Role of Ethylene in *Lactuca sativa* cv 'Grand Rapids' Seed Germination

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ABSTRACT

Promotion of thermoinhibited (30°C) lettuce (*Lactuca sativa* cv 'Grand Rapids') seed germination by ethylene is similar to the action of the gas in other hormonal systems. Ethylene was more active than propylene and ethane was inactive. An inhibitor of ethylene production, aminoethoxyvinylglycine, reduced ethylene evolution and germination. Inhibitors of ethylene action such as, 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole, 2,5-norbornadiene, and silver thiosulfate inhibited germination and the effect was reversed by the addition of ethylene to the gas phase. The action of ethylene appears to be due to the promotion of radial cell expansion in the embryonic hypocotyl. The action of N6-benzyladenine and fusiccocin, which also overcome thermoinhibition, appears to be due to a promotion of hypocotyl elongation. None of the germination promoters studied appeared to function by lowering the mechanical resistance of the endosperm to embryonic growth. Data presented here are consistent with the view that ethylene plays a role in lettuce seed germination under thermoinhibited and normal conditions.

Ethylene has been shown to promote seed germination (1). However, seeds of many plants do not respond to ethylene, and some of the promotive effects are small (38). The most thoroughly studied system is the lettuce seed (actually an achene or cypsela). In lettuce seeds, germination may be considered as a growth, or cell expansion process, in which the expansion of the hypocotyl region of the embryo results in the penetration of the endosperm by the radicle. In lettuce, the endosperm has two functions. The radicle end acts as a restriction or barrier for embryo growth. The remaining portion surrounding the cotyledons is enzymically digested to provide nutrients for the growing seedling following germination. In this paper, the part of the endosperm which restrains growth of the embryo and is penetrated by the radicle is called barrier endosperm.

Factors which prevent or promote germination are associated with the control of embryonic growth. As long as sufficient phytochrome is in the far red absorbing form, and the temperature is less than 25°C, fully imbibed seeds germinate within 24 h (8, 21, 27). As the temperature is raised to 30°C, germination is inhibited. This effect is often called thermodormancy, but a more appropriate term is thermoinhibition, since the embryos themselves germinate readily if the endosperm is removed or punctured. In other words, it appears as if the effect of temperatures above 25°C is to inhibit the ability of the embryo to develop sufficient force to penetrate the barrier endosperm. Lettuce seed germination can also be prevented at nonthermoinhibited temperatures, below 25°C, by placing them in an osmoticum such as mannitol. This method of inhibiting germination is called

osmotic restraint (22). Both thermoinhibition and osmotic restraint can be overcome by removing the endosperm (10), or treating seeds with ethylene (2), cytokinin (28), or FC[1] (6).

Because of its simplicity and rapid response, ethylene enhanced germination of thermoinhibited lettuce seeds is a convenient system to study ethylene action. However, other than the observation that ethylene promotes lettuce seed germination, little is known concerning its endogenous role in germination, which cells of the endosperm or embryo respond to the gas, or its mechanism of action. The experiments described here were designed to evaluate the concept that promotion of germination was similar to other physiological effects of the gas in terms of dose response, analog action, and effects of inhibitors of ethylene action. The role of ethylene as an endogenous mediator or intermediate in the action of other germination promoters was also studied. In addition, the action of ethylene on the structural integrity of the endosperm and the role of the endosperm as a physical barrier to germination was evaluated.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* cv 'Grand Rapids') seeds were purchased from Burpee Seed Co., Warminster, PA.

STS was prepared by mixing equal volumes of 2 mM $AgNO_3$ and 8 mM $Na_2S_2O_3 \cdot 5H_2O$. Ethylene was measured by GC using an alumina column and a flame ionization detector. Ethylene production was measured by sampling the gas phase of 25 ml Erlenmeyer flasks containing 1 g (dry weight) of seeds on a moist filter paper disk.

Germination studies were performed by placing 4 groups of 25 seeds in Petri plates containing a 9-cm diameter Whatman No. 3 filter paper soaked with 4 ml of the appropriate solution. Seeds were imbibed for 2 to 4 h in the dark at 30°C, removed and exposed to fluorescent lights in the laboratory for at least 5 min, and then placed in the temperatures or gas phases indicated. This procedure was used to ensure that embryos were fully imbibed, and that their phytochrome was in the far red absorbing form at the start of the experiments. Seeds were gassed by placing them in 4 L plastic paint cans (Freund Can Co., Chicago, IL) or 10 L desiccators fitted with rubber vaccine stoppers. Except where noted, germination was measured after 24 h. Germination was scored by counting the seeds whose radicles emerged from the seed coat. Data are expressed as the mean of the four 25 seed samples and were separated by LSD as calculated by the Duncan's multiple range test. All experiments were replicated two or more

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[1] Abbreviations: FC, fusicoccin; AVG, aminoethoxyvinylglycine; DIHB, 3,5-diiodo-4-hydroxy-benzoic acid; Ethrel, 2-chloroethyl-phosphonic acid; MCEB, 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole (DU 17623, TH 6241); N6-BA, N6-benzyladenine; NBA, 2,5-norbornadiene; STS, silver thiosulfate.

times.

Embryos and endosperms were prepared by cutting 1 mm of tissue from the cotyledonary end of imbibed seeds. The embryos were forced out of the endosperms by pushing a pointed rubber eraser against the lower cotyledon end of the seed. Care is needed in this process since the embryos are easily damaged in the dissection process. Isolated embryos were considered germinated when the hypocotyl elongated and root hairs appeared on the root.

Hyperbaric studies (1–5 bar at 25°C) were performed by placing Petri plates in a soil water extraction apparatus manufactured by Soil Moisture Equipment Co., Santa Barbara, CA.

The penetration force required to puncture the barrier endosperm was measured in centinewtons (1 centinewton = 1.02 g) with a force gauge (Correx 30 g gauge, Haagstreit Bern, Wagner Instruments, Greenwich, CT) fitted with a 0.4 mm steel rod with a spherical tip. The endosperm was held with forceps and the steel rod inserted inside against the barrier endosperm. The force gauge was then rotated until the barrier endosperm was penetrated by the steel rod (Fig. 1).

RESULTS

Figure 2 shows that thermoinhibition at 30°C was overcome by 10 μl/L ethylene. However, at higher temperatures, such as

33°C, the ability to override thermoinhibition was lost.

The action of two ethylene analogs with low (propylene), and no (ethane) hormonal activity are shown in Figure 3. Propylene had about 1% the activity of ethylene, and ethane had none.

The germination promoting activity of ethylene may be associated with its ability to increase the growth potential of the embryo. There are two methods of demonstrating this effect. The first consists of using the established method of placing excised embryos in a series of mannitol solutions. As shown in Figure 4, ethylene increased germination of embryos incubated in mannitol solutions of increasing osmotic potential.

A second approach measured the ability of ethylene, and the cytokinin N6-BA, to promote the germination of seeds exposed to hyperbaric pressure. As shown in Table I, 5 bars of pressure at 25°C inhibited germination. The hyperbaric effect is interpreted here as loading mechanical pressure against the endosperm and subsequently the embryo. This experiment can be thought of as the mechanical equivalent of placing embryos or seeds in solutions of mannitol or other osmotica. The germination inhibiting effect of hyperbaric conditions could be circumvented by slitting the top part of the endosperm, or excising the embryo. This observation suggests that the hyperbaric effect was not due to toxic effects of increased CO_2 or O_2 partial pressures. As shown in Table I, N6-BA and ethylene (supplied as ethrel), also promoted germination of intact seeds.

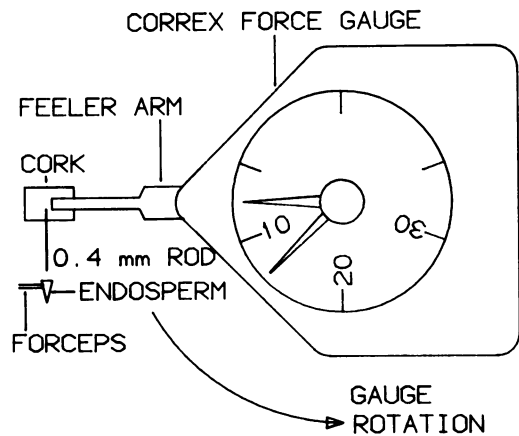The experiment shown in Figure 5 was designed to test the

FIG. 1. Technique used to measure breakstrength of lettuce barrier endosperm tissue. Endosperms are held over penetrating rod by means of a forcep. Penetrometer measurements are made by rotating the force gauge until the rod penetrates the radicle end of the endosperm.
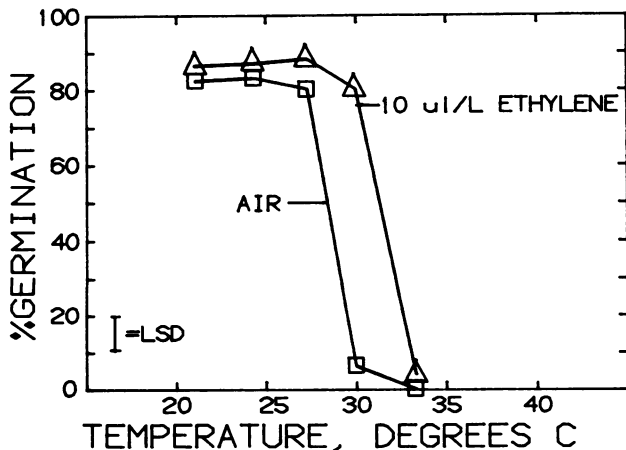

FIG. 2. Ability of ethylene to overcome the effect of thermoinhibition in lettuce seeds. Percent germination in this and subsequent figures (except Fig. 13) was measured at 24 h following imbibition.
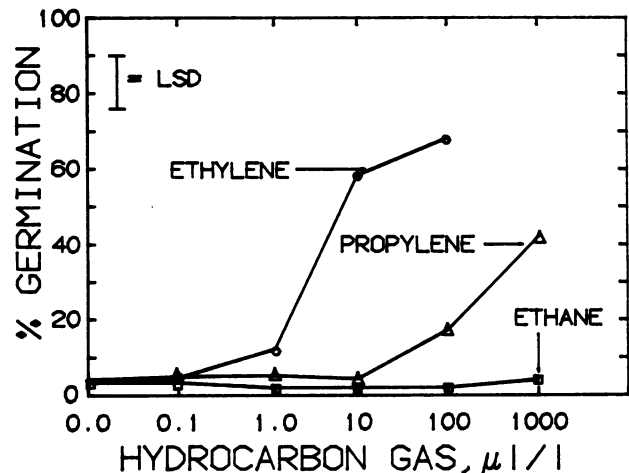

FIG. 3. Ability of ethylene and ethylene analogs to overcome thermoinhibition in lettuce seeds.
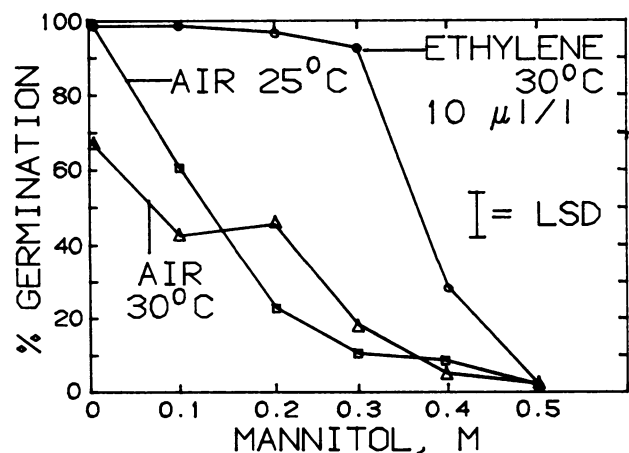

FIG. 4. Effect of ethylene on the inhibitory effect of mannitol on the germination of lettuce embryos.

Table I. *Germination of Lettuce Seeds under Hyperbaric (5 bar) Conditions*

Effect of cutting a vertical 1 mm slit on the apical end of the seed, freeing the embryo from the endosperm, or treating the seeds with 50 μM N6-BA, and 1 mM ethrel on germination.

Treatment	Germination	
	1 bar	5 bar
	%	
Intact control	81 a[a]	16 d
Split endosperm	81 a	84 a
Free embryo	64 bc	60 b
N6-BA	86 a	90 a
Ethrel	83 a	51 c

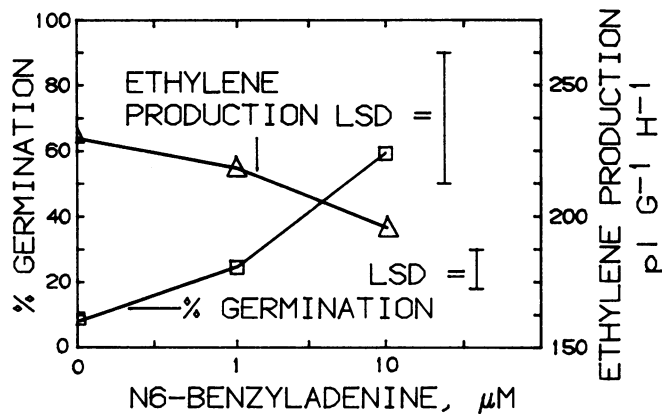[a] Identical letters indicate means were similar at the 5% level.

FIG. 5. Effect of N6-BA on ethylene production and germination of lettuce seeds at 30°C. Ethylene production was measured for the 8 h period following imbibition with various concentrations of N6-BA shown.
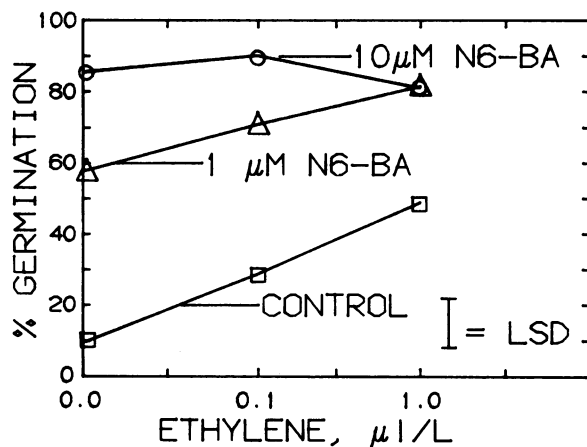

FIG. 6. Interaction of ethylene and N6-BA on lettuce seeds germinating at 30°C. Curve labeled control treated with ethylene only.

idea that N6-BA promotes germination through an enhancement of ethylene production. The data shown indicates that ethylene does not mediate the effect of N6-BA since N6-BA did not increase ethylene production in the 8 h period following imbibition. In addition, no evidence was obtained for an enhancement or potentiation of ethylene activity by N6-BA. A combination of low concentrations of ethylene and N6-BA resulted in an additive as opposed to synergistic effect on germination (Fig. 6).

Ethylene, N6-BA, and FC may stimulate germination by pro-

moting growth of the hypocotyl region of the embryo axis. However, as shown in Table II, they have different effects on hypocotyl or root growth. Ethylene increased radial expansion of the hypocotyl while N6-BA and FC increased elongation. Ethylene, and to a smaller extent N6-BA, inhibited root elongation. FC had the opposite effect and promoted root growth.

Thermoinhibition of seed germination may be the result of the inability of the embryo to develop enough force to penetrate the barrier endosperm. The role of the endosperm as a controlling factor during germination was evaluated by measuring the effect of temperature and hormonal treatments on the structural integrity of the barrier endosperm. As shown in Figure 7A, most of the mechanical resistance of the barrier endosperm was lost as the seeds germinated in water. In the curve labeled water, endosperms were removed from the nongerminated seeds after 24 h. The endosperms of these seeds showed little or no resistance to the penetrometer. By 48 h, all of the seeds had germinated and hence the penetration force is considered as zero for these seeds. When germination was blocked by placing the seeds on 0.5 M mannitol, the endosperms lost some of their structural integrity by 24 h. However, no additional loss of endosperm integrity was

Table II. *Effect of Ethylene, N6-BA, and FC on the Growth of the Germinating Lettuce Hypocotyls and Roots at 30°C*

Controls were germinated by placing seeds at 20 and 30°C.

Seedling Part	Control		Size after 24 h Treatment		
	20°C	30°C	Ethylene 10 μl/L	N6-BA 10 μM	FC 10 μM
			mm		
Hypocotyl length	0.73 c[a]	0.80 c	0.89 c	1.36 b	1.90 a
Hypocotyl diameter	0.63 c	0.76 b	0.99 a	0.80 b	0.76 b
Root length	1.93 d	3.65 b	2.49 cd	2.94 bc	5.35 a

[a] Identical letters indicate means within a horizontal row were similar at the 5% level.
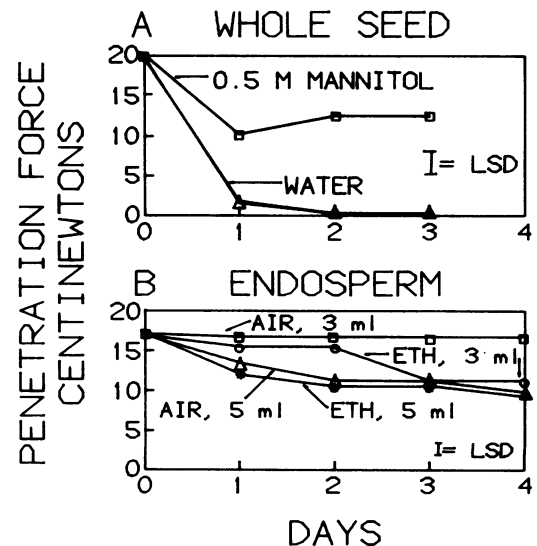

FIG. 7. Changes in the mechanical integrity of barrier endosperm during germination. A, Force required to penetrate endosperms isolated from intact seeds incubated on water or 0.5 M mannitol at 20°C. Seeds incubated on water germinated 80% by d 1 and 100% by d 2. Seeds incubated on mannitol did not germinate during the course of the experiment. Endosperms isolated from seeds on day indicated. B, Force required to penetrate endosperms isolated on d 0 and incubated at 20°C. Endosperms were incubated on filter paper moistened with either 3 ml or 5 ml of water. Endosperms were incubated in air or 10 μl/L ethylene as indicated.

observed during continued incubation. The data shown in Figure 7B described results with isolated endosperms incubated under moist (filter paper soaked with 3 ml of water) or excess liquid water (5 ml of water) conditions. The treatment with 5 ml of water resulted in some softening of the endosperm by d 1. Ethylene had no effect on the endosperm under these conditions. The endosperm retained most of its structural integrity when incubated on 3 ml. Unlike the results with 5 ml of water, ethylene reduced the structural integrity by d 3.

A comparison of the effect of ethylene and other hormones on the structural integrity of endosperm tissue under moist or fluid water conditions is shown in Table III. Ethylene was the only compound that reduced the breakstrength of barrier endosperm tissue. However, it was only active after 2 d of incubation and when the endosperms were incubated at 20°C in 3 ml of water.

Ethylene production by seeds incubated at 25 and 30°C was monitored to see if thermoinhibition was a result of an inhibition of ethylene production at the higher temperature. As shown in Figures 8 and 9, more ethylene was produced by seeds incubated at 30°C in an 8 h period following inhibition.

An inhibitor of ethylene production, AVG, was used to see if endogenous ethylene production promoted or controlled germination at normal temperatures. As shown in Figure 9, 1 mM AVG caused about a 50% reduction in ethylene production.

Table III. *Effect of N6-BA, Gibberellic Acid, and Ethylene on the Breakstrength of Isolated Endosperms*

Breakstrength measurements were made after 3 d incubation at either 20 or 30°C. Endosperms were placed on filter papers with either low (3 ml) or high (5 ml) moisture levels.

	Endosperm Breakstrength			
	Temperature			
Treatment	20°C		30°C	
	Moisture levels			
	3 ml	5 ml	3 ml	5 ml
	centinewtons			
Initial, day 0		17.4 a[a]		
Control, 3 days	15.8 abc	9.4 h	16.7 ab	12.5 ef
N6-BA, 10 μM	14.3 cde	9.1 h	16.4 ab	9.8 gh
GA₃, 10 μM	14.8 bcd	9.9 gh	15.8 abc	11.7 fg
Ethylene, 10 l/L	10.5 gh	10.3 gh	17.3 a	13.6 def

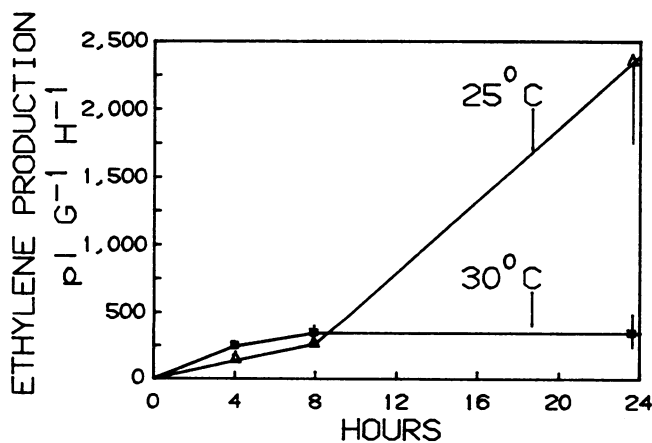[a] Identical letters indicate means are similar at the 5% level.

FIG. 8. Ethylene production by lettuce seeds under thermoinhibition (30°C) and nonthermoinhibited conditions (25°C). Germination of nondormant seeds had commenced by 14 h. The high rate of ethylene production at 24 h for the 25°C treatments represents the high rate of ethylene production from growing seedlings.
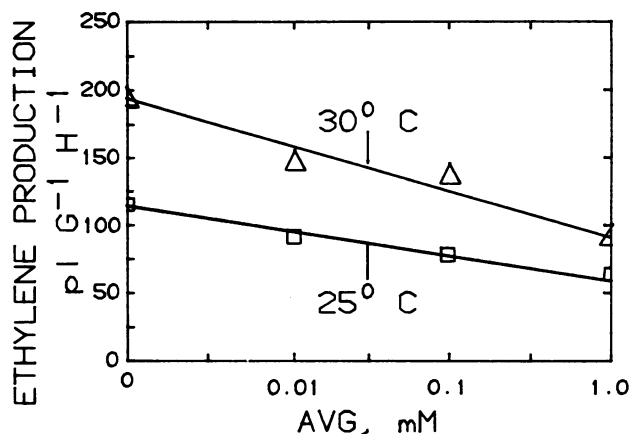

FIG. 9. Effect of AVG on ethylene production by thermoinhibited and nonthermoinhibited lettuce seeds. Data shown represent ethylene production during an 8 h period following imbibition.
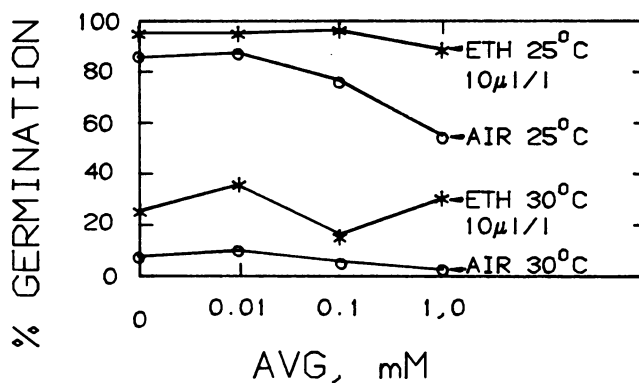

FIG. 10. The ability of ethylene to overcome the inhibition of germination by AVG.
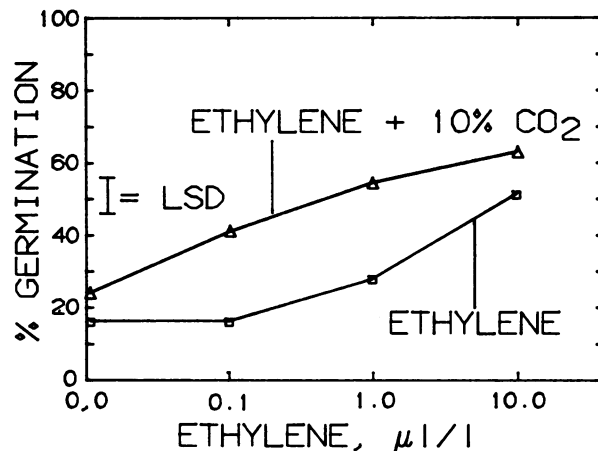

FIG. 11. The enhancement of ethylene action on lettuce seed germination by CO₂.

AVG also reduced the germination of normal and thermoinhibited seeds (Fig. 10). Most, but not all, of this inhibition could be overcome by the addition of ethylene to the gas phase.

The endogenous role of ethylene in germination was also studied by examining the effects of inhibitors of ethylene action. The inhibitors used for these studies were: CO₂, STS, NBA, DIHB, and MCEB.

As shown in Figure 11, CO₂ promoted rather than inhibited ethylene action in germination.

STS had more of an inhibitory effect on thermoinhibited seeds than on ones incubated at 25°C (Fig. 12). Most, but not all, of
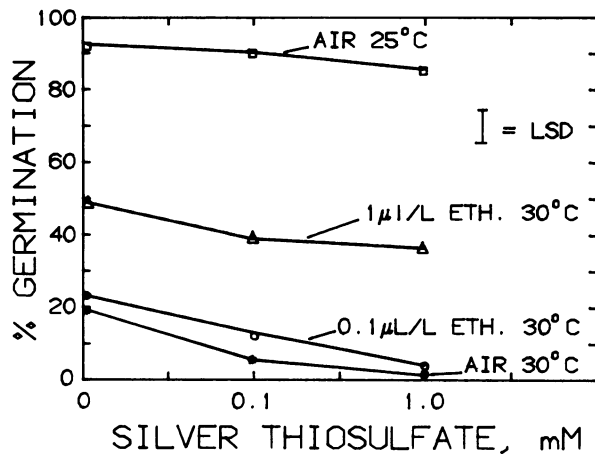
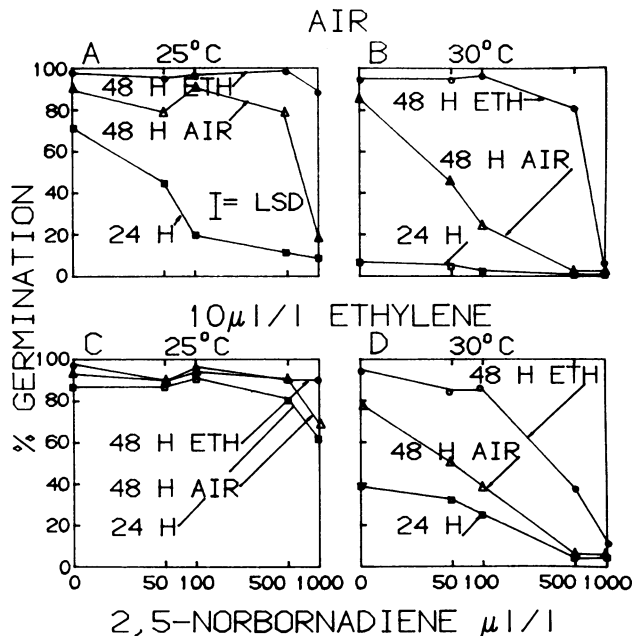FIG. 12. Inhibition of lettuce seed germination by STS and a partial reversal of the effect by ethylene.

FIG. 13. Reversal of the inhibitory effect of NBA on lettuce seed germination by ethylene. A, Air and 25°C; B, air and 30°C; C, 10 μl/L ethylene and 25°C; D, 10 μl/L ethylene and 30°C. See "Results" for discussion.

the inhibitory effect could be reversed by addition of ethylene.

The effect of NBA is shown in Figure 13. In the graphs labeled A and B, seeds were exposed to NBA for 24 h. In the graphs labeled C and D, seeds were exposed to a combination of NBA and 10 μl/L ethylene for 24 h. This experiment was performed at 25°C (A and C) and 30°C (B and D). The resultant germination at these temperatures is shown in the curves labeled 24 H. After this 24 h period, the NBA was removed by venting the seeds in air for 1 h. One-half of these seeds were then incubated in air at 25°C for an additional 24 h to see if they recovered from the inhibitory effect of NBA (see data in curve labeled 48 H AIR). The other half of the seeds were incubated in 10 μl/L ethylene at 25°C to maximize the recovery effect (48 H ETH). As shown in Figure 13A, NBA inhibited germination at 25°C. After venting the seeds and subsequent incubation in air or ethylene, germination was restored. When the seeds were thermoinhibited (Fig. 13B) NBA blocked the small amount of germination observed. As in the prior experiment, the ability of the seeds to germinate was regained when the seeds were vented and moved to 25°C. In

all of these experiments, ethylene had a greater effect on restoring germination than the air treatment. The data in Figure 13C shows that 10 μl/L ethylene overrode the inhibitory effect of NBA at 25°C. Figure 13D shows that NBA blocked ethylene enhanced germination of thermoinhibited seeds. As before, venting and treating the seeds with ethylene restored the ability of seeds to germinate. NBA at 1000 μl/L appeared to permanently inhibit germination when applied at 30°C as opposed to 25°C.

DIHB was not an effective inhibitor in the ethylene-lettuce seed system. Figure 14 shows that DIHB did not inhibit germination at 25°C and it appeared as if ethylene was unable to reverse what inhibition did occur at 30°C. One possible explanation for this observation is that the endosperm prevented the movement of DIHB to the embryo.

As shown in Figure 15, MCEB inhibited germination at both 25 and 30°C. At 25°C, 10 μl/L ethylene was able to overcome most of the inhibitory effect of 10 μM MCEB. Higher concentrations were either toxic or irreversibly bound. In the experiment shown in Figure 16, 10 μM MCEB caused a 50% inhibition of germination at 25°C. Adding increasing amounts of ethylene to the gas phase resulted in full reversal of the inhibitory effect. However, ethylene was unable to overcome the effect of 50 μM MCEB. In a final experiment, seeds were incubated at 30°C, in water or 25 μM MCEB for 3 h or 24 h. Seeds given the 3 h MCEB treatment were transferred to filter paper moistened with water
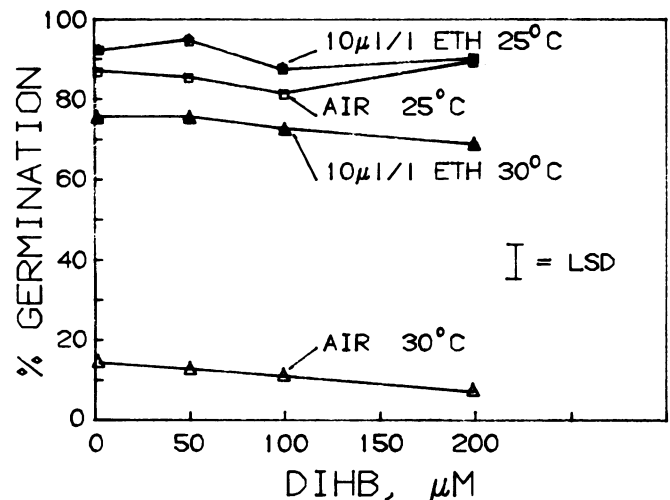

FIG. 14. Effect of a combination of DIHB and 10 μl/L ethylene on lettuce seed germination.
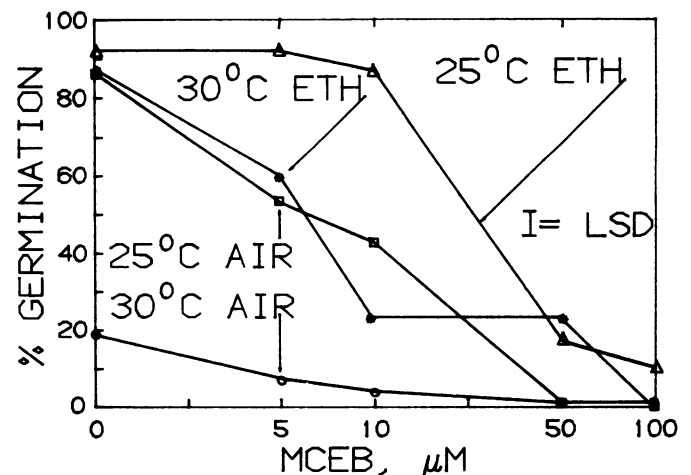

FIG. 15. Effect of a combination of various concentrations of MCEB and 10 μl/L ethylene on lettuce seed germination at 25 and 30°C.
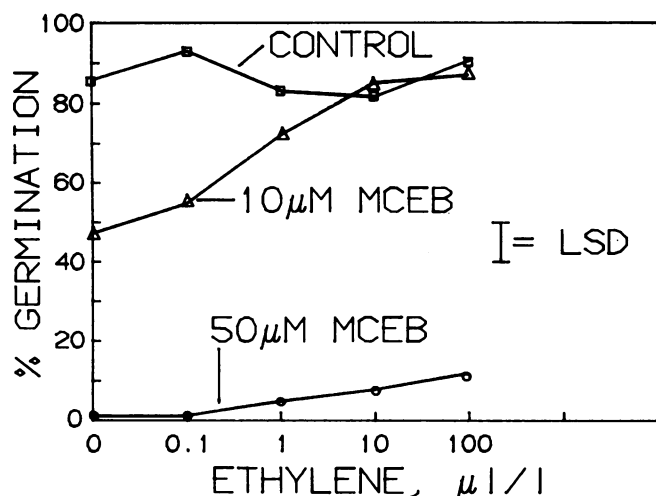
FIG. 16. Reversal of the inhibitory effect of MCEB on lettuce seed germination at 25°C by various concentrations of ethylene.
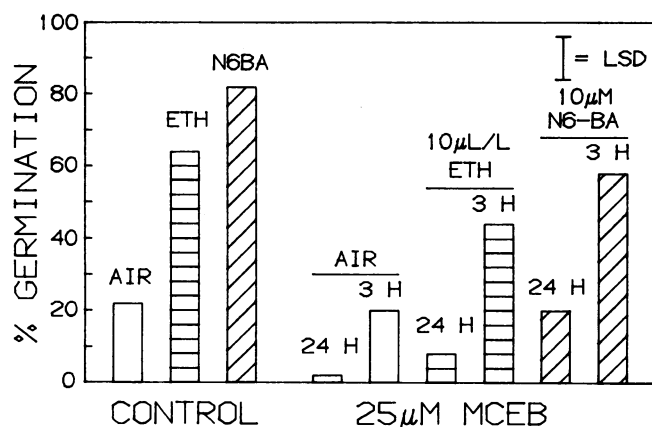

FIG. 17. Effect of 10 μl/L ethylene and 10 μM N6-BA on seeds exposed to a 3 or 24 h treatment with 25 μM MCEB at 30°C. Germination measured at 24 h. The three treatments on the left hand side of the Figure represent control seeds not treated with MCEB. In the remaining treatments, MCEB was present for either 3 or 24 h. In the case of 3 h treatments, the seeds were transferred to filter paper saturated with water or N6-BA after 3 h.

or N6-BA after 3 h and germination measured after 24 h. As shown in Figure 17, 10 μl/L ethylene and 10 μM N6-BA promoted germination of the control seeds incubated at 30°C. If the seeds were left on MCEB during the entire 24 h period, then ethylene and N6-BA only partially reversed the inhibition. Transferring seeds to water after 3 h of MCEB treatment resulted in an increase in germination to control levels. However, part of the ethylene and N6-BA effect was still lost.

DISCUSSION

The ability of ethylene to override thermoinhibition takes place in a narrow 5°C range. The ability of ethylene to promote germination was lost at temperature above 30°C (Fig. 2). This is similar to an earlier report where ethylene promoted germination at 32°C but not 36°C (12). Results presented earlier by others, and confirmed here, indicate that thermoinhibition was not due to an inhibition of ethylene production (12). However, the effect of temperatures above 30°C is not due to thermal denaturation. A number of workers have shown that transferring thermoinhibited seeds to cooler temperatures (4) resulted in germination. Imbibed lettuce seeds will germinate when held at 45°C for 32 h

or 50°C for 5 h if the seeds are subsequently incubated at 15°C (36). These workers also reported that temperatures of 75°C or greater were lethal and that some thermolabile factor played a role in germination. The effect of thermoinhibition is also lost if the seeds are treated with cytokinins or FC (6). Additional evidence that ethylene and cytokinins have different modes of action stems from the observation that ethylene does not override the inhibiting effect of 35°C while cytokinins do (32). Thermoinhibition can also be prevented by removing the endosperm (10) or weakening it with NaOCl (11) or HCl (21).

The response of seeds to ethylene and ethylene analogs was similar to the action of ethylene in the other systems in which it has a hormonal function. In an earlier study on seed germination (38), ethylene was found to be the most active of a series of hydrocarbon gases tested. As shown in Figure 3, ethylene was 100-fold more active than propylene and ethane had no activity.

However, the dose response curve for germination appears to be shifted toward higher concentrations of the gas. One of the typical attributes of ethylene is the fact that 0.1 μl/L ethylene is the concentration normally needed for a half-maximal effect. In the case of seed germination (Fig. 3), threshold effects occurred at 0.1 μl/L, half-maximal effects at 1 μl/L, and saturation at 10 to 100 μl/L ethylene (7, 8, 13, 17). The explanation for this shift in the dose response curve may lie in the physical nature of germination since germination is scored only after the radicle penetrates the endosperm. Thus, while 0.1 μl/L ethylene may promote some radial expansion of the hypocotyl, it is not sufficient to result in the penetration of the barrier endosperm.

Table II suggests that compounds which promote the germination of thermoinhibited seeds do so by increasing either the radial or longitudinal growth of embryonic cells destined to become the hypocotyl. In this regard, the hypocotyl can be thought of as a source of pressure which drives the radicle through the restraining endosperm. Root growth does not appear to be essential for germination since it normally starts after the radicle penetrates the endosperm, and ethylene and to a lesser extent N6-BA inhibited root elongation.

The target for ethylene action appears to be the embryonic hypocotyl since the gas inhibits root elongation and had no effect on isolated endosperms during the initial 24 h in which it promoted germination. Ethylene stimulated embryo growth against the osmotic restraint of mannitol (Fig. 4) and like cytokinin, overcame some of the inhibition of germination induced by 5 bar of hyperbaric pressure (Table I). Earlier work has shown that cytokinin also promoted germination when seeds were placed under osmotic restraint (6, 28). The observation that cytokinin was a more effective promoter of germination than ethylene (Table I; Fig. 17), suggests that longitudinal as opposed to radial expansion of the hypocotyl is a more effective method of initiating germination. Other workers have suggested that kinetin promoted germination by stimulating cotyledon expansion (36). In addition, evidence presented in Figures 5 and 6 indicated that ethylene does not play a role in, or mediate, cytokinin action. The observation that ethylene controls germination by promoting radial expansion of the hypocotyl may explain why ethylene promotes germination in only some plants. It is conceivable that the action of ethylene is limited to those situations in which ethylene mediated hypocotyl expansion can be used to push the radicle through the seed coat.

The structural integrity of the endosperm is an important factor in the germination of lettuce seeds. Georghiou et al. (18) and Psaras (29) noted preemergence changes in the barrier endosperm such as mobilization of storage materials and vacuolation of the cytoplasm. Another indication that barrier endosperm cells may have a special function in germination is the observation that they are structurally intact after the rest of the endosperm has disintegrated. Gibberellic acid was found to cause

structural alterations in barrier endosperm cells if it was applied to isolated endosperms (30).

The force required to penetrate intact barrier endosperm tissue, 17 to 19 centinewtons, is similar to values obtained earlier by others. Nabors and Lang (26) reported that 13.7 to 19.6 centinewtons were needed to penetrate endosperm tissue with a 0.4 mm diameter steel rod. Using an Instron fitted with a 0.2 mm steel rod, Tao and Khan (37) reported 60 centinewtons were needed to penetrate lettuce endosperm tissue. The force required to penetrate pepper endosperm tissue with a 0.4 mm steel rod was reported to be 8 centinewtons (40).

As shown in Figure 7A, a seed attempting to germinate under osmotic restraint causes some loss in the structural integrity of the barrier endosperm. At this point it is not known if the effect was due to a mechanical stretching of the endosperm by the imbibed embryo or softening caused by enzymes secreted by the endosperm or radicle. Ethylene reduced the structural integrity of the barrier endosperm. However, this effect is probably unrelated to its ability to promote germination. This conclusion is based on the observation that 3 d were required for an effect, and that the effect was observed at 20°C but not 30°C (Fig. 7B). The nature of the endosperm softening effect observed with ethylene is unknown.

Endogenous ethylene production may play a role in lettuce seed germination. AVG inhibited both ethylene production and germination. However, others have reported that AVG reduced ethylene production but not germination in peanut (20) and *Amaranthus* (23) seeds. As shown in Figure 10, exogenously applied ethylene overcame some of the inhibitory effect of AVG.

Additional support for the idea that ethylene plays a role in germination can be derived from experiments with inhibitors of ethylene action. CO_2 was the first compound shown to be a competitive inhibitor of ethylene action (9). Since then the following compounds have been shown to have antiethylene effects: MCEB (39), NBA (34), DIHB (25), and silver ions (5).

DIHB appeared to have no effect on germination at 25°C. Either the action of this compound is not as specific as originally proposed by Larque-Saavedra *et al.* (25), or the compound failed to penetrate the endosperm and reach the embryo under the conditions of these experiments. The endosperm is a significant barrier to many solutes and this problem has been discussed earlier by others (16). However, triiodobenzoic acid, a compound similar to DIHB, was shown to prevent lettuce seed germination and its effect was reversed by ethrel (31).

Problems with penetration may also explain the results obtained with STS. At 25°C, STS did not appear to inhibit germination. At 30°C some inhibition was observed, and depending upon the concentration used, could be overcome by 0.1 μl/L or 1 μl/L ethylene.

The observation that CO_2 enhanced instead of inhibited germination is opposite to that one would expect for a competitive inhibitor of ethylene action. This effect has been reported earlier, and in fact CO_2 has a promotive effect on seed germination in general. CO_2 has been shown to promote germination in lettuce (2, 17, 27), clover (3), cocklebur (15), and peanuts (24). However, CO_2 did reduce the ability of ethylene to promote witchweed germination (14). CO_2 also blocked the ability of ethylene to promote lettuce seed germination if the seeds were imbibed in 0.2 M NaCl (27). Under these conditions germination is observed only when ethylene is added to the gas phase.

NBA was reported to be an effective inhibitor of ethylene action by Sisler and Pian (34). They reported that NBA prevented senescence and seed (species not indicated) germination. NBA has also been used to prevent abscission (33) and germination of *Amaranthus caudatus* seeds (23). NBA appeared to be an effective inhibitor of lettuce seed germination (Fig. 13). At both 25 and 30°C, it blocked germination at concentrations of 100 μl/L

and higher. The inhibitory effect of NBA was lost when seeds were treated simultaneously with ethylene. The NBA effect was reversible. Placing seeds treated with NBA in air or ethylene, resulted in an increased rate of germination. Concentrations of 1000 μl/L NBA at 30°C may be toxic since seeds did not exhibit full rates of germination after exposure to these levels of the gas.

MCEB also proved to be an effective and partially reversible inhibitor of germination. At a concentration of 10 μM it reduced germination by 50% and increasing the level of ethylene resulted in full germination. Higher concentrations of MCEB were not fully reversible by ethylene. As shown in Figure 17, some of the MCEB effect was lost if seeds were incubated in water. Additional work is needed to differentiate between the removal of MCEB by diffusion or metabolism by the seed. Unlike other inhibitors of ethylene action, the structure of MCEB does not suggest a mode of action dependent upon competitive binding.

The data in Figure 17 indicated that MCEB also blocked the action of N6-BA. We also observed that MCEB also blocked the effect of FC (data not shown). These observations suggest that MCEB may not be a specific inhibitor of ethylene action but a general one on cell expansion. Hence, any treatment which promotes cell expansion will override the effect of MCEB.

The results obtained here suggest that ethylene plays a role in lettuce seed germination because inhibitors of ethylene production reduced germination and ethylene reversed the effect of some inhibitors of ethylene action. While the mode of action of thermoinhibition is not known, it does not appear to be due to an inhibition of ethylene production. Ethylene had some softening effect on the endosperm tissue. However, this action was not correlated with its effect as a germination promoter. The site of ethylene action appears to be embryonic cells destined to become the hypocotyl. The mode of action may be associated with the ability of ethylene to promote radial cell expansion (19, 35) and thereby overcome the restraining effect of the endosperm.

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