6-1974

Implications for Microtubule Mediated Ethylene Effects in Pea Stem Sections

David A. Steen

LOMA LINDA UNIVERSITY

Graduate School

IMPLICATIONS FOR MICROTUBULE MEDIATED ETHYLENE EFFECTS IN PEA STEM SECTIONS

by

David A. Steen

A Dissertation in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in the
Field of Biology

June 1974

Each person whose signature appears below certifies that this dissertation in his opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

Arthur V. Chadwick, Chairman
Arthur V. Chadwick, Assistant Professor of Biology

Elwood S. McCluskey
Elwood S. McCluskey, Assistant Professor of Physiology and Biophysics

Ian M. Fraser
Ian M. Fraser, Professor of Pharmacology

Norman L. Mitchell
Norman L. Mitchell, Associate Professor of Biology

Conrad D. Clausen
Conrad D. Clausen, Assistant Professor of Biology

To the love of my life,

Linda

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my guidance committee members who have freely given of their time in counsel, encouragement, and advice. I am especially grateful to Art Chadwick for his friendship and help with ideas and research, to Elwood McCluskey for his careful attention to detail, and for his guidance throughout my graduate school experience, to Ian Fraser and Norman Mitchell for the time which they spent thinking about my project and helpfully criticizing my paper, and to Conrad Clausen for his friendship, encouragement and help with data processing and writing.

So many others have become involved in this project and have graciously given of their time and particular skill which has cheered me on my way. I wish to thank all of my fellow graduate students for their encouragement and support; especially Bruce Buttler, Ron Carter, Dave Crabtree, Lanny Fisk, Gary Hanson, Hal Lund, and John Rosario. Each has contributed in his own unique way. I very much appreciate the technical assistance given by Elmer Chinnock and Michael Titford in electron microscopy and I thank Brian Bull and the Loma Linda Medical Center Department of Pathology for giving me access to the electron microscope. I am indebted to Tom and Dan Ingalls, Don Garcia and Alfred Haindl for their help in tissue processing. I thank Carol Fisk for photographic assistance, Paul Yahiku

for statistical advice and data processing help, and Ben Nava for studying electron micrographs.

Altogether many individuals have given of themselves to make this project complete. I thank you all.

Computer time for calculations and statistical analysis was provided by the Scientific Computation Facility at Loma Linda University, sponsored in part by NIH Grant RR-00276.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURES PAGE

INTRODUCTION

Exposure to ethylene causes pea subhook tissue to swell, resulting in a reduction in the rate of elongation (2,10,12,13). Both ethylene application (1,57,58) and supraoptimal auxin concentrations (63,64) cause altered orientation of newly deposited cellulose microfibrils in a longitudinal rather than the usual radial direction. Presumably as a result of this change in orientation, ethylene alters the birefringence pattern of the cell walls, producing a characteristic banding pattern in treated parenchyma cells (10,13,26,55).

According to the multinet hypothesis, isodiametric expansion is prevented in normally elongating cells by radially oriented microfibrils in the cell wall (35,56). The finding that the orientation of microfibrils usually parallels that of microtubules (44,51) has led to the suggestion that microtubules may be responsible for deposition of cellulose microfibrils (8,32,44,51,53). In addition, colchicine (8,53) depolymerizes microtubules in plant and animal cells and is reported to disrupt newly deposited cellulose microfibrils in plant cell walls (31) causing a mottled birefringence pattern in parenchyma cells (26). Low temperature causes depolymerization of microtubules (47,62) in contrast to the stabilizing effects of D_2O (16,33,34) which also causes swelling and a banded birefringence pattern (17).

The effects of ethylene on microtubules have not been investigated and in light of the apparent link between microfibrils and microtubules, it now seems imperative to do so. This paper presents evidence suggesting that microtubules are indeed reoriented as a consequence of ethylene treatment and that some of the pronounced effects caused by the gas can be reversed by low temperature or colchicine. A model is presented in which ethylene stabilizes microtubular structure which may cause the observed change in the orientation of microtubules and microfibrils leading to radial cellular expansion.

MATERIALS AND METHODS

Hook Curvature Studies. Seeds of Pisum sativum L. (cv. Alaska) were soaked for 6 hr in running water, and planted in moist vermiculite in wide mouth glass jars. After growing 3 to 4 days in darkness at 24 C, the seedlings were 3 to 4 cm tall. The jars were sealed with airtight covers and ethylene was added to make appropriate final concentrations. Some jars were kept at 6 C for 48 hr in either light or dark and others were incubated for various times at 24 C in the light or dark. After the predetermined period of time, epicotyls were cut from the seed and shadowgraphed. Hook angles were measured with a protractor (40).

Straight Growth Tests. Seeds were soaked as previously described, germinated in plastic bins containing moist vermiculite and grown in darkness at 24 C for 7 days. Under dim green light, 10 mm subhook sections were excised from the third internode of selected seedlings (plants whose third internode was less than 30 mm). Ten sections were floated in 10 ml of standard growth media (2% sucrose (w/v), 5.0 μm $CoCl_2$, 5.0 mM phosphate buffer (pH 6.8), 1.0 μM IAA, and appropriate concentrations of colchicine and ethylene) in 125 ml Erlenmeyer flasks which were sealed with vaccine caps and gently shaken in the dark at 24 C for 12 hr. In some experiments, some of the flasks were incubated for 48 hr at 6 C. After incubation, ethylene levels were determined by gas chromatography, stem sections were weighed on an analytical balance and lengths were measured to the nearest 0.1 mm.

Long Term Low Temperature Experiments. Pea seeds were surface sterilized with a 5% clorox solution, rinsed and soaked in sterile water for 6 hr and planted in moist vermiculite in autoclaved 1 liter glass jars. The jars were sealed with airtight lids and appropriate concentrations of ethylene were introduced through a millipore filter. Jars were incubated at 6 C for up to 60 days. Every 3 to 5 days the jars were ventilated and fresh ethylene was introduced. Observations were made and pictures were taken

periodically.

D_2O Growth Studies. Peas were treated as above except that D_2O was substituted for various amounts of H_2O and growth was at 24 C. Samples of air were withdrawn periodically to check for D_2O-induced ethylene production. Observations were made and pictures were taken periodically.

Tissue Preparation For Electron Microscopy. Presoaked seeds were planted in moist vermiculite in 1 liter wide-mouth jars. After 4 days in the dark the jars were sealed and ethylene was added to some for 12 hr. Five mm subhook sections were then excised from normal looking plants with terminal internode lengths of at least 15 mm. The sections were cut longitudinally with a sharp razor blade into approximately equal halves and placed in 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) at 24 C for 24 hr. Sections were then rinsed with buffer for 1 hr and rinsed again in buffer. After thorough dehydration in an ethanol series, the tissue was infiltrated with a propylene oxide to epon series. Sections were embedded in epon, cured overnight, and sectioned with glass knives. The grids were post-stained with uranyl acetate and lead citrate. Cell wall regions were photographed at various magnifications with a Siemens 1A transmission electron microscope. Three separate tissue batches were prepared and photographed in this manner.

RESULTS

Hook Curvature Studies. When applied to etiolated pea seedlings, ethylene causes the hook to tighten, increasing the angle of curvature (Table 1), unless the seedlings are under low temperature conditions. I found that at 6 C, marked hook expansion occurs in both the control and ethylene treated hooks and no significant difference between the means was detectable even when treated with high concentrations of the gas for as long as 7 days.

When pea seedlings are exposed to a light regime, a pronounced hook expansion results, an effect reversible by ethylene. My data show that under low temperature conditions however, the pea seedlings respond by an even greater degree ($P<.001$) of hook opening, and ethylene only partially attenuates this response.

Straight Growth Tests. The dose response curve for ethylene at 24 C shows increasing inhibition of elongation with increasing gas concentrations (Fig 1). This inhibition due to ethylene was almost entirely reversed when the sections were incubated at 6 C. The inset shows that within 15 min the flasks had cooled to very nearly the incubation temperature which demonstrates that most of the growth occurred at the low temperature.

Straight growth tests with a colchicine containing medium show that inhibition due to colchicine increases with

increasing drug concentrations (Fig 2A). When stem sections are incubated in various combinations of colchicine and ethylene, the inhibition due to ethylene is significantly ($P<.01$ by ANOVA) reversed (Fig 2B). Instead of a synergistic effect of the two elongation inhibitors, there appears to be an antagonistic effect, with colchicine opposing the inhibition due to ethylene.

Long Term Growth At 6 C. Alaska variety seeds germinate and grow much more slowly at low temperatures than they do at 24 C (Table 2). Concentrations of ethylene which have a characteristic and marked effect at 24 C show little or no effect on cold grown seedlings (Fig 3, 4 and Table 2). Horizontal nutation of stems is absent, subhook swelling and growth inhibition due to ethylene is drastically reduced. Ethylene treated roots are geotropic in the cold and show practically no swelling, in contrast to the striking effects caused by similar treatment at 24 C (Fig 4).

D_2O Growth Studies. Deuterated water causes delayed germination (Table 2), swelling, ageotropic roots (Fig 5 and 6), and in 75% D_2O treated seedlings, a curious release of axillary buds (Fig 6).

Electron Microscopy. Longitudinal and transverse sections were examined to determine microtubule orientation in elongating pea stem parenchyma cells. The normal orientation of microtubules is radial, i.e., they are seen to run

circumferentially (around the cell) in the cytoplasm near the plasmalemma (51). This orientation was confirmed in most cases by both longitudinal (Fig 7) and transverse (Fig 8) sections of control tissue. In tissue treated with ethylene however, the microtubule orientation was found to be altered so that the patterns in transverse and longitudinal sections were reversed with respect to control tissue (Fig 9 and 10). This condition was observed in 70% of the fields photographed in which microtubules were discernible. Microtubules in both orientations were seen in 11% of the fields and the remaining 19% (most of which were fields of ethylene treated tissue) showed microtubules orientation opposite to the stated conditions.

DISCUSSION

Radial cellular expansion is prevented by circumferentially oriented microfibrils in the cell wall of many plant tissues (35,56). Agents which cause swelling in etiolated pea stem sections, such as benzamidazole (13,26, 30,54), benzyladenine (26), kinetin and other cytokinins (29), colchicine and vinblastin sulfate (10), supraoptimal auxin concentrations and ethylene (13,26), all reorient microfibrils, as evidenced by changes in the optical birefringence patterns (10,13,26,54). The characteristic banded pattern produced by ethylene is indistinguishable

from that observed in cells treated with benzamidizole, benzyladenine, kinetin, or supraoptimal auxin concentrations. Microfibril orientation is altered by a different mechanism however when cells are treated with colchicine and vinblastin sulfate. In this case the optical birefringence pattern appears diffuse or mottled (26,53) apparently due to the depolymerization of microtubules (51,53). All these agents which cause swelling do so by altering cellulose microfibrillar deposition; the auxins, ethylene, benzyladenine and benzamidizole alter the microfibrils to a longitudinal direction by an orderly redirecting of cellulose deposition (1,54, 63,64) and the others do so by random deposition (10,26). These findings clearly indicate that swelling is mediated by microfibrillar orientation.

In view of the fact that microtubule orientation usually parallels that of newly deposited cellulose microfibrils (44,51), it had been suggested that microtubules may be responsible for microfibrillar deposition (8,32,44, 51, 53). Further support for this suggestion is my evidence that ethylene treated tissue has microtubules which, like the microfibrils, are reoriented to a predominantly longitudinal direction (Fig 9 and 10). Therefore a key to microfibrillar orientation and radial swelling in cells seems to lie in the structure and orientation of microtubules.

Microtubules are protein polymers in which the spiral-

ing monomers from hollow, unbranched, cylindrical structures about 240 Å in diameter (51). Inoué and Sato (37) have proposed a dynamic state of equilibrium between pools of monomers and the microtubule polymers which undergo cyclic breakdown and reformation. Under certain conditions such as low temperature (62), high hydrostatic pressure (52), and treatment with colchicine and other drugs (16,36,38), the dynamic equilibrium is shifted towards the monomer state resulting in a breakdown of microtubules. Converesly, high temperature (47,62), low hydrostatic pressure (47), and D_2O (16,46,47) result in stabilization of microtubular structure due to a shift towards increased polymerization. Thus, conditions which alter the stability of microtubules also alter birefringence patterns and microfibrillar deposition.

The similarity of effects of ethylene and D_2O (Table 3) suggest that ethylene may be affecting microtubules in a manner similar to that of heavy water, that is by stabilization of the microtubular structure.

If ethylene is in fact stabilizing microtubules when it causes swelling and altered microfibrillar deposition, this could explain the pronounced reversal of ethylene effects by low temperature (Table 1). By causing hooks to open, low temperature has the same effect as light application or hypobaric treatment [which removes the gas from the tissue (40)]. These latter conditions are known to open

hooks by attenuation of the ethylene effects. Reversal of inhibition due to ethylene at low temperatures (Fig 1) and slight reversal of the inhibition by some levels of colchicine (Fig 2) are added evidence that ethylene may act by stabilization of microtubules.

Gross and Spindle (33,34) have suggested hydrogen bonding to be the force responsible for stabilization or freezing of the mitotic apparatus, a structure composed largely of microtubules. This suggestion was based upon the rapid and reversible arrest of mitosis following application of D_2O, apparently as a result of overstabilization of microtubules and the evidence that D_2O forms stronger intermolecular deuterium bonds.

Evidence that the major bonding force may be other than hydrogen bonds comes from the effects of temperature on microtubules (47,62). If hydrogen bonding supplied the major impetus, the bonds should be weaker at higher temperatures and stronger at lower temperatures (45). One of the most consistent observations in connection with microtubules is that, in fact, the opposite is true. This condition is precisely what one would expect were hydrophobic bonds the major source of interaction between subunits (43,50). The observed effects of D_2O would be explanable on the basis of an increased strength of hydrophobic bonding resulting from the reduction of entropy imposed by slightly stronger D-O

attractive forces.

If hydrophobic bonds constitute the major stabilizing force in microtubule polymerization, a mechanism for ethylene action suggests itself. Each subunit may contain one or more divalent cations to which ethylene could bind (14). In the absence of ethylene a dynamic equilibrium is maintained between the polymer and monomers by the presence of hydrophilic sites (divalent cations) on the individual subunits. When ethylene is bound to this site, the equilibrium is shifted in favor of the polymer by the resulting enhancement of hydrophobic bonds. Furthermore, CO_2, a potent competitive inhibitor of ethylene action which is thought to bind to the ethylene site (14,39), may act by reducing the strength of the hydrophobic bond. This mechanism of CO_2 action is what would be predicted due to the polarity of the molecule and its affinity for water. Binding of CO_2 on a microtubule polymer then would shift the equilibrium toward a depolymerized state by the reduction of hydrophobic bonding.

Such a model would suggest that cold hardy plants ought to be less susceptible to ethylene or D_2O and a difference ought to be observed in the structure of their microtubules. The microtubules of cold hardy plants have not yet been investigated (65), but winter rye plants were much less susceptible to D_2O (59) than were most other types of seed plants (27).

A recent finding (which may possibly be related to hydrophobic bonding and its reaction to ethylene) was that yeast alcohol dehydrogenase, a metal containing enzyme, is stabilized when in an ethylene atmosphere (28). It is possible, though only speculative, that this stability is caused by an increase in the strength of hydrophobic bonds.

Further investigation ought to be done to determine the connection between reorientation and stabilization of the microtubular structure, to determine the susceptibility of cold hardy plants to ethylene and D_2O and to determine what differences there are, if any, in microtubular structure.

SUMMARY

This paper presents evidence that:

1. Cold reverses many of the ethylene effects such as hook tightening, swelling, horizontal nutation, root ageotropism and inhibition of elongation.
2. Colchicine reduces the effects of ethylene as determined by the straight growth test.
3. The effects of ethylene and D_2O are, in many cases, similar.
4. Microtubule orientation is markedly altered by ethylene treatment.

Based on this evidence, a working model is suggested

in which ethylene stabilizes the microtubule polymer (when it binds to a metal containing site on the microtubule subunit) by strengthening hydrophobic bonding.

Table 1. Hook curvatures of etiolated seedlings treated with and without added ethylene at 24 C and 6 C.

TREATMENT				HOOK ANGLE (degrees)				
				CONTROL		ETHYLENE		
Temp C	Light Cond.	Incubation Time	C_2H_4 ppm	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$	N	P value
24	Dark	24 hr	10	125 ± 5	42	164 ± 5	48	.001
24	Dark	12 hr	1.0	120 ± 8	31	166 ± 5	41	.001
24	Light	24 hr	10	85 ± 5	29	178 ± 8	22	.001
6	Dark	7 day	100	125 ± 5	27	120 ± 6	19	.5
6	Dark	48 hr	10	104 ± 4	62	108 ± 4	73	.5
6	Light	48 hr	10	45 ± 4	24	94 ± 6	22	.001

Table 2. The effects of low temperature, ethylene and D_2O on germination and morphological development. This data represents the general trend of at least 6 experiments (3 for D_2O). After 9 days all observed effects are $\pm$ 3 days.

Days from Planting	24 C			6 C	
	Control	Ethylene	D_2O	Control	Ethylene
3	Epicotyls less than 1 cm, Roots 3 to 4 cm, both geotropic		Roots less than 1 cm		
6	Epicotyls 20 to 30 cm, Roots more than 15 cm, geotropic	Roots less than.1 cm	Roots 1 cm		
9		Roots 0.5 cm	Roots 1.5 cm	Roots less than 1 cm	Roots less than 1 cm
12		Roots 1.2 cm	Epicotyls less than 1 cm, swollen, Roots ageotropic	Roots 1 to 4 cm	Roots 1 to 2 cm
15		Epicotyls 1 cm, swollen, Roots 3 cm, ageotropic	Epicotyls 1 cm, swollen, Roots 3 cm, ageotropic	Roots 3 to 5 cm	Roots 2 to 3 cm
24		Epicotyls 2 cm, swollen, Roots 4 cm, ageotropic	Epicotyls 2 cm, swollen, Roots 4 cm, ageotropic	Epicotyls 1 to 2 cm No swelling Geotropic	Epicotyls 0.5 cm No swelling Geotropic
50				Epicotyls 2 to 3 cm No swelling Geotropic	Epicotyls 1 to 2 cm Moderate swelling Geotropic

Table 3. Comparison of effects of ethylene and D_2O as found in the literature and experimental results.

OBSERVATIONS	ETHYLENE	D_2O
Reduced elongation	(7,8,12,13,18,49)	(4,5,6,22)
Swelling	(9,11,13,23,49,60)	(Fig 5)
Horizontal nutation	(12,13,18,49)	(6)
Ageotropic roots	(12,18,19,Fig 5)	(Fig 6)
Banded birefringence pattern	(13,17,26,54,63,64)	(17)
Leaf epinasty	(7,13,24,25,67)	(6)
Anthocyanin production	(20,21,42)	(5,6)
Reversible effects	(18)	(6)
Delayed germination	(Table 2)	(4,5)
Chloroplast bleaching	(61)	(5)
Flowering effects	(15)	(5)
Reduced cell division	(3,41)	(33,34,66)
Species specific effects	(48)	(4,22)

Figure 1. Effects of ethylene on elongation of 10 mm subapical sections when incubated at 24 C for 12 hr (●); and at 6 C for 48 hr (■). Vertical lines represent ± 1 standard error. Data are means of at least 30 replicates. Inset shows rate of cooling of flasks.

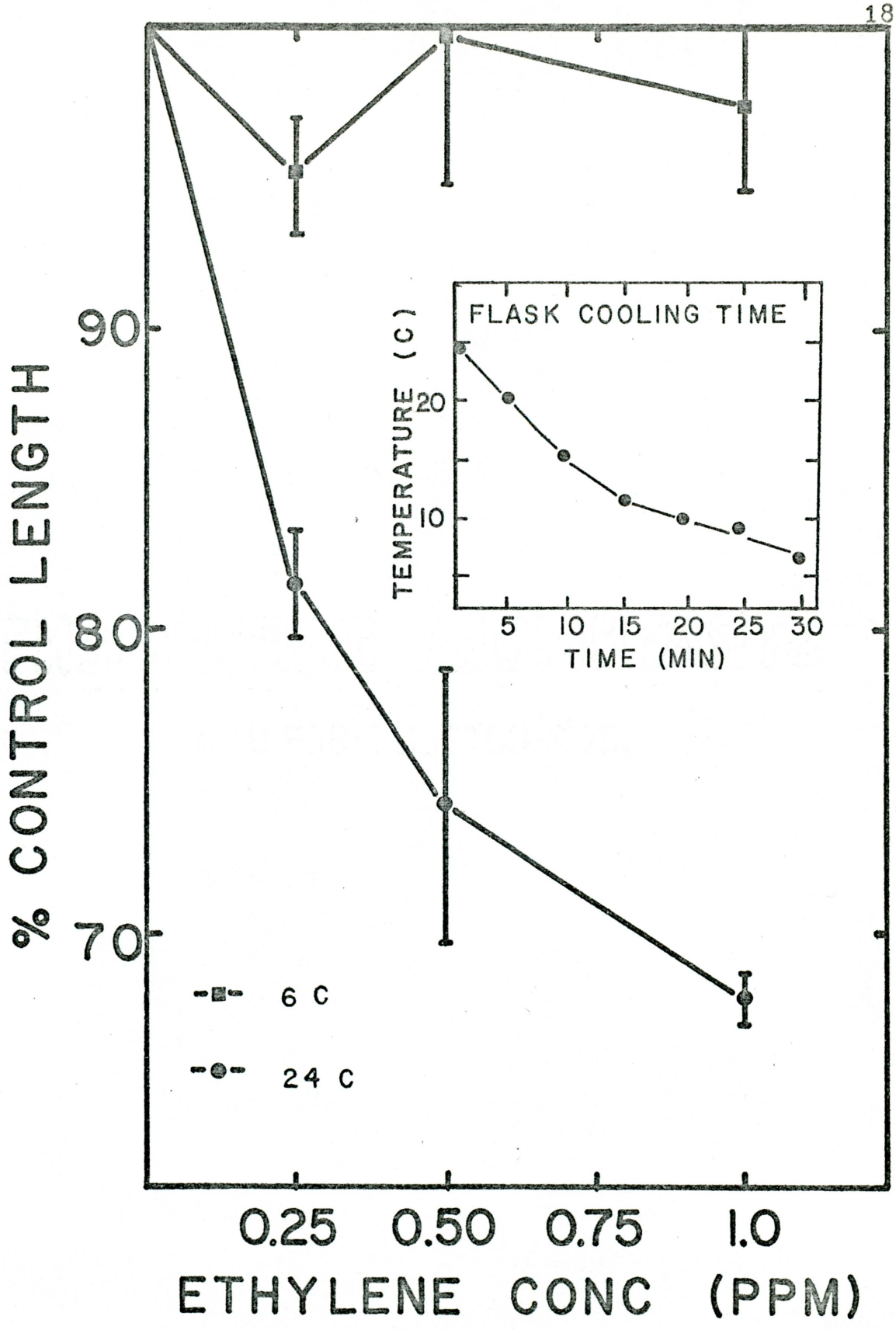

% CONTROL LENGTH
90
80
70
6 C
24 C
FLASK COOLING TIME
TEMPERATURE (C)
20
10
5
10
15
20
25
30
TIME (MIN)
0.25
0.50
0.75
1.0
ETHYLENE CONC (PPM)

Figure 2. A. Effects of various concentrations of colchicine on elongation in 10 mm subhook sections.

B. Effects of various combinations of ethylene and colchicine concentrations on elongation of 10 mm subhook sections. Dotted lines represent + 1 standard error. (X) is the number of replicates.

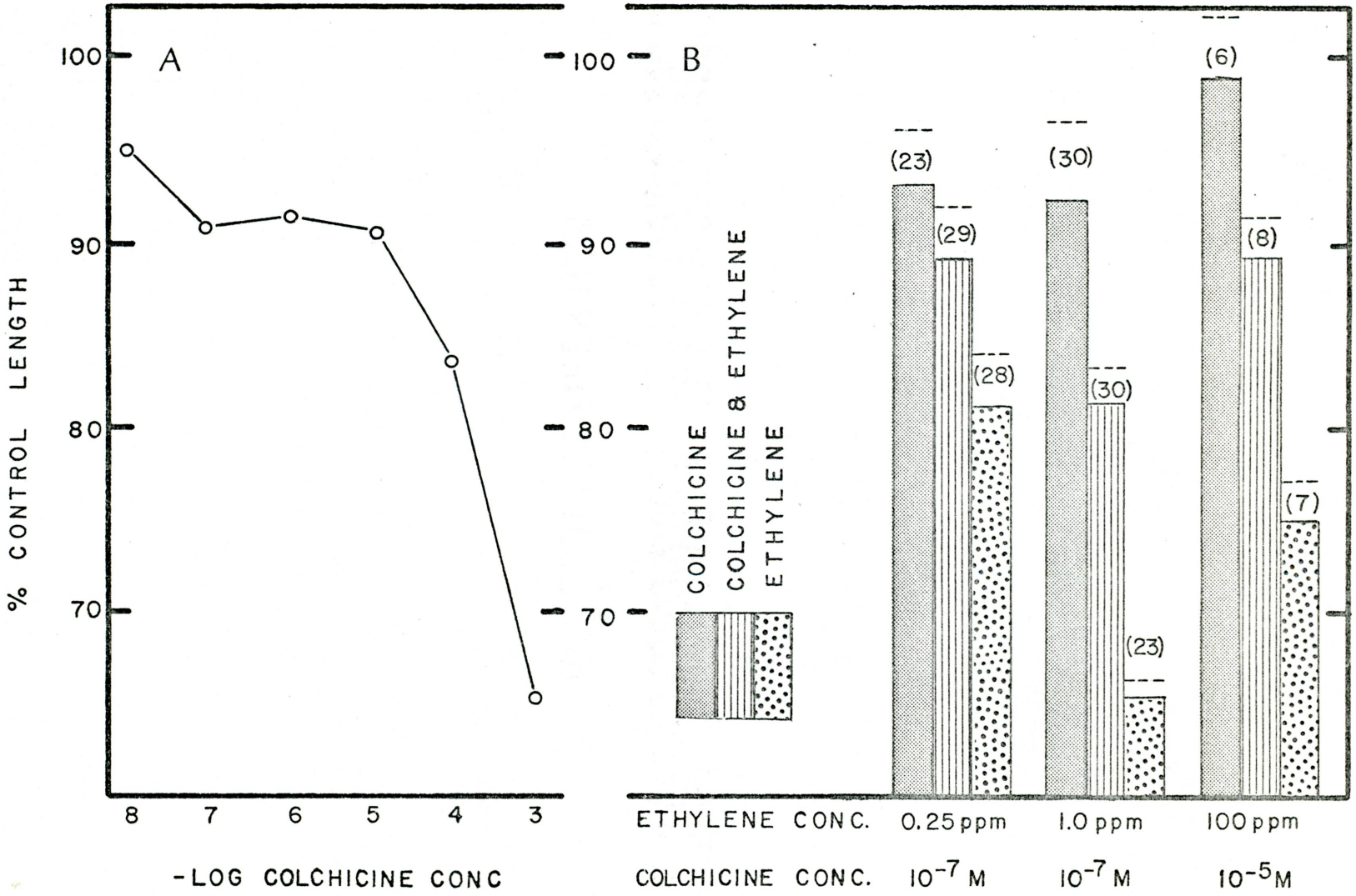

A
100
90
80
70
% CONTROL LENGTH
8
7
6
5
4
3
-LOG COLCHICINE CONC
B
100
90
80
70
COLCHICINE
COLCHICINE & ETHYLENE
ETHYLENE
(23)
(29)
(28)
(30)
(30)
(23)
(6)
(8)
(7)
ETHYLENE CONC. 0.25 ppm 1.0 ppm 100 ppm
COLCHICINE CONC. 10^{-7} M 10^{-7} M 10^{-5} M

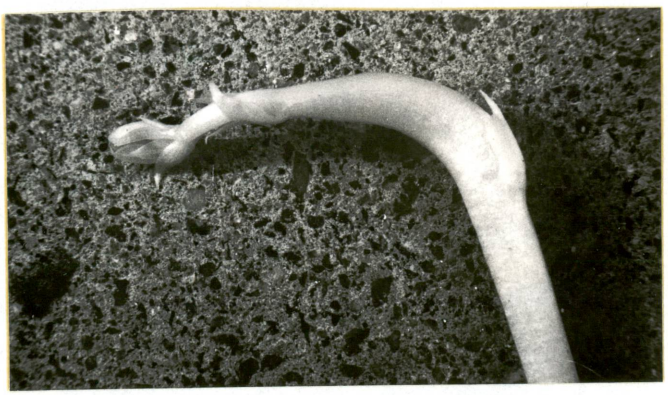
Figure 3. A: Control hook taken from 7 day old etiolated stem. Terminal internode is about 20 mm.

B & C: Intact stem tissue treated for 3 days with 100 ppm ethylene. Notice swelling and horizontal nutation.

D: Intact stem tissue treated with 100 ppm ethylene for 24 days at 6 C. Note absence of swelling and horizontal nutation.

A

B



C

D

Figure 4. Upper: Seedlings which were germinated and grown for 7 days in the presence of 5.0 ppm ethylene at 24 C. Epicotyls are swollen, roots are ageotropic, and growth retarded. Epicotyls of control seedlings grow to about 20 cm in 7 days. Each large square is 1 square inch.

Lower: Seedlings which were germinated and grown at 6 C for 30 days. 100 ppm ethylene was introduced initially which dropped to 15 ppm at the conclusion of the 30 days. In order to prevent contamination, the jars were not aired during the experimental period. Seedlings in both pictures are oriented in their growing position.

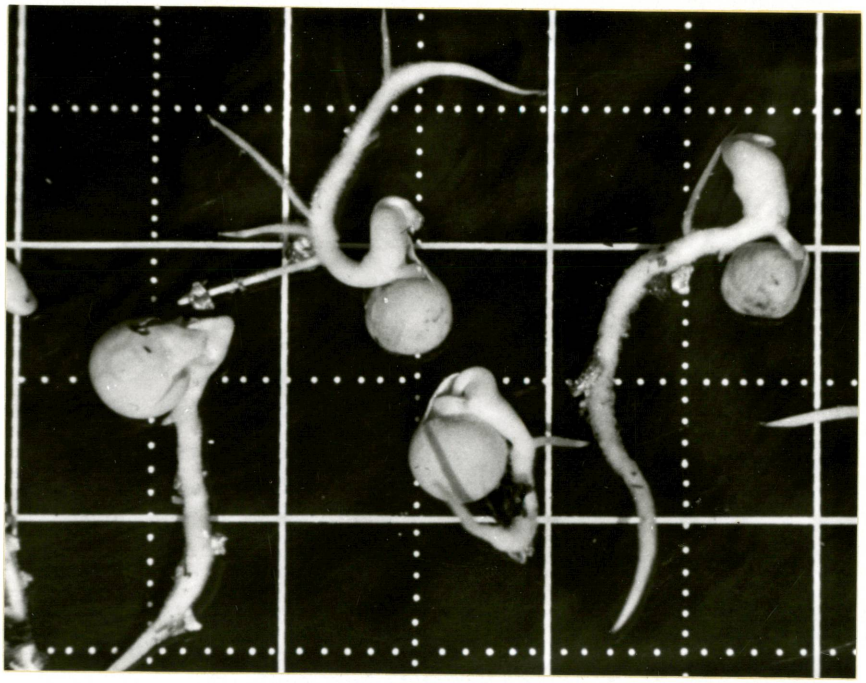


Figure 5. Upper: Closeup of a seedling which was germinated and grown in 75% D_2O for 28 days showing pronounced swelling and reduced plumule.

Lower: Closeup of a seedling which was germinated and grown in a 5 ppm ethylene atmosphere for 7 days at 24 C. Swelling is pronounced and the plumular hook is tight. The root is ageotropic and with prolific root hairs.

Figure 6. Upper: Seedling which was germinated and grown in 50% D_2O for 15 days at 24 C in the dark. Epicotyl is swollen, roots ageotropic, and growth is markedly inhibited.

Lower: Closeup of a seedling which was germinated and grown in 75% D_2O for 28 days showing axillary buds which have been released from apical dominance. Nearly all of the seedlings which germinated in 75% D_2O exhibited this phenomenon.

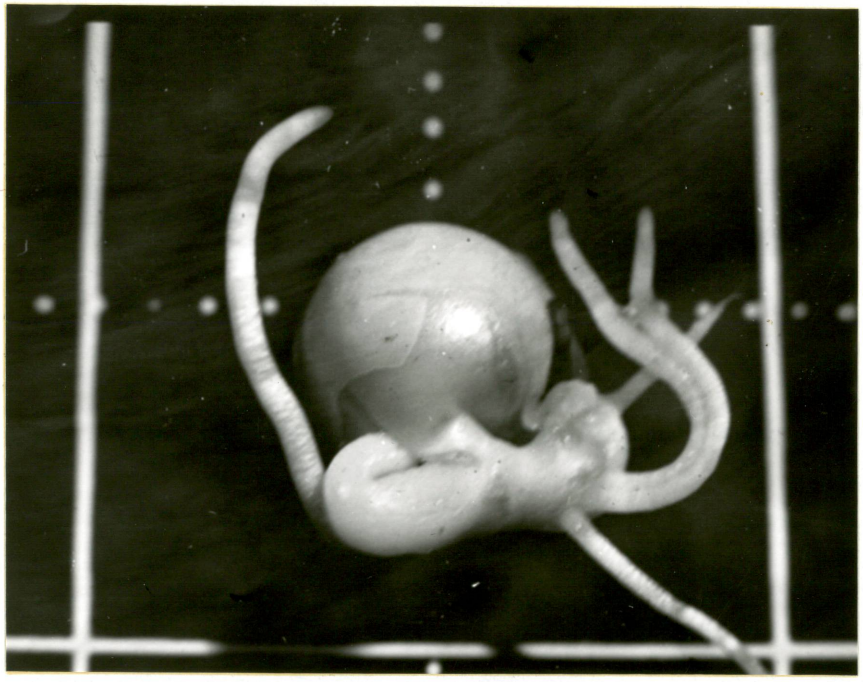


Figure 7. Longitudinal section through the subapical zone of a pea stem from a control plant showing the cell wall (CW) region of parenchyma cells. Note the microtubules (arrowed) commonly found in groups of three just beneath the plasmalemma. X100,000.

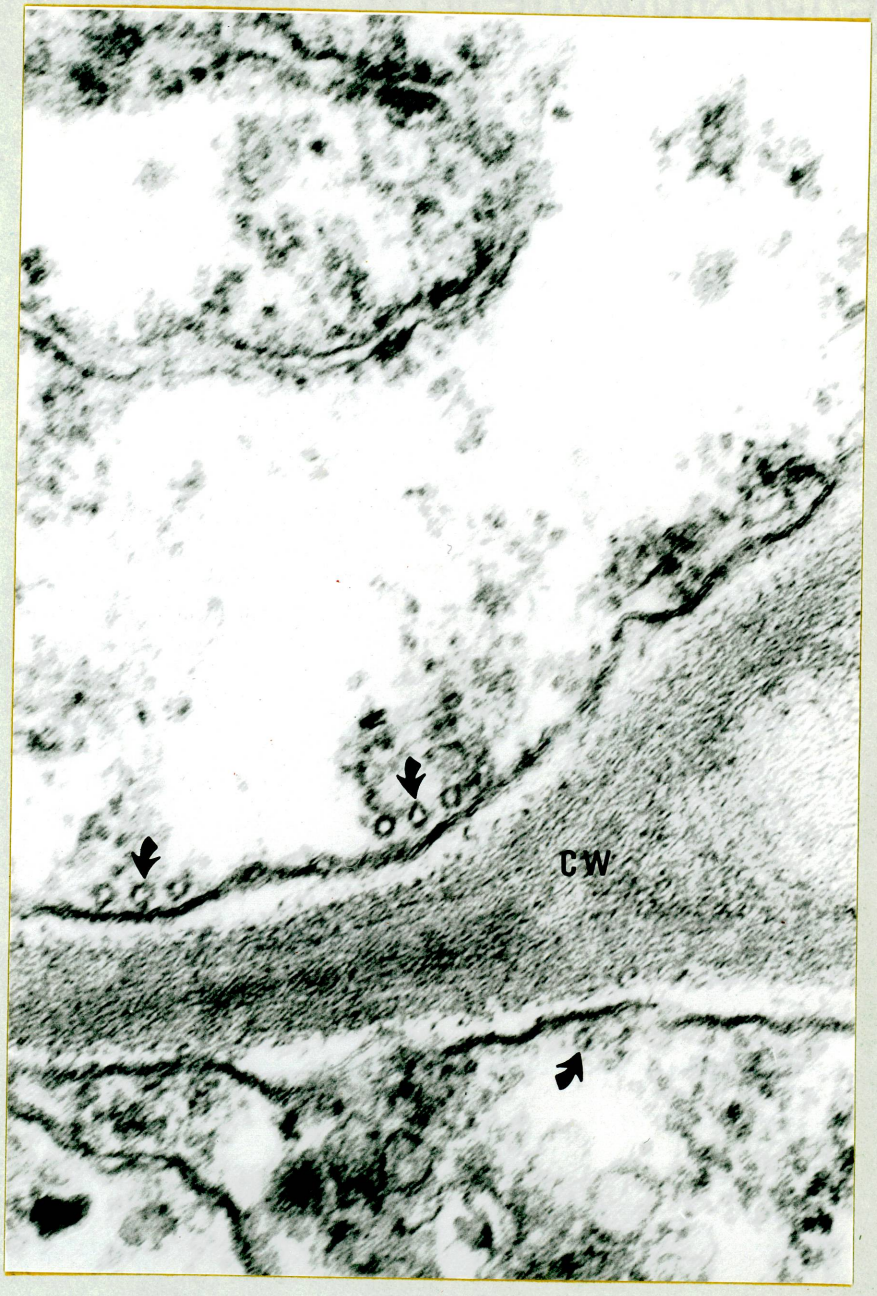

CW

Figure 8. Transverse section through the subapical zone of a pea stem from a control plant showing the cell wall (CW) region of parenchyma cells. Microtubules (arrowed) are found running circumferentially just beneath the plasmalemma. Note the orientation of newly deposited microfibrils which mirror the orientatation of the microtubules. X100,000.

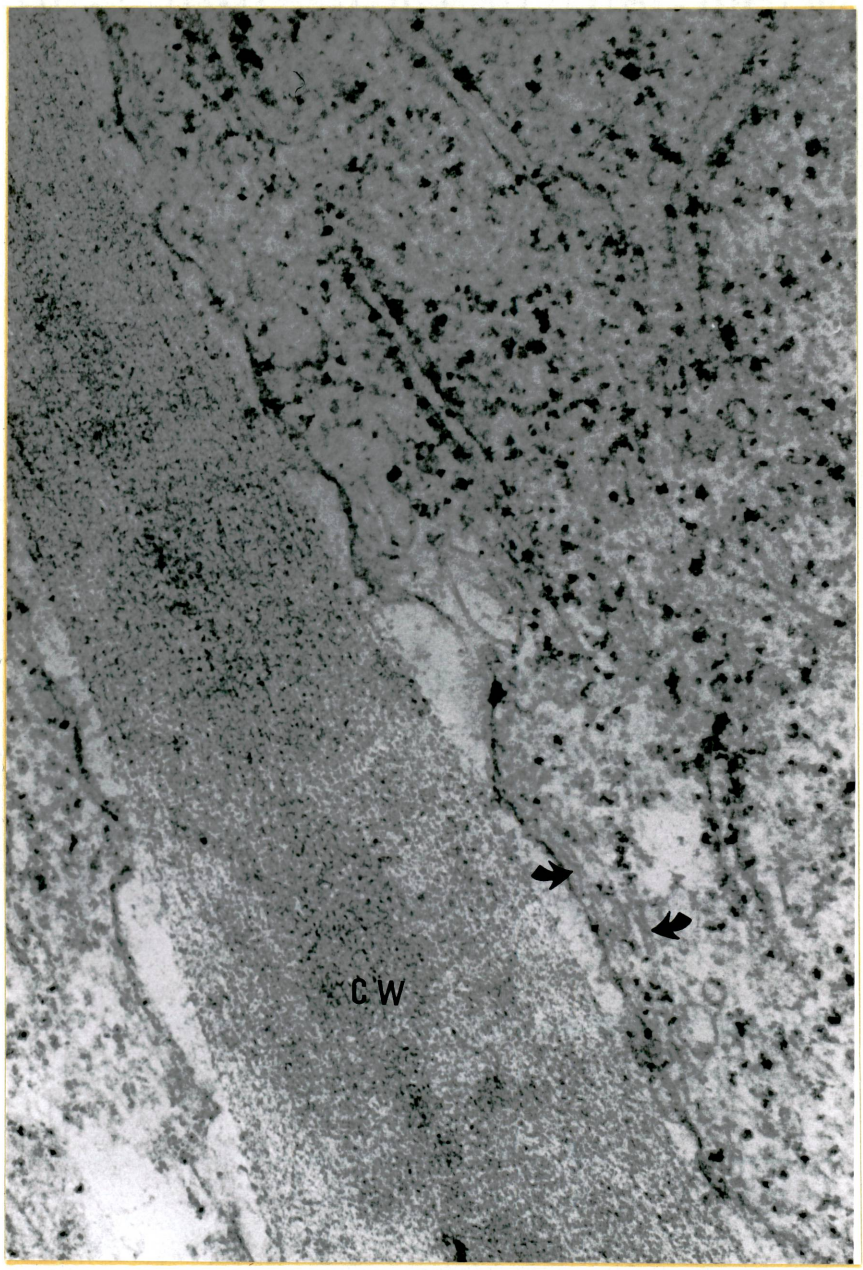

CW

Figure 9. Longitudinal section through the subapical zone of a pea stem from an ethylene treated plant showing the cell wall (CW) region of parenchyma cells. Microtubules (arrowed) appear in a longitudinal orientation paralleling the long axis of the cell. X130.000.

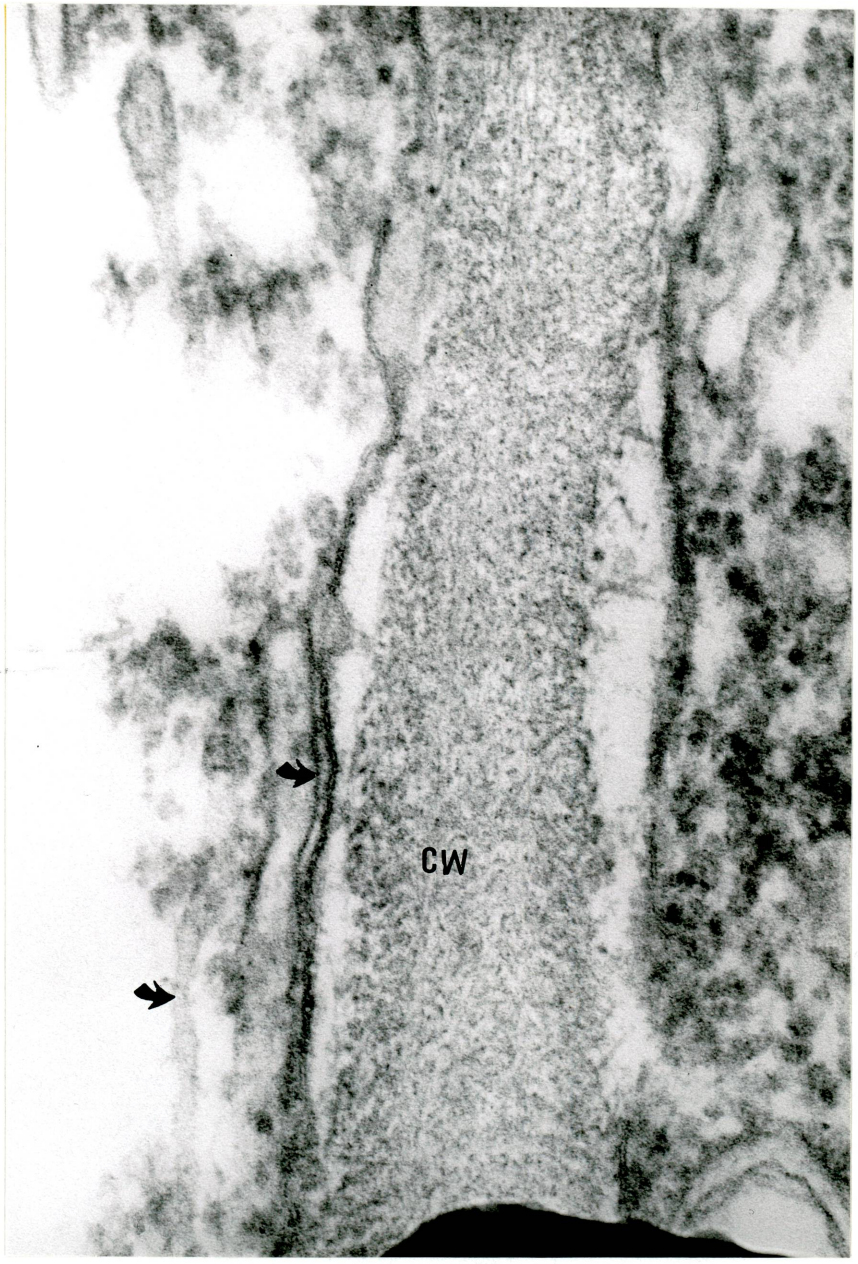
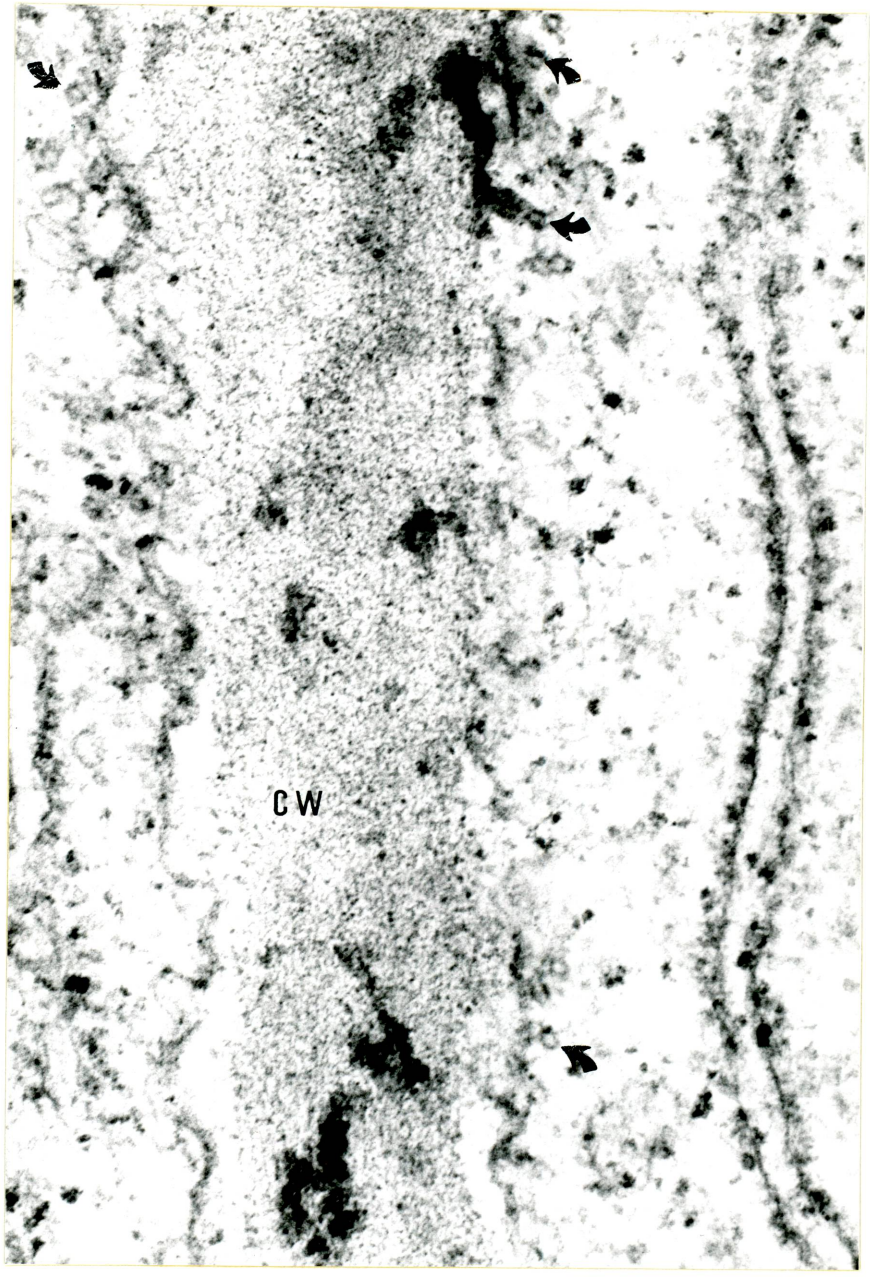

CW

Figure 10. Transverse section through the subapical zone of a pea stem from an ethylene treated plant showing the cell wall (CW) region of parenchyma cells. Microtubules (arrowed) are shown in cross section parallel to the orientation of newly deposited cellulose microfibrils in the cell wall. X130,000.


CW

LITERATURE CITED

1. Apelbaum, A. and S. P. Burg. 1971. Altered cell microfibrillar orientation in ethylene-treated Pisum sativum stems. Plant Physiol. 48:648-652.

2. Apelbaum, A. and S. P. Burg. 1972. Effects of ethylene and 2,4-Dichlorophenoxyacetic acid on cellular expansion in Pisum sativum. Plant Physiol. 50:125-131.

3. Apelbaum, A. and S. P. Burg. 1972. Effects of ethylene on cell division and deoxyribonucleic acid synthesis in Pisum sativum. Plant Physiol. 50:117-124.

4. Bhandarkar, M. K., S. Bhattacharya and B. K. Gaur. 1971. Physiological studies with heavy water. II Germination and growth inhibition of barley by D_2O. Physiol Plant. 24:517-521.

5. Bhatia, C. R. and H. H. Smith. 1968. Adaptation and growth response of Arabidopsis thaliana to deuterium. Planta(Berl.) 80:176-184.

6. Blake, M. I., F. A. Crane, R. A. Uphaus, and J. J. Katz. 1964. Effects of deuterium oxide on the growth of peppermint (Mentha piperita L.) I. J. Pharm. Sci. 53:79-83.

7. Borgström, G. 1939. Theoretical suggestions regarding the ethylene responses of plants and observations on the influence of apple-emanations. Kg. Fysiograf. Sallskap. Lund Forh. 9:35-74.

8. Borisy, G. G. and E. W. Taylor. 1967. The mechanism of action of colchicine: Binding of colchicine-^{3}H to cellular protein. J. Cell Biology 34:525-533.

9. Burg, S. P. 1970. Ethylene in plant growth. Proc. Nat. Acad. Sci. 70:591-597.

10. Burg, S. P., A. Apelbaum, W. Eisinger, and B. G. Kang. 1971. Physiology and mode of action of ethylene. HortSci. 6:359-364.

11. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Sci. 148:1190-1196.

12. Burg, S. P. and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. 55:262-269.

13. Burg, S. P. and E. A. Burg. 1967. Auxin stimulated ethylene formation: Its relationship to auxin inhibited growth, root geotropism and other plant processes. In: F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Rung Press Ltd., Ottawa, Canada. pp.1275-1294.

14. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42:144-152.

15. Burg, S. P. and M. J. Dijkman. 1967. Ethylene and auxin participation in the pollen induced fading of Vanda orchid blossoms. Plant Physiol. 42:1648-1650.

16. Burgess, J. and D. H. Northcote. 1969. Action of colchicine and heavy water on the polymerization of microtubules in wheat root meristem. J. Cell Sci. 5:433-451.

17. Chadwick, A. V. and D. A. Steen. 1973. Effects of deuterium oxide, low temperature and ethylene on pea growth. Plant Physiol. 51(Suppl.):31.

18. 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.

19. Chadwick, A. V. and S. P. Burg. 1970. Regulation of root growth by auxin-ethylene interaction. Plant Physiol. 45:192-200.

20. Craker, L. E., L. A. Standley, and M. J. Starbuck. 1971. Ethylene control of anthocyanin synthesis in sorghum. Plant Physiol. 48:349-352.

21. Craker, L. E. and P. J. Wetherbee. 1973. Ethylene, light, and anthocyanin synthesis. Plant Physiol. 51:436-438.

22. Crane, F. A., M. I. Blake, R. A. Uphaus, and J. J. Katz. 1969. Effects of deuterium replacement on seed development: Influence on excised embryos. Canadian J. Bot. 47:1465-1467.

23. Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1935. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. 7:231-248.

24. Denny, F. E. 1935. Testing plant tissue for emanations causing epinasty. Contrib. Boyce Thompson Inst. 7:341-347.

25. Denny, F. E. and L. P. Miller. 1935. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. 7:97-102.

26. Eisinger, W. R. and S. P. Burg. 1972. Ethylene-induced pea internode swelling: Its relation to ribonucleic acid metabolism, wall protein synthesis, and cell wall structure. Plant Physiol. 50:510-517.

27. Flaumenhaft, E., S. Bose, H. L. Crespi, and J. J. Katz. 1965. Deuterium isotope effects in cytology. Int. Rev. Cytol. 18:313-361.

28. Fuchs, Y. and E. Gertman. 1973. Stabilization of enzyme activity by incubation in an ethylene atmosphere. Plant and Cell Physiol. 14:197-199.

29. Fuchs, Y. and M. Lieberman. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. Plant Physiol. 43:2029-2036.

30. Galston, A. W., R. Baker and J. W. King. 1953. Benzimidazole and the geometry of cell growth. Physiol. Plant. 6:863-872.

31. Green, P. 1962. Mechanism for plant cellular morphogenisis. Sci. 138:1404-1405.

32. Green, P. B. 1964. Cell walls and the geometry of plant growth. In: Meristems and differentiation. Brookhaven Symp. Biol. 16:203-217.

33. Gross, P. R. and W. Spindel. 1960. Heavy water inhibition of cell division: An approach to mechanism. Annals New York Acad. Sci. 90:500-522.

34. Gross, P. R. and W. Spindel. 1960. The inhibition of mitosis by deuterium. Annals New York Acad. Sci. 84:745-754.

35. Houwink, A. L. and B. A. Roelofsen. 1954. Fibrillar architecture of growing plant cell walls. Acta Bot. Neerl. 3:385-395.

36. Inoué, S. 1952. The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. Explt. Cell Res.(Suppl.) 2:305-318.

37. Inoué, S. and H. Sato. 1967. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. J. Gen. Physiol. 50(Suppl.):259-292.

38. Inoué, S., H. Sato and M. Ascher. 1965. Counteraction of colcemid and heavy water on the organization of the mitotic spindle. Biol. Bull. 129:409-410.

39. Kang, B. G., C. S. Yocum, S. P. Burg and P. M. Ray. 1967. Ethylene and carbon dioxide; mediation of hypocotyl hook opening response. Sci. 156:958-959.

40. Kang, B. G. and S. P. Burg. 1972. Ethylene as a natural agent inducing plumular hook formation in pea seedlings. Planta(Berl.) 104:275-281.

41. Kang, B. G. and S. P. Burg. 1973. Influence of ethylene on nucleic acid synthesis in etiolated Pisum sativum. Plant and Cell Physiol. 14:981-988.

42. Kang, B. G. and S. P. Burg. 1973. Role of ethylene in phytochrome-induced anthocyanin synthesis. Planta(Berl.) 110:227-235.

43. Kauzmann, W. 1959. Some factors in the interpretation of protein denaturation. Adv. Prot. Chem. 14: 1-63.

44. Ledbetter, M. C. and K. R. Porter. 1963. A microtubule in plant cell fine structure. J. Cell Biol. 19:239-250.

45. Lehninger, A. L. 1970. The molecular basis of cell structure and function. Worth Publishers, Inc., New York. 833 pp.

46. Marsland, D. and A. M. Zimmerman. 1965. Structural stabilization of the mitotic apparatus by heavy water in the cleaving eggs of Arabacia punctulata. Exptl. Cell Res. 38:306-313.

47. Marsland, D., L. G. Tilney and M. Hirshfield. 1971. Stabilizing effects of D_2O on the microtubular components and needle-like form of helizoan axopods: A pressure-temperature analysis. J. Cell Physiol. 77: 187-194.

48. Morgan, P. W., E. Beyer, and H. W. Gausman. 1967. Ethylene effects on auxin physiology. In: F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Rung Press Ltd., Ottawa, Canada pp.1255-1273.

49. Neljubow, D. 1901. Ueber die horizontale nutation der stengel von Pisum sativum und einiger anderen pflanzen. Botan. Centr. 10:128-139.

50. Nemethy, G. and H. A. Scheraga. 1962. The structure of water and hydrophobic bonding in proteins. III. The thermodynamic properties of hydrophobic bonds in proteins. J. Phys. Chem. 66:1773-1789.

51. Newcomb, E. H. 1969. Plant Microtubules. Ann. Rev. Plant Physiol. 20:253-288.

52. Peas, D. C. 1946. High hydrostatic pressure effects upon the spindle figure and chromosome movement. II. Experiments on the meiotic divisions of Tradescantia pollen mother cells. Biol. Bull. 91:145-169.

53. Pickett-Heaps, J. D. 1967. The effects of colchicine on the ultrastructure of dividing cells, xylem wall differentiation and distribution of cytoplasmic microtubules. Devel. Biol. 15:206-236.

54. Probine, M. C. 1964. Chemical control of plant cell wall structure and of cell shape. Proc. Royal Soc. (London) 161:526-537.

55. Ridge, I. 1973. The control of cell shape and rate of cell expansion by ethylene: Effects on microfibril orientation and cell wall extensibility in etiolated peas. Acta Bot. Neerl. 22:144-158.

56. Roelofsen, B. A. and A. L. Houwink. 1953. Architecture and growth of the primary cell wall in some plant hairs and in the Phycomyces sporangiophore. Acta Bot. Neerl. 2:218-225.

57. Sargent, J. A., A. V. Atack, and D. J. Osborne. 1973. Orientation of cell growth in the etiolated pea stem. Effects of ethylene and auxin on cell wall deposition. Planta(Berl.) 109:185-192.

58. Sargent, J. A., A. V. Atack and D. J. Osborne. 1974. Auxin and ethylene control of growth in epidermal cells of Pisum sativum: A biphasic response to auxin. Planta(Berl.) 115:213-225.

59. Siegel, S. M., L. A. Halpern and C. Giumano. 1964. Germination and seedling growth of winter rye in D_2O. Nature 201:1244-1245.

60. Steen, D. A. and A. V. Chadwick. 1973. Effects of cycloheximide on indoleacetic acie-induced ethylene production in pea root tips. Plant Physiol. 52:171-173.

61. Taylor, O. C. 1971. Effects of combustion pollutants on plants and ecology. 1971 Intersociety Energy Conversion Engineering Conference Proceedings. 323-328.

62. Tilney, L. G. and K. R. Porter. 1967. Studies on the microtubules in heliozoa. II. The effects of low temperature on these structures in the formation and maintenance of the axopodia. J. Cell Biol. 34:327-343.

63. Veen, B. W. 1970. Control of plant cell shape by cell wall structure. Koninkl. Nederl. Akad. van Wet. (Amsterdam) 73:118-121.

64. Veen, B. W. 1970. Orientation of microfibrils in parenchyma cells of pea stem before and after longitudinal growth. Koninkl. Nederl. Akad. van Wet. (Amsterdam) 73:113-117.

65. Waber, J. and W. S. Sakai. 1974. The effects of growth in 99.8% Deuterium oxide on the ultrastructure of winter rye. Plant Physiol. 53:128-130.

66. Zimmerman, P. W. and A. E. Hitchcock. 1933. Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gasses. Contrib. Boyce Thompson Inst. 5:351-369.

67. Zimmerman, P. W. and F. Wilcoxin. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. 7:209-229.

APPENDIX

INTRODUCTION

The purpose and rationale of this paper have been stated previously in the introduction to the manuscript for publication. The purpose of this appendix is: to present additional evidence which was not included in the main body because, either it was not significant enough for publication or it has already been published; and to add details to the materials and methods which may be too insignificant to mention in a publication but which would be helpful to any who try to build on this work.

LITERATURE REVIEW

A report in the Proceedings of the Academy of Natural Sciences in Philadelphia in 1858 (39) describing the effects of "illuminating gas" on plants in the vicinity of a broken natural gas line is probably the first observation in the literature on the effects of ethylene, a component of natural gas. The active ingredient however, was not discovered until 1901 when Neljubow, while conducting experiments in the laboratory on etiolated pea seedlings, noticed a strange behavior of the seedlings when grown in air contaminated with natural gas (56). He isolated ethylene as the culprit and the effects on etiolated seedlings which he described, later became known as the "triple response" (31). The triple response, which involves subapical swel-

ling, inhibition of elongation, and horizontal nutation, is characteristic and so sensitive to low levels of ethylene that for many years etiolated pea seedlings were used to determine the presence of ethylene.

Many of the early ethylene effects on plant physiology were investigated at the Boyce Thompson Institute where it was learned that most vegetative tissues produce ethylene to some extent (33,34) and that ethylene production is enhanced by application of the growth hormone indole-3-acetic acid (64). It was also while working at the institute that Crocker, Hitchocock and Zimmerman proposed ethylene as a plant growth hormone since so many of its effects were similar to those of IAA (31). Recent reports however have established that swelling and inhibition of elongation resulting from auxin application are caused entirely by ethylene resulting from IAA-induced ethylene production (10, 18,26).

From the late 50's and on, investigators turned again toward physiological ethylene effects --- reviewed by Pratt and Goeschl (58) --- in an effort to find a mechanism or mechanisms of action for the catalog of effects which by this time included abscission of buds, fruits (43), and leaves (1); inhibition of elongation (18); mediation of geotropic responses (26,27); swelling (13,18); root hair proliferation (26,53); senscence (4,5); epinasty (19); and

effects on flowering (17,24), to name a few. Most of the reports dealing with mechanisms of action can be divided into a few general areas which are here discussed briefly. There is also an excellent review on ethylene and its mechanism of action (3).

ETHYLENE AND AUXIN TRANSPORT: Ethylene completely blocks lateral auxin transport in peas (18) but not in Avena coleoptiles (52), which explains why ethylene destroys tropistic responses in peas and not in Avena. Basipetal movement of auxin is reversibly inhibited after prolonged exposure to the gas (20), and leaf abscission has been closely tied to ethylene mediated inhibition of polar auxin transport (9,28). Morgan showed that most species show some degree of decline in auxin transport in response to ethylene (55). Furthermore Burg and Burg have (18) suggested that ethylene effects on auxin uptake, transport, and destruction may account for tropistic responses.

ETHYLENE AND NUCLEIC ACID METABOLISM: DNA content and growth rate in the apical region of soybean seedlings are reduced when exposed to the gas (45). Ethylene inhibits incorporation of ^{3}H-thymadine in the plumule, subapex and root of intact etiolated pea seedlings (8,50) but RNA synthesis remains unchanged (50). Bean leaf tissue however shows no change in DNA content or rate of synthesis during a 7 hour exposure to ethylene (44). Ethylene does inhibit

cell division in lateral buds of etiolated pea plants and petunias after they have been released from apical dominance (22). Cell division is also inhibited in fig fruits (54), potatoes (38) and fern spores and gametophytes after exposure to the gas (35,36).

ETHYLENE AND PROTEIN SYNTHESIS AND STABILITY: Ethylene inhibits incorporation of ^{14}C-leucine and ^{14}C-proline (but not ^{14}C-glucose) into a pronase- or base-extractable cell wall fraction after 4 hours of incubation. This inhibition is synchronous with the onset of swelling (37). In contrast, after a 3 hour lag time, ethylene induces cellulase production which is localized in the separation layer of the abscission zone (2,4,46). Abscission retardants such as IAA, cytokinins, CO_2, cycloheximide, and actinomycin D inhibit cellulase induction and with the exception of the antibiotics, they are ethylene competitors or antagonists.

Chlorophyll biosynthesis during light application in dark grown cucumber cotyledons is enhanced by ethylene treatment during the dark phase (6) and, depending on the light and treatment involved, ethylene both promoted and inhibited anthocyanin synthesis in sorghum (29,30,51).

Fuchs and Gertman (40) just recently reported a stabilizing effect of ethylene on the enzymatic activity of yeast alcohol dehydrogenase, a relatively unstable enzyme at low concentrations. Thus, ethylene has been found to

induce or inhibit protein synthesis as well as to have a stabilizing effect on protein structure.

ETHYLENE AND CELL ULTRASTRUCTURE: Veen (61,62) reported that supraoptimal concentrations of IAA cause swelling along with a change in the optical birefringence pattern and re-orientation of newly deposited cellulose microfibrils in parenchyma cells. Since Burg and others (10,26,31,49,60) have established that supraoptimal auxin concentrations induce ethylene formation and that ethylene alone is the cause of swelling, it is not surprising that ethylene also causes a change in the birefringence pattern of parenchyma cells in pea subapical sections (14,19,37) and a change in orientation of newly deposited wall microfibrils (7). It has been suggested that since microtubule orientation usually parallels the orientation of newly deposited wall microfibrils, the microtubules may be responsible for microfibrillar orientation (42,53,57) in newly deposited wall material.

This paper presents evidence that ethylene alters microtubule orientation and furthermore, that the stability of microtubule polymers are enhanced by ethylene application. The spectrum of effects brought on by ethylene treatment are probably due to one or more of the above mechanisms acting singly or in concert to bring about modifications in plant morphology and development.

MATERIALS AND METHODS

Straight Growth Tests. Seeds of Pisum sativum L. (cv. Alaska) were soaked in running tap water for 6 hr in the dark. Plastic pans which measure 30 x 22 x 12 cm were filled to within 3 cm of the top with soaked and drained vermiculite which had been freshly transferred to the pan after draining to prevent water induced settling and packing of the vermiculite particles. The surface of the vermiculite was leveled but not packed and then the freshly soaked seeds were sprinkled on top until they were evenly layered to about 2 seeds deep. More soaked and drained vermiculite was spread on top (smoothing but not packing) till all seeds were covered by about 1 cm of vermiculite.

The pans were left in the dark room with a fan running to circulate room air but aimed so as not to dry the vermiculite. Every two or three days the seedlings were watered with about 250 ml of tapwater per pan.

After 7 days, when a majority of the seedlings had achieved a third internode length of between 10 and 30 mm, the plants were harvested and processed one handful at a time by cutting them off close to the substrate surface with a razor blade. Ten mm sections were excised from selected seedlings (those which had a normal appearing third internode without swelling or horizontal nutation and a length of between 10 and 30 mm). All subsequent manipulations

were carried out as previously described.

Optical Birefringence Studies. After the 12 hr straight growth incubation period, when stem sections had been weighed and measured, several sections each from selected flasks were put in small vials containing a 1:1 mixture of H_2O_2 and glacial acetic acid. After 24 hr, the tissue was macerated in the same solution and optical birefringence patterns of parenchyma cell walls were observed and photographed using a polarized light microscope fitted with a Polaroid film pack. The microscope stage was rotated until the long axes of cells were oriented at a 45 degree angle to one of the axes of the birefringence pattern seen in bubbles in the same field as the observed cell. Stem sections taken from at least 3 separate experiments were observed and parenchyma cells were catagorized as to their birefringence pattern. A minimum of 100 cells were counted for each type of treatment.

Germination and Growth of Intact Seedlings in D_2O. Seeds were surface sterilized as previously described, rinsed and soaked in various concentrations of sterile D_2O for 6 hr. Imbibed seeds were then planted in sterile vermiculite which had been soaked with the appropriate concentration of D_2O in sterile glass jars with airtight covers. Appropriate concentrations of C_2H_4 were introduced with syringes and the jars were aired periodically.

All manipulations were done at 24 C under dim green light to avoid photomorphic or phototropic responses. When seedlings had grown to their limit in the jars or when their future existence was threatened by fungi and rot, the plants were taken out, photographed, weighed and measured.

Straight Growth Tests With D_2O and CO_2. Preliminary experiments to determine the effects of D_2O and CO_2 were conducted at 24 C for 12 hr as previously described under straight growth tests except that predetermined volumes of D_2O were substituted for H_2O. CO_2 gas was introduced through the sealed caps with a syringe.

RESULTS

Optical Birefringence Experiments. Optical birefringence patterns of the cell walls of etiolated subhook sections have been described for control, ethylene treated, and colchicine treated tissues (19,37,41). The descriptions are in agreement with my results (Fig 11 and Table 4). The majority of cells from control tissue exhibit a more or less uniform appearing birefringence pattern whereas the majority of ethylene or D_2O treated cells have a similar and characteristic banding pattern which we recently reported (25). Colchicine treated cells have a very faint and almost nonexistent birefringence pattern which appears mottled or with lighter more diffuse banding. When treated with combi-

nations of colchicine and ethylene or D_2O and CO_2, the majority of cells again look like control cells.

D2O And Ethylene Growth Studies. Seedlings grown in the presence of various concentrations of D_2O exhibit many of the characteristics of ethylene treatment (Fi 12 and Table 5). Inhibition of germination and elongation are directly proportional to the concentration of heavy water as they are with ethylene. Swelling, horizontal nutation of stems and ageotropic roots are characteristic of both types of treatment. The additive effects of D_2O and ethylene become obvious when the growth rate is high enough to demonstrate this effect (i.e. 25% D_2O).

Straight Growth Tests in D2O and CO2. Preliminary results indicate that inhibition is a direct function of D_2O concentration (Fig 13). A maximum inhibition of about 80% is achieved with 99.8% D_2O. When 10% CO_2 was introduced in some experiments, it weakened the severity of the D_2O induced inhibition.

DISCUSSION

The use of the optical birefringence pattern in connection with microtubule (47,48) and microfibril (11,12,19,37, 61,62) orientation studies make it a useful tool for determining the effects of various chemicals on the change in orientation of cell ultrastructure. Much more difficult

means such as electron microscopy must be used however to determine precise orientations with respect to the cell axis.

To my knowledge, there are no other reports in which birefringence data is presented as a differential count of representative forms of a dynamic process (Table 4). Most of them simply present data in the form of a picture, never mentioning the polymorphic appearance of the parenchyma cell. I believe that the data are much more informative using the differential count. Although the small sample size used for differential counts preclude any strong conclusions, it is interesting to note general trends. The decided majority of morphs from each treatment are in agreement with previously published descriptions but an interesting reversal to control like forms occurs from treatment with a combination of colchicine and ethylene or with D_2O and CO_2. This is precisely what would have been predicted from the results of straight growth tests using combinations of colchicine and ethylene (Fig 2) and from the preliminary results in Fig 13. These findings should be repeated and put together with the results of additional straight growth tests to confirm the validity of the observed trends.

Added evidence for the marked similarities of effects of ethylene and D_2O (Table 3) are given in Table 5 and Fig 12. At high D_2O concentrations there is an apparent inability of ethylene to exert any added inhibition; which

could be caused by a maximal effect on the same system due to D_2O. If D_2O and ethylene are both acting by enhancing hydrophobic bonding then high concentrations of either one or the other could produce maximal effects with no added effects due to the other.

Carbon dioxide, a potent competitive inhibitor of ethylene action, is thought to bind to the same metal containing site to which ethylene binds (21). Preliminary results of straight growth tests with various combinations of D_2O and 10% CO_2 show that CO_2 may affect D_2O induced phenomena in the same way. This aspect ought to be investigated further.

SUGGESTION FOR FURTHER RESEARCH

The relationship between CO_2 and the various D_2O induced effects should be investigated using straight growth tests, swelling, and optical birefringence pattern studies to see if in fact CO_2 is a competitor of D_2O action.

The relationship between microtubule and microfibril orientation needs to be studied with emphasis on how microtubule orientation is changed and how that change brings about microfibrillar orientation changes. One way of determining the mechanism of microtubule reorientation is to treat with ethylene and then take successive specimens at intervals and observe what happens by electron microscopy. It would be time consuming and tedious but should show what happens, much like the successive frames of a movie.

Table 4. Differential counts (%) of various optical birefringence patterns as seen in macerated parenchyma cells under polarized light microscopy.

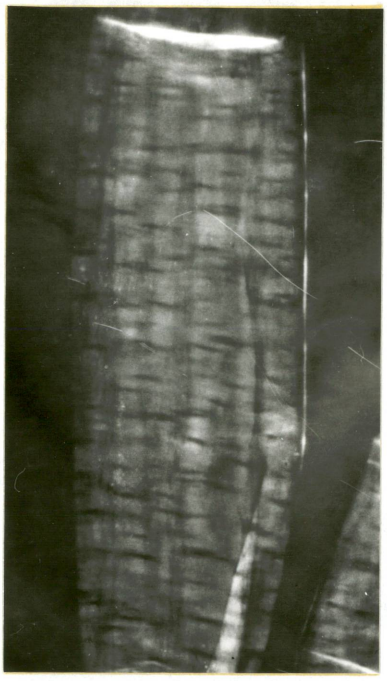
Treatment	Birefringence Pattern			
				
Control	90	9	1	0
94% D_2O	18	3	79	0
50% D_2O	17	10	73	0
10^{-6}M Colchicine	10	48	1	41
10 ppm Ethylene	9	22	69	0
10^{-6}M Colchicine 10 ppm Ethylene	66	24	10	0
50% D_2O 10% CO_2	64	27	9	0

Table 5. Effects of D_2O and ethylene on germination and development of etiolated seedlings.

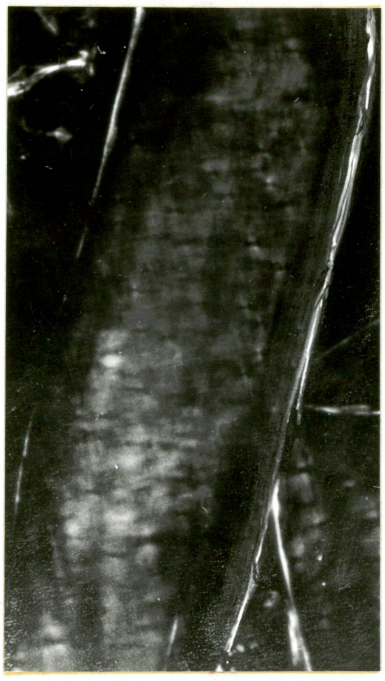
Treatment	Days of growth	LENGTH (cm)		% Germination	% With Axial Buds
		Epicotyls	Roots		
Control	6	6.6 ± .2	14.2 ± .3	100	0
Control + C_2H_4 0.2 ppm	6	5.8 ± .4	12.4 ± .5	100	0
25% D_2O	6	4.6 ± .3	11.9 ± .7	100	0
25% D_2O + C_2H_4 0.2 ppm	6	2.2 ± .2	9.8 ± .4	98	0
50% D_2O	15	2.5 ± .2	10.0 ± .5	90	67
50% D_2O + C_2H_4 10 ppm	15	2.8 ± .2	9.2 ± .5	93	49
75% D_2O	28	1.1 ± .1	4.2 ± .3	85	100
75% D_2O + C_2H_4 10 ppm	28	1.1 ± .1	3.8 ± .2	85	100
99.8% D_2O	28	-	.99 ± .1	66	-
99.8% D_2O + C_2H_4 10 ppm	28	-	.84 ± .1	80	-

Figure 11. Birefringence patterns as seen in macerated parenchyma cells of excised tissue treated as follows. A: Control; B: 10^{-7}M Colchicine for 12 hr; C: 10 ppm ethylene for 12 hr; D: 50% D_2O for 12 hr.

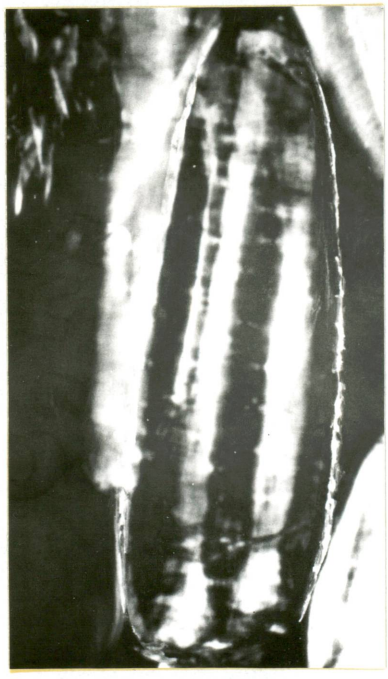
A



B



C



D

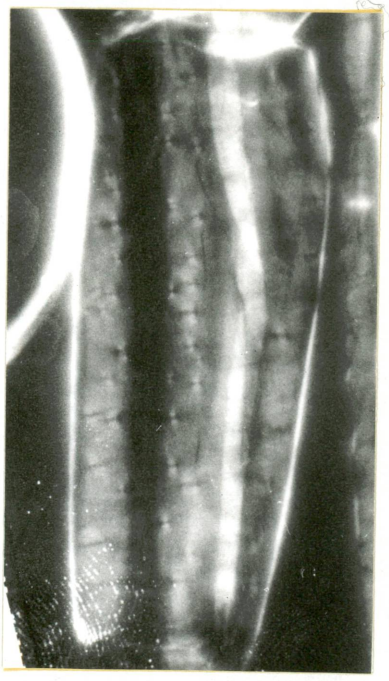
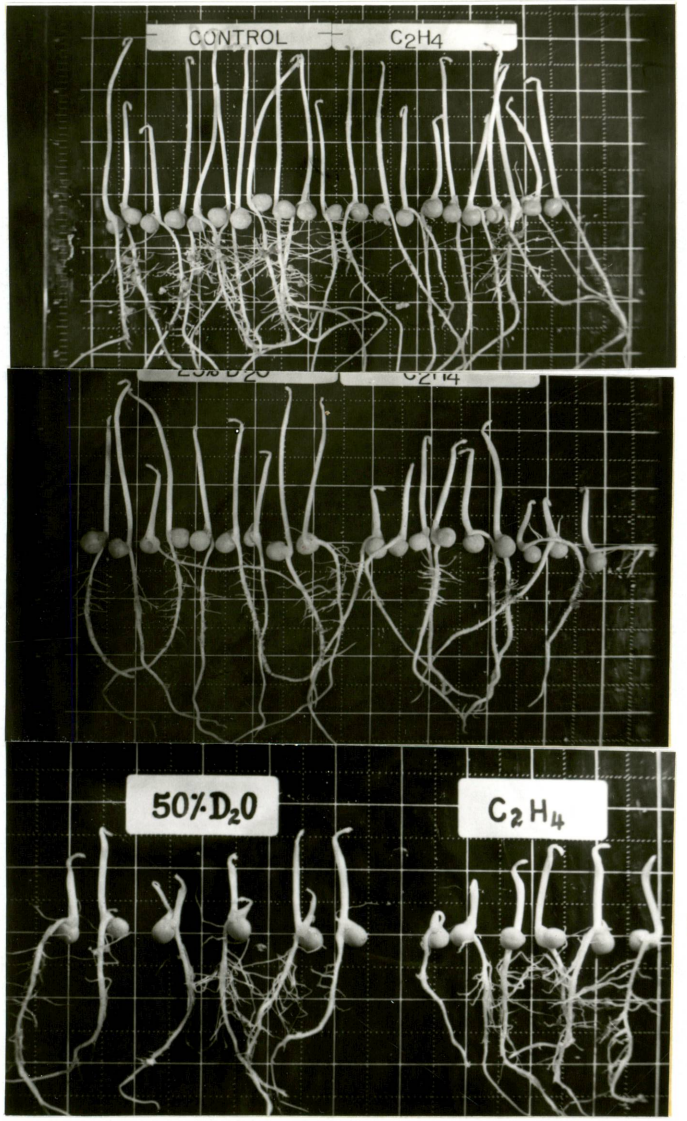
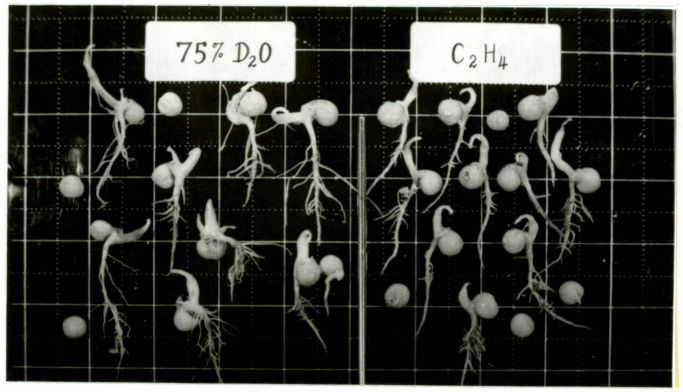


Figure 12. Effects of various concentrations of ethylene and D_2O on morphological development. Solid lines are 1 inch apart.

A: Control with and without 0.2 ppm ethylene, 6 days
B: 25% D_2O with and without 0.2 ppm ethylene, 6 days
C: 50% D_2O with and without 10 ppm ethylene, 15 days
D: 75% D_2O with and without 10 ppm ethylene, 28 days
E. 100% D_2O with and without 10 ppm ethylene, 28 days


CONTROL
C_2H_4
A
B
50% D_2O
C_2H_4
C


75% D_2O
C_2H_4
D

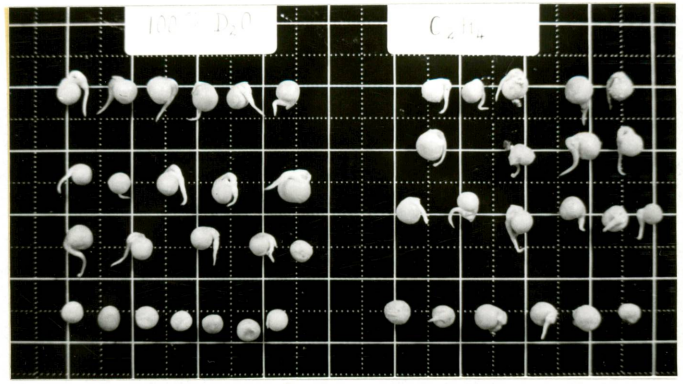
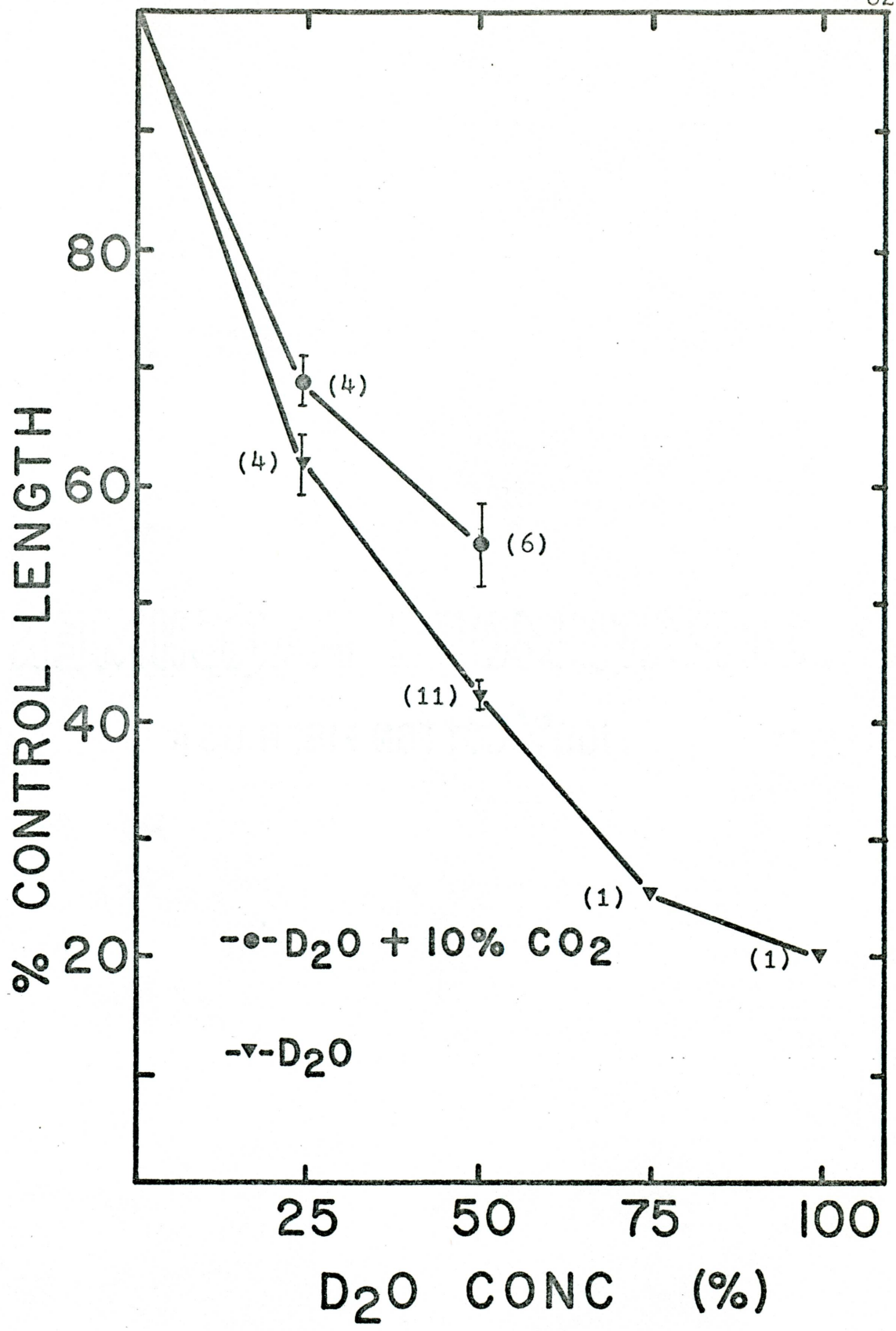

C_2H_4
E

Figure 13. Effects of various concentrations of D_2O on elongation of 10 mm subapical sections. CO_2 was added in some experiments. Vertical lines represent $\pm$ 1 standard error and number of flask replicates are in brackets.


% CONTROL LENGTH
80
60
40
20
(4)
(4)
(6)
(11)
(1)
(1)
-●-D2O + 10% CO2
-▼-D2O
25
50
75
100
D2O CONC (%)

LITERATURE CITED*

1. Abeles, F. B. 1967. Mechanism of action of abscission accelerators. Physiol. Plant. 20:442-454.

2. Abeles, F. B. 1969. Abscission: Role of cellulase. Plant Physiol. 44:447-452.

3. Abeles, F. B. 1972. Biosynthesis and mechanism of action of ethylene. Ann Rev. Plant Physiol. 23:259-292.

4. Abeles, F. B., G. R. Leather, L. E. Forrence and L. E. Craker. 1971. Abscission: Regulation of senescence, protein synthesis, and enzyme secretion by ethylene. HortSci. 6:371-376.

5. Abeles, F. B., R. E. Holm and H. E. Gahagan. 1967. Abscission: The role of aging. Plant Physiol. 42: 1351-1356.

6. Alscher, R. G. and P. A. Castelfranco. 1972. Stimulation by ethylene of chlorophyll biosynthesis in dark-grown cucumber cotyledons. Plant Physiol. 50:400-403.

7. Apelbaum, A. and S. P. Burg. 1971. Altered cell microfibrillar orientation in ethylene-treated Pisum sativum stems. Plant Physiol. 48:648-652.

8. Apelbaum, A. and S. P. Burg. 1972. Effects of ethylene on cell division and deoxyribonucleic acid synthesis in Pisum sativum. Plant Physiol. 50:117-124.

9. Beyer, E. M. 1973. Abscission: Support for a role of ethylene modification of auxin transport. Plant Physiol. 52:1-5.

10. Borgström, G. 1939. Theoretical suggestions regarding the ethylene responses of plants and observations on the influence of apple-emanations. Kg. Fysiograf. Sallskap. Lund Forh. 9:35-74.

11. Borisy, G. G. and E. W. Taylor. 1967. The mechanism of action of colchicine: Binding of colchicine-^{3}H to cellular protein. J. Cell Biol. 34:525-533.

12. Borisy, G. G. and E. W. Taylor. 1967. The mechanism of action of colchicine: Colchicine binding to sea urchin eggs and the mitotic apparatus. J Cell Biol. 34:535-548.

* Appendix only. For literature of manuscript see page 37

13. Burg, S. P., A. Apelbaum, W. Eisinger, and B. G. Kang. 1971. Physiology and mode of action of ethylene. HortSci. 6:359-364.

14. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37:179-189.

15. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Sci. 148:1190-1196.

16. Burg, S. P. and E. A. Burg. 1965. Gas exchange in fruits. Physiol. Plant. 18:870-884.

17. Burg, S. P. and E. A. Burg. 1966. Auxin-induced ethylene formation and its relation to flowering in the pineapple. Sci. 152:1269.

18. Burg, S. P. and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. 55:262-269.

19. Burg, S. P. and E. A. Burg. 1967. Auxin stimulated ethylene formation: Its relationship to auxin inhibited growth, root geotropism and other plant processes. In: F. Wightman and G. Setterfield, eds., Biochemistry and Physiology of Plant Growth Substances. Rung Press Ltd., Ottawa, Canada. pp.1275-1294.

20. Burg, S. P. and E. A. Burg. 1967. Inhibition of polar auxin transport by ethylene. Plant Physiol. 42:1224-1228.

21. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42:144-152.

22. 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.

23. Burg, S. P. and E. A. Burg. 1969. Interaction of ethylene, oxygen, and carbon dioxide in the control of fruit ripening. Qual. Plant. Mater. Veg. 14:185-200.

24. Burg, S. P. and M. J. Dijkman. 1967. Ethylene and auxin participation in the pollen induced fading of Vanda orchid blossoms. Plant Physiol. 42:1648-1650.

25. Chadwick, A. V. and D. A. Steen. 1973. Effects of deuterium oxide, low temperature and ethylene on pea growth. Plant Physiol. 51(Suppl.):31.

26. 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.

27. Chadwick, A. V. and S. P. Burg. 1970. Regulation of root growth by auxin-ethylene interaction. Plant Physiol. 45:192-200.

28. Craker, L. E., A. V. Chadwick, and G. R. Leather. 1970. Abscission: Movement and conjugation of auxin Plant Physiol. 45:790-793.

29. Craker, L. E., L. A. Standley, and M J. Starbuck. 1971. Ethylene control of anthocyanin synthesis in sorghum. Plant Physiol. 48:349-352.

30. Craker, L. E. and P. J. Wetherbee. 1973. Ethylene, light, and anthocyanin synthesis. Plant Physiol. 51: 436-438.

31. 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: 231-248.

32. Crocker, W., L. I. Knight and R. C. Rose. 1913. A delicate seedling test. Sci. 37:380.

33. Denny, F. E. 1935. Testing plant tissue for emanations causing epinasty. Contrib. Boyce Thompson Inst. 7:341-347.

34. Denny, F. E. and L. P. Miller. 1935. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. 7:97-102.

35. Edwards, M. E. and J. H. Miller. 1972. Growth regulation by ethylene in fern gametophytes II. Inhibition of cell division. Amer. J. Bot. 59:450-457.

36. Edwards, M. E. and J. H. Miller. 1972. Growth regulation by ethylene in fern gametophytes III. Inhibition of spore germination. Amer J. Bot. 59:458-465.

37. Eisinger, W. R. and S. P. Burg. 1972. Ethylene-induced pea internode swelling: Its relation to ribonucleic acid metabolism, wall protein synthesis, and cell wall structure. Plant Physiol. 50:510-517.

38. Elmer, O. H. 1932. Growth inhibition of potato sprouts by the volatile products of apples. Sci. 75:193.

39. Fahnstock, G. W. 1858. Proc. Acad. Nat. Sci. Phila. 1. (Cited in Burg, S. P. 1970. P. N. A. S. 70:591-597.)

40. Fuchs, Y. and E. Gertman. 1973. Stabilization of enzyme activity by incubation in an ethylene atmosphere. Plant and Cell Physiol. 14:197-199.

41. Green, P. 1962. Mechanism for plant cellular morphogenisis. Sci. 138:1404-1405.

42. Green, P. B. 1964. Cell walls and the geometry of plant growth. In: Meristems and differentiation. Brookhaven Symp. Biol. 16:203-217.

43. Hall, W. C., G. B. Truchelot, C. L. Leinweber, and F. A. Herrero. 1957. Ethylene production by the cotton plant and its effects under experimental and field conditions. Physiol. Plant. 10:306-317.

44. Holm, R. E. and F. B. Abeles. 1967. Abscission: The role of RNA synthesis. Plant Physiol. 42:1094-1102.

45. Holm, R. E. and F. B. Abeles. 1967. The role of ethylene in 2,4-D-induced growth inhibition. Planta 78:293-304.

46. Horton, R. F. and D. J. Osborn. 1967. Senescence, abscission, and cellulase activity in Phaseolus vulgaris. Nature 214:1086-1088.

47. Inoué, S. 1952. The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. Exptl. Cell Res. Suppl. 2:305-318.

48. Inoué, S. and H. Sato. 1967. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. J. Gen. Physiol. 50(Suppl.):259-292.

49. Kang, B. G. and S. P. Burg. 1971. Mechanism of auxin induced ethylene production. Plant Physiol. 47:504-509.

50. Kang, B. G. and S. P. Burg. 1973. Influence of ethylene on nucleic acid synthesis in etiolated Pisum sativum. Plant and Cell Physiol. 14:981-988.

51. Kang, B. G. and S. P. Burg. 1973. Role of ethylene in phytochrome-induced anthocyanin synthesis. Planta(Berl.)110:227-235.

52. Laan, P. A. van der. 1934. Effects of ethylene on growth hormone formation in Avena and Vica. Rec. Trav. Botan. Neerl. 31:691-742.

53. Ledbetter, M. C. and K. R. Porter. 1963. A microtubule in plant cell fine structure. J. Cell Biol. 19:239-250.

54. Maxie, E. C. and J. C. Crane. 1968. Effects of ethylene on growth and maturation of the fig Ficus carcia L. fruit. J. Amer. Soc. Hort. Sci. 92:255-267.

55. Morgan, P. W. 1967. Preliminary report in Proc. 64th South Agric. Workers Conf., pp 271-272.

56. Neljubow, D. 1901. Ueber die horizontale nutation der stengel von Pisum sativum und einiger anderen pflanzen. Botan. Centr. 10:128-139.

57. Newcomb, E. H. 1969. Plant Microtubules. Ann. Rev. Plant Physiol. 20:253-288.

58. Pratt, H. K. and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.

59. Sievers, A. F. and R. H. True. 1912. U. S. Dept. Agr. Bur. Plant Ind. Bull. 232:1-38.

60. Steen, D. A. and A. V. Chadwick. 1973. Effects of cycloheximide on indoleacetic acid-induced ethylene production in pea root tips. Plant Physiol. 52:171-173.

61. Veen, B. W. 1970. Control of plant cell shape by cell wall structure. Koninkl. Nederl. Akad. van Wet. (Amsterdam) 73:118-121.

62. Veen, B. W. 1970. Orientation of microfibrils in parenchyma cells of pea stem before and after longitudinal growth. Koninkl. Nederl. Akad. van Wet. (Amsterdam) 73:113-117.

63. Zimmerman, P. W. and A. E. Hitchcock. 1933. Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gasses. Contrib. Boyce Thompson Inst. 5:351-369.

64. Zimmerman, P. W. and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contr. Boyce Thompson Inst. 7:209-229.

LOMA LINDA UNIVERSITY

Graduate School

IMPLICATIONS FOR MICROTUBULE MEDIATED ETHYLENE EFFECTS IN PEA STEM SECTIONS

by

David S. Steen

An Abstract of

a Dissertation in Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the

Field of Biology

June 1974

ABSTRACT

The marked effects of ethylene on pea stem growth have been investigated. Low temperature and colchicine, both known microtubule depolymerization agents, reverse the effects of ethylene in straight growth tests. Low temperature (6 C) also profoundly reduces the effects of the gas in terms of swelling, hook curvature, and horizontal nutation. Electron microscopy shows that microtubules are reoriented after treatment with ethylene for as little as 12 hours. The findings indicate that some of the ethylene responses may be due to a stabilizing effect on microtubules in plant cells.