Communication

Role of Ethylene in the Geotropic Response of Bermudagrass (*Cynodon dactylon* L. Pers.) Stolons[1]

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ABSTRACT

We studied the relationship between ethylene and gravity-induced upward bending of bermudagrass (*Cynodon dactylon* L. Pers.) stolons. Ethylene production begins within 3 hours of the onset of gravistimulation, and increases thereafter until the 15th hour, after which it declines. There is a close positive relationship between ethylene production and upward bending during the first 12 hours of gravistimulation. Incubation of stolons with AgNO₃ did not prevent ethylene evolution but delayed upward bending. In addition, ethylene production was 10-fold greater and peaked earlier in gravistimulated nodes incubated with 1-aminocyclopropane 1-carboxylic acid. The gravitational stimulation could be due to an increase in both 1-aminocyclopropane 1-carboxylic acid synthase and the ethylene forming enzyme. The results suggest that ethylene promotes the activity of indoleacetic acid.

Bermudagrass (*Cynodon dactylon* L. Pers.) stolons normally grow horizontally when attached to the plant. However, their excision results in the reorientation of apical tissue growth from horizontal to vertical (12). Since the work of Neljubow on pea (discussed by Abeles [1]), it has been known that ethylene can alter the orientation of stem growth from vertical to horizontal in pea and cotton (7) and conversely, from horizontal to vertical in strawberry clover (8) and peanut (22). Clifford *et al.* (6) found that bending was not inhibited by ethylene inhibitors, although they measured more ethylene production in geostimulated peduncles of dandelion. Later Wheller *et al.* (19) demonstrated that ethylene increases in basal tissues coincident with bending and that the addition of ethylene overcomes to some extent the effect of ethylene inhibitors. Kelly and Bradford (11) treated a diagravitopic mutant (23) insensitive to auxin with ethylene, and discovered that it reoriented its growth from horizontal to vertical (5). They postulated that the reduced sensitivity to auxin resulted both in an altered regulation of ethylene synthesis and in a changed response to gravity by the mutant. Thus, the role of ethylene in diagravitropism seems to be complicated and diverse and not very well understood (14). The purpose of

this paper is to evaluate the role of gravitationally induced ethylene production in the response of stolons to gravity.

MATERIALS AND METHODS

The experimental materials were stolons of bermudagrass (*Cynodon dactylon* L. Pers.) collected from plants grown in a field plot. For measuring curvature, apical stolon explants, 10 cm long, were excised from plants and their cut ends fitted through tight rubber stoppers into 8 mL flasks containing the solution to be tested. The cut end of each explant was immersed in the test solution. The prepared explants were kept for 12 h in a vertical position to eliminate the effects of wound ethylene (unpublished personal observations) and then were arranged horizontally on a framework, which consisted of a wooden base, above which two vertical wooden frames were tightly mounted. Sixteen holes were drilled into each frame, so that each flask could be placed horizontally and fit tightly. Curvature measurements of 32 stolons were made from photographs taken manually every 3 h during a 23 h time period.

To minimize biological variability between stolons, values are given as the percentage of curvature with respect to the final curvature of each stolon: final curvature percentage (FCP) = observed curvature/final curvature X 100. This normalization allowed us to determine when differences in the rate of bending were significantly different.

Ethylene Measurements

Groups of 25 apical nodal segments of bermudagrass stolons were weighed, and then arranged in 7-mL, gas-tight vials containing washed vermiculite to provide moisture and support them in the proper orientation. Only the apical node of each stolon, including 5 mm of surrounding tissue, was used. The vials were kept upright and unsealed in humidified plastic boxes for 12 h at 20°C in the dark to allow dissipation of wound ethylene. Vials then were placed horizontally according to the treatment and subjected to gravistimulation for time periods of 3, 7, 11, 15, 19, and 23 h. The flasks were sealed 3 h before assay for ethylene. All experiments were performed in darkness and at a room temperature of 28°C.

A 1-mL gas sample was withdrawn from each flask, and ethylene was assayed with a gas chromatograph equipped with a stainless steel column filled with Chromosorb 105 and fitted with a flame ionization detector. Results are expressed in μL of ethylene per kg fresh mass of tissue per h. Experiments

were performed twice, and in each, four replicates at each sampling time were measured. The least significant difference, LSD, at 0.05 is given. The treatments are as follows.

Treatment 1

Stolons were not subjected to gravistimulation but their ends were immersed in distilled water.

Treatment 2

Stolons with their ends immersed in distilled water were placed horizontally to induce gravistimulation.

Treatment 3

The stolons were gravistimulated with their ends dipped in 1 mM ACC,[3] the immediate precursor of ethylene (3).

Treatment 4

Gravistimulated stolon ends were immersed in 0.5 mMM AgNO₃, an inhibitor of ethylene action (4). To improve Ag mobility within the tissue, AgNO₃ was complexed with sodium thiosulfate (17) (1 mM AgNO₃ + 4 mM Na₂S₂O₃) and then diluted to its final concentration.

Treatment 5

Gravistimulated stolon ends were immersed in 100 mM IAA.

RESULTS

In preliminary experiments, it was found that almost all of the upward bending of stolons took place within 23 h, the duration of the experiments described here. The data in Figure 1 show an increase in ethylene production by geostimulated stolons compared to nongravistumulated control plants, whose rate of ethylene production remained at a level of 0.6 μL kg^{-1} h^{-1} throughout the experiment. The increase in ethylene production by horizontal plants was induced after a 3-h lag period and was threefold higher after 15 h of gravistimulation. This was followed by a steady decline over the next 8 h to a level just higher than that in nongravistimulated controls. AgNO₃ had no effect on the induction of gravitationally induced ethylene production. The hypothesis that a part of the stimulation in ethylene production was due to an increase in activity of ethylene forming enzyme was tested by treating explants with IAA and ACC. In both cases, the increase in ethylene production was enhanced by the addition of these compounds (Fig. 1). In gravistimulated nodes incubated with ACC, ethylene production was 10-fold greater and peaked earlier than when nodes were incubated with water (Fig. 1). IAA treatment enhanced ethylene production but did not change the ethylene evolution pattern, because ethylene production peaked after 15 h of gravistimulation similarly to ethylene evolution of gravistimulated stolons incubated with water.

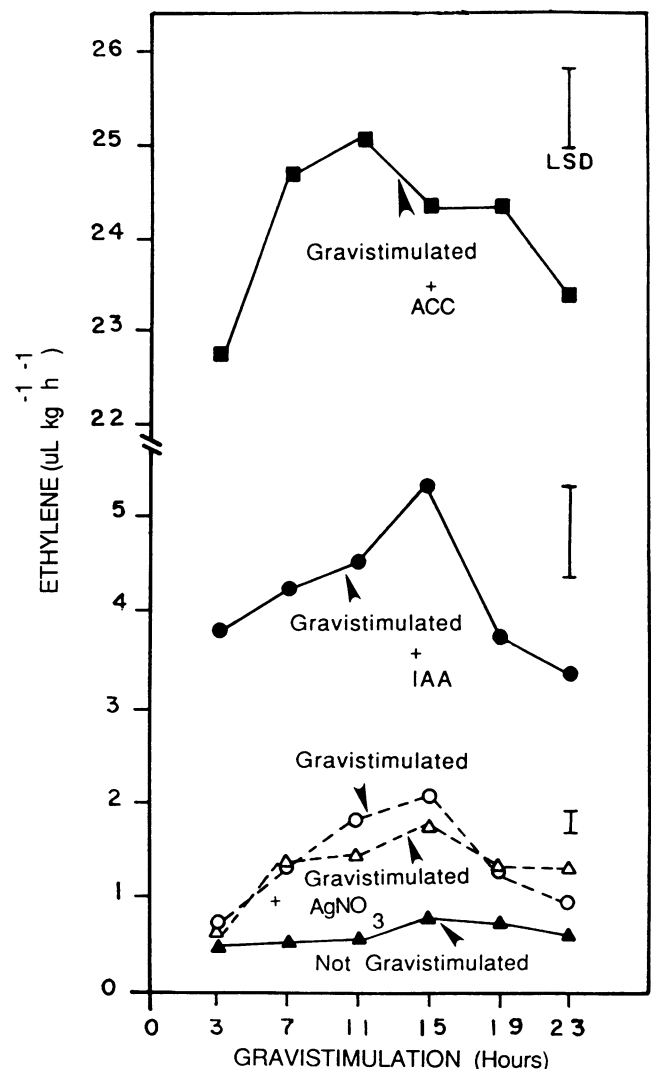[3] Abbreviation: ACC, 1-aminocyclopropane 1-carboxylic acid.

Figure 1. Ethylene evolution in gravistimulated stolons of C. dactylon L. Pers.

Figure 2 shows that there exists a positive correlation (r = 0.99, P = 0.05) between the rate of ethylene production and the degree of curvature of the stolons.

Figure 3 demonstrates that the degree of curvature was promoted in the presence of ethylene, generated either by its release from ethephon [(2-chloroethyl)phosphonic acid] or by the plant when treated with its precursor ACC. The slopes of both ACC and ethephon treated stolons (b = 0.39) were greater than those of gravistimulated stolons incubated with water (b = 0.26). The degree of curvature was also increased by treating gravistimulated stolons with an ethylene enriched atmosphere (data not shown). Inhibition of ethylene action with AgNO₃ resulted in a partial inhibition of curvature shown by the small value of the slope (b = 0.11) for the treatment.

DISCUSSION

There was a 3-h latent period before gravity induced ethylene production was detected (Fig. 1). This latent period could have been due to the utilization of whole tissue in the
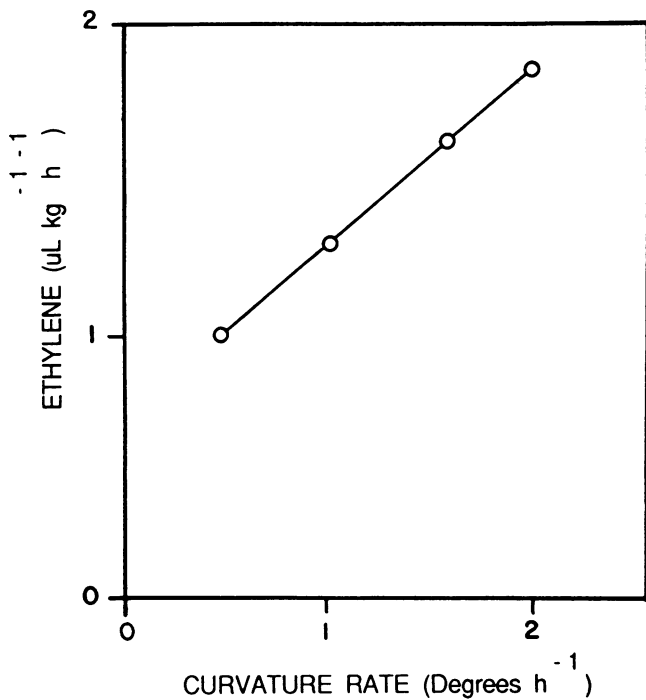
Figure 2. Ethylene and curvature rate relationship in gravistimulated stolons of C. dactylon L. Pers.
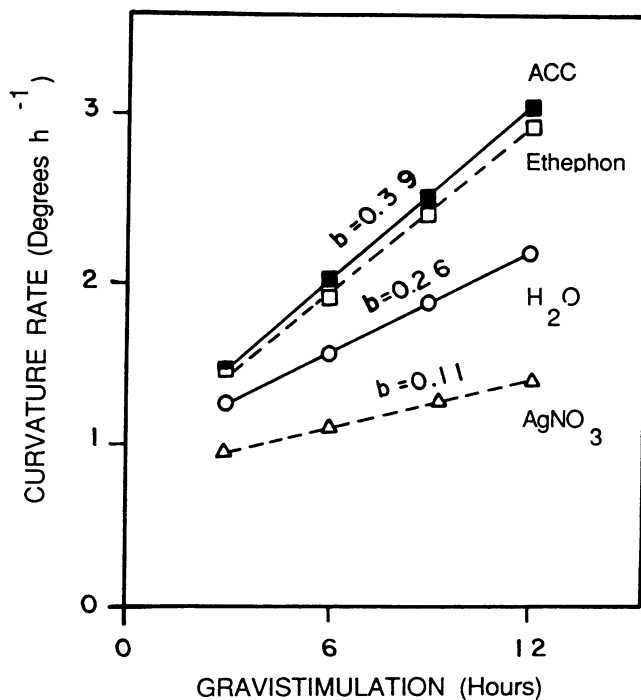

Figure 3. Curvature rate of stolons during gravistimulation of C. dactylon L. Pers. stolons subjected to different treatments.

experiments. If we had differentiated between top and bottom by evacuating the tissue under vacuum we might have reduced the 3-h lag period (6). However, we worked with the whole stolon to minimize disturbance of the tissue. A latent period

in ethylene production also was found in studies with other plant parts or plants either in horizontal orientation (2, 9, 20) or in inverted orientation (13, 15, 16).

Ethylene production in gravistimulated stolons increased until 15 h and thereafter decreased (Fig. 1). There was a positive correlation between curvature rate and ethylene evolution, although only for the first 12 h of gravistimulation. In previous kinetic studies (19) it was shown not only that ethylene increases in basal tissue along with bending of geostimulated seedlings, but also that the addition of ethylene overcomes to some extent the effect of ethylene inhibitors. For other grasses, there also is a great increase of ethylene evolution in nodes placed horzontally (10, 20), but this burst in ethylene was considered to be the result either of gravistimulation-induced stress, or of elevated levels of free IAA that occur in the pulvini, especially on the lower side.

Ethylene evolution was not affected by Ag^+. Delayed curvature nevertheless did occur (Fig. 3). It is possible that in the presence of Ag^+, a higher concentration of ethylene would be necessary to trigger the upward bending of stolons.

Though it was found that IAA treatment did not induce upward bending in gravitationally induced stolons (12), IAA enhanced ethylene production (Fig. 1). These results might have been due to IAA enhancement of sucrose movement toward the treated plant part (18), because it is known that high sucrose concentrations maintain diagravitropic growth of bermudagrass stolons (12). Future work on the role of sucrose in geobending and its relationship with ethylene and IAA is warranted.

The results shown in Figure 1 indicate that gravitational stimulation increased both ACC synthase and ethylene forming enzyme. IAA will activate ACC synthase so that the internal supply of ACC will not be a limiting factor (21) and therefore the rise of ethylene production might be due to higher levels of ethylene forming enzyme. This was confirmed when the system was saturated, so that ACC was no longer a limiting factor. In this case, the additional rise in ethylene production must be due to increased levels of ethylene forming enzyme or other part of the ethylene biosynthetic pathway.

Our results indicate that ethylene promotes the activity of IAA. However, the hypothesis that ethylene is causally related to increased IAA activity, and therefore upward bending, requires further work.

ACKNOWLEDGMENTS

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