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Effect of Ethylene on Cell Division and Deoxyribonucleic Acid Synthesis in *Pisum sativum*[1]

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ABSTRACT

Ethylene and supraoptimal levels of 2,4-dichlorophenoxyacetic acid inhibit the growth of the apical hook region of etiolated *Pisum sativum* (var. Alaska) seedlings by stopping almost all cell divisions. Cells are prevented from entering prophase. The hormones also retard cell division in intact root tips and completely stop the process in lateral buds. The latter inhibition is reversed partially by benzyl adenine. In root tips and the stem plumular and subhook regions, ethylene inhibits DNA synthesis. The magnitude of this inhibition is correlated with the degree of repression of cell division in meristematic tissue, suggesting that the effect on cell division results from a lack of DNA synthesis. Ethylene inhibits cell division within a few hours with a dose-response curve similar to that for most other actions of the gas. Experiments with seedlings grown under hypobaric conditions suggest that the gas naturally controls plumular expansion and cell division in the apical region.

Growth of etiolated pea seedlings (38) and many other plants is suppressed by ethylene at least in part because the gas slows the rate of cellular expansion and causes roots and shoots to expand isodiametrically rather than longitudinally (4, 8–11). In pea seedlings several processes dependent upon cell division are also inhibited by ethylene; for example, primary root elongation and lateral root formation (10, 11), lateral bud development (7), and expansion of the plumular leaf (18, 19). Therefore, the effect of the gas on cellular division and DNA synthesis was investigated to determine whether these processes also might be involved in ethylene-induced growth inhibition. Applied ethylene and auxin generally affect the growth of pea seedlings in a similar manner because auxin induces ethylene production (1, 4, 9, 23, 24, 32). The present study compares the effects of 2,4-D and ethylene on growth and cell division, and investigates the possibility that endogenous ethylene, produced primarily in the plumule and other auxin-rich meristematic cells (8, 18), naturally controls the cell division frequency in these tissues.

MATERIALS AND METHODS

Seeds of *Pisum sativum* (var. Alaska) were soaked for 6 hr in tap water, planted in moist vermiculite, and grown in complete darkness at 23 C and 80% relative humidity. Unless otherwise indicated, 7-day-old plants with third internodes, approximately 1.5 cm long, were selected for experiments on plumular, stem, or bud growth, while 3-day-old seedlings with primary roots, 1 to 2 cm long, were used for studies on root growth. Pots of seedlings also were grown under hypobaric conditions by placing them in a 10-liter desiccator which was evacuated continuously at approximately 0.5 standard cubic foot per hr with a vented exhaust oil-seal pump. The pressure within the desiccator was maintained at 120 mm Hg by continuously admitting pure O_2 to the desiccator through a Matheson No. 49 regulator (5). The incoming O_2 was saturated at the reduced pressure by passing it through water.

Growth Measurements. All tissue was handled under dim green light. A 5-mm stem region was demarcated with two ink spots just below the hook; this is referred to as the subhook region, and the region above it as the hook region. Groups of potted plants were either gassed with ethylene in air tight chambers, sprayed with 2,4-D, treated with a combination of the two hormones or irrigated with 2,4-D solution. The chambers were aerated for several minutes each day to prevent accumulation of CO_2, ethylene, or other gases, after which the appropriate concentration of ethylene was replaced as required. In some cases seedlings treated with ethylene for various periods of time were transferred to a ventilated space to study their recovery from the action of the gas. Every 24 hr 10 plants from each treatment were harvested and the region between the two spots and that above the upper spot excised, weighed, and the length measured.

Bud growth was studied by the method of Wickson and Thimann (7, 49) using 3-cm sections cut from the second node of 7-day-old etiolated seedlings. Ten sections were placed with their buds uppermost in a Petri dish containing 10 ml of 2% sucrose, 5 mM potassium phosphate buffer, pH 6.8, and 5 μM $CoCl_2$ with or without 0.1 mM BA.[3] The Petri dishes were sealed in desiccators, and in some cases sufficient ethylene was added to yield a final concentration of 50 μl/l. Tissue was incubated for 48 hr at 25 C with a 16-hr light cycle (1600 μw per cm^2), and then fixed and embedded. The buds were sectioned and stained to determine the number of mitotic figures.

Tissue Staining and Determination of Cell Division Frequency. Tissue was fixed in FAA, dehydrated with TBA, embedded in Paraplast, sectioned, and stained with Delafield Hematoxylin-Safranine. Mitotic figures excluding prophase were located and counted with a camera lucida drawing attachment. Variability is expressed as standard deviation. In some cases a Feulgen staining procedure was used to distinguish prophase from other stages of cell division.

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[3] Abbreviations: BA: benzyl adenine; FAA: ethyl alcohol-formalin-acetic acid, 6:3:1; TBA: *tert*-butyl alcohol.

Determination of DNA Synthesis. Twenty sections were incubated in 10 ml of medium containing 5 mM potassium phosphate buffer, pH 6.8, 2% sucrose, 1 μM IAA, and 5 μM $CoCl_2$ to which thymidine methyl-^{3}H (specific radioactivity 18 mc/mmole) and 0.1 mM sodium penicillin G was added. Either the entire hook, the 5-mm subhook region, or a 5-mm root tip was used. At the end of the incubation period, the sections were rinsed with distilled water and floated for 15 min in distilled ice water to remove residual isotope from the free space. DNA synthesis was determined using two methods: extraction of DNA and historadioautography. Ten sections from each treatment were used for DNA extraction and 10 sections for historadioautography. To extract DNA, tissue was ground in a glass homogenizer with cold methanol and aliquots removed for determination of total isotope uptake and incorporation into DNA. Aliquots were extracted twice with cold methanol containing 50 mM formic acid, then washed three times with cold 5% trichloroacetic acid containing 0.1 mM thymidine monophosphate, and the residue was collected on a glass fiber filter (43). Total isotope uptake was measured using 0.2 ml of the initial homogenate, and incorporation of thymidine methyl-^{3}H into DNA was determined by placing the precipitate-containing filter paper discs in scintillation vials containing 10 ml of Bray's solution. For historadioautography, tissue was fixed in FAA, dehydrated by means of TBA, embedded in Paraplast, and sectioned. The ribbons were floated onto slides coated with egg albumin adhesive, dried, the paraffin was removed with xylene, and the slides brought to water by means of an ethanol series. The slides were again dried and then dipped in Kodak NTB2 nuclear track emulsion, exposed in darkness at 4 C for 2 days, and developed in Kodak D-19 developer (29). After sections were rinsed in stop bath and fixed, they were stained with 0.1% aqueous Toluidine Blue-O for 30 sec, brought to xylene, and permanently mounted in Piccolyte resin mounting medium. Incorporation of thymidine methyl-^{3}H into DNA was determined by counting silver grains over labeled nuclei per 1000 cells ± standard deviation.

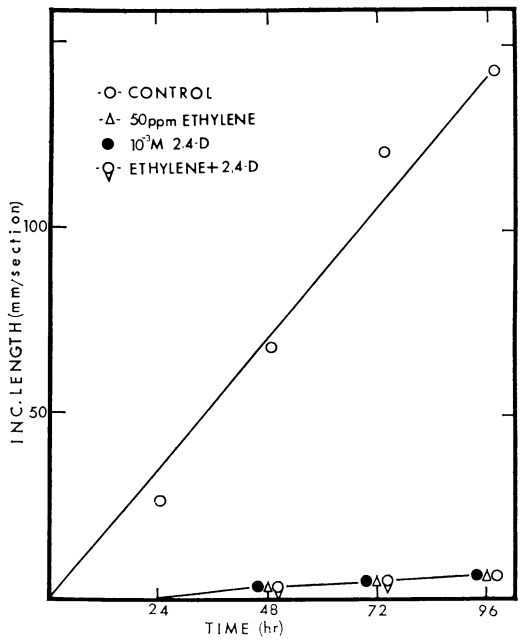

FIG. 1. Effect of ethylene and 2,4-D on the elongation of the hook region in etiolated pea seedlings. Similar results were obtained with 0.1 mM 2,4-D substituted for 1 mM.

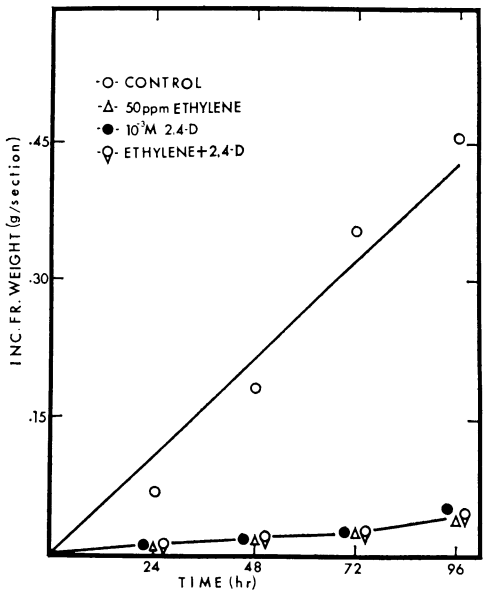

FIG. 2. Effect of ethylene and 2,4-D on the increase in fresh weight of the hook region in etiolated pea seedlings. Similar results were obtained with 0.1 mM 2,4-D substituted for 1 mM.

RESULTS

Effect of Ethylene on the Growth of the Apical Hook Region. The hook of control plants elongates and increases in fresh weight at a rapid, linear rate during a 4-day experimental period (Figs. 1 and 2) whereas 50 μl/l ethylene, 0.1 or 1 mM 2,4-D, and a combination of the two hormones continuously inhibits plumular growth by about 94%. As 2,4-D stimulates ethylene evolution, the similar responses to 2,4-D and ethylene, and lack of a synergistic effect, indicate that 2,4-D inhibits growth of the apex primarily by inducing ethylene production. No changes in the gross morphology of the plumule, other than hook tightening, were caused by any of the treatments. Growth continued to be inhibited for more than 10 days and no new internodes formed as long as ethylene was present. However, when plants were removed from ethylene after 1 or 2 days treatment and placed in a ventilated space, they appeared to recover from the inhibition; within 8 to 10 hr a variable but substantial increase in apical growth was observed and subsequently the seedlings appeared to resume a normal rate of elongation.

Effects of Ethylene on Cell Division. The cell division frequency is reduced by 95 to 98% in the hook region of plants treated with 50 μl/l ethylene or either 0.1 or 1 mM 2,4-D for 24 to 48 hr (Table I). The maintenance of a high cell division frequency in the apex is contingent upon the continued attachment of the plumule to the plant. In three replicate experiments it was found that within 12 hr after plumules were excised from 7-day-old seedlings and placed in a solution containing 2% sucrose, 5 mM potassium phosphate buffer, pH 6.8, and 5 μM $CoCl_2$, the cell division frequency was reduced from an initial value of 547 to only 30 per 5 median 10 μ thick longisections. After 36-hr incubation only 19 cell divisions were detected in the excised hooks, whereas 470 were counted in the plumules of intact seedlings at that time. Throughout this period of time the isolated plumules continued to grow by cellular expansion at a rapid rate, reforming a subhook zone more than 1 cm in length. Because isolated plumules failed to maintain a high cell division frequency, no attempt was made to utilize them in subsequent studies.

Table I. *Effect of Ethylene and 2,4-D on Cell Division in the Apical Hook Region of Etiolated Pea Seedlings*

Treatment	Number of Cell Divisions[1]		
	Initial	24 hr	48 hr
Control	470 ± 32	525 ± 66	430 ± 45
Ethylene (50 μl/l)		27 ± 4	16 ± 9
2,4-D (0.1 mM)		21 ± 4	29 ± 11
2,4-D (1 mM)		12 ± 3	8 ± 3

[1] Number of mitotic figures (meta-, ana-, and telophase) in the median 50 μ (5 median longisections, 10 μ per section) of the hook region, including the leaves. Data are presented as average of four replicate experiments, with standard deviation.

Ethylene inhibits cell division within a few hr (Fig. 3). After 2-hr treatment with 50 μl/l ethylene, the number of metaphase figures is reduced by 27%; after 6 hr, by 50%; and within 10 hr the inhibition is almost complete. Only metaphase figures were counted because this is the earliest stage of cell division recognizable by the Delafield Hematoxylin-Safranine staining procedure, and therefore ought to be the stage most rapidly depleted in the ethylene treated seedlings even if cells which have started to divide complete division in the presence of the gas. Recovery from ethylene treatment occurs rather slowly; when plants exposed to the gas for 1 to 2 days were placed in an ethylene-free atmosphere, the cell division frequency did not increase substantially for 18 to 20 hr. At that time it was still very low and highly variable, averaging only about twice the number in plants maintained continuously in ethylene. Within a few days it may recover completely, however, for growth of the hook appears to occur at a normal rate by then. Cell division has the same remarkable sensitivity to ethylene as numerous other processes in pea seedlings; a threshold response with 0.05 μl/l, half maximal at 0.2 μl/l, and a complete effect with a few μl/l ethylene (Fig. 4).

Ethylene interferes with cell division by blocking a stage before prophase (Table II). Prophase and metaphase figures decrease in frequency at about the same rate as the mitotic index and more rapidly then anaphase and telophase, at least initially. A Feulgen staining procedure was used in these studies and zones of cells scored in the apex and plumular leaf. No attempt was made to obtain median longitudinal sections so the data do not present as accurate a time sequence as that depicted in Figure 3 where all cells in the median 10 μ zone of five sections were counted. In using the Feulgen method it was advantageous to select zones of farily active cell division in the

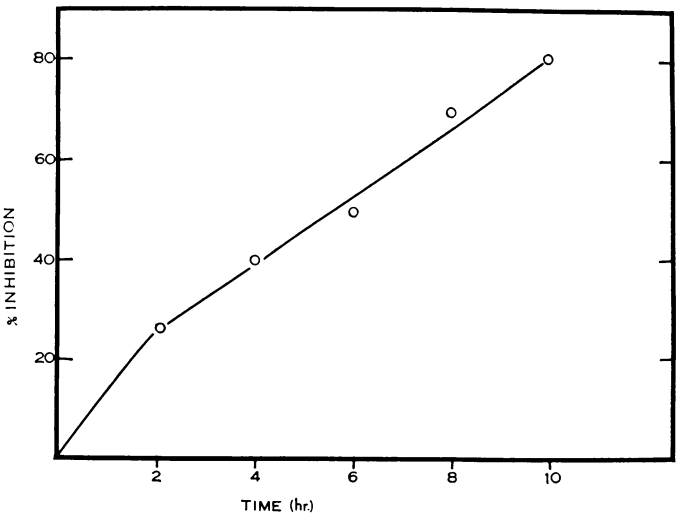

FIG. 3. Time courses for the inhibition of cell division in the hook region by 50 μl/l ethylene. Data represent the number of metaphase figures in the median 50 μ (5 median longisections, 10 μ per section) of the hook region.

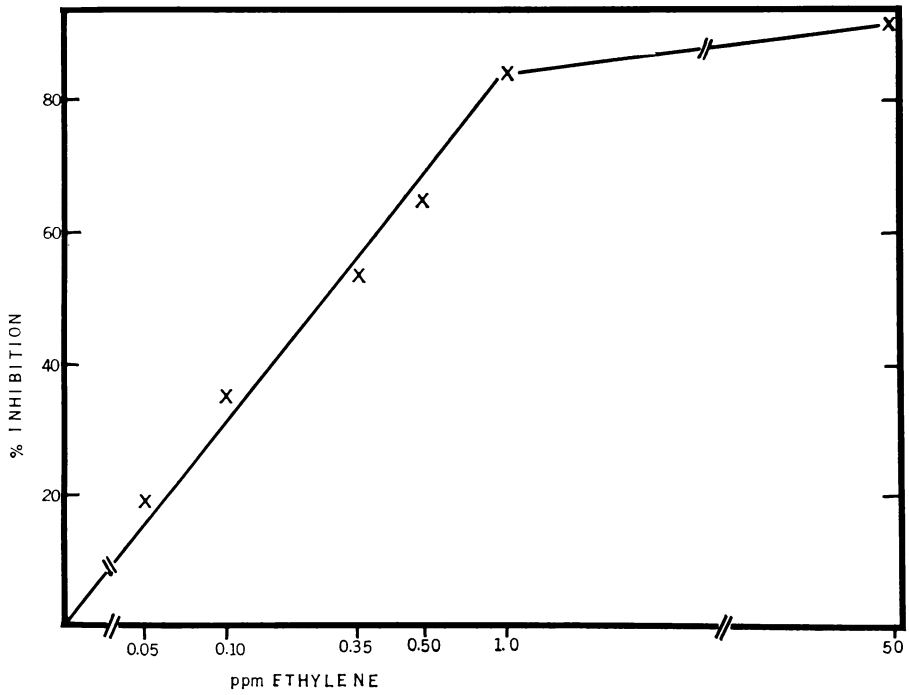

FIG. 4. Effect of various concentrations of ethylene on cell division in the hook region. Data represent number of mitotic figures (meta-, ana-, and telophase) in the median 50 μ (5 median longisections, 10 μ per section) of the hook region 24 hr after treatment.

Table II. *Effect of 100 μl/l Ethylene on the Percentage of Cells in Various Stages of Mitosis in the Apical Hook Region of Etiolated Pea Seedlings*

Treatment	Mitotic Index	Percentage of Cells in[1]			
		Pro-	Meta-	Ana-	Telophase
hr					
0	3.45	1.96	0.41	0.91	0.17
2	3.36	1.82	0.36	0.95	0.23
4	2.90	1.67	0.35	0.74	0.14
8	2.62	1.53	0.34	0.63	0.12

[1] Apices were fixed immediately after the treatment was concluded. Percentages are based on counts of 6000 cells; 3000 cells in three zones of the leaf and 3000 in three zones of the apical bud in each case. Sections were stained by the Feulgen method.

Table III. *Effect of Ethylene and 2,4-D on Cell Division in the Primary Root Apex of Pisum sativum*

Treatment	Number of Cell Divisions[1]		
	Initial	24 hr	48 hr
Control	124 ± 16	146 ± 24	169 ± 23
Ethylene (50 μl/l)		53 ± 5	69 ± 9
2,4-D (0.1 mM)		8 ± 4	10 ± 3

[1] Number of mitotic figures (meta-, ana-, and telophase) in the median 40 μ (5 median longisections, 8 μ per section) of 1-mm root tips taken behind the cap. Data are presented as average of four replicate experiments, with standard deviation.

ethylene-treated tissue in order to obtain a more accurate estimate of the effects of the gas on various stages of division; however, this approach tends to underestimate the rate and intensity of the response to ethylene.

Ethylene also inhibits mitosis in lateral buds, and here BA partially reverses the action of the gas. When nodal sections containing lateral buds are incubated for 48 hr, apical dominance is broken (7, 49); under these conditions in four replicate experiments 170 ± 42 cell division figures were found in five median 8-μ longisections of control buds and 200 ± 25 when 0.1 mM BA was added. Gassing the isolated sections with 50 μl/l ethylene prevented all growth of the buds and no cell division figures were detected. When BA and ethylene were added together, the action of ethylene was partially reversed and 48 ± 3 cell divisions were detected after 48 hr.

Ethylene and 2,4-D also inhibit cell division in the primary root apex (Table III). Cell divisions occur frequently in the 1-mm region just behind the root cap of control plants, but the number is reduced by 60% in roots exposed to ethylene for 24 or 48 hr. Irrigating the seedlings initially with 100 μM 2,4-D almost completely stops cell division in the root apex, reducing the frequency by about 95% (Table III). However, the 2,4-D treatment markedly stimulates cell division in the upper portions of the root (Fig. 5), and these divisions ultimately give rise to lateral root initials. Root growth was not measured, but previous studies with intact 3-day-old pea roots have shown (11) that ethylene and 0.1 mM IAA alone or in combination inhibit elongation by 60% and the rate of increase in fresh weight by 50% during 8 to 24 hr.

No cell divisions could be detected in the subhook region of control plants or plants treated with ethylene. Occasionally, 96 hr after application of 1 mM 2,4-D, a few divisions were noted in areas of the stem which had split due to excessive swelling, but the frequency of these mitotic figures associated with wounded tissue was very low.

Effect of Ethylene on DNA Synthesis. Ethylene inhibits the incorporation of ^{3}H-thymidine into DNA both in meristematic and nonmeristematic tissues (Tables IV and V). In no case did the gas significantly alter total isotope uptake (Table IV) calculated on a fresh weight basis, so the reduced incorporation into DNA must be caused by a lowered rate of DNA synthesis. DNA extraction studies show that, in the apical hook region (Table IV), ethylene treatment reduces the rate of ^{3}H-thymidine incorporation into DNA by 74%, while in roots it reduces the rate by 50%. Even in the subhook region, where no cell divisions occur, ethylene inhibits the incorporation of ^{3}H-thymidine into DNA by almost 80% (Table IV). Historadioautographic studies on incorporation of ^{3}H-thymidine into DNA (Table V) confirm the results obtained by direct extraction of DNA. In the hook region ethylene reduces the number of labeled nuclei per 1000 cells by 78%, in the root apex by 50% and in the subhook region by 98%. The data suggest that the frequency of cell division is determined by the degree of inhibition of DNA synthesis in ethylene-treated apices and roots (Tables IV, V).

Control of Leaf Expansion and Cellular Division by Endogenous Ethylene. When plants are exposed to hypobaric conditions, the outward diffusion of endogenously produced volatiles is enhanced because the diffusion coefficient of these volatiles in air, and hence their rate of escape from the tissue, is inversely related to the density of the air. Consequently, if no change in the rate of ethylene evolution occurs, the endogenous concentration of ethylene at equilibrium is diminished in direct proportion to the reduction in atmospheric pressure (5). To avoid O_2 depletion and desiccation under hypobaric conditions, the plants were continuously ventilated with pure water-saturated O_2 at 120 mm Hg. the lowest pressure under which we have been able to grow pea seedlings at a completely normal rate (2). When 3-day-old seedlings are transferred for 4 days to hypobaric conditions the hook opens and the plumular leaf expands (Fig. 6). In four replicate experiments the hook region of 7-day-old control plants contained 498 ± 26 mitotic figures (meta-, ana-, and telophase) in

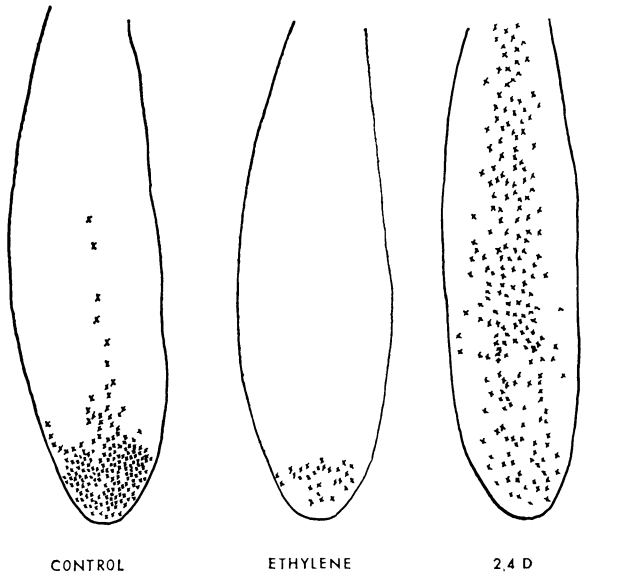

FIG. 5. Location of cell division figures (excluding prophase) in the median 7 μ longisection of a 5-mm root tip from a 3-day-old seedling. Ethylene (50 μl/l) or 2,4-D (0.1 mM) was applied for 24 hr to 2-day-old seedlings.

Table IV. *Effect of 50 μl/l Ethylene on Incorporation of ^{3}H-Thymidine into DNA in the Apical Hook, Root Tip, and Subhook Regions*

Twenty sections were incubated in 10 ml of media containing 5 mM potassium phosphate buffer, pH 6.8, 5 μM $CoCl_2$, 2% sucrose, 0.1 mM sodium penicillin G and ^{3}H-thymidine (18 mc/mmole). Five μc/ml of isotope was used with the apical hook and subhook regions, and 1 μc/ml with the root tip. The hook regions from 8-day-old control plants, or plants of similar age pretreated with ethylene for 24 hr, were incubated for 5 hr and then a 1-mm region near the cut surface was excised under a dissecting microscope. The 1-cm subhook region was cut from 8-day-old control plants of similar age pretreated with ethylene for 24 hr and incubated for 4 hr before the entire section was used for DNA analysis. A 5-mm zone of primary root tip from 4-day-old control seedlings, or plants of similar age pretreated with ethylene for 24 hr, was incubated for 1 hr, after which 1-mm sections were cut from behind the root cap using a dissecting microscope. Ten sections were used for each analysis, and data represent the average of four replicate experiments with standard deviation.

	Fresh Weight	Total ^{3}H-Thymidine Uptake	DNA
	mg/10 sections	*cpm × 10^{-3}*	
Apical hook			
Control	33	10.7 ± 2.0	1.36 ± 0.2
Ethylene	38	13.5 ± 2.3	0.26 ± 0.09
Root tip			
Control	5.0	59.0 ± 8.0	1.25 ± 0.2
Ethylene	5.9	70.0 ± 9.1	0.65 ± 0.08
Subhook region			
Control	465	835 ± 104	11.6 ± 2.1
Ethylene	580	970 ± 121	2.4 ± 0.7

Table V. *Historadioautographic Studies on the Incorporation of ^{3}H-Thymidine into DNA of the Root Tip, Hook, and Subhook Regions of Ethylene-treated Tissue*

After exposure to ^{3}H-thymidine, as in DNA extraction studies (Table III), 10 stem or root sections were fixed and embedded. Stem regions were sectioned 10 μ and roots 8 μ thick. Radioautographs were prepared and silver grains over labeled nuclei were counted. Data are presented as average of four replicate experiments, with standard deviation.

Region	Labeled Nuclei	
	Control	Ethylene (50 μl/l)
	no. per 1000 cells	
Hook	148 ± 10	33 ± 8
Subhook	95 ± 16	2 ± 10
Root tip	138 ± 8	64 ± 5

five 10-μ median longisections, whereas the treated plants had 1280 ± 40 cell divisions in the same tissue. The distribution of cell division figures within the hook region of plants grown under hypobaric conditions differed from that in control seedlings. In control plants the number of cell divisions in the plumular leaf was almost the same as that in the hook elbow (Fig. 7), whereas plants grown under hypobaric conditions had numerous cell divisions in the plumular leaf but only a slight increase in the hook elbow as compared with the control. The large number of cell divisions in the plumular leaf probably accounts for its expansion, and the fact that cell division frequency increases when the atmospheric pressure is reduced suggests that mitosis in the plumule is limited in rate by an accumulated volatile. Since ethylene is specifically synthesized in the apex (8, 19), and as very low concentrations of ethylene inhibit cell division, this olefin must be the volatile controlling cell division in the plumule. Lateral buds in pea, however, are not released from apical dominance under hypobaric conditions (Fig. 6). This suggests that, while endogenous ethylene may limit the rate of cellular division in expanding apices and buds, it is not responsible for the lack of cell divisions in lateral buds which have not been released from apical dominance, at least in the case of pea seedlings.

DISCUSSION

Auxins such as 2,4-D and IAA inhibit cell division in root, shoot, and lateral bud primary meristems but stimulate mitotic

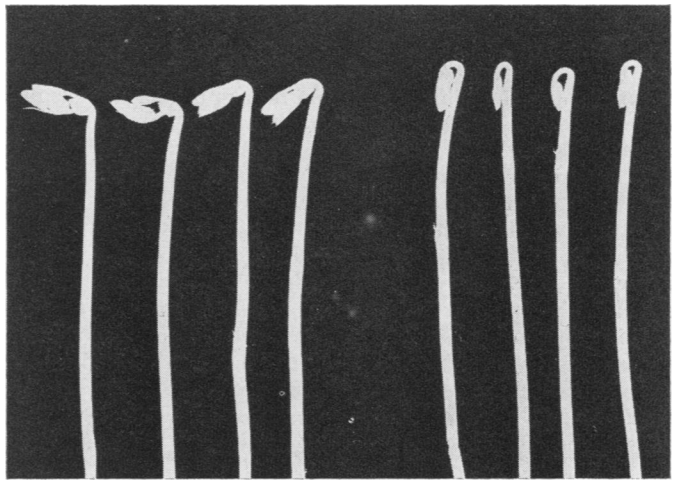

FIG. 6. Control of leaf expansion and hook opening by endogenous ethylene. Three-day-old seedlings which had developed in air were grown for 4 additional days at 120 mm Hg pressure of O_2, or under normal atmospheric conditions. Note the leaf expansion and hook opening in plants grown under hypobaric conditions (left) compared to control plants of the same age (right).

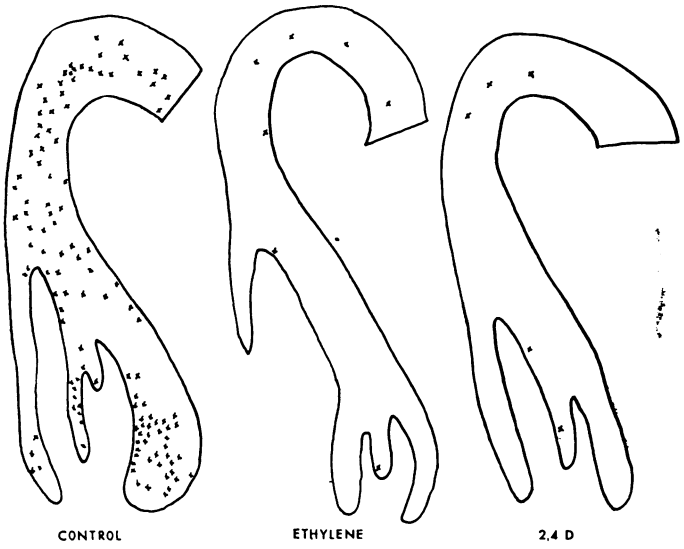

FIG. 7. Location of cellular divisions in the medium 10 μ longisection of the apical hook of 8-day-old etiolated pea plants. Ethylene (50 μl/l) or 2,4-D (0.1 mM) was applied to 7-day-old seedlings for 24 hr.

activity in expanding or mature, nondividing cells of roots and shoots (14, 20, 28, 44, 46). Auxins also stimulate cambial activity in stems (46) and are required for mitotic activity and DNA synthesis in tissue cultures (39). We find that auxins inhibit cell division in the primary meristem of the pea root, lateral bud and shoot, and that they stimulate mitotic activity in the elongating zone of the root. We did not observe a stimulation of cell division in the subhook region of pea plants sprayed once with 0.1 or 1 mM 2,4-D, presumably because insufficient auxin penetrated the cutinized surface. However, when pea seedlings are decapitated and 0.5% IAA is applied in lanolin paste to the stump, cell divisions and massive synthesis of DNA, RNA, and protein are induced within 1 to 2 days (17, 42). The synthesis of new DNA is directly associated with cell division, for both are specifically prevented by 5-fluorodeoxyuridine (17). These cell divisions, just like those induced by auxin in pea roots, give rise to lateral root initials (42). Similarly, cell divisions leading to root initiation can be induced by placing pea stem cuttings in auxin solutions (46). Thus, with respect to the effect of auxin on cell division, the pea plant generally behaves in a typical manner. The degree to which auxin inhibits cell division in the primary meristem of *Pisum sativum* roots is closely similar to that reported in *Zebrina pendula* (28), *Allium cepa* (44), and *Vicia faba* roots (14) exposed to comparable concentrations of IAA or 2,4-D (10–100 μM). The data presented in the present communication indicate that this effect of auxin on root meristems, as well as the inhibitory effect on stem and lateral bud meristems, is mediated by auxin-induced ethylene. The similar actions of 50 μl/l ethylene and 0.1 or 1 mM 2,4-D on growth and cell division in the hook region, and lack of any synergistic response, suggest that 2,4-D inhibits cellular division and growth in the plumule by inducing ethylene production. A very high concentration of auxin, especially a nondegradable synthetic auxin like 2,4-D, can exert a herbicidal effect independent of and/or in addition to the action of auxin-induced ethylene (1, 4, 10, 11, 23, 36). This action of 2,4-D undoubtedly accounts for the fact that 1 mM 2,4-D depresses the rate of cell division in the plumule to a value slightly but significantly lower than that observed with ethylene or 0.1 mM 2,4-D (Table I). Herbicidal effects occur in roots at even lower auxin concentrations than in stems (28), so that in roots the growth inhibition caused by auxin-induced ethylene production almost invariably is accompanied by a direct inhibitory action of auxin (10, 11). It is not surprising, therefore, that in roots 0.1 mM 2,4-D is more inhibitory to cell division than ethylene alone (Table I); in fact, 1 mM 2,4-D cannot be used in these experiments because it kills the roots. Growth of pea buds released from apical dominance is completely prevented by ethylene or a concentration of IAA which induces substantial ethylene production, and these inhibitions are reversed by kinetin (7, 49). We find ethylene, like auxin, to prevent cell division in pea buds released from apical dominance, and the cell division factor BA to reverse partially the effect of the gas. Thus in the plumule, root and shoot of pea seedlings the inhibitory action of auxin on cell division in the primary meristem is partly or wholly due to auxin-induced ethylene production except that at very high auxin concentrations a direct herbicidal action also contributes to the effect.

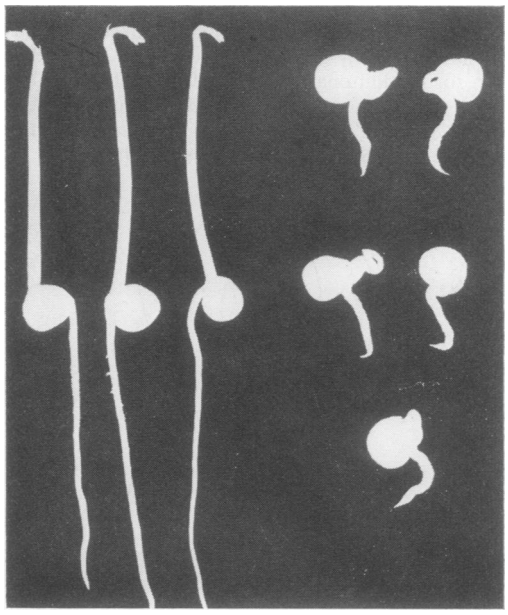

FIG. 8. Effect of ethylene on pea seed germination and growth. Seeds were imbibed for 4 hr, sown in pots, and immediately placed for 4 days in a desiccator with 50 μl/l ethylene continuously present (right). Control plants (left) of the same age have root and shoot systems of equal size; seedlings treated with ethylene have almost no shoot systems, whereas root growth has been depressed to a lesser extent. Note swelling and plageotropic growth of the ethylene-treated roots.

Stimulation of cell division by auxin in expanding, or mature, nondividing tissues does not seem to depend upon, or be influenced by, auxin-induced ethylene production in most cases. Ethylene stimulates adventitious root formation in several species which tend to form adventitious roots spontaneously, or readily in response to auxin (13, 50, 51). In part, this response to the gas may be due to the fact that ethylene irreversibly inhibits polar auxin transport (6, 22, 37). The rooting response only appears after ethylene is removed, and might be caused by subsequent accumulation of auxin in tissue formed above the transport block during the period of recovery. Whether or not ethylene participates directly in adventitious root formation after auxin application cannot yet be decided, but it is highly unlikely that this generally is true, for ethylene application rarely stimulates root formation whereas auxins frequently have this effect. Similarly, the induction of cell divisions in the elongating zone of roots and shoots by auxins cannot be due to auxin-induced ethylene production since ethylene alone does not cause this effect. On the other hand, auxin and ethylene cause intumescence formation on stems and roots (13, 15, 20, 47, 50) and both regulators induce periderm expansion in roots (3, 13, 50). Ethylene has little effect on the growth of tissue cultures (32) except to stimulate it in a few cases (45). In summary, those tissues in which auxin stimulates cell division sometimes respond to ethylene in like manner but, in general, the stimulation by auxin cannot be ascribed to or even associated with auxin-induced ethylene production. On the other hand, ethylene does not seem to inhibit cell divisions in tissues which are stimulated to divided by auxin.

When IAA inhibits cell division in the apical meristem of *Vicia faba* roots it reduces the extent of metaphase delay and prevents entry of cells into prophase (14, 48). In roots of *Vicia faba* treated with 100 μM IAA for 3 hr, a fall in mitotic index occurs 3 hr later and the cell division frequency reaches a very low value within 24 hr. Studies with ^{3}H-thymidine reveal that under these conditions G2 cells are not affected, but cells labeled in S fail to reach prophase (48). *Vicia faba* roots (31) are very sensitive to applied ethylene, responding in exactly the same manner as pea roots (11). The mechanism by which ethylene inhibits cell division is connected intimately with the effect of the gas on DNA synthesis. That the suppression of DNA synthesis by ethylene does not result from

rather than cause the inhibition of cell division is suggested by the fact that ethylene inhibits DNA synthesis even more markedly in the subhook region where cell division is not occurring than it does in meristematic tissue (Tables IV, V). The timing of the ethylene inhibition of cell division in the pea plumule (Fig. 3) is closely similar to that for auxin inhibition of cell division and DNA synthesis in roots (14, 48). Moreover, both 2,4-D and ethylene have been found to inhibit DNA synthesis in the soybean apex to the same degree (25) and it has been reported that tomato plants sprayed with ethylene have a reduced content of DNA (40). These facts, and the observation that ethylene blocks cells division at some stage prior to prophase (Table II) are all compatible with the idea that auxin inhibits mitosis in primary meristems by inducing ethylene formation. It is not clear, however, whether ethylene not only prevents DNA synthesis but also (like auxin) blocks the progression of S cells into prophase.

In roots, shoots, and lateral buds released from apical dominance, ethylene inhibits growth to the extent that it inhibits cell division and DNA synthesis (7, 10, 11, 49; Fig. 1, 2; Tables I, III–V). Since ethylene almost completely stops cell division in the shoot apex but is only partially effective in inhibiting cell division in the root, it nearly stops shoot development but only partially inhibits root development in pea seedlings grown continuously in the presence of the gas for 4 days after seed imbibition (Fig. 8). Ethylene only inhibits the rate of cellular expansion in the intact shoot of etiolated peas by about 65% and similtaneously prolongs the duration of the expansion phase of growth (2), so this action of the gas cannot account for a nearly total inhibition of shoot development. Thus in the plumule, root and lateral buds of pea seedlings, growth inhibition caused by ethylene must be due mainly to suppression of cell division.

The observation that ethylene and auxin inhibit cell division in meristematic tissue, coupled with our recent finding that the gas prolongs the phase of cell expansion (2), help to explain why ethylene or applied auxin inhibits the growth of fig fruits (33, 35) treated during the stage of cell division (phase I), whereas both hormones enhance growth if applied in phase II. Similarly, ethylene inhibits the growth of rice coleoptiles if applied during the stage of cell division, but prolongs their growth markedly if applied later (30). Fern gametophytes form fewer but larger cells when they are grown in the presence of ethylene, and spore germination is prevented because the cells do not divide (35). Both ethylene and auxin prevent potato buds from sprouting (16, 21), and both inhibit growth of the soybean apex but stimulate expansion of its hypocotyl (25). Thus, the combined effects of ethylene on cell division and the duration of cell expansion explain many of the overall growth responses to the gas.

Ethylene is produced mainly in the apical hook of the etiolated pea seedling (8, 19). The endogenous content of the gas is sufficiently high in the pea and bean hook to prevent hook opening, but when ethylene production is depressed under the influence of red light, these hooks unfold (8, 18, 26, 27). The hook opening response cannot depend upon any effect of ethylene on cell division because it occurs in excised pea apices, in which cell divisions are almost completely lacking. The amount of ethylene required to prevent light-induced hook opening has been determined (26), and must approximate 0.2 μl/l, the quantity of ethylene needed to maintain the etiolated hook in a nearly closed configuration. The light-induced hook opening response has the same sensitivity to ethylene as most other processes affected by the gas (9) including cell division. Apparently the hook normally contains somewhat less than a saturating concentration of ethylene, because applied gas causes the hook of etiolated control plants to tighten significantly (8, 26), and CO_2, a competitive inhibitor of ethylene action (8), opens the hook almost completely (19, 26). Since cell division and hook opening are equally responsive to applied ethylene, it follows that if the plumule normally contains enough ethylene to maintain the hook in a closed configuration, sufficient gas must also be present to influence the frequency of cell division. This prediction is supported by the finding that, when endogenous ethylene is removed under hypobaric conditions, the cell division frequency increases, the plumular leaves expand and the hook opens. These changes are similar to those caused by red light when it inhibits ethylene production, and by CO_2 when it displaces ethylene from its receptor (8, 19, 26). The data support the view that light-induced plumular expansion (19) and the frequency of cell division in the apex are controlled by the action of endogenously produced ethylene. Possibly the division frequency of root apices also is limited by ethylene production, for CO_2 stimulates root growth (41). In both cases endogenous auxin could exert feedback control over the cell division frequency by regulating ethylene production.

LITERATURE CITED

1. Abeles, F. B. and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963-969.
2. Apelbaum, A. and S. P. Burg. 1972. Effects of ethylene and 2,4-D on cellular expansion in *Pisum sativum*. Plant Physiol. 50: 125-131.
3. Borgstrom, G. 1939. Theoretical suggestions regarding the ethylene responses of plants and observations on the influence of apple-emanations. Kung. Fysiog. Salls. I. Lund Fohr. 9: 135-174.
4. Burg, S. P. and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. U.S.A. 55: 262-269.
5. Burg, S. P. and E. A. Burg. 1966. Fruit storage at subatmospheric pressure. Science 153: 314-315.
6. Burg, S. P. and E. A. Burg. 1967. Inhibition of polar auxin transport by ethylene. Plant Physiol. 42: 1224-1228.
7. Burg, S. P. and E. A. Burg. 1968. Ethylene formation in pea seedlings: its relation to the inhibition of bud growth caused by indole-3-acetic acid. Plant Physiol. 43: 1069-1074.
8. Burg, S. P. and E. A. Burg. 1968. Auxin stimulated ethylene formation: its relation to auxin inhibited growth, root geotropism and other plant processes. *In:* F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Runge Press Ltd., Ottawa, Canada. pp. 1275-1294.
9. Burg, S. P. and E. A. Burg. 1968. Ethylene formation, plant senescence and abscission. Plant Physiol. 43: 1503-1511.
10. Chadwick, A. V. and S. P. Burg. 1967. An explanation of the inhibition of root growth caused by indole-3-acetic acid. Plant Physiol. 42: 415-420.
11. Chadwick, A. V. and S. P. Burg. 1970. Regulation of root growth by auxin-ethylene interaction. Plant Physiol. 45: 192-200.
12. Chouinard, A. L. 1956. Nuclear differences in *Allium cepa* root tissues as revealed through induction of mitosis with indoleacetic acid. Can. J. Bot. 33: 628-646.
13. Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1935. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. Plant Res. 7: 231-248.
14. Davidson, D. and R. D. MacLeod. 1966. Changes in mitotic indices in roots of *Vicia faba*. I. Antagonistic effects of colchicine and IAA. Chromosoma (Berl.) 18: 421-437.
15. Doubt, S. L. 1917. The response of plants to illuminating gas. Bot. Gaz. 63: 209-224.
16. Elmer, O. H. 1932. Growth inhibition of potato sprouts by the volatile products of apples. Science 75: 193.
17. Fan, D. and G. A. Maclachlan. 1967. Massive synthesis of RNA and cellulose in the pea epicotyl in response to indoleacetic acid with and without concurrent cell division. Plant Physiol. 42: 1114-1123.
18. Goeschl, J. D., H. K. Pratt, and B. A. Bonner. 1967. An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. Plant Physiol. 42: 1077-1080.
19. Goeschl, J. D. and H. K. Pratt. 1968. Regulatory roles of ethylene in the etiolated growth habit of *Pisum sativum*. *In:* F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Runge Press Ltd. Ottawa, Canada. pp. 1229-1243.
20. Gorter, C. J. and W. van der Zweep. 1964. Morphogenetic effects of herbicides. *In:* L. J. Andus, ed., The Physiology and Biochemistry of Herbicides. Academic Press, Inc., New York. pp. 235-275.
21. Guthrie, J. D. 1939. Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthalene-acetic acid. Contrib. Boyce Thompson Inst. Plant Res. 10: 325-328.

22. GUTTENBERG, H. VON AND E. STEINMETZ. 1947. Der Einfluss des Aethylens auf Wuchstaff und Wachstum. Pharmazie 1: 117–121.
23. HOLM, R. E. AND F. B. ABELES. 1967. 2,4-Dichlorophenoxyacetic acid-induced ethylene evolution: its role in soybean hypocotyl swelling. Plant Physiol. 42: S30–31.
24. HOLM, R. E. AND F. B. ABELES. 1968. The role of ethylene in 2,4-D-induced growth inhibition. Planta 78: 293–304.
25. HOLM, R. E., T. J. O'BRIEN, J. L. KEY, AND J. H. CHERRY. 1970. The influence of auxin and ethylene on chromatin-directed ribonucleic acid synthesis in soybean hypocotyl. Plant Physiol. 45: 41–45.
26. KANG, B. G. AND P. M. RAY. 1969. Ethylene and carbon dioxide as mediators in the response of the bean hypocotyl hook to light and auxins. Planta 87: 206–216.
27. KANG, B. G., C. S. YOCUM, S. P. BURG, AND P. M. RAY. 1967. Ethylene and carbon dioxide; mediation of hypocotyl hook-opening response. Science 156: 958–959.
28. KIERMAYER, O. 1964. Growth responses to herbicides. *In:* L. J. Audus, ed. The Physiology and Biochemistry of Herbicides. Academic Press, Inc., New York. pp. 207–233.
29. Kodak Pamphlet No. P-64. 1966. Kodak materials for nuclear physics and autoradiography. Eastman Kodak Company, Rochester, New York. p. 10.
30. KU, H. S., H. SUGE, L. RAPPAPORT, AND H. K. PRATT. 1970. Stimulation of rice coleoptile growth by ethylene. Planta 90: 333–339.
31. LAAN, P. A. VAN DER. 1934. Der Einfluss von Aethylen auf die Wuchsstoffbildung bei *Avena* and *Vicia*. Ext. Rec. Trav. Bot. Neerl. 31: 691–742.
32. LARUE, T. A. G. AND O. L. GAMBORG. 1971. Ethylene production by plant tissue cultures. Plant Physiol. 48: 394–398.
33. MAXIE, E. C. AND J. C. CRANE. 1968. Effect of ethylene on growth and maturation of the fig, *Ficus carica* L., fruit. Amer. Soc. Hort. Sci. 92: 255–267.
34. MAXIE, E. C. 1967. 2,4,5-Trichlorophenoxyacetic acid: effect on ethylene production by fruit and leaves of fig trees. Science 155: 1548–1550.
35. MILLER, P. M., H. C. SWEET, AND J. H. MILLER. 1970. Growth regulation by ethylene in fern gameotophytes. I. Effects on protonemal and rhizoidal growth and interaction with auxin. Amer. J. Bot. 57: 212–217.
36. MORGAN, P. W. AND W. C. HALL. 1962. Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorgum. Physiol. Plant. 15: 420–427.
37. MORGAN, P. W. AND H. W. GAUSMAN. 1966. Effect of ethylene on auxin transport. Plant Physiol. 41: 45–52.
38. NELJUBOW, D. 1901. Uber die Nutation der Stengel von *Pisum sativum* and einiger anderen pflanzen. Bot. Centrabl. Beinhefte. 10: 128–139.
39. PATAU, K., N. K. DAS, AND F. SKOOG. 1957. Induction of DNA synthesis by kinetin and IAA in excised tobacco pith. Physiol. Plant. 10: 949–966.
40. PRATT, H. K. AND J. D. GOESCHL. 1969. Physiological roles of ethylene in plants. Annu. Rev. Plant Physiol. 20: 541–584.
41. RADIN, J. W. AND R. S. LOOMIS. 1969. Ethylene and carbon dioxide in the growth and development of cultured radish roots. Plant Physiol. 44: 1584–1589.
42. SCOTT, F. M. 1938. Anatomy of auxin treated etiolated seedlings of *Pisum sativum*. Bot. Gaz. 100: 167–185.
43. SMILLIE, R. M. AND G. KROTKOV. 1960. The estimation of nucleic acids in some algae and higher plants. Can. J. Bot. 38: 31–49.
44. STERN, H. 1956. The physiology of cell division. Annu. Rev. Plant Physiol. 7: 91–114.
45. STOUTMEYER, V. T. AND O. K. BRITT. 1970. Ethrel and plant tissue cultures. Bioscience (Abstr.) 20: 914.
46. THIMANN, K. V., (ed.) 1952. The action of hormones in plants and invertebrates. Academic Press, Inc., New York.
47. WALLACE, R. H. 1926. Production of intumescences upon apple twigs by ethylene gas. Bull. Torrey Bot. Club 53: 385–401.
48. WEBSTER, P. L. AND D. DAVIDSON. 1967. Studies of IAA effects on the S phase of the mitotic cycle. Amer. J. Bot. 54: 633–634.
49. WICKSON, M. AND K. V. THIMANN. 1958. The antagonism of auxin and kinetin in apical dominance. Physiol. Plant. 11: 62–74.
50. ZIMMERMAN, P. W. AND A. E. HITCHCOCK. 1933. Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gases. Contrib. Boyce Thompson Inst. Plant Res. 3: 359–481.
51. ZIMMERMAN, P. W. AND F. WILCOXON. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. Plant Res. 7: 209–229.