

# Ethylene Evolution from Maize (*Zea mays* L.) Seedling Roots and Shoots in Response to Mechanical Impedance

Jorge I. Sarquis, Wayne R. Jordan, and Page W. Morgan\*

Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77840

## ABSTRACT

The effect of mechanical impedance on ethylene evolution and growth of preemergent maize (*Zea mays* L.) seedlings was investigated by pressurizing the growth medium in triaxial cells in a controlled environment. Pressure increased the bulk density of the medium and thus the resistance to growth. The elongation of maize primary roots and preemergent shoots was severely hindered by applied pressures as low as 10 kilopascals. Following a steep decline in elongation at low pressures, both shoots and roots responded to additional pressure in a linear manner, but shoots were more severely affected than roots at higher pressures. Radial expansion was promoted in both organs by mechanical impedance. Primary roots typically became thinner during the experimental period when grown unimpeded. In contrast, pressures as low as 25 kilopascals caused a 25% increase in root tip diameter. Shoots showed a slight enhancement of radial expansion; however, in contrast to roots, the shoots increased in diameter even when growing unimpeded. Such morphological changes were not evident until at least 3 hours after initiation of treatment. All levels of applied pressure promoted ethylene evolution as early as 1 hour after application of pressure. After 1 hour, ethylene evolution rates had increased 10, 32, 70, and 255% at 25, 50, 75, and 100 kilopascals respectively, and continued to increase linearly for at least 10 hours. When intact corn seedlings were subjected to a series of hourly cycles of pressure, followed by relaxation, ethylene production rates increased or decreased rapidly, illustrating tight coupling between mechanical impedance and tissue response. Seedlings exposed to 1 microliter of ethylene per liter showed symptoms similar to those shown by plants grown under mechanical impedance. Root diameter increased 5 times as much as the shoot diameter. Pretreatment with 10 micromolar aminoethoxyvinyl glycine plus 1 micromolar silver thiosulfate maintained ethylene production rates of impeded seedlings at basal levels and restored shoot and root extension to 84 and 90% of unimpeded values, respectively. Our results support the hypothesis that ethylene plays a pivotal role in the regulation of plant tissue response to mechanical impedance.

Successful crop establishment is often hindered by tillage pans and/or high bulk densities in the soil profile that restrict root penetration, thus limiting the soil volume that can be explored for water and nutrients (8, 24). Turgor pressure in enlarging cells of the growing regions provides the driving force to overcome resistance to growth imposed by the rigid cell wall and the soil matrix (14). Root cells may alter their maximum growth potential by increasing turgor through osmoregulation. Maize roots are capable of exerting axial and

radial growth pressures of 1.45 and 0.66 mPa, respectively (19).

Two general approaches have been used to study tissue response to mechanical impedance. One uses either beds of glass beads of small diameter as growth medium (10, 20) or hollow containers in which tissue is brought in contact with a solid barrier (15, 27). However, these systems do not simulate actual soil conditions. A more realistic approach is based on the application of pressure either directly to the growing tissue (1) or to the growth medium (2, 5, 12). We have adopted and modified the triaxial cell system described by Collis-George and Yoganathan (5) to simulate a variable mechanical stress during which ethylene production by the plants can be continuously monitored.

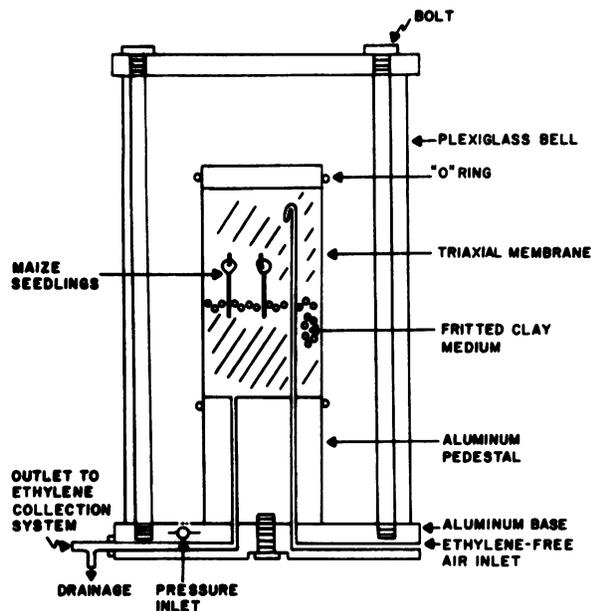
Mechanical impedance generally decreases elongation and increases radial expansion of plant tissue (2, 5, 10, 12, 23). Increasing evidence suggests that the mechanisms by which the coleoptile or root senses an external mechanical impedance and transduces this stimulus into growth pressure involve hormonal regulation (6). A correlation between mechanically induced change in growth pattern and a higher than normal evolution of ethylene from the tissue led to the hypothesis that tissue response is mediated by ethylene (10, 13, 15). In support of this notion, applied concentrations of ethylene as low as  $0.02 \mu\text{L L}^{-1}$  reduced pea epicotyl elongation rate within 5 to 10 min (11). Promotion of lateral expansion in etiolated pea stems may begin after about 1 h of treatment and achieves a maximum by 3 h (9). Maximum growth response is concentration and tissue dependent. In peas, peanuts, beans, and cotton seedlings, elongation and radial expansion are most sensitive to concentrations between  $0.9$  and  $5 \mu\text{L L}^{-1}$  (11). In maize, half-maximal response occurred at  $0.6 \mu\text{L L}^{-1}$  and the response saturated at  $6 \mu\text{L L}^{-1}$  (26). Inhibition of elongation took place within 20 min. The higher the ethylene concentration, the shorter the elapsed time before elongation rates deviated from pretreatment rates and the longer the time required to recover upon removal of the gas (26). However, whether ethylene regulates the changes in growth observed under physical impedance remains controversial (20, 27).

Specific involvement of IAA and ABA in response to mechanical impedance was studied by Lachno *et al.* (16, 17). IAA levels increased 3.5 times in impeded roots. Rauser and Horton (21) showed that a supraoptimal concentration of ethylene inhibited pea root extension less than IAA at concentrations between 5 and  $20 \mu\text{M}$ , indicating that inhibition of root growth may be mechanistically different in each case. Bradford and Yang (4) confirmed that stress-induced ethylene production is independent of IAA effects.

In this report we address the following questions. First, how do primary maize roots and shoots respond to a treatment of mechanical impedance generated by the application of a known pressure to the growth medium? Second, are the kinetics of ethylene evolution under mechanical impedance consistent with a regulatory role of ethylene in the response of plant tissue to mechanical stress? Third, does exogenous ethylene cause changes in growth of intact seedlings that are similar to those observed under mechanical impedance? Finally, is the growth response to mechanical impedance modified by the presence or absence of inhibitors of ethylene synthesis and/or action?

### MATERIALS AND METHODS

Corn seeds (*Zea mays* L., cv Tx 5855) were surface sterilized in 1% NaClO for 10 min, rinsed, and soaked under running water overnight before planting in vermiculite wet to saturation with a  $10^{-4}$  M CaCl<sub>2</sub> solution. After 48 h of germination in the dark, seedlings were selected for uniform root length ( $35 \pm 2$  mm) and transferred in groups of five into triaxial cells (net volume, 100 cm<sup>3</sup>) filled with fritted clay (Absorb-N-Dry) previously screened with a 1.0-mm mesh to

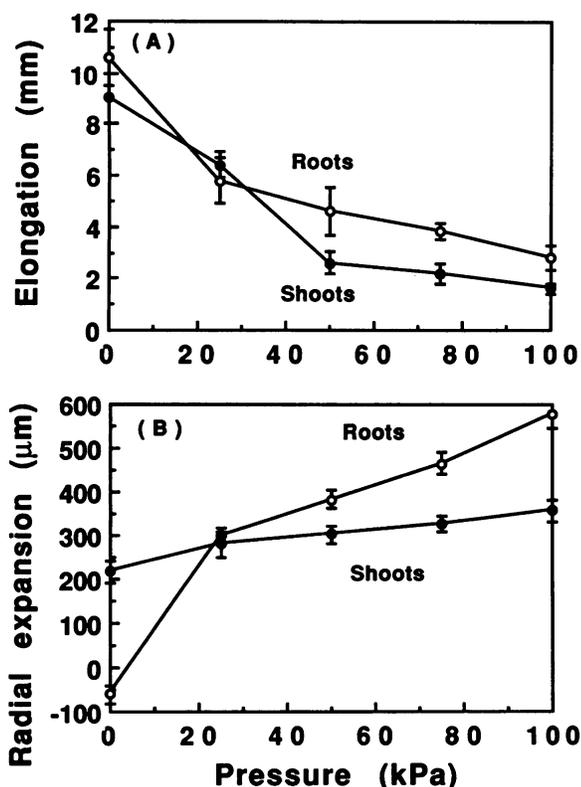


**Figure 1.** Triaxial cell modified to allow continuous flushing with ethylene-free air and connection to an ethylene-collecting system for precise monitoring of ethylene production by intact seedlings. Plants are grown inside a latex membrane filled with fritted clay growth medium and mounted on an aluminum pedestal screwed onto a base plate. An acrylic disc tops the membrane and "O" rings seal both ends of it. A plexiglass bell mounted on an aluminum plate is then screwed onto the base plate. The air inside this chamber can then be pressurized around the triaxial membrane and its contents. Ethylene-free air is carried to the top of the triaxial membrane by an extension of the inlet to ensure complete rinsing of the growth medium. Air flow rate through the void space in the growth medium is not altered by the pressure on the membrane and its contents.

eliminate the smallest size fraction and to obtain a bulk density of  $0.69 \text{ g cm}^{-3}$  (Fig. 1). Calculated total porosity of this material was  $0.73 \text{ g cm}^{-3}$  based on a measured value of  $2.50 \text{ g cm}^{-3}$  for particle density. At the time of transfer, the fritted clay was saturated with  $10^{-4}$  M CaCl<sub>2</sub> solution or with a test solution and allowed to drain. All seedling handling operations were performed under a dim green light (maximum transmission at 525 nm and negligible transmission <475 or >575 nm). After the triaxial cells were assembled (see Fig. 1), they were wrapped in aluminum foil and transferred to a growth chamber at 30°C. A tank of compressed breathing air and a manifold equipped with a mercury manometer allowed each of five cells to be individually pressurized to the desired level. This pressure did not alter water potential of the fritted clay but compressed the medium around the seedling tissue and thus increased the resistance to elongation. Similarly, applied pressure did not alter at all gas flow rates. Seedlings removed from the triaxial cells for growth measurement did not exhibit physical cuts, necrosis, darkening of the tissue, or exudation of fluids. Root and shoot length were measured to the closest millimeter mark past by the elongating tissue with a millimeter scale under a stereoscopic microscope to avoid overestimation and to minimize time required to complete measurements. Root diameter was measured 5 mm behind the tip with a microscope equipped with an ocular micrometer. Shoot diameter was measured with a precision vernier. Growth measurements were made in a humidified chamber under green dim light. All experiments were repeated at least three times.

To monitor ethylene evolution from intact seedlings, the triaxial cells were modified to allow their connection to a flow system which permitted continuous flushing of the gas phase of the growth medium with fresh ethylene-free air at a rate of  $3.5 \text{ L h}^{-1}$ . This air caused only a slight decrease in volumetric water content of the fritted clay during the experimental period which was equivalent to a decrease of about 0.01 mPa in water potential. The stream of air carried the ethylene evolved from the plants through a Poropak R column (50–80 mesh, Alltech) cooled to  $-95^\circ\text{C}$  with acetone and liquid N<sub>2</sub> which worked as an ethylene trap (7). After a 10-min collection period, the air flow was rerouted and the column was heated with boiling water to release the ethylene into a gas chromatograph (Beckman 72–5) equipped with an activated alumina column and a flame ionization detector. The data are reported as ethylene production rates on a fresh weight basis. Seedlings were allowed to acclimate to the growth chamber environment for 2 h before pressure was applied. By this time the rapid burst in ethylene evolution caused by handling had subsided (data not shown).

To test the effect of exogenous ethylene on the growth of corn seedlings growing under otherwise optimal conditions, glass columns filled with vermiculite equilibrated to  $2.5 \text{ g water g}^{-1}$  vermiculite were used that were provided with a bottom and a top port, used as a gas inlet and outlet, respectively. The inlet was connected to a stream of air containing  $1 \mu\text{L L}^{-1}$  ethylene, flowing at a rate of  $1.5 \text{ L h}^{-1}$  controlled by an electronic flow meter. The outlet was connected to an exhaust outside the growth chamber. The concentration of ethylene going into and out of each glass column was moni-



**Figure 2.** Effect of 10-h exposure of corn seedlings to mechanical impedance. A, Root and shoot elongation; B, root and shoot radial expansion. Seedlings were germinated in the dark for 48 h, measured, and then transferred into a fritted clay medium in a triaxial cell. Mechanical impedance was simulated by applied pressure. Diameters were measured 5 mm behind the tip. Vertical bars, sd.

tored periodically by gas chromatography. Measurements from columns with only the vermiculite medium but no seedlings indicated no absorption of ethylene by the vermiculite.

To test the effect of ethylene inhibitors, 48-h-old seedlings were placed on a plastic grid to stand upright with the roots immersed in aerated test solutions in a dark chamber for 2 h. After the seedlings were transferred into the triaxial cell, the fritted clay was watered with fresh test solution. Inhibitors tested included AVG,<sup>1</sup> which inhibits the formation of ACC by ACC synthase (28); AIB, which inhibits the conversion of ACC into ethylene by the EFE complex (22); STS, which inhibits the formation of a functional ethylene-receptor complex (3, 25), and cobaltous ion, which also inhibits EFE complex activity (18). The concentrations used in each case were determined from dose-response curves (data not shown) and represent minimal concentrations required to achieve highest suppression of ethylene synthesis, except for STS which was used at the highest concentration that did not

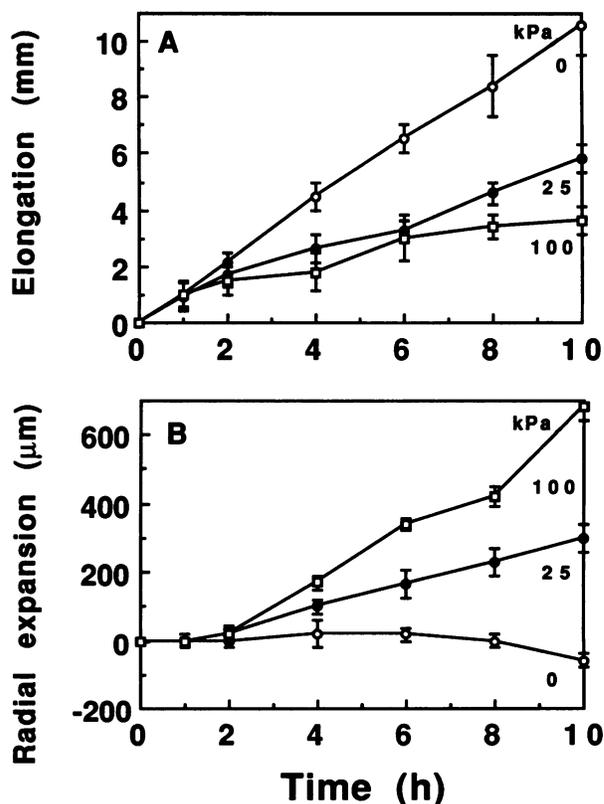
<sup>1</sup> Abbreviations: AVG, aminoethoxyvinyl glycine; ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene-forming enzyme; AIB,  $\alpha$ -aminoisobutyric acid; STS, silver thiosulfate.

inhibit growth. The concentrations used were as follows: 10  $\mu$ M AVG, 20 mM AIB, 1  $\mu$ M STS, and 0.1 mM  $\text{CoCl}_2$ .

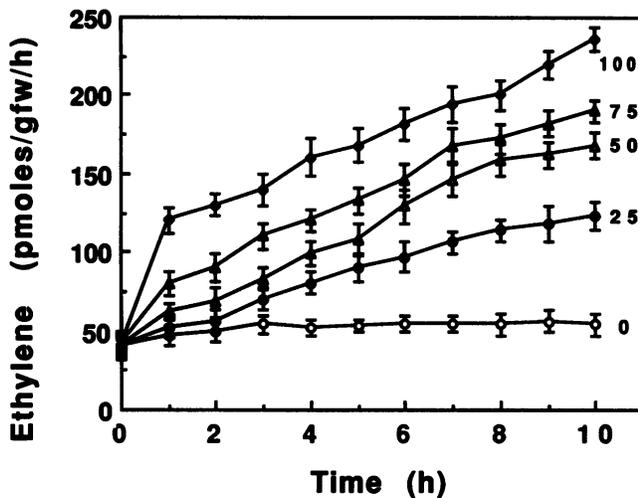
## RESULTS

Root elongation rates were unaffected by transfer to the triaxial cells. The elongation of maize primary roots and preemergent shoots was affected by applied pressures as low as 25 kPa (Fig. 2A). A steep decline in elongation occurred at low pressures, and both shoots and roots responded to additional pressure in a linear manner. A 50% reduction in elongation of both organs occurred at pressures <40 kPa; however, at pressures >40 kPa, elongation of the shoot was more severely inhibited than that of the primary root.

When transferred into the triaxial cells, primary roots and shoots had a diameter of  $1300 \pm 55$  (mean  $\pm$  SD) and  $1890 \pm 97$   $\mu$ m, respectively. Radial expansion was promoted in both organs by mechanical impedance (Fig. 2B). Primary roots typically became thinner when allowed to grow unimpeded, and shoot diameter increased about 10% under the same conditions. In contrast, 25 kPa of pressure caused a 23% increase in root tip diameter over the initial value. Maximum root tip diameter increase was 44%, and it occurred under 100 kPa of pressure. Shoots showed a slight increase in diameter promoted by mechanical impedance, although not nearly as dramatic as that observed in root tips. At pressures <25 kPa, the increase in root tip diameter per unit of pressure



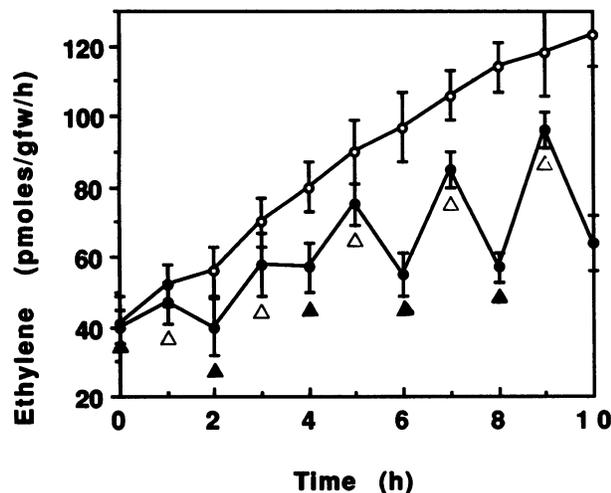
**Figure 3.** Time course of the growth of primary roots under pressure. A, Elongation; B, radial expansion. Vertical bars, sd.



**Figure 4.** Time course of ethylene evolution from intact corn seedlings growing under pressures of 0, 25, 50, 75, or 100 kPa. Ethylene produced was continuously flushed by a stream of air onto an ethylene-collecting system directly connected to a gas chromatograph. Vertical bars, sd.

was greater than at pressures between 25 and 100 kPa. Changes in elongation rate and radial expansion were not observed until 4 h after initiation of the stress treatment (Fig. 3). On the other hand, all levels of pressure promoted ethylene evolution as early as 1 h after initiation of treatment. After 1 h, ethylene evolution rates from seedlings subjected to 25, 50, 75, and 100 kPa had increased 10, 32, 70, and 255% over the base level, respectively (Fig. 4). After this time, the rate of ethylene evolution increased linearly for at least 10 h at all pressures tested.

When seedlings were subjected to a series of hourly cycles of pressure, ethylene production rates increased or decreased rapidly illustrating tight coupling between mechanical imped-

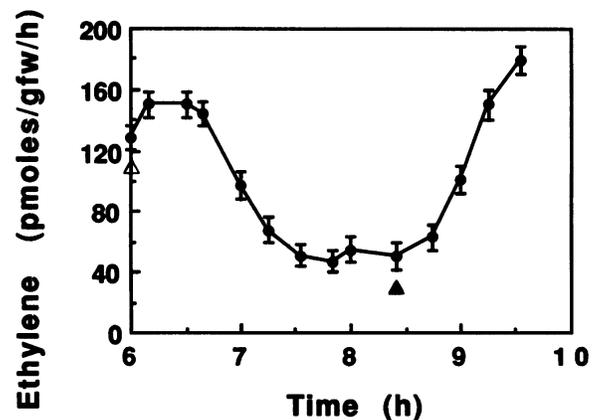


**Figure 5.** Ethylene production rate from corn seedlings subjected to 25 kPa continuously (O) or hourly cycles of 0 and 25 kPa (●). ▲, pressure turned on; △, pressure turned off. Vertical bars, sd.

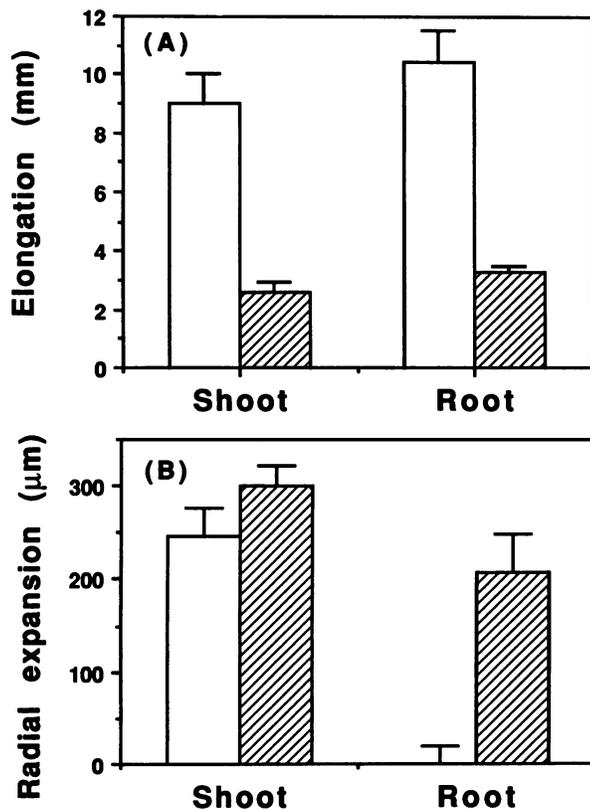
ance and tissue response (Fig. 5). The increase in ethylene production rate was greater with each succeeding cycle and the new base rate increased after the second cycle. However, the highest ethylene evolution rate at the end of each cycle was never quite as high as it was from seedlings kept continuously under pressure. A detailed time course of ethylene production when the pressure was reduced to zero after 6 h at 50 kPa showed a transient increase in production rate, followed by a decrease to the base rate in <1.5 h (Fig. 6). After the ethylene production rate had remained unchanged for about 1 h, pressure was reapplied, and ethylene production increased rapidly to a higher rate than before in 1 h. This increase after reapplication of pressure was significant in about 30 min.

When maize seedlings were allowed to grow unimpeded but continuously exposed to a concentration of  $1 \mu\text{L L}^{-1}$  ethylene for 10 h, the changes in growth were qualitatively similar to those observed under mechanical impedance (Fig. 7). Exposure to ethylene caused a 71% reduction in shoot elongation and a 68% reduction in root elongation with respect to controls after 10 h. Shoot radial expansion among controls was moderate, and radial expansion did not occur in control root tips. Fumigation with ethylene did not increase shoot diameter significantly over controls, but it caused a fivefold increase in root tip diameter. However, maximum root radial expansion was substantially less than that observed under all pressures tested.

Results from experiments to study the effect of inhibitors of ethylene synthesis or action on the growth of impeded tissue are summarized in Table I. Whereas ethylene production by impeded tissue was almost 3 times that of controls after 5 h under 50 kPa, treatment with either  $10 \mu\text{M AVG}$ ,  $10 \mu\text{M AVG} + 1 \mu\text{M STS}$ , or with  $10 \mu\text{M AVG} + 20 \text{ mM AIB} + 20 \mu\text{M CoCl}_2$  caused ethylene production rates to be maintained near that of controls. Treatment of impeded seedlings with either 20 mM AIB or 0.1 mM  $\text{CoCl}_2$  allowed ethylene production rates to increase to about twice the control rate. In contrast, impeded seedlings treated with  $1 \mu\text{M STS}$  produced 10 times as much ethylene as controls after 5 h of treatment.



**Figure 6.** Immediate effect of stress relief and reinitiation on ethylene evolution rate from intact corn seedlings grown under 50 kPa of pressure. ▲, pressure turned on; △, pressure turned off. Vertical bars, sd.



**Figure 7.** Effect of exogenous ethylene on the growth of corn primary roots and shoots. A, Elongation; B, radial expansion. Seedlings were grown in a vermiculite medium flushed by a continuous air stream containing  $1 \mu\text{L L}^{-1}$  ethylene for 10 h. □, Controls; ▨, treated with ethylene. Vertical bars, sd.

Impeded shoots and roots elongated only 50% as much as controls after 5 h, whereas treatment with inhibitors of ethylene synthesis or action allowed maintenance of growth to various extents (Table I). This effect was more pronounced in the root than in the shoot. The combination of  $10 \mu\text{M}$  AVG with  $1 \mu\text{M}$  STS was particularly effective in counteracting ethylene effects on tissue elongation, maintaining shoot and root extension at 84 and 90% of the unimpeded values, respectively. AVG,  $10 \mu\text{M}$ , in combination with  $20 \text{ mM}$  AIB and  $20 \mu\text{M}$   $\text{CoCl}_2$  was somewhat less effective; in this case shoot and root elongation were restored to 62 and 82% of unimpeded values, respectively. Use of  $20 \text{ mM}$  AIB alone resulted in a better maintenance of growth than  $0.1 \text{ mM}$   $\text{CoCl}_2$ .  $\text{CoCl}_2$  was the only inhibitor that caused substantial reduction in fresh weight accumulation, suggesting a possible toxic effect, which was also expressed in inhibition of elongation. STS at  $1 \mu\text{M}$  was more efficient than AIB in counteracting ethylene-induced effects but was not as effective as when applied in combination with  $10 \mu\text{M}$  AVG.

Diameters of impeded roots of control seedlings increased  $235 \mu\text{m}$  after 5 h under pressure (Table I). AVG, STS, AVG + STS, AIB, and AVG + AIB +  $\text{CoCl}_2$  treatments all suppressed mechanically induced root radial expansion by 46 to 55%.  $\text{CoCl}_2$  suppressed root radial expansion by only 30% when used alone. Mechanically induced shoot radial expansion was suppressed between 20 and 44% by AVG, AIB, STS, or their combinations. When applied alone,  $\text{CoCl}_2$  suppressed shoot radial expansion to less than that of the nonpressurized control, an effect that again suggests toxicity.

## DISCUSSION

Effects of mechanical impedance on plant tissue elongation and radial expansion observed in this study were qualitatively similar to those reported for a range of crop species. We adopted and modified the approach followed by Barley (2),

**Table I.** Effect of Inhibitors of Ethylene Synthesis or Action on the Growth of Maize Seedlings under Mechanical Impedance

Seedlings were incubated in the test solutions for 2 h in the dark before transfer to a fritted clay medium in a triaxial cell where they were watered with fresh test solution. Growth measurements were made 5 h after pressure application. AVG inhibits ACC synthesis; AIB and  $\text{Co}^{2+}$  inhibit conversion of ACC into ethylene, and  $\text{Ag}^+$  inhibits ethylene action. Concentrations used were  $10 \mu\text{M}$  AVG,  $20 \text{ mM}$  AIB,  $0.1 \text{ mM}$   $\text{Co}^{2+}$ , and  $1 \mu\text{M}$  STS; when combined with AVG and AIB,  $\text{Co}^{2+}$  was used at a concentration of  $20 \mu\text{M}$ .

Treatment	Elongation		Diameter Increase		Fresh Wt	Ethylene <sup>a</sup>	
	Shoot	Root	Shoot	Root		0 h	5 h
	mm		$\mu\text{m}$		mg/5 seedlings	pmol/g fresh wt/h	
No pressure applied							
Control	$4.5 \pm 0.8$	$5.1 \pm 1.0$	$109 \pm 25$	$0 \pm 20^b$	$165 \pm 30$	$49 \pm 6$	$42 \pm 7$
50 kPa pressure applied							
No inhibitor	$2.2 \pm 0.4$	$2.6 \pm 0.5$	$152 \pm 20$	$235 \pm 20$	$116 \pm 23$	$49 \pm 5$	$110 \pm 8$
AVG	$2.6 \pm 0.4$	$3.7 \pm 0.3$	$122 \pm 18$	$110 \pm 25$	$161 \pm 28$	$29 \pm 7$	$39 \pm 8$
$\text{Co}^{2+}$	$1.1 \pm 0.1$	$2.0 \pm 0.1$	$40 \pm 10$	$164 \pm 25$	$84 \pm 18$	$54 \pm 9$	$82 \pm 10$
STS	$2.4 \pm 0.2$	$3.8 \pm 0.4$	$86 \pm 18$	$132 \pm 28$	$131 \pm 26$	$52 \pm 10$	$425 \pm 33$
AVG + STS	$3.8 \pm 0.6$	$4.6 \pm 0.5$	$100 \pm 23$	$108 \pm 23$	$160 \pm 15$	$28 \pm 10$	$47 \pm 9$
AIB	$2.1 \pm 0.2$	$3.2 \pm 0.2$	$90 \pm 13$	$120 \pm 32$	$130 \pm 30$	$49 \pm 9$	$80 \pm 9$
AVG + AIB + $\text{Co}^{2+}$	$2.8 \pm 0.4$	$4.2 \pm 0.4$	$105 \pm 17$	$130 \pm 20$	$130 \pm 18$	$21 \pm 12$	$45 \pm 8$

<sup>a</sup> Ethylene measurements reported correspond to 0 and 5 h after initiation of pressure treatment.

<sup>b</sup> Typically, root diameter of control seedlings either increased or decreased very slightly.

Goss *et al.* (12), and Collis-George and Yoganathan (5), based on the concept of shear strength, a measure of the force required to cause soil failure under external pressure such as that caused by expanding roots or emerging shoots.

Using a triaxial cell system (Fig. 1), we demonstrated an inhibition of axial growth and a stimulation of radial expansion induced by a high resistance simulated by the application of pressure to the growth medium (Fig. 2).

We confirmed a strong correlation between mechanical impedance and increased ethylene production. Furthermore, our results illustrate that the kinetics of ethylene production and morphological changes induced by the stress are consistent with a regulatory role for ethylene in the tissue response to mechanical impedance, because increases in ethylene production rates preceded measurable morphological changes by at least 1 h (Figs. 3 and 4). In fact, even at the lowest pressure applied, there was a measurable increase in ethylene production rate, but no decrease of elongation rate was observed before 2 h of treatment, even at the highest pressure tested. When responses to 100 kPa are compared, ethylene production was 3 times higher than in controls after 1 h, but elongation and radial expansion were indistinguishable from the control at 2 h. Admittedly, the elongation measurements may not be as precise as ethylene measurements. However, the technique used to measure length (see "Materials and Methods") minimized the opportunity for overestimation; therefore, any potential error would shift all treatment curves in Fig. 3 in the same direction. Also, we do not believe that the magnitude of any potential error in root measurements would significantly alter our conclusions.

When seedlings were grown in moist vermiculite aerated continuously for 10 h with an air stream containing  $1 \mu\text{L L}^{-1}$  of ethylene (Fig. 7), elongation was reduced by about 70% in both shoots and roots; a comparable reduction in shoot length was observed under 45 kPa, but it took twice as much pressure to reduce root length to the same extent. With regard to radial expansion, fumigation with  $1 \mu\text{L L}^{-1}$  ethylene produced results similar to those obtained under very low applied pressures. These observations suggest that, although exogenous ethylene does duplicate mechanically induced morphological changes, differences in tissue permeability as well as sensitivity may be involved in the differential response of shoots and roots to applied pressure or ethylene treatment.

We found that AVG, AIB, AVG + STS, and AVG + AIB +  $\text{CoCl}_2$  all allowed growth of impeded seedlings to proceed to some extent. AVG combined with STS was particularly effective, allowing maintenance of shoot and root elongation at 84 and 90% of unimpeded values, respectively, and mechanically induced radial expansion was reduced by 46 to 55%.

These results do not support a recent report (20) in which it was concluded that ethylene did not play an important role in tissue response to mechanical impedance. We believe the discrepancy between our results and this report may be explained by the differences in the way mechanical impedance was simulated in each case, the way inhibitors were applied, and the manner and timing of ethylene production measurements. It is important to recognize that there is a difference between compressing the growth medium around growing tissue and confronting the tissue with an impenetrable me-

dium. In the latter case, if elongation is absolutely blocked, then, regardless of whether ethylene production decreases or increases, elongation growth cannot occur. Because seedlings in the soil are both laterally and vertically constrained, the application of pressure to the medium that the roots are trying to penetrate may better represent actual soil conditions. If inhibitors are applied at the same time as the stress, the time it takes for the inhibitors to be absorbed will allow continued ethylene production and action at least for some time, which may be enough to cause a response. Pretreatment of the plant material with inhibitors will overcome this problem. Moss *et al.* (20) reported observations of ethylene production made 40 h after inhibitor treatment but did not record the status before the initiation of the impedance treatment or the short-term responses. We applied inhibitors and monitored ethylene production even before pressure was applied. Ethylene production rate in impeded tissue treated with inhibitors was at the level of control seedlings or lower even before initiation of the pressure treatment (Table I).

Thus, in the system used here, increased mechanical resistance promoted ethylene production and the effect was reversed by inhibitors of ethylene synthesis or action.

Using a barrier raised to meet laterally unsupported roots as a system to simulate mechanical impedance, Whalen (27) found inhibition of ethylene synthesis and accumulation of ACC in maize primary roots within 5 h. Because roots could bend and grow laterally without restriction, growth rates of control and impeded roots appeared to be the same after 100 and 300 min, and no swelling was reported. It appears that the role of ethylene was not fully tested in this study, because the experimental set up allowed growth to continue after the roots bent away from the restriction, whereas in the soil they are constrained both vertically and laterally.

The effects of inhibitors were consistent with the hypothetical role of ethylene in the alteration of growth by mechanical impedance and their action on ethylene physiology (Table I). STS alone increased ethylene synthesis, presumably by a heavy metal toxicity (3, 29), but it reduced the effects of impedance on elongation and radial expansion. The latter effect was as anticipated from its inhibition of ethylene action, presumably at a receptor (3, 29). When STS and AVG were combined, the silver toxicity was not expressed as increased ethylene production and the prevention of impedance effects was maximal. The interpretation is that partially blocking both ACC synthase and the ethylene receptor site affords more protection against ethylene than doing either alone. AIB and  $\text{CoCl}_2$ , which act on the EFE, were least effective at protecting against impedance stress.

Our results support the hypothesis that ethylene plays a pivotal role in the regulation of plant tissue response to mechanical impedance, because morphological changes are preceded by increased ethylene production and the inhibition of ethylene synthesis and/or action at least partially restores an unimpeded pattern of growth.

#### ACKNOWLEDGMENTS

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