Plant Physiol. (1972) 50, 719–722

Ethylene Synthesis in Lettuce Seeds: Its Physiologial Significance[1]

Received for publication May 16, 1972

A. N. BURDETT
Photobiology Group, Department of Biological Sciences, Simon Fraser University, Burnaby 2, British Columbia, Canada

ABSTRACT

The germination and pregermination ethylene production of Grand Rapids lettuce seeds (*Lactuca sativa* L.) incubated at 20 C after a red light treatment are inhibited if the seeds are first imbibed at 30 C for 36 hours. In this study, low concentrations of ethylene were found to enhance the germination of seeds pretreated at 30 C more than that of untreated controls. In the presence of high concentrations of ethylene, pretreated seeds and controls germinated at a similar rate. These results are consistent with the view that a prolonged imbibition at 30 C inhibits germination at a lower temperature through its effect on the ethylene production of the seeds. As a further test of the hypothesis, estimates were made of the pregermination ethylene content of untreated seeds and pretreated seeds incubated in the presence of sufficient ethylene to make them germinate as rapidly as untreated seeds. The values obtained were 0.65 and 0.74 nanoliter of ethylene per gram (dry weight) of seeds, respectively.

A prolonged imbibition at a temperature which is too high for germination has an inhibitory effect on the capacity of Grand Rapids lettuce seeds to germinate at lower temperatures (2). Since the effect is not reversed by red light, it cannot be attributed to a rapid loss of Pfr at elevated temperatures (2). Previously, it was reported that the inhibitory effect of a 36-hr imbibition at 30 C on the germination of seeds subsequently exposed to red light and incubated at 20 C was reversed by exogenous ethylene and associated with a decrease in the ethylene production of the seeds (2). It was suggested, therefore, that the influence of a pretreatment at 30 C on germination was due to its effect on ethylene production. However, it was not determined whether the level of endogenous ethylene is sufficient, even in unpretreated seeds, to affect germination significantly. As this question is relevant in assessing both the necessity of ethylene for germination and its role as an intermediary in the regulation of germination by environmental conditions, it was investigated in the study reported here.

MATERIALS AND METHODS

The seeds (*Lactuca sativa* L. cv. Grand Rapids) were of the same lot as those used in earlier studies on ethylene and lettuce seed germination and were stored and selected before use as previously described (2, 3).

Experiments on the uptake and release of ethylene were carried out at 20 C with imbibed seeds prepared in the following way. Three hundred-milligram lots of desiccated seeds were placed in 4-cm^{-2} dishes of aluminum foil and incubated for 2 hr with 3 ml of water. Excess water was then removed with a syringe, approximately 1.25 ml of water remaining for each gram (dry weight) of seeds.

The time course of exogenous ethylene release from the imbibed seeds was followed after first incubating them for 3 hr with 500 μl/liter of ethylene in a desiccator lined with wet filter paper. They were then transferred to another desiccator lined with wet filter paper which contained a 200-cm^{-2} paper wick moistened with 5 ml of a 0.25 M solution of mercuric perchlorate in 2 N perchloric acid (prepared as described by Young *et al.* (11) to absorb ethylene released by the seeds. At intervals, one lot of seeds was placed in a 25-ml flask which was sealed with a ground glass stopper incorporating a stopcock. Four hours after collection of the last sample, a 5-ml gas sample was extracted from each flask, by means of a hypodermic needle and syringe, through a serum cap placed over the stopcock outlet. The ethylene content of the samples was analyzed by gas chromatography as previously described (2). The initial exogenous ethylene content of the seeds was then estimated by subtracting from the total amount of ethylene released during the final incubation the quantity of ethylene evolved by controls which were not exposed to ethylene. The validity of this procedure depends on two assumptions. First, that virtually all of the exogenous ethylene within the seeds is released within 4 hr; and second, that the ethylene production of the seeds is not enhanced by the ethylene treatment, an effect observed in some other tissues (5, 9). Support for these assumptions was provided by the observation that, after a 4-hr incubation in the absence of ethylene, seeds treated with 500 μl/liter of ethylene for 1 hr did not release significantly more ethylene than controls which were not exposed to exogenous ethylene. During a 3-hr period, the mean rate of ethylene release by four replicates of ethylene-treated seeds and controls was 0.91 (SE 0.10) and 0.87 (SE 0.13) nl/g (dry weight), respectively.

A similar procedure was followed to determine the exogenous ethylene content of seeds incubated for 3 hr in the presence of ethylene at one of a range of concentrations. However, all the samples were sealed in a flask 2 min after they were removed from the ethylene. After determining the exogenous ethylene released by the seeds during the following 4 hr, their ethylene content at the end of the incubation with ethylene was estimated by extrapolation from the curve showing the time course of ethylene release (Fig. 2).

Dose response curves for the effect of ethylene on germination were obtained with seeds prepared in the following ways. Those referred to below as pretreated seeds, were placed in lots of 50 on 2.2-cm^2 filter papers. Then, they rested on a larger filter paper covering a glass shelf standing in a dish. The large filter paper was folded over the edges of the shelf so that water

[1] The research was supported in part by Canadian National Research Council Grant A2908.

added to the dish was carried to the seeds. After a 36-hr dark imbibition at 30 C, the seeds received a 1-min red light exposure from a filtered incandescent source with a maximal energy at 660 nm (half-peak band width 10 nm) and an irradiance of 6×10^{-3} J cm^{-2} sec^{-1}. Seeds receiving what is referred to as a preimbibition light treatment were imbibed to a water content of 20% by holding them in a water-saturated atmosphere for 2 hr at 20 C. They were then given the same irradiation as the pretreated seeds. However, unlike the latter, they were redried before being used for the germination assay.

The germination assay was carried out with lots of 50 seeds each placed in a 125-ml Erlenmeyer flask containing two Whatman No. 1 filter papers. After adding 1.8 ml of water, the flasks were sealed with rubber stoppers. The controls excepted, 1 ml of one of a range of air-ethylene mixtures was injected through the stopper into each flask to give an approximate ethylene concentration of from 0.1 to 10 μl/liter. The concentration was estimated more precisely by analyzing samples from the flasks by gas chromatography. The germination of the seeds was recorded after a 16-hr dark incubation at 20 C. Emergence of the radicle, determined by inspection with the naked eye, was the criterion of germination adopted.

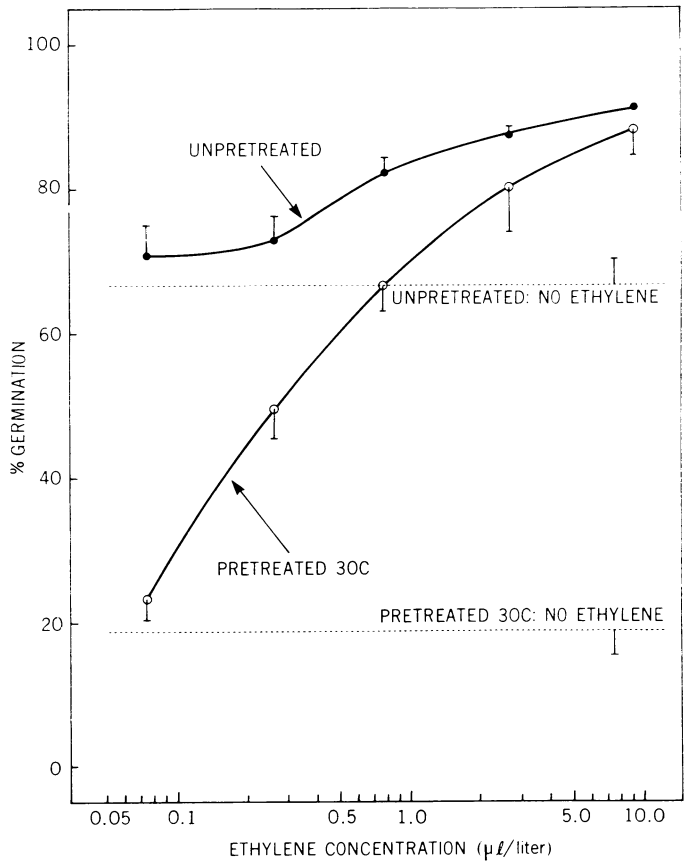

FIG. 1. Effect of ethylene on the germination of pretreated (○) and unpretreated (●) seeds after a 16-hr incubation at 20 C. The pretreated seeds were imbibed in the dark at 30 C for 36 hr and then exposed to red light at the beginning of the germination assay. The unpretreated seeds received a preimbibition red light treatment before the start of the experiment. Each point represents the mean germination percentage for 10 replicates of 50 seeds. The length of the vertical bar indicates the standard error of the mean.

RESULTS

Previously it was suggested that a 36-hr imbibition at 30 C inhibits the germination of Grand Rapids lettuce seeds during a subsequent incubation at a lower temperature through its inhibitory effect on their capacity to produce ethylene (2). The following consequences are predicted by this hypothesis.

1. Low concentrations of exogenous ethylene will enhance the germination of seeds pretreated at 30 C more than that of controls.
2. In the presence of high concentrations of ethylene, pretreated seeds and controls will germinate at essentially the same rate.
3. During the period when it exerts its effect on germination, the ethylene content of untreated seeds will be the same as that of pretreated seeds incubated in the presence of sufficient ethylene to make them germinate as rapidly as untreated seeds.

The dose response curves illustrated in Figure 1 confirm the first two predictions. The remaining experiments reported here were carried out to test the third.

It has been reported that, irrespective of the time at which it is supplied, the germination of lettuce seeds is not affected by exogenous ethylene until 7 to 9 hr after the beginning of the treatment (2). In the present study, therefore, it has been assumed that, insofar as it is influenced by ethylene, the germination of a population of seeds is largely dependent, at least for the first few hours after its onset, on the ethylene content of the seeds during the last 9 hr of the pregermination period.

It was found impossible to determine the ethylene content of the seeds during this period by analyzing gas from their intercellular spaces, a method used successfully with other tissues (1, 4), owing to the difficulty of obtaining uncontaminated samples. Instead, the endogenous ethylene content

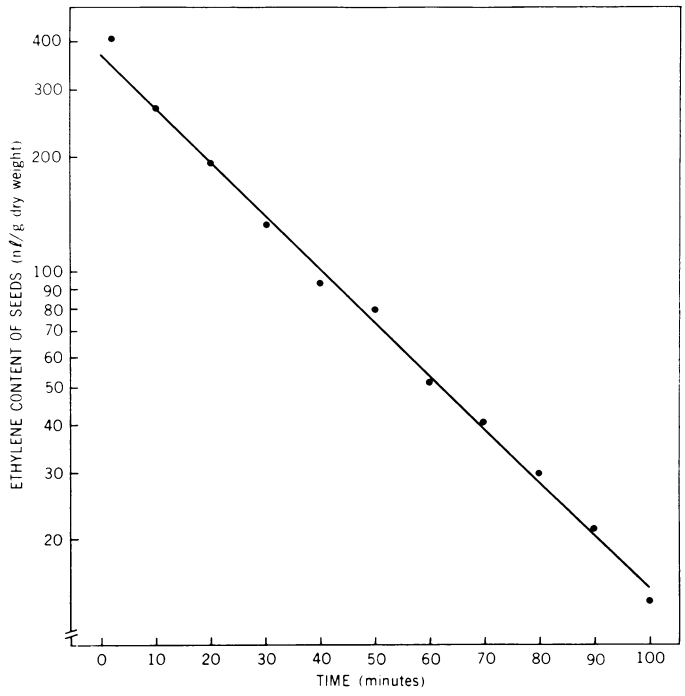

FIG. 2. Time course of exogenous ethylene release from imbibed seeds incubated at 20 C in the presence of an ethylene absorbant after a 3-hr incubation at the same temperature with 500 μl/liter ethylene. Each point indicates the mean of 2 determinations.

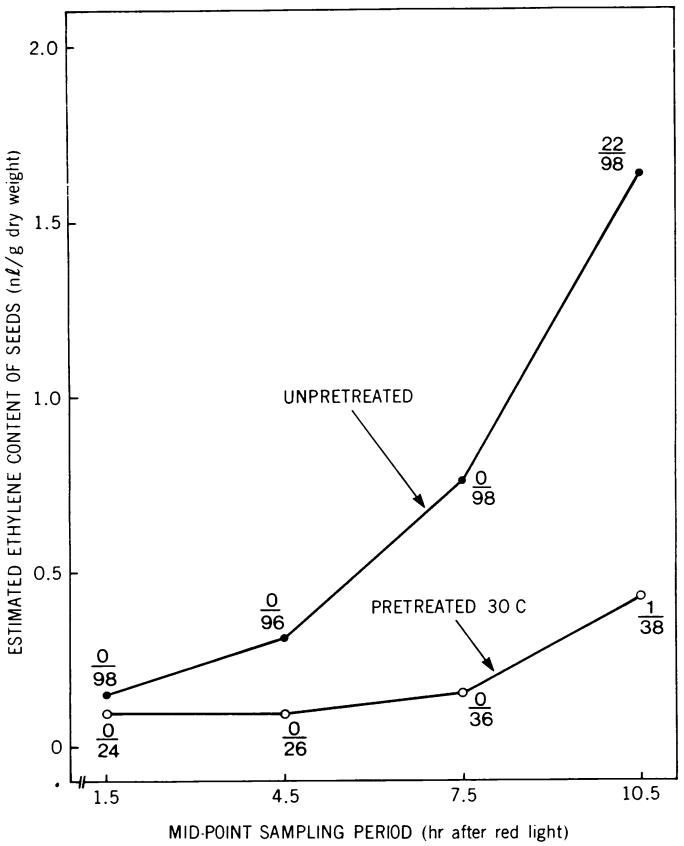

FIG. 3. Time course of the endogenous ethylene content of pretreated (○) and unpretreated (●) seeds incubated at 20 C and exposed to red light after 1 hr. The data are calculated from those of Figure 2 in reference 1. For the purpose of this calculation, a value of 1.11 mg (dry weight)/seed was used to convert ethylene production rates from pl/seed/hr to nl/g (dry weight)/hr. Each point represents the mean ethylene content of four lots of 135 seeds calculated from the mean rate of ethylene release during a 3-hr sampling period. The figures adjacent to the points indicate: upper: the mean germination percentage of the seeds used for the determination of the rate of ethylene release as recorded at the end of the sampling period; lower: the mean germination percentage attained 24 hr after the red light treatment by two replicates of 135 seeds incubated in Petri dishes except for the duration of the sampling period when they were placed in gas collecting bottles.

of the seeds was calculated from the rate at which it is released, using a rate constant determined for the purpose.

Figure 2 shows that there is a first order time-dependent decrease in the exogenous ethylene content of imbibed seeds held in the presence of an ethylene absorbent after they had been incubated with 500 μl/liter of ethylene. Calculated from the slope of the curve, the rate constant for the release of ethylene from the seeds at 20 C is 1.92 hr^{-1}.

This constant was used to calculate the time course of the pregermination ethylene content of untreated seeds and seeds pretreated at 30 C from previously reported data on the rate at which they release ethylene at 20 C. The validity of the derived data (Fig. 2 in ref. 2) shown in Figure 3 depends on the assumption that the rate constant, here used, applies when the ethylene content of the seeds is as low as 0.1 nl/g (dry weight). While this supposition remains to be demonstrated, it is consistent with the report that the diffusion of ethylene from fruit tissues is directly proportional to the internal-external concentration gradient over a 10,000-fold range extending as low as 0.04 μl/liter (4). There is also a possibility of error due to a time-dependent change in the rate constant during the pregermination period. However, since water uptake by lettuce seeds is reported to cease after the first 1 or 2 hr of imbibition (7), subsequent changes in the rate constant due to a change in the surface-volume ratio of the seeds can be excluded.

The germination data included in Figure 3 show that germination began between the 9th and 12th hr of the experiment. Taking the onset of germination to have occurred at 10.5 hr, the mean internal ethylene content of the seeds during the last 9 hr of the pregermination period was calculated from the area under each of the curves. The values thus obtained are 0.17 and 0.65 nl/g (dry weight) for the pretreated and unpretreated seeds, respectively.

The dose response curves in Figure 1 indicate that an external ethylene concentration of 0.76 μl/liter was required to make seeds pretreated at 30 C germinate as rapidly as controls incubated without ethylene. The results of an experiment to determine the exogenous ethylene content of seeds incubated with this concentration of ethylene are illustrated in Figure 4. As would be expected both for ethylene in the intercellular spaces and, if it obeys Henry's law, that dissolved in the tissue, the ethylene content of the seeds was directly proportional to the external concentration over the range used. On the assumption that the same proportionality occurs at ethylene concentrations below this range, the straight line through the points in Figure 4 was extrapolated to zero. The curve was then used to estimate the exogenous

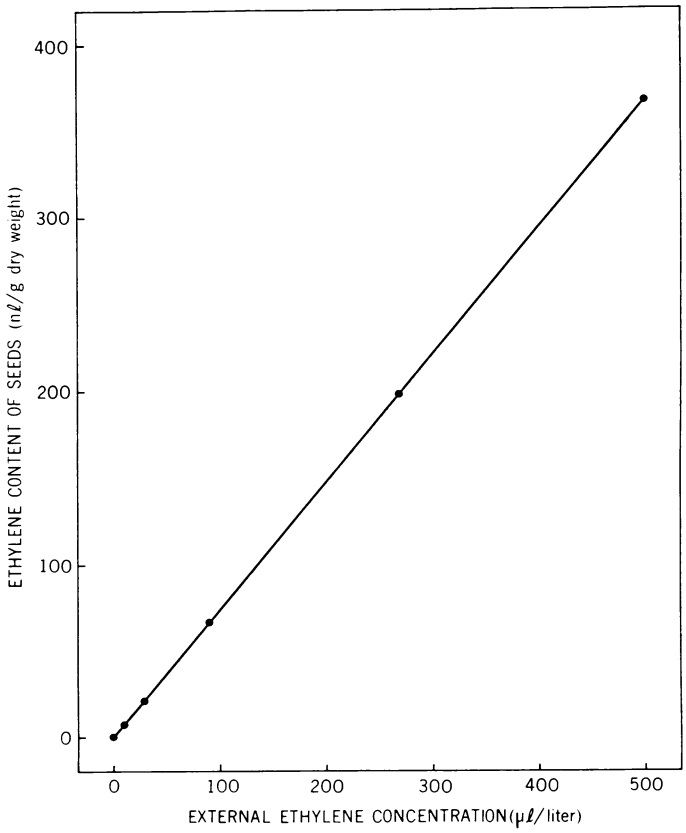

FIG. 4. The exogenous ethylene content of imbibed seeds after a 3-hr incubation at 20 C in the presence of one of a range of external ethylene concentrations. Each point represents the mean of two determinations.

ethylene content of seeds incubated with ethylene at a concentration of 0.76 μl/later. The concentration indicated is 0.57 nl/g (dry weight). This was added to the estimated concentration of endogenous ethylene in pretreated seeds to provide an estimate of their total pregermination ethylene content when incubated with 0.76 μl/liter of ethylene. The value of 0.74 nl/g (dry weight) thus obtained quite closely approximates the estimated endogenous ethylene concentration of 0.65 nl/g (dry weight) in unpretreated seeds.

DISCUSSION

Previously it was reported that the germination and pregermination ethylene production of Grand Rapids lettuce seeds incubated at 20 C after a red light treatment was inhibited if the seeds were first imbibed at 30 C for 36 hr (2). This study provides two independent lines of evidence suggesting a causal relationship between the effects of a pretreatment at 30 C on the ethylene production and germination of the seeds. First, it was found that seeds pretreated at 30 C showed a greater germination response to low concentrations of ethylene than did controls. In the presence of 10 μl/liter of ethylene, a concentration which is close to optimal for the promotion of lettuce seed germination (3), pretreated seeds and controls germinated at a similar rate. Second, a close similarity was found in the estimated ethylene content, during the period when it exerts its effect on germination, in untreated seeds and seeds pretreated at 30 C and then incubated with sufficient ethylene to make them germinate as rapidly as untreated seeds.

Although the first line of evidence is circumstantial and the validity of the second is dependent on several assumptions discussed above, together, they provide reasonable grounds for the view that a pretreatment at 30 C reduces germination primarily through its inhibitory effect on the ethylene production of the seeds. If this interpretation is correct, it follows that ethylene is a normal prerequisite of germination in lettuce seeds. Since it has been shown that the inhibitory effect of a prolonged imbibition at 30 C on the ethylene production of lettuce seeds is reversed by an incubation at a lower temperature (2), it also follows that ethylene is an intermediary in the regulation of germination by environmental conditions.

There is evidence that ethylene may have a similar role in the germination and dormancy regulation of other seeds. Ethylene was shown to break dormancy in clover seeds and varietal differences in dormancy were found to be negatively correlated with the pregermination ethylene production of the seeds (6). An inverse relationship between the ethylene production of germinating rape and peanut seeds and their degree of dormancy has also been observed (8, 10).

Acknowledgment—I thank Dr. A. H. Burr of this department for advice concerning the interpretation of the kinetics.

LITERATURE CITED

1. Beyer, E. M. and P. W. Morgan. 1970. A method for determining the concentration of ethylene in the gas phase of vegetative tissues. Plant Physiol. 46: 352–354.
2. Burdett, A. N. 1972. Antagonistic effects of high and low temperature pretreatments on the germination and pregermination ethylene synthesis of lettuce seeds. Plant Physiol. 50: 201–204.
3. Burdett, A. N. and W. E. Vidaver. 1971. Synergistic action of ethylene with gibberellin or red light in germinating lettuce seeds. Plant Physiol. 48: 656–657.
4. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37: 179–189.
5. Burg, S. P. and M. J. Dijkman. 1967. Ethylene and auxin participation in pollen induced fading of Vanda orchid blossoms. Plant Physiol. 42: 1648–1650.
6. Esashi, Y. and A. C. Leopold. 1969. Dormancy regulation in subterranean clover seeds by ethylene. Plant Physiol. 44: 1470–1472.
7. Ikuma, H. and K. V. Thimann. 1964. Analysis of germination processes by means of temperature and anaerobiosis. Plant Physiol. 39: 756–767.
8. Ketring, D. L. and P. W. Morgan. 1969. Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type seeds. Plant Physiol. 44: 326–330.
9. Mapson, L. W. and J. E. Robinson. 1966. Relation between oxygen tension, biosynthesis of ethylene, respiration and ripening changes in banana fruit. J. Food Technol. 1: 215–225.
10. Takayanagi, K. and J. F. Harrington. 1971. Enhancement of germination rate of aged seeds by ethylene. Plant Physiol. 47: 521–524.
11. Young, R. E., H. K. Pratt, and J. B. Biale. 1952. Manometric determination of low concentrations of ethylene. Anal. Chem. 24: 551–555.