Ethylene as a Factor Regulating the Growth of Pea Epicotyls Subjected to Physical Stress[1]

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Summary. Pea epicotyls (*Pisum sativum*, cv. Alaska) were enclosed in chambers in which their elongation was restricted by means of a foam neoprene stopper or by a medium of glass beads. These treatments increased evolution of ethylene and resulted in reduced length and increased diameter of both the internodes and the cells of the internodes. These responses increased with increasing degrees of restriction. A time-sequence study of the emergence of epicotyls through 90 mm of glass beads showed that an accelerated evolution of ethylene preceded a reduction in elongation. As the epicotyls elongated through the glass bead medium and less resistance was encountered, evolution of ethylene declined and rapid elongation was resumed. The morphological and anatomical effects of a 120-mm column of glass beads were duplicated by applied ethylene concentrations of 0.2 ppm or less. Evolution of CO_2 was inhibited slightly by the ethylene treatments. The data indicate that production of ethylene by pea epicotyls is increased by nonwounding physical stress, and that the ethylene acts as an endogenous growth regulator, decreasing elongation and increasing diameter in response to increasing increments of stress.

When etiolated pea epicotyls are treated with low concentrations of ethylene, the new growth is reduced in length, increased in diameter, and deviates from its normal vertical direction. This triple response, described by Neljubow (16) and by Crocker, Knight, and Rose (9) has been used as a bioassay to indicate the production of ethylene by plant material, especially fruits (5). Denny and Miller (10), using leaf epinasty bioassays, showed that a variety of vegetative tissues also produce ethylene. These observations, together with the known responses of plants to ethylene, led Crocker, Hitchcock, and Zimmerman (8) to suggest that ethylene might be an endogenous plant growth regulator. However, very little direct evidence has been presented to support their suggestion. Michener (14), Burg (5), and Burg and Burg (7) cited numerous reports of the effect of ethylene on plant growth and of ethylene production by plant tissues. Most reports, including that by Denny and Miller (10), pertain to excised, senescing, diseased, or otherwise injured tissues. Treatment with auxins has been shown to increase production of ethylene by seedlings or their excised parts (1, 15, 21). Since the control seedlings in these experiments also produced ethylene, and since Meheriuk and Spencer (13) reported that ethylene was produced by whole germinating oat and pea seedlings, it is evident that ethylene is evolved by healthy, growing plants.

The original objective of this investigation was to determine whether ethylene is produced by the epicotyl portion of intact etiolated pea seedlings. In preliminary experiments, test plants were grown with the epicotyl enclosed in stoppered glass cylinders and all were found to produce some ethylene, but epicotyls that had grown enough to press against the upper stoppers showed an unexpected increase in evolution of ethylene. Since there were no unobstructed controls of the same age in these tests, more carefully controlled experiments were then initiated to determine whether such a physical stress influences production of ethylene and thereby affects plant growth habit. All experiments reported were repeated one or more times with consistent results.

Materials and Methods

Plant Material. Pea seeds (*Pisum sativum* L., cv. Alaska) were soaked for 6 hours in aerated distilled water, rinsed, and planted 1 cm deep in 6.5 cm of vermiculite in perforated plastic trays. The vermiculite was moistened with distilled water and drained, and the trays were incubated until the seedlings reached the desired size. Those with straight epicotyls and with plumular hook angles between 65 and 80° were selected for experimental use. The seedlings were grown and all experiments were conducted in the dark at 20°. Necessary observations of the

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plants were made under low intensity green light, a single 15-w white fluorescent tube covered with 3 layers each of yellow, green, and blue DuPont Cellophane.

Test Chambers. Glass cylinders (35 mm I. D. by 200 mm long) were fitted with foam neoprene stoppers (fig 1). The stoppers were 12 mm thick and 37 mm in diameter. The lower one had 6 radially-oriented slits extending inward about 6 to 8 mm from the edge. These served to enclose and support the epicotyls within the chamber, leaving the cotyledons and root system below. The chambers were supported in a rack, with the roots extending into a tray of aerated distilled water. The stoppers were adjustable, so that the effective heights of the chambers could be varied and elongation of epicotyls could be obstructed without detectable wounding. Alternatively, obstruction was provided by filling the chambers to various depths with 4-mm glass beads. The chambers were tested for leakage and permeability and were found to lose less than 1.0 % of the ethylene and CO_2 by diffusion under steady state conditions.

Measurement of Gas Exchange. Two holes in the upper stopper of each chamber (fig 1) provided access for a 4-mm I. D. glass inlet tube extending to the bottom of the chamber and for a 2-mm I. D. capillary glass outlet tube. A measured constant flow of air (75–125 ml per hr) was passed through each chamber, so that the concentration of CO_2 did not exceed about 0.5 % and ethylene was held below 0.03 ppm. The outlet was fitted with a short rubber tube; this was pinched over at each sampling, serving as a septum through which samples could be drawn with a hypodermic syringe. Samples of the effluent air were taken as needed and analyzed with sensitive gas chromatographs (12). At least 2 blank chambers were used in each experiment to provide corrections for ethylene and CO_2 in the input air.

Results

Obstruction with Neoprene Barrier. Selected seedlings (6 days old, 55–60 mm tall) were placed in the slits of the lower stoppers of the experimental chambers (fig 1) with 40 mm of the epicotyl extending above the surface of the stopper. In 5 chambers the upper stopper was placed 20 mm above the epicotyls (obstructed), and in another 5 it was placed 60 mm above (controls). Comparable seedlings were placed in additional chambers (both obstructed and control) so that 12 epicotyls could be harvested and weighed at each gas sampling period for calculation of respiration on a fresh weight basis.

Disturbance of the seedlings during transplanting caused a temporary cessation of growth and probably led to an initially high ethylene reading. Elongation resumed in about 3 hours and proceeded at about 0.8 mm/hour, accompanied by a low rate of ethylene production. Differential treatment actually started when the obstructed epicotyls touched the upper stopper about 18 hours after transferring (fig 2). Two hours later, half the obstructed epicotyls had a slight curvature, showing the development of physical stress, but the evolution of ethylene was as yet unaffected. Within 6 hours of the beginning of the stress treatment nearly all the obstructed epicotyls were moderately curved, and ethylene produc-

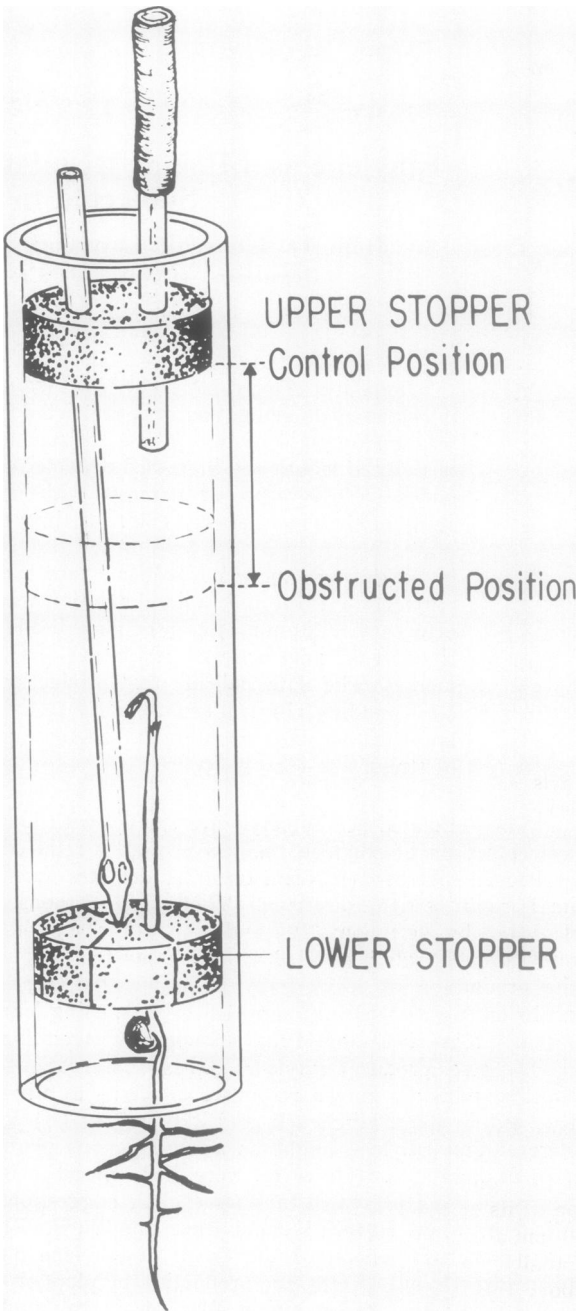

FIG. 1. Diagram of test chamber (35 mm I. D., 200 mm long) with foam neoprene stoppers. The lower stopper holds and encloses 6 seedlings. The upper stopper provides access for inlet and outlet tubes and can be lowered to obstruct elongation of epicotyls.

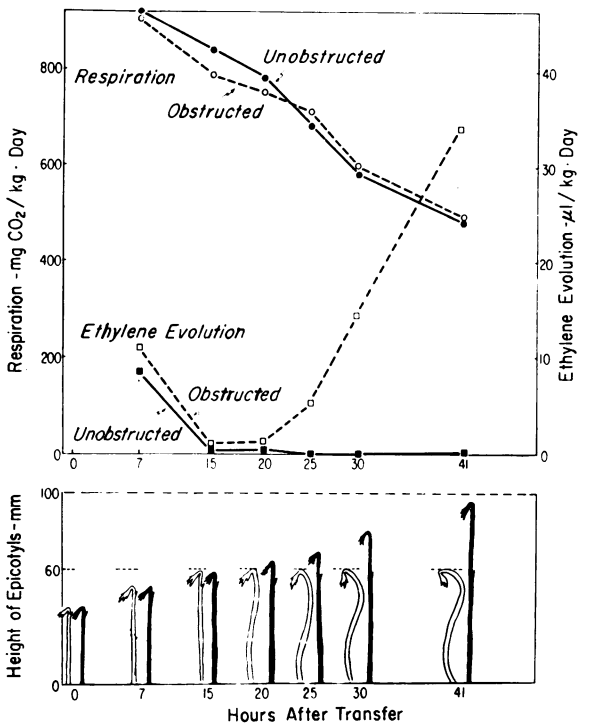

FIG. 2. Growth pattern and gas exchange of pea epicotyls with and without obstructions. Unshaded figures (below) represent growth habit of epicotyls which encountered a foam neoprene stopper (position indicated by short, dashed lines) about 18 hours after transfer. Black figures represent the unobstructed, or control, epicotyls. Upper figure shows ethylene evolution and CO_2 evolution by the same epicotyls. Each figure and point is based on the mean of 5 chambers containing 6 epicotyls each.

tion was 3 to 4 times greater. As curvature increased further, the rate of ethylene production rose dramatically.

The experiment was terminated when the tallest of the control epicotyls reached within 5 mm of the top of its chamber. Since all epicotyls reached about 60 mm above the lower stopper at the time stress was actually applied, only the portion of the epicotyl above this height was harvested as representing the growth that occurred in both the control and treated chambers during the differential treatments. Obstruction caused a 42 % decrease in elongation and a 33 % decrease in fresh weight, as compared to the controls, but there was no difference in the rate of CO_2 production as calculated on a fresh weight basis.

Obstruction with Varied Depths of Glass Beads. To test the effects of a restricting but penetrable medium on growth and production of ethylene, selected seedlings (4.5 days old, 22–26 mm tall) were mounted in the test chambers so that 10 mm of the epicotyl extended above the surface of the lower stopper. (In seedlings of this age, the second internode has not appeared below the plumular hook nor has it begun rapid elongation.) The chambers were then filled loosely with glass beads to heights of 60, 90, or 120 mm; the beads were not packed down. The air inlet tubes extended to the bottom of each chamber, allowing the whole column of beads to be aerated. For comparison, plants in chambers without beads were treated with air containing 0.2, 0.4, or 0.6 ppm ethylene. Four chambers (24 seedlings) were used for each treatment; corresponding controls received no beads and no added ethylene.

Elongation of the epicotyls resumed about 6 hours after transfer, and by 35 hours ethylene production had increased significantly in the lots under beads (fig 3), although there was as yet no obvious difference in length between the controls and the seedlings under treatment. The rate of ethylene evolution was greater under increasing depths of beads. The lag between initiation of treatment and increased production of ethylene (24 hrs longer than in the experiment reported above) probably reflected lack of initial resistance to growth, since the beads were placed in the chambers very loosely. In other experi-

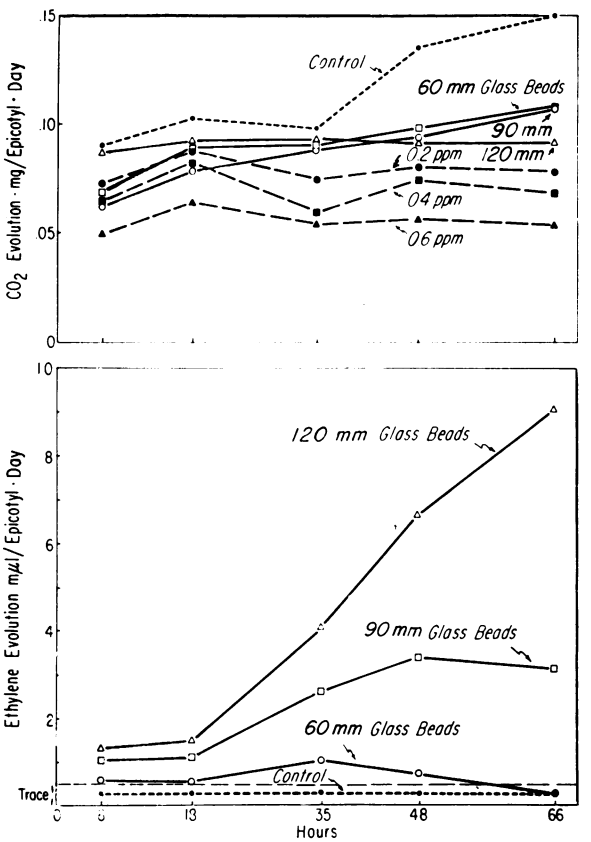

FIG. 3. Evolution of CO_2 and ethylene by pea epicotyls grown in chambers loosely filled with glass beads to levels of 60, 90, or 120 mm, or treated with 0.2, 0.4, or 0.6 ppm ethylene. Control plants were grown in chambers without beads or added ethylene. Each point represents the mean of 4 chambers, each containing 6 plants.

ments this lag was reduced to 8 hours or less (fig 6) by compacting the beads with light vibration.

Respiration data were somewhat erratic; the standard deviations of the values plotted (fig 3) are high. Recalculation of the final readings (66 hr) on a fresh weight basis shows only the 0.6-ppm treatment to be statistically different from the controls. However, the trend showing that greater stress (more beads) or ethylene treatment led to a lower respiration is repeatable and agrees with the findings of Harvey (11).

After 66 hours of treatment, all epicotyls were harvested by cutting 5 mm above the lower stopper, thus limiting consideration to that portion of the epicotyl that had elongated during the treatment. Epicotyl diameter was taken as the average of the midpoints of the first and second internodes. Treatment with either glass beads or applied ethylene caused a decrease in elongation of the epicotyls (fig 4), and this decrease was directly correlated with decreases in either fresh or dry weight (fig 4C and D) and with increased diameter (fig 4A). These responses appear to be the same whether caused by application of stress or of ethylene.

Effect of Obstruction on Growth. It has been reported that ethylene modifies plant growth by altering cell expansion (14). To verify this and to compare the effects of ethylene with those of stress, the size and shape of the second internode as a whole was compared with the dimensions of its component cells. In the experiment reported immediately above, the entire elongation of the second internode

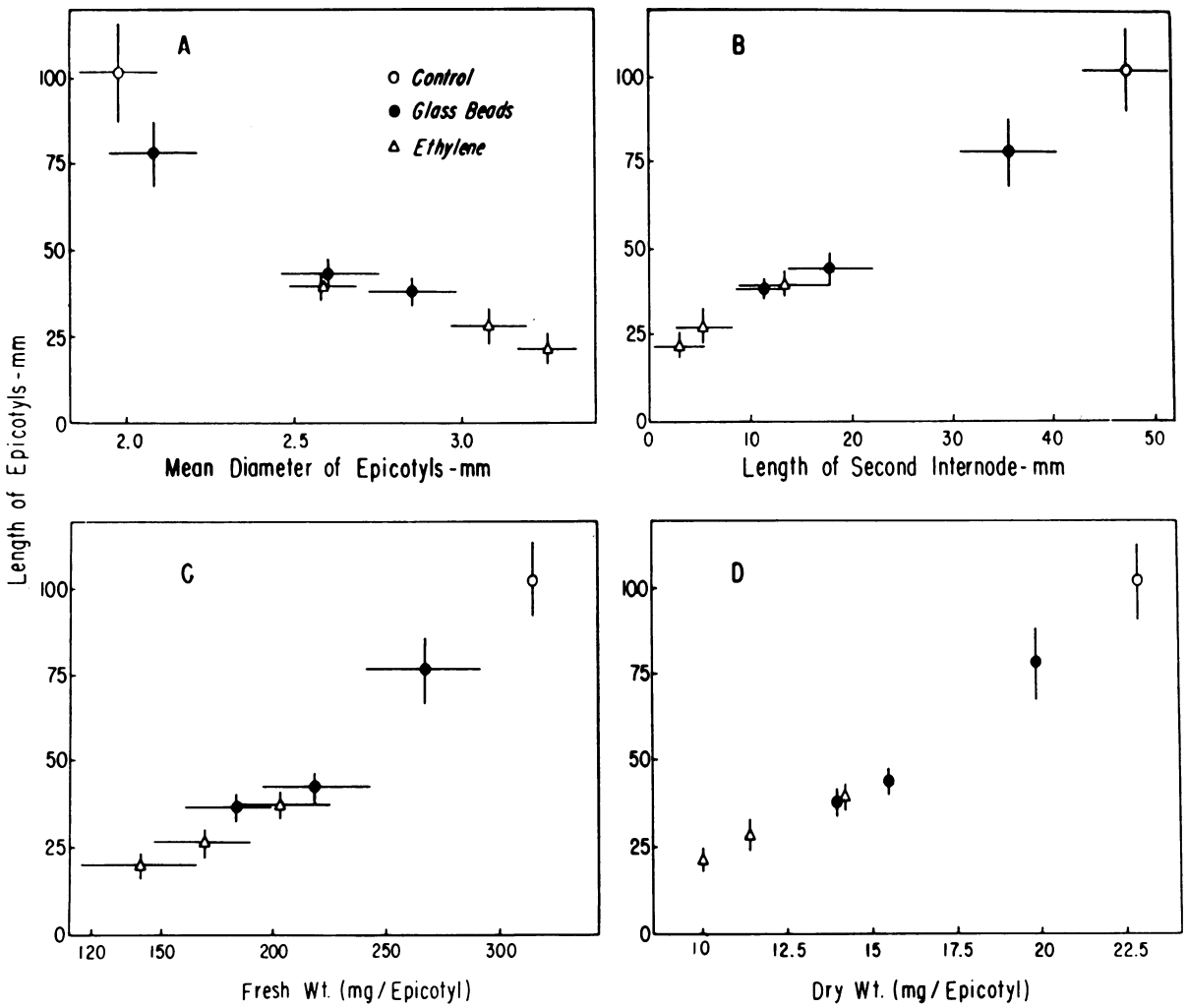

FIG. 4. Effect of glass beads or ethylene on growth of pea epicotyls (the same plants as in fig 3). Control plants were grown in chambers without beads or ethylene. In vertical descending order the treatments were: ○ = control; ● = 60, 90, or 120 cm of beads; △ = 0.2, 0.4, or 0.6 ppm ethylene. The diameter (A), length of second internode (B), and gains in fresh (C) and dry weight (D) of the epicotyls are related to the lengths of the same epicotyls. Each point in A, B, and C represents the mean of data from 24 plants; vertical lines are standard deviation for length, and horizontal lines are standard deviations for each measured variable. Dry weights (D) were determined by weighing 12 randomly selected epicotyls.

occurred during the period of treatment, and the change in length of that internode was linear in relation to that of the whole epicotyl (fig 4B). For cell measurements, 10 internodes each were selected at random from the controls, the 0.2-ppm ethylene treatment, and the 120-mm bead treatment. Stem pieces (5 mm) were cut from the center of each sample internode, fixed in 10 % $KMnO_4$, and embedded in Carbowax (17); longitudinal and transverse sections were cut at 10 μ. Cortical parenchyma cells were measured with a microscope ocular micrometer along transects extending from the pith region to the epidermis and passing midway between one of the lateral vascular bundle and the dorsal fiber bundle.

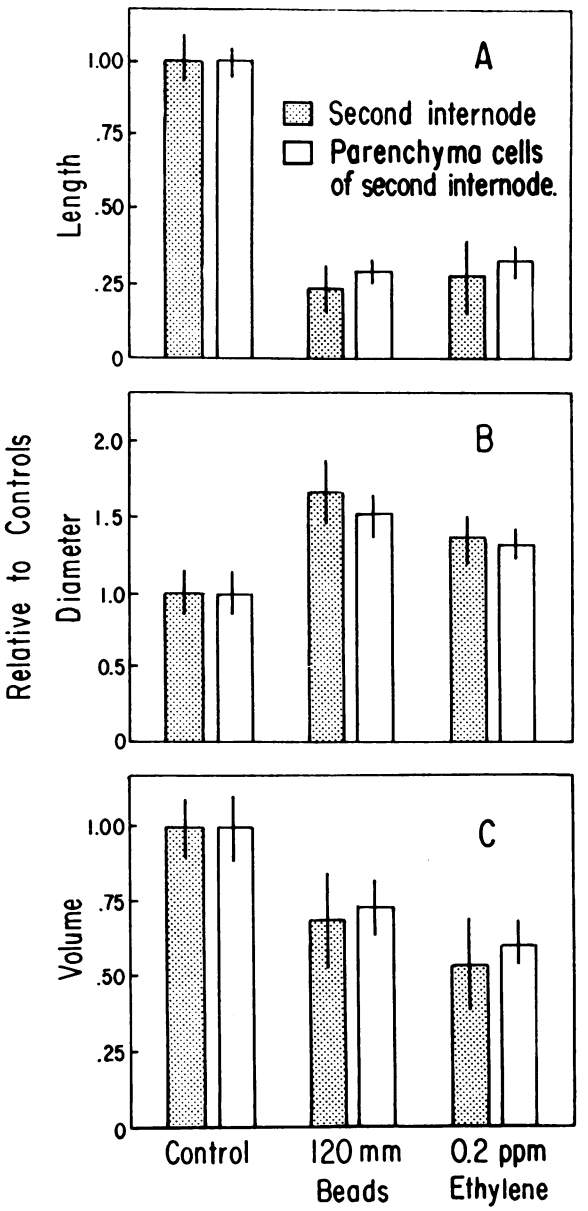

FIG. 5. Effect of glass beads or ethylene on relative length, diameter, and volume of second internodes as compared to relative length, diameter, and volume of cortical parenchyma cells of the same internodes (taking the control values as 1.0). The plants are the same as those of figure 3. Each bar represents measurements of at least 10 cells from the middle of each of 10 internodes; the standard deviation of each value is shown by the short vertical line in the top of each bar.

Treatments with glass beads and with applied ethylene reduced the length and increased the diameter of the second internode and of its constituent parenchyma cells (fig 5A, B). The relative reduction in length of the second internode was statistically the same as the relative reduction in length of its cells in both treatments. Increases in stem diameter were accounted for almost completely by increases in the diameter of the cortical parenchyma cells. The number of cells appeared not to be changed by either treatment, but cell volume was reduced about equally by both (fig 5C).

Relation of Ethylene Production to Epicotyl Elongation during Growth through a Column of Glass Beads. In this experiment, the growth and ethylene production were followed as the seedlings grew through and emerged from a deep bead column. Selected seedlings (4.5 days old, 22–26 mm tall) were mounted in the test chambers so that 5 mm of the epicotyl extended above the lower stopper, and the chambers were filled to 90 mm with glass beads; the beads were settled firmly into place by gently tapping each chamber. Epicotyl lengths were measured at the time of each ethylene sampling by lighting the chamber from behind with low-intensity green light and measuring the distance from the lower stopper to the top of the silhouette of each epicotyl.

Elongation resumed 6 hours after transfer, and at that time both treated and control epicotyls were evolving ethylene at the same rate (fig 6). Ethy-

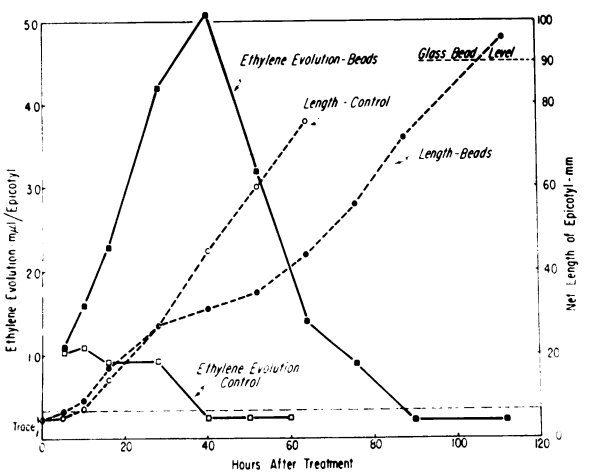

FIG. 6. Elongation and ethylene production of pea epicotyls grown in glass chambers filled to a depth of 90 mm with glass beads (beads vibrated into place). Each point represents data from one chamber containing 6 epicotyls.

lene production by the treated plants increased rapidly thereafter, the increase clearly preceding any decline in growth rate. Despite the physical restriction, growth of the treated epicotyls kept pace with that of the controls for several hours. However, as ethylene production continued to increase, the growth rate decreased markedly. Growth was slowest when the epicotyls were about 30 mm long (leaving 60 mm of beads above) and ethylene production was near its peak. Production of ethylene then declined markedly and rapid elongation was resumed. By the time the epicotyls reached 60 mm, both ethylene evolution and growth rate had returned to normal, agreeing with other tests which had shown that 30 mm of beads did not apply sufficient stress to restrict growth or induce high ethylene production.

This experiment illustrated the conditions which would be met by seedlings growing in the soil. The lag between transfer and the onset of increased ethylene production was reduced by settling the beads more firmly. This firmer packing may also account for the greater maximum rate of ethylene production, compared to the 90-mm bead depth illustrated in figure 3. Although the diameter of these epicotyls was not measured, it was obvious that the lower third was enlarged considerably, the upper third was similar in diameter to that of the controls, and the middle third showed a gradation between them.

Discussion

Nature of the Physical Stress. To examine the nature of the stress in the glass bead experiments, the beads were weighed and the cross-sectional areas of the column of beads and of the pea epicotyls were measured. Less than 1 g was found to rest on each epicotyl under a bead depth of 120 mm. In preliminary tests, as much as 3 g was placed on an epicotyl with only a small transient increase in ethylene production and little, if any, reduction of elongation. The effect of the glass beads appears, therefore, to be due to increasing friction and immobility of the beads at increasing depths rather than to bead weight per se. The stress (load) on the epicotyls in both the glass bead and obstruction experiments was essentially self-induced as the epicotyls grew against the physical resistance.

Physiological Effects of Physical Restriction and of Ethylene. The physical resistance to epicotyl elongation brought about an increase in evolution of ethylene, a decrease in elongation, and an increase in radial expansion. It is important to note that the increase in ethylene evolution was not simply the by-product of a general increase in metabolism, because the respiratory rates of obstructed and unobstructed epicotyls were almost equal at a time when their ethylene evolution rates differed greatly (fig 2). The increased production of ethylene appears to be a direct response to the physical stress. A crucial question which remains is whether the decreased length and increased diameter are induced as concurrent but independent events by the physical resistance or whether the altered growth habit is mediated by the increased concentration of ethylene. Consideration of the following results suggests that ethylene intervenes as a regulating mechanism in this system.

Firstly, the initial rate of elongation of epicotyls under 90 mm of glass beads was nearly identical to that of the unrestricted controls until the production of ethylene had increased severalfold (fig 6). With the continued increase in the production of ethylene, the elongation rate began to decrease. A resumption of normal growth did not occur until after the production of ethylene decreased to nearly the initial rate.

Secondly, the morphological and anatomical features which resulted from the 120-mm glass bead treatment were closely duplicated by treatment with 0.2 ppm of ethylene. The apparent colinear effects of various levels of glass beads and of various concentrations of ethylene on the growth habit of the epicotyls (fig 4) suggest that the effect of 90 and 60 mm of beads would also have been approximated by ethylene at concentrations less than 0.2 ppm. Indeed subsequent studies have shown that a 25% reduction of elongation, such as resulted from 60 mm of beads, can be induced by 0.02 ppm of ethylene. This agrees with the findings of Crocker, Hitchcock, and Zimmerman (8) that 0.025 ppm would cause a significant reduction in elongation.

The third consideration must be stated first as a question: Was there sufficient ethylene within the tissue of these plants to have accounted for the alteration of growth habit that was observed? Inherent in the answer to this question is the answer to the older question as to whether ethylene is an endogenous growth regulator. Michener in 1938 concluded that physiologically significant concentrations of ethylene probably do not exist in the tissues of a well-ventilated growing plant. This interpretation was based in part on the comparatively insensitive techniques available at that time, even though Zimmerman and Wilcoxon (21) had shown that growing plants produce some ethylene. Michener's assessment of the amount and effectiveness of ethylene within the tissues of these plants was probably influenced by the rather high concentrations (2.0–1000 ppm) used in his experiments. Direct measurement of ethylene within small amounts of tissue may be technically possible at present, but the known methods of sampling with hypodermic syringes or by evacuation may introduce physiological artifacts and certainly constitute terminal measurements.

We may draw on recent studies of ethylene production in ripening fruits for a means of estimating internal concentrations. It has been found that resistance to gaseous diffusion at the surface of fruits results in an accumulation of ethylene within the tissue until the rate of evolution equals the rate of production and a substantial steady state concentra-

tion gradient is established (6, 12). The internal concentration of ethylene in fruits can be estimated from the ethylene production rate by a constant (2 ppm/μl kg^{-1} hr^{-1}) which is based on the maximum surface permeability (6). Since the elongating portion of pea epicotyls has a surface: volume ratio up to 15 times greater than that of the fruits to which the constant applies, then the constant must be reduced by a factor of 15. Pea epicotyls which were grown under 120 mm of beads and were producing ethylene at a rate of 2.1 μl/kg per hour (calculated from data on fig 3 and fig 4 taken at 66 hr) would contain at least 0.28 ppm ethylene. By the same procedure of estimation, the mean internal concentration during the course of the bead treatment would have been at least 0.14 ppm. Such values are tentative, since we have no data to show that the maximum surface permeability of etiolated pea epicotyls is the same as that of fruits. However, these values compare quite favorably with the 0.2 ppm ethylene which was applied to seedlings without beads and which had essentially the same effect on growth as 120 mm of beads. At 66 hours, when these seedlings presumably contained 0.28 ppm ethylene, the concentration of ethylene in the chamber had risen to 0.025 ppm, or about 10 % of the theoretical concentration within the tissue. Had the chambers been ventilated at a 10-fold faster rate, the ambient concentration would have been reduced to 0.0025 ppm and, by a principle established in fruit tissues (6), the internal concentration would have dropped from 0.28 to 0.26 ppm, an insignificant change. The flow rates used in these experiments were chosen to give ambient concentrations which were relatively easy to measure yet low enough for the most part to be physiologically ineffective as externally applied treatments. The ambient concentration had importance, therefore, only as a means of measuring production rates. We may conclude, therefore, that sufficient ethylene was present within the plants to have caused the observed alteration of growth habit.

It may be of some importance that the rate of production of ethylene in the control plants decreased with time (fig 2, 6) despite the increases in fresh and dry weight, number of cells, and total evolution of CO_2. That the production of ethylene did not increase concomitantly with the growth of the plants suggests that there may be a localized zone of production. Preliminary experiments in this laboratory suggest that essentially all of the detectable ethylene is evolved from that part of intact etiolated pea epicotyls above the plumular hook.

Although physical stress had little effect on respiration on a fresh weight basis (fig 2, 3), there appeared to be a trend toward a lower rate of CO_2 evolution with increasing concentrations of ethylene (up to 0.6 ppm). Only part of the data showing this trend are statistically significant, but the result is consistent with the reduced growth, as indicated by the reduction of fresh and dry weight (fig 4) and reduced cell volume (fig 5C). Even though ethylene increased the diameter of the cells (fig 5B), its inhibitory effect on elongation was great enough to reduce the volume of the cells at the concentrations used in these experiments. Burg and Burg (7) reported that the volume of cells was not reduced when sections of pea epicotyls were treated simultaneously with ethylene and a growth hormone (presumably auxin).

Physiological Implications of Endogenous Ethylene. The foregoing observations bear on the interpretation of data describing seedling emergence under conditions of physical stress such as might occur in compacted or crusted soils. Reduced elongation and increased diameter of stems and roots of plants grown in restrictive conditions have long been known (4). Barley (2, 3) reported these responses in corn roots grown in a medium of fine glass beads under various amounts of pressure. More pertinent is the report by Sedgley and Barley (18) that a 35-g weight placed on the epicotyl of *Vicia faba* induced a 12 % decrease in elongation and a 4 % increase in diameter. However, a possible role for ethylene was not suggested. Williams (19, 20) germinated a number of cultivars of clover under various weights (10–50 g). By counting the number of seedlings that did not lift a given weight, he determined a median emergence force for each cultivar and concluded that emergence force in the different cultivars was related to size of seed.

Alternatively, it is possible that the emergence force per unit cross-sectional area of hypocotyl was the same for all cultivars Williams used. The increased total emergence force of larger-seeded varieties would then be proportional to the increase in cross-sectional area of the hypocotyls. If the diameter of each variety of clover increases with increasing physical obstruction, as it does in peas, then the total emergence force of a given variety is not a constant, and it, too, increases with increasing obstruction. A seedling germinated under 20 g of weight might develop just 20 g of emergence force and another seedling under a 40-g weight, by an ethylene-induced increase in diameter, would be able to exert 40 g of emergence force. In this connection, it is well established that the strength (critical load) of a homogeneous cylinder of any material increases by the fourth power of the radius. Therefore, even a small increase in the cross-sectional area of a plant axis would result in a substantial increase in its strength and ability to resist bending or deformation under restrictive conditions. The increase in diameter would increase both the total force which the plant could exert (proportional to r^2) and the ability of the axis to support the resulting load (proportional to r^4). Such an hypothesis does not conflict with the conclusion that emergence force is related to seed size, because each species or variety of plant has some inherent limitation on emergence force. The present findings suggest a role for ethylene in determining the extent to which a plant approaches that limitation.

Literature Cited

1. Abeles, F. B. and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963–69.
2. Barley, K. P. 1962. The effects of mechanical stress on the growth of roots. J. Exptl Botany 13: 95–110.
3. Barley, K. P. 1963. Influence of soil strength on growth of roots. Soil Sci. 96: 175–80.
4. Borgström, G. 1939. The transverse reactions of plants. C. W. K. Gleerup, Lund. 230 p.
5. Burg, S. P. 1962. The physiology of ethylene formation. Ann. Rev. Plant Physiol. 13: 265–302.
6. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37: 179–89.
7. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Science 148: 1190–96.
8. Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1935. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. 7: 231–48.
9. Crocker, W., L. I. Knight, and R. C. Rose. 1910 Effect of various gases and vapors upon the etiolated seedling of the sweet pea. Science 31: 635–36.
10. Denny, F. E. and L. P. Miller. 1935. Production of ethylene by plant tissues as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. 7: 97–102.
11. Harvey, E. M. 1915. Some effects of ethylene on the metabolism of plants. Botan. Gaz. 60: 193–214.
12. Lyons, J. M., W. B. McGlasson, and H. K. Pratt. 1962. Ethylene production, respiration, and internal gas concentrations in cantaloupe fruits at various stages of maturity. Plant Physiol. 37: 31–36.
13. Meheriuk, J. and M. Spencer. 1964. Ethylene production during germination of oat seeds and *Penicillium digitatum* spores. Can. J. Botany 42: 337–40.
14. Michener, H. D. 1938. The action of ethylene on plant growth. Am. J. Botany 25: 711–20.
15. Morgan, P. W. and W. C. Hall. 1962. Effect of 2,4-dichlorophenoxyacetic acid on the production of ethylene by cotton and grain sorghum. Physiol. Plantarum 15: 420–27.
16. Neljubow, D. 1901. Ueber die horizontale Nutation der Stengel von *Pisum sativum* und einiger anderen Pflanzen. Botanisches Centralblatt 10: 128–39.
17. Riopel, J. M. and A. R. Spurr. 1962. Carbowax for embedding and serial sectioning of botanical material. Stain Technol. 37: 357–62.
18. Sedgley, R. H. and K. P. Barley. 1963. The effect of a small axial pressure on the growth of *Vicia faba* var. Minor Beck. Australian J. Biol. Sci. 16: 19–27.
19. Williams, W. A. 1956. Evaluation of the emergence force exerted by seedlings of small seeded legumes using probit analysis. Agron. J. 48: 273–74.
20. Williams, W. A. 1963. The emergence force of forage legume seedlings and their response to temperature. Crop Sci. 3: 472–74.
21. 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. 7: 209–29.