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Short Communication

Dormancy Regulation in Subterranean Clover Seeds by Ethylene[1]

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In the past there have been scattered reports of breaking of dormancy in seeds by ethylene (4, 11) or by ethylene chlorohydrin (5) which apparently acts on plants through a release of ethylene (12). The advent of gas chromatographic techniques has made it possible to show that many seeds produce ethylene naturally during the germination process (8, 9, 10); these reports raise the interesting possibility that the production of ethylene by the imbibed seed may contribute to the breaking of dormancy. This possibility has been examined for peanuts by Toole *et al.* (10) and Ketring and Morgan (6), who concluded that the ethylene production may actually contribute to the breaking of dormancy. From studies with lettuce, Abeles and Lonski (1) reached the opposite conclusion since ethylene was produced mainly by those seeds which were going to germinate, and there were only small increases in percentage germination in the presence of added ethylene. We have been studying the role of ethylene in germination of seeds of several species, and our evidence indicates that in some seeds there is a substantial response to ethylene in breaking of dormancy, and further that the ethylene produced by the seed can have a regulatory role in determining whether or not germination will be achieved.

Our work on Subterranean clover (*Trifolium subterraneum* L.) started as an effort to determine whether the breaking of dormancy in that species by carbon dioxide (2) or by various types of poisons (3) might be a result of enhanced ethylene formation following the treatments. Using a variety with strong dormancy (Dinninup), we found that germination could be enhanced by application of low ethylene concentrations over a wide range of germination conditions. For example, 3 separate experiments at various temperatures are presented in Fig. 1, and in each case stimulatory effects are seen with the application of 0.001 or 0.01 ppm ethylene. In this and subsequent tests the criterion of germination was the emergence of the white radicle through the

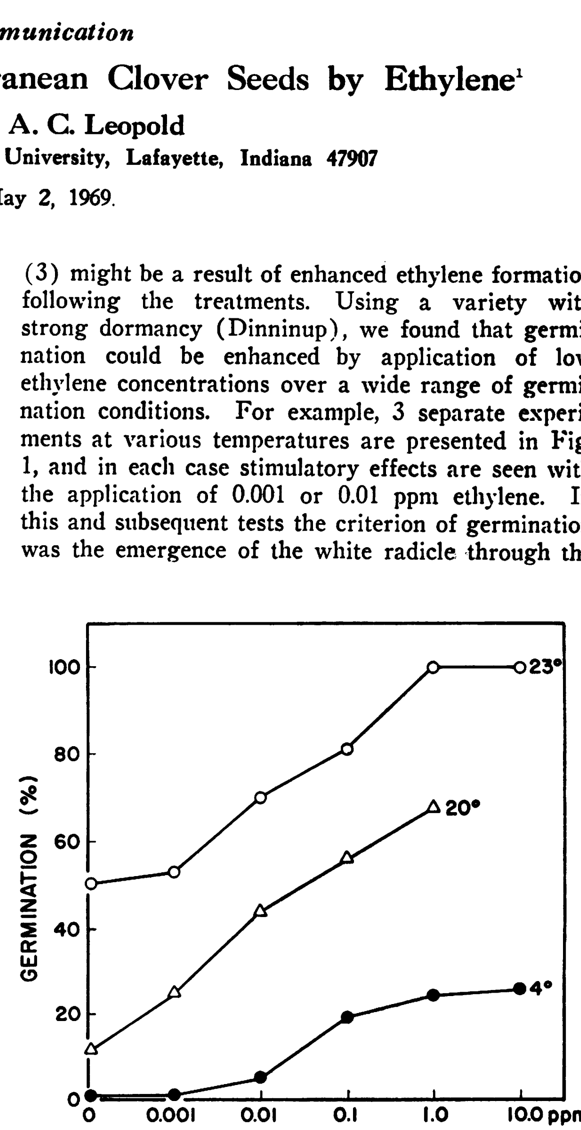

FIG. 1. The effects of ethylene on the germination of 'Dinninup' clover seed at 3 different temperatures. System: Seed (1 g) in 5 cm Petri dish with 2 ml water was inserted into a 1200 cc beaker covered wtih Parafilm through which ethylene was injected to give indicated concentrations. Percent germination was determined after 23, 18, or 56 hr in dark at 23, 20, or 4°.

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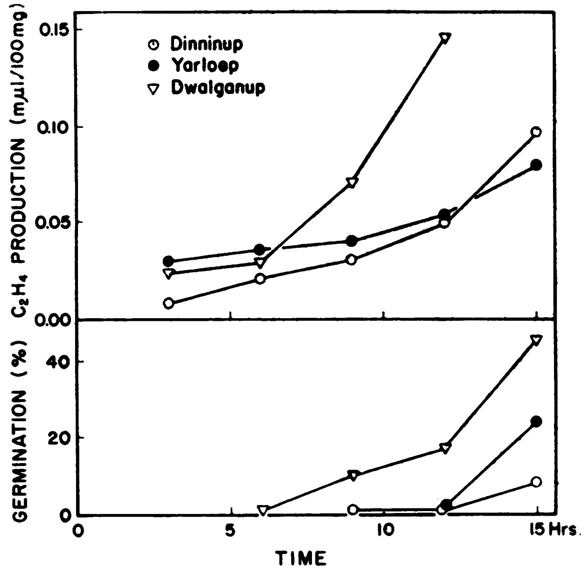

FIG. 2. The time course of ethylene production during germination of 3 varieties of clover. System: Seed (300 mg) in 7 cc vial with 1 ml water was held in dark at 20°. Treatments are in triplicate. Ethylene values are cumulative.

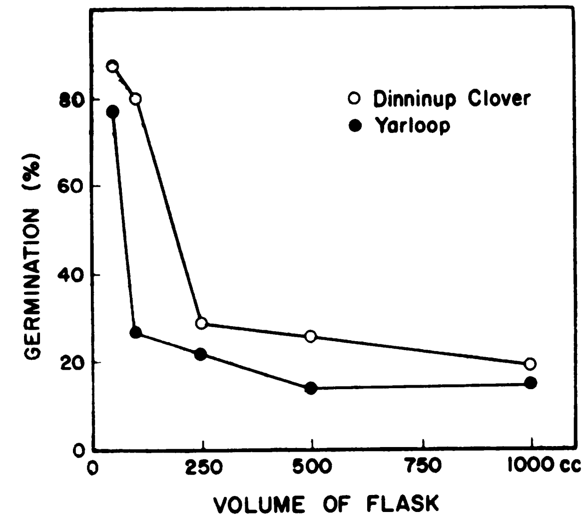

FIG. 3. The effect of container size on the germination of 2 varieties of clover. System: Seed (1 g) in 50 ml beaker with 1.5 ml water was covered with double layer of parafilm (50 ml volume) or enclosed in larger beaker which was covered with double parafilm to provide container size indicated. Germination is recorded after 2 days at 23° in dark. Treatments are in triplicate.

seed coat. Control groups of seeds in unsealed dishes reach nearly full germination in 24 hr at 20°. Referring to the figure, it is evident that maximal stimulations by the ethylene concentrations tested range from 25 to 50 % increases over the controls.

The production of ethylene by the seed following imbibition of water has been followed in time and compared to the emergence of the radicle as an index of germination. Such data are presented in Fig. 2 for 3 varieties of clover, including 2 dormant varieties (Dinninup and Yarloop) and 1 non-dormant variety (Dwalganup). Two principal features are evident from the Fig.: first, more ethylene is produced by the non-dormant variety, and second, the production of ethylene markedly preceeds the first emergence of the radicle.

Since ethylene is capable of stimulating germination, and ethylene is formed by the seed soon after the start of imbibition, the next question is whether the ethylene production by the seed is sufficient to cause the seed to germinate. A simple means of posing this question experimentally is to compare the germination of seeds of dormant clover when enclosed in flasks of graded volumes. Such an experiment is presented in Fig. 3, in which 2 dormant varieties (Dinninup and Yarloop) were each placed in beakers ranging from 50 to 1000 ml volumes; in each instance 500 mg of seeds were placed in a 50 ml beaker with 1.5 ml water and a disc of filter paper, and this beaker was sealed inside graded series of larger beakers by covering with a double layer of parafilm (American Can Company, Neenah, Wisconsin). The data in Fig. 3 show that in large enclosures germination is below 20 % in each instance, whereas in volumes of 100 or 50 ml the seeds achieve about 80 % germination.

The stimulation of germination by limited enclosures could be due to the production of either ethylene or carbon dioxide by the seeds, for it is well established that carbon dioxide has a pronounced stimulatory effect on clover seed germination (2, 7). The stimulatory effect of CO_2 in clover is illustrated by the data in table I. It is evident that associated with the CO_2 stimulation of germination there is a stimulation of ethylene production by the seed. The inter-

Table I. *The Stimulatory Effects of Carbon Dioxide on Germination of Yarloop Clover, and the Associated Stimulation of Ethylene Production*

System: Seed (300 mg) in 7 ml vials were held at 20° in the dark. Ethylene was determined after 11 hr, germination after 20 hr. Treatments are in triplicate.

Treatment	Germination	Ethylene production
	%	*nl/100 mg*
Control, open vial	6	...
Control, closed vial	37	0.054
1 % CO_2	41	0.056
2 % CO_2	53	0.063
3 % CO_2	62	0.070
4 % CO_2	60	0.068

Table II. *The Influence of Ethylene and Carbon Dioxide on the Germination of Dinninup Clover*

System: Seed (500 mg) on a 4.5 cm paper disc in a 5 cm open Petri dish suspended in a 250 cc beaker covered with double Parafilm. Ethylene or CO_2 was injected to make concentrations of 1 ppm or 2.5 % respectively. Saturated $HgClO_4$ or 10 % NaOH (10 ml) was pipetted into the 250 ml beaker or an enclosed 50 ml beaker fitted into the larger one, in each case with a 4.5 cm filter paper wick. Germination is recorded after 24 hr in dark at 25°. Treatments are in triplicate.

	Percent germination			
Treatment	Air	Ethylene 1 ppm	CO_2 2.5 %	Ethylene and CO_2
No addenda	12	62	27	83
$HgClO_4$	4	5	17	
NaOH	2	55	4	
$HgClO_4$ and NaOH	0			

actions of ethylene and CO_2 stimulations of germination are examined further in the data presented in table II, in which the effects of each agent are compared with and without removal of the ambient ethylene by mercury perchlorate (synthesized according to 13) and of the CO_2 by NaOH. These data indicate that the stimulatory effect of ethylene is entirely removed by the presence of mercury perchlorate, and the effect of CO_2 is entirely removed by the alkali; however a 50 % stimulation of germination by ethylene is achieved whether alkali is present or not, and a 13 or 15 % stimulation by CO_2 is achieved whether mercury perchlorate is present or not. We suggest then that the 2 stimulations are relatively independent actions.

In Subterranean clover, then, it appears that ethylene produced by the imbibed seed can contribute to the regulation of dormancy in the seed. The self-regulatory effect of ethylene may have an ecological benefit to the seed, encouraging germination when the seed is enclosed in the soil medium (where the ethylene produced by the seed may be retained in the vicinity of the seed) and depressing germination when it is exposed on the soil surface even though a supply of moisture may be temporarily available.

Acknowledgment

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Literature Cited

1. Abeles, F. B. and J. Lonski. 1969. Stimulation of lettuce seed germination by ethylene. Plant Physiol. 44: 277–80.
2. Ballard L. A. T. 1958. Studies of dormancy in the seeds of subterranean clover. I. Breaking dormancy by CO_2 and by activated carbon. Australian J. Biol. Sci. 11: 246–60.
3. Ballard, L. A. T. and A. E. G. Lipp. 1967. Seed dormancy: breaking by uncouplers and inhibitors. Science 156: 398–99.
4. Balls, A. K. and W. S. Hale. 1940. The effect of ethylene on freshly harvested wheat. Cereal Chem. 17: 490–94.
5. Deuber, C. G. 1931. Chemical treatments to shorten the rest period of tree seeds. Science 73: 320–21.
6. 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: 326–30.
7. Lipp, A. E. G. and L. A. T. Ballard. 1959. The breaking of seed dormancy of some legumes by carbon dioxide. Australian J. Agr. Res. 10: 495–99.
8. Meheriuk, M. and M. Spencer. 1964. Ethylene production during germination of oat seeds and *Penicillium* spores. Can. J. Botany 42: 337–40.
9. Spencer, M. and A. O. Olson. 1965. Ethylene production and lipid mobilization during germination of castor beans. Nature 205: 699–700.
10. Toole, V. K., W. K. Bailey, and E. H. Toole. 1964. Factors influencing dormancy of peanut seeds. Plant Physiol. 39: 822–32.
11. Vacha, G. A. and R. B. Harvey. 1927. The use of ethylene, propylene and similar compounds in breaking the rest period of tubers, bulbs, cuttings, and seeds. Plant Physiol. 2: 187–94.
12. Warner, H. L. 1970. The synthesis and mode of action of ethylene in etiolated pea seedlings. Ph.D. Dissertation, Purdue University, Lafayette, Indiana. 117 p.
13. Young, R. E., H. K. Pratt, and J. B. Biale. 1951. Identification of ethylene as a volatile product of the fungus *Penicillium digitatum*. Plant Physiol. 26: 304–10.