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Growth Regulator Changes in Cotton Associated with Defoliation Caused by *Verticillium albo-atrum*[1]

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ABSTRACT

Cotton plants, variety Acala 4-42 family 77 (*Gossypium hirsutum* L.,), were stem puncture-inoculated with either a defoliating isolate (T9) or a nondefoliating isolate (SS4) of *Verticillium albo-atrum* (Reinke and Berth.). As symptoms developed, growth regulators were assayed in diseased plants to discern their importance in the disease syndrome.

An *Avena* coleoptile straight growth bioassay demonstrated the presence of several growth-regulatory compounds in cotton tissue extracts. Indoleacetic acid was among the compounds whose effects on coleoptile growth were influenced by disease development. Coleoptile growth due to indoleacetic acid was greater in extracts of diseased stems and leaves than in extracts of comparable healthy tissues. During the defoliation period the T9 and SS4 isolates appeared equally effective in increasing indoleacetic acid and reducing indoleacetic acid decarboxylation. Preceding defoliation, however, in plants showing equivalent symptoms the degradation of auxin was reduced more by infection with T9, the defoliating isolate. The reduced auxin degradation appeared to be releated to concomitant increases in caffeic acid and other indoleacetic acid-oxidase inhibitors in the affected tissues.

Abscisic acid in tissue extracts strongly inhibited coleoptile growth. During the defoliation period gas-liquid chromatographic and ultraviolet absorption measurements revealed that abscisic acid levels were approximately doubled in T9-infected leaves but were relatively unaffected in leaves infected with the nondefoliating isolate and in stems infected with either isolate.

The onset of epinasty and especially defoliation was also accompanied by increased ethylene production in diseased plants. Ethylene in gas samples taken from jars confining plants infected with SS4 or T9, respectively, was increased 2- and 5-fold over uninoculated controls. Ethylene supplied exogenously to healthy plants in concentrations as low as 0.2 microliter per liter induced both the epinasty and defoliation symptoms characteristic of *Verticillium* infection. Ethylene treatment did not, however, induce other symptoms of *Verticillium* infection and did not affect endogenous levels of abscisic acid.

Defoliation of T9- but not SS4-infected plants apparently is related to the differential alterations in abscisic acid and ethylene levels induced by each isolate, and perhaps to differential alterations in initial rates of indoleacetic acid decarboxylation. These growth regulator alterations apparently are reflections of altered host metabolism rather than direct contributions of the invading fungus.

Cotton plants infected with *Verticillium* manifest a number of different symptoms. The initial symptom is usually an epinasty of the terminal leaves. Subsequent symptoms may include chlorosis, necrosis, and abscission of leaves, plant stunting, and vascular discoloration. Apparently a number of different physiological disturbances occur during disease development, but the mechanisms underlying pathogenesis are not completely understood.

Two of the more obvious suppositions made from symptomatology are that the chlorotic and necrotic reactions are mediated by phytotoxins of host or fungal origin, or both, and that the epinastic and defoliation reactions are mediated by imbalances in growth regulators. The latter assumption draws its support from two indirect lines of evidence. First, the effects of growth regulators such as indoleacetic acid and ethylene on the epinasty and abscission of healthy leaves are well documented (7, 14). Abscisic acid, recently characterized in cotton and other plant species, also has pronounced effects in accelerating leaf abscission (4). Second, evidence is also available (22) for disease-induced effects on growth regulators, especially on IAA and ethylene. ABA[3] levels, too, can be altered in diseased plants, as evidenced in a single report on virus-infected lupins (26). Hence, the object of this study was to characterize and determine the potential of possible *Verticillium*-induced alterations in growth regulators in cotton.

Although the exact biochemical mechanisms involved may not be discerned, quantitative assays of growth regulators can reveal chemical changes perhaps closely allied to symptom production in *Verticillium*-infected cotton. Growth regulator levels, therefore, were compared in healthy and infected plants in relation to symptom development and especially in relation to the defoliation response.

MATERIALS AND METHODS

Growth and Inoculation of Plants. Cotton plants of the variety Acala 4-42, family 77 (*Gossypium hirsutum* L.), were inoculated with either a defoliating isolate (T9) or a nondefoliating isolate (SS4) of *Verticillium albo-atrum* (Reinke and Berth.) using a stem puncture inoculation technique. The inoculum consisted of a suspension of conidia washed from cultures grown 7 to 10 days on potato-dextrose agar. The conidia of both isolates (95–100% viable) were adjusted with distilled water to a concentration of 10^6 conidia/ml based on hemocytometer counts or absorbance at 540 mμ. A droplet (approximately 0.05 ml of the adjusted conidial suspensions was placed on hypocotyls of greenhouse-grown plants, 6 to 8 weeks of age, and introduced into the vascular tissue with a dissecting needle. Inoculations were performed during afternoon daylight hours, and with few exceptions the entire droplet of inoculum would be drawn into the plant once the needle penetrated the xylem. Control plants were similarly treated but with distilled water or an autoclaved suspension of conidia.

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[3] Abbreviation: ABA: abscisic acid.

The stem puncture inoculation technique, a modification of a method described previously (11), offered several advantages over root or soil inoculations for physiological studies related to symptom development. First, direct introduction of the fungus into the vascular tissues avoided the irregularities associated with root penetration and ensured uniform development of disease symptoms in aerial portions of the plant. Second, all symptoms were well developed during the 2nd week after inoculation rather than 3 to 4 weeks after inoculation, as with root inoculations. Third, the symptoms, though produced more rapidly, were identical to those developed from the more natural entrance of the fungus through the roots.

Both the SS4 and T9 isolates caused similar degrees of stunting, epinasty, chlorosis, and necrosis, beginning 5 days after inoculation. Leaf abscission, however, occurred only in plants inoculated with the T9 isolate. Abscission began 6 to 8 days after inoculation and persisted as long as new leaves were formed. At 14 days, defoliation was usually 50 to 80% complete.

Extraction of Tissues and Isolation of Growth Regulators. Unless indicated otherwise, stems (excluding hypocotyls) and unabscised leaves were harvested separately from plants at intervals during a 2-week period following inoculation. The tissues, in different stages of disease development, were rinsed with distilled water and blotted dry, and 10-g fresh weight samples were removed for dry weight determinations. Forty-gram fresh weight samples of each tissue were extracted with 400 ml of 60% aqueous ethanol in a blender for 5 min. The 60% aqueous ethanol proved a satisfactory general solvent for acidic growth regulators and avoided the removal of interfering amounts of lipids and pigments that occurs with less polar solvents.

The blended ethanol-tissues breis were filtered through washed Whatman No. 1 paper in a Buchner funnel, and the residue was rinsed with a few milliliters of 95% ethanol. The combined filtrates were concentrated to approximately 100 ml under partial vacuum at 40 C. In controlled extractions, this procedure recovered between 45 and 60% of the IAA and ABA added as internal standards.

The concentrated tissue extracts were brought to pH 2 with HCl and extracted with three separate 50-ml volumes of diethyl ether in a separatory funnel. Acids were removed from neutral components in the combined ether extracts by extraction with three separate 50-ml volumes of 5% $NaHCO_3$ (w/v). The bicarbonate extracts were then combined and acidified to pH 2, and the acids were re-extracted with ether. The neutral and acidic fractions thus prepared were concentrated under an air stream, and quantities equivalent to those extracted from 1 to 10 g of tissue, dry weight, were line-loaded on 4- × 26-cm strips or 20- × 26-cm sheets of Whatman No. 1 paper. Recrystallized IAA (Sigma Chemical Company) and a mixture of ABA and its *trans*-isomer, *t*-ABA (Research Division, Reynolds Tobacco Company), were spotted as markers. The loaded chromatograms were developed in sealed glass tanks with a descending solvent of isopropanol-concentrated ammonium hydroxide-water (10:1:1, v/v/v). Because of previous reports of growth-regulatory materials in paper (15, 29), all chromatography paper was eluted with the developing solvent and dried prior to loading.

Growth regulators separated on developed chromatograms were located and characterized with ultraviolet irradiation, sprays of brom cresol green (23), Ehrlich's (23) and Salkowski's reagents (12), and an *Avena* coleoptile straight growth bioassay.

***Avena* Coleoptile Bioassay.** Centers of physiological activity on developed chromatograms were located by incubating serial 0.05 R_F segments of the strip chromatograms with 10 coleoptile sections on 5 ml of incubation solution. The coleoptile sections were cut from seedlings of Victory oats (*Avena sativa* L.) grown for 90 hr under red light in the laboratory. Sections 10 mm long were cut on a razor guillotine beginning 3 mm below the tip of coleoptiles 2 to 3 cm long. All sections were floated for 1 hr in distilled water preliminary to incubation, and all bore an internal leaf segment.

The incubation solution contained 10 g of sucrose and 0.1 mg of IAA per liter of distilled water and was buffered at pH 5.5 with 0.05 M potassium phosphate. Incubation was carried out for 24 hr at 23 to 24 C in capped 25-ml glass vials in the dark with shaking. The IAA in the incubation medium induced the coleoptile sections to grow to about half their potential (2–3 mm) and aided detection of growth inhibitors while permitting detection of growth promotors as well. Duplicate segments of each chromatogram preceding the origin were incubated as controls.

After incubation, the length of each coleoptile image, magnified 10-fold by a shadowgraph, was measured with a flexible ruler. An average length for coleoptile sections was determined for each chromatogram segment, and growth histograms were constructed for each chromatogram. Qualitative comparisons of individual growth regulators on different chromatograms were based on the magnitude of the coleoptile responses induced by each.

Abscisic Acid. Absorption spectroscopy and gas-liquid chromatography were employed in addition to the bioassay for more effective comparison of ABA levels in healthy and diseased cotton plants. Crude acidic fractions of tissue extracts were used for the bioassay, but the more sensitive measurements required preliminary purification of ABA.

Quantities of crude acidic fractions equivalent to 10 g dry weight of tissue were reduced to dryness under a stream of nitrogen. The dried residues were resuspended in 5 ml of 20% aqueous ethanol and applied quantitatively to the top of a 1- × 5-cm activated charcoal-Celite column (1:2, w/w), and the column was rinsed with 5 ml of water. ABA retained on the column was eluted with 200 ml of 60% aqueous acetone. The decolorized eluates were concentrated to approximately 50 ml under partial vacuum at 40 C, acidified to pH 2 with HCl, and extracted with 3 separate 50-ml volumes of ether. The ether extracts were combined and concentrated under a nitrogen stream, and quantities equivalent to 1 to 10 g dry weight of tissue were prepared for gas-liquid chromatography or purified further by thin layer chromatography.

Methods described by Davis *et al.* (10) were used for gas chromatographic measurements of ABA. The ABA recovered from charcoal was converted to its trimethylsilyl derivative with bis(trimethylsilyl)acetamide. ABA in cotton tissues was estimated quantitatively by comparing the retention times and peak heights of the trimethylsilyl derivatives of ABA from cotton and from standards of authentic ABA.

ABA was quantitatively estimated also by comparing the absorbance of purified samples at 254 mμ. After elution from charcoal, ABA was line-loaded by silica gel thin layer sheets containing a fluoresecent indicator (Eastman Chromagram) to be developed with an ascending solvent of benzene-acetone-glacial acetic acid (70:30:1, v/v/v). Developed and dried chromatograms were examined under an ultraviolet lamp (maximum emmission at 254 mμ), and the dark-absorbing band corresponding to ABA was eluted with 1% glacial acetic acid (v/v) in methanol. The eluate was concentrated and line-loaded on an additional thin layer sheet to be developed with an ascending solvent of benzene-ethyl acetate-glacial acetic acid (50:5:2, v/v/v). The ABA band purified by this second separation was again eluted quantitatively with acidified methanol, and the absorbance of each eluate was measured at its absorption maximum, 254 mμ. Determined for comparison was the absorbance of eluates of similarly chromatographed quantitative ABA standards.

Ethylene. Plants in different stages of disease development were sealed in polyethylene bags or glass jars with a capacity of approximately 24 liters. Enclosure was limited to eight plants (approximately 200 g fresh wt), the uncrowded capacity of each container. The test was conducted under continuous illumination with fluorescent lights (900 ft-c minimum) in a greenhouse. Il-

lumination of the enclosed plants appeared to favor ethylene production, perhaps by preventing the buildup of inhibitory concentrations of CO_2 in the containers (2, 6). An external water bath maintained temperatures inside the containers within 3 C of the ambient greenhouse temperature (21–28 C).

After 15 to 24 hr of enclosure, an airtight syringe was used to remove gas samples from the containers through special sampling ports fitted with rubber serum bottle stoppers. The gas samples, usually 2 ml, were assayed directly for ethylene by gas-liquid chromatography according to Maxie *et al.* (16). Ethylene in the gas samples was also characterized by comparison with authentic ethylene using the mercuric perchlorate manometric procedure of Young *et al.* (30).

The jars used to confine plants for measurements of ethylene production were also used in the greenhouse to measure the effects of exogenously supplied ethylene on healthy cotton plants. Enclosed plants were exposed to different ethylene-air mixtures passed through the jars at a rate of 50 ml/min. Exposure to ethylene was continuous until defoliation exceeded 50% or up to a maximum of 14 days.

IAA Decarboxylation. Because the oat coleoptile bioassay showed altered levels of IAA in diseased plants, attempts were made to characterize the alterations further by measuring IAA decarboxylation in healthy and diseased tissues. Rates of auxin degradation in tissues harvested as for growth regulator assays, were determined according to Daly and Deverall (9).

One gram of stem sections cut 1 to 2 mm thick, or 0.5 g of leaf discs cut 7.2 mm in diameter, was incubated with 5 ml of a solution of ^{14}C-IAA labeled in the carboxyl group. The incubation solution was prepared by dissolving calculated quantities of labeled and unlabeled IAA in 0.1 M potassium monobasic phosphate at pH 4.5 so as to yield a final auxin concentration of 10^{-4} M and a final activity of 80,000 to 100,000 cpm/5 ml of solution.

Incubation was conducted in darkened, stoppered 100-ml lipless beakers at 23 to 24 C with shaking for 6 hr. The $^{14}CO_2$ liberated during incubation was trapped on paper strips moistened with 0.1 ml of 10% KOH (w/v) suspended in the beakers. The paper strips were replaced at intervals during incubation and transferred directly to scintillation counting vials for measurement of radioactivity. Each vial contained 5 ml of a solution prepared from 6 g of 2,5-diphenyloxazole (PPO), 0.1 g of *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 500 ml of Triton X-100 per liter of toluene. A Nuclear-Chicago ambient temperature scintillation counter was used to measure radioactivity in each vial to less than 1% counting error. Counting efficiency was approximately 65%.

Control beakers containing only the incubation solution were included in each experiment to correct for spontaneous decarboxylation. All individual treatments were replicated in three separate beakers, and the results of all replicates were averaged.

RESULTS

***Avena* Coleoptile Bioassay.** Ethanol extracts of cotton tissues contained several different compounds affecting coleoptile growth. Upon fractionation of the extracts, nearly all of the growth-regulatory materials were isolated in acidic fractions. Chromatograms of acidic fractions subjected to bioassay showed several centers of physiological activity; both promotors and inhibitors of coleoptile growth were in evidence. Chromatograms of neutral fractions, on the other hand, exhibited but a single center of physiological activity, near the solvent front. In this region coleoptile growth was strongly promoted.

It was evident also that compounds affecting coleoptile growth were more concentrated and perhaps more numerous in leaf extracts than in stem extracts. When equivalent dry weight quantities of leaf and stem tissues were tested, the reduced coleoptile response on chromatograms from stem extracts was especially evident when acidic fractions were tested (Fig. 1).

The region of growth promotion on chromatograms of neutral fractions bore certain characteristics in common with indoleacetonitrile, including cochromatography, growth promotion, and positive reaction with Ehrlich's reagent. However, the region of growth promotion was not completely separated from traces of yellow and green contaminating pigments which kept identification from being more than tentative. Attempts to further charac terize this growth regulator in the neutral fractions were abandoned when tests comparing healthy and diseased tissues yielded similar results. Even in late stages of disease development and during leaf abscission, the response of coleoptiles to the neutral growth promotor from diseased stems or leaves remained unaltered.

Although the neutral growth promoter from cotton appeared unaffected by disease development, coleoptile response was affected markedly in several locations on the chromatograms of acidic fractions, particularly from extracts of diseased leaves. In all cases the disease-induced changes in coleoptile response were most prevalent during the 2nd week after inoculation, when symptoms of *Verticillium* infection were pronounced. During this period, infection with either the defoliating or nondefoliating isolate induced alterations in growth regulators in stems and leaves of infected plants. Although the inherent potential for defoliation differed in T9- and SS4-infected plants, no differential changes in growth regulators i.e., changes related to the defolia-

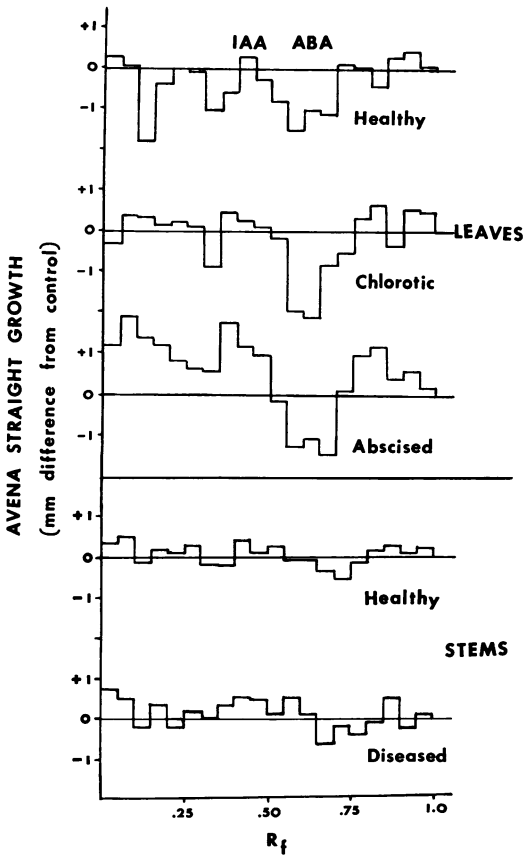

FIG. 1. Growth histograms obtained from incubation of oat coleoptile sections with segments of developed chromatograms. Each chromatogram represents a quantity of acidic fractions from leaf or stem extracts, equivalent to 2 g dry weight of tissue. Diseased tissues were harvested from T9-infected plants 14 days after inoculation. IAA and ABA, respectively, mark the locations of indoleacetic acid and abscisic acid. Growth differences exceeding $\pm$ 0.3 mm are significant.

tion response, were evident in stems up to day 14, or in leaves before abscission (Fig. 1). The resultant growth histograms representing unabscised leaves from T9- or SS4-infected plants were nearly identical, as were those from T9- or SS4-infected stems.

One of the compounds among those shown by the bioassay to be affected by disease development was identified as IAA. The magnitude of coleoptile growth promotion due to IAA was consistently greatest when extracts of diseased tissues were tested. This reflection of increased auxin in diseased tissues was slight but consistent in diseased stems and unabscised leaves, and strikingly apparent in leaves once abscission occurred (Fig. 1). The IAA in quantities of cotton tissue extracts equivalent to 2 g dry weight induced coleoptile elongation to the same degree as 1 to 100 μg of separately chromatographed authentic IAA.

IAA was the only growth regulator consistently affected in diseased stems, whereas diseased leaves also showed marked alterations in other unidentified growth regulators (Fig. 1). Increases in growth promotion were detected near the origin and solvent front of chromatograms representing diseased leaf tissues. A growth inhibitor from healthy leaves at R_F 0.1 was not detected in chlorotic leaves and was replaced by a growth-promoting material once abscission occurred. An additional growth inhibitor in healthy leaves at R_F 0.3 appeared to be reduced once infection had occurred and was no longer detected after abscission. These additional growth regulators in cotton leaves are presumably acids but were not characterized further.

ABA Measurements. Regions of pronounced growth inhibition corresponding to ABA were evident on all chromatograms of acidic fractions (Fig. 1). The magnitude of growth inhibition by this abscission accelerator, however, was not related consistently to disease development or defoliation. Omitting IAA from the incubation solution did not accentuate a possible relationship between ABA levels and *Verticillium*-induced defoliation. Analytical techniques more sensitive than the bioassay, such as gas-liquid chromatography, were therefore employed for more precise measurement of ABA in healthy and diseased plants.

Both absorption measurements at 254 mμ and gas-liquid chromatography detected changes in ABA levels that were closely aligned to the defoliation response and perhaps too subtle to detect by bioassay. The alterations in ABA were detected only in leaves infected with the defoliating isolate (Table I and Fig. 2). ABA levels were approximately doubled in T9-infected leaves and relatively unchanged in SS4-infected leaves and in stems infected with either isolate. The increased levels of ABA were not apparent until 5 to 7 days after inoculation, when symptoms were pronounced and leaf abscission was imminent. Unlike IAA levels, the increase in ABA was not further affected once leaf abscission occurred.

Higher absolute levels of ABA were detected in tissue extracts by gas chromatography than by ultraviolet absorption. However, the results of both assays performed on the same or separate extracts were always in relative agreement (Table I). Perhaps the direct measurements of ABA in crude acidic fractions by gas chromatography avoided possible yield losses associated with incomplete separation of ABA on or elution from thin layer chromatograms during purification for absorption measurements.

Table I. *Abscisic Acid in Healthy and in SS4- or T9-infected Cotton Tissues*

Plant Part	Method of Assay	Healthy	Diseased[1]	
			SS4	T9
		μg/g dry wt		
Leaves	GLC[2]	4.0	3.8	8.6
	TLC[3]	1.2	1.6	2.8
Stems	TLC	0.6	0.3	0.4

[1] Tissues harvested 12 days after inoculation.
[2] Assayed by gas-liquid chromatography.
[3] Assayed by ultraviolet absorption at 254 mμ after purification by thin layer chromatography.

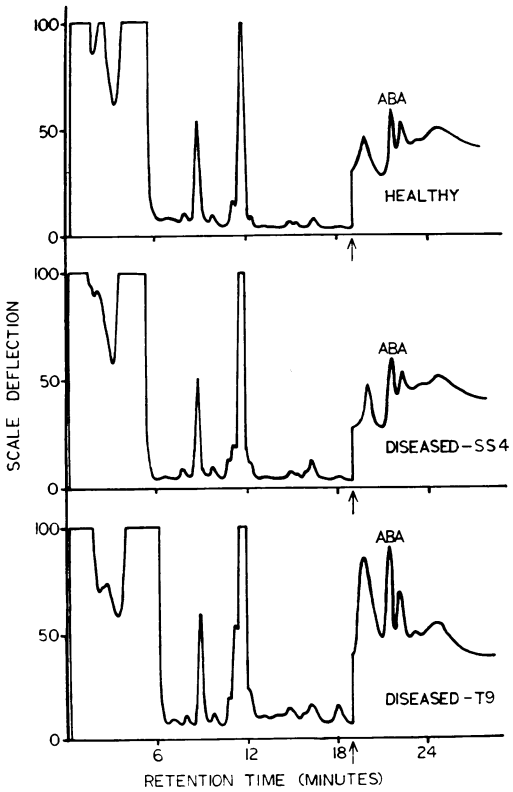

FIG. 2. Gas-liquid chromatograms obtained from injection of 3 μl (3% of the total sample) of silylated crude acidic fractions equivalent to 10 g dry weight of cotton leaves into a Varian aerograph model 204 equipped with a flame-ionization detector and linear temperature programmer. A 5-ft × ⅛-inch column of 5% QF-1 coated on 60 to 80 mesh acid-washed dimethylchlorosilane-treated Chromosorb W was used. Column temperature was set at 60 C for 6 min and increased to 195 C at a rate of 9.1 C/min. Attenuation was set at × 128 and changed to × 32 at 19 min.

The benzene-ethyl acetate-glacial acetic acid solvent used in the final purification of ABA on thin layer chromatograms was doubly useful in that it cleanly separated ABA (*cis-trans*-ABA) from *t*-ABA (*trans-trans*-ABA). With this solvent, it was possible to discern that *cis-trans*-ABA was the main isomeric form extracted from cotton. *t*-ABA was detected occasionally in trace quantity on heavily loaded chromatograms, but since ABA can be converted to *t*-ABA by certain irradiations (17), the traces of *t*-ABA were probably derived from *cis-trans*-ABA. On all gas-liquid chromatograms, no peaks corresponding to *t*-ABA were detected.

Gas chromatography detected certain other compounds that were also affected in defoliating plants. The chromatograms showed additional peaks on each side of the ABA peak that were increased in approximately the same proportion as ABA in T9-infected leaves (Fig. 2). The growth-regulatory nature of these additional compounds, closely allied to the defoliation response and perhaps related to ABA, was not determined, however.

Ethylene. When gas samples were removed from chambers

confining cotton plants and assayed gas chromatographically, ethylene was readily detected. Ethylene levels in the chambers were usually greater when diseased plants were enclosed and were consistently greater than healthy controls when compared on a fresh weight basis. In day-to-day measurements, ethylene production increased in plants inoculated with *Verticillium* while remaining at a uniform level in uninoculated controls (Fig. 3). The increases in ethylene were apparent in both T9- and SS4-inoculated plants beginning approximately 5 days after inoculation. After day 5, however, and especially during the defoliation period, it was possible to discern differential increases in ethylene in plants infected with the defoliating and nondefoliating isolates. Increases in ethylene, therefore, appeared closely allied to the appearance of both epinasty and leaf abscission. During the defoliation period, ethylene production in SS4- and T9-inoculated plants, respectively, was increased approximately 2- and 5-fold over healthy controls. Because ethylene production appeared closely allied to symptom development in diseased plants, ethylene was supplied exogenously to healthy plants to determine its capacity for symptom production. Plants exposed to ethylene in concentrations as low as 0.2 μl/liter developed both the epinasty and defoliation characteristic of *Verticillium* infection. The degree of epinasty and rate of defoliation were both dependent on the concentration of the ethylene administered. Ethylene at 10.4 μl/liter induced marked epinasty and nearly complete defoliation within 48 hr. The approximate threshold for a visible response to ethylene was near 0.2 μl/liter.

The ethylene-induced epinasty was morphologically similar to that caused by *Verticillium*, and both ethylene- and *Verticillium*-induced defoliation developed acropetally. Leaves from plants defoliated by ethylene, however, lacked the interveinal chlorosis and incipient necrosis shown by diseased leaves. Leaves from *Verticillium*- and ethylene-defoliated plants differed also in that ABA levels were unaltered in the latter. No appreciable changes in endogenous ABA were observed in leaves of plants exposed for 48 hr to 10.4 μl/liter ethylene.

IAA Decarboxylation. The greater coleoptile response to IAA in *Verticillium*-infected cotton than in healthy plants suggested that an auxin imbalance might be of significance in the disease syndrome. To characterize further the effects of *Verticillium* infection on auxin levels in cotton, rates of IAA decarboxylation were compared in healthy and diseased plants.

When healthy plants were examined, stem sections decarboxylated IAA readily, whereas leaf discs did not. Compared with stem tissues on a fresh weight basis, leaf tissues released only trace quantities of $^{14}CO_2$ during the 6-hr incubation period. The low rates of auxin degradation detected in leaf discs apparently

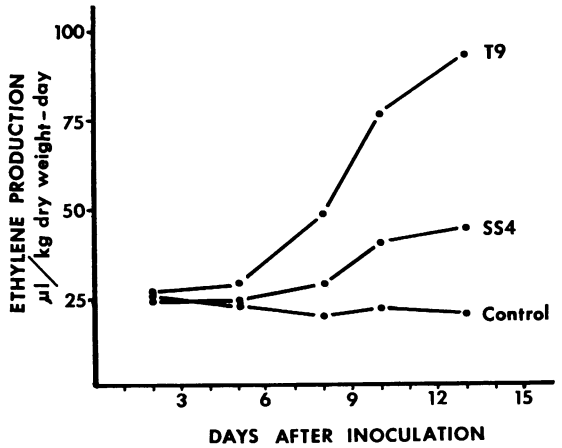

FIG. 3. Ethylene production by healthy and T9- or SS4-infected plants at intervals after inoculation.

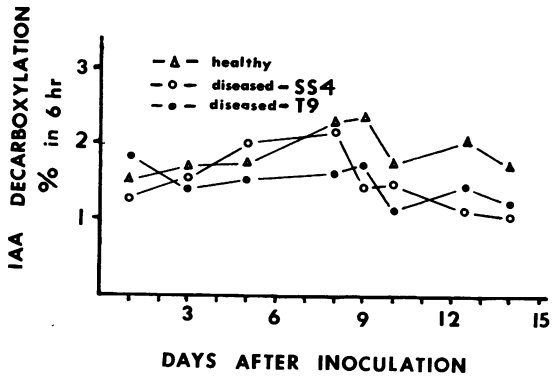

FIG. 4. Percentage of IAA decarboxylation in 6 hr by stem sections from T9- or SS4-infected plants at intervals after inoculation.

were not related to the accessibility of the substrate to oxidative leaf enzymes, since smaller leaf discs or discs partially or completely homogenized were not more effective in releasing $^{14}CO_2$. Infection with either the T9 or SS4 isolate had no effect on the inherently low decarboxylation rates detected in leaves, regardless of the symptoms expressed.

Both isolates of *Verticillium*, however, were effective in reducing IAA decarboxylation in stems (Fig. 4). Reduced auxin degradation in diseased stems was first apparent 3 to 6 days after inoculation. During the 2nd week after inoculation with either isolate, rates of auxin degradation were reduced 25 to 50% below those in healthy controls. The magnitude of the reduction during this period appeared independent of the defoliation response. During the days immediately following inoculation, however, in most experiments, though not all, it appeared that decarboxylation levels were reduced most by infection with the defoliating isolate (Fig. 4).

DISCUSSION

Although certain fungal metabolites can accumulate in infected host plants, it appears unlikely that the increases in growth regulators in infected cotton are contributed directly by the *Verticillium* fungus. In an earlier study (28), equivalent fresh weight quantities of mycelium or conidia from cultures of T9 and SS4 were grown on potato-dextrose agar as for inoculum production or on potato-dextrose broth, and these were examined for growth regulators. Neither isolate contained detectable quantities of ABA, and neither was effective in decarboxylating IAA. Both isolates produced IAA and ethylene, but never in more than trace quantities (28). It thus appears likely that the increases in growth regulators in *Verticillium*-infected cotton are reflections of altered host metabolism.

If *Verticillium* can assume some control over the metabolism of its host, it is possible that it achieves part of its control via effects on host enzymes. IAA decarboxylation, for example, may be an enzymic process which reflects a form of *Verticillium*-induced enzyme dysfunction (Fig. 4). Although enzyme inactivation and accessibility might be involved, enzyme inhibition is perhaps the best explanation of reduced IAA decarboxylation in diseased cotton stems in light of our knowledge of oxidative enzymes and their substrates. *Verticillium* apparently affects several different physiological mechanisms and, hence, the levels of numerous metabolites in its host. Certain phenolic compounds, for example, are both increased in *Verticillium*-infected cotton and are among the compounds which affect auxin metabolism via effects on oxidative enzymes. Studies in our laboratory (28) paralleling the growth regulator assays have verified increases in such compounds as scopoletin, gossypol, and caffeic acid in *Verticillium*-infected cotton. These same compounds added to decarboxylation

media reduced auxin degradation in healthy cotton tissues. At 10^{-4} M, scopoletin and gossypol were mildly inhibitory, but caffeic acid decreased $^{14}CO_2$ release by stem sections as much as 30% below that in untreated controls. Because the inhibitory action of phenolics as inhibitors of IAA-oxidase are well documented (20–22), it is reasonable that increased auxin levels in diseased cotton may result in part from the accentuated inhibition of IAA-oxidase by concomitant increased levels of phenolic compounds in the affected tissues.

While effects on auxin degradation might explain initial auxin increases in diseased plants, the pronounced increase in auxin in abscised leaves (Fig. 1) need not be related to effects on IAA decarboxylation. During the abscission process, polar transport of auxin would be impeded and eventually interrupted. Without polar transport, IAA would be expected to accumulate in tissues distal to the abscission zone until actual death of the leaf occurred.

The possible regulatory association of phenolics and IAA metabolism might also be extended to ABA metabolism since Walton and Sondheimer (27) recently demonstrated that ABA stimulated the activity of phenylalanine-ammonium-lyase in bean plants. This enzyme regulates the synthesis of *trans*-cinammic acid, from which compounds such as scopoletin and caffeic acid can be derived. Thus, a possible relationship between ABA and phenolic synthesis in diseased cotton warrants consideration.

In addition to the imbalances in IAA, ABA, and ethylene, of further possible significance to leaf abscission might be the changes observed in certain unidentified compounds in diseased tissues (Figs. 1 and 2). With disease development, coleoptile response reflected an increase in growth promoters which tended to overshadow or replace the unidentified growth-inhibitory materials found in healthy cotton leaves (Fig. 1). It would be paramount to a more complete understanding of *Verticillium*-induced defoliation to determine the identity and significance of these additional compounds affected in diseased plants.

To maintain a proper perspective, one must recognize that certain, if not all, the hormonal alterations in defoliating cotton may be of secondary importance. Ethylene production, for example, has been reported to be increased in plants by both disease and mechanical injury (24). In the present study, leaf abscission, which is a physiological process and not mechanical injury *per se*, was the only visible disease symptom not shared by both T9- and SS4-inoculated plants during the 2-week period following inoculation. Although there is no evidence for increased ethylene production resulting from abscission injury, ethylene production may be a reflection of the total physiological injury within the plant.

Since several plant components can influence leaf abscission in controlled tests in the laboratory, and since the levels of several growth regulators are altered in infected cotton, it is probable that natural abscission and *Verticillium*-induced leaf abscission are regulated by more than one chemical entity. If several components regulate abscission, new questions arise as to the particular role each plays in relation to the other components involved. In the present study the endogenous levels of ABA in cotton plants were not affected by ethylene treatment. Apparently, ethylene-induced leaf abscission does not occur via effects on ABA. However, the possibility that defoliation by ABA is mediated via effects on ethylene has been proposed (1).

While little is known about possible ABA-ethylene interactions, striking associations exist between IAA and ethylene. Ethylene administered to plant tissues can affect the transport (5, 13, 18) and synthesis (25) of auxin, and, conversely, auxin can induce ethylene production (3, 5, 8, 19). Since ABA clearly inhibits auxin-induced growth (Reference 4 and Fig. 1), it, too, is capable of interaction with auxin. Defoliation of T9- but not SS4-infected plants therefore may involve differential growth regulator interactions which stem from the differential hormone imbalances induced by each isolate.

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