Water-Stress-Induced Ethylene Production in Wheat

A Fact or Artifact?

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ABSTRACT

Effects of water stress on ethylene evolution from excised leaf segments and intact plants of wheat (Triticum aestivum L. cv Katepwa) were studied. Excised leaf segments of 8 day or 6 week old plants were dried until they lost 8% of their fresh weight (water potential about -2.3 megapascals). These and nondried control leaf segments (water potential about -1.0 megapascal) were sealed in glass tubes, and their ethylene production rates were compared by head space analysis via gas-chromatography. The dried leaves of both ages produced significantly more ethylene than the corresponding controls. However, when 6 week old intact plants were water-stressed by withholding water supply, and their ethylene production measured using a continuous-flow system, no increase in ethylene was detected despite a drop in water potential to -2.9 megapascals over 6 days. Even the leaf segments excised from plants that had been subjected to water stress for 2, 4, or 6 days produced no more ethylene (in sealed tubes) than the leaves from well-watered plants. In fact, the ethylene production by these segments decreased with the increase in the severity of stress experienced by the plants. The results show that the commonly reported overproduction of ethylene by excised leaves subjected to rapid drying represents an artifact, which has little relevance to the water stress responses of intact wheat plants.

In wheat, all the reports of enhanced ethylene production consequent upon water stress are based upon experiments with either excised and partially dried leaf segments (3, 20, 23-26) or, occasionally, whole seedlings subjected to sudden water loss by lowering the osmotic potential of the root medium while blowing warm air over the shoot (24, 25). In all these cases, the leaves or the whole seedlings were sealed inside airtight containers, a condition that is certain to cause major changes in the important components (CO₂, O₂, C₂H₄, etc.) of the atmosphere around the tissue (5, 9, 16), and thus possibly affect the rates of ethylene production (6, 15, 16, 19). Using these techniques, several complex interactions among hormones and environmental conditions, and regulatory aspects of the ethylene biosynthetic pathway have been studied (13, 20, 24-26). The results of such studies have often been interpreted in terms of the regulation of drought responses of whole plants (20, 25).

As a part of our ongoing research on the role of plant hormones in stress-induced reproductive disfunction in wheat, we measured ethylene production by intact water-stressed plants using a continuous-flow system that allows precise control of the gaseous environment. We found no evidence of enhanced ethylene production from stressed plants. This contradiction with what appeared to be a widely documented and accepted response to water stress (17, 27), coupled with the above mentioned technical deficiencies of the previous work, led us to the present work to reexamine the effect of water deficit on ethylene production in wheat.

MATERIALS AND METHODS

Plant Growth

In experiments with 8 d old plants, wheat (Triticum aestivum L. cv Katepwa) seedlings were grown in vermiculite in 27 × 52 × 5.5 cm trays, maintained in a greenhouse (April–May 1990) with supplementary lighting from four 400 W sodium lamps (16 h photoperiod). The trays were watered daily. The plants (two per pot) used at 6 weeks of age were grown in plastic pots (9.5 cm diameter × 15 cm height) containing a 50:50 mixture of sand and Pro-Mix (Premier Brands Inc., Stamford, CA). The plants spent the initial 1 week in the above greenhouse (at various times throughout the year), followed by transfer to a controlled environment chamber (Conviron model EF 7) with 20/18 ± 1°C (day/night) temperature, 16 h photoperiod, and a canopy level PPFD of 260 μmol m⁻² s⁻¹ of fluorescent and incandescent...
illumination. The pots were watered on alternate days unless otherwise stated.

**Desiccation of Excised Leaves and Measurement of Ethylene Evolution**

The method of drying excised leaf segments and measurement of ethylene evolution from these segments was similar to that used by Wright (23). The aerial part above the coleoptile (8 d old seedlings) or the three top-most leaves (6 week old plants) were excised in the morning, and cut into 11 cm long segments. The segments were immediately weighed and then allowed to dry on the laboratory bench at the room temperature until they lost 8% of their initial fresh weight. The segments were placed inside 70 mL clear-glass tubes, which were then sealed with rubber sleeve stoppers (Fisher Scientific). Parallel control tissues were similarly sealed immediately after excision and weighing. The tubes were maintained in a controlled environment chamber in the light as stated above. One milliliter gas samples were periodically drawn over 24 h from the tubes with a gas-tight syringe, and their ethylene content measured by injection onto a Porapak Q (Supelco, Canada) packed column maintained at 50°C in a Hewlett-Packard 5830A gas-chromatograph equipped with a flame-ionization detector. One milliliter of air was injected back into the tubes after each injection. Known amounts of ethylene injected into the sealed tubes gave retention of more than 95% after 24 h.

**Water Stress Imposition on Intact Plants and Measurement of Ethylene Evolution**

Intact mature plants were stressed by withholding water for the required number of days such that the plants were 6 weeks old on the last day of each treatment. The corresponding controls were maintained on normal watering schedule.

Ethylene evolution from the whole plants was measured using a continuous flow system by enclosing the top three leaves and the associated part of the culm in a 1.4 L, cylindrical clear-glass cuvette (Fig. 1). The cuvette comprised two parts: the base which was permanently fixed to the culm throughout an experiment, and the removable top that was attached to the base through a ground-glass connection only during the sampling period (see below). The base was sealed around the culm with a rubber stopper (autoclaved to release hydrocarbons) and Polyfilla, a cellulose filler (Le Page’s Ltd., Canada) (Fig. 1, inset). The cuvette was connected to a 100 mL min⁻¹ flow of air, from which hydrocarbons, including ethylene, were removed by platinum-catalyzed thermal oxidation (8). Ethylene released by the plant was thus continuously swept out by the air stream. One hour before sampling, the cuvette-top was sealed to the base to permit gaseous equilibrium. Ethylene was then collected from the gaseous effluent for 30 min by passage through a U-tube containing 0.5 g silica gel (60–100 mesh) kept in a dry ice-acetone bath. The U-tube was removed and connected to a specially installed injection port on a Hewlett-Packard 5830A gas chromatograph described above. Ethylene was desorbed from silica gel by placing the U-tube in a boiling water bath for 10 min, and injected by opening a four-way valve. Additional details of this method for ethylene collection and injection were as published (5, 8). The U-tube trap collected 4.3 nL ethylene in 10 min when plants previously sprayed with 1 mg L⁻¹ 2-chloroethylphosphonic acid (an ethylene releasing compound) were enclosed in the cuvette. Since this amount was more than five times higher than the highest amount of ethylene ever collected (in 30 min) from a test plant, the trap was considered an accurate sampling tool. This trap can, in fact, retain up to 7.5 µL ethylene with 100% efficiency (5). No ethylene was absorbed by the U-tube trap in the absence of a plant, indicating that the incoming air was essentially ethylene free.

Alternatively, the whole plants were subjected to water deficit for various days as described above, and ethylene evolution from the top three leaves was measured in a closed system. The leaves were cut into 11 cm long segments,
weighed, and their ethylene production rates were measured using sealed tubes as described above.

**Measurement of Water Status**

The leaf water status was measured using parallel plants treated in the same manner as those used for ethylene measurements. Penultimate leaves of 6 week old plants or randomly chosen leaves of 8 d old seedlings were used for these measurements. The water potential measurements in experiments on excised leaves correspond to the time of enclosure of the leaves in the tubes. The leaf water potential was measured using a Wescor HR-33T dew-point hygrometer coupled with C-30 (at 20 ± 0.1°C) or C-52 (at the room temperature) sample chambers. Relative water content (fresh water content as percentage of the water content at full turgor) was measured by the method of Barrs and Weatherley (4).

**Replication**

All treatments within an experiment were given and all measurements were made in three or more replicates, and each experiment was repeated at least three times. Results from one representative experiment in each case are presented, unless otherwise stated in the figure legend.

**RESULTS**

**Ethylene Production in Sealed Tubes by Leaf Segments Dried after Excision**

When excised 8 d old leaves were dried on the laboratory bench, and ethylene production measured using a sealed tube following procedure used by many previous workers (23), the dried leaves produced considerably more ethylene than the nondried controls (Fig. 2A). The ethylene production peaked at 6 h after the enclosure. Overall, the results were qualitatively in accord with the previous reports (3, 23, 26) that drying of detached wheat leaves causes a short-lived burst of ethylene production. Similar results were also obtained with leaves from 6 week old plants (Fig. 2B), except that these leaves produced nearly four times less ethylene per unit fresh weight than the younger leaves (cf. Fig. 2A and 2B). The burst of ethylene production from the older leaves lasted about 3 h longer than that from the younger ones. The water potentials of dried (about -2.2 MPa) and control (about -1.0 MPa) samples were comparable between the younger and the older leaves (Fig. 2A and B).

**Effect of Water Deficit on Ethylene Production by Intact Plants in Continuous-Flow System**

Further experiments were done with the 6 week old plants since, on the basis of the results in Figure 2, they fairly represented the general pattern of drying-induced ethylene evolution reported in wheat. This permitted the use of continuous-flow system for ethylene measurements since, unlike the fragile younger seedlings, the culms of the older plants could withstand sealing to the cuvette base.

When watering was stopped 6 d before the plants reached 6 weeks of age, the leaf water potential or relative water content did not change for the initial 3 d, following which these parameters declined gradually to reach -2.9 MPa and 52%, respectively, by the sixth day (Fig. 3). Despite this substantial drop in the plant water status, no obvious differences in ethylene evolution between stressed and well-watered plants were observed in the continuous flow system (Fig. 4). The level of ethylene emanating from any one plant fluctuated considerably with time (up to sixfold), but this fluctuation was of similar magnitude in stressed and control plants. Most of the readings, however, were concentrated below 0.6 nL/h/plant in both the treatments (Fig. 4). The analysis of these data for individual plants did not reveal any diurnal pattern of ethylene evolution.

**Ethylene Production in Sealed Tubes by Leaf Discs Taken from Water-Stressed Whole Plants**

In another set of experiments, intact plants were water-stressed by withholding water (see Fig. 3 for water status), and the top three leaves were excised on the second, fourth, and sixth day of this treatment. Ethylene evolution from these leaves was measured using the closed system employed by the

![Figure 2](www.plantphysiol.org) Ethylene evolution (total accumulation) from excised leaf segments of wheat during 24 h following inclusion in sealed tubes. Water potentials (WP) for dried and control samples just prior to sealing are shown. Data are the means of three replicates ± SE. A, Eight-day-old seedlings; B, 6-week-old plants.
previous workers (3, 23, 26). The leaves taken from the stressed plants consistently produced less ethylene than the leaves from the well-watered controls, with the amount of ethylene generally decreasing with the increase in the severity of stress (Fig. 5).

**DISCUSSION**

The results clearly demonstrate that water-stressed intact wheat plants do not produce any more ethylene than the well-watered plants (Fig. 4). Moreover, water stress indeed appears to diminish the capacity for ethylene production, as demonstrated by the comparatively lower rates of ethylene production in the sealed tubes by the leaves excised from stressed plants (Fig. 5). The previous findings of drying-induced over-production of ethylene from excised leaves (3, 20, 23–26) still hold true in our similar experiments (Fig. 2). The reasons for these differences between the excised and intact tissues remain to be confirmed, but it can be surmised that the response of excised tissues may represent a shock reaction to sudden desiccation, probably superimposed on the effects of wounding, senescence, and confinement (6, 27). This could also explain why Wright observed increased ethylene evolution from rapidly dried (30 min) intact wheat seedlings enclosed in a sealed container for 6 h, 45 min (24, 26).

Several studies have used confined excised leaves to study interactions among hormones and environmental factors, as well as the regulation of ethylene biosynthesis under water stress (13, 20, 24–26). Our present results, however, show that drying-induced ethylene production by excised leaves represents an artifact, with little direct relevance to the whole plant hormone relations under water stress conditions. Hence, although the use of excised leaves is convenient and has led to some interesting findings, it probably cannot serve as a valid model since the very existence of water-stress-induced ethylene production in whole plants is in question. The extrapolation to whole plants of the results obtained with excised leaves (20, 25) should, therefore, be viewed with caution.

Production of ethylene and 1-(malonylamino)cyclopropane-1-carboxylic acid (a metabolite arising from ethylene biosynthesis) have been suggested as indicators of stress (13, 28). However, the evidence comes exclusively from excised leaves, and has never been demonstrated with intact plants despite apparent attempts to do so (13).

Finally, these findings with wheat do not imply that water stress does not enhance ethylene production in other species. In fact, ethylene evolution from intact cotton petioles and *Vicia faba* plants does appear to increase under water stress.

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**Figure 3.** Relative water content (RWC) and water potential (WP) of the uppermost fully expanded (penultimate) leaf of wheat plants subjected to water stress by withholding water. Each point represents the mean of four replicates ± SE.

**Figure 4.** Ethylene evolution in the continuous-flow system, from intact wheat plants subjected to water stress by withholding water at time 0, as compared with the well-watered controls (see Fig. 3 for water status). The data are pooled measurements from six stressed and five control plants. The regression lines and equations for the stressed (S) and control (C) plants are shown.

**Figure 5.** Ethylene evolution (total accumulation) from leaf segments excised from 6-week-old plants subjected to water stress by withholding water for 2, 4, or 6 d prior to the removal of the leaves (see Fig. 3 for water status). Controls were well watered throughout. The leaf segments were sealed in glass tubes immediately after excision, and ethylene concentration measured periodically during the following 24 h. Each value is the mean ± SE of three replicates.
(10, 12, 21). Monocots and dicots may have fundamental differences in this regard, particularly if increase in ethylene production is related to processes such as abscission that are specific to dicots (14, 18).

NOTE ADDED IN PROOF

Morgan et al. (Plant Physiol 1990 94: 1616–1624) have also recently reported that water deficit does not promote ethylene production by intact plants of bean, cotton, and miniature rose.

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LITERATURE CITED

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