Short Communication

An Effect of Water Stress on Ethylene Production by Intact Cotton Petioles[1]

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The concept that ethylene is an endogenous growth regulator has evolved in the past few years (16). This concept has been strengthened by recent findings that internal concentrations of endogenous ethylene in vegetative tissues reach physiologically active levels (3, 12, 13). These internal concentrations have been directly related to the corresponding production rates of excised tissues (13), although parallel data on production rates of intact plant tissues are not available. Using excised abscission zones from primary bean leaves, Jackson and Osborne (9) presented evidence that the timing of abscission of explant petioles can be related to the extent of ethylene production adjacent to the separation zone. They suggest that the ethylene production is coupled to a particular stage of senescence. Further, they proposed that in natural leaf abscission ethylene initiates the biochemical sequences leading to separation, but the mechanism is not clear. Beyer and Morgan (4) have recently shown that ethylene production by and internal levels in detached cotton cotyledons increases as auxin transport declines. Amounts of exogenous ethylene necessary to induce abscission and inhibit auxin transport were similar. They propose that the rise in ethylene production and decline in auxin transport capacity are causally related and that reduced auxin transport is one of the ethylene mediated actions which precede induction of hydrolytic enzymes in the separation layer. Their measurement of ethylene involved whole cotyledons and was not restricted to the petiole. McAfee and Morgan (13) found internal ethylene levels and production rates were several times higher in petioles than leaf blades. Since auxin must be translocated through the petiole to the abscission zone, this observation strengthens the proposed role of ethylene in auxin transport inhibition preceding cotton leaf abscission (4). Alternatively, high rates of ethylene production by petiole tissue near the abscission zone may directly trigger the biochemical changes preceding separation independent from the proposed effect on auxin supply to the abscission zone.

Aside from abscission related to senescence, little attention has been directed to other problems of natural abscission, particularly those involving environmental stresses. Plant water deficits may induce both leaf and boll abscission from cotton under field conditions (5, 6, 18). In most instances, actual separation follows relief from the deficit and rehydration of the abscission zone. This communication describes the effect of a brief period of water deficit on ethylene production by intact cotton petioles.

MATERIALS AND METHODS

Cotton plants (*Gossypium hirsutum* L. var TM-1) used in this study were grown in pots containing sand in a greenhouse and were between 75 and 90 days of age. Each pot contained two plants of equal size, each with 15 to 17 leaves on the main stem. The use of paired plants made possible the determination of leaf water potential on one plant, while ethylene production was measured on the second, undisturbed plant. The plants were transferred from the greenhouse to a controlled environment chamber at least 5 days before ethylene measurements were begun. Chamber conditions were as follows: light intensity, 1400 ft-c; temperature, 27 C constant; relative humidity, 65% constant. The day length varied among experiments and is shown in Figure 2.

Leaf water potential values were determined with a pressure chamber technique (17). Ethylene production rates of intact petioles were determined by sealing a section of the petiole in a small glass chamber (Fig. 1) and periodically withdrawing a gas sample for analysis. The ground glass flanges of the chamber were lubricated with silicone stopcock grease. The outside seam was further sealed with Permagum after the petiole was enclosed. The chamber with the enclosed petiole was clamped to a ring stand for support and to prevent unnecessary movement of the chamber during sampling. Total internal volume of the chamber was approximately 23 cm³. After sealing, ethylene was allowed to accumulate for periods of 2 to 4 hr. Known concentrations of ethylene could be maintained in the chamber for at least 4 hr when a glass rod was substituted for a petiole. An 8-ml gas sample was removed for ethylene analysis using a Beckman GC-4 gas chromatograph. After sampling the chamber was flushed with a stream of dry air to remove all ethylene prior to the next collection period. The fresh weight of the enclosed petiole segment was determined at the end of each experiment.

RESULTS AND DISCUSSION

Ethylene production rates and leaf water potentials (Ψ) during three separate experiments are shown in Figure 2. Under the growth chamber conditions, well watered plants of this size developed internal water deficits of up to −15 bars after exposure to the light for several hours. Severe water deficits occurred rapidly when water was withheld for relatively short periods of time. When the plants were well watered (Ψ greater than −15 bars), consistently higher ethylene production rates were obtained from the upper petioles throughout the day. The rate of ethylene production by petioles on well watered plants during the dark period appeared to be less than that observed during the light period. Other studies (Mr. John Lipe, personal communication) have demonstrated the existence of

a definite diurnal pattern of ethylene production by intact cotton bolls. It was further observed that the magnitude of the diurnal variation was reduced under conditions of constant light or dark or constant temperature, as was used in the present study.

We investigated the possibility that higher production levels during the light period may be due to higher temperatures within the glass chamber. Fine wire thermocouples were attached to the underside of the petiole section sealed in the chamber and to an exposed portion of the same petiole. During the light period, the temperature of the enclosed petiole section was never more than 1.5 C higher than the adjacent, nonenclosed section. It is doubtful that this small temperature difference could result in variations in ethylene production rates as large as those observed during the diurnal cycle.

Ethylene production rates of petioles from intact cotton plants during the dark period were similar to those observed by McAfee and Morgan (13) for excised cotton petioles. Their data, collected from petioles of 21-day-old plants in the dark, also indicated slightly higher production rates by the uppermost petioles which were correlated with higher internal concentrations of ethylene. We compared ethylene production by excised and intact petiole segments from 60-day-old greenhouse plants (Table I). Excised petiole segments were taken 1.5 cm from the junction with the main stem and were 5.7 cm in length. The glass chambers were positioned to enclose a similar petiole segment on the intact plant. The excised segments were placed upright in 2 ml of 2% agar (Difco) gel contained in a 25-ml glass test tube. A drop of 1% agar solution was then placed on the apical end of each petiole to prevent tissue desiccation (13). The tubes were plugged with cotton for 6 hr to allow the wound response to subside (13). The glass chambers on intact petioles remained unstoppered for an iden-

FIG. 2. Ethylene production rates of intact cotton petioles and leaf water potential. Arrows on the time scale indicate water additions. Dark periods indicated by stippled portion of figure. Upper, middle, and lower parts of the figure represent data from experiments 1, 2, and 3 respectively. Leaf water potential (●), ethylene production by petiole at 3rd node (■), ethylene production by petiole at 12th node (▲).

Table I. *Comparison of Ethylene Production Rates by Intact and Excised Segments of Petioles From 60-day-old Cotton Plants*

Trial	Nodal Position	Ethylene Production[1]	
		Excised petioles	Intact petioles
		μl/kg fresh wt·hr	
1	5	0.62 ± 0.10	0.44 ± 0.19
2	5	0.85 ± 0.24	0.34 ± 0.22
3	12	1.16 ± 0.24	0.52 ± 0.31
4	12	1.70 ± 0.29	0.52 ± 0.39

[1] Each value represents four separate determinations with five petiole segments in each determination.
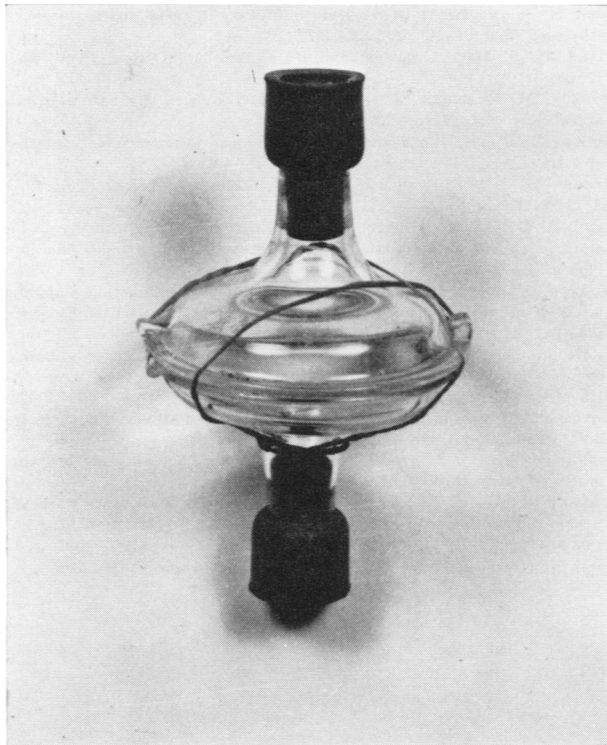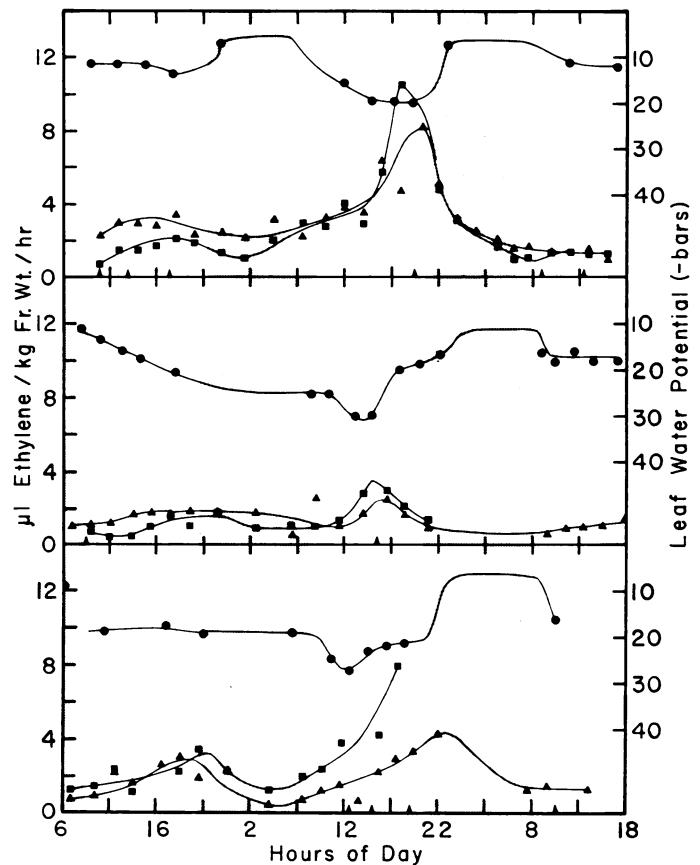

FIG. 1. Glass chamber for collection of ethylene released from intact petioles of cotton plants. Petioles projected through indentations in the flanges shown on the left and right sides of the apparatus. Gas samples were taken through the rubber serum stoppers which were removed during ventilation.

tical period of time. After 6 hr the tubes and chambers were sealed with serum vial stoppers and allowed to accumulate ethylene for an additional 6-hr period. An 8-ml gas sample was removed from each tube and chamber for ethylene analysis. The tissue segments were cut, and the chambers were attached to plants before sunrise. The tubes and plants with chambers were kept in the dark during the subsequent 6 hr equilibration and ethylene accumulation periods.

Petioles excised from the 12th node produced approximately twice as much ethylene as those from the 5th node. This result confirms and extends the observations by McAfee and Morgan (13). In the present study the differences between nodal posi-

tions were considerably larger and are probably due to a greater differential in leaf age than was present in the previous study (60- versus 21-day-old plants). Differences of similar magnitude due to nodal position were not observed with the intact segments. This result is in accord with data presented in Figure 2 which illustrates that differences in production rates between nodal positions were greatly reduced at the end of the dark period in the growth chamber. Compared with intact segments, ethylene production was slightly higher for excised segments from the petiole at the 5th node, and nearly three times as large for segments from the 12th node. These differences may be due to an inadequate period of time (6 hr) allowed for the wounding response to subside. In addition, the data may indicate that ethylene production in response to wounding may be related to the physiological state and age of the petiole.

As severe water deficit developed, sharp increases in ethylene production rates of all petioles resulted (Fig. 2). Contrary to the case for well watered plants, production rates of the older petioles exceeded those of the uppermost, younger petioles. A quantitative relationship between the magnitude of internal plant water deficit and production rate was not apparent. Ethylene production rates fell rapidly after the internal water deficit was relieved by watering. In experiments 2 and 3, abscission zones rapidly formed after water application and separation occurred within 6 hr.

There was no clear relation between the magnitude of the stress-induced ethylene production peak and leaf abscission. In experiment 1 (upper portion of Fig. 2) a water deficit near −20 bars was associated with an ethylene production rate greater than 11 μl/kg fresh weight·hr for the petiole of the 3rd node, yet the leaf at the 3rd node did not abscise. Although more severe water deficits occurred in the 2 following experiments, maximum production rates reached only 2.9 and 6.2 μl/kg fresh weight·hr (middle and lower portions of Fig. 2). Leaves at the 3rd node abscised in both of these experiments. Beyer and Morgan (4) have presented evidence suggesting that ethylene production rates of 2 to 3 μl/kg fresh weight·hr are adequate to reduce auxin transport and hasten abscission of cotton cotyledonary leaves. The data presented here suggest that the level of ethylene production per se may not be the controlling factor in leaf abscission, presuming it exceeds some minimum critical level, but that the physiological state of each leaf governs the response to increased endogenous ethylene production. After rewatering, ethylene production rates of the remaining petioles fell near or below prestress levels and remained low the following day.

Our data illustrate that increased ethylene production rates of petioles are associated with greater than normal plant water deficits. We have also found that leaf abscission and plant water deficit are related in a linear manner (14). The nature of this water stress-induced abscission is not clear, but a model has recently been formulated which may bear on the problem (4). According to this model, the hormonal balance of the abscission zone is maintained, under normal conditions, within a range which precludes development of the separation layer. If this hormone balance is forced out of normal maintenance limits, the sequence of steps leading to eventual separation may be irreversibly triggered. Although the identity of all the hormones comprising this balance are probably not known, ample evidence is available that IAA may play a key role in leaf abscission (8, 11). Using cotton explants, Addicott and Lynch (1) concluded that distally applied IAA retards formation of the abscission zone. Since IAA is presumably elaborated in the leaf blade (10), the petiole functions as a channel through which sufficient quantities of IAA are transported to maintain a favorable hormone balance in the abscission zone. Should this basipetal auxin transport be slowed or blocked for a time, the hormone balance may be sufficiently altered to trigger abscission. The ability of ethylene to modify basipetal auxin transport in petioles of cotton (2, 4, 15) and pea (17) has been well documented. In the present case of water stress-induced abscission, basipetal auxin transport may be altered by ethylene inhibition of the transport process in the petiole. It is possible that moisture stress contributes both to a physical inhibition of auxin transport and stimulation of ethylene production which then further reduces auxin supply and induces synthesis of hydrolytic enzymes in the abscission zone.

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