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# Physiology of Oil Seeds

## II. DORMANCY RELEASE IN VIRGINIA-TYPE PEANUT SEEDS BY PLANT GROWTH REGULATORS[1]

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### ABSTRACT

**Germination, ethylene production, and carbon dioxide production by dormant Virginia-type peanuts were determined during treatments with plant growth regulators. Kinetin, benzylaminopurine, and 2-chloroethylphosphonic acid induced extensive germination above the water controls. Benzylaminopurine and 2-chloroethylphosphonic acid increased the germination of the more dormant basal seeds to a larger extent above the controls than the less dormant apical seeds. Coumarin induced a slight stimulation of germination while abscisic acid, 2,4-dichlorophenoxyacetic acid, and succinic acid 2,2-dimethylhydrazide did not stimulate germination above the controls. In addition to stimulating germination, the cytokinins also stimulated ethylene production by the seeds. In the case of benzylaminopurine, where the more dormant basal seeds were stimulated to germinate above the control to a larger extent than the less dormant apical seeds, correspondingly more ethylene production was induced in the basal seeds. However, the opposite was true of kinetin for both germination and ethylene production. When germination was extensively stimulated by the cytokinins, maximal ethylene and carbon dioxide evolution occurred at 24 and 72 hours, respectively. Abscisic acid inhibited ethylene production and germinaton of the seeds while carbon dioxide evolution was comparatively high. The crucial physiological event for germination of dormant peanut seeds was enhancement of ethylene production by the seeds.**


We earlier reported that induction of ethylene production is directly correlated with release of dormancy of Virginia-type peanut seeds (7). Subsequently, we have studied the effect of ethylene and substances that might conceivably induce ethylene synthesis or breakdown to ethylene (IAA, GA, and CEPA[2]) on the germination of dormant Virginia-type peanut seeds (8). Only ethylene and CEPA enhanced extensive germination above the water control. Gibberellic acid stimulated germination somewhat, and this appeared to correlate with its ability to induce a moderate degree of ethylene production by the seeds. However, ethylene was most directly related to initiation of growth of these dormant peanut seeds. Ethylene has been implicated as a regulator of dormancy in other seeds also (3, 13).

Since growth regulators other than GA, IAA, and ethylene are known to influence germination of dormant seeds, the response of dormant, Virginia-type peanut seeds to such substances might clarify the nature of the dormancy regulative system in such seeds. Kinetin and its analogues are known to stimulate seed germination (9, 11). Kinetin has been shown also to stimulate ethylene production by seedlings (4). Abscisic acid is an effective inhibitor of seed germination (2). Coumarin is the classic seed germination inhibitor, but it has been shown to induce ethylene production by bean hypocotyl hooks (12). Alar, a plant growth retardant, inhibits ethylene production by ripening apples (10). Ethylene production by plant tissues and organs is stimulated by treatment with 2,4-D (1, 5, 6). We undertook to determine how these substances affect germination, ethylene production, and carbon dioxide production of dormant Virginia-type peanut seeds. This approach allowed comparison of the behavior of respiratory metabolism and ethylene production in growth regulator treatments with a wide range of effects on germination. This study also attempts to explain more fully the over-all regulatory system in peanut seed dormancy. The findings reflect on whether ethylene participates in dormancy release or is simply a product of germination which follows dormancy release.

### MATERIALS AND METHODS

The procedures for determining ethylene and the method of germination were reported previously (7). Carbon dioxide production was determined in a manner similar to ethylene production. A 1-ml sample of the gas phase was injected into a 6-ft × ⅛-inch silica gel column connected to a thermal conductivity gas chromatographic detector. Helium was the carrier gas. The amount of carbon dioxide was determined by comparing the area under the curve with the area of known amounts of carbon dioxide plotted on a standard curve. The detector response was linear. The germination potential of this 1969 harvest of NC-13 Virginia-type peanut seeds was 95 to 100% and 85 to 90% for apical and basal seeds, respectively. This was determined by treating imbibed seeds with 100 μl of ethylene per liter of air for 48 hr. Standard deviations from the mean of data shown in the text were rounded off to the nearest whole number.

Kinetin was obtained from Calbiochem. Benzylaminopurine and 2,4-D were obtained from Nutritional Biochemical Corp., CEPA (Amchem 68-62)[3] from Amchem Products, Inc., Alar

[1] Cooperative investigations of the Plant Science Research Divison, Agricultural Research Service, United States Department of Agriculture and the Texas Agricultural Experiment Station, Texas A&M University.

[2] Abbreviations: Alar: succinic acid 2,2-dimethylhydrazide; CEPA: 2-chloroethylphosphonic acid.

[3] Mention of a trademark or proprietory product does not constitute endorsement by the United States Department of Agriculture or Texas A&M University and does not imply its approval to the exclusion of other products that also may be suitable.

(Alar-85) from Uniroyal Chemical, coumarin from K & K Laboratories, and abscisic acid by courtesy of Shell Development Company, Modesto, California. The coumarin was recrystallized twice before use.

## RESULTS AND DISCUSSION

**Effect of Plant Growth Regulators on Germination.** Kinetin at $10^{-4}$ and $5 \times 10^{-5}$ M stimulated the germination of dormant peanut seeds (Fig. 1). At $10^{-7}$ M kinetin, there was no appreciable germination above the water control. The inherently less dormant apical seeds were induced to germinate to a greater extent above the control than the inherently more dormant basal seeds. The apical seeds reached their maximal germination potential (see "Materials and Methods") at $10^{-4}$ M kinetin and nearly attained it at $5 \times 10^{-5}$ M kinetin, but the basal seeds did not germinate to their potential at either concentration of kinetin.

The cytokinin benzylaminopurine, at the same concentrations as kinetin, also stimulated the germination of dormant peanut seeds (Fig. 2). The relationship between extent of germination above the controls for apical and basal seeds was reversed because of higher germination of apical controls. However, both seed types reached their germination potential at the lower concentration of benzylaminopurine. There was also earlier attainment of maximal germination above the control for both seed types. Thus, benzylaminopurine was more effective than kinetin in promoting germination of the dormant seeds.

CEPA is equivalent to ethylene in promotion of germination of dormant peanut seeds and may also be used as a standard to which other growth regulators may be compared. As we previously reported, CEPA induced excellent germination of dormant peanut seeds (Fig. 1 and Ref. 8). The germination values are in the range of potential germination values, yet

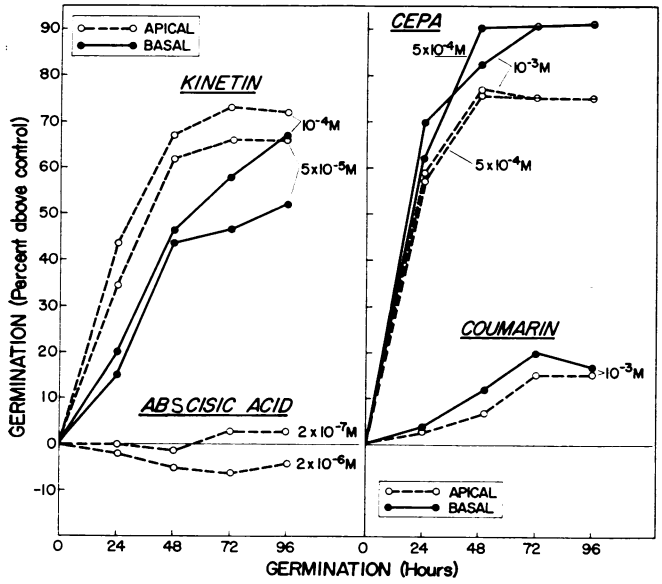


FIG. 1. The effect of kinetin, CEPA, coumarin, and abscisic acid applied for 96 hr on the germination of dormant NC-13 peanut seeds. Control germination values for apical seeds at 96 hr of germination were 22 ± 0, 23 ± 1, 16 ± 8, and 11 ± 1% for kinetin, CEPA, coumarin, and abscisic acid, respectively. Similarly, control germination values for basal seeds were 5 ± 2, 4 ± 3, 13 ± 5, and 8 ± 0%. The same control served for both concentrations of growth regulator where applicable. Each point in this and subsequent figures represents the mean of duplicate samples each containing 50 seeds.

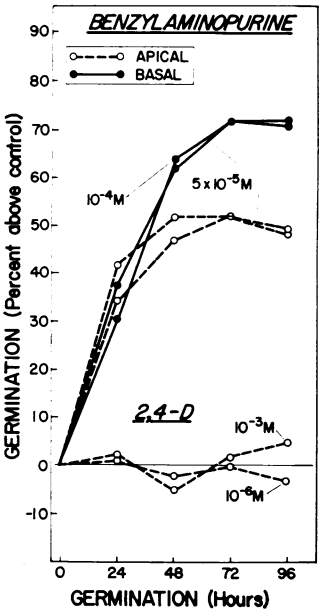


FIG. 2. The effect of benzylaminopurine and 2,4-D applied for 96 hr on the germination of dormant NC-13 peanut seeds. Control germination values for apical seeds at 96 hr were 48 ± 0 and 21 ± 1% for benzylaminopurine and 2,4-D, respectively. Similarly, the control germination values for basal seeds were 14 ± 4 and 8 ± 2%. The same control served for both concentrations of growth regulator.

the germination of basal seeds was the highest observed for these more dormant seeds in this study. The controls germinated to the same extent as for kinetin (legend, Fig. 1).

An unexpected result was that coumarin produced a slight stimulation of germination of dormant peanut seeds above the control (Fig. 1). Standard deviations of the mean did not indicate any significant difference between apical and basal seed germination at $10^{-3}$ M coumarin. The apical seed germination at $5 \times 10^{-4}$ M coumarin was the same as at $10^{-3}$ M, but there was approximately a 50% decrease in basal seed germination at the lower concentration.

Abscisic acid at $2 \times 10^{-6}$ M and higher concentrations retarded dormant peanut seed germination (Fig. 1). Germination of seeds in a random sample that would normally germinate was inhibited by $2 \times 10^{-6}$ M ABA, and the inhibition declined at $2 \times 10^{-7}$ M ABA. Although both basal and apical seeds were tested, data are presented only from the latter because these seeds are usually the easiest to induce to germinate.

Neither 2,4-D (Fig. 2) nor Alar at $10^{-3}$, $5 \times 10^{-4}$, or $10^{-6}$ M induced dormant peanut seeds to germinate. Both apical and basal seeds were tested for both compounds, but only the results for the effect of 2,4-D on apical seeds are presented. During the 96 hr of the experiment, the cotyledons of 2,4-D-treated seeds showed apparent localized areas of proliferation or swelling. Subsequently, 2,4-D-treated and and control seeds were exposed to 100 μl/liter ethylene, which released dormancy. Germination was 83 and 78% for apical and basal control seeds, respectively, while the values were 80 and 51% for 2,4-D-treated seeds. Seedlings that emerged after 2,4-D and subsequent ethylene treatment had short, thickened hypocotyl-radicles while control seedlings were normal in appearance. The only normal seedlings produced by 2,4-D-treated seed occurred at $10^{-6}$ M 2,4-D, where neither ethylene production nor cotyledonary swelling was induced.

**Effect of Plant Growth Regulators on Ethylene and Carbon Dioxide Production.** Why do some growth substances stimulate

dormant peanut seeds to germinate and others not? Our previous study (8) indicated that the partial effectiveness of gibberellic acid was correlated with its ability to induce some ethylene production by the peanut seeds. This became much more evident with our use of the substances in these experiments; thus, the time at which the maximal ethylene production occurs may be critical.

Figure 3 shows that kinetin, at $10^{-4}$ and $5 \times 10^{-5}$ M, stimulated both ethylene and $CO_2$ production by the peanut seeds. There was more ethylene production by the apical seeds than by the basal seeds; this response was positively correlated with the germination of the two seed types in response to kinetin (Fig. 1). At $5 \times 10^{-5}$ M kinetin, where the ethylene maximum was less for both apical and basal seeds and delayed until 48 hr for basal seeds, there was less germination of both seeds types (Fig. 1). At $10^{-7}$ M kinetin, there was very little stimulation of either ethylene production or germination of the dormant seeds. Carbon dioxide production showed similar trends to ethylene, but the maximum was delayed until 72 hr when considerable elongation of the radicle and hypocotyl had occurred. That the respiratory maximum may be delayed beyond 72 hr while germination is stimulated to an earlier maximum is discussed with the CEPA data.

At both $10^{-4}$ and $5 \times 10^{-5}$ M, benzylaminopurine stimulated ethylene production by the basal seeds to a slightly greater extent or at least equal to the apical seeds and the maximal production for both seed types occurred at 24 hr (Fig. 4). This increased ethylene production by basal seeds was correlated with the greater effectiveness of benzylaminopurine than kinetin in promoting germination of the more dormant basal peanut seeds (Figs. 1 and 2). The enhanced release of basal seed dormancy by benzylaminopurine is apparently the result of the ability of this growth substance to induce ethylene production by basal seeds in amounts at least equivalent to ethylene produced by the less dormant apical seeds. This agrees with our previous data, which showed that a higher concentration of exogenous ethylene gas was required in order to induce germination of the basal seeds to the same extent as that of the apical seeds (8). The optimal concentration of benzylaminopurine for enhancing ethylene production (and thus germination of the seeds) was about $5 \times 10^{-5}$ M, since $10^{-7}$ M did not stimulate either ethylene production or germination of the seeds. The $CO_2$ production of the seed types

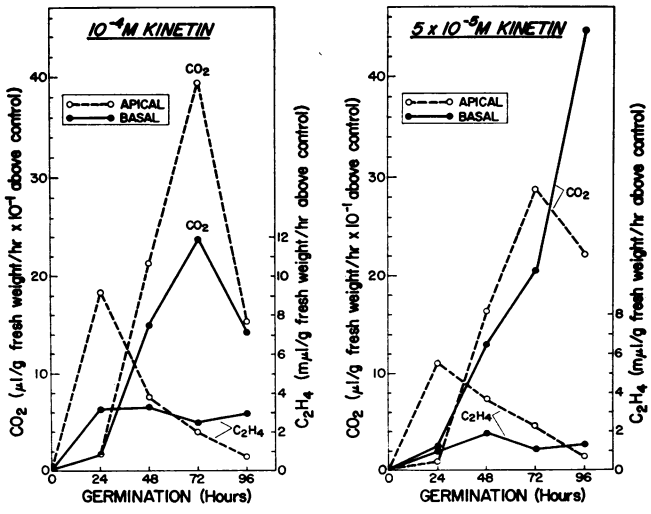


FIG. 3. The effect of kinetin applied for 96 hr on the ethylene and carbon dioxide production by dormant NC-13 peanut seeds following imbibition.

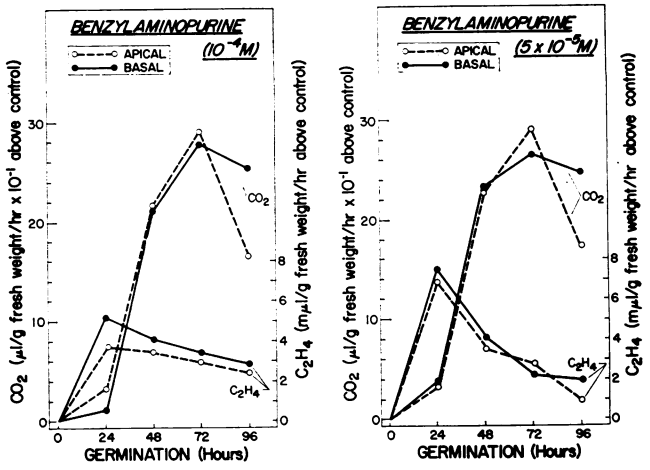


FIG. 4. The effect of benzylaminopurine applied for 96 hr on the ethylene and carbon dioxide production by dormant NC-13 peanut seeds following imbibition.

was similar and showed the same trends as for kinetin (Figs. 3 and 4).

The effect of CEPA on respiratory metabolism, as indicated by $CO_2$ production (Fig. 5), was entirely different from that of the cytokinins. The absolute values were much lower during the first 72 hr; any major stimulation was delayed until the 72 to 96 hr period, 24 to 48 hr after maximal germination above the control was attained. The CEPA treatments had a smaller, delayed effect on $CO_2$ production than the cytokinins, but they had a greater stimulatory effect on germination (Figs. 1, 5 and Ref. 8). At $10^{-6}$ M CEPA there was only a small stimulation of $CO_2$ evolution (Fig. 5) and germination (8). The data for the higher CEPA concentrations indicate that it is possible to obtain substantial increases of germination above the control in the presence of ethylene well before correspondingly large increases in respiration occur.

Coumarin gave only a slight stimulation of germination, but the apical and basal seed germinations were comparable and the ethylene production by the basal seeds was, in this instance, more than that by the apical seeds (Fig. 6). This is in agreement with the data for benzylaminopurine (Figs. 2 and 4).

Abscisic acid had no effect or failed to stimulate both ethylene production (Fig. 7) and germination (Fig. 1). Carbon dioxide production patterns were similar over the concentrations of ABA used. Comparison of the $CO_2$ values for apical seeds treated with $2 \times 10^{-7}$ M ABA with those for CEPA indicates that respiratory activity in the presence of ABA was not the limiting factor in germination of the seeds. The factor which appeared limiting in the presence of ABA was the low level of ethylene production by the seeds.

Alar did not enhance dormant peanut seed germination, and this agrees with its failure to induce ethylene production by the seeds except in low amounts for apical seeds at $10^{-6}$ M concentration (Fig. 6).

The synthetic auxin 2,4-D, at $10^{-8}$ and $5 \times 10^{-4}$ M, stimulated ethylene production by the seeds (Fig. 8), as in other systems (1, 5, 6), but this stimulation occurred in the absence of a significant increase in germination at the same concentrations (Fig. 2). The timing of induced ethylene production was different for 2,4-D than for the cytokinins, where germination was stimulated. With 2,4-D, enhanced ethylene synthesis began to appear at 48 hr and increased with time (Fig. 8) while for cytokinins ethylene synthesis peaked at 24 to 48 hr and declined with time (Figs. 3 and 4). Thus, application of 2,4-D

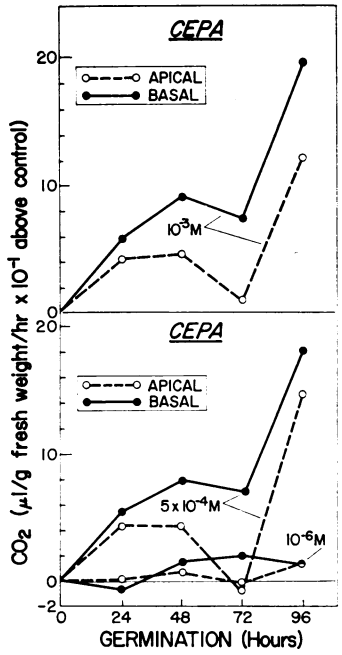


FIG. 5. The effect of CEPA applied for 96 hr on the carbon dioxide production by dormant NC-13 peanut seeds following imbibition.

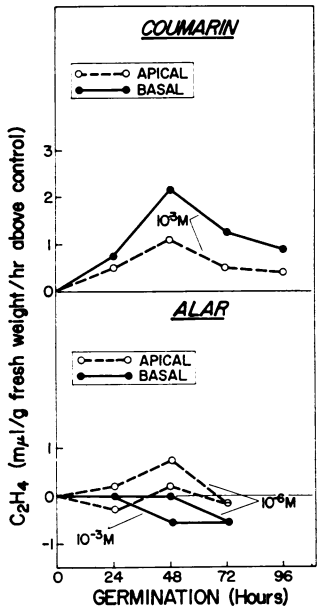


FIG. 6. The effect of coumarin and Alar applied for 96 hr on the ethylene production by dormant NC-13 peanut seeds following imbibition.

produced metabolic changes which result in swelling of seed tissue, abnormalities of subsequently germinating seedlings, delayed enhancement of ethylene production, but no significant stimulation of germination. IAA also does not stimulate germination of dormant peanut seeds (8), but it results in no visible seedling damage or ethylene production. Our findings suggest that processes by which ethylene production is stimulated in dormant peanut seeds are different for 2,4-D and the germination-inducing cytokinins. Further, 2,4-D may block the ability of the ethylene ultimately produced to release dormancy. Auxins stimulated ethylene production of intact plants while retarding leaf abscission, a specific response to ethylene (5).

The data here for $CO_2$ evolution indicate that, during release of dormancy, the action of ethylene is not dependent on and its synthesis is not the result of a major increase in respiratory metabolism. Except for basal seeds treated with kinetin, cytokinins caused a peak release of ethylene 48 hr before the peak evolution of $CO_2$ (Figs. 3 and 4). With CEPA, which is rapidly converted to ethylene, maximal germination preceded the major rise in $CO_2$ evolution, and the shape of the $CO_2$ release curve was completely different from the germination response curve. ABA, particularly at $2 \times 10^{-7}$ M, stimulated $CO_2$ evolution more promptly and to a greater extent than CEPA, yet ABA either inhibits germination or has no effect. It is clear that the seeds induced to germinate by CEPA and the cyto-

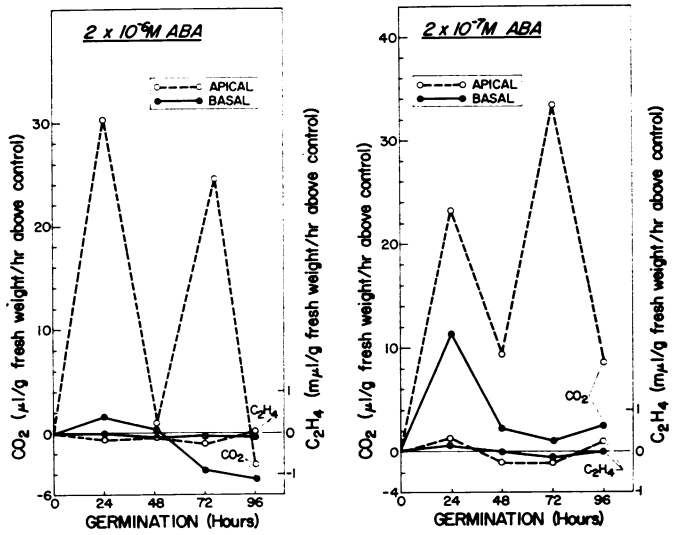


FIG. 7. The effect of abscisic acid applied for 96 hr on the ethylene and carbon dioxide production by dormant NC-13 peanut seeds following imbibition.

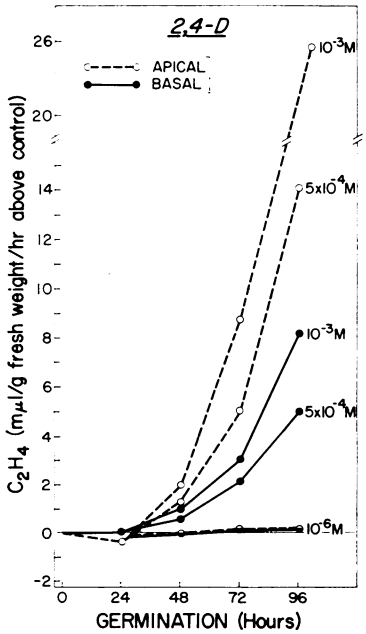


FIG. 8. The effect of 2,4-D applied for 96 hr on the ethylene production by dormant NC-13 peanut seeds following imbibition.

kinins produced $CO_2$ and that there was a stimulation of this process over the control seed, but respiratory activity does not seem to be the controlling process.

In our studies thus far, the controlling process in dormant peanut seed germination appears to be an enhancement of the natural low capacity to produce ethylene which exists in the dormant seeds. In the presence of various growth regulators, where there was no apparent adverse effects on the seeds, ethylene production ranged from 0.1 to 2 mμl per g fresh weight per hr for substances that were partially or completely ineffective in stimulating their germination (IAA and GA [8]; Alar and coumarin, Fig. 6). The time at which the maximal ethylene enhancement occurred with these substances was 48 to 96 hr of germination. Considering the substances that effectively stimulated germination of the dormant seeds (kinins, Figs. 1 and 2), the enhancement of ethylene production ranged from 2 mμl per g fresh weight per hr at $5 \times 10^{-5}$ M kinetin for basal seeds at 48 hr to 9 mμl of ethylene per g fresh weight per hr at $10^{-4}$ M kinetin for apical seeds at 24 hr of germination (Fig. 3). The least enhancement of ethylene production provided the smallest stimulation of germination and *vice versa*. Similar analogies may be drawn with benzylaminopurine. The minimal enhancement of ethylene production was about 3 mμl per g fresh weight per hr for benzylaminopurine. Thus, there is a lower limit of enhancement of about 2 to 3 mμl of ethylene production per g fresh weight per hr and an upper time limit of 48 hr of germination for this enhancement to occur if a substance is to effectively stimulate germination of the dormant seeds. This agrees with the concentration and time dependence that we previously reported for germination of dormant peanut seeds in the presence of ethylene gas (8).

The following observations support the concept that ethylene is a substance directly involved in the release of dormancy of these seeds rather than a product resulting from germination. (*a*) There is apparently a threshold ethylene production rate of 2 to 3 mμl of ethylene per g fresh weight of seeds that must be attained before dormancy is broken and germination will occur. We had previously estimated an internal concentration of 0.4 μl/liter ethylene from a production rate of 3 mμl of ethylene per g fresh weight per hr (7). (*b*) Substances that will enhance the ethylene production to these rates within 24 to 48 hr of germination without damage to the seeds will effectively stimulate germination of the dormant seeds (Figs. 1, 2, 3, and 4). (*c*) In the instances when the more dormant basal seeds were caused to germinate equal to their potential germination and in a manner approaching that of the less dormant apical seeds, ethylene production by the basal seeds was enhanced to equal or exceed that of the less dormant apical seeds (Figs. 2 and 4). Also the treatment was either ethylene or an ethylene substrate (Ref. 8 and Fig. 1 herein).

Nevertheless, it is well accepted that more than one growth substance may be involved in a given plant process. These data indicate that the cytokinins may mediate dormancy release primarily through stimulation of ethylene synthesis, but an additional, more direct role is not excluded. Dormancy does not appear to be due to a shortage of auxin (Fig. 2 and Ref. 8), and gibberellin initially plays a relatively minor or indirect role (8). Abscisic acid may prevent germination and thus cause dormancy by preventing ethylene synthesis by the seeds (Figs. 1 and 7).

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