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Stimulation of Lettuce Seed Germination by Ethylene

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Abstract. **Ethylene increased the germination of freshly imbibed lettuce (*Lactuca sativa* L. var. Grand Rapids) seeds. Seeds receiving either red or far-red light or darkness all showed a positive response to the gas. However, ethylene was apparently without effect on dormant seeds, those which failed to germinate after an initial red or far-red treatment. Carbon dioxide, which often acts as a competitive inhibitor of ethylene, failed to clearly reverse ethylene-enhanced seed germination. While light doubled ethylene production from the lettuce seeds, its effect was not mediated by the phytochrome system since both red and far-red light had a similar effect.**

A number of reports have demonstrated that phytochrome is able to regulate ethylene production from seedling tissue (3, 12, 15). It is also known that ethylene can increase seed germination (5, 19, 22, 23). In a preliminary report (3) the ability of ethylene to increase lettuce seed germination was described. We concluded that phytochrome controlled ethylene production in these seeds since higher levels of ethylene production were associated with seeds exposed to red light. The data presented here indicate that phytochrome does not control ethylene evolution from lettuce seeds but rather that seeds treated with red light produce more ethylene than controls because germinating seeds produce 10 times the amount of ethylene than dormant ones. This interpretation removes any contradictions to the observation that in systems in which the phenomenon occurs, red light decreases ethylene evolution.

Materials and Methods

Seeds of *Lactuca sativa* L. cv. Grand Rapids were stored at −15° until required for use. About 100 seeds were spread on 3 layers of moist filter paper in 9-cm petri dishes and placed in the dark at 30° ± 2° for a 5-hr imbibition period. After this imbibition period, the seeds were exposed to various light sources or temperatures and then either stored in 10-liter desiccators for ethylene and CO_2 treatments or in gas collection bottles for ethylene production measurements.

The far-red light was provided by a 150-watt incandescent lamp fitted with a 165-mm² Corning filter No. 2600CS 7-69. For red light, Corning filter No. 3961 CS 1-56 was used. All light treatments were given for 15 min, which was adequate to elicit the phytochrome response. Except for the exposure to red or far-red light, all manipulations were performed under a green light (Corning filter CS 5-75), which had no effect on seed germination.

Gas chromatography was used to determine the ethylene content of the gas phase surrounding the lettuce seeds. Two-milliliter gas samples were injected into an oxygen-hydrogen flame ionization gas chromatograph fitted with a one-fourth inch, 60-cm activated alumina column run at 100°. Sensitivity of the chromatograph permitted the determination of 25 pl (picoliters = 10^{-12} liters) ethylene per ml. Carbon dioxide used in these experiments contained less than 0.01 ppm ethylene. One ppm of ethylene equals 1.25×10^{-9} g or 1 nl (nanoliter = 10^{-9} liters) ethylene per ml gas phase.

The CO_2 and ethylene were added to the gas phase surrounding the seeds by creating a partial vacuum inside the desiccators, injecting the gases by means of a syringe through a rubber vaccine cap covering the desiccator outlet, and then removing the vaccine cap to equilibrate the contents to atmospheric pressure. Ethylene production was determined by placing the lettuce seeds on moist filter paper in gas collection bottles (5 cm in diameter and 2.5 cm high, fitted with a neck to accommodate a 25-mm diameter rubber vaccine cap) and sampling the gas phase with a syringe.

Results

The ability of ethylene to increase the rate of lettuce seed germination is shown in table I. The

Table I. *Effect of Ethylene on the Percentage of Lettuce Seed Germination After 24 Hr*

	ppm Ethylene			
Light treatment[1]	0	1	10	100
	%	%	%	%
Far-red	3	4	6	8
Red	33	45	56	56
Dark control	13	22	20	20

[1] 500 Seeds per treatment.

Table II. *Effect of 1 ppm Ethylene and 10 % and 15 % CO_2 on the Percentage of Lettuce Seed Germination After 24 Hr*

Light treatment[1]	Control	Ethylene	CO_2	CO_2 + Ethylene
10 % CO_2				
	%	%	%	%
Far-red	2	8	3	7
Red	37	51	36	49
Dark control	11	27	21	29
15 % CO_2				
Far-red	2	6	4	10
Red	37	58	41	48
Dark control	2	6	4	10

[1] 1600 Seeds per treatment.

gas increased the rate of germination of seeds given a prior treatment of darkness, red, or far-red light.

High concentrations of CO_2 usually block the biological activity of ethylene. However, as shown in table II, CO_2 alone increased germination, especially at the 15 % concentration, and failed to give a uniform reversal of ethylene action.

Ethylene appears to affect only the initial stages of germination. Figure 1 shows germination was essentially completed by 24 hr and that, while ethylene increased the total percent germination, the period of time over which germination occurred was not extended.

The ability of ethylene to act only during the initial stages of germination was demonstrated in another way. In this experiment seeds were permitted to germinate as a result of various light treatments, and after no further germination was observed the remaining seeds were then treated with ethylene. As shown in figure 2, ethylene had no further effect on the germination of the lettuce seeds. However, the ungerminated seeds are still viable because they

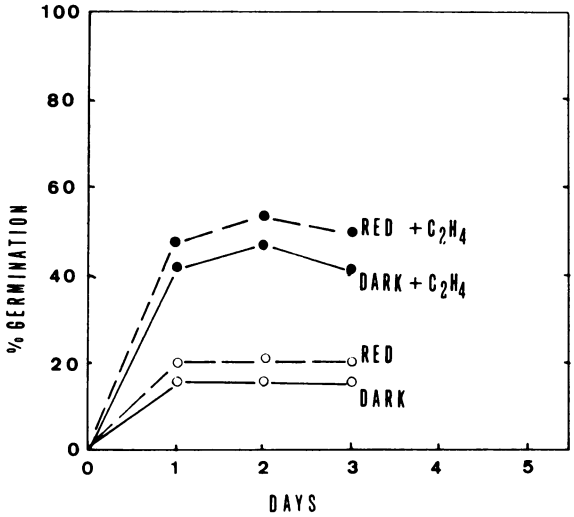

FIG. 1. Effect of 10 ppm ethylene added after imbibition on the germination of Grand Rapids lettuce seeds.

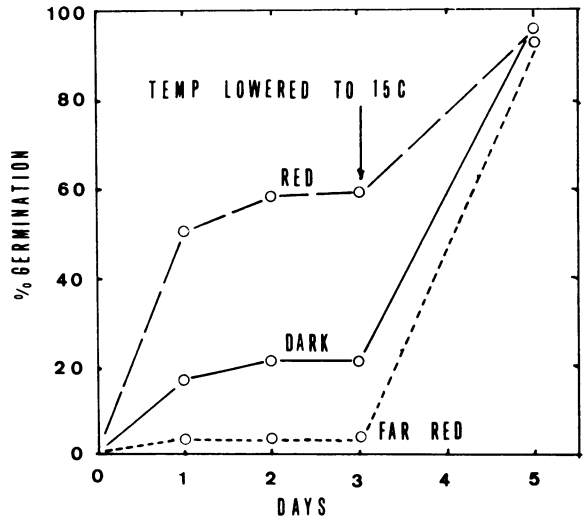

FIG. 2. Effect of 10 ppm ethylene added 3 days after imbibition on the germination of Grand Rapids lettuce seeds.

germinate when exposed to a 15° cold treatment (fig 3). The cold treatment was applied by lowering the temperature to 15° after the third day and holding the seeds at that temperature until germination was measured on the fifth day.

Other workers have shown that ethylene production may be controlled by phytochrome (12, 16). Since lettuce seed germination appears to be regulated by phytochrome and ethylene appears to regulate the rate of germination, a series of experiments were initiated to determine if phytochrome regulated ethylene production from the lettuce seeds.

The data in table III indicate that, while light did increase the rate of ethylene production, both red and far-red light appear to be equally effective. This experiment was performed by first giving all the seeds a 5-hr imbibition period at 30° in the dark followed by a red, far-red, or dark control treatment. The seeds were then allowed to germinate and the germinated seeds were discarded every 24 hr. After 72 hr, when germination was essentially complete,

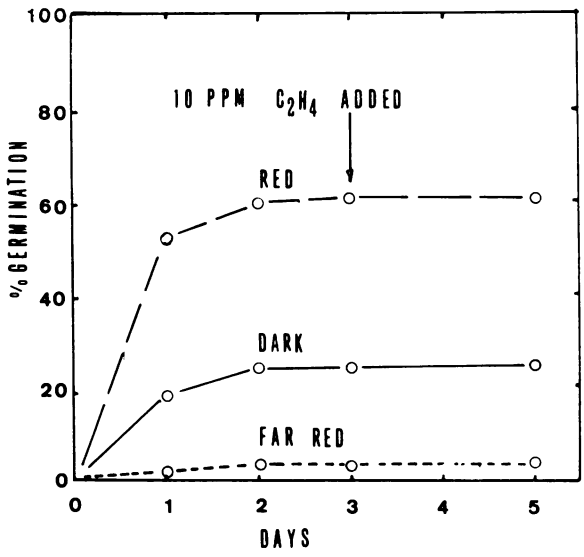

FIG. 3. Effect of a 15° treatment 3 days after imbibition on the germination of Grand Rapids lettuce seeds.

5 samples of 200 ungerminated seeds were placed in gas collection bottles. Ethylene production and any further germination was recorded 24 hr later. As shown in table III, most of the lettuce seeds remained dormant, and the rate of ethylene production from seeds treated with both red and far-red light was about the same and twice that of the dark controls.

Another way to determine if ethylene production from lettuce seeds is controlled by phytochrome is to compare the rate of ethylene production from germinating seeds given a prior exposure to red, far-red, darkness, and cold. In this experiment all the seeds were given a 5-hr 30° imbibition period. The seeds were then divided into 4 sets of 2000 seeds each. One set was illuminated with 15 min of red light, while another was illuminated with far-red light. The 2 other sets of seeds were held in the dark during this time. After this 15-min treatment period, the seeds from each treatment were placed in gas collection bottles, 100 seeds per bottle, the bottles sealed, and ethylene production and germination determined 24 hr later. In the case of seeds treated with cold, the seeds were transferred to a 4° incubation chamber and left there for 24 hr. Lots of 100 seeds were then placed in gas collection bottles, the bottles sealed, stored at 30° and germination and the ethylene content of the gas phase determined 24 hr later. The seeds were kept in the dark during these manipulations.

Table III. *Ethylene Production from Non-Germinating Lettuce Seeds During a 24-Hr Period*

Treatment	Additional germination	C_2H_4 in the gas phase	C_2H_4 per non-germinating seed
	%	*ppm*	*pl*
Far-red	0.2	0.10	23
Red	0.6	0.094	21
Dark	0.2	0.044	9.9

The following assumptions were made in calculating the data presented in table IV. First, that the germination-stimulating 4° treatment slowed the physiological processes of the seeds to an insignificant rate and that they continued at a normal rate when the seeds were returned to 30°. Secondly, that the rates of ethylene production calculated for the ungerminated seeds given various treatments in table III could be used in determining the rates of ethylene production from the germinating seeds. The data in table IV indicate that ethylene production from germinating seeds is an order of magnitude greater than from dormant ones and that light, red or far-red, had no effect on the rate of ethylene production.

Table IV. *Ethylene Production From Germinating Lettuce Seed During a 24-Hr Period*

Treatment	Germination	C_2H_4 in the gas phase	C_2H_4 per germinating seed
	%	*ppm*	*pl*
Far-red	7	0.090	259
Red	43	0.287	272
Dark control	25	0.145	232
Dark 4°	50	0.300	260

Discussion

Stimulation of seed germination by ethylene has been reported earlier by a number of workers (3, 5, 19, 22, 23). Ethylene chlorohydrin, which often mimics the effect of ethylene, has also been reported to increase the germination of tree seeds (10). Data presented here indicate that ethylene could also increase lettuce seed germination. However, CO_2 did not act as a competitive inhibitor as it does in a number of other processes regulated by ethylene, such as root (8) and stem (7) growth inhibition, fruit ripening (17), celery blanching (18), flower wilting (20), abscission (4), hook opening (16), and peroxidase formation (11). A number of earlier workers have reported that CO_2 increases the germination of lettuce (21), legume (13), and peanut (22) seeds. The observation that CO_2 fails to act as a competitive inhibitor in seed germination suggests that the mechanism of ethylene action in seed germination may be different from other phenomena mentioned above.

The data presented here indicate that ethylene does not act by overcoming dormancy. Treatment of dormant seeds with ethylene has no effect on germination (fig 2), but a 15° cold treatment did result in essentially complete germination (fig 3). In addition ethylene does not appear to act by enhancing phytochrome action; if it has, ethylene would

have decreased germination of seeds receiving far-red light as well as increasing the germination of seeds receiving red light.

Germination is a complex phenomenon involving imbibition, mobilization, cell enlargement, cell division, and cell differentiation. Ethylene is known to influence a number of these processes, including enzyme secretion (15), cell enlargement and differentiation (8) and hook opening (12, 16). The role of ethylene in lettuce seed germination is not clear, but the data presented here suggest that its action is limited to initial steps in the germination process because it was effective only when applied to freshly imbibed seeds (fig 1 *versus* fig 2).

Ethylene has been shown to act as an intermediate in the action of auxin (2, 8) gibberellin, abscisic acid (1), malformin (9), and 2,4-dichlorophenoxyacetic acid (14). Goeschl *et al.* (12) and Kang *et al.* (16) reported that ethylene may serve as an intermediate in phytochrome-mediated processes such as the hook opening response. Since lettuce seed germination is influenced by ethylene and phytochrome has been shown to regulate germination, experiments on the control of ethylene production from lettuce seeds by red and far-red light were performed. In an abstract published earlier (3), we reported that phytochrome did regulate ethylene production since seeds treated with red light produce more ethylene than controls. The data presented here, however, indicate that seeds treated with red light produce more ethylene only because germinating seeds produce 10 times as much ethylene as dormant ones (table III *versus* table IV). While light did appear to increase ethylene production from lettuce seeds, it apparently made no difference if red or far-red light was used (table III), and we conclude that the germination-stimulating effect of red light is not due to the control of ethylene production.

Acknowledgment

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