Plant Physiol. (1972) 50, 382–387

Physiology of Oil Seeds

IV. ROLE OF ENDOGENOUS ETHYLENE AND INHIBITORY REGULATORS DURING NATURAL AND INDUCED AFTERRIPENING OF DORMANT VIRGINIA-TYPE PEANUT SEEDS[1, 2]

Received for publication February 29, 1972

D. L. KETRING AND P. W. MORGAN
Department of Plant Sciences, Texas A&M University, College Station, Texas 77843

ABSTRACT

To further elucidate the regulation of dormancy release, we followed the natural afterripening of Virginia-type peanut (*Arachis hypogaea* L.) seeds from about the 5th to 40th week after harvest. Seeds were kept at low temperature (3 ± 2 C) until just prior to testing for germination, ethylene production, and internal ethylene concentration. Germination tended to fluctuate but did not increase significantly during the first 30 weeks; internal ethylene concentrations and ethylene production remained comparatively low during this time. When the seeds were placed at room temperature during the 30th to 40th weeks after harvest, there was a large increase in germination, 49% and 47% for apical and basal seeds, respectively. The data confirm our previous suggestion that production rates of 2.0 to 3.0 nanoliters per gram fresh weight per hour are necessary to provide internal ethylene concentrations at activation levels which cause a substantial increase of germination. Activation levels internally must be more than 0.4 microliter per liter and 0.9 microliter per liter for some apical and basal seeds, respectively, since dormant-imbibed seeds containing these concentrations did not germinate. Abscisic acid inhibited germination and ethylene production of afterripened seeds. Kinetin reversed the effects of ABA and this was correlated with its ability to stimulate ethylene production by the seeds. Ethylene also reversed the effects of abscisic acid. Carbon dioxide did not compete with ethylene action in this system. The data indicate that ethylene and an inhibitor, possibly abscisic acid, interact to control dormant peanut seed germination. The inability of CO_2 to inhibit competitively the action of ethylene on dormancy release, as it does other ethylene effects, suggests that the primary site of action of ethylene in peanut seeds is different from the site for other plant responses to ethylene.

We have studied the regulation of dormancy release in Virginia-type NC-13 peanut seeds (8, 9). Seeds that germinate produce ethylene prior to hypocotyl-radicle emergence. All treatments of dormant seeds which promote germination also enhance ethylene production within 24 hr of the start of treatment. This enhancement of ethylene production is substantially above the low levels that naturally occur in dry or dormant-imbibed seeds. All of the data are compatible with our hypothesis that ethylene produced by the seeds promotes germination. Although we recognize that this hypothesis is not absolutely proven at this time, we do not think it is only a coincidence that application of exogenous ethylene breaks dormancy of peanut seeds and other seeds as well.

The purposes of the present paper are 3-fold. First, we present additional evidence bearing on the question of whether endogenous ethylene produced by peanut seeds escaping dormancy has a regulatory role or its presence is merely an indication that the seeds are no longer dormant and are, in fact, germinating. Secondly, we present data showing that exogenous ABA mimics natural dormancy and its action is competitively reversed by cytokinins, and most important, ethylene. Competitive action of ethylene with a natural growth inhibitor has not previously been reported. The interaction between substances that have opposing effects on plant growth has been found to be one means of dormancy regulation in both seeds and buds (1, 3, 6, 10, 11). Thus, our data indicate that such a promoter-inhibitor system may regulate germination of dormant peanut seeds. Our third purpose is to present results of an extensive study of ethylene physiology of afterripening peanut seeds with the view that additional data on this stage of development will be of general value to the understanding of afterripening of peanut seeds and perhaps other seeds as well.

MATERIALS AND METHODS

The basis for the experiments reported here were described in detail in previous communications (7–9). Briefly, dormancy of Virginia-type NC-13 peanut (*Arachis hypogaea* L.) seeds is a natural phenomenon which varies in intensity (degree or depth of dormancy) from year to year. Immediately after harvest and curing (drying of seeds to a safe storage moisture level of about 6%), afterripening begins. At 3 C, the storage temperature we use, afterripening will not be complete (90% germination) for several months (8, Fig. 5). But each lot of seeds is quantitatively different in depth of dormancy to some degree from previous lots, and dormancy is constantly changing as afterripening progresses. Thus, from a practical standpoint, it is impossible to do all of the desirable experiments with seeds that have the same quantitative degree of dormancy. Further, detailed dose-response curves have quantitative value only for a given seed lot at a given stage of afterripening. Within the limitations of such a system, we have conducted a sequential study on an annual basis in which we have built each series

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

[2] Mention of a trademark name or a 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.

of experiments on the previous year's findings. Where necessary the specific purpose of experiments is given below along with earlier supporting information.

The procedures for determining ethylene, the method of germination and seed source have been reported (7–9). The procedure for treatments with CO_2 are the same as those used for ethylene (8). The method of Beyer and Morgan (2) was used to determine the internal ethylene concentrations of the seeds. Internal ethylene concentrations of both germinated and ungerminated seeds were measured at 96 hr from the start of imbibition, when a maximum number of seeds had germinated. This may be the least favorable time to measure internal ethylene of germinated seeds, since maximum ethylene production occurs earlier, but this procedure allowed sufficient time to be more certain that the remaining imbibed seeds were dormant. These imbibed dormant seeds were a key part of our study since their ethylene content and production ability *cannot* be viewed as a by-product of germination. They have not germinated and experience indicated they would not germinate unless they were subjected to ethylene.

Samples of seeds were tested for germination beginning about the 5th week after harvest. Sampling was done weekly or biweekly for the first 22 weeks after harvest to detect any rapid changes in germinability that might have occurred. The intervals between sampling were then increased to 3 to 4 weeks until termination of the experiments at 40 weeks after harvest.

Seeds used for the initial 18 weeks were hand shelled shortly after curing and stored in sealed containers at 3 ± 2 C within 5 weeks after harvest. The remainder of the seeds were bulk stored in the shell at the same temperature but in a plastic bag placed inside a large, covered plastic container. These seeds were brought from bulk storage in small lots, and hand shelled and placed in sealed containers at 3 ± 2 C when the original supply of seeds was nearly depleted. This was done for convenience only but may have affected our results (see "Results and Discussion").

RESULTS AND DISCUSSION

Effect of ABA on Ethylene Production and Germination. Previously, treatment of a relatively dormant seed lot with ABA reduced the residual ethylene production and germination that usually occurs in dormant peanut seeds (9). The inhibitory activity of ABA was more fully expressed when it was applied to afterripened peanut seeds with relatively high germination (Fig. 1). ABA reduced ethylene production and germination in a nearly parallel manner at concentrations between 2 and 100 μM. As seeds afterripen with time, there are increases in the percentage of a population that will produce ethylene and germinate (Figs. 5A and 6). Our results (Fig. 1) suggest that during natural afterripening there are quantitative changes in either or both growth-inhibiting (ABA) and stimulating substances (C_2H_4) that regulate dormancy of peanut seeds.

Effect of Cytokinin and ABA on Ethylene Production and Germination. Since ABA clearly reinstated dormancy or inhibited germination (Fig. 1) and cytokinins break dormancy or promote peanut seed germination (9, Fig. 2), the ability of a synthetic cytokinin, benzylaminopurine, to competitively overcome ABA-mediated dormancy was tested. The concentration of benzylaminopurine used was based on previous results (9) that showed a 50 μM concentration was effective in breaking peanut seed dormancy. At an ABA level that significantly lowered ethylene production and germination of afterripened seeds (Fig. 1) and reduced ethylene production and germination of dormant seeds below the water controls (Fig. 2), benzylaminopurine overcame any ABA effect and stimulated both

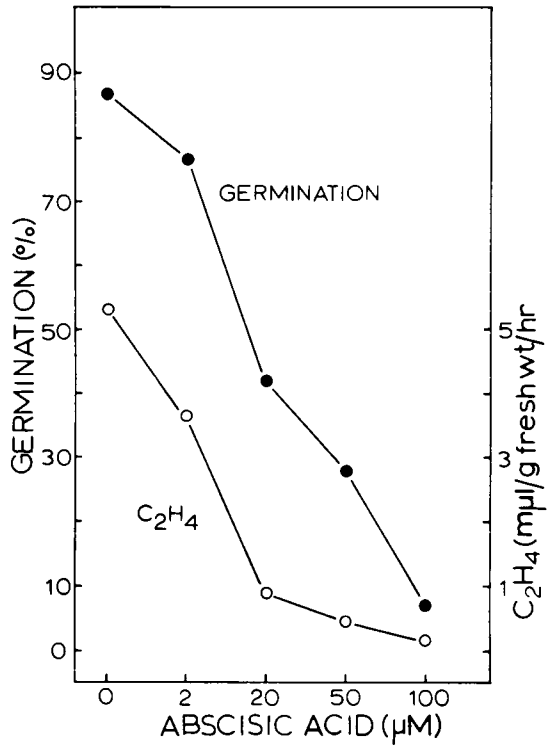

FIG. 1. Effect of ABA on germination and ethylene production by afterripened Virginia-type, apical peanut seeds.

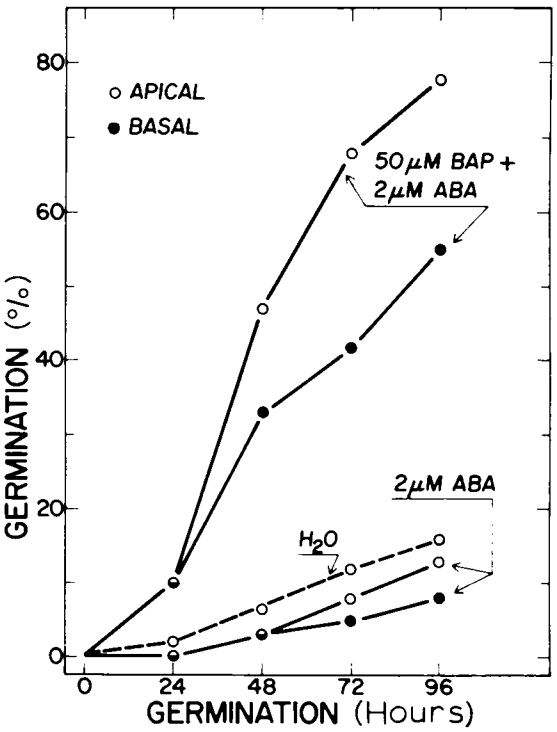

FIG. 2. Effect of ABA and ABA plus benzylaminopurine (BAP) on germination of dormant, Virginia-type peanut seeds. Basal seeds are inherently more dormant. One-half open symbols in all figures indicate a common point.

ethylene production and germination of dormant peanut seeds in a closely correlated manner (Figs. 2 and 3). The initial cytokinin-induced increase in both ethylene production and

germination was fully evident at 24 hr, a time at which CO_2 production (Fig. 3, upper) had not risen above that for the water or ABA-treated seeds. Occurrence of the cytokinin-mediated stimulation of ethylene evolution and germination at a time when CO_2 evolution was the same in control, ABA and ABA plus cytokinin-treated seeds is evidence against the argument that ethylene production occurs after dormancy release and simply parallels respiratory metabolism. In earlier work the ability of cytokinins to break peanut seed dormancy was attributed to their large stimulation of ethylene production (9).

Effect of Ethylene and ABA on Germination. If benzylaminopurine mediates reversal of the germination inhibition by ABA (Fig. 2) via its stimulation of ethylene production and consequently increases internal ethylene concentrations of the seeds, then externally applied ethylene should also reverse the effects of ABA. Table I shows that 8.5 μl/l C_2H_4 induced 91% germination of dormant apical seeds that were previously treated for 48 hr with 20 μM ABA, a concentration which will provide at least 50% inhibition of germination of afterripened seeds (Fig. 1). We had previously found in a detailed study of ethylene effects on dormant peanut seeds that 8 μl/l C_2H_4 was sufficient to induce equal germination of both apical and basal seeds (8). In the presence of ABA or because this was a more domant seed lot, a higher concentration of C_2H_4 was required to induce a similar amount of germination in the more dormant basal seeds (Table I). Thus, ethylene competitively reversed the inhibitory effects of exogenous ABA on germination of dormant peanut seeds that were imbibed in ABA. Also, at sufficient levels, ethylene removed the quantitative difference in dormancy, natural as well as ABA induced, between apical and basal seeds.

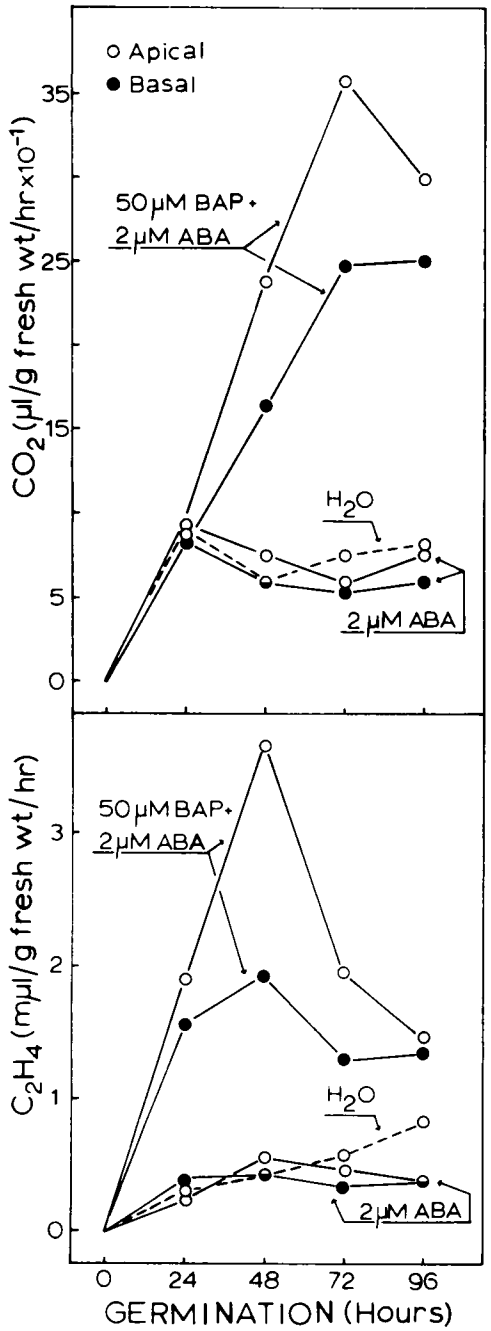

FIG. 3. Effect of ABA and ABA plus benzylaminopurine (BAP) on carbon dioxide (upper) and ethylene production (lower) by dormant, Virginia-type peanut seeds.

Table I. *Effect of Ethylene on Germination of Dormant Virginia-type Peanut Seeds*

Seeds were treated with 20 μM ABA. Seeds were imbibed 16 hr in ABA, excess solution was poured off, and germination was 0% at 48 hr. At this time C_2H_4 was added, and the germination was recorded after an additional 48 hr. The data are the means of duplicate samples of apical and basal seeds.

C_2H_4	Germination	
	Apical	Basal
μl/l	%	
8.5	91	55
50.0	97	92

Effect of Ethylene and CO_2 on Germination. In many plant responses to C_2H_4, CO_2 acts as a competitive inhibitor of ethylene action (4), and this effect of CO_2 has been used as evidence that the response is under control by ethylene. It is well known that a variety of dormant seeds are stimulated to germinate, at least somewhat, in the presence of CO_2 and this also has been documented for peanut seeds. Our purpose is to show its effect in relation to the C_2H_4 response of dormant peanut seeds. We obtained nearly the same results with 10 or 15% CO_2.

At 1 μl/l C_2H_4 and 15% CO_2 there was an additive promotion of germination by the two gases (Fig. 4); the promotion by CO_2 was almost equal to that of ethylene at this concentration. At 5 μl/l C_2H_4 there was no detectable effect of either 10 or 15% CO_2. A CO_2 effect was also absent at 10 μl/l ethylene. Ethylene at 30 μl/l raised germination to above 90% for both apical and basal seeds in the presence of 10% CO_2.

It is clear that CO_2 does not competitively inhibit C_2H_4 action in this system. In fact, CO_2 acts to promote germination somewhat but the promotion was obscured by much greater ethylene stimulation of germination between 1 and 5 μl/l C_2H_4. There is a definite possibility that the capacity of endogenous ethylene to break dormancy would be promoted by any endogenous CO_2 produced after imbibition.

These findings have several implications. The general role of CO_2 in seed germination needs to be re-evaluated. Other workers have observed promotion of seed germination by CO_2 but made no particular connection of these observations to ethylene physiology until recently (12). The data are compatible with the hypothesis that ethylene participates in natural dormancy release and raises the possibility that C_2H_4 and CO_2 have different relationships before and after dormancy release. However,

Egley and Dale (5) found that CO_2 (10%) competitively inhibited the C_2H_4 stimulation of germination of dormant witchweed seeds. In this system, CO_2 alone inhibited germination below control values, which suggests that the action of native ethylene was being blocked. The site of ethylene action or C_2H_4-CO_2 competition may be significantly different in dormant witchweed and peanut seeds.

Natural Decline of Dormancy. For the first 14 weeks after harvest, the mean germination from five experiments was 21% and 14% for apical and basal seeds, respectively (Fig. 5). This is similar to our previous report (8). Ethylene production for this period was less than 1.0 nl/g fresh wt·hr for both apical and basal seeds (Fig. 6A). It should be emphasized that the ethylene production observed was primarily due to germinating seeds in the test samples. Dormant-imbibed seeds have a comparatively low rate of ethylene production (7). Internal ethylene concentrations were 0.03 μl/l for dry seeds and 0.25 μl/l for dormant-imbibed seeds (Fig. 5B and C).

We expected that germination would continuously rise from the 14th week onward, rapidly for the inherently less dormant apical seeds (8), but this did not occur. There was a moderate increase in germination between the 16th and 22nd week and then a decline at 24 to 30 weeks after harvest. Apparently these seeds were more dormant than those used previously, since the previous lot afterripened more rapidly (8).

The germination decline during weeks 24 to 30 may have been caused by a change in type of storage employed for the seed used from weeks 20 to the completion of the test (see "Materials and Methods"). However, two experiments with the freshly shelled seeds at 20 and 22 weeks revealed no change in germination and ethylene production between these seeds and those shelled earlier. Therefore, either a natural "secondary dormancy" or an effect of storage in the shell which requires 3 to 4 weeks after shelling for expression is responsible for the decline in germination during the 24- to 30-week period after harvest.

Internal ethylene concentrations of dry seeds were relatively low and showed only minor changes during the entire afterripening period (Fig. 5B). Apparently, the changes in dry seeds during afterripening, which allow increased ethylene production and internal concentrations to occur, can be expressed only after imbibition.

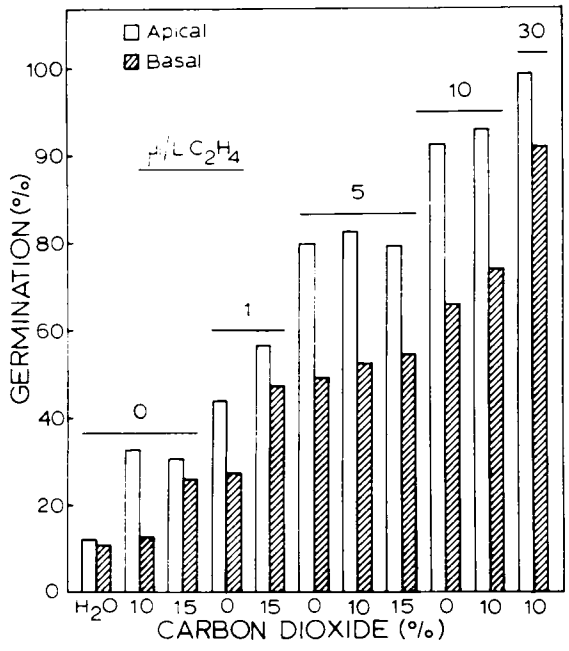

FIG. 4. Effect of CO_2 and ethylene applied separately and together on germination of dormant, Virginia-type peanut seeds. Each datum is the mean of two to four replicate samples.

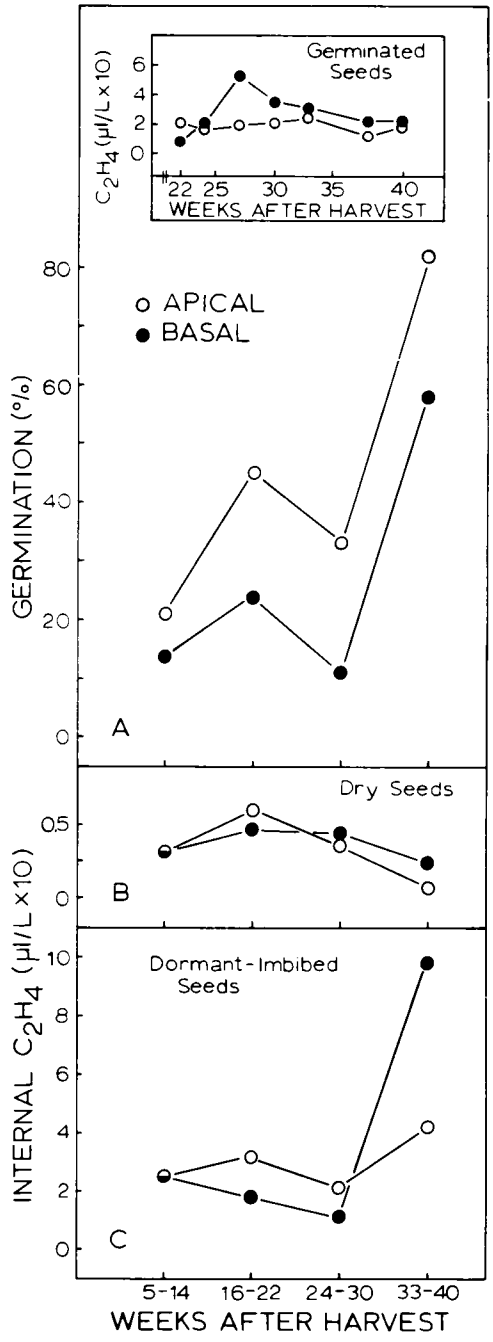

FIG. 5. The natural changes in germination (A) and internal ethylene concentrations of dry (B), dormant-imbibed (C), and germinated seeds (A, inset) during afterripening of dormant, Virginia-type peanut seeds. Each point represents the mean of at least three experiments during each time interval.

Internal ethylene concentrations of dormant-imbibed seeds were followed to allow the ethylene producing capacity of these seeds which failed to germinate in 96 hr to be compared to the germination pattern of the population. During the first three periods (5–14, 16–22, and 24–30 weeks), ethylene levels were relatively stable, *i.e.*, 0.2 ± 0.1 μl/l (Fig. 5C). Germination did not increase significantly during this time (Fig. 5A). It became evident that a large increase in germination of these

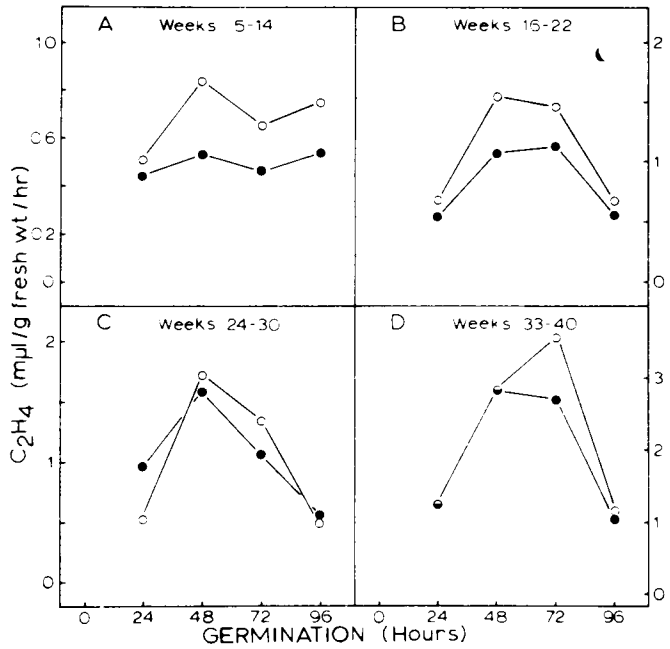

FIG. 6. The natural changes in ethylene production during afterripening of dormant, Virginia-type peanut seeds. The data are for heterogenous samples that contained some seeds which germinated (Fig. 5A) and some which remained dormant. Solid symbols are for basal and open symbols are for apical seeds. Note changes in scales for ethylene production with weeks after harvest.

seeds might not occur in the cold before the seed lot was exhausted by sampling. Therefore some of the seed containers were placed at room temperature at 30 weeks after harvest. The mean germination for three experiments during the period 33 to 40 weeks after harvest was 82% and 58% for apical and basal seeds, respectively (Fig. 5A). This was an increase of 49% and 47% above the previous period for apical and basal seeds, respectively. At the same time that these increased increments of germination occurred, the internal ethylene concentration of dormant-imbibed apical seeds doubled and that of basal seeds increased 9-fold. Thus, while the dormancy of the total population of seeds was decreasing significantly, there were major increases in ethylene in those seeds that were still "too dormant" to germinate. The ethylene concentrations of 0.4 and 0.9 μl/l observed at this time in apical and basal seeds, respectively, must still be somewhat below activation levels since these seeds did not germinate. Experience with dormant seeds showed that, with few individual exceptions, all seeds that would germinate had done so by 96 hr and those still ungerminated at this time could be brought to nearly 100% germination by exposure to 100 μl/l ethylene. Therefore, high internal ethylene concentrations are necessary to induce the most dormant seeds in a sample to germinate and a higher concentration is required for the inherently more dormant basal seeds than the apical seeds.

We reason that the internal ethylene levels in the seeds which germinated during the interval of rapid decline in dormancy (33–40 weeks) rose above the threshold level to release dormancy within 96 hr after imbibition. This is suggested in Figure 6 where ethylene production by a population of seeds was increased from around 1 to 2 to nearly 3.0 nl/g fresh wt·hr at 48 hr of germination during the period of rapid decline in dormancy (33–40 weeks). That some of the ethylene production monitored in Figure 6 derived from seeds between the time of imbibition and dormancy release is supported by the significant levels of the gas found in other seeds of the same sample which were still dormant (Fig. 5C) and the fact that both internal ethylene levels and production rates of imbibed seed were much lower during the initial weeks of the experiment. During these first 5 to 14 weeks few seed germinated (Fig. 5A), and ethylene production (Fig. 6A) and internal levels were at their lowest levels.

Internal levels of ethylene in germinated seed at 96 hr were predictably low (Fig. 5A, insert); the time course of ethylene production consistently shows declines before this time (Fig. 6). The data in Figure 5A, insert, do allow a defense of the vacuum extraction technique which releases and collects internal gases so that they can be analyzed for ethylene content. The methods produced uniform data for various types of seeds extracted (Fig. 5A, insert; 5B, and 5C) and only during the period of dormancy release did major changes in values obtained occur. Thus, the doubling and 9-fold increases in ethylene levels must be recognized as a valid indication of a rise in the capacity of imbibed seed to produce ethylene.

These data show that a rise in ethylene production capacity is necessary to release dormancy in peanut seeds; concomitantly, there was an increase in internal ethylene concentration of the seeds. It is possible to attain germination levels in the 50 and 25% range with ethylene production rates of 1 to 2 nl/g fresh wt·hr at 48 hr of germination and internal ethylene concentrations of 0.3 and 0.2 μl/l for apical and basal seeds, respectively (Fig. 5A, 6, B and C, 5C). In a heterogenous seed population, the seeds which germinate under these conditions are most likely the less dormant ones. However, when a large increase in germination was achieved during natural release of dormancy, in this case 2.5- and 5-fold for apical and basal seeds, respectively, ethylene production rose to nearly 3.0 nl/g fresh wt·hr at 48 hr of germination for both seed types. This agrees with our previous data for seeds treated with cytokinins to break dormancy (9). At the same time, internal ethylene concentrations increased 2-fold to 0.4 μl/l and 9-fold to 0.9 μl/l for apical and basal seeds, respectively. The lower amount of internal ethylene (0.4 μl/l) agrees with our previous estimate from mixed seed types (7), but the value of 0.9 μl/l for the inherently more dormant basal seeds is at least twice the previous estimate.

The control of dormancy in some seeds and buds has been shown to be due to different levels of plant growth stimulators and inhibitors in the organ and/or adjacent tissues (1, 3, 6, 10, 11). The data above suggest that such a system is operative in dormant peanut seeds. Ethylene appears to participate as a primary stimulant and the gas interferes with ABA to overcome its inhibitory action. Whether ABA is the natural germination inhibitor (dormancy agent) in peanut seeds is being investigated. Ethylene is the key stimulatory factor, whether it is supplied exogenously or a treatment is applied to the seeds which increases their ability to synthesize the gas.

Neither the presence nor absence of CO_2 precludes ethylene activity. In fact, at low ethylene levels CO_2 adds to the activity of the regulator rather than inhibiting it. When germination of dormant seeds is stimulated, the peak in respiratory activity is later than the peak in ethylene production. Thus, neither respiratory activity expressed as CO_2 release, nor the usual ability of CO_2 to competitively inhibit ethylene action, absent here, are as closely associated with dormancy release, expressed by germination, as is the level of ethylene in the imbibed seeds. Here, ethylene is initiating growth rather than inhibiting it. Since CO_2 does not compete with ethylene in this system, it is quite possible that at least two different types of active sites for ethylene exist or the site is different from that in other systems.

The ability of ethylene and cytokinins (which stimulate ethylene production) to reverse the inhibition of ethylene pro-

duction and germination exerted by ABA strongly suggest that ethylene participates in dormancy release and is not simply a by-product of metabolism which arises in seeds once dormancy is released or germination starts. This conclusion is further supported by the rise in internal ethylene levels in dormant-imbibed, nongerminating seeds which correlate with major declines in dormancy of the total population. Thus, ethylene production capacity rises before afterripening is complete. Those seeds in a given group which germinate represent that portion in which the balance in the level of natural germination inhibitors and the capacity to produce ethylene had shifted to favor germination.

Taken as a whole our data favor the hypothesis that the germination of dormant peanut seeds is controlled through plant growth regulator action on ethylene production by the dormant seeds. The plant growth regulator may be a cytokinin or more directly ethylene itself. One important event is a rise in ethylene production that provides stimulatory levels of ethylene in the tissue. Declines in levels of germination inhibitors, such as ABA, may be another element of control.

Acknowledgment—This paper is dedicated to my daughter, Carol Ann Ketring Buckley, whose enthusiastic approach to life, typical of the majority of today's young adults, broached caution, which sometimes results in tragedy.

LITERATURE CITED

1. Addicott, F. T. and J. L. Lyon. 1969. Physiology of abscisic acid and related substances. Annu. Rev. Plant Physiol. 20: 139–164.
2. Beyer, E. M., Jr., and P. W. Morgan. 1970. A method for determining the concentration of ethylene in the gas phase of vegetative plant tissue. Plant Physiol. 46: 352–354.
3. Black, M. 1970. Seed germination and dormancy. Sci. Prog. 58: 379–393.
4. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144–152.
5. Egley, G. H. and J. E. Dale. 1970. Ethylene, 2-chloroethylphosphonic acid, and witchweed germination. Weed Sci. 18: 586–589.
6. Evenari, M. 1957. The physiological action and biological importance of germination inhibitors. Symp. Soc. Exp. Biol. 11: 21–43.
7. 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 peanut seeds. Plant Physiol. 44: 326–330.
8. Ketring, D. L. and P. W. Morgan. 1970. Physiology of oil seeds. I. Regulation of dormancy in Virginia-type peanut seeds. Plant Physiol. 45: 268–273.
9. Ketring, D. L. and P. W. Morgan. 1971. Physiology of oil seeds. II. Dormancy release in Virginia-type peanut seeds by plant growth regulators. Plant Physiol. 47: 488–492.
10. Wareing, P. F. 1969. The control of bud dormancy in seed plants. Symp. Soc. Exp. Biol. 23: 241–262.
11. Wareing, P. F. and G. Ryback. 1970. Abscisic acid: a newly discovered growth-regulating substance in plants. Endeavour 29: 84–88.
12. Wegm, F. B., O. E. Smith and J. Kumamoto. 1972. Interaction of carbon dioxide and ethylene in overcoming thermodormancy of lettuce seeds. Plant Physiol. 49: 869–872.