Water Stress Enhances Ethylene-mediated Leaf Abscission in Cotton

Wayne R. Jordan, Page W. Morgan, and Thomas L. Davenport

Department of Plant Sciences, Texas A&M University, College Station, Texas 77843

ABSTRACT

Abscission of cotyledonary leaves from cotton (Gossypium hirsutum L. cv. Stoneville 213) seedlings occurred following relief from water stress. The amount of abscission was related to the magnitude of the plant water deficit. Leaf abscission promoted by exogenous ethylene was enhanced in seedlings subjected to water stress. Treatment with ethylene (2.0 to 3.2 microliters of ethylene per liter of air for 24 hours) raised the threshold plant water potential required to induce abscission from −17 to −7 bar, indicating that the stress caused the tissue to become predisposed to ethylene action. Based on the abscission response curve for seedlings treated with ethylene while under water stress, this apparent predisposition was developed as the plant water potentials reached the −7 to −10 bar range. The abscission-promoting effects of ethylene in combination with water stress were reversed with 15% CO₂ at plant water potentials above −12 bar, but the CO₂ reversal was lost at lower water potentials. These results are compatible with the concept that ethylene plays a regulatory role in leaf abscission induced by water stress.

The involvement of ethylene as an endogenous regulator in natural leaf abscission has been strongly implicated in recent studies (5, 11). Ethylene appears to play multiple roles in leaf abscission resulting in a reduction in auxin transport to the abscission zone (4, 5, 7), and by the induction of hydrolytic enzyme synthesis (1, 2, 10, 13, 16, 17, 19, 21) and enzyme secretion into walls of cells comprising the separation layer (3). It is unclear at this time whether all these actions of ethylene are causally related.

Evidence indicates that ethylene acts as an aging hormone and is related to leaf abscission, at least in part, through its ability to accelerate senescence in these tissues (9). This view was recently supported by additional findings, and the term "phytogerontological effect" was coined to describe the aging effect of ethylene (3). Recognizing that the action of ethylene which precedes induction of enzyme synthesis may be achieved by regulation of the supply of hormones to the abscission zone, Beyer and Morgan (5) have shown that ethylene modification of auxin transport is apparently involved in both natural abscission and abscission hastened by exogenous ethylene.

In addition to natural abscission related to senescence, recent reports have implicated ethylene in leaf abscission induced by environmental stress. Young and Meredith (22) presented evidence that ethylene production by freeze-injured citrus leaves was involved in freeze-induced leaf abscission. McMichael et al. (14) reported increased ethylene production rates by intact cotton petioles during a period of plant water deficit. The authors (14) were unable to demonstrate a clear relation between the magnitude of the stress-induced ethylene production and level of water deficit or abscission. They concluded that the level of ethylene production per se may not be the controlling factor in leaf abscission, but that the physiological state of each leaf governs the response to increased endogenous ethylene production. In a second paper, McMichael et al. (15) demonstrated that the extent of leaf abscission from cotton plants following water stress was related to the severity of the stress in a linear manner.

In view of these findings, it would seem attractive to propose a role for ethylene in stress-induced abscission. This report provides evidence for the involvement of ethylene in leaf abscission induced by plant water deficit.

MATERIALS AND METHODS

Plant Materials. Cotton seedlings (Gossypium hirsutum L. cv Stoneville 213) were grown in a controlled environment chamber in a mixture of sand, peat, and vermiculite (3:2:1) contained in 33 x 10 x 10 cm plastic trays. Growth conditions were as follows: 1800 ft-c; 15 hr photoperiod; temperature 27 ± 1 °C day, 22 ± 1 °C night; ambient relative humidity. The plants were watered daily with a modified Hoagland’s solution (14). Water stress was induced by withholding nutrient solution until the desired stress was reached, usually over a period of 4 to 6 days. A range of plant water stress was attained by thinning the number of plants to between 10 and 20 plants per tray. The level of stress was quantified by plant water potential determinations made with a pressure bomb (18) near the end of the dark period on the day treatments were initiated.

Ethylene and CO₂ Fumigations. The water-stressed plants were fumigated with ethylene or ethylene plus CO₂ in 268-liter Plexiglas chambers for 24 hr. The chambers were kept in the dark at 23 ± 1 °C during the fumigation period. CO₂ was removed with 10% KOH on filter paper wicks in those treatments not involving fumigations with CO₂. Ethylene concentrations were determined by gas chromatography (5) at 6 and 24 hr after injection. The concentration measured within the first 6 hr was maintained within ±5% during the 24-hr treatment period. Following the treatment period the chambers were opened, the leaves tested for abscission, and the pots fully watered.

Abscission Measurements. All leaves were tested for abscission by applying slight pressure (about 5 to 10 g) by hand to each petiole near the blade. After this initial testing, all trays were fully watered and placed in a growth room under the fol-

1 A contribution of the Texas Agricultural Experiment Station. Supported in part by Cotton Incorporated.
RESULTS

The effects of water stress and water stress in combination with exogenous ethylene on cotyledonary leaf abscission are illustrated in Figure 1. Each curve represents data from one tray containing 16 to 20 plants and is typical for the indicated levels of water stress observed in several other experiments. For plants not treated with ethylene there was little abscission during the first 4 days after water stress was relieved. There was no apparent effect of water stress alone on cotyledonary leaf abscission from these plants within the range of plant water potentials from −6.7 (controls) to −12.3 bar. Normally, natural abscission after 10 days was between 5 and 20% for plants of this age, and appeared to be independent of plant water potential in the range of −5.0 to −15.0 bar.

There was an obvious stimulation of abscission in treatments exposed to ethylene (2.6 µl/l of air for 24 hr) which was directly related to the level of water stress. There was no ethylene-induced abscission in plants not water stressed (−6.7 bar) although pronounced epinasty of the cotyledons was observed. Both the initial rate and the final amount of abscission were higher for the ethylene-treated plants with the lowest water potentials (greatest stress). In addition, there was a substantial amount of abscission observed at the end of the fumigation period but before the pots were rewated.

Data from a more intensive study of the relation between plant water potential and ethylene-induced abscission are presented in Figure 2. Abscission was measured 24 hr after fumigation and relief from water stress. A plant water potential of −15 to −17 bar appeared to be the threshold level of water stress for induction of abscission in the absence of added ethylene. Addition of ethylene to water-stressed plants (either 2.0 or 3.2 µl/l of air) raised this threshold to about −7 bar, while fumigation with 1.0 µl of ethylene per liter of air was without effect over the same plant water potential ranges. Greater than 90% abscission with ethylene treatment was observed at the most severe level of water stress attained in these experiments. Abscission from nonfumigated control plants appeared to be related in a linear manner to the plant water potential below −15 bar.

Since CO₂ has been recognized as a competitive inhibitor of many ethylene-mediated responses including leaf abscission (2, 6, 8, 12, 20), we studied the ability of CO₂ to inhibit the ethylene stimulation of leaf abscission from water-stressed seedlings (Fig. 3). Abscission induced by water stress and ethylene at 3.4 µl/l of air was similar to that observed earlier (Fig. 2). Abscis-
sion of leaves from seedlings fumigated with ethylene in the presence of 15% CO$_2$ was related to the degree of water stress. Ethylene-promoted abscission was reversible with CO$_2$ only so long as the plant water potential remained above $-12$ bar. Once the stress exceeded $-12$ bar, the effect of CO$_2$ was reduced and finally lost at water potentials approaching $-20$ bar. Similar results were obtained in a separate experiment in which plants were fumigated with 2.0 g/L of ethylene per liter of air in the presence or absence of 15% CO$_2$.

**DISCUSSION**

Abscission of leaves from cotton plants can be induced by an unfavorable plant water balance. The amount of abscission appears to be directly related to the magnitude of the internal plant water deficit (15). Although an association between ethylene production rates of intact cotton petioles and plant water deficits has been presented (14), the mechanism by which water stress induces the abscission process has not been investigated. In this paper, we present evidence for a possible role of ethylene in leaf abscission induced by plant water deficit.

From data in Figure 1, it appears that an internal water deficit causes cotyledonary leaves to become predisposed to the abscission-inducing effects of exogenous ethylene. This "pre-disposition" resulted in increased rates of abscission and in a significant amount of abscission before the stress was relieved. Plants either fumigated (for 24 hr) but not stressed, or stressed but not fumigated, abscised leaves before waterlogging very rarely. This result is in agreement with a previous report (15).

Although not related in a simple manner, these observed changes induced by water stress are strongly related to the magnitude of the water deficit (Fig. 2). While the nature of these changes is unknown, the data clearly demonstrate a rapid response to exogenous ethylene at plant water potentials near $-10$ bar. The threshold water potential required to induce significant abscission was raised from $-17$ to $-7$ bar by ethylene treatment, suggesting a direct role for ethylene in abscission induced by water stress. This conclusion is supported by the ability of CO$_2$ to inhibit leaf abscission induced by a combination of water stress and exogenous ethylene at water potentials above $-12$ bar. Competition between CO$_2$ and water-stress-dependent ethylene-induced abscission appears less likely if the effect of ethylene is to increase sensitivity to ethylene (or leaf senescence) rather than raising the level of endogenous ethylene.

If tissue modifications are induced by water deficits of approximately $-10$ bar, then these changes must also be reversed or prevented by CO$_2$, further suggesting that abscission induced by water stress may be mediated through increased ethylene production by or retention in the tissues. Since CO$_2$ is an effective inhibitor of ethylene action only within rather narrow ranges of ethylene concentration (6), an explanation may be offered for the inability of CO$_2$ to reverse the ethylene promotion at lower water potentials. If ethylene production rates increase as a function of plant water deficit as suggested by McMichael et al. (14), the internal tissue levels resulting from endogenous production and exogenous application may have become too high for effective reversal by CO$_2$. Other recent studies support the conclusion that ethylene participates in water stress-induced abscission. Reduced pressure decreased the amount and rate of leaf, flower bud, and young fruit abscission from cotton plants subjected to water deficits of about $-30$ bar (J. A. Lipe and P. W. Morgan, unpublished data). This result was interpreted to indicate that a partial vacuum hastened removal of increased levels of ethylene resulting from water stress and thereby reduced or delayed abscission.

This paper presents evidence that ethylene is involved in leaf abscission induced by water stress. Changes appear to be initiated at plant water potentials near $-10$ bar which predispose cotyledonary leaves to the abscission-inducing action of ethylene. At least two types of changes could account for this response; the ethylene production rates of the tissue may increase as a function of the internal plant water deficit, or the tissue sensitivity to ethylene may be increased during the development of internal water deficits. Data presented in this paper do not afford a definitive separation of these two possibilities.

**LITERATURE CITED**