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Does Water Deficit Stress Promote Ethylene Synthesis by Intact Plants?[1]

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ABSTRACT

The effect of plant water deficit on ethylene production by intact plants was tested in three species, beans (*Phaseolus vulgaris* L.), cotton (*Gossypium hirsutum* L.) and miniature rose (*Rosa hybrida* L., cv Bluesette). Compressed air was passed through glass, plant-containing cuvettes, ethylene collected on chilled columns, and subsequently assayed by gas chromatography. The usual result was that low water potential did not promote ethylene production. When plants were subjected to cessation of irrigation, ethylene production decreased on a per plant or dry weight basis of calculation. No significant promotion of ethylene production above control levels was detected when water deficit-treated bean or cotton plants were rewatered. The one exception to this was for cotton subjected to a range of water deficits, plants subjected to deficits of −1.4 to −1.6 MPa exhibited a transient increase of ethylene production of 40 to 50% above control levels at 24 or 48 hours. Ethylene was collected from intact leaves while plants developed a water deficit stress of −2.9 megapascals after rewatering, and no significant promotion of ethylene production was detected. The shoots of fruited, flowering cotton plants produced less ethylene when subjected to cessation of irrigation. In contrast, the ability of bench drying of detached leaves to increase ethylene production several-fold was verified for both beans and cotton. The data indicate that detached leaves react differently to rapid drying than intact plants react to drying of the soil with regard to ethylene production. This result suggests the need for additional attention to ethylene as a complicating factor in experiments employing excised plant parts and the need to verify the relevance of shock stresses in model systems.

Ethylene production by plants is increased by a number of biotic and abiotic stresses (1). The phenomenon is so common it is referred to as stress ethylene production. Plant water deficit is one stress which has been extensively associated with elevated release of ethylene (3–6, 13, 15, 16, 18, 24, 25, 31, 32). The impact of water stress on ethylene synthesis is of interest because the ethylene could be responsible for senescence and abscission induced by water deficits (13, 25, 26).

Although the promotion of ethylene synthesis by water deficits appears firmly established, there are a number of concerns. First, to have a convenient experimental system, water deficits have often been imposed rapidly by drying detached leaves or fruits (3–6, 16, 18, 24, 31). In contrast, under natural drought the soil water is depleted slowly and plants progress through a series of drying cycles during which ψ_w[3] falls during the day and rises at night as plants recover due to reduced evaporative demand (30). Second, the experimental measurement of ethylene has usually required that detached plant parts be concentrated in a sealed container from which air samples are withdrawn and analyzed for ethylene. The air supply in these containers has often been static which is of some concern because oxygen is needed for the conversion of ACC to ethylene (2) and CO_2 can either promote or reduce the production of ethylene (33). Finally, there are a few reports in the literature where investigators failed to observe a promotion of ethylene release with water deficit treatment of intact plants (6, 9, 13, 19).

With the development of flowing air systems capable of scrubbing ethylene from relatively large samples of air and subsequently analyzing the amount conventionally by GC (11), a tool was available to reinvestigate the effect of water stress on ethylene production. We have specifically asked whether intact plants subjected to a natural decrease in water supply due to cessation of daily irrigation exhibit an increase in their production of ethylene. Our results are markedly different from those obtained with detached plant parts subjected to rapid desiccation shock and analyzed in closed containers.

MATERIALS AND METHODS

Plant Culture

Experiments with beans were conducted at the University of Antwerp. Seeds of *Phaseolus vulgaris* L. cultivar Limburg were planted in vermiculite (No. 5, SIBLI, Liege, Belgium) in 10 cm diameter pots in trays of tap water. Temperature was maintained at 23°C constantly and 150 μmol photons·m^{-2}·s^{-1} light provided by Philips TL 33RS fluorescent tubes on a

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[3] Abbreviations: ψ_w, plant water potential; ACC, 1-aminocyclopropane-1-carboxylic acid; D, dark period; GC, gas chromatography; L, light period.

16 h photoperiod. Plants were thinned to 6 per pot and, if grown for more than 2 weeks, were watered weekly with a commercial soluble fertilizer solution and tap water as needed.

Experiments with cotton and miniature roses were conducted at Texas A&M University. Seeds of *Gossypium hirsutum* L. cultivar Stoneville 213 were planted in a mixture of peat moss, vermiculite, and sand supplemented with dolomite (limestone), commercial fertilizers (NPK), and trace elements. Plants were germinated and grown in EGC environment rooms with fluorescent and incandescent lamps which provided 800 to 1000 μmol photons$\cdot m^{-2}\cdot s^{-1}$ at the upper canopy level. Light banks were moved to remain a constant distance above the leaf surfaces. Plants were watered with tap water as needed and with nutrient solution every other day. Other conditions were: 12 h photoperiod, day 30°C and 70% RH, night 25°C and 80% RH. Air flow was vertical with a complete exchange of the air in a chamber each 10 min. Levels of CO_2 in makeup air were 580 ppm day and 350 ppm night.

Miniature rose (*Rosa hybrida* L. cv Bluesette) plants were grown from cuttings from plants originating from stocks maintained in *in vitro* shoot tip culture by M. De Proft for several years. At Texas A&M University they were transferred by R. H. Smith from *in vitro* culture medium to rooting medium and subsequently to soil and hence to the growth room described for cotton. Individual potted plants were 45 to 60 d old when used for experiments.

Detached Leaf Experiments

Bean leaves were excised and placed in turn into groups. Control samples were immediately weighed and placed in 0.5 L canning jars with lids left unsealed. At the same time other leaves were weighed, spread out on paper toweling and dried under a fan for 30 or 45 min at room temperature (23°C), weighed again, and placed in jars. All the jars were then sealed and ethylene samples were taken hourly. After each assay containers were aired out briefly and then resealed for the next hour. Ethylene was analyzed by injecting 1 mL samples into a gas chromatograph equipped with a H_2-flame ionization detector and an activated alumina column (6 mm × 2 m; 80–100 mesh, Waters Associates) which was calibrated daily with an ethylene standard. Similar experiments were conducted with cotton leaves and variations noted in figure and table legends.

Whole Plant Experiments

Bean plants, 6 per pot, were subjected to either reduction of the water supply by application of PEG (Fluka Chemie AG, labo grade, mol wt 6000–7500) solution to the roots or termination of irrigation until visible wilting occurred. In either case, to begin stress treatments plants were removed to a second growth room where 14 to 16 pots were placed under fluorescent tubes (70 μmol photons$\cdot m^{-2}\cdot s^{-1}$, 16 h photoperiod), a fan positioned to blow over the plants, and the room temperature raised to 28°C. One set of pots (six total; three control and three stressed) was placed in cuvettes in an ethylene collection system while a second set was maintained in the environment room. The sets were rotated each 24 h so that data were collected from each set twice to minimize any detrimental effect of enclosure.

For these and subsequent tests at Antwerp the ψ_w of leaves was evaluated with a HR-33T Dewpoint Microvoltmeter (Wescor, Inc., Logan, UT). Leaf discs were placed in planchets in individual cells, allowed to reach a stable reading, and ψ_w determined based on standard curves produced for each cell with solutions of NaCl of known molal concentration. At the end of the experiments, fresh and dry weights of plant tops were determined and relative water content calculated.

The flowing air ethylene collection system has been described (11). Briefly, compressed breathing air containing 300 $\mu L\ L^{-1}$ CO_2 passed through a heated catalyst to oxidize organic contaminants, then at controlled flow rates through six 10-L glass, plant-containing cuvettes in two growth cabinets (three cuvettes in each cabinet) illuminated with fluorescent tubes and six Phillips E/86 MLR 160 W flood lamps (550 μmol photons$\cdot m^{-2}\cdot s^{-1}$, 16 h photoperiod) with temperature controlled at 23°C continuous. The air stream passed from individual plant cuvettes to an adjacent laboratory where valves allowed concentration of ethylene on Poropak S (6 mm × 10 cm, 80–100 mesh) columns at acetone-liquid nitrogen temperature (−90°C). The ethylene collected during 10 min (flow rate 200 mL/min) was transferred, after boiling water heating of the column, to a GC where it was analyzed with a 3 mm × 3 m column packed with Porapak R (50–80 mesh) (Waters Associates) and a H_2-flame ionization detector calibrated daily with an ethylene standard. Flow was continuous, but air passed through the concentration columns only during sample collection. The system was usually allowed to equilibrate for 4 h before ethylene was assayed, but some exceptions are noted.

The collection system depends on the fact that at equilibrium ethylene production by the enclosed plants will equal loss due to exit of air and that decreases or increases in the rate of ethylene release will increase or decrease the amount of ethylene collected until a new equilibrium is established. For the 7 and 10 L cuvettes the 200 mL min^{-1} flow rate provided one air exchange each 35 or 50 min. By measuring ethylene several times a day it was possible to distinguish amounts of ethylene from plants receiving different treatments. In all experiments at both Antwerp and College Station appropriate blanks were analyzed to ensure that ethylene did not come from nonplant sources.

At College Station, cotton plants growing three per 12 × 8.5 cm clay pot were subjected to water deficit stress by withholding water. When the desired water potential was reached, pots of control and stressed plants were enclosed in a flowing air ethylene collection system similar to the one in Antwerp except that it accommodated only four cuvettes which were placed on a laboratory bench with fluorescent and incandescent lamps surrounding them. Cuvettes were 13.5 × 44 cm tall glass chromatography cylinders (7 L volume) with the rim ground to seal to a glass plate. The plate was held to the seal by clamps made with threaded rods and inlet and outlet air lines passed through holes in the glass plate. The light intensity achieved was 450 μmol photons $m^{-2}\ s^{-1}$, and temperature in the containers was 27°C day and 22°C night. Air flow was maintained at 200 mL min^{-1}, and the GC was calibrated with standard dilutions made from reagent ethylene. Standards were replaced as needed.

Experiments at College Station achieved water deficit

stresses more quickly due to lower RH, more rapid air flow, and higher light intensity in the growth room. Some experiments were done with plants or individual attached leaves on plants which were maintained in the growth room in which they were grown, and the air flow and ethylene collection apparatus and GC equipment were positioned nearby. These experiments employed larger plants grown individually in 2 gallon glazed crock pots (24 × 24.5 cm). Individual leaves were enclosed in 2 L canning jars with lids modified to accept split rubber stoppers with holes to accommodate the petioles and inlet and outlet tubing for air flow. Each flask was clamped in place and sealed with silicone rubber (Dow Corning 3110 RTV). For protection, the exposed portions of petioles were tightly wrapped in Parafilm (American National Can). Flow was then established for the time necessary to remove the physical stress-induced ethylene after which data were collected. Two leaves were enclosed on each plant and the flow rate was 117 mL min^{-1}. The enclosed leaves did not dry out or senesce during the experiments. In addition, one series of experiments was done with mature cotton plants enclosed in 40 L chromatography cylinders sealed to glass base plates placed on top of metal plates on top of the pots. Blanks were assayed to assure the ethylene-free status of the plant cuvette scrubbing air which contained 335 ± 5 μL L^{-1} CO_2.

Water potentials of cotton and miniature rose plants used in experiments at College Station were determined by thermocouple psychrometry using the isopiestic method (8) with equipment modified slightly to include metal heat sinks in an insulated chest. Each thermocouple was calibrated with standard NaCl solutions and data were not recorded until stable, reproducible values were observed. The system is accurate to ±0.5 bars.

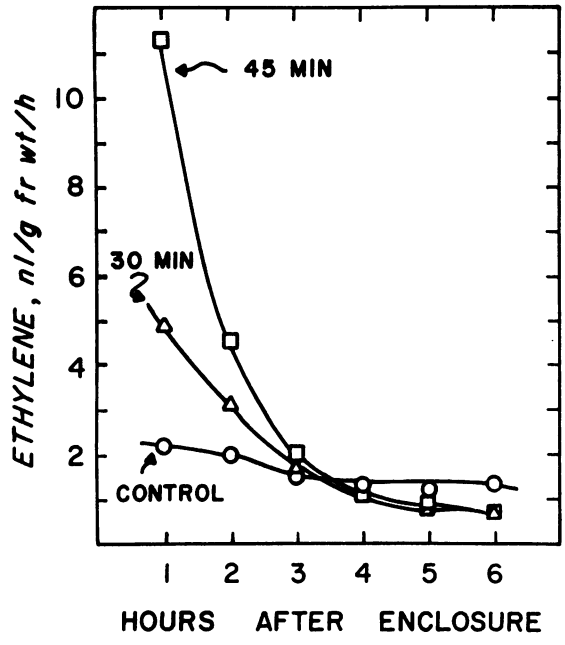

Figure 1. Effect of low water potential imposed by laboratory drying of detached bean leaves on ethylene production. Leaves were detached and enclosed (but not sealed) immediately (control) or dried on a laboratory bench under a fan for 30 or 45 min before enclosure. All containers were sealed at the same time. Ethylene was sampled from static containers which were opened for air exchange and reclosed after each assay. Data are averages of three experiments each with three repetitions/treatment. Average moisture loss with drying treatment was control, 0; 30 min, 8.95%; 45 min, 11.29%.

Table I. *Effect of Low Water Potential Imposed by Laboratory Drying of Detached Cotton Leaves on Ethylene Production Rate 2 h after Enclosure*

Groups of leaves (No. 3 from the apex beginning with the first expanded leaf) from approximately 60-d-old plants were subjected to drying and then enclosed and ethylene measured hourly. Data are averages of four replications per experiment.

Experiment	Dry[a]	Control	Ethylene	
			Dry[a]	Control
	% fresh wt loss		nL·g dry wt^{-1}·h^{-1}	
1	9.2	0	28.6 ± 2.6	9.4 ± 0.9
2[b]	6.7	0	27.07	9.8
3	9.1	0	28.8 ± 1.2	9.4 ± .02

[a] Dried on lab bench for 30 min. [b] In two replications of experiment 2 drying was continued until moisture loss > 16% and ethylene production of these leaves was only slightly higher than controls. These results are omitted from the data given.

RESULTS

Detached Leaves

In order to confirm earlier reports, we determined the effect of drying detached bean leaves for 0, 30, or 45 min on subsequent ethylene production in a sealed container. Moisture loss from open air drying increased ethylene production on the average up to fivefold in the first hour (Fig. 1). The effect decreased with time until at 6 h the leaves subjected to drying produced slightly less ethylene than control leaves. The degree of promotion was proportional to the degree or duration of drying, at least to a point, as has been observed by others (4, 6, 31). With cotton, a water loss of 6 to 10% promoted ethylene release about threefold (Table I), but losses >16% had much less effect.

Intact Bean Plants

To impose water deficit stress, one approach was to irrigate the rooting medium with low ψ_w PEG solution, thus rapidly reducing the availability of water for uptake. Ethylene production by PEG-treated plants fell below that of control plants at every measurement period in three experiments (Fig. 2). There was a daily pattern of ethylene production with the lowest level detected before the lights came on and the highest level detected toward the end of the light period.

A second approach was simply to stop watering half of the pots and, when these plants began to wilt, to compare their ethylene production with that of the well watered plants. The water deficit-treated plants usually appeared wilted in the cuvettes and the amount of water they transpired was enough lower that their cuvettes, in contrast to the controls, were not

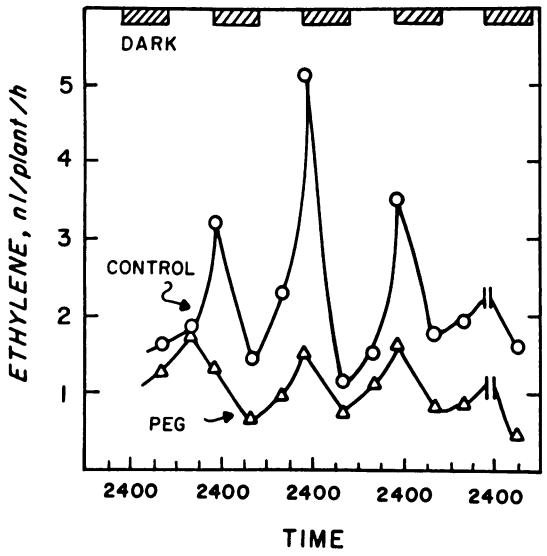

Figure 2. Effect of low water potential imposed by immersing roots of 2-week-old bean plants in osmoticum (PEG ψ_w −2.6 MPa) on ethylene production. Osmoticum or water (for control plants) was drained away after 15 min. Control plants but not PEG-treated plants watered daily for duration of experiment. Data are averages of three experiments each with three repetitions of each treatment and six plants per repetition (pot). Experiments were conducted with two sets of plants alternated each 24 h; data points are connected for clarity with no inference of continuous monitoring of the same plants. Average moisture content at the end of the experiments was 89.3% for control and 86.3% for PEG-treated (in one experiment ψ_w observed at −1.01 MPa control and −1.85 MPa PEG-treated at end of experiment).

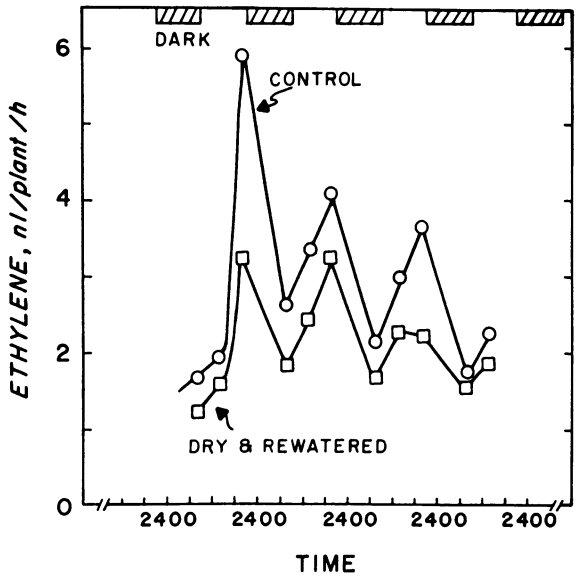

Figure 3. Effect of water deficit imposed by termination of irrigation on ethylene production of 2-week-old bean plants. Data are averages of two experiments each involving two separated groups of control and stressed (unwatered) plants alternately enclosed or placed in controlled environment growth room. Experiment 1 included three repetitions of both treatments; experiment 2 included two repetitions of control and four repetitions of stress-treated pots which had been rewatered and drained immediately before enclosure. Results of stress and stress + rewatering treatments were similar and averages are presented for clarity. Average moisture content at the end of experiment 1 was 89.5% control and 87.0% stress and for experiment 2 it was 91.1% control and 89.5% stress-rewatered. The average ψ_w in experiment 1 taken from each set of plants before they were put into the cuvette was −0.8 MPa control and −1.15 MPa stress. At the start of experiment 2, before rewatering, ψ_w was −0.7 MPa control and −0.9 MPa stress and for the second group of plants it was −0.4 MPa control and −1.25 MPa stress.

wet with condensed moisture. As with the PEG-induced drought, the average production of ethylene by the plants subjected to cessation of irrigation was consistently less than that of control plants (Fig. 3). The daily pattern of ethylene production was similar in both types of experiments (Figs. 2 and 3). In one of two experiments, the water deficit-treated plants were rewatered immediately before they were enclosed and ethylene collection started, but these plants still produced less ethylene than control plants in the same experiment (Fig. 3). In addition, in two experiments plants subjected to water deficit were rewatered after being placed in cuvettes, but no stimulation of ethylene production over control plants was detected (data not given).

Intact Miniature Rose Plants

Miniature rose plants were subjected to cessation of irrigation until water potentials had decreased to about −2.0 MPa and ethylene production was only 33 and 44% of control ethylene production at 24 and 48 h, respectively (Table II).

Intact Cotton Plants

Since cotton and other plants are known to exhibit diurnal cycles of ethylene production (14, 22, 29; and Figs. 2 and 3), the pattern of release by plants in this system was determined to allow selection of appropriate sampling times. With a 12 h photoperiod, ethylene production increased during the light period and decreased during the dark period (Fig. 4, D-L-D-L). The decline was delayed if the light period was extended (Fig. 4, D-L-L-L), and the day/night differences decreased more if the previous night was replaced by a light period and the lights were kept on during the experiment (Fig. 4, LIGHT). Continuous darkness lowered the ethylene production rate and eliminated most of the diurnal pattern (Fig. 4, DARK). Based on these data, most ethylene observations were made during the afternoon and early evening, and, unless otherwise noted, enclosure was at 8 to 9 AM.

Table II. *Effect of Low Water Potential on Ethylene Production Rate of Intact Miniature Rose Plants at 24 and 48 h after Enclosure*[a]

Treatment	Ψ_w	24 h		48 h	
	MPa	C_2H_4, nL·g dry wt^{-1}·h^{-1}	%	C_2H_4, nL·g dry wt^{-1}·h^{-1}	%
Control	−1.06	1.35	100	1.25	100
Water stress	−2.00	0.45	33	0.55	44

[a] Each datum is an average of two repetitions from a single experiment.

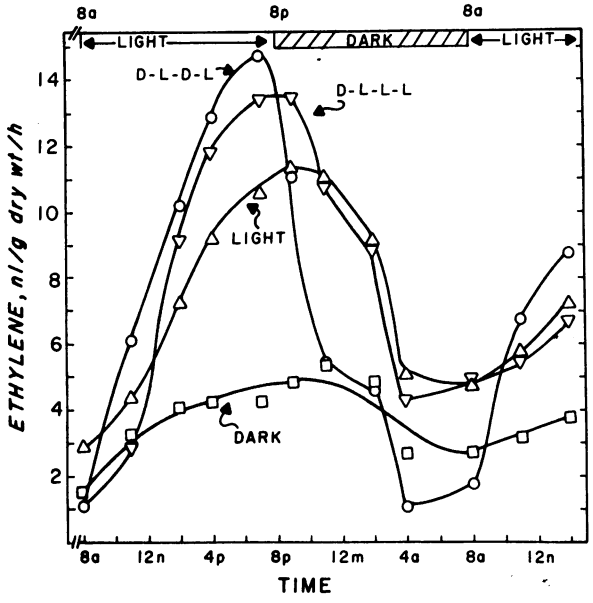

Figure 4. Diurnal pattern of ethylene production by 22-d-old cotton plants in continuous air flow ethylene collection system. Plants were exposed to a 12 h light (8 AM to 8 PM) to 12 h dark cycle except as noted. Plants were enclosed at 6 PM the evening before the first data collection, and from that time onward exposed to continuous light (LIGHT) or dark (DARK) or the normal sequence of D-L-D-L. The fourth treatment received darkness after enclosure but continous light from the beginning of data collection onward (D-L-L-L).

A series of experiments was conducted in which plants were exposed to a range of water potentials and ethylene production assayed several times per day. At 2, 5, 8, and 12 h after enclosure, ethylene production rates of stressed, nonrewatered plants never exceeded those of control plants (Fig. 5A, inset). Since the rate of ethylene production increased up to about 24 h after enclosure and then stabilized (Fig. 5A, inset), the ethylene production rate at 24 and 48 h was plotted *versus* the ψ_w of the plants when they were enclosed (Fig. 5A, B). Control plants (−0.8 to −0.9 MPa) produced around 14 nL ethylene·g^{-1} dry weight·h^{-1}. In every case, as the ψ_w became more negative the rate of ethylene production decreased (Fig. 5A, open symbols). The lowest ethylene production rate, 3.5 to 5 nL g^{-1} dry weight·h^{-1}, occurred at about −2.9 MPa. The relationship between ψ_w and ethylene production was essentially linear. When plants were rewatered shortly before enclosure, at 24 h all but one pot of plants was releasing ethylene at rates above the line relating ethylene production rate to ψ_w of nonrewatered plants (Fig. 5A, solid symbols). In three different experiments plants with ψ_w around −1.4 MPa were producing ethylene at rates averaging 50% above the controls at 24 h. At 48 h after enclosure the same general relationships held; a line could trace the decline in ethylene production with decreasing ψ_w for nonrewatered plants and rewatered plants produced ethylene at rates above this line (Fig. 5B). Generally, most of these rates were about the same as control rates except for two pots of plants which had been stressed to around −1.6 MPa; these were producing ethylene at 40% above the average control rates.

The relationship of ethylene production assayed daily at 4 PM during water deficit stress and after rewatering is shown in Figure 6. Ethylene production rates of two pots of plants subjected to cessation of irrigation were less than 40% of the control rates. After rewatering, the water deficit stress-treated plants recovered to slightly above the control ethylene production rate before dropping to the control rates.

In order to analyze ethylene production while plant ψ_w was actually falling, intact leaves (two per plant) were enclosed and ethylene collected from the air stream sweeping through the leaf chambers (Fig. 7). The initial rate of ethylene production by leaves in both control and unwatered plants after enclosure decreased about twofold; this decrease may reflect the normal decline in ethylene production of cotton leaves as they age (our unpublished data) and that enclosure in a flask is a less than ideal condition for the leaves. Nevertheless, the decline in the ethylene production rate was greater in the unwatered plants than in control plants (Fig. 7). The ethylene production rate of the water deficit-treated plants decreased from around 4 to 1.4 nL·g^{-1} dry weight·h^{-1} while their ψ_w decreased from about −0.85 MPa to about −2.9 MPa. After rewatering, the stressed plants showed almost complete recovery of their ψ_w and ethylene production rates recovered to the control levels (Fig. 7).

Since the response to water stress could be strongly age-related in cotton, we subjected mature plants with fruit and

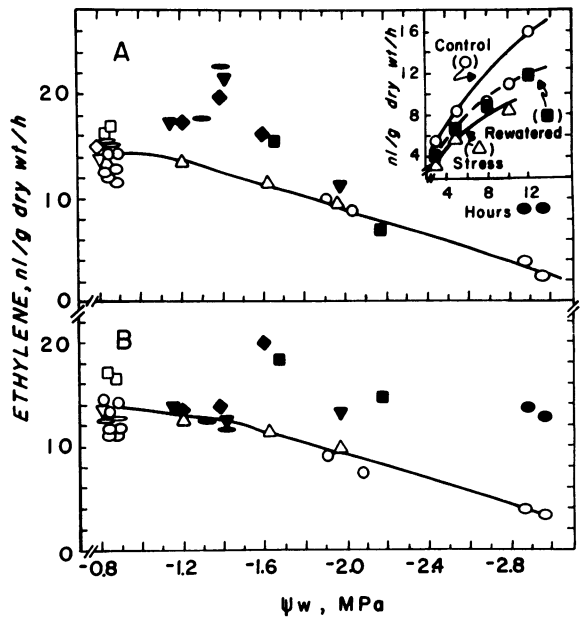

Figure 5. Effect of low water potential on ethylene production rates of 30-d-old intact cotton plants. Different symbols are used for each experiment. Rate calculation based on dry weight at end of experiment and ethylene production 24 h (A) and 48 h (B) after enclosure. Solid symbols are for plants rewatered shortly before enclosure (0 h). Water potentials were measured before either enclosure or rewatering. Water potentials of rewatered plants recovered to the −0.9 MPa range 24 h after rewatering in separate trials. Inset, average ethylene production for control (ψ, −0.85 MPa), not irrigated (ψ, −1.76 MPa), and rewatered (ψ, −1.53 MPa, before rewatering) during the first 12 h after enclosure.

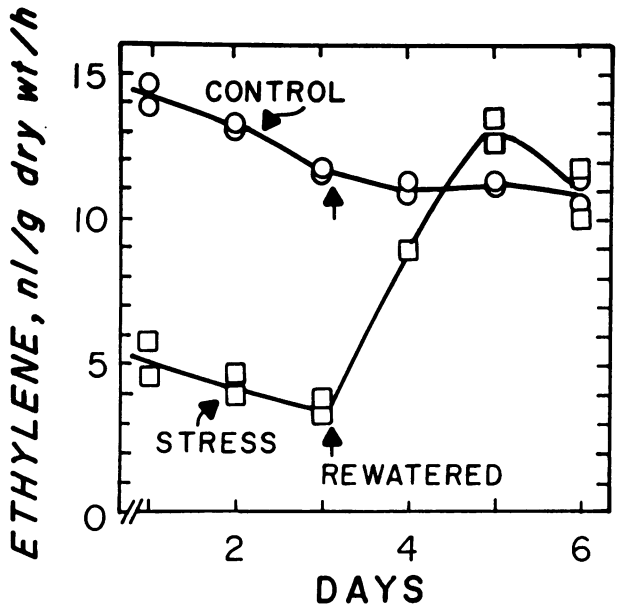

Figure 6. Effect of low water potential and rewatering on ethylene production rate of 30-d-old intact cotton plants. Two pots of plants subjected to cessation of irrigation and two pots watered daily were enclosed and afternoon rates of ethylene production measured for 3 d after which the plant cuvettes were opened, pots rewatered, and then the system closed and ethylene production measured for 3 additional days. Cessation of irrigation occurred 3 d before d 1 in the figure. At the time of rewatering, ψ_w of control plants was −0.87 MPa; for nonirrigated plants it was −2.9 MPa.

flowers to stress by withholding water. The ethylene production rate decreased progressively in nonwatered plants (Fig. 8). This experiment excluded the roots from the cuvette from which ethylene was collected and yet the results were similar to other experiments with entire plants (Fig. 6).

DISCUSSION

The question we have considered is whether intact plants subjected to water deficit stress increase their ethylene production rates significantly. The brief answer is no. We have examined three different species and found no evidence for a major increase in ethylene production with water deficit stress applied in most cases in ways which duplicate a natural drought. In fact, water deficit stress most commonly decreased ethylene production, if data are calculated on a per plant or unit dry weight basis. Water deficits reduce fresh weight; thus, values calculated for stressed plants on a fresh weight basis will be higher than if calculated on a per plant basis. The point is not the degree of decrease, which appeared consistently in every experiment, but the absence of an increase.

We are confident that we have not failed to detect a response that actually does exist. First, three appropriate species were tested. Both beans and cotton produce ethylene which regulates, at least in part, natural leaf abscission (7, 20). Both species demonstrated the common acceleration of ethylene release from detached leaves when they are air dried (Fig. 1; Table I). Miniature rose exhibits extensive leaf abscission after water deficit stress (our unpublished data); yet, neither it nor beans nor cotton demonstrated a promotion of ethylene release during severe stress (Table II; Figs. 1–8). Second, we verified that an increase in ethylene production does not occur after rewatering (although most of the reports that drying does promote ethylene synthesis involved assay of ethylene before rewatering or show a reduction in ethylene production upon rewatering [4–6, 13, 15, 31, 32]). With beans, we conducted several experiments in which plants were enclosed in the air flow cuvettes before rewatering so that a rapid burst of ethylene production after rewatering would not go unnoticed, but no promotion was detected in those experiments (data not given) or in experiments with plants rewatered and then enclosed (Fig. 3). With cotton, a series of time course experiments with stressed and stressed then rewatered plants failed to detect any promotion of ethylene production in the latter plants above control levels (Fig. 5), except that at intermediate levels of stress (−1.4 to −1.6 MPa) there were transient, modest increases in ethylene production at 24 and 48 h after rewatering (Fig. 5). The increase at −1.4 MPa at 24 h occurred in different experiments than the increase at −1.6 MPa at 48 h, and the increase above the control was only 40 to 50% in contrast to the several-fold elevation resulting from laboratory bench drying of detached leaves (Fig. 1; Table I). Third, observations were made to cover a broad time interval during imposition of stress on through to recovery. Initially, we made four observations per day covering about 14 h and including one prior to lights on (Figs. 2, 3). Ethylene production of cotton was assayed frequently for 24 h and additional observations were made until 48 h with plants in stress and stressed ones that had just been rewatered (Fig. 5). Daily observations were made with entire plants (Figs. 6, 8) and isolated leaves (Fig. 7) for extended periods. Timing of daily observations

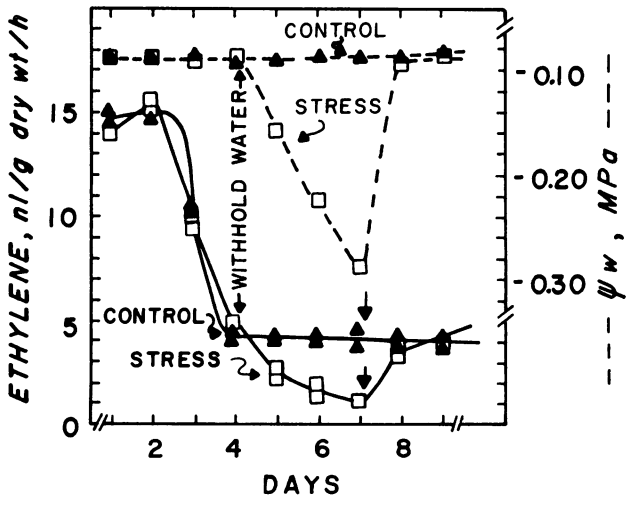

Figure 7. Ethylene production rate of individual leaves on 75-d-old plants subjected to cessation of irrigation or daily irrigation. Since only two older leaves were enclosed, plants experienced near normal water loss by transpiration during the period of drought and daily observations of ψ_w are plotted before and after rewatering (on d 7, vertical arrows). Leaves 10 and 11 from the base of the plant were enclosed on both the control and the nonirrigated plants.

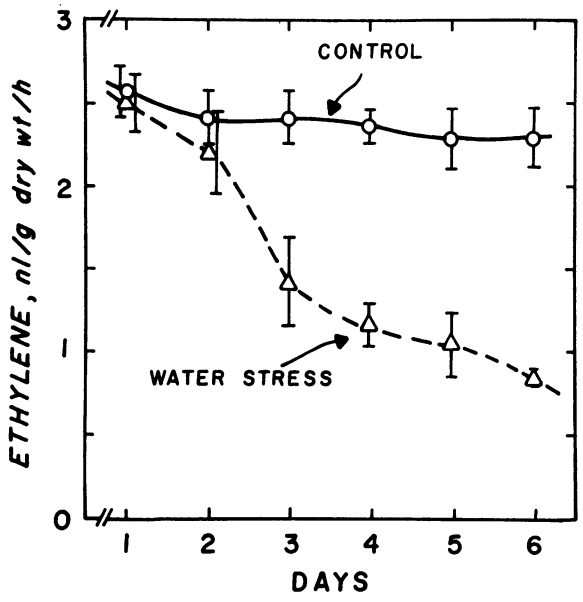

Figure 8. Ethylene production rate of flowering cotton plants subjected to daily irrigation or cessation of irrigation on d 1. Individual plants grown in 2 gallon crocks were 75 to 90 d old and bore mature fruit and flowers when enclosed in 40 L chromatography cylinders sealed to plates at the base of the stems. Data are averages of three experiments. Air flow rate maintained at 400 mL min^{-1}.

was during the experimentally determined daily peak of ethylene release (Fig. 4). Finally, cotton plants from vegetative to mature fruiting stages were tested. Apparently either no promotion of ethylene synthesis occurs or it is so transient in time or limited to such a small part of the total plant that it could not be detected. Neither of these possibilities is suggested by the ethylene response of detached leaves to laboratory bench drying. Furthermore, the dynamics of the air flow system increases the likelihood that any significant release of ethylene would be detected in experiments in which multiple daily observations were made (Figs. 2, 3, 5).

Why do detached leaves respond to drying stress differently than intact plants? We suggest two possible explanations. First, detached leaves are subjected to several stresses—excision, handling, disruption of transport systems, gravitropic disorientation, and rapid loss of water. Strong interactions may occur between some of the former stresses and drying, and these interactions may promote ethylene synthesis. Second, the loss of water from a detached leaf may be so much different than the stress experienced by an intact plant growing in gradually drying soil that different kinds of damage occur. In other words, drying detached leaves on a laboratory bench appears to impose a drying shock rather than a drought stress. There is evidence that membranes are involved in ethylene synthesis (17, 28), and the drying shock treatment may alter membranes more quickly than drought treatments.

It is interesting to note that most reports of promotion of ethylene production by water deficit stress have involved detached plant parts (3–6, 13, 15, 16, 18, 24, 31, 32) and that several studies with intact plants found no promotion of ethylene production (6, 9, 19), in agreement with our findings. El-Beltagy and Hall (13) observed that water deficit stress promoted ethylene production if assayed in detached leaves but not if air samples were taken directly from internal lacunae of leaves on stressed plants. In some cases, intact plants were subjected to some water deficit-inducing-treatment and then leaves or fruits were removed and ethylene production measured after static collection (16) or by vacuum extraction (4, 13, 15). The fact that these approaches detected an increase in ethylene production (4, 13, 15, 16) while in our study collecting ethylene from a constant flow air stream passing over stressed plants did not detect an increase, suggests that some interaction between detachment and plant water deficit occurs rather rapidly. Some of the experiments with intact plants involved exposure to PEG rather than to drying soil (15, 32), but ethylene was measured from detached leaves in one study (15) and not compared to nonstressed controls in the other (32).

The earliest, widely cited study reporting evidence that plant water deficits promote ethylene production was that of McMichael *et al.* (25) in which two-piece glass chambers were employed to enclose a portion of mature leaf petioles. Ethylene levels in the chambers were assayed after 2 to 4 h collection periods for 36 h, and chambers were flushed after each assay. Ethylene production was monitored from two petioles of three test plants during a drought and recovery period. Thus, control plants *versus* water deficit stressed plants were not compared but, rather, a peak of ethylene was observed during the light period in which ψ_w fell to below −1.5 MPa. This peak contrasted to broad peaks at lower rates during the light periods before stress and after rewatering. The control was to determine that reagent ethylene in the chambers in which peitoles were replaced with glass rods was not lost in 4 h. Later, Davenport (9) repeated the technique with modifications which included providing continuous air exchange in the petiole chambers during nonsampling periods and providing a control chamber by enclosing a glass rod. He concluded that the ethylene observed by McMichael came from the rubber serum caps or the permagum sealant used. Davenport (9) failed to find a promotion of ethylene release both with the petiole chamber technique and by comparing levels of ethylene vacuum extracted from control and drought-treated cotton seedlings. His samples were of cotyledonary blades and petioles of seedlings while McMichael *et al.* (25, 26) studied mature, fruited plants. Although cotton petioles have been shown to have higher ethylene production rates than leaf blades (23), an observation not confirmed in other species (4), there is no reason to anticipate that water deficit stress-mediated ethylene production would be limited to that tissue. This is especially true since the bulk of the published data are from leaf blades (4–6, 13, 15, 18, 24, 31, 32) and since collection of ethylene from part of a petiole and leaf blade in this study (Fig. 7) revealed the same effect of water stress as whole plant studies (Figs. 2, 3, 5, 6). The data obtained by McMichael *et al.* (25) are unique in that rather severe stresses were induced in a short time since they used two large plants growing in sand in a single container. In addition, there was an interesting feature in that the rates of production of ethylene by young and old petioles reversed during low ψ_w. Our results do not resolve the question of the

reliability of the previous observations (25), but the main point is that we did not find a promotion of ethylene production, even with mature, fruited plants (Fig. 8), when water deficit stress was imposed more slowly.

If a gradually imposed water deficit does not significantly increase ethylene production rates, why do leaves, flower buds, and fruits abscise extensively from stressed and rewatered plants (13, 25, 26)? It seems likely that changes in sensitivity to ethylene play a major role. Water deficit stress causes a large increase in abscission induced by exogenous ethylene (21). Recently, ethylene was implicated in induction of aerenchyma formation in maize roots by nutrient deficiency (12). Although deficiency treatments reduced ethylene production rates, they increased sensitivity to ethylene to an even greater degree (12). Water deficit stress reduces auxin transport capacity (10). When plants subjected to water deficit stress were treated with auxin transport inhibitors and the ethylene releasing compound ethephon, ethephon had the greatest ability to promote abscission (27). This suggests that the effect that low ψ_w had on auxin transport was near maximal but that ethylene was limiting for induction of abscission. In a system with such a close or delicate balance it would be possible for transient increases in ethylene production (Fig. 5) to be important to the induction of abscission. On that basis, the results in this paper do not necessarily alter the theoretical mechanism of drought-induced abscission, but they do suggest that more attention should be given to the mechanisms involved in responses to ethylene.

The findings in this paper suggest two general conclusions. First, the enhancement of ethylene production in detached plant parts is further emphasized as a potential artifact which might impact a variety of studies. Second, the value of intact plant experiments using treatments which approximate natural stresses is emphasized. Such an approach seems essential if normal plant behavior is in view. Efforts to confirm the relevance of shock stresses in model systems to the behavior of plants in nature are needed.

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