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OZONE AND VASCULAR TISSUE DIFFERENTIATION IN PLANTS

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OZONE AND VASCULAR TISSUE DIFFERENTIATION IN PLANTS

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ABSTRACT

The purpose of this research is to ascertain the possible influence of ozone on the process of vascular tissue differentiation in plants and the protein changes associated with it. Test materials were wounded plant internodes and callus tissues grown, exposed, and studied under laboratory conditions. Ozone was more effective in reducing xylem regeneration in those internodes grown in Indoleacetic acid than in Dichlorophenoxyacetic acid. Preliminary findings of the protein and enzyme patterns in callus tissues exposed to ozone suggest that it has an influence on them. It is concluded that plant internodes and callus tissues can be used to study the effects of ozone on certain processes related to plant growth and development.

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SECTION I

CONCLUSIONS

1. Ozone reduces xylem regeneration in wounded internodes.

2. The reduction in the number of new xylem elements formed around the wounds was greater following immediate rather than delayed exposure of the internodes to ozone.

3. Ozone-induced xylem regeneration was reduced more in internodes cultured in sucrose plus Indole-3-acetic acid (IAA) than in sucrose or 2, 4-Dichlorophenoxyacetic acid (2,4-D) plus sucrose.

4. Basal applications of equimolar concentrations of Indole-3-acetic acid (2 ppm) and 2, 4-Dichlorophenoxyacetic acid (2.5 ppm) does not induce a significant number of new xylem in wounded isolated internodes of Coleus.

5. Basal applications of either Indole-3-acetic acid or 2, 4-Dichlorophenoxyacetic acid in the presence of sucrose induces significant numbers of new xylem around the wounds of isolated Coleus internodes.

6. Regeneration of new xylem failed to occur in internodes cultured in glass distilled water.

7. Xylem regeneration occurred in internodes cultured in liquid media providing the proper constituents are added.

SECTION II

RECOMMENDATIONS

Along with the research on the role of oxidants in plant cell growth and plant vitality, it is recommended that more attention be given to cell and organ differentiation and the subtle biochemical changes associated with these processes.

The following lines of research should be followed in order to understand further the impact of ozone on vascular tissue differentiation in plants:

1. The effect of ozone on phloem differentiation
2. The effectiveness of growth regulators in the presence of ozone
3. The effect of ozone on cellulose and lignin production
4. The effect of ozone on plant membrane integrity
5. The effect of ozone on the quality and quantity of proteins and enzymes in growing and differentiating plant tissues.

SECTION III

INTRODUCTION

The purpose of this research was to determine whether plant tissues upon exposure to ozone will show alterations in the quality and quantity of vascular tissues produced in them when grown on a variety of media. Xylem regeneration around wounds was studied in ozonated and nonozonated internodes of Coleus blumei (Benth) grown on media containing 4% sucrose in combination with different growth regulators. Studies were also made to determine the effect of ozone on peroxidase and the total protein content in plant callus tissues from stems of Parthenocissus tricuspidata (veitchi) which were also grown on a variety of media. One basic assumption of this study was that the role of IAA in the biochemistry of xylogenesis could possibly be influenced by a strong oxidant such as ozone which is a common air pollutant. Ordin (1962) found that ozone did indeed inactivate IAA in cell elongation studies. Furthermore, studies with isolated internodes have shown that IAA influences differentiation and regeneration of xylem, Jacobs (1952, 1954) and of phloem, Lamotte and Jacobs (1963). Additionally, IAA in combination with sucrose enhances differentiation and regeneration in callus, Wetmore and Rier (1963) and Rier and Beslow (1967). A combination of sucrose and IAA stimulated more regeneration in isolated Coleus internodes than separate additions of either components, Beslow and Rier (1969). A logical extension of the assumption in the above process is that perhaps ozone could modify IAA-influenced xylogenesis. Furthermore, current information in regards to changes in

protein constituency and enzymic variations in relation to plant growth and development support an examination of them in these studies on ozone and xylogenesis.

This report will show that ozone does influence the degree of xylogenesis in internodes grown in media containing IAA and sucrose and that the substitution of 2,4-D for IAA has a mitigating effect on the action of ozone in this process.

Experiments were done to determine the protein pattern of ozonated and non-ozonated callus from stems of Boston Ivy, Parthenocissus tricuspidata (var. veitchi) which was grown on a maintenance medium containing 1.5% sucrose and 0.1 ppm IAA, Wetmore and Rier (1963). Variations in the medium were made by increasing the IAA concentration to 2 ppm, or by substituting an equimolar concentration of 2,4-D (2.4 ppm) for IAA. Previous studies have shown that modifications of the medium influenced xylogenesis. Changes in sucrose concentration will also influence the quantities of xylem in these tissues, Rier and Beslow (1967). Furthermore, it has been shown in the present report that ozone does have an effect on xylogenesis. It was thought that the protein patterns and perhaps some of the enzymic systems have been correlated with morphogenesis in plants and plant parts, Frenkel and Hess (1974), Juo and Stotzky (1970), and Leshem et al (1970).

SECTION IV

MATERIALS AND METHODS

ASSEMBLY OF OZONATION APPARATUS

The ozone generator (Welsbach Ozonator - Model T 408) used in these experiments was appropriately connected to a cold water source for cooling, Aviator's Breathing oxygen, and an exposure chamber. The flow of oxygen from a cylinder was controlled by a regulator on the tank and by a pressure valve on the generator. Oxygen was allowed to flow into the ozonator until a steady rate could be maintained. Subsequently, the voltage on the ozonator was adjusted to give an ozone concentration of 50 pphm, as measured by a Welsbach Ozone Meter, Model H-100 LC, and recorded on a Modified Strip Chart Recorder. The excess ozone was passed into a KI solution where it was reconverted into oxygen for disposal.

WOUNDING OF INTERNODES

Cuttings were taken from Coleus and placed in perlite. Roots developed on them within 5 to 7 days and they were subsequently potted in soil. All plants were watered daily for one month and fertilized once weekly. Lateral shoots were removed as soon as they appeared. At the end of the growth period, the apical half of the plant was removed, washed in 10% chlorox, rinsed in three changes of sterile distilled water, and debladed. The second internode was removed and wounded by cutting a V-notch in one corner in such a manner as to sever a major vascular bundle. The apical end of

the segment was cut at an angle for identification.

After wounding, ten segments were placed in petri dishes containing various media and held in an upright position with polyethylene sponge discs. The aqueous media contained one of the following: (1) 2 ppm IAA, (2) 2.4 ppm 2,4-D, (3) 4% sucrose, (4) 2 ppm IAA plus 4% sucrose, and (5) 2.4 ppm 2,4-D plus 4% sucrose. Internodes were cultured in glass distilled water for comparison. All internodes were cultured under room conditions. A total of 2160 internodes were grown and used in this study.

OZONATION AND STAINING

A schedule of the culture and ozonation of the internodes were made to reveal the degree of xylogenesis under the following conditions:

A. Ozone exposures were made at the noon hour. (It has been determined that perhaps the ozone level of the atmosphere is highest during the noon hours, Heggestad and Middleton, 1959).

B. Immediately upon wounding, internodes were exposed to ozone at 50 pphm for one hour daily for one week and killed.

B_1. Control internodes were grown in the absence of ozone for one week and killed.

C. Internodes were cultured as in B for one week in the presence of ozone at 50 pphm for one hour daily and for an additional week in the absence of ozone prior to killing.

D. Internodes were grown for two weeks. During the first week they received no ozone. During the second week they were exposed to ozone at 50 pphm for one hour daily.

E. Segments were ozonated for the entire two week culture period.

F. Conditions C_1, D_1, and E_1 were controls in which the internodes were grown in the absence of ozone for two weeks.

Cultures under the above conditions were grown in each of the media listed above.

Preparation of materials for histological examination followed the procedure established by Fuchs (1963). The segments were killed and fixed in formalin acetyl alcohol (FAA), hydrated, and stained in fuchsin stain at 60°C for 10 to 14 hours. The staining solution contained 10 grams of sodium hydroxide in 100 ml of distilled water and 1 gram of basic fuchsin. After staining the segments were placed in several changes of tap water and taken through dehydration to xylene. Each segment was cut longitudinally at the corner immediately behind the wounded vascular bundle and placed on the slide, wounded side facing up. This was done so that the entire wound area was visible for microscopic examination.

With the aid of a microscope and a tally denominator, counts of newly regenerated xylem vessels were made in the wound area between two major vascular bundles and to a distance approximately 2.0 to 2.5 mm above and below the wound. Computations were made in regards to the number of vessels regenerated in relation to the experimental conditions. Statistical means and standard deviations were obtained and t-tests were run to determine the significance between the controls and experimental treatments. Graphs were made of these calculations.

CULTURE AND OZONATION OF CALLUS TISSUES

Subcultures were made of stem callus tissue stocks of Parthenocissus tricuspidata var. veitchi that were grown on a maintenance medium. The experimental media consisted of Kaden's media plus 1.5% sucrose and 2 ppm IAA and 4% sucrose and 2 ppm IAA (Rier and Beslow). They were placed in 2 ounce square bottles with screw caps. Cultures were maintained at 25°C with a photoperiod of 8 hours light and 16 hours darkness.

Following an adjustment period of 24 hours in the incubator, tissues were selected and exposed to ozone. Bottles containing tissues were surfaced sterilized with 70% ethyl alcohol and placed in sterile chamber for ozonation. Their caps were carefully removed and placed in the chamber where they remained during ozonation. The cultures received 50 pphm ozone for three hours once each of four successive weeks and returned to the incubator following each treatment. Control tissues remained in the incubator throughout the experimental period of six weeks.

EXTRACTION OF PROTEIN FROM CALLUS TISSUE

At the end of the growth period the ozonated tissues and controls were removed from the bottles and washed with distilled water. Approximately 10 grams of the tissue were placed in the refrigerator and allowed to cool. Extraction of protein and disc electrophoresis followed the methods of Caponetti (personal communication) with modifications. Tissue was ground in a prechilled mortar which was kept cold on a stirrer cooler

(Model SK 12 manufactured by Thermoelectrics Unlimited, Inc.). Proteins were extracted with 6 ml of 0.1M Hepes buffer at pH 7.4, 8 drops of Cleland's reagent or dithiothreitol (Dtt), and 1 gram of polyvinylpyrrolidone (PVP-AT) powder per 10 grams of tissue at a temperature of minus 2°C. After extraction the tissue was forced through 4 layers of cheesecloth, previously soaked in Hepes buffer. The strainate was centrifuged twice at 4°C at 20,000 x g for 30 minutes each. The supernatant was eluted from a 15 x 1.5 cm column packed with Sephadex G50.

The Sephadex beads were previously soaked in distilled water for 3 days. The column was flushed with the eluant, 0.05M Tris-HCl buffer, pH 8.0 to remove the water and allowed to settle for 12 to 24 hours. Approximately 100 ml of the extract and eluant was collected and dialyzed against several changes of 0.05M Tris-Glycine buffer, pH 8.3, for 12 hours. The dialyzate was concentrated by rolling in dry Aquacide II powder. The final volume of 1 or 2 ml was placed in small vials in the refrigerator.

ELECTROPHORESIS OF EXTRACTS

The apparatus used for disc electrophoresis and the power supply were manufactured by Buchler Instruments. A volume of 0.35 ml of the extract was applied to gels for separation. The gels were made in tubes 13 cm x 1/2 cm. The separation gel was 8 cm long with a pH of 8.9 and a pore size of 13 percent. The stacking gel was 2 cm long with a pH of 5.5 and a pore size of 4.5 percent. The extract was placed on the stacking gel and carefully layered with resevoir buffer, 0.01M Tris-Glycine, pH 8.3.

A tracking or indicator dye, bromophenol blue, was placed in one of the tubes prior to adding buffer. Electrophoresis was done in the cold at about $4^{o}C$ and run at 3 mA per tube.

STAINING FOR PROTEINS AND PEROXIDASE

Gels were removed from the tubes and stained either for total protein with 0.1% Amido Schwartz overnite and destained with several changes of 7% acetic acid or were stained for peroxidase. The peroxidase stain (Yoneda and Endo, 1969) contained equal volumes of:

0.5M Na-Acetate buffer pH 4.0

0.1% Benzidine HCl

0.3% H_2O_2

The sodium acetate buffer was substituted for Tris-acetic acid buffer used in this study.

All gels were compared for similarity or differences in the banding pattern for total protein and peroxidase of callus tissue grown on the various media in the presence or absence of ozone.

SECTION V

DISCUSSION

XYLEM REGENERATION IN INTERNODES

Regeneration of xylem elements was negligible in segments cultured in aqueous media containing IAA or 2,4-D. The additions of sucrose to each medium increased the amount of regeneration. Medium containing sucrose and distilled water produced a minimal response (Fig. 1). Xylem regeneration was increased three-fold by a combination of sucrose and IAA (Fig. 2) and about twelve-fold by a combination of sucrose and 2,4-D (Fig. 3).

Ozone effectively reduced the amount of xylem regeneration in Coleus segments when cultured in either of two media, sucrose or sucrose plus IAA (Fig. 4). Suppression of the regenerative response was greatest when the segments were cultured in these two media and exposed to ozone immediately after wounding. For instance, regeneration in segments grown under conditions A of the culture schedule was reduced by 34% when grown in sucrose and by 46% when they were grown in sucrose plus IAA. Under condition C, regeneration was reduced by 26% when the segments were grown in sucrose and 65% when they were grown in sucrose plus IAA. When segments were exposed to ozone for two weeks, condition E, regeneration was reduced by 22% in internodes cultured in sucrose and 56% in those cultured in sucrose plus IAA. If cultures of internodes were initiated and grown in sucrose for 1 week without ozonation and exposed to ozone during

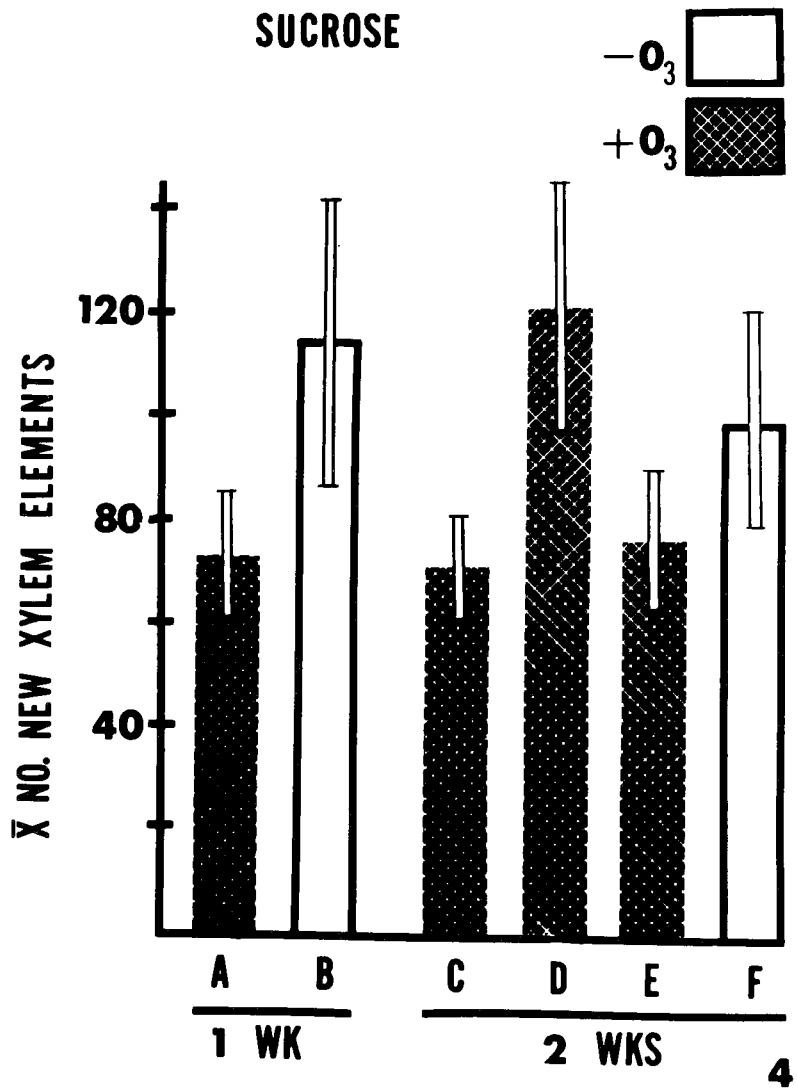

Fig. 1. The mean number of xylem elements regenerating in internodes cultured in 4% sucrose in response to conditions of exposure to ozone. Vertical bars (inserts) represent standard error.

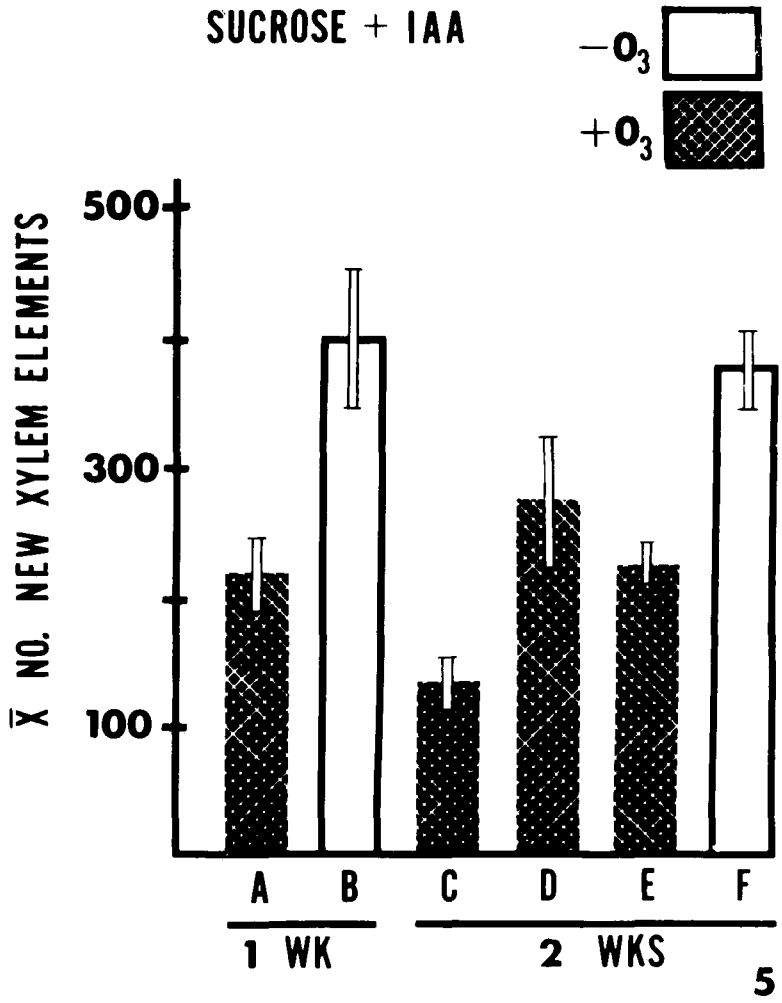

Fig. 2. The mean number of xylem elements regenerating in internodes cultured in 4% sucrose plus 2 ppm IAA and the affect of varying conditions of ozone exposure. Vertical bars (inserts) represent standard error.

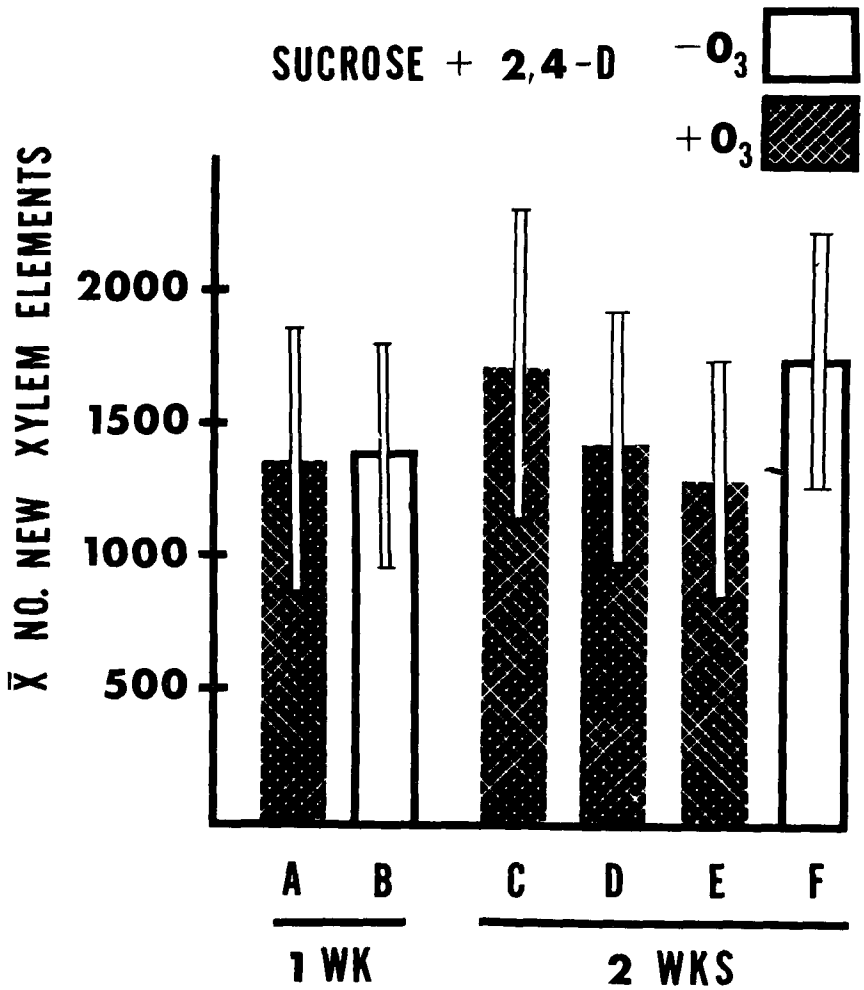

Fig. 3. The mean number of new xylem elements regenerating in internodes cultured in 4% sucrose plus 2.5 ppm 2,4-D under varying conditions of exposure to ozone. Vertical bars (inserts) represent standard error.

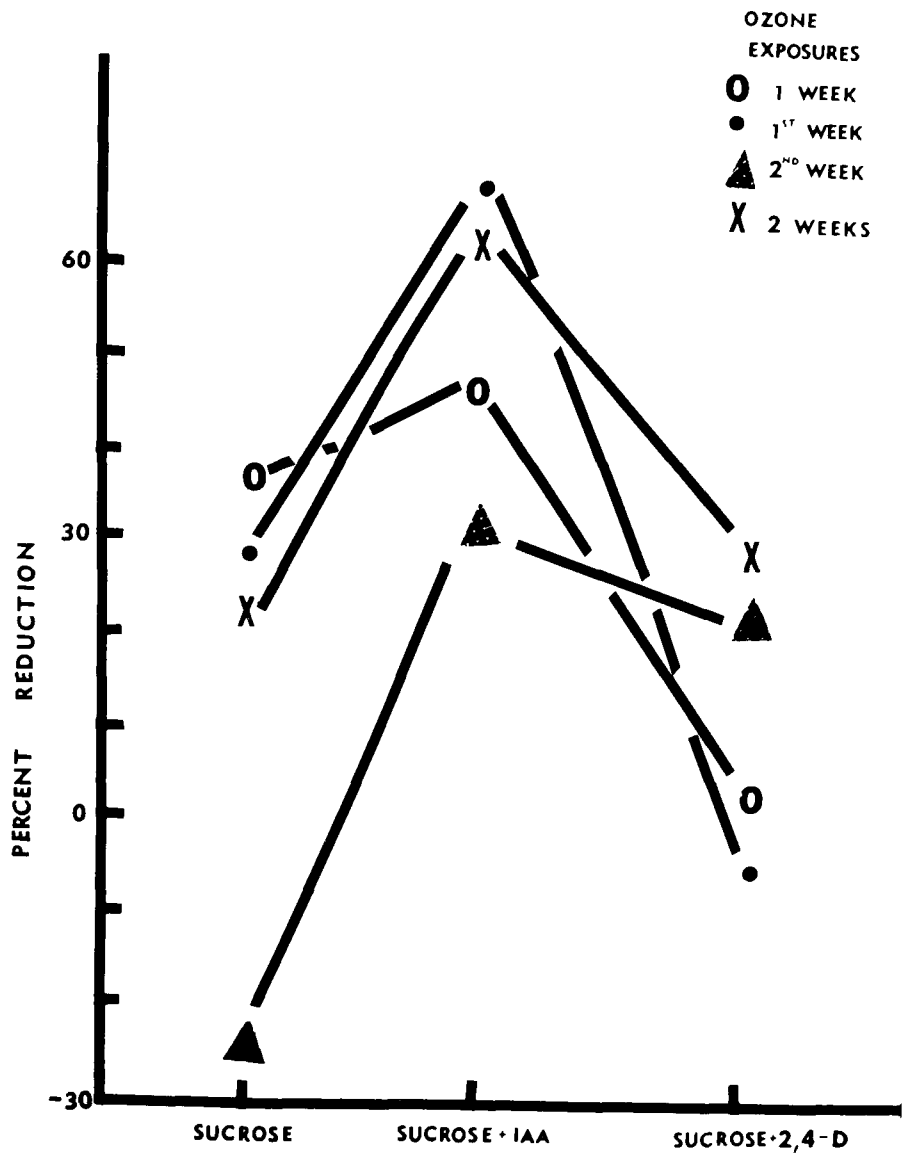

Fig. 4. Percent of reduction in the regeneration of xylem elements for each exposure period. Internodes were cultured in sucrose, sucrose plus IAA, or sucrose plus 2,4-D. Negative values represent increases.

the second week as in condition D, there was no reduction in regeneration. There was actually a 26% increase in regeneration. Under these same conditions (D) if they were grown in the sucrose plus IAA medium, regeneration was reduced by 30 percent.

Suppression of regeneration was not as significant in segments which were cultured in the medium containing sucrose and 2, 4-D regardless of the timing of the ozone exposure (Fig. 4). For example, in those segments treated under exposure condition B, regeneration was reduced by 1.5 percent. There was a 7% increase in regeneration in segments receiving treatment C and a 17% decrease in those receiving treatment D (exposure to ozone was during the second week of growth). When the segments received treatment E (exposure was for two weeks) regeneration was reduced by 21 percent. There was little difference in the number of xylem elements regenerated in segments in culture for 1 or 2 weeks in the absence of ozone regardless of the composition of the media.

Growth and development in plants is dependent upon cell division, cell enlargement, and cytodifferentiation. A delay or alteration in either of these processes will ultimately change the structure and physiology of the plant. Studies have shown that ozone will indeed influence cell enlargement, Ordin (1962). Information is scant in regards to its effect in cytodifferentiation or tissue differentiation. Fully and properly differentiated cells and tissues in plants maximize their effectiveness in competing with the environment and functioning efficiently.

Specifically, the vascular tissues, xylem and phloem, are the paramount conductors of nutrients and water in plants. Reduction in the amount of these tissues could reduce the capacity of the plant to produce fruit, wood and other products.

There are many reports of the killing effect of ozone on plant life, Heggestad and Middleton (1959), Ledbetter, et al. (1960), Hill, et al. (1961) among others. The issue in this research, however, raises a more insiduous question. What are the effects of ozone on plant life when from all appearances it is surviving and healthy? The work in this report suggests that possibly some damage is done to the biochemical systems for xylem differentiation. The ultimate test for this will have to come in the whole intact plant.

The effect of ozone on the processes of xylem regeneration and the suppression of this affect by 2,4-D is possibly related to chemical and physical changes in the plant. These changes involve membrane permeability changes caused by ozone and IAA, the transport of auxins and sucrose, cell division, processes of differentiation, and the affect of the polarity of the auxins involved.

OZONE ENHANCEMENT OF MEMBRANE PERMEABILITY

Membrane permeability changes caused by ozone can affect the rate of regeneration. Evans and Ting (1973) observed increases in the permeability of water and solutes in leaf tissues exposed to ozone. Such enhanced permeability could account for the uptake of ozone which would increase the peroxidation of lipids in membranes and decrease

fatty acid synthesis, Tomlinson and Rich (1969, 1971). This decrease in lipid biosynthesis resulted from the relatively easy oxidation of sulfhydryl groups by ozone, Tomlinson and Rich (1967, 1968, 1969, 1970a, 1970b, 1971) and Treashow et al. (1969). The extent to which ozone caused such changes in the membrane of parenchyma cells of Coleus was probably minimal unless the gas was absorbed along with components of the media. Whatever changes in the membranes did occur probably affected the rate of diffusion of auxin as well as sucrose resulting in reduced regeneration. The diffusion of 2,4-D and sucrose was apparently not affected by changes in permeability since regeneration was only slightly reduced in cultures containing these substances.

REDUCTION OF CELL DIVISION BY OZONE

Another manner in which ozone influences regeneration is by interrupting the processes of cell division which leads to a reduction in the number of cells ordinarily undergoing division. The manner in which ozone could have interrupted these processes is by oxidizing nucleic acids and proteins. Davis (1959) demonstrated the sensitivity of the bases of nucleic acids to ozone. He found that thymidine was more sensitive to ozone than cytosine or uracil. This shows that ozone also penetrates nuclear membranes. Oxidations of nucleic acids would limit the synthesis of proteins but the protein molecules can also be oxidized. In fact, increases in free pool amino acids of leaves exposed to ozone suggested that such oxidations readily occur, Tomlinson and Rich (1967a), Lee (1968), Ting and Mukerji (1971), and Craker and Starbuck (1972). Additionally, Mudd et al. (1969) demonstrated the sensitivity of amino acids to ozone,

cysteine being more sensitive than others. This amino acid accumulates in excess in the walls of parenchyma cells undergoing meristematic activity and in the walls of new xylem elements, Rier and Beslow (1967). Its oxidation by ozone could greatly limit differentiation and reduce the regeneration of new xylem. Since cell division is a necessary prerequisite of differentiation, Fosket (1968), reduction in the amount of xylem in Coleus internodes may not necessarily have been caused by extensive oxidation of cysteine but such oxidations could greatly limit the number of parenchyma cells which differentiate directly into new wound vessel members. The lack of pronounced reduction in segments cultured in 2,4-D plus sucrose is probably due to the fact that the initiation of the regenerative process by 2,4-D was too rapid and had proceeded to a level not readily affected by ozone by the time of exposure. Sucrose and 2,4-D had previously negated the effect of smog, which contained ozone and hexene, in reducing growth of coleoptile segments. This shows that at least the processes of cell division and elongation was not affected by ozone, Koritz and Went (1953).

OXIDATION OF LIGNIN FORMING ENZYMES BY OZONE

Ozone can interfere with the processes of lignin formation, a process which is dependent upon auxins in the differentiation of new xylem. For instance, the auxin activated peroxidase enzyme, which stimulates lignin formation has been shown to be inactivated by ozone. It has been successfully inactivated in vitro, Todd (1958) but not in vivo, Dass (1972). In Dass's study, electrophoretic peroxidase actually increased, by one band, in bean leaves exposed to ozone. Another enzyme, one involved in cell wall

synthesis, phosphoglucomutase, is also inactivated in vitro but not in vivo, Ordin (1965), by ozone. These unsuccessful in vivo oxidations show that for ozone to be greatly effective in reducing lignin formation, it would have to penetrate the cells. These authors did not observe such penetration. Extensive reduction in the number of lignified elements was not observed in Coleus nor in maple seedlings treated with 2 to 3 ppb ozone when tested for changes in lignin content following exposure, Hibben (1969).

OZONE AFFECT ON THE INCORPORATION OF SUCROSE INTO CELLULOSE

Ordin and Skoe (1964) showed that ozone caused increased uptake of labelled glucose by isolated coleoptile segments but incorporation of the label into cell wall components was considerably reduced. They attributed this reduction to the sensitivity of glucose pathways leading to cell wall formation to ozone. Glucose pathways leading to cellulose formation were also found to be sensitive to ozone. This was indicated by pronounced inhibition of cellulose synthesis. In this manner, ozone reduced these normally rapidly elongating segments. Ozone could have similarly inhibited the synthesis of cellulose in cells elongating in the wound area of Coleus internodes. Such inhibition could prevent differentiation in a number of cells resulting in reduced regeneration. Reduction in the number of elements in segments grown in sucrose show that ozone could have affected the utilization of sucrose in a manner similar to the change in the utilization of the labelled glucose. In addition to reductions caused by sucrose alone, reduction in cellulose and other processes of xylem regeneration could also result from changes in the

utilization of IAA.

OZONE INACTIVATION OF IAA

The most probable factor responsible for reduction of xylem regeneration in Coleus segments was inactivation of the IAA molecule. Ordin and Propst (1962) observed complete inactivation of IAA within one hour of ozone treatment and found that the inactivation was caused by breaks in the indole ring part of the molecule. Inactivation of IAA by ozone is more responsible than permeability changes in reducing regeneration because many of the processes involved in regeneration are initiated by auxin. Reduction in the amount of IAA, due to inactivation by ozone would subsequently reduce the activity of these processes, cell division, lignin and cellulose formation. Since an hour was required for complete inactivation of the molecule, many of the remaining active molecules probably acted with sucrose to stimulate regeneration regardless of ozone treatment. If this were not so, the amount of regeneration in internodes grown in the presence of sucrose, IAA, and ozone would be comparable to that in sucrose alone Such similarity was never observed. Reduction in regeneration was more pronounced in segments grown in sucrose and IAA than in any other media because ozone could have affected the utilization of both materials. Minimal reductions in regeneration in segments grown in media containing 2, 4-D and sucrose indicate that 2, 4-D was not inactivated by the ozone.

REDUCTIONS IN REGENERATION CAUSED BY THE RATE OF DIFFUSION OF IAA OR 2,4-D

The protective effects of 2,4-D and sucrose together against reductions in xylem regeneration can also be due to the increased rate of absorption and translocation of 2,4-D over IAA. Hill (1965) showed how the difference between rates of translocation of the two auxins affected the production of callus on isolated potato stems. Low concentrations of 2,4-D, 0.5 ppm or less, produced callus only at the top of the stem away from the medium. Higher concentrations induced callus over the entire stem. On the other hand, IAA, regardless of concentration, produced callus only on the end of the stem closest to the medium whether the stem was in the normal upright or inverted position. This shows that IAA moved only in its established acropetal manner, a very slow movement, and that 2,4-D moved in a very rapid acropetal fashion. This type of polarity was expressed in Coleus internodes. That 2,4-D was translocated more extensively into the apical region than IAA was noted by the appearance of newly differentiated xylem close to the apex of the stem. New xylem did not regenerate to such a height in internodes cultured in IAA plus sucrose. Rapid translocation of the auxin provided a means of establishing the processes of xylem regeneration prior to exposure to ozone. Slow diffusion of IAA allows more of it to become inactivated by ozone which decreases the amount of xylem regeneration.

There is an apparent interaction between ozone, IAA, sucrose, and 2,4-D. These interactions, though not fully understood, can modify, quantitatively, the amount of

xylem regeneration around wounded Coleus internodes. The rates of translocation of auxins and sucrose may be significant factors influencing the amount of xylem regeneration in wounded internodes and the inactivation if IAA may have been the major factor reducing xylem regeneration in this study.

PROTEIN PATTERNS IN OZONATED CALLUS TISSUES

Preliminary findings suggests that bands for proteins and peroxidase from callus tissue can be visualized in acrylamide gel electrophoresis. This is the first report of its kind on electrophoresis of ozonated callus tissue. The present assessment of the banding pattern and number of bands suggests that variation exists as a function of media composition and ozone exposure. A tendency for variation exists in the pattern and number of bands in cultures containing IAA rather than in those containing 2,4-D. Given the findings of several workers on studies made to correlate protein composition and enzymic patterns with growth and development, research would be fruitful in regards to ozone and its effects on growth and development. A callus tissue system is perhaps a good one for studying the effects of ozone on differentiation.

SECTION VI

REFERENCES

Beslow, D. T. and J. P. Rier. Sucrose Concentration and Xylem Regeneration in Coleus Internodes in vitro. Plant and Cell Physiol. 10:69-77, 1969.

Caponetti, James D., Personal Communication. Department of Botany, University of Tennessee, Knoxville, Tennessee, 1973.

Craker, L. E. and T. S. Starbuck. Metabolic Changes Associated with Ozone Injury to Bean Leaves. Can. J. Plant Sci. 52:589-597, 1972.

Dass, H. C. Enzymatic Changes in Intact Leaves of Phaseolus vulgaris Following Ozone Fumigation. Atmos. Environ. 6:759-763, 1972.

Davis, I. The Survival and Mutability of Escherichia coli in Aqueous Solutions of Ozone. Ph. D. Thesis, University of Pennsylvania Medical School, Philadelphia. 1959.

Evans, L. S. and I. P. Ting. Ozone Induced Membrane Permeability Changes. Amer. J. Bot. 60:155-162, 1973.

Fosket, D. E. Cell Division and the Differentiation of Wound Vessel Members in Cultured Stem Segments of Coleus. Proceedings of the National Academy of Sciences, 59:1081-1096, 1968.

Frenkel, C. and C. E. Hess. Isozymic Changes in Relation to Root Initiation in Mung Bean. Can. J. Bot. 52:295-297, 1974.

Fuchs, C. Fuchsin Staining with NaOH Clearing for Lignified Elements of Whole Plants or Plant Organs. Stain Technol. 38:141-144, 1963.

Heggestad, H. E. and J. T. Middleton. Ozone in High Concentration as a Cause of Tobacco Leaf Injury. Sci. 129:208-210, 1959.

Hibben, G. R. Sugar Maple - Ozone Toxicity. Phytopath. 59:1423-1427, 1969.

Hill, R. A. Polarity Studies in Roots of Ipomea batatas in vitro. Master's Thesis, Howard University, Washington, D. C. 1965.

Jacobs, W. P. The Role of Auxin in the Differentiation of Xylem Around a Wound. Amer. J. Bot. 39:301-309, 1952.

Jacobs, W. P. Acropetal Auxin Transport and Xylem Regeneration - A Quantiative Study. Amer. Nat. 88:327-337, 1954.

Juo, P. and G. Stotzky. Changes in Protein Spectra of Bean Seed During Germination. Can. J. Bot. 48:1347-1350, 1970.

Koritz, H. G. and F. W. Went. The Physiological Action of Smog on Plants. I. Initial Growth and Transpiration Studies. Plant Physiol. 28:50-62, 1953.

Lamotte, C. and W. P. Jacobs. A Role of Auxin in Phloem Regeneration in Coleus Internodes. Develop. Biol. 8:80-98, 1963.

Lee, T. T. Effect of Ozone on Swelling of Tobacco Mitochondria. Plant Physiol. 43:133-139, 1968.

Leshem, Y., A. W. Galston, R. Kaur-Sawhney, and L. M. Shih. Auxin Macromolecular Repressors and the Development of Isoperoxidases in Cultured Tobacco Pith. In: Plant Growth Substances 1970, Carr, D. J. (ed.). New York, Springer-Verlag Publishers, 1972. pp. 228-233.

Mudd, J. B., R. Leavitt, A. Ongun, and T. T. McManus. Reactions of Ozone with Amino Acids and Proteins. Atmos. Environ. 3:699-682, 1969.

Ordin, L. and A. Altman. Inhibition of Phosphoglucomutase Activity in Oat Coleoptiles by Air Pollutants. Physiol. Planatarum. 18:790-797, 1965.

Ordin, L. and B. Propst. Effect of Airborne Oxidants on Biological Activity of Indoleacetic Acid. Bot. Gaz. 123:170-175, 1962.

Ordin, L. and B. P. Skoe. Ozone Effects on Cell Wall Metabolism of Avena Coleoptile Sections. Plant Physiol. 39:751-753, 1964.

Rier, J. P. and D. T. Beslow. The Incorporation of Cysteine ^{35}S in Callus Tissue of Parthenocissus tricuspidata. Plant and Cell Physiol. 8:799-781, 1967.

Rier, J. P. and D. T. Beslow. Sucrose Concentration and the Differentiation of Xylem in Callus. Bot. Gaz. 128:73-77, 1967.

Ting, I. P. and S. K. Mukerji. Leaf Ontogeny as a Factor in Susceptibility to Ozone: Amino Acid and Carbohydrate Changes During Expansion. Amer. J. Bot. 58:497-504, 1971.

Todd, G. W. Effects of Low Concentrations of Ozone on the Enzymes Catalase, Peroxidase, Papain, and Urease. Physiol. Planturum. 11:457-463, 1958.

Tomlinson, H. and S. Rich. A Comparison of the Effects of Ozone and Sulfhydryl Reagents on Plants. Phytopathol. 57:834. (Abstr.) 1967.

Tomlinson, H. and S. Rich. The Ozone Resistance of Leaves as Related to their Sulfhydryl and Adenosine Triphosphate Content. Phytopathol. 58:808-810, 1968.

Tomlinson, H. and S. Rich. Relating Lipid Content and Fatty Acid Synthesis to Ozone Injury of Tobacco Leaves. Phytopathol. 59:1284-1287, 1969.

Tomlinson, H. and S. Rich. Disulfides in Bean Leaves Exposed to Ozone. Phytopathol. Notes. 60:1842-1843, 1970.

Tomlinson, H. and S. Rich. Lipid Peroxidation, a Result of Injury in Bean Leaves Exposed to Ozone. Phytopathol. 60:1531-1532, 1970.

Tomlinson, H. and S. Rich. Effects of Ozone on Sterols and Sterol Derivatives in Bean Leaves. Phytopathol. 61:1404-1405, 1971.

Wetmore, R. H. and J. P. Rier. Experimental Induction of Vascular Tissues in Callus of Angiosperms. Amer. J. Bot. 50:418-430, 1963.

Yoneda, Y. and T. Endo. Effects of Low Concentration of Hydrogen Peroxide on Indoleacetic Oxidase Zymogram in Pharbitis nil. Plant and Cell Physiol. 10: 235-237, 1969.

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16. ABSTRACT

This study is concerned with the influence of ozone on the process of vascular tissue differentiation in plants and the concomitant changes in plant proteins. The test materials consisted of wounded plant intermodes and callus tissues grown, exposed, and studied under controlled laboratory conditions. Ozone was more effective in reducing xylem regeneration in those internodes grown with indole-3-acetic acid than with 2, 4-dichlorophenoxyacetic acid. From the results, it was concluded that plant internodes and callus tissues can be used to study the effects of ozoen on certain processes related to plant growth and development.

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