Plant Physiol. (1972) 49, 869–872

Interaction of Carbon Dioxide and Ethylene in Overcoming Thermodormancy of Lettuce Seeds

Received for publication October 19, 1971

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ABSTRACT

The combination of ethylene with CO_2 will completely overcome the thermodormancy of lettuce (*Lactuca sativa* L.) seeds at 35 C. This combination is effective if it is added to seeds either at the start or after several days of imbibition. The action of ethylene is dependent upon the CO_2 level present in the atmosphere surrounding the seeds. When CO_2 is trapped by KOH the ethylene effect is essentially nil.

The inhibition of lettuce (*Lactuca sativa* L.) seed germination at higher temperatures (thermodormancy) has been attributed to several factors: (*a*) restraint of the embryo's growth by the endosperm (5), which causes the absence of over-all embryo expansion (10); (*b*) reduction of the permeability of the seed coats (endosperm and integumentary membrane) to oxygen and carbon dioxide (5, 7); (*c*) accumulation of metabolic products in the endosperm or in the embryo which inhibit initial germination stages (5); (*d*) nonfunctioning of the active form of the phytochrome (21); (*e*) formation of a temperature dependent inhibitor during the initial germination stages (4); (*f*) inhibition of radicle growth (16); and (*g*) diffusion of a growth-inhibiting substance from the seed into the aqueous medium (22).

Several treatments have been used to overcome thermodormancy in lettuce seeds: (*a*) removing all seed coverings (7); (*b*) subjecting the moist seed to low temperature to initiate germination (5); (*c*) exposing briefly to low temperature during the course of imbibition at higher temperature (4); (*d*) germinating seeds under an increased oxygen (5) or carbon dioxide (26) pressure; and (*e*) chemically treating with thiourea (25), kinetin (14), and humic acid (11).

Ethylene has been reported to accelerate and stimulate the germination of certain seeds (27). Similarly, carbon dioxide stimulates seed germination (2). Abeles and Lonski (1) reported that at 30 C, ethylene and/or CO_2 increased the germination of "Grand Rapids" lettuce seeds which had been given prior treatments of darkness, red or far red light. Stewart and Freebairn (24) inhibited lettuce seed germination at 25 C by first heating the dry seeds at 97 C, and then showed that ethylene at 100 μl/l overcame this inhibition.

The objective of this report is to demonstrate that lettuce seed thermodormancy can be overcome by the combination of ethylene and CO_2.

MATERIALS AND METHODS

Fifty lettuce seeds (*Lactuca sativa* L. "Mesa 659"), were imbibed under air in 50-ml Erlenmeyer flasks on two layers of Whatman No. 1 filter paper (4.25 cm in diameter) moistened with 2 ml of glass redistilled water. Germination was carried out under 80 ft-c of continuous 20 w cool white fluorescent light with the main body of the flask submerged in a 35 ± 0.1 C constant temperature water bath. All experiments mentioned in this report were done at 35 C, a temperature at which untreated seeds do not germinate. The germination (protrusion of the radicle) percentage was determined after 48 hr or as otherwise indicated. Each experiment was carried out in triplicate and these were repeated at least 10 times. The flasks were sealed with rubber serum caps and ethylene and/or carbon dioxide were injected through the serum caps to give 16 μl/l ethylene and 16% CO_2 by volume in the flasks. An equal volume of air was withdrawn from the flask before injection of the gas(es). Laboratory compressed air was used for the air purges. Potassium hydroxide (20% w/v) was used to trap the CO_2 from the sealed flasks and mercuric perchlorate (G. Fredrick Smith Chemical Co.) (0.25 M) in 2.0 M perchloric acid (28) was used to trap the ethylene.

Gas samples of 0.50 ml were withdrawn through the serum caps and analyzed on a Beckman GC-4 dual hydrogen flame gas chromatograph equipped with 285 cm long, one-eighth inch outside diameter stainless steel columns packed with Porapak Q. 50 to 80 mesh (Waters Associates, Inc., Framingham, Mass.). The carbon dioxide and ethylene content of each sample were analyzed simultaneously. Gases were passed through a thermal conductivity detector before entering the dual hydrogen flame detectors. The actual amounts of ethylene and carbon dioxide in each sample were determined by comparison of the unknown with the elution pattern of standard gas mixtures.

RESULTS

In the course of our studies on lettuce seed thermodormancy, the effects of ethylene gas were compared with those of some other chemicals which release ethylene. Ethephon (Ethrel, Amchem 68–240), in a small test tube, was placed inside the germination flask which was sealed with a rubber serum cap. To release ethylene, the pH of the Ethephon solution was raised by injecting 0.2 ml of 1.0 N NaOH through the serum cap into the test tube. The results in Table I show that ethylene released from Ethephon had little effect on germination. On the other hand, 2-trimethylsilylethanol ($Si[CH_3]_3CH_2CH_2OH$) which releases ethylene in acid pH was effective in inducing germination. In this case 0.2 ml of 1.8 M H_2SO_4 was used to lower the solution's pH. Analysis of the atmosphere in the flasks showed that no CO_2 was present in the Ethephon-treated flasks. The CO_2 had been effectively trapped by the alkaline solution. Similarly, removing CO_2 from the 2-trimethylsilylethanol-treated flasks reduced its effect on the germination of lettuce seeds.

These preliminary experiments indicated the importance of

CO_2 produced by seeds for induction of germination in the presence of ethylene. Therefore experiments using gas traps were undertaken to determine the regulation of lettuce seed germination by both ethylene and carbon dioxide. The results of Table I show that ethylene gas caused only slight stimulation in the absence of carbon dioxide. Ethylene, in the presence of the amount of endogenous CO_2 normally produced by the seeds in the sealed flask, caused 49% germination in 48 hr. On the other hand, ethylene in the presence of increased CO_2 concentration caused 95% germination in 48 hr.

The addition of CO_2 alone to flasks had no significant immediate effect on lettuce seed germination compared to the air control. However, if flasks were examined after remaining sealed for 8 days at 35 C, the average germination was 53% compared to 0 to 2% for the air control flasks. When ethylene was prevented from accumulating in the CO_2-treated flasks by a mercuric perchlorate trap, germination was essentially prevented even after 8 days exposure to CO_2.

Earlier work by Abeles and Lonski (1) showed that ethylene increased the germination of Grand Rapids lettuce seeds at 30 C under different light conditions. They suggested that ethylene does not act by overcoming dormancy and that its action is limited to initial steps in the germination process since treatment of dormant seeds with ethylene had no effect on germination in their experiments.

To determine the effect of ethylene plus CO_2 on germination after various periods of imbibition, the following experiment was performed. Seeds were imbibed in closed flasks at 35 C without adding gases other than air. The flasks were opened and purged with air every 24 hr to remove any accumulated gases or volatiles until treated. At 0, 3, or 6 days after the start of imbibition, either ethylene, ethylene plus CO_2, or ethylene minus CO_2 was added to the flasks. A KOH trap was used to insure the latter treatment. Any seeds that had germinated prior to adding the gases were removed at treatment time. The germination percentage was observed for 3 days after each treatment.

Table I. *Effect of Ethylene (16 μl/liter) and Carbon Dioxide (16%) on Lettuce Seed Germination at 35 C*

Treatment	Germination after 48 Hr	
	%	
Ethylene	49	
Ethylene − CO_2	0–4	
Ethylene + CO_2	95	
Control (air)	0–3	
CO_2	0–2	53[1]
CO_2 − ethylene	0–2	3[1]
Ethephon (pH 10)	0–4	
2-Trimethylsilylethanol	40	
2-Trimethylsilylethanol − CO_2	0–2	

[1] % Germination determined after 8 days.

Our results (Fig. 1) indicate that a combination of ethylene plus CO_2 added at any time after the start of imbibition results in essentially full germination within 24 to 48 hr. This is true even if ethylene plus CO_2 is added to dormant seeds after 10 days of imbibition (data not shown). When ethylene is added to flasks but CO_2 is continually trapped in KOH, little or no germination results. These observations indicate that CO_2 is required when ethylene is used to break thermodormancy of lettuce seeds.

The results in Figure 1 also indicate that ethylene will induce germination of dormant seeds when added either initially or even to those that had been maintained in a dormant state up to 6 days prior to exposure to the gas. When ethylene was added after 3 or 6 days of imbibition (Fig. 1 B and C) there was approximately a 24-hr lag period before seeds started to germinate. There was no lag period when ethylene was added at the start of the imbibition period (Fig. 1A), although all treatments resulted in about the same germination percentage (40–50%) after 3 days. The 24-hr lag in germination response when dormant seeds were treated with ethylene after 3 or 6 days is probably related to the time required to restore the endogenous CO_2 level which was decreased when purged with air.

The CO_2 level in germination flasks was followed during a 6-day period. After the sample for gas chromatographic analysis was removed, flasks were purged with air and new serum caps were used each time the flasks were opened. The amount of CO_2 accumulating during each 24-hr period is shown in Figure 2. It is readily seen that approximately twice as much CO_2 accumulates during the first 24 hr as accumulates during any of the subsequent 24-hr periods. The level of 0.89% at the end of the 3rd day and 0.71% at the end of the 6th day are both approximately one-half of the 1.62% level measured at the end of the 1st day. This agrees with the findings of Evenari *et al.* (9) who reported that the respiration rate of Grand Rapids lettuce seeds decreased after the 22nd hr of imbibition at 30 C, a temperature which inhibited germination.

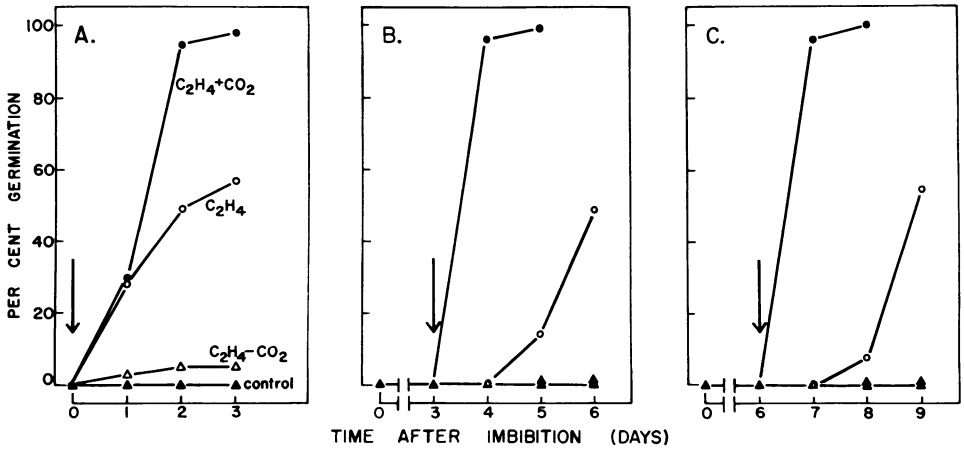

FIG. 1. Effect of ethylene (16 μl/liter), ethylene plus 16% CO_2 and ethylene minus CO_2 (KOH trap) on the germination of thermodormant lettuce seeds at 35 C. Gases were added (→) at the start of imbibition (A), 3 days (B), and 6 days (C) after imbibition. Control refers to seeds imbibed under air.

In another experiment seeds were imbibed and flasks were purged every 24 hr until ethylene was added after either 3 or 6 days of imbibition, after which the CO_2 level was measured on either the 4th or 7th day, respectively. In each case the addition of ethylene did not change the level of CO_2 produced during the following 24-hr period from that produced in control flasks. This suggests that ethylene does not enhance respiration. Under these conditions the 24-hr lag in germination was still present. In another treatment seeds were imbibed for either 3 or 6 days, flasks were purged with air and then treated with ethylene plus enough additional CO_2 to make the concentration in the flask equal to that measured on the first day of imbibition (1.62%). Under these conditions, there was no germination lag. When seeds were imbibed and flasks were not opened daily, the CO_2 level accumulated in an additive fashion (Fig. 2). For example, the CO_2 level in the flasks was 3.45% after 3 days and 5.14% after 6 days. Ethylene added to either of these flasks induced germination without any lag period. These results again support the concept that a minimum level of CO_2 is necessary for ethylene to cause its effect.

Further experiments were conducted to determine if CO_2 and ethylene exert their action(s) independently of each other. Seeds were imbibed for 3 days in the presence of ethylene and a KOH trap or in the presence of CO_2 and a mercuric perchlorate trap. Then the flasks were opened and purged with air. The few seeds which had germinated during the first 3 days were removed. Ethylene and a KOH trap were then added to those flasks exposed initially to CO_2 and mercuric perchlorate. Carbon dioxide and a mercuric perchlorate trap were added to those initially exposed to ethylene and KOH. Germination counts were made during the subsequent 3 days. The results (Fig. 3) indicate that neither of these gases can stimulate any substantial germination in the absence of the other, even if the seeds had been incubated for 3 days in the presence of the other gas. When either ethylene or CO_2 was added after 3 days (see arrow), but no KOH or mercuric perchlorate traps were used to prevent a buildup of the endogenously produced gases, germination increased within 48 hr (dotted lines, Fig. 3). It is interesting that when CO_2 is added after 3 days of exposure to ethylene minus CO_2, germination begins within 48 hr. However, if CO_2 is added without any pre-exposure to ethylene (Fig. 4) there is approximately a 5-day lag period before germination begins.

Seeds in the presence of ethylene alone or CO_2 alone will remain dormant as long as they remain at 35 C, but they can be induced to germinate if the temperature is lowered to 25 C or if they are exposed to a combination of ethylene and carbon dioxide.

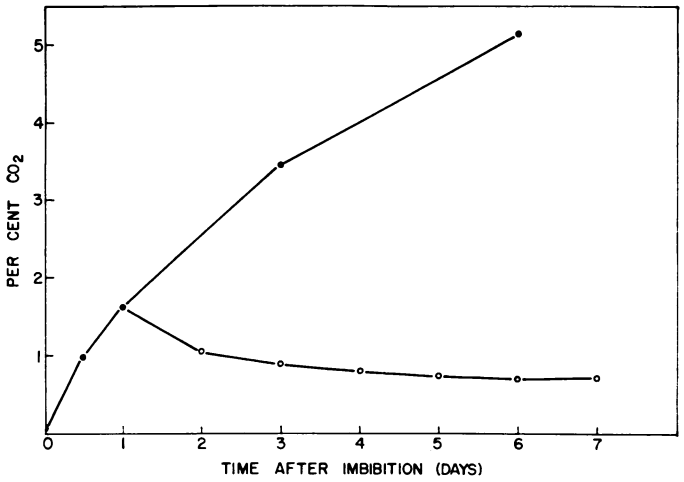

FIG. 2. Carbon dioxide production of thermodormant lettuce seeds measured at 24 hr intervals (○—○) or after accumulating for 3 or 6 days (●—●).

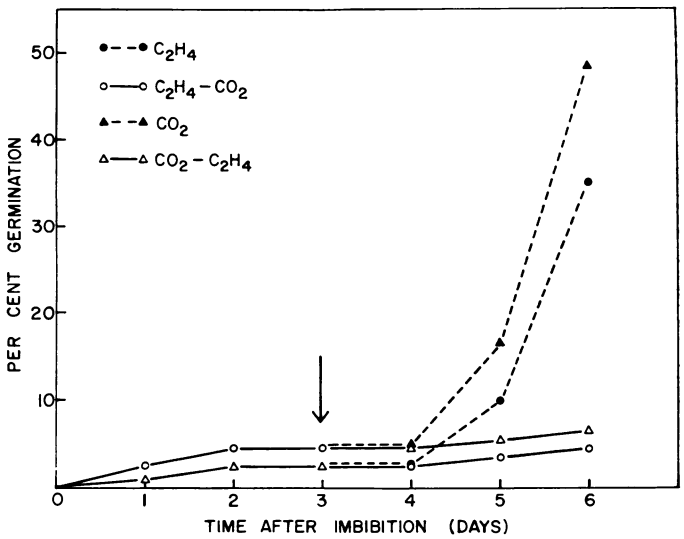

FIG. 3. The germination response of lettuce seeds to C_2H_4, C_2H_4 minus CO_2, CO_2 or CO_2 minus C_2H_4 after exposure for 3 days to CO_2 minus C_2H_4 or C_2H_4 minus CO_2. Arrow (→) indicates the time of changing the atmosphere surrounding the seeds.

DISCUSSION

The results reported here clearly show that ethylene and CO_2 can overcome lettuce seed thermodormancy if they are both present in the atmosphere surrounding the seeds. The action of ethylene is dependent upon the CO_2 level present. Further, it is shown that ethylene can induce germination of dormant seeds at any time after imbibition if a critical level of CO_2 is present. This is in contrast to the report of Abeles and Lonski (1), who suggested that the action of ethylene was limited to the initial steps in germination.

In 1936 Thornton (26) reported that CO_2 could overcome thermodormancy in freshly harvested lettuce seeds. He apparently used a closed system which would allow an accumulation of endogenous ethylene during the course of imbibition. We found that CO_2 (16%) was without effect if the ethylene produced by seeds was continually removed by mecuric perchlorate (Fig. 4). Similarly the response to added CO_2 depends

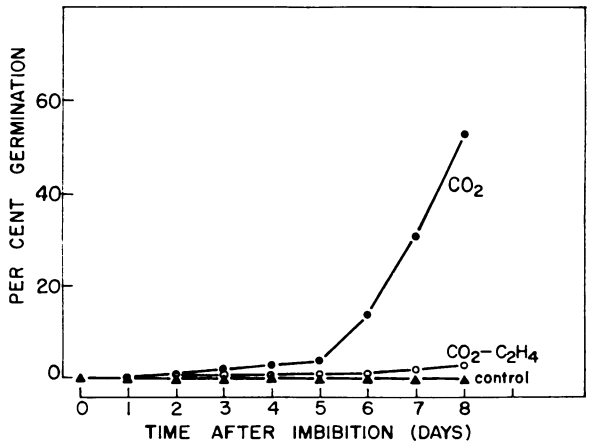

FIG. 4. Germination of thermodormant lettuce seeds treated with 16% CO_2 or CO_2 minus C_2H_4 (mercuric perchlorate trap).

upon the buildup of endogenous ethylene that occurs when one or more seeds begins to germinate.

As has been pointed out (20), the mechanism of action of ethylene in physiological growth systems has defied elucidation and explanation; and so it is with its effect in overcoming thermodormancy of lettuce seeds. It is, however, interesting to compare its effects with other hormones which have an effect on thermodormancy. Porto and Siegel (19) found that the germination of heat-treated lettuce seeds was restored by kinetin. Gibberellic acid, on the other hand, was ineffective in reversing the heat inactivation (12, 24). Stewart and Freebairn (24) suggested that GA was unable to overcome the temporary inhibition resulting from heat treatment of lettuce seeds because the heat had stopped ethylene synthesis. They advanced a hypothesis that GA primarily induces its response by stimulating ethylene production and the ethylene produced then in turn stimulates the seeds to germinate. However, their data do not appear to show any difference in response between water control and GA-treated seeds under normal conditions (no heat treatment). Similarly, lettuce seeds remain thermodormant in the presence of GA (12, 17), but not in the presence of kinetin (12). We have found that the absence of ethylene from the atmosphere surrounding kinetin treated lettuce seeds will not retard their germination, but the addition of ethylene to those seeds will increase the fraction that germinates. In addition, the absence of carbon dioxide was found to delay and depress the germination of those seeds treated with kinetin (unpublished data). Other workers (18) suggested that the cytokinins may mediate dormancy release in peanut seeds primarily through the stimulation of ethylene synthesis.

Ballard (2) studied the effect of CO_2 on the germination of subterranean clover seeds and found that the most active concentration was approximately 2.5%, though as little as 0.3 to 0.5% (by volume) was effective in overcoming dormancy in these seeds. Ballard also showed that respiratory CO_2 evolved by dormant seeds, if allowed to accumulate in sealed vessels, could itself initiate germination. The possibility that ethylene formed by these seeds during imbibition and in the presence of CO_2 could overcome dormancy has been clarified by Esashi and Leopold (8). They showed that the stimulations of subterranean clover seeds by ethylene and CO_2 are relatively independent actions. Ballard (3) suggested that CO_2 is probably incorporated by dark fixation and initiates the normal citric acid cycle and other reactions. He found that dormant seeds accumulated ^{14}C from $^{14}CO_2$ more strongly than did nondormant seeds.

Intact lettuce seeds were able to fix ^{14}C from $NaH^{14}CO_3$ into soluble compounds (13). Analysis of extracts after 3 hr imbibition showed that about one-third of the total fixed ^{14}C appeared in malic acid, and 12 to 15% in each of the following: glutamine, aspartic acid, citric acid, and glutamic acid. Their data suggested that ^{14}C was fixed primarily by carboxylation into organic acid and that the tricarboxylic acid cycle and transamination mechanisms functioned during the earliest phases of germination.

The role of ethylene in seed germination is still unknown but our results indicate that an interaction with CO_2 is necessary. Furthermore, there is no indication that CO_2 acts as a competitive inhibitor of ethylene action, and this confirms the work by Abeles and Lonski (1). Ethylene has been reported to stimulate the synthesis of some enzymes (15). Carbon dioxide has been reported to control the synthesis of fats (23), and to increase the protein synthesis rate (6). Ethylene might be involved in stimulating CO_2 fixation in thermodormant lettuce seeds which is required for lipid synthesis or organic acids and protein synthesis in the early stages of germination.

Acknowledgment—This work was supported in part by a grant from the California-Arizona Lettuce Seed Research Program. Seeds were provided through the courtesy of Keystone Seed Company, Hollister, California. The authors wish to express their thanks to Dr. M. Fahmy, University of California, Department of Entomology, for supplying 2-trimethylsilyl-ethanol, B. L. Horst for his helpful discussion, and J. Moore for making the illustrations.

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