94		4.45		6.76		6.13		15.59		17.46		16.25		16.25		16.25	
	0.1 AT	7.27	7.18	4.49	5.94	4.99	6.08	5.91	13.96	14.06	13.54	15.22	17.00	16.20	16.13	14.40	11.33	13.60	7.33
	0.25 AT	5.21	7.39	5.83	6.95	4.79	5.46	14.18	14.78	15.46	16.48	15.40	6.75	—	14.45	—	12.48	—	8.18
	0.5 AT	4.49	7.18	4.97	6.22	5.40	5.98	14.08	2.18	14.25	8.04	14.08	15.13	—	15.05	—	14.20	—	13.61

parently can be explained by examining the corresponding fluctuation in reducing sugars in these tissues.

A preliminary experiment was conducted to determine the effects of AT on the inorganic makeup of Stoneville 2B plants. The plants were treated with 750, 1,500, 3,000 and 6,000 mg/l (p.p.m.) AT. The blades were harvested 24 and 96 hours after treatment and analyzed according to methods and procedures previously summarized (11). Potassium, phosphorus and calcium were determined colorimetrically or turbidmetrically following wet-ashing with H_2SO_4 and H_2O_2. Total N was determined by the micro-Kjeldahl procedure. Unsprayed plants growing on the same bench were harvested as controls at the two sampling intervals.

Compared with the controls, the K content decreased in the 750 and 1500 mg/l treated tissues, increased at the 3,000 mg/l level but decreased sharply at the 6,000 mg/l concentration. Phosphorus content increased slightly but consistently up to the 3,000 mg/l treatment. Total nitrogen increased slightly up to 1500 mg/l concentration but decreased slightly at 3,000 mg/l and dropped to a low level in the 6,000 mg/l treated tissues. The calcium content remained fairly constant in both treated and untreated plants.

Due to the limited number of plants available, formal data are not presented for this phase. The results are considered preliminary and no conclusion can be made at this time. A more comprehensive study of the effects of AT on the inorganic makeup of the cotton plant is now in progress (14).

Growth Effects

Cotton Plant

Stoneville 2B cotton was grown in the greenhouse in 2-gallon jars containing fertile soil. When the plants were growing vigorously and were at about 10-leaf stage they were divided into 12 lots of three plants each. On July 25, 1953, they were sprayed with the concentrations of AT shown in Table 4. They were observed weekly until September 25 when the main stems were measured and other pertinent information recorded.

The growth of the main stem was reduced as the concentration of AT was increased. Other symptoms of inhibition were recorded, particularly at the higher concentrations of AT (Table 4).

A similar experiment was conducted on July 30 with younger cotton at the four-true-leaf stage of growth. All plants eventually died at concentrations above 625 mg/l. The effects of AT otherwise were comparable with the results with the older plants shown in Table 4.

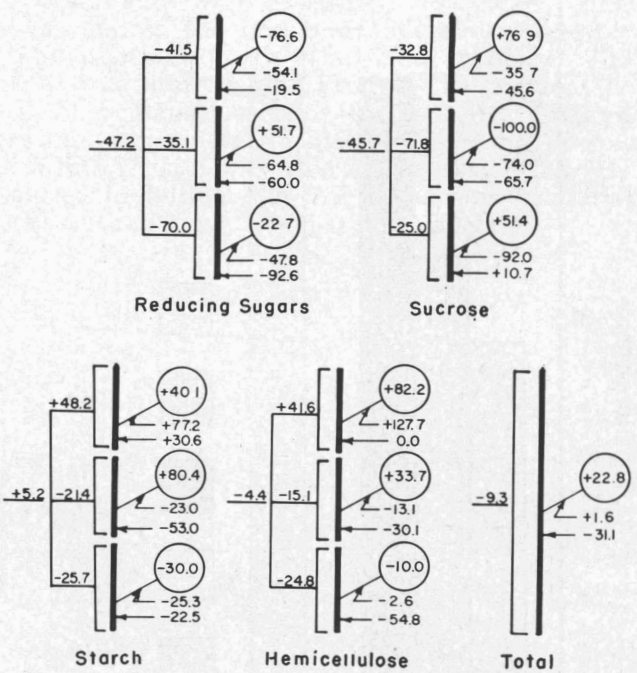

Figure 2. Percentage change in carbohydrates on the dry weight basis of leaf blades, petioles and main stalk of cotton after 48 hours following treatment with 0.1 percent AT. Plant fractionated into upper, middle and basal thirds. A plus value indicates an increase from the original content, a minus value a decrease in content.

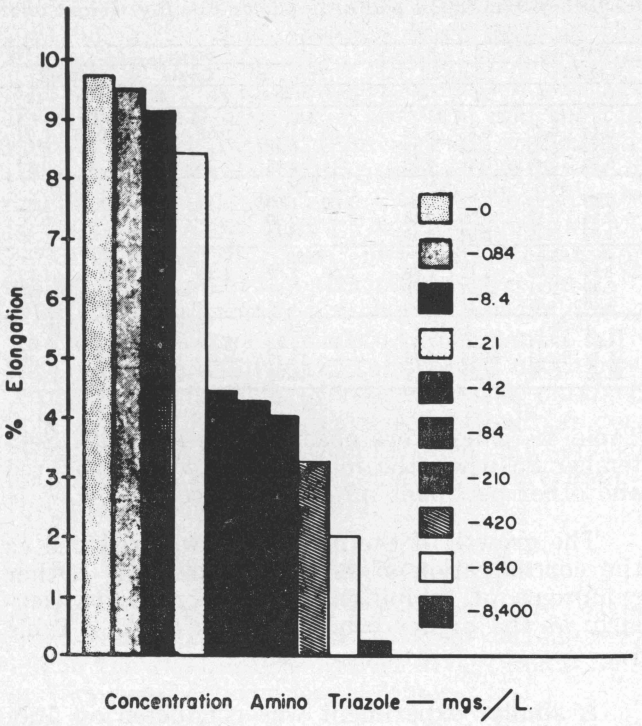

Figure 3. Mean growth of Avena coleoptile sections (as percentage elongation) as a function of AT concentration.

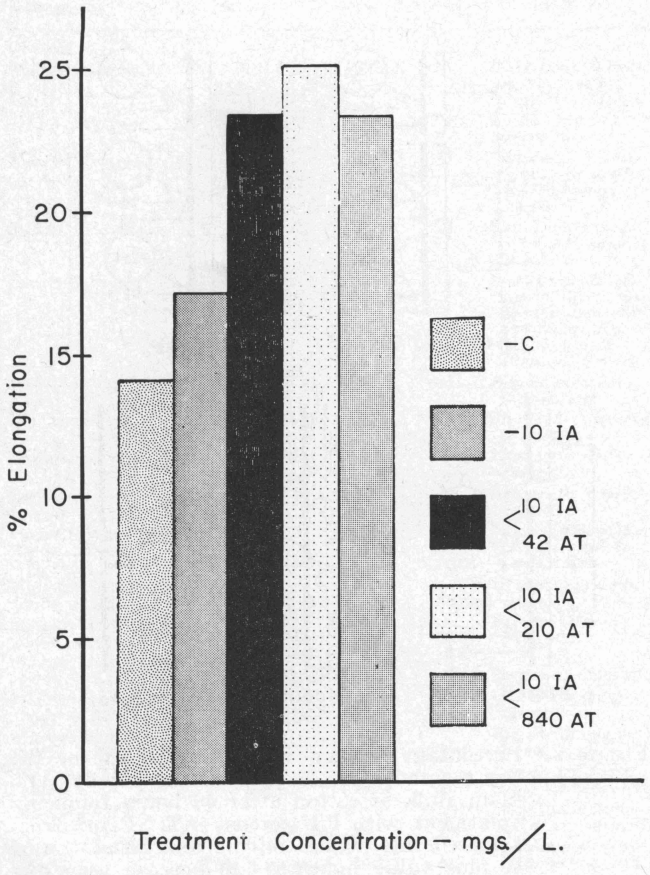

Figure 4. Effect of AT on IAA-induced growth of Avena coleoptile sections.

Table 4. Effects of Amino Triazole on growth of the cotton treated at the 8 to 10 leaf stage 2 months after treatment

Concentration AT. mg./l.	Average height main stem, inches	Remarks
0 (controls)	52.2	Cotton normal for spring-summer greenhouse-grown cotton.
9	51.0	Plants normal in color, fruiting, branching and other growth characteristics.
19	51.6	Plants normal in color and fruiting, vegetative and fruiting branches less than controls.
39	48.0	Plants apparently normal except for lack of axillary branches.
78	45.0	Size reduction noticeable, otherwise normal vegetatively and reproductively. Sparse branching at base of plant.
156	42.0	Old leaves with necrotic areas, otherwise plants almost normal except for reduced size.
312	36.4	Old leaves partially mottled. New leaves smaller and lighter colored than controls, shortened internodes in upper plant. Fruiting slightly inhibited.
625	36.0	Old foliage partially mottled and dried. New leaves almost normal in color but reduced in size. Reduced flowering and fruiting only at top of plant.
1250	31.0	Old leaves partially mottled and dried. New leaves small in size but almost normal in color. Growing point region and axillaries chlorotic. Small, abnormal fruiting at top of plant.
2500	27.6	Most of old leaves had abscised, mottled or dried. Growing point inactive, extremely short internodes, white axillaries. Leaves small and abnormal. No flowering or fruiting.
5000	24.0	Terminal meristem dead, axillaries white and dead. Little growth after application. Main stem still alive at base. No intact fruits. Squares abscised.
10,000	23.3	Terminal meristem dead and main stem dead almost to base. Axillaries white and dead. Leaves and squares abscised. Plant practically dead for all purposes.

Avena Coleoptile Sections

Seed of the Victory variety of oats were surface sterilized and soaked for 1 hour in distilled water. They were germinated in stainless steel trays containing moist sterilized vermiculite. Germination and growth of the seedlings took place in the dark or under weak red light at 25 to 26° C. and a relative humidity of 85 to 90 percent. When the coleoptiles were 2.5 to 3 cm. in length, usually 80 to 84 hours after planting, uniform coleoptiles were selected and a single section 5.0 mm. long was cut 2 to 3 mm. from the tip with a double-bladed tool. All sections were composited and lots of 20 sections distributed to sterile Petri dishes containing 20 ml. of the test solution. Thirty sections were selected at random after cutting and their lengths measured with a wide-field binocular microscope fitted with an eye piece micrometer to determine the average initial length and accuracy of cutting. All solutions were adjusted to either pH 4.5 or 5.0 and buffered with 0.03 M phosphate buffer. The sections were incubated in the dark at 25 to 26° C. for 16 hours. Each treatment consisted of 40 to 60 sections. The final lengths of the sections were determined under a microscope at the end of the incubation period. The mean growth of the sections is expressed as percentage elongation and compared with the distilled water controls (Figures 5, 6, 7 and 8).

The effects of AT on the elongation of Avena sections were tested in serial concentration from 0.84 to 8,400 mg/l. (Figure 3). Compared with

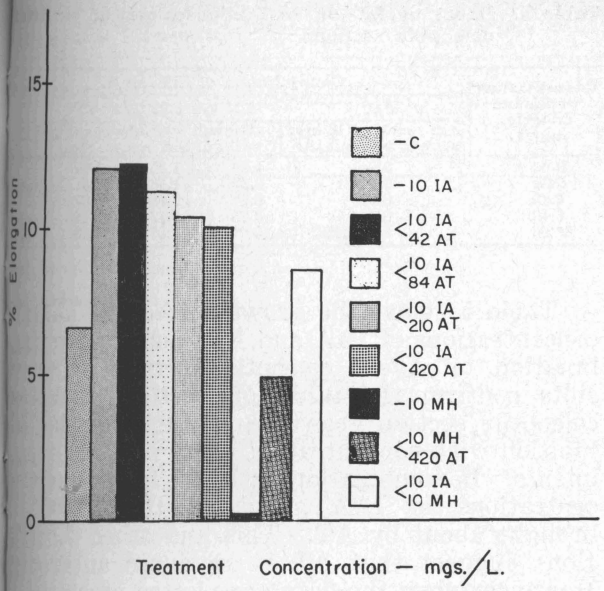

Figure 5. Effects of AT, IAA and MH, alone and in combination, on growth of Avena coleoptile sections.

distilled water controls, all concentrations inhibited growth; the inhibition increasing with increasing concentration.

In the second experiment, IAA-induced section growth, alone and in combination with AT, was compared with distilled water controls (Figure 4). The stimulating effect of IAA alone on Avena coleoptile section growth was demonstrated. Combination of IAA with AT further enhanced elongation; the optimum combination in these experiments was with 210 mg/l AT.

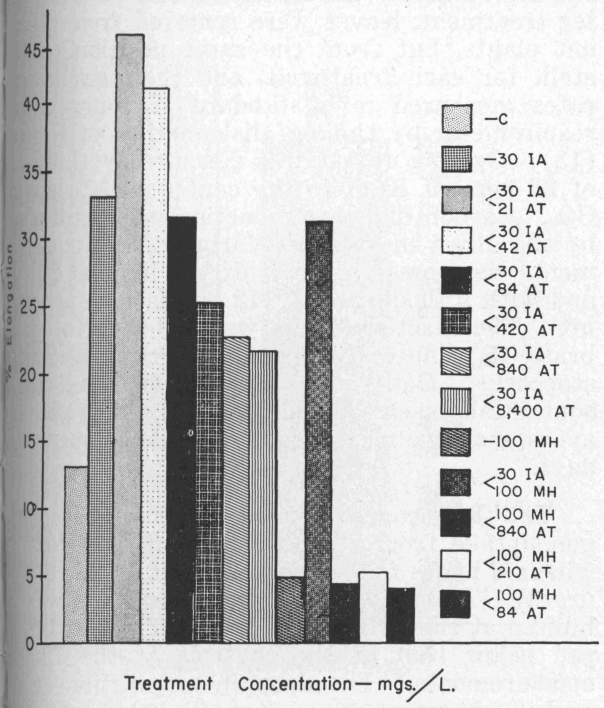

Figure 6. Effects of AT, IAA and MH, alone and in combination, on growth of Avena coleoptile sections.

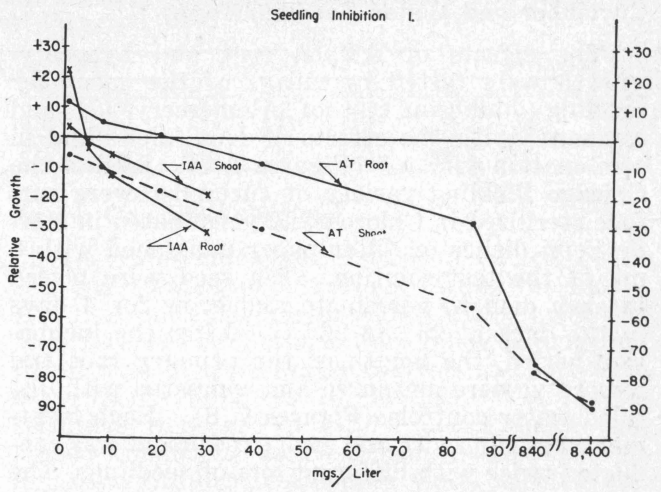

Figure 7. Comparative effects of AT and IAA on cucumber seedling root and shoot (hypocotyl) growth as percent of distilled water controls.

The effects of IAA, AT and maleic hydrazide (MH), alone and in various combinations, on coleoptile section growth are summarized in Figures 5 and 6. Older coleoptile sections were used in the experiment shown in Figure 5 than in the other coleoptile experiments; the relative differences in response of the various lots of sections to treatments were essentially the same. IAA alone, and in the proper combination with AT, stimulated section growth. MH, at 10 and 100 mg/l., was inhibitory to growth. Either IAA or AT, in combination with 10 mg/l. MH, reduced the inhibiting effect of MH. At 100 mg/l. level of MH, IAA still was effective in alleviating MH induced inhibition, but AT in various combinations with 100 mg/l. MH was ineffective.

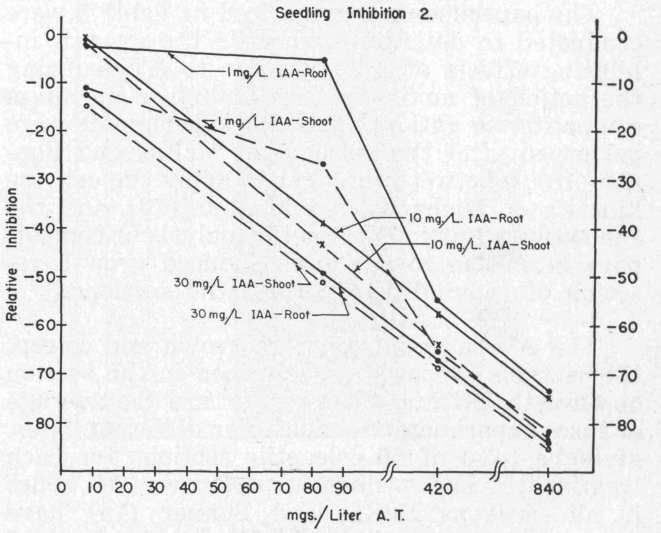

Figure 8. Combined effects of IAA and AT on cucumber seedling root and shoot (hypocotyl) growth as percent of distilled water controls.

Cucumber and Cotton Seedling Tests

The effects of AT on root and hypocotyl growth were tested by means of the cucumber seedling inhibition test of Alamercery (7) and compared with the effects of IAA, alone and in combination with AT (Figures 7, 8). Seed of the Chicago Pickling variety of cucumber were surface sterilized in Chlorox and germinated in sterile Petri dishes on filter paper moistened with 8 ml. of the test solution. Ten seed were placed in each dish to germinate and grow for 4 days in the dark at 25° to 26° C. After the incubation period, the length of the primary root and hypocotyl were measured and compared with distilled water controls (Figures 7, 8). Each treatment was run twice and each experiment was conducted twice with different lots of seedlings. The data presented as percentage of the controls (Figures 9, 10) are the average of 40 seedlings per treatment.

At concentrations up to 21 mg/l., AT slightly stimulated the elongation of the primary root (Figure 7). Above 21 mg/l. AT, root growth was inhibited and hypocotyl growth was reduced at all concentrations of AT. Equivalent concentrations of IAA in the same tests suppressed root growth more than AT, but had less effect on hypocotyl growth.

The growth effects of varying concentrations of IAA and AT in combination on cucumber seedlings are summarized in Figure 8. All concentrations and combinations reduced root and hypocotyl growth and the inhibition increased with increased concentrations.

Essentially the same results were obtained with cotton seed in similar tests.

Competitive Inhibition Studies

The experiments summarized in Table 5 were conducted to determine whether the growth inhibiting effects of AT were due to AT reducing the action of auxin by functioning as an auxin competitor or anti-auxin. These experiments were patterned after the methods of McRae and Bonner (18), who were the first to apply the enzyme kinetics of Michaelis and Menten (19) and the Lineweaver-Burk (15) kinetic analysis of competitive inhibition to the auxin-induced growth reaction of isolated Avena coleoptile sections.

The Avena seedlings were grown and coleoptile sections prepared as described in the section on Growth Effects. The results are the average of three experiments conducted on different dates, giving a total of 60 coleoptile sections for each treatment. The incubation period was 16 hours in all cases as McRae and Bonner (18) have shown that the velocity of IAA-induced growth of Avena sections is constant up to 18 hours following application.

Table 5. Effect of AT on IAA-induced growth of Avena coleoptile sections

Concentration of Amino Triazole, mg./l.	Concentration of IAA, mg/l.				
	0.00	0.1	0.25	0.5	1.0
	mm./section/16 hrs. growth without IAA	Growth with IAA minus growth without IAA mm./section/16 hrs.			
0.00	0.64	1.09	1.39	1.65	1.77
0.84	0.62	0.99	1.48	1.61	1.78
4.20	0.52	0.67	0.88	1.07	1.40
8.40	0.44	0.75	1.12	1.14	1.24
84.00	0.39	0.79	1.07	1.16	1.32

Table 5 shows the growth effects of varying concentrations of IAA and AT, alone and in combination, on Avena coleoptile sections. The results confirm the inhibitory action of AT on coleoptile section growth and the increasing inhibition with concentration noted in other experiments. In combination with AT, increasing concentrations of IAA alleviated the inhibitions brought about by AT. This and other observations suggest that AT is an auxin antagonist. However, when the data are plotted according to the Lineweaver-Burk treatment as a test of competitive inhibition the requisites of a true anti-auxin are not fulfilled. Thus it appears that AT inhibits growth or interacts with IAA in a manner which is qualitatively but not quantitatively expected of an anti-auxin.

Effects on Respiration

Cotton Leaves

Intact, fully expanded main-stalk cotton leaves were treated with 84, 840 and 8,400 mg/l AT, distilled water (controls) and 10 mg/l IAA by the dip method. Three, 24, 48 and 72 hours after treatment, leaves were removed from different plants, but from the same position on the stalk for each treatment, and their respiratory rates measured in a standard Warburg microrespirometer by the leaf-disk method of Klinker (13). Oxygen uptake was measured with 0.2 ml. of 20 percent KOH in the center well to absorb CO_2. Carbon dioxide production was determined by the direct method of Warburg. All measurements were made in very diffuse light at 25° C., and with a flask rate of 112 oscillations per minute. The reaction flasks were allowed to equilibrate 15 minutes before closing the manometer stopcocks. Gas exchange was measured for 1 hour in all cases. Table 6 and Figure 9 give the average of two measurements made on successive days.

The three concentrations of AT increased oxygen uptake from 20 to 60 percent over controls within 3 hours following treatment (Table 6, Figure 9). After 48 hours, oxygen uptake was inhibited at the two higher levels of AT and dropped below that of the controls at the 72-hour measurements. The drop in respiration at the higher concentrations after 48 hours coincided with the rapid blade dehydration and the initiation of visual abscission. AT gave a greater ini-

Table 6. Effects of Amino Triazole on respiration of cotton leaf-blade tissue as micrograms O_2 uptake per hour per mg. dry weight (Qo_2) or micrograms Co_2 output per hour per mg. dry weight (Qco_2)

Hours after treatment	Control			84 mg/l. AT			840 mg/l. AT			8,400 mg/l. AT			10 mg/l. IAA		
	Qo_2	Qco_2	R.Q.	Qo_2	Qco_2	R.Q.	Qo_2	Qco_2	R.Q.	Qo_2	Qco_2	R.Q.	Qo_2	Qco_2	R.Q.
3	1.58	1.79	1.13	1.90	2.75	1.45	2.54	1.85	1.37	2.08	2.46	1.18	1.75	1.60	0.92
24	1.81	1.90	1.05	2.14	4.27	2.00	2.45	3.56	1.45	1.93	3.25	1.68	1.90	3.22	1.69
48	1.51	2.32	1.53	2.21	2.78	1.25	2.34	2.33	0.99	0.51	1.21	2.37	1.95	2.21	1.13
72	1.15	1.31	1.13	2.81	3.89	1.38	1.04	0.65	0.62	0.62	1.00	1.61	2.21	3.01	1.36

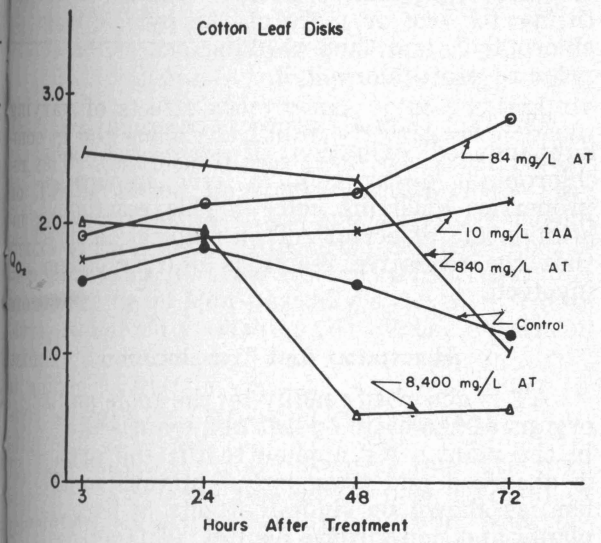

Figure 9. Rate of oxygen uptake of cotton disks from intact leaves pretreated with distilled water, IAA and AT in microliters per milligram per hour (Qo_2).

tial stimulation of cotton-blade respiration than did IAA. Carbon dioxide production followed closely the trend of oxygen consumption.

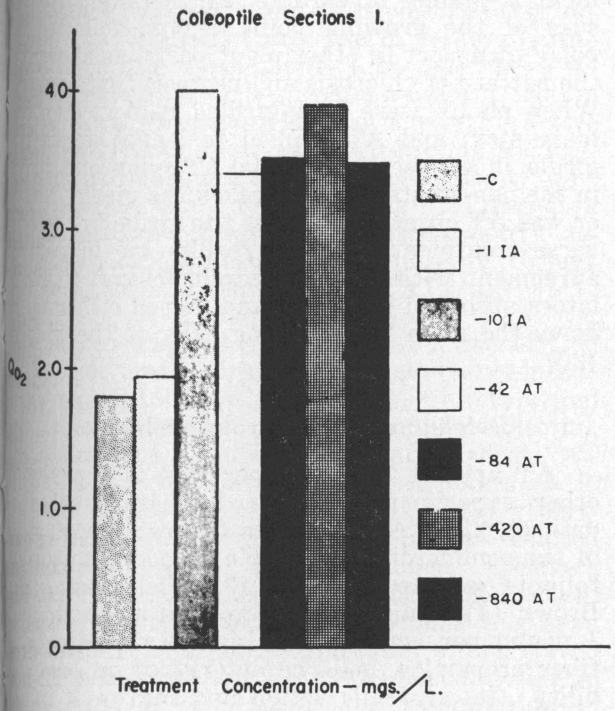

Figure 10. Comparative effect of IAA and AT on oxygen uptake of Avena coleoptile sections as microliters per milligram per hour (Qo_2).

Avena Coleoptile Sections

The rates of oxygen uptake of oat coleoptile sections treated with various concentrations of AT, IAA and maleic hydrazide (MH), singly and in different combinations, were followed and compared with distilled water controls in short-term experiments (Figures 10, 11, 12). The Avena seedlings were grown for 84 hours under controlled conditions as described in the previous section. Uniform coleoptiles were selected and one 5.0 mm. section was cut 2 to 3 mm. from the tip with a double-bladed cutting tool. After cutting, the sections with leaves removed were randomized and lots of 20 sections were placed in the main compartment of the reaction flasks. Two-tenths ml. of 20 percent KOH was placed in the center well to absorb CO_2, and 2 ml. of the test solution (distilled H_2O, AT, IAA, MH or combinations) were buffered to pH 4.5 with 0.01 M phosphate buffer added to the main compartments of the flasks. Other details of carrying out the respiration measurements were the same

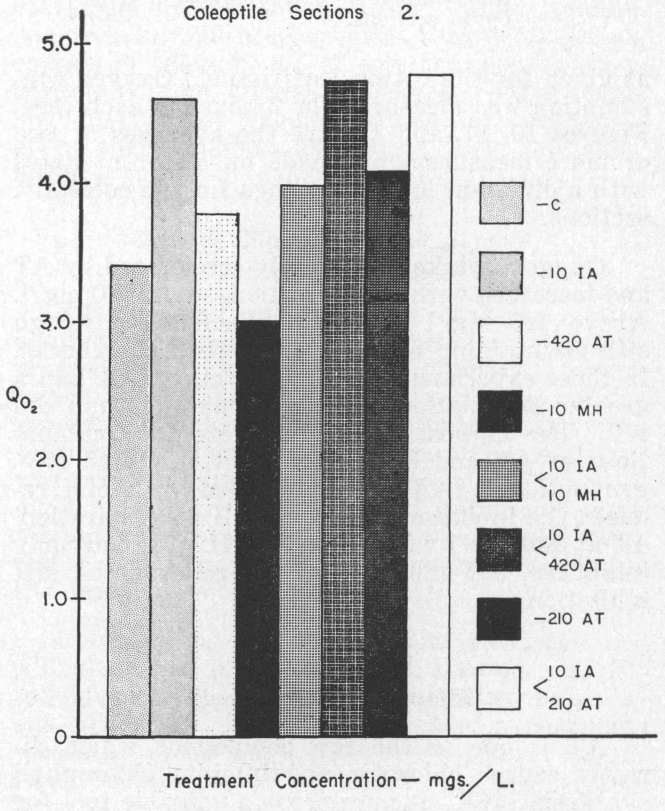

Figure 11. Effect of IAA, AT and MH, alone and in combination, on oxygen uptake of Avena coleoptile sections.

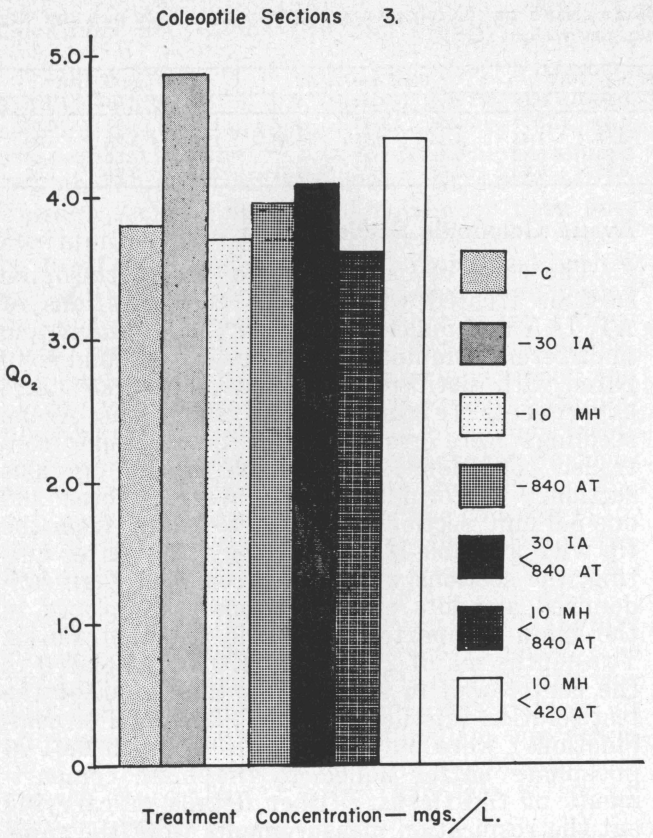

Figure 12. Effects of IAA, AT and MH, alone and in combination, on oxygen uptake of Avena coleoptile sections.

as given for the cotton leaf tissue. Oxygen consumption was measured for 2 hours in each case. Figures 10, 11, and 12 give the averages of two or more measurements made on different dates with a different lot of seedlings for the coleoptile sections.

Oxygen uptake was greatly accelerated by AT and increased with concentrations up to 420 mg/l. Above 420 mg/l respiration declined, although still maintaining a higher level than the checks. In these experiments, 10 and 30 mg/l IAA had a greater stimulation on oxygen uptake than did AT. The highest Qo_2 values were for combinations of AT and IAA. MH alone inhibited oxygen uptake. IAA in combination with MH reduced the inhibitory effect of MH on respiration. AT at 840 mg/l with 10 mg/l MH were mutually inhibitive, but 420 mg/l of AT relieved the MH inhibition.

DISCUSSION

Effects on Chlorophyll

AT is one of the few compounds which directly causes chlorosis and inhibits chlorophyll synthesis (16). It may prove a valuable tool for investigators interested in studying chlorophyll synthesis in higher plants. Work with cotton seedlings indicates that AT is not entirely inhibiting to the step from protochlorophyll to th chlorophyll stage but has its effect earlier in th biogenesis of chlorophyll. The inhibitory effe does not appear to be due to immobilization o ions essential for chlorophyll synthesis. Althoug chemically different, the similar structure of th triazole ring and the pyrrole rings of chlorophyl suggests that Amino Triazole might be substituting for one or more of the pyrrole rings o chlorophyll, and thus blocking one of the stage prior to protochlorophyll.

The fact that AT protects chlorophyll agains light-induced oxidation *in vitro*, yet enhance chlorophyll destruction *in vivo* implicates th properties of living cells in this reaction. Th stimulating effect of AT on respiration indicate that the oxidative reactions of metabolism are involved.

Absorption and Translocation

AT is absorbed readily by the roots and aeria organs of the cotton plant and translocated with in the plant. AT applied to the soil apparentl is absorbed and transported upward in the xylem, as shown by similar effects in bark-girdle plants and non-girdled plants. This agrees wit other work on growth regulators and other materials absorbed by the root system which mov rapidly upward in the transpiration stream o water conducting tissues (12, 20).

Foliar application of AT shows that the tissues exterior to the xylem, presumably the phloem, are involved in translocation. The movement is mainly upward to the meristematic tissues of the growing point region, followed by polar transport to other plant parts, as shown by the pattern of chlorosis and necrosis that appears. When plants were bark-girdled half-way up the main stem and AT applied to leaves above the girdle, in no case were typical AT symptoms found in the non-treated tissues below the girdle. Neither was AT observed to cross the girdle in the converse treatment. These results are in general agreement with other research on growth regulators absorbed by leaves and moved either up or down the stem in the living cells of the phloem (20).

Defoliation and Regrowth Inhibition

AT applied at the proper rate is superior to other experimental cotton defoliants tested to date (1, 22). AT compares favorably with most of the commercially available standard cotton defoliants reported upon in 1953 field tests (21). Brown (4) found that AT applied up to rates of 1 pound per acre had no significant effects o fiber properties, boll characteristics or seed via bility. Its systemic action and regrowth inhibiting properties enhance its desirability as a cotton defoliant. Compatibility with commercial defoliants also makes it possible to use AT as an

additive to increase defoliation and to check axillary bud growth. It has proved more effective in suppressing regrowth than maleic hydrazide (9), which although an auxin antagonist, has little immediate effect on cotton defoliation unless added with defoliating chemicals. AT also was superior to MH in checking regrowth in Arizona and Mississippi in 1953 (22).

The physiological mode of action of AT in inducing abscission has not been investigated fully. However, its stimulatory effect on respiration, its ability to cause tissue injury (the degree depending on concentration) and desiccation, and its antagonizing action on auxin parallel closely the known properties of other successful abscission-inducing chemicals. Although inducing abscission, AT inhibits growth, and effect that discounts the belief that basically abscission results from stimulated growth of cells in the abscission zone.

Effects on Chemical Composition

The analytical results suggest that the total soluble sugars (reducing sugars plus sucrose) of the aerial organs of the cotton plant were decreased by the effects of AT on respiration rather than by translocation to the root system. Two facts support this view: 1) the concentrations of AT used in the carbohydrate studies greatly stimulated respiratory gas exchange and 2) most of the carbohydrate transport occurred acropetally.

However, substantial decreases in reserve carbohydrates in the basal parts of the shoot suggest accelerated hydrolysis and transport to other organs. The significant increase in reserve carbohydrates in the upper plant parts could have resulted from either or both impaired translocation out of these tissues of the products of active photosynthesis or from polymerization of the sugars transported there from the basal plant. The latter appears more tenable in light of the data and the known effects of AT on chlorophyll destruction and synthesis and the observed injury and desiccation of leaf tissue by AT.

Comparisons of the effects at AT and MH on the carbohydrate status of treated plants needs further study. Both are growth inhibitors and are derivatives of a common starting material, the hydrazines. MH causes carbohydrate accumulation in the leaves of treated plants (8, 9, 17). In studies on cotton, McIlrath (17) reported that foliar carbohydrate accumulation resulted from the collapse of the sieve tubes. Greulach (8) reported that excessive accumulation of sucrose and starch in MH-treated tomato and bean leaves was not due to MH blocking either starch synthesis or breakdown, but was probably due to lower rates of respiration and assimilation in treated plants and to interference with translocation because of MH injury to the sieve tubes. Even though AT inhibits growth, it, temporarily at least, is a potent respiratory stimulator. The net decrease in soluble carbohydrates roughly parallels the respiratory stimulation and it appears logical to connect sugar disappearance to the oxidative reactions of respiration.

An explanation of the AT-induced redistribution of carbohydrates from basal to upper plant parts necessarily involves translocation. The pathway of carbohydrate transport and accumulation corresponds to the principal route of AT movement and its accumulation in the apex of the plant. Whether AT directly elicits the acropetal translocation of carbohydrates or whether it is merely passively accompanying the upward flow of carbohydrates is unknown. Stimulated oxidative processes in the upper plant by AT also should be considered as a possible factor in inducing the flow of sugars to these centers. Possible injury to the sieve tubes by AT was not investigated. Therefore it is not known definitely whether AT interferes with polar transport from the leaf by injury to the phloem, as reported for MH. Injury appears unlikely, however, as the girdling experiments and the apparent acropetal translocation in the pholem indicate that it was functional after the application of AT.

Because of the limited number of plants used, the effects noted for AT on the potassium, phosphorus, nitrogen and calcium makeup of the cotton plant cannot be considered conclusive until confirmed by subsequent work. These preliminary results indicate, however, that, with the exception of calcium, AT affects the inorganic and organic content of treated leaves. Experiments now in progress should disclose further details about these changes as well as their reproducibility.

Growth Effects and Competitive Inhibition Studies

AT inhibited shoot growth of intact cotton plants when applied at two stages of growth, suppressed axillary bud growth of defoliated mature cotton plants, inhibited Avena coleoptile section growth and the growth of cucumber and cotton seedlings. Primary root growth of the seedlings was promoted by concentrations of AT below 21 mg/l.; but in all other cases AT applied alone inhibited growth at all the lowest concentrations tested and increased concentrations accentuated the degree of inhibition.

Comparisons of the effects of IAA, MH and AT, alone and in combinations, showed unexpected interactions in the growth response of the Avena coleoptile. In two of the three experiments where 10 or 30 mg/l. IAA were applied in different combinations with AT in concentration from 21 to 8,400 mg/l., the auxin-induced stimulation of growth was further enhanced by the proper combination with AT. In the third experiment (Figure 5), where older sections were

used inadvertently, only the inhibitory effect of AT was expressed. The explanation for the stimulating effect of AT (which by itself is inhibitive), used in combination with relatively high concentrations of IAA (10-30 mg/l.) only can be surmised. However, these concentrations of IAA, although inducing greater growth than distilled water, appear higher than usually found to give maximum growth in Avena coleoptile sections. Possibly the antagonizing action of AT on IAA reduced superoptimal concentrations of auxin to levels more effective for maximum growth.

In the competitive inhibition experiments where auxin concentrations below 1 mg/l. were tested in combination with AT, increasing concentrations of IAA alleviated the inhibitory effect of AT; and conversely, although a few exceptions are apparent, increasing concentrations of AT usually reduced the stimulating effect of IAA. The 2,4-D salt of AT was less effective as a herbicide than either 2,4-D or AT applied singly (2). Thus it appears that AT and auxin are mutually antagonistic, and although AT does not fulfill the quantitative requirements of a true anti-auxin according to the McRae-Bonner application of the Lineweaver-Burk treatment for competitive inhibition, it does interact with auxin in a manner of practical significance. The role of AT may be similar to that of 2,4-Dinitrophenol and maleic hydrazide as discussed by McRae and Bonner (18).

At comparable concentrations, MH was a more effective inhibitor of Avena section growth than AT; yet, as a suppressant of axillary bud growth AT has proved superior (9). IAA in combination with MH completely overcame the inhibiting effect of 10 and 100 mg/l. MH on Avena section growth. Relatively high concentrations of AT could relieve partially the inhibition of 10 mg/l. MH, but higher concentrations of the two were only mutually inhibitive.

The interaction of AT and IAA on growth is complicated further by the results obtained with cucumber and cotton seedlings. Below 21 mg/l., AT stimulated root growth, a response also obtained by others (2), yet hypocotyl growth was reduced by all concentrations of AT. On the other hand, IAA was more inhibitory to roots and less inhibitory to hypocotyl growth than AT. In combination, IAA and AT were inhibitory to both responses at all concentrations tested.

Respiratory Effects

AT stimulated the respiratory rates of cotton leaf blade tissue and Avena coleoptile sections. Even the higher concentrations, found to be inhibitory for growth, initially stimulated the rate of respiratory gas exchange. The disappearance of sugar and the initiation of abcission in AT-treated tissue paralleled the AT-induced respiratory effects and they appear to be closely associated in metabolism if not dependent phenomena. Chemicals effective in inducing abscission also are respiratory stimulators, although apparently this is not the sole requisite of a successful defoliant.

IAA stimulates respiration yet prevents abscission. On the other hand, MH and other compounds capable of antagonizing the effects of auxin, do not significantly increase defoliation unless added in conjunction with defoliants (9). MH inhibits respiration. Both DNP and AT stimulate respiration but inhibit growth. DNP is an "uncoupling agent" (6, 18). Experiments in progress suggest a similar function for AT, but these results are tentative.

AT gave a greater stimulation of cotton leaf blade tissue respiration than did IAA, yet the converse occurred for Avena coleoptile respiration. This might be expected as dark-grown coleoptiles elongate rapidly in response to exogenous auxin and their growth rate parallels their respiratory rate (6).

The proper combination of AT and IAA enhanced slightly the oxygen uptake of Avena sections, but MH inhibition of respiration was relieved by IAA or AT applications in a manner qualitatively but not quantitatively similar to their effects noted on growth.

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