VARIATION IN THE PHYSIOLOGICAL PROCESSES OF EASTERN WHITE PINE (*PINUS STROBUS* L.) DIFFERING IN SENSITIVITY TO OZONE, SULFUR DIOXIDE, AND NITROGEN DIOXIDE

BY

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INTRODUCTION

Along with the development of civilization and industrialization, man has continuously released various waste materials into his surrounding environments. However, only recently has he ever became aware of air pollution problem and its feedback threat to his future. Due to world weather patterns and other physical meterological factors, air pollution can no longer be treated as a local problem. It is an immediate problem man faced in common without the limitation of political boundaries.

Every entity of this planet is subjected to air exposure. The quality of air environment should then be considered very crucial to man, all flora fauna, and materials. From an economic point of view, air pollution has caused production reduction of vegetation, and deterioration of animal health and natural beauty. Between the conflicts of modern industrialization and clean beautiful air environments, we must make decisions which are guided by long range allowable air quality standards to settle this dispute. The goals of this compromise can only be achieved through a wide range of research and knowledge on the effects of pollutants on vegetation, animals, and materials.

Vegetation is generally considered to be more sensitive to air pollution than animals. Striking plant sensitivity to pollutants and remarkable individual variation have been

reported in certain species. There are several advantages on the study of pollutant effects on plants over the use of instrumental monitoring of pollutants. Because such research are 1) economically feasible, 2) providing a direct information of pollutant effects on biological system, 3) requiring less maintenance and attendance, 4) by using native plant species, field injury data could be readily available in any part of the world, and 5) when applying to perennial tree species, a pollution history in that region can be recorded and studied through the works of dendrochromatology.

Macroscopic visible symptoms on injured leaf surfaces have served as important criteria in the past in the identification and evaluation of pollution injury on plants. Recently, an increased awareness of subtle plant physiological or biochemical responses to pollution stress has developed. This new field of physiological studies have provided a better understanding of various aspects of pollutant toxic effects on vegetation; such as the discovery and proof of hidden injury, the revelation of different modes of action by different pollutants, and the comprehension of various pollutant mechanisms in plants.

The complexity of field pollutant-dose-receptor-response relationship is gradually disclosed as more constituents of polluted air have been found, various combination ratios among these components in a given meteorological condition have been detected, and great variations of plant response

have been determined due to the changes of environmental factor. The use of genetically identical plant materials in environment-controlled pollutant fumigations minimizes the variation of plant response that resulted from the fluctuation of environmental factors and/or the heterogenecity of plant materials.

The purpose of this study was to investigate the response of ten eastern white pine (Pinus strobus L.) clones differing in pollutant sensitivity under simulated pollutant exposures and compare the data with field observations. This study also attempted to discern the inherent differences of these clones to three commonly existing gasous pollutants, i.e. ozone, sulfur dioxide, and nitrogen dioxide, at various concentrations and combinations with respect to photosynthetic, transpiratory, and respiratory rate, chlorophyll content, degree of foliar injury, needle growth, and yield. Holistic disease concept was emphasized in this study to demonstrate the interdependence of various plant responses and the importance of this concept in the determination of plant sensitivity rankings to air pollutants.

The specific objectives of this research were by using genetical identical ramets from 10 eastern white pine clones to 1) continue the determination of their relative sensitivity to the designated pollutants used singly and in combination, 2) observe the influence of long-term low dose pollutant fumigation on clonal growth, 3) determine the influence

of air pollutant fumigation on current year needle chlorophyll contents, and 4) investigate the photosynthetic and respiratory rates of these clones as influenced by ozone, sulfur dioxide, and nitrogen dioxide.

LITERATURE REVIEW

Ever since Haagen-Smit et al. (1952) and Richards et al. (1958) discovered that various anthropogenic air components in the Los Angeles basin were phytotoxic to plant growth in the 1950's, a broad range of research interest has been stimulated on pollution-induced plant diseases. In this review, only ozone, sulfur dioxide, and nitrogen dioxide related studies will be included.

Major Sources of Gaseous Pollutants

Ozone has long been recognized as the most widely distributed air pollutant in the United States (National Science Academy, 1978; Heggestad, 1969). The existence of hydrocarbons, primarily from automobile exhaust, in the ambient air has been shown to disrupt natural photochemical cyclic reaction and raise ozone concentratins from 0.02-0.04 parts per million (ppm) characteristic of natural background concentration to 0.15-0.25 ppm in many areas (Stephens, 1969; Rasmussen and Went, 1965; Leighton, 1961).

Sulfur dioxide is one of the most notorious man-made pollutants to cause extensive damage on vegetation, properties, and health. The major source of sulfur dioxide is the combustion of sulfur-bearing fuel oil or coal by various power generating plants, industrial facilities, and building heating processes (Lacasse and Treshow, 1978). The natural background of sulfur dioxide concentration is between 0.002

ppm to 0.008 ppm. In large cities, sulfur dioxide concentration averages of 0.20 to 0.30 ppm have been monitored and have been reported to reach 0.50-0.60 ppm over a period of hours. In some European cities, such as London or the Ruhr district of Germany, sulfur dioxide concentrations as high as 1.40 ppm to 1.50 ppm for short period of time has been recorded (Mudd and Kozlowski, 1975).

There are several oxides of nitrogen found in polluted air. They are occurred primarily through processes that involved high temperature combustion of fossil fuels and organic wastes (Wood, 1968). Oxides of nitrogen play a key role in photochemical oxidant formation under favor conditions (Spicer, 1977). The natural background of nitrogen oxides has been less than 0.1 ppm but occasionally it has exceeded 1.0 ppm as has been recorded in urban or industrial areas (Anon, 1962). The highest concentration of nitrogen dioxide + nitric oxide in combination has been reported to reach to 3.93 ppm in Los Angeles area for a short period of time (Anon, 1962).

Visible Injury of Air-Pollutant-Induced Plant Diseases

Air pollution causes various adverse effects on plants. Visible injury on plant surfaces is one of the most easily observed manifestations. Traditionally, visible injury has been classified as chronic or acute. Chronic injury has usually been considered to result from prolonged, low concentra-

tion pollutant exposures and has been used to describe the symptoms predominantly considered as a result of destruction of chlorophyll and/or chlorophyll synthesis over a long period of time (Thomson et al., 1974). Cell death is not involved in this catagory (Heck and Brandt, 1977; Smith, 1974; Taylor, 1968).

Chronic ozone injury on broad-leaf plants has been observed as stippling, yellowing or bronzing of leaf surfaces, premature defoliation, and in some cases a general unthrifty appearance of entire plant which resulted in reduced growth and yield. Chronic ozone induced injury to conifers has been described as chlorotic mottle with white, yellow to tan coloration and loss of all but the current year's needles causing trees to appeared tuft (USEPA,1976; Heggestad and Middleton, 1959; Rich, 1964; Miller, 1973).

Chronic sulfur dioxide injury on broad-leaf plants has generally been reported as shining water-soaking on leaf surfaces, foliar chlorosis or bleaching, interveinal discoloration, mottling, and reduced growth. Chronic sulfur dioxide symptoms on coniferous trees has been reported to be similar to those in broad-leaf plants except the interveinal discoloration changed to tip-burn symptom (USEPA, 1976; Anon, 1962).

Chronic foliar nitrogen dioxide injury on broad-leaf and coniferous plants has been reported as chlorotic lesions and growth suppression (USEPA, 1976).

Acute injury usually results from exposures to high con-

centration of pollutant for a rather short period of time. It always involves cell death. Generally, the affected area is restricted by a rather distinguishable line of demarcation between necrotic and macroscopic asymptomatic portions of leaf tissue (USEPA, 1976).

Acute ozone injury on broad-leaf plants generally includes shining or oily water soaking in early stages of symptom development following by flecking. The shapes of such monofacial or bifacial necrosis vary with ozone dosage and plant species. In conifers, acute foliar injury usually consists of tip-burn or necrotic banding along the needles, reduced terminal and annual increment growth, and needle death (USEPA, 1976; Mudd and Kozlowski, 1975; Linzon and Costonis, 1971).

Acute sulfur dioxide injury on broad-leaf plants consists of water-soaking, marginal and interveinal scorch with a distinct line between affected and non-affected areas, cell death, and sudden premature defoliation. In conifers, acute sulfur dioxide injury includes necrotic banding, tip-burn, premature defoliation, and cell or tissue death (USEPA, 1976).

Acute nitrogen dioxide injury includes water soaking, rapid development of irregular-shaped intercostal lesions, and defoliation. Injury on needles of conifers from acute nitrogen dioxide exposures are needle tip discoloration which progress toward the base with a distinct demarcation between

healthy and injured tissues, premature defoliation, and tissue death (Taylor et al., 1975).

Many plants yield characteristic foliar symptoms to specific pollutant exposures. Visible symptoms have thus served as the markers in the identifying air pollution induced injury (USEPA, 1976; Hill, 1971; Hepting, 1964, 1968; Treshow, 1975). In the pioneering stages of air pollution investigations, the majority of research only dealt with symptom descriptions and identification of plant threshold dosages to specific pollutant. Super high pollution concentrations, which were not realistic under actual field conditions, were frequently employed in these fumigations in order to induce visible foliar symptoms.

Recently, substantial evidence has accumulated which has demonstrated that air pollutants may induce significant plant yield losses without any visible foliar symptoms (Bell and Clough, 1973). Awareness of this possible adverse effect on plant growth without visible symptoms and the lack of our ability to estabilish a direct relationship between plant sensitivity to specific pollutant and its associated foliar symptom expressions, research interest has been shifted from the determination of plant threshold dosages to plant physiological or biochemical responses. For example, in the measurement of net photosynthesis rate of ponderosa pine during the course of an ozone fumigation, Coyne and Bingham (1980) indicated that plant physiological response was superior to

the estimation of injury by symptom expressions because it detected functional impairment before chlorotic mottle symptoms were visible. Furthmore, this technique provided a quantitative value which avoided the subjective judgements necessary in the describing of symptom severity.

The subtle effects of air pollutants on plants can be multiple and simultaneous. They have been reported on various cellular organelles and cell functions, such as buffering capacity (Pell, 1980; Jager and Klein, 1977), enzyme activity (Horseman and Wellburn, 1975; Ziegler, 1972, 1973, 1977), chlorophyll synthesis (Rao and LeBlanc, 1965; Puckett et al., 1973), mitochondria activity (Racker, 1965; Lee, 1967, 1968), chloroplast structure (Thomson et al., 1966; Malhotra, 1976; Malhotra and Khan, 1980), cell membrane integrity (Pell and Weissberger, 1976), photosynthesis (McLaughlin et al., 1979, 1980; Carpon and Mansfield, 1976; Biscoe et al., 1973; Hill and Littlefield, 1969; Black and Unsworth, 1979; Dugger et al., 1962), respiration (Pell and Brennan, 1973; Srivastava et al., 1975a, 1975b), and transpiration (Throne and Hanson, 1972; Evans, 1975; Schramel, 1975; Biggs and Davis, 1980; Dunning and Heck, 1977).

The metabolic effects of air pollution on plants has been thoroughly reviewed by Mudd (1975), Ziegler (1975), and Tingey (1974). In this review, in order to illustrate the pathogenesis of pollutant-induced plant diseases, various plant reactions will be discussed in series and logically

interrelated. However, it is important to keep in mind that many of these physiological and biochemical responses may be induced simultaneously and the actual sequence of events and mechanisms by which air pollutants affect plant metabolism have not been fully elucidated.

Injury Mechanisms of Gaseous Pollutants

Because the different chemical characteristics among sulfur dioxide, nitrogen dioxide, and ozone, the injury mechanisms to plants by these pollutants are believed to be different.

Gaseous sulfur-related and nitrogen-related pollutants must be dissolved with water into ionic forms in order to react with cellular constituents (Thomas et al., 1956; Taylor et al., 1975; Ziegler, 1975). Since plants are capable of reducing sulfur dioxide and nitrogen dioxide into less toxic forms (such as sulfate, nitrate, and nitrite) and finally incorporating these ions into normal metabolic cycles (Salisbury and Ross, 1978; Thomas and Hill, 1937; Thomas et al., 1950), certain amounts of sulfur dioxide and nitrogen dioxide can thus be metabolized and/or translocated without causing any adverse effects on plant growth (Jensen, 1973; Lockyer et al., 1976; Cowling and Koziol, 1978).

When pollutant influx rate is more than what plant can ultilize, further uptakes of pollutants will be accumulated in the vacuoles as sulfate and nitrate. This instant dynamic

plant response at first causes an increase of osmotic pressure in cell sap before it tops cell capacity and breakdowns the cellular buffering systems (Ziegler, 1975; Taylor et al., 1975). In supporting this hypothesis, Jager and Klein (1977) fumigated pea (Pisum sativum L.) seedlings with sulfur dioxide at 0.10, 0.15, and 0.25 ppm for 18 days. They found that under all tested conditions, plant cellular buffering capacity to H-ions and OH-ions was decreased.

The breakdown of cellular buffering system can initiate a series of destructive chain reactions in plant cellular metabolisms, such as inactivation of enzymes, destruction of protein structures, loss of selective cell permeability, and decompartmentation of individual cells, that finally lead to the dysfunction of cellular organelles (Pucket et al., 1974; Ziegler, 1975; Srivastava et al., 1975a, 1975b; Wellburn et al., 1972). In other words, when pollutant uptake is beyond plant internal physiological threshold dosage, irreversable injury will be induced and resulted in characteristic foliar symptoms.

The actual physiological threshold dosage of each pollutant to a given plant is dependent on plant's pollutant absorption rate (Bennett et al., 1975), metabolization and translocation rate (Garsed and Read, 1978), activity and concentrations of enzymes participating in cellular repairment (Horseman and Wellburn, 1975), and cellular buffering capacity (Jager and Klein, 1977; Taylor et al., 1975). The con-

centration and duration of pollutant exposure is thus important in the determination of plant injury degrees. It is believed that more pollutants can be buffered at low concentration fumigation for an extended time than applying the same amount of pollutant at a higher concentration in a shorter period of time.

For pollutant per se, its phytotoxicity to plants has been attributed to 1) water solubility (Bennett and Hill, 1973a, 1973b, 1974), 2) chemical reactivity and toxicity (Evans and Miller, 1972, 1975; Berry, 1971; Miller and McBride, 1975; Miller, 1973), 3) sensitivity of exposed plant tissues (Barnes, 1972), 4) cellular repairability (Pell, 1980), and 5) environmental and physiological conditions of plant tissues (Heck, 1968; Cotrufo, 1974; Cotrufo and Berry, 1970; Davis and Wood, 1972, 1973a, 1973b).

Unlike sulfur dioxide and nitrogen dioxide, ozone is an unstable trioxygen molecule with strong oxidizing properties. Chemical oxidation is the principle injury mechanism for ozone exposures. The injury mechanisms of ozone on plant tissues are thus considered to be different from sulfur dioxide and nitrogen dioxide. Although the injury mechanisms are different among these pollutants, the final results of their destruction on organelle integrity and interference on cell metabolisms by these pollutants are similar (Mudd and Kozlowski, 1975).

Ozone has been suspected to react with the first reac-

tive molecule which it encountered within plant tissues (Stephens, 1969). With the aid of electron microscopy, Pell and Weissberger (1976) identified that plasmalemma was the primary site of ozone injury. Biochemically, ozone can attack cellular unit membranes by oxidation of unsaturated fatty acids, inactivation of SH-enzymes, disruption of S=S bonds, or irreversible oxidation of cofactors. The chemical properites and physiological functions of cell membranes are thus destroyed (Chang and Heggestad, 1974).

Because most of known cellular biochemical reactions take place within unit-membrane-enclosed organelles (Curtis et al., 1976), any disruption or disorganization of unit membranes by ozone, sulfur dioxide, or nitrogen dioxide will certainly affect electron transport systems, membrane permeability, enzyme activity, photosynthesis, respiration, transpiration, and other plant cellular metabolisms.

A. Effects on Photosynthesis

Many researchers have studied the performance of photosynthesis under the influence of pollutant exposures because potential growth suppressions due to air pollution present a major concern (Wilkinson and Barnes, 1973).

Decrease of photosynthesis has often been observed in pollutant fumigated plants either at sublethal or high concentrations. Bennett and Hill (1973a) reported a threshold dosage of ozone as low as 0.05 ppm to induce reduction of net

photosynthesis on barley (Hordeum sativum Jess.) and oat (Avena sativa L.). Miller et al. (1969) fumigated three-year-old ponderosa pines with 0.15, 0.30, and 0.45 ppm ozone for 9 hours daily for 30 days; at the end of experiment, net photosynthesis rates were reduced by 10, 70, and 85%, respectively. For higher ozone concentration, Coyne and Bingham (1978) found an 18% reduction of photosynthesis on first-trifoliated-leaf-stage snap bean after exposure to 0.72 ppm ozone for four hours daily for 18 days.

In sulfur dioxide exposures, Black and Unsworth (1979) reported that net photosynthesis of three-week-old Vicia faba cv. Dylan was inhibited by all tested sulfur dioxide concentrations exceeding 0.015 ppm when compared with control plants. Keller (1977) exposed three-year-old spruce (Picea abies (L.) Karst.) clones to 0.05, 0.1, and 0.2 ppm sulfur dioxide for 10 weeks. After six weeks of 0.2 ppm fumigation, carbon dioxide uptake rate was decreased to 25%, 50%, and 65% as compared to the first day of the experiment in sensitive, intermediate, and tolerant clone, respectively. Such reduced carbon dioxide uptakes were found long before visible symptoms appeared on all clones.

Carpon and Mansfield (1976) conducted an experiment to expose six-week-old tomato plants (cv. Moneymaker) to 0, 0.10, 0.25, and 0.50 ppm nitric oxide and nitrogen dioxide singly and in combinations for 20 hours and measured the rates of photosynthesis. They found that inhibition of pho-

tosynthesis occurred before there was any external visible injury. Both gases reduced net photosynthesis to approximately the same extent with 28% reduction in 0.50 ppm nitric oxide and 32% in nitrogen dioxide. Srivastava et al. (1975a, 1975b) exposed snap bean (Phaseolus vulgaris L.) primary leaves to nitrogen dioxide and examined gas exchange rates. Net photosynthesis and dark respiration were both inhibited by nitrogen dioxide concentrations between 1.0 and 7.0 ppm. The degree of inhibition was increased by increasing nitrogen dioxide concentrations and increasing exposure time. Hill and Bennett (1970) also observed an inhibition of net photosynthesis by nitrogen oxides in alfalfa and oats. Four to eight week old alfalfa (cv. Ranger) and oats (var. Park) were exposed to 0.10 ppm nitric oxide, nitrogen dioxide singly and in combination for up to 250 minutes. They observed that the inhibitory effects of nitrogen dioxide and nitric oxide on net photosynthesis of oats and alfalfa were well below those required to cause visible injury. There appeared to be a threshold concentration of about 0.6 ppm for each pollutant to each plant species.

Physio-biochemically, the observed reduction of net photosynthesis by pollutant fumigations can be attributed to one or more of the following factors: 1) interference between carbon dioxide fixation enzymes and pollutants (Hill and Bennett, 1970; Ziegler, 1972, 1973, 1975), 2) disruption of electron transport system (Rhoads and Brennan, 1978; Chang

and Heggestad, 1974), 3) destruction of chlorophyll and/or chloroplasts (Malhotra, 1976; Rao and LeBlanc, 1965; Thomson et al., 1974), and 4) closing of stomata (Engle and Gabelman, 1966).

Oxidation was proposed by Puckett et al. (1973), Heath (1975), and Howell (1974) as the mechanism for chlorophyll destruction in sulfur dioxide and ozone exposures, respectively. Howell (1974) in his study of ozone exposure on beans, he suggested that ozone can destroy chlorophyll molecules either by reaction with polyphenols or by destruction of cellular membranes. Puckett et al. (1973) suggested that the inhibition of lichen photosynthesis by sulfur dioxide was due, at least in part, to the destruction of chlorophyll by an irreversible oxidation process.

In field conditions, Austrian pine (Pinus nigra Arad.), eastern white pine (Pinus strobus L.), Scotch pine (P. silvestris L.), and Douglas fir (Pseudotsuga menziesii) have been reported exhibiting different degrees of chlorophyll decomposition under the influence of polluted environments (Gowin and Goral, 1977). Higher degree of chlorophyll destruction and pheophytin content were found in needles of trees growing under chronic industrial pollution as compared to those grown in pollution free areas (Gowin and Goral, 1977).

B. Effects on Respiration

Besides photosynthesis, respiration is the other major plant physiological process commonly affected by pollutant exposures.

MacDowall (1965) investigated the stages of ozone injury to respiration on ozone-sensitive tabacco (Nicotiana tabacum L. cv. White Gold) leaves. He observed an injurious dose of ozone initially inhibited respiration of fumigated leaves before weather flecks appeared. However, when visible injury subsequently developed, oxygen uptake was characteristically stimulated in treated leaves and in mitochondria prepared from such visibly injured tissues. Unfortunately, there were no direct evidence to demonstrate that the increased respiration in the early stages of air pollutant fumigation was either due to the uncoupling of phosphorylation or the energy-required repairing processes, or both.

C. Effects on Transpiration

Transpiration is another plant metabolism frequently affected by pollutant exposures (Todd and Propst, 1963). Air pollutants have been shown to induce or suppress stomatal opening depending upon plant species, pollutant species, pollutant dosage, and environmental factors. Although the studies of the effects of ozone and nitrogen oxides on plant transpiration were rather limited in the literature, the reported studies have tended to agree that inhibitory effects

were induced by these two pollutants on transpiration (Lee, 1967; Srivastava et al., 1975a, 1975b).

Transpiration in response to sulfur dioxide fumigations have been reported to vary with sulfur dioxide concentration, relative humidity, plant species, and plant age (Biscoe et al., 1973; Black and Black, 1979; Mansfield and Majernik, 1970, 1972). Majernik and Mansfield (1970, 1972) fumigated broad bean (Vicia faba L. var. Windsor Harlington) to various sulfur dioxide dosages in several studies. They observed stimulated stomatal opening as a general broad bean reaction to sulfur dioxide exposure. A linear positive ralationship was found between the degrees of stimulated stomatal opening and 0.25 to 1.00 ppm of sulfur dioxide. They also found that in the conditions of low relative humidity (less than 40% R.H. at 18°C), sulfur dioxide suppressed stomatal opening while with a higher water vapor content in the atmosphere (greater than 40% R.H. at 18°C) there was an appreciable stimulation of stomatal opening. Black and Black (1979) exposed three week old field bean (Vicia faba cv. Dylan) to 2-20 ppm sulfur dioxide for two hours and observed a comparable concentration independent of 20-25% increase on leaf diffusive conductance on both adaxial and abaxial surface. Such stomatal opening was associated with a sharp reduction in the proportion of living epidermal cells adjacent to the stomata.

Others, (Sij and Swanson, 1974; Ziegler, 1975; McLaughlin et al., 1979) have proposed that sulfur dioxide effects

on stomata were resulted from general changes in physiology of leaf tissues rather than on guard cells per se. They suggested that the primary sulfur dioxide effect on plant was at cytological or biochemical level rather than stomata basis.

Effects of Pollutant Combinations on Vegetation

Recently, in order to have a better understanding of total potential impact of pollutants on plants, increased attention has been directed toward examining plant responses under pollutant combination exposures. The effects of pollutant combinations on plants as compared to single pollutant exposures could be classified into one of the following three categories: 1) more than additive (greater than the sum of the individual pollutant effects), 2) additive (equal to the sum of the effects of individual pollutant), or 3) less than additive (less than the sum of the individual effects).

It is important to realize that several other principles in addition to those applied in single pollutant exposures are extremely critical in the determination of plant responses to pollutant combinations. These include concentration, ratio, and exposure sequence of pollutant components in the mixture. For example, Middleton et al. (1958) exposed Phaseolus vulgaris L. to a combination of sulfur dioxide and ozone with different ratios and observed foliar injury two hours after the fumigations. When the ratio was 4:1 of sulfur dioxide : ozone, ozone appeared to interfere with the

injury from sulfur dioxide, at a 5:1 ratio, sulfur dioxide did not interfere with the amount of foliar injury caused by ozone, when the ratio was raised to 6:1, both gases induced their own typical symptoms.

A. Two-Pollutant Combinations

Menser and Heggestad (1966) first reported a more than additive interaction between ozone and sulfur dioxide. They exposed Nicotiana tabacum var. Bel-W3 (ozone-sensitive), Bel-B (ozone-tolerant), and Consolation 402 to 0.03 ppm ozone and 0.25 ppm sulfur dioxide singly and in combination for two and four hours. No visible symptoms were apparent after single pollutant exposures. However, pollutant combination treatment caused 15%, 9%, and 12% of leaf area injury in two hour fumigation and 41%, 23%, and 43% of leaf area injury in four hour fumigation for Bel-W3, Bel-B, and Consolation, respectively. Tingey et al. (1973b) exposed soybean (Glycine max (L.) Merr.) cvs. Hood and Dare to 0.05 ppm ozone and 0.05 ppm sulfur dioxide singly and in combination for eight hours a day, five days a week for three weeks. They observed no significant plant growth effects on 0.05 ppm ozone, or 0.05 and 0.20 ppm sulfur dioxide treatments. But the pollutant combination significantly reduced top fresh weight, root fresh and dry weight, and shoot-root ratios in both cultivars.

In tree species, Karnosky (1977) reported a more than

additive effect of pollutant combination induced by the exposure of 0.05-0.20 ppm ozone and 0.35-0.65 ppm sulfur dioxide for three hours on trembling aspen (Populus tremuloides Michx.) symptom expression.

Not all studies on ozone + sulfur dioxide interactive effects on plants have yielded data supportive of synergism. Tingey et al. (1971a, 1973a) exposed radishes to 0.05 ppm sulfur dioxide and 0.05 ppm ozone singly and in combination for eight hours daily, five days a week for five weeks. They reported that pollutant combination reduced leaf fresh weight, dry weight, and root width to the amount equal to the sum of the reductions observed on individual pollutant treatments. However, total plant fresh weight, root length, and root fresh and dry weight were less than the sum of the reductions of single pollutant treatments. In another experiment, Tingey and Reinert (1975) exposed tobacco and alfalfa plants to 0.05 ppm ozone and 0.05 ppm sulfur dioxide singly and in combination for eight hours a day, five days a week for various time periods. They reported an additive effect of pollutant combination treatment on the reductions of tobacco leaf, stem, and root dry weights and a less than additive effect on alfalfa foliage and root dry weight measurements.

Less than additive effects of ozone + sulfur dioxide combinations have also been reported in gas exchange studies. Rosen et al. (1978) exposed one year old Ives grapevines

(Vitis labrusca L. cv. Ives) to 0.5 ppm ozone and sulfur dioxide singly and in combination for two hours. They found that ozone exposure increased stomatal resistance by 30% and sulfur dioxide induced an increase of 190% as compared to control plants. The exposure of pollutant combination did not significantly alter stomatal response although visible symptoms appeared on grape leaves after the fumigation. In forest tree species, Carlson (1979) fumigated black oak, sugar maple, and white ash with 0.50 ppm ozone and 0.50 ppm sulfur dioxide singly and in combination. After one week of fumigation, the rate of photosynthesis was 52, 73, and 100% at ozone alone, 52, 46, and 80% at sulfur dioxide alone, and 56, 59, and 62% at ozone + sulfur dioxide treatment for black oak, sugar maple, and white ash, respectively.

Several studies have reported more than additive effects of sulfur dioxide + nitrogen dioxide combination on foliar injury as well as physiological responses. Tingey et al. (1971b) reported no foliar injury on tobacco, pinto bean, tomato, radish, oats, and soybean plants when they were exposed to sulfur dioxide concentrations at less than 0.50 ppm or 2 ppm of nitrogen dioxide for four hours. However, the sublethal pollutant combination of sulfur dioxide and nitrogen dioxide at concentrations ranging from 0.05-0.25 ppm for each pollutant did cause leaf injury on all of six plant species. Bennett et al. (1975) exposed oats, pinto bean, radish, sweet pea, and Swiss chard to 0.125-1.0 ppm sulfur

dioxide and nitrogen dioxide singly and in combination (1:1 by volume) for one and three hours. None of the species displayed any visible injury from exposure to the tested nitrogen dioxide or sulfur dioxide alone treatments at concentrations less than 0.5 ppm, but enhanced phytotoxicity of pollutants were observed in pollutant combination in all species by inducing foliar symptoms.

For photosynthesis performance, White et al. (1974) exposed alfalfa to 0-0.50 ppm of sulfur dioxide and nitrogen dioxide singly and in combination for one and two hours. They reported an 2-3%, 0%, and 9-15% photosynthesis inhibition of 0.25 ppm sulfur dioxide, nitrogen dioxide, and sulfur dioxide + nitrogen dioxide treatment, respectively. In their studies, the degrees of more than additive effects decreased as individual pollutant concentration (1:1 by volume) increased from 0 to 0.50 ppm.

After review of the literature, little is known about the effects of ozone and nitrogen dioxide combination on plants. Matsushima (1971) found less than additive foliar injury on Lycopersicum esculentum Mill. and Capsicum frutescens L. when exposed to 0.4 ppm ozone and 15 ppm nitrogen dioxide for 50 minutes.

B. Three-Pollutant Combinations

There were several reports in the literature concerning about plant responses to three-pollutant combinations. Fuji-

wara et al. (1973) reported that, at equal concentrations, ozone singly induced most severe foliar injury on pea (Pisum sativum L.) and spinach (Spinacia oleracea L.) followed by sulfur dioxide and no effect, if any, induced by nitrogen dioxide exposure. The addition of nitrogen dioxide to ozone + sulfur dioxide exposure had little effect on the foliar injury noted. Reinert and Gray (1980) examined the effects of ozone, sulfur dioxide, and nitrogen dioxide, alone and in all combinations at 0.2 and 0.4 ppm of each pollutant for three and six hours on radish cv. Cherry Belle. Ozone reduced root dry weight more at 0.4 ppm than at 0.2 ppm. Sulfur dioxide depressed root/shoot ratio at both 0.2 and 0.4 ppm; however, in sulfur dioxide + nitrogen dioxide treatment there was a significantly greater than additive depression of root/shoot ratio at 0.4 ppm. The ozone reduction in root weight was additive in the presence of sulfur dioxide and nitrogen dioxide.

Kress (1978) has provided the first report of forest tree responses to three-pollutant interactions. He conducted fumigations of 0.05 ppm ozone, 0.14 ppm sulfur dioxide, and 0.10 ppm nitrogen dioxide singly and in all possible combinations for six hours per day for 28 consecutive days on seedlings of loblolly pine and American sycamore. In loblolly pine, when all three pollutants were combined, the resultant foliar injury was significantly different from that of ozone alone treatment. However, the observed effect was not signi-

ficantly different from that of ozone + sulfur dioxide treatment. Exposures of sycamore demonstrated that foliar injury was not a reliable indicator of plant sensitivity to pollutants under these experimental conditions since no treatment was consistently significantly different from any other. Treatment with all three pollutants produced an 45% and 25% growth reduction on sensitive and tolerant sycamore lines, respectively. Significant growth reductions were also observed in the ozone + sulfur dioxide treatment where 34% and 17% growth reductios were observed on the sensitive and tolerant lines, respectively.

Effects of Air Pollutants on Eastern White Pine

Because of its wide natural distribution in the industrialized northeastern United States, eastern white pine (Pinus strobus L.) has been subjected to more air pollution injury than any other tree species native to North American (Gerhold, 1977). There have been several extensive reviews on the sensitivity of white pine to pollutants (Gerhold, 1977; Nicholson, 1977). In this review, only certain aspects pertaining to this study will be included.

A. White Pine Foliar Symptoms Induced by Pollutants

Eastern white pine emergence tipburn (also called white pine needle blight or white pine blight) was first reported occurred in 1905 in pine plantation in Concord, New Hampshire (Dana, 1908). Affected trees have been observed throughout

the range of the species on all types of growth environments (such as latitude, soil type, soil moisture content, shade, and exposure conditions ----etc.) (Baldwin, 1954). Needle injury on pine fascicles was first shown as a yellowish to faint pinkish spot located at some distance back from the tip of needles. As disease progressed, these spots enlarged into orange-red bands or developed into needle tipburn along the fascicle. Necrotic areas were sharply distinct from the green base. The dead tips were at first pinkish yellow, but after several days the color appeared to a brownish red (Dana, 1908). The sensitivity of white pines to this disease was later proven to be genetically controlled (Berry, 1961; Linzon, 1961). The etiology of emergence tipburn was finally proven by Berry (1961), and Berry and Ripperton (1963) in their field studies to be ambient oxidants.

In laboratory, Costonis and Sinclair (1969) fumigated four to five year old potted eastern white pine with 0.03 ppm ozone for 48 hours and 0.07 ppm for four hours, symptoms of characteristic emergence tipburn injury were induced on pine needles of sensitive trees. They described the initial macroscopic symptoms of ozone injury as minute, silver flecks which later coalesced into larger chlorotic flecks visible to the naked eyes. On ozone sensitive trees, only current year needles were retained by mid-summer instead of normal three years of needle age. Individual trees varied greatly in the sensitivity to ozone injury.

The threshold of ozone dosage at which visible injury to Pinus strobus occurred has differed greatly in the literature. Berry (1961) fumigated 0.065 ppm ozone for one hour on sensitive trees and induced typical ozone symptoms on needles. Other reports indicated ozone concentration of 0.05 ppm for one hour (Berry and Ripperton, 1963), 0.06 ppm for four to eight hours (Davis and Wood, 1972), 0.1 ppm for eight hours (Berry, 1971), 0.25 ppm for four hours (Davis and Wood, 1973a), or 0.5-1 ppm for four hours (Botkin et al., 1971, 1972) was the threshold dosage for foliar injury.

Ozone was not the only atmospheric phytotoxicant to eastern white pine. Linzon (1971) conducted a five-year field survey and showed that eastern white pine was extensively injured by sulfur fumes at distance up to 25 miles from the smelting area at Sudbury, Ontario. Affected trees displayed extensive foliar injuries which were exhibited initially as grayish-green or whitish areas over the entire injured portion of the needles. These lesions then progressed through color changes to reddish brown color. Bark abnormalities, radial and volume growth losses, and mortality were some of the further stages of decline symptoms in sensitive trees. Outside this 25 mile inner zone, plants were subjected to infrequent invasions of damage-producing sulfur dioxide fumes with results of little or no tree injury. Unfortunately, during his experiment period, the other major gaseous pollutants, such as ozone, nitrogen dioxide, and flo-

ride, were not measured. The symptoms described above were thus probably from sulfur dioxide or sulfur dioxide and other pollutant combinations.

Based on artificial exposures, Costonis (1970, 1971, 1973) described the symptom expressions of sensitive, intermediate, and tolerant eastern white pines to sulfur dioxide exposures. Current year needles of sensitive trees between four to five weeks old were acutely injured by sulfur dioxide at dosages ranging from 0.04-0.06 ppm for one hour to 0.10-0.20 ppm for two hours. Acute sulfur dioxide injuries initially appeared as collapsing or slightly sunken of affected needle tissues on stomatal-bearing faces of the needles. Necrosis of needle tissues progressed from the point of initial injury to the base of needles. In acute injury, all needles in a fascicle were usually not equally affected by sulfur dioxide exposure nor was the injury always uniform from fascicle to fascicle or from tree to tree. It was very common to find all stages of lesion development on a given plant. In the late stages of acute sulfur dioxide injury, necrotic bandings with distinctive demarcation lines between healthy and affected tissues were very common. Dead tissues turned to reddish-brown. Severe premature defoliation might occur later.

Chronic sulfur dioxide injury on eastern white pine has been reported by Berry in 1964 which included general chlorosis of entire needle length, scattered pigmented necrotic

lesion, premature defoliation, and unthrifty appearance of entire plant (Berry, 1964).

Differences in the development of white pine foliar injury induced by sulfur dioxide and ozone was microscopically distinguishable within the first 24 hour after injury onset (Houston, 1974; Costonis, 1970, 1971). After the first 72 hours of lesion development, it is very difficult to distinguish between lesions induced by either pollutant. Like ozone injury, eastern white pine varied greatly in their sensitivity to sulfur dioxide.

There were several other studies concerning about the threshold sulfur dioxide dosage in inducing visible symptoms on white pine needles. Berry (1973) reported injurying foliage of white pine at a concentration of 0.25 ppm for one hour. In other fumigation experiments, sulfur dioxide dosage as low as 0.03 ppm for one hour (Costonis, 1971), and 0.025 ppm for six hours (Houston, 1974) have been reported to injury extremely sensitive clones of eastern white pine.

Published information on nitrogen oxides effects on eastern white pine has not been extensive. Skelly et al. (1972) examined symptom expressions of eastern white pine located near a point source of sulfur dioxide and nitrogen oxides. At their research site, the highest one-hour nitrogen oxides concentration in the ambient air has been recorded greater than 0.585 ppm and the highest two-hour sulfur dioxide concentration was 0.690 ppm at 400-600 yard downwind

monitoring stations from the industrial plant. They reported that eastern white pine seedlings in that area exhibited symptoms as tufted needle growth with severe needle tip-burn, chlorotic mottle, and stunted growth. The tip-burn was expressed as a very delineated area of orange-colored tissue. A striking tree-to-tree variation on pollution sensitivity was noted on observed trees with 6% exhibiting symptoms approaching the classic chlorotic dwarf condition and 20% considering to be free of any symptoms of air pollutant effects. Although possible pollutant combination effects were not measured in this study, it was very likely to be involved in this case.

Nicholson (1977) exposed two-year-old grafted white pine to 0.10 and 0.30 ppm nitrogen dioxide for six hours. Chlorotic spot, chlorotic mottle, and necrotic tip-burn were accounted for the majority of visible injuries on needle surfaces.

B. Effects of Pollutant Exposures on White Pine Photosynthesis

Botkin et al. (1971) exposed branches of eastern white pine to 0.9 to 1.0 ppm ozone for three hours and found that net photosynthesis was reduced by approximately 80%. Recovery phenomenon was observed after ozone treated plants were once again placed into ozone-free air. In the following year, Botkin et al. (1972) reported that ozone threshold dosage on white pine net photosynthesis suppression was approxi-

mately 0.50 ppm for a minimum of four hours. Above this dosage, three categories (sensitive, intermediate, and tolerant) of ozone sensitivity were detectable in white pine plants. In intermediate and tolerant clones, suppression of photosynthesis was reversible if an ozone-free recovery period was made possible. They also reported that the visible symptom expression on current year white pine needles was not a good index for the timing or the severity of ozone induced photosynthetic suppression. Barnes (1972) exposed eastern white pine seedlings to 0.05 and 0.15 ppm ozone for periods of five to 18 weeks. He found that ozone at 0.05 ppm had variable effects on photosynthesis depending upon foliar age. In 0.15 ppm treatment, ozone had a more consistent depressing effect on photosynthesis. Among measured responses, the most consistent effect of ozone on white pine seedlings was the stimulation of respiration. It was almost double the control rates at the 36th day and nearly 40% higher at the 77th day of fumigation.

McLaughlin et al. (1979) labelled foliage of three different pollution sensitivity classes of field grown eastern white pine trees with isotopic carbon dioxide-14 four times during the growing season under the influence of ambient air pollutant, particularly ozone. Photosynthate allocation patterns indicated that contribution of photosynthate by old needles to new needle growth occurred and this process was most rapid in tolerant trees which retained nee-

dles from two prior years and least significant in sensitive trees. There were no distinct differences in foliar retention of ^{14}C between these three sensitivity classes. Generally, higher levels of transfer of ^{14}C from foliage into branches were noted in the tolerant trees throughout the growing season. They indicated that growth limitations in sensitive trees were a function of pollutant-induced reduction in photosynthate availability which resulted from reduced needle length and premature defoliation.

In the field study of chronology of eastern white pine chlorotic symptom development among three pollutant sensitivity classes during an entire growing season, Mann et al. (1980) reported that no differences in photosynthetic rates were observed between diseased and healthy trees although total chlorophyll content was different. They suggested that decreased growth as evidenced in shoot elongation and vigor of chlorotic trees appeared to be related to premature defoliation and retention of a reduced quantity of photosynthetically active tissue rather than to a reduced photosynthetic efficiency of the existing needles.

Eckert and Houston (1980) exposed white pine ramets with 0.05 ppm sulfur dioxide for two hours. The rates of photosynthesis in sensitive and tolerant clones were depressed significantly below control plants. Photosynthesis rates in sensitive clones were decreased to a greater extent (27%) than in tolerant clones (10%) during two hour fumigation.

Needle length of sensitive and tolerant treated plants at the end of the growing season were found shorter than control needles. They suggested that substantial loss of photosynthate production which resulted from supressed photosynthesis might attribute to needle length reduction in apparently tolerant as well as sensitive white pines during sulfur dioxide fumigations.

C. Effects of Pollutant Combinations on White Pine

While studies were solving white pine needle blight in the late 1950's, work was also being initiated in Ohio in 1959 by Dochinger to determine the cause of chlorotic dwarf. Chlorotic dwarf was first described by Swingle (1944) in white pine plantations throughout Ohio since 1936. Swingle (1944) in his original report stated that the chlorotic dwarf condition was very similar in some respects to white pine blight as described by Spaulding (1909). Trees with chlorotic dwarf disease were characterized by stunted tops and roots, short and mottled needles, and premature defoliation. On genetically sensitive trees, new needles emerged normally but soon became light green color and mottling with chlorotic spots. As disease progressed, needles often yellowed by early season as chlorotic flecks or mottling coalesced. The older needles turned prematurely yellow and were shed before the current needles reached to full development.

Five years after their initial work, a comprehensive

report on the etiology of eastern white pine chlorotic dwarf was made by Dochinger and Seliskar (1970). They showed that typical symptoms of chlorotic dwarf disease could be induced from the injury of gaseous pollutants upon the foliage of genetically sensitive white pine clones by 0.10 ppm ozone and sulfur dioxide singly or in combination (Dochinger and Seliskar, 1965, 1970). When senstive trees were protected in charcoal-filtered chambers, the injurious effects of air pollutants were completely recovered but not in non-filtered chambers. The typical symptoms of chlorotic dwarf induced by the exposure of 0.025 ppm sulfur dioxide and 0.05 ppm ozone was later reported by Houston (1974). Like white pine blight, eastern white pine showed a wide range of differential sensitivity to chlorotic dwarf (Dochinger and Seliskar, 1970; Dochinger and Heck, 1969).

Response of eastern white pine to ozone + sulfur dioxide mixtures has been reported to be more than additive by Dochinger and Seliskar (1970), Banfield (1972), and Houston (1974) based on foliar injury evaluation. Costonis (1973), however, observed a less than additive effect at lower pollutant dosages.

In the mixture of ozone and nitrogen dioxide, Nicholson (1977) fumigated 12 grafted white pine clones to 0.10 ppm ozone and 0.30 ppm nitrogen dioxide singly and in combination for six hours. The most prevalent symptoms were chlorotic mottle, necrotic tip-burn, and pigmented banding. The sensi-

tivity rankings of these clones were found generally consistent between the evaluations of visible injury and the mean needle length of the current year needles. However, the rankings of these clones obtained from artificial environment-controlled fumigations were slightly different from field rankings.

Research at Radford Army Ammunition Plant

A series of studies have been conducted in the forested area surrounding the Radford Army Ammunition Plant (RAAP) as well as supported studies being conducted in laboratory. The RAAP is situated in a forested geographic bowl and is isolated from other major air pollutant sources with a known history as a point source of pollutant emissions into the surrounding areas. Because of the characters of nitrogenous-ammunition production and the self-supplied, coal-burning electricity systems at RAAP along with the known existance of ozone in the ambient air of this region of the United States, all three major phytotoxic air pollutants (i.e. ozone, sulfur dioxide, and nitrogen dioxide) are presented with varying degrees in the ambient atmosphere at this facility.

Eastern white pine was chosen as the major indicator species for studying pollutant abatement at RAAP after consideration of the following facts: 1) this species is indigenous to the area and found abundantly within the installation, 2) it has been shown in previous studies to exhi-

bit strong correlation between average white pine annual increment growth and RAAP production rates, 3) this species has shown great variation in air pollutant sensitivity on a clonal basis, but individual trees have responded with a high degree of uniformity, and 4) this species can be clonally propagated to insure uniform genetic characteristics.

Skelly et al. (1972) conducted height growth studies in a 13-year-old stand of white pine at RAAP. Macroscopic asymptomatic trees as well as chlorotic dwarf trees were included in their studies. They used a simple linear regression analysis to evaluate the relationship of white pine annual radial increment growth to annual RAAP production levels (an indicator of air pollution concentrations). A significant inverse relationship between plant growth and air pollution concentration was found in white pine and such findings were the basis for continued studies. In the following year, Stone and Skelly (1973, 1974) studied the annual radial increment growth in a mixed white pine and yellow poplar (Liriodendron tulipifera L.) stand. A significant negative relationship between plant growth and RAAP coal burning load was again found in these studies. Phillips et al. (1977a, 1977b) extended similar types of observations into three loblolly pine stands using multiple linear regression analysis with plant annual radial increment growth as dependent variable and annual RAAP production levels, total annual rainfall, annual seasonal rainfall, and plant age as indepen-

dent variables to evaluate pollution impact on plant growth. A significant (P=0.01) inverse relationship was demonstrated in two of three stands examined with regard to plant growth and previous production history at RAAP. The third stand was sufficiently depressed in growth due to its proximal location to pollutant sources so that growth differences could not be defined. Phillips et al. (1977a) also re-evaluated the white pine stand which was studied by Stone and Skelly (1973) in an effort to identify growth reduction in trees differing in pollutant sensitivity. Four classes of foliar symptom expression were used to categorize the sample trees for computer analysis of their respective growth rates in response to their previous exposure to pollutants. Analysis using regression analysis revealed that there was no significant growth rate differences between symptom classes and production peaks, i.e. the growth of macroscopic asymptomatic trees was reduced as much as trees with symptoms during the time of sampling.

Later work reported by Nicholson (1977) indicated that growth in this white pine stand has continued to increase since 1972 as a result of reduced rates of production and concurrent efforts in pollution abatement of the major sources of sulfur dioxide and nitrogen oxides at RAAP. Nicholson (1977) used selected white pines from previously studied stands as ortets for the propagation of clonal lines of differing sensitivities to ozone, sulfur dioxide, and

nitrogen dioxide. Grafts were made using 2:0 root-stock and scion from 12 ortets of white pine growing at RAAP. The 12 ortets represented four symptom severity classes (3 ortets/ class) as classified by Phillips et al. (1977a, 1977b) ranging from trees with greater than 25% of their crowns exhibiting necrotic tip-burn to those with healthy crowns. Five ramets/clone were used in each six hours treatment of 1) ozone 0.10 ppm, 2) ozone 0.30 ppm, 3) nitrogen dioxide 0.10 ppm, 4) nitrogen dioxide 0.30 ppm, 5) ozone 0.10 + nitrogen dioxide 0.10 ppm, 6) ozone 0.10 + nitrogen dioxide 0.30 ppm, and 7) control. All ramets were returned to charcoal-filtered greenhouse immediately after treatment. Ramets were evaluated prior to fumigation and then two, seven, and 14 days thereafter for visible symptoms. Analysis of variance showed that there were significant differences at P=0.05 level between clones, classes, and treatments. After 14 days, three of the 12 clones tested showed the more than additive effects of ozone + nitrogen dioxide in treatment 5 and seven clones showed the same reaction in treatment 6.

Recently, Skelly and Yang (1980) re-examined the radial increment growth of 50 eastern white pine growing in the same pine stand as an update and re-evaluation of previous studies. A significant (P=0.05) negative relation was found in multiple linear regression tests with annual increment growth as the dependent variable and annual seasonal rainfall, total annual rainfall, tree age, and annual coal consumption in

RAAP power houses (as a surrogate for pollution) as the independent variables. Such latest results uphold the previous studies regardless of the pollutant concentration fluctuation in the recent years.

In a continued effort of developing eastern white pine as a bioindicator for air pollutant monitoring and probing the mechanisms of plant tolerance, several physiological-biochemical studies were conducted using artificial environment-controlled fumigations. Epidermal characters of ozone-sensitive and ozone-tolerant white pine clones were microscopically examined by Trimble (1980). The mean value of stoma number per unit needle area was not significantly different (as 51 stomata per unit area of sensitive clone to 53 stomata per unit area of tolerant clone) between clones regardless of their ozone sensitivity.

MATERIALS AND METHODS

Plant Materials

The eastern white pines (*Pinus strobus* L.) used in this study were vegetatively propagated from 10 different ortets. These ortets were located in a natural, uneven aged, mixed stand of conifers and hardwood situated on a sharply sloping northeast exposure within Radford Army Ammunition Plant (RAAP) boundary.

The sensitivities of these ortets to air pollution have been previously classified into four classes based upon crown visible symptom expression, vigor, and shape in the field (Phillips et al., 1977a, 1977b). The symptom severity classes were defined as follows: class I = greater than 25% of the crown exhibiting necrotic tip-burn, class II = less than 25% of the crown exhibiting necrotic tip-burn, Class III = crown chlorotic but not necrotic, and Class IV = crown exhibiting no symptom.

In March of 1978 and 1979, scionwood was collected from designated ortets. At this time, terminal buds were still tight and no indication of eminent bud break was observed. The scions were cut from the outer edge of upper crown to avoid the shaded poor growth and to provide scionwood with apical dominance. The scions were immediately sealed in plastic bags, transported, and stored in a cold room (5°C)

until needed for grafting. Each year's grafting process was completed within 1.5 months after scionwood collection.

Two-year-old eastern white pine rootstocks were obtained from Virginia Division of Forestry Nursery at Crimora, VA. Seedlings were potted one year prior to grafting in 0.9-liter black plastic pots containing Spasoff soil mixture, a 2:2:1 v/v ratio of Weblite (an expanded shale product of the Webster Brick Company, Roanoke, VA 24016), vermiculite, and peat. One gram of Osmocote 14-14-14 (N-P-K) slow release fertilizer (Sierra Chemical Company, Milpitas, CA 95035) was added to soil surface of each pot after potting. Thereafter, one gram of Osmocote was applied to each plant every six to seven months throughout the experimental period.

The newly potted pine seedlings were kept in charcoal-filtered air supplied greenhouse. One year later, following the successful establishment of root system, vigorous seedlings were selected for use as rootstock in the grafting program. Current year's terminal bud from previously described scionwoods was grafted to these rootstocks according to the side veneer method (Hartmann and Kester, 1975). Due to the large demand for genetically uniform plant materials, 250 to 300 scions from each ortet were grafted each year. Newly grafted ramets were moved to charcoal-filtered greenhouse and kept under mist for four to six weeks. Successfully grafted ramets were held in the same greenhouse during that summer and fall. They were then transfered to an

outdoor cold frame in the winter to promote natural dormancy but with protection from winter stress. Thus, grafts were prepared one year ahead of fumigation to eliminate most possible effects of grafting injury.

In the following mid-April, grafted plants were once again placed in the greenhouse to induce uniformed bud breaks and shoot elongation. Dormancy was broken in the greenhouse with a regime of 20°C-30°C night-day temperature, 55-70% relative humidity, and 14-hour photoperiod. The greenhouse was equipped with high pressure sodium lamps (Harvey Hubbell, Inc., Lighting Division, Christiansburg, VA 24073) providing supplemental lighting of 21,000-24,000 lux and 330-360 $uE/m^2/sec$. In the summer, greenhouse was shaded to prevent the occurrence of high temperatures. Throughout the study, plants were watered to saturation one hour before and after daily fumigation.

Generally, two weeks after plants were moved into greenhouse the initiation of bud break was noted. The ramets of the same clone with the same date of bud break were separated from clonal pool as a group and later randomly assigned to pollutant treatments. At the time of the beginning of fumigation, current year's needles were 21-25 days old.

In 1979, fumigations were undertaken to determine the relative clonal sensitivity of eastern white pines to air pollutants as indicated by changes in symptom expression, needle growth, and needle dry weight. These fumigations were

included in four sets: 1) 0.05 and 2) 0.10 parts per million (ppm, v/v) ozone, sulfur dioxide, and nitrogen dioxide singly and in all possible combinations, 3) 0.10, 0.20, and 0.30 ppm ozone alone, and 4) 0.10, 0.20, and 0.30 ppm sulfur dioxide alone (Table 1). Each set of fumigation was conducted four hours daily for 35 consecutive days. The set of 0.05 ppm fumigation and ozone alone fumigation (i.e. 0.10, 0.20, and 0.30 ppm) were carried out daily between 0800-1200 hours. The other two set of fumigations were conducted between 1400-1800 hours. Control chamber received only charcoal-filtered air with 0 ppm of pollutant. Except for daily four hour fumigation, plants were maintained in the charcoal-filtered air supplied greenhouse.

In 1980, four different sets of fumigations were administrated. They were 1) 0.10 ppm ozone, sulfur dioxide, and nitrogen dioxide singly and in all combinations and 0.10, 0.20, and 0.30 ppm 2) ozone, 3) sulfur dioxide, and 4) nitrogen dioxide alone (Table 2). These fumigations were conducted four hours daily for 50 consecutive days. The fumigation procedures were the same as in 1979.

The studies were statistically constructed as a randomized block design. Treatments were randomly distributed daily among Continuous Stirred Tank Reactor (CSTR) chambers in order to minimize chamber effects. For each treatment, the four ramets of each clone were randomly placed in each of the four quadrant sectors of each CSTR chamber on a daily

basis to minimize any differences within chamber. Due to the limited availability of clonal materials from all ortets, the specific clones used in these fumigations were not identical (Tables 1, 2). In total, 10 clones were used in this study.

Fumigation Facilities and Procedures

Twelve CSTR chambers designed by Heck et al. (1978) were used for indoor environment-controlled laboratory fumigation purposes. Lighting, relative humidity, and temperature in CSTR chamber were independently controlled. The fumigation was carried out with a complete air change every 1.4 minute in each CSTR chamber. Light was supplied by high pressure sodium lamps. The lamp was adjusted individually above the top of each CSTR chamber to produce a range of 21,000-28,000 lux and 360-410 uE/m^2/sec photosynthetically active radiation (400-700nm) (PAR) at plant height within all chambers. Light was measured with a photon and quantum sensor (Lambda Instrument Corporation, Lincoln, NE 68504) throughout all 12 chambers. Humidity was produced by a steam generator (Sussman Hot Shot Electric Boiler, Automatic Steam Products Corp., Long Island, NY 11101) which was equipped with adjustable valves. The relative humidity within CSTR chambers during fumigation was maintained between 60-70%. Chamber air temperature during the same period was 28-32°C. Relative humidity and temperature were monitored by Abbeon Relative Humidity and Temperature Indicator Model M2A48 (Abbeon Calibration

Table 1. Fumigation program of eastern white pine (Pinus strobus L.) in 1979*.

Treatment**	Pollutant concentration	Clone used
	--- ppm ---	
O_3, SO_2, NO_2,	0.05	I-2,
O_3+SO_2, O_3+NO_2, SO_2+NO_2,		II-1, II-3,
O_3+SO_2+NO_2,		III-3,
CK.		IV-2.
O_3, SO_2, NO_2,	0.10	I-1,
O_3+SO_2, O_3+NO_2, SO_2+NO_2,		II-1, II-3,
O_3+SO_2+NO_2,		III-2, III-3,
CK.		IV-1.
O_3	0.00	I-3, I-4,
	0.10	II-1, II-3,
	0.20	III-2, III-3,
	0.30	IV-1, IV-2.
SO_2	0.00	I-3, I-4,
	0.10	II-1, II-3,
	0.20	III-2, III-3,
	0.30	IV-1, IV-2.

*Fumigations were conducted four hours daily for 35 consecutive days.
**Four observations per treatment.

Table 2. Fumigation program of eastern white pine (Pinus strobus L.) in 1980*.

Treatment**	Pollutant concentration	Clone used
	-- ppm --	
O_3, SO_2, NO_2,	0.10	I-1,
O_3+SO_2, O_3+NO_2, SO_2+NO_2,		II-1, II-3,
O_3+SO_2+NO_2,		III-2, III-3,
CK.		IV-1.
O_3	0.00	II-1,
	0.10	III-2,
	0.20	IV-2.
	0.30	
SO_2	0.00	II-1,
	0.10	III-2,
	0.20	IV-2.
	0.30	
NO_2	0.00	II-1,
	0.10	III-2,
	0.20	IV-2.
	0.30	

*Fumigations were conducted four hours daily for 50 consecutive days.
**Four observations per treatment.

Inc., Santa Barbara, CA 93101). Temperature and relative humidity were also determined periodically with dry bulb-wet bulb hydropsychrometer and recorded by Speedomax Compact Multipoint Recorder Model 547 (Leeds and Northrup, North Wales, PA 19454).

The air passing through these chambers was filtered with activated charcoal to remove nitrogen oxides, sulfur dioxide, ozone, and other impurities. Pollutants were added to this filtered air from individual sources and adjusted by rotometers to the desired concentration in each chamber. In 1980, a coil cooling unit was added to the air intake system. The intaking air was passed through cooling coils prior to the charcoal filters in order to decreased temperature in the CSTR chambers during summer months.

Air pollutant concentrations (v:v) in each CSTR chamber were continuously sampled at 50 cm height above the chamber floor and then passed through an auto-switched solenoid system which were connected to three different monitors. The concentrations of each pollutant were recorded by Speedomax Model H Recorder. This setup provided a simultaneous monitoring of three different pollutants at each chamber at any given time.

Ozone was generated by a Welsbach Laboratory Ozonator Model T-408 (Welsbach Ozone Systems Corporation, Philadelphia, PA 19129). The ozone enriched air was carried by 0.64 cm teflon tubings to 10.16 cm ducts supplied with charcoal-

filtered air just prior to the entry into CSTR chambers. A rotometer was installed in each teflon line to facilitate controlling ozone flow. Ozone concentrations could also be adjusted by regulating voltage output rheostat on the ozone generator. The ozone concentrations were monitored by a Bendix Model 8002 Chemiluminescent Ozone Analyzer (Bendix Process Instruments Division, Lewisburg, WV 24901). In the first year (1979), ozone monitor was calibrated by a known source of ozone generated by a Bendix Model 8852 Dynamic Calibration System. In the second year (1980), the same ozone monitor was calibrated by a Photocal 3000 Automated Ozone Calibrator (Columbia Scientific Industries, Austin, TX 78766). Both calibration procedures were performed using three to five data points following the calibration procedures recommended by the United States Environmental Protection Agency (USEPA).

Sulfur dioxide was obtained as commercial high-pressured bottled gas diluted in nitrogen (1.03% sulfur dioxide purity). It was delivered to each CSTR chamber by teflon tubing and its flow was controlled by a series of rotometers. The sulfur dioxide concentrations in 1979 were monitored by a Meloy Sulfur Dioxide Analyzer Model SA 285E (Meloy Laboratories, Inc., Springfield, VA 22151) which was calibrated with the Bendix Dynamic Calibration System using a sulfur dioxide permeation tube. In 1980, the sulfur dioxide concentrations were monitored by a Pulsed Fluorescent Ambient Sulfur Dioxide

Analyzer Model 43 (Thermo Electron Corporation, Environmental Instruments Division, Hopkinton, MA 01748) which was calibrated as in the 1979 procedures.

Nitrogen dioxide was supplied by industrial high pressure bottled nitrogen dioxide gas bottle diluted in either nitrogen or air (1.05% nitrogen dioxide purity). The nitrogen dioxide concentrations were monitored with a Bendix Model 8101B NO-NO2-NOx Chemiluminescent Analyzer. For the first year's fumigation, the calibration of monitor was done with the Bendix Dynamic Calibration System using a nitrogen dioxide permeation tube. In the second year, the same nitrogen dioxide monitor was modified according to new USEPA 1980 requirements and calibrated as the first year's methods.

Measurement of Parameters

In 1979, foliar symptom expressions and needle length of current year needles were measured weekly during the 35-day fumigation period. All fascicles on the new growth were visibly evaluated for pollutant-induced symptoms. Injury was recorded as the percent of total needle area symptomatic. The total injured area was counted as a combination of chlorotic spot, chlorotic band, necrotic spot, necrotic band, tip-burn, and any other pollutant induced symptoms. Needle length was determined by the average of the oldest 10 fascicles (the lowest part of the current year's growth).

In 1979, needle dry weight of three chosen clones were

measured at the end of 35 days fumigation. The five oldest fascicles of main shoot of each treated plant were sampled for this purpose. The dry weight of these fascicles was measured with a Mettler 3-digital Electronic Balance Model PT 320 after freeze-drying fresh tissues for 24 hours with a Labcones Freeze Dryer-5 Model 75050 (Labconco Co. Kansas City, MO 64132).

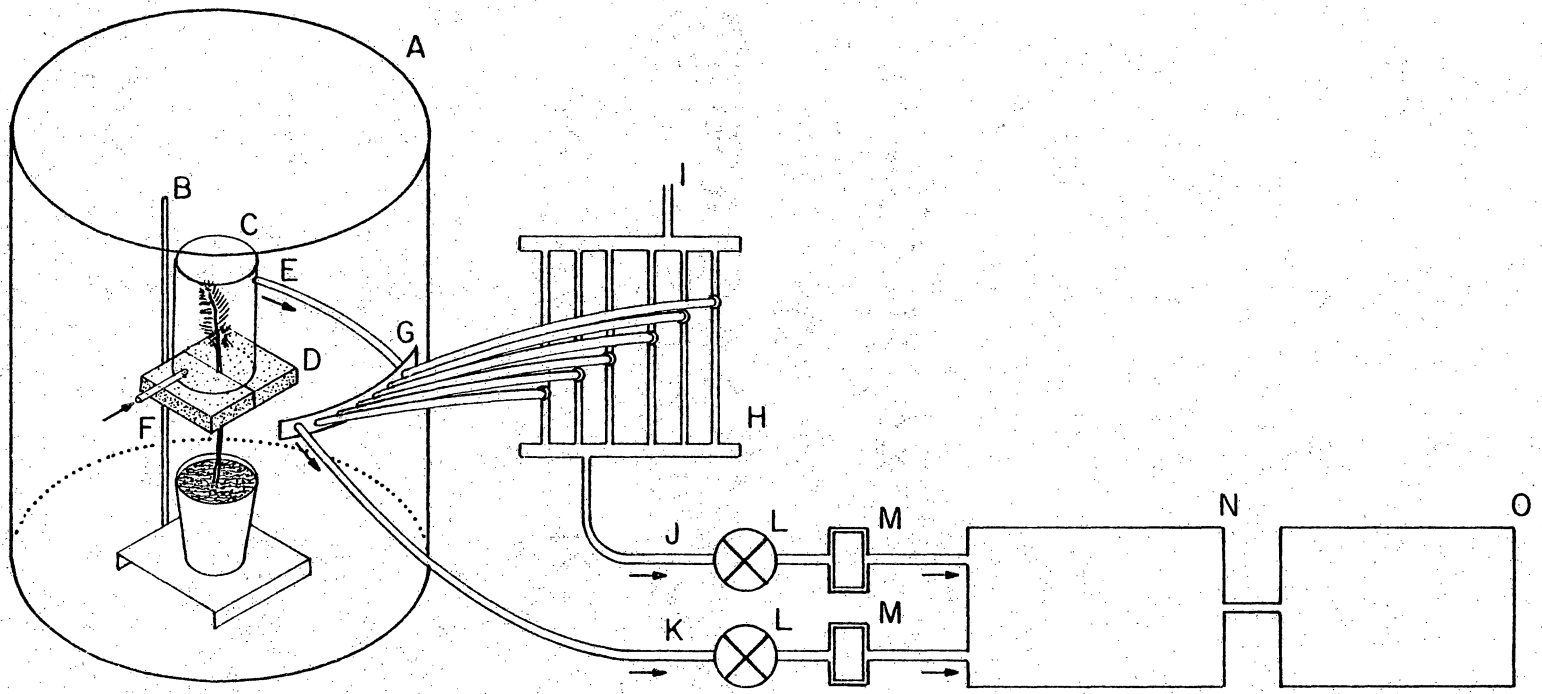
In 1980, the foliar symptom expression and needle length of current year needles in the set of 0.10 ppm fumigation were measured weekly and at the end of 50-day fumigation. Net photosynthesis, photosynthetic transpiration, dark respiration, dark respiratory transpiration, and chlorophyll content of current year needles were measured at 10 days interval during the fumigation of 0.10, 0.20, and 0.30 ppm ozone alone, sulfur dioxide alone, and nitrogen dioxide alone. Needle dry weight of treated plants at the end of fumigation was determined following the same methods as in 1979.

Gas exchange rates of current year needles during daily four-hour exposures were measured with an Infrared Non-dispersive Dual-gas (i.e. carbon dioxide and water vapor) Analyzer (IRGA) Model AR 600R (Anarad, Inc., Santa Barabara, CA 93105). At each sampling day, the main branchlet of current year's growth was enclosed within a glass minichamber which was modified from one-liter Pyrex beaker with two 0.64 cm I. D. air outlet arms. Gas exchange measurements were made zero hour prior to, hourly, and one hour after daily four-

hour pollutant exposures. Plants were returned to greenhouse one hour after the termination of daily pollutant exposure. There were three measurements of each clone in each treatment. The minichambers were situated on top of two pieces of styrofoam and mounted on a ring stand within CSTR chamber. The detail setup of this minichamber and flow chart of gas flow were illustrated in Figure 1.

Since the minichamber design was an air-sealed compartment with open flow system (Wolf et al., 1969), the loose seam between two styrofoam pieces and the bottom air inlet arm of minichamber were necessary in order to provide the entrance of needed air for gas exchanges in the minichamber. Air was withdrawn separately at the same rate with diaphragh pumps from top air outlet of minichamber (as the sample air) and from CSTR chamber (as the reference air). The air from these two lines was connected to IRGA after passing through rotometers for differential carbon dioxide and water vapor measurements.

The flow rate of sample and reference line was set at 1.2 liter/minute as compared to approximately 0.9 liter net volumn of minichamber after confinement of single pine branchlet. Such flow rate was determined experimentally in this study to be sufficient to ensure that the rate of photosynthesis and transpiration within minichamber was not limited by diffusion through unstirred air in the chamber. Pollutant concentrations and temperatures in the minichamber



LEGEND

A: CSTR Chamber
B: Ring Stand
C: Minichamber
D: Styrofoam Support
E: Air Outlet
F: Air Inlet
G: Air Sampling Ports
H: Six-lined Manifold
I: Exhaust for By-passing Chambers
J: Sample Line
K: Reference Line
L: Diaphragh Pumps
M: Flow Meters
N: Dual-gas IRGA
O: Dual-pan Recorder

Figure 1. Schematic Diagram of Apparatus and Flow Chart of Gas Flow for Measuring Photosynthesis and Transpiration.

were tested against the conditions in the CSTR chamber. They were 0.01 ppm lower and 0.1°C higher than CSTR's when the ozone concentration and air temperature in CSTR were 0.30 ppm and 32°C, respectively.

The addition of manifold installation in this study (Figure 1) with a larger diaphragh pump drawing air from unmeasuring minichambers at the same flow rate as measuring minichamber tremendously increased the number of measurements within each time unit. The measurements on IRGA was transmitted to a dual-pen recorder. Conversion of these data to the calibrated results was done by modifying with the standard curve of each gas.

For the calculation of photosynthesis and transpiration rates, total needle area of each branchlet enclosed in minichamber during gas exchange measurements was obtained by multiplying needle length by perimeter. Perimeter was calculated by assuming that a five-needled fascicle approximated a solid cylinder (Kozlowski and Schumacher, 1943; Madgwick, 1964; Wood, 1971). Taper only occurred at the extreme fascicle tip and was treated as negligible on surface area calculation. Fascicle radii were obtained by measuring needle cross sections with a Spenser Hemacytometer. Actual needle length and numbers of needles of each branchlet at each measurement were recorded and averages were obtained.

The designs of such air-sealed minichamber allowed continuous recording of small rapid fluctuations and changes in

photosynthesis and transpiration rate with considerable accuracy (Šesták et al., 1971). It also provided a leak- and damage-free micro-environment for gas measurements. A similar chamber has been used in field for cereals and grasses photosynthesis studies and was proven particularly useful because its quick, simple gas exchange measurements (Wolf et al., 1969).

The rates of dark respiation and dark respiratory transpiration were measured following the same procedures as in photosynthesis and photosynthetic transpiration but at dark periods (2200-0400 EST). These measurements were taken at 10-day intervals from the same branchlet but five days before photosynthesis measurements. At each sampling date, day time exposures were terminated at hour 1900. Plants were moved once again from greenhouse and exposed to designated pollutant concentrations from hour 2300 to hour 0300 in the following day. The CSTR chambers were kept in darkness by sealing off any possible leaks of what in CSTR chamber room.

Chlorophyll content of current year needles was measured every 10 days during 50-day fumigation followed the procedures of Vernon (1960): Two grams of fresh needle segments was homogenized with an Omni-mixer (Ivan Sorvall Inc., Newtown, CT 06470) in 80% cold acetone for 5-10 minutes in darkness and under low temperature. The extract was filtered through Whatman number one filter paper and collected in an 100 ml volmetric flask. The filtrate was then re-extracted

following the same method twice with 80% cold acetone in Omni-mixer for three minutes. New extracts were combined with the first extract and centrifuged at 5,000 rpm for five minutes. The supernatant was poured into a volmetric flask and made up to 100 ml volume with 80% acetone and stored in darkness. The absorbance of this solution between 400-700 nm wavelength was measured with a Unicam SP 800 spectrophotometer (Unicam Instruments Limited, Cambridge, England). All absorbance readings were corrected for individual cuvette absorbance. The concentration of chlorophyll a, b, and a + b, were determined by following the formula given by Vernon (1960).

chlorophyll a (mg/liter) = 12.7 (A663) - 2.69 (A645)

chlorophyll b (mg/liter) = 22.9 (A645) - 4.68 (A663)

where A663 indicated the amount of absorbance at 663 nm and A645 indicated the amount of absorbance at 645 nm.

RESULTS

A. Foliar Symptoms Induced by Ozone

Visible symptoms induced by ozone on eastern white pine current year needles ranged from general chlorosis, pigmented mottling, necrotic banding to necrotic tip-burn. Mottling varied from yellow, red to tan color depending on white pine clone and stage of symptom development. The predominant symptoms resulting from low-dose ozone fumigations were pigmented mottling and necrotic tip-burn. Premature defoliation only occurred in the very sensitive clones.

Ozone injured areas were categorically distinguishable in each test fumigation set among sensitive (class I and II), intermediate (class III), and tolerant (class IV) classes regardless of pollutant treatments (Figures 2, 3, 4, 5). Sensitive clones always exhibited more severely injured needles than intermediate and tolerant clones. In 1979, the exposure of 0.10 ppm ozone for 35 consecutive days (Figure 3) caused more foliar injury than 0.05 ppm exposure (Figure 2). However, such a positive relationship between ozone dosages and symptom expressions was less obvious when ozone concentrations were above 0.10 ppm (Figure 5).

B. Foliar Symptoms Induced by Sulfur Dioxide

Sulfur dioxide induced visible symptoms on white pine current year needles occurred as either yellowish chlorosis,

chlorotic mottling, pigmented mottling ranging from yellow, pink, red, brown to tan, necrotic banding, necrotic tip-burn, or premature defoliation. In general, these symptoms were indistinguishable from ozone-induced symptoms except in the early stage of symptom development. At that time, symptoms of sulfur dioxide exposure were predominately chlorosis or chlorosis-related discoloration while necrotic symptoms were prevalent in ozone exposures. At the end of four hours daily, 35-day-long fumigation, more severe foliar injury was induced by 0.10 ppm sulfur dioxide both on sensitive and intermediate clones when compared with 0.05 ppm sulfur dioxide treatment (Figures 2, 3). No symptoms were visible on tolerant clones following any of the above exposures.

When sulfur dioxide concentrations were increased to 0.10, 0.20, and 0.30 ppm (Figure 6), the types of symptoms were the same as those induced by lower concentrations (Figures 2, 3, 4), however, the total injured areas were not proportional to pollutant concentrations used (Figure 6). For clone IV-1, the only symptom observed was necrosis of 5% of the surface area at the end of 0.3 ppm exposure (Figure 6).

C. Foliar Symptoms Induced by Nitrogen Dioxide

Nitrogen dioxide alone at the concentrations tested (Tables 1, 2) rarely caused visible symptoms on white pine current year needles. Among six tested clones, clone II-3 was the most sensitive clone to nitrogen dioxide exposure

followed by clone I-1 (Figures 3, 4). Fifteen and five percent of surface areas, respectively, of these two clones were injured by 0.10 ppm nitrogen dioxide at the end of experiment. They were the only two clones exhibited foliar injury that resulting from nitrogen dioxide treatment. Nitrogen dioxide induced symptoms were characterized by mottling, necrotic banding, or necrotic tip-burn. None of the tested clones had any nitrogen dioxide related symptom at 0.05 ppm exposure (Figure 2).

D. Timing of The First Visible Symptom

Symptom development on pine needles involved an intensification of the first visible symptom throughout the entire experiment period. Initial minor lesion might become dramatic discoloration by color changes or coalesce the affected area to a severe injured area. Generally speaking, the first ozone or sulfur dioxide induced visible symptom on sensitive clones was observed between 0-2 weeks at 0.05 ppm exposure and 0-1 week at 0.10 ppm exposure after the initiation of fumigation. When concentrations higher than 0.10 ppm were used, macroscopic symptoms were visible at the first week after the beginning of treatment. For intermediate sensitive clones, the first pollutant-related symptom was noticed at 2-3 weeks at 0.10 ppm and 1-2 weeks at concentrations higher than 0.10 ppm after the first day of fumigation. No visible injury was found in intermediate and tolerant clones exposed

to 0.05 ppm pollutants (Figure 2). Pollutant combinations, in most cases, induced the first foliar symptom several days earlier than single pollutant treatment.

E. Foliar Symptoms Induced by Pollutant Combinations

The types of symptoms caused by pollutant combinations of ozone, sulfur dioxide, and/or nitrogen dioxide virtually included all types of symptoms induced by individual pollutant. Pollutant combinations either caused less than additive, additive, or more than additive effects in terms of total injured area depending upon species of pollutant, pollutant concentration, and clone of eastern white pine (Figures 2, 3, 4). In general, more than additive effects were more common in intermediate clones and tolerant clones (such as clone III-3 in Figure 3, and clone III-3 and VI-1 in Figure 4). However, clone III-2 exhibited less than additive effects at ozone + sulfur dioxide, sulfur dioxide + nitrogen dioxide, and ozone + sulfur dioxide + nitrogen dioxide exposures while exhibited more than additive effect at ozone + nitrogen dioxide exposure (Figures 3, 4). For sensitive clones, pollutant combinations prevalently caused less than additive effects either at 0.05 ppm or 0.10 ppm concentration (Figures 2, 3, 4). The more than additive effects of pollutant combination on sensitive clones was only found in clone I-2 at 0.05 ppm ozone + sulfur dioxide and sulfur dioxide + nitrogen dioxide exposures (Figure 2), and in clone I-1 at

0.10 ppm ozone + nitrogen dioxide exposure (Figure 3). The additive effect of pollutant combination on total injury area was found in clone II-1 at 0.05 ppm sulfur dioxide + nitrogen dioxide (Figure 2) and in clone II-3 at 0.10 ppm ozone + nitrogen dioxide treatment (Figure 4).

Needle Elongation Affected by Pollutants

Under pollution-free environments, the growth of current year needles of white pine clones were very similar (Figures 7-11).

When exposed to ozone, sulfur dioxide, and/or nitrogen dioxide singly at chronic concentrations, plant responses varied with pollutant species, white pine clone, and pollutant dosage. The suppression of needle growth due to pollutant fumigation was more evident in sensitive clones than in intermediate or tolerant clones (Figure 9). Such response was especially obvious with high pollutant concentrations (Figures 10, 11).

Based on needle length measurements, clone I-1 was ranked very sensitive (P=0.05) to ozone exposure, clone IV-1 to sulfur dioxide (Figure 8), and clone I-2, II-3, and III-2 to both ozone and sulfur dioxide alone treatment (Figure 9). The exposure of nitrogen dioxide alone at test conditions exerted no significant effects in needle length at the end of 35 days fumigation except there was an adverse effect in clone II-3 due to 0.10 ppm exposure (Figure 9). The benefi-

cial effect of chronic pollutant exposure on needle growth was found in clone III-3 at 0.05 ppm sulfur dioxide fumigation (Figure 7).

When fumigation period was extended from 35 days (in 1979 experiment) to 50 days (in 1980 experiment), the inhibitory effect of 0.10 ppm ozone alone was again observed in clone I-1 , and sulfur dioxide alone on clone II-1 and II-3 (Figure 9). Neither clone IV-1 nor clone III-2 exhibited sensitive reactions to sulfur dioxide alone and ozone alone exposure, respectively, as in 1979. In nitrogen dioxide exposures, none of the needle length of tested clones was found different from controls (Figure 9).

At higher concentrations (i.e. 0.10, 0.20, and 0.30 ppm) of single pollutant exposures, ozone significantly reduced needle length in clone II-1 at 0.20 and 0.30 ppm, and clone II-3 at 0.10 and 0.20 ppm concentration (Figure 10) at the end of a four-hour daily, 35 consecutive days fumigation. In intermediate clones, the only significant decrease of needle length due to above ozone treatments was found in clone III-2 at 0.30 ppm concentration.

Exposure to 0.10, 0.20, and 0.30 ppm sulfur dioxide significantly reduced needle length by 1/4 to 1/3 of control plants in clone I-3, I-4, II-1, and II-3 (Figure 11) at the end of long-term fumigation. Sulfur dioxide caused decreases in needle length on clone III-2 and III-3 but only the suppressions at 0.10 ppm on clone III-2, and 0.10 and 0.20 ppm

on clone III-3 were statistically significant (Figure 11).

In clone IV-1 and IV-2, none of ozone nor sulfur dioxide treatments (up to 0.30 ppm) significantly (P=0.05) decreased needle length at the end of a 35 consecutive day fumigation (Figures 10, 11).

In pollutant combinations, no benificial or adverse effect of 0.05 ppm pollutant combination was found in needle elongation of clone II-1, II-3, and IV-2. Significant reduction of needle length was observed in clone I-2 by ozone + nitrogen dioxide, and in clone III-2 by sulfur dioxide + nitrogen dioxide exposure (Figure 7).

All pollutant combinations induced significant reductions in needle length in clone II-3 and III-2 at 0.05 ppm concentration (Figure 8). However, only the reductions in clone I-1 for ozone + sulfur dioxide and clone IV-1 for ozone + sulfur dioxide + nitrogen dioxide treatments were statistically significant (Figure 8).

In 1980, at the end of four hours daily, 50 consecutive days exposure at 0.10 ppm concentration, all of pollutant combination exposures (i.e. ozone + sulfur dioxide, ozone + nitrogen dioxide, sulfur dioxide + nitrogen dioxide, and ozone + sulfur dioxide + nitrogen dioxide) significantly reduced needle length in clone I-1, II-1, and II-3 (Figure 9) when compared with control. Triple pollutant combination significantly reduced needle length in clone III-2 and III-3 by the end of 50 day exposure. None of pollutant combination

exposures significantly affected needle growth in clone IV-1 at P=0.05 level (Figure 9).

Needle Dry Weight

The average needle dry weight of three chosen clones after been exposed to 0.10 ppm ozone, sulfur dioxide, and/or nitrogen dioxide for four hours daily for 35 consecutive days was presented in Table 3. In clone II-1, all pollutant-treated plants had less biomass production than controls. The maximum reduction of biomass was found in ozone + sulfur dioxide combination treatment. Ozone alone, sulfur dioxide alone, and ozone + sulfur dioxide + nitrogen dioxide combination exposure caused significant (P=0.01) biomass reduction in this clone (Table 3).

In clone III-2, nitrogen dioxide alone, ozone + nitrogen dioxide, and sulfur dioxide + nitrogen dioxide exposure did not cause statistically significant changes in needle dry weight as compared with controls. Triple pollutant combination was found at 5% level significance while ozone alone, sulfur dioxide alone, and ozone + sulfur dioxide exposures at 1% level significance in causingneedle dry weight reduction in clone III-2 (Table 3).

None of the pollutant-treated plants were observed to have less biomass production (P=0.05) than controlled fumigation in clone IV-2 (Table 3).

In the higher dose of single pollutant (i.e. at 0.10,

0.20, and 0.30 ppm concentration), ozone significantly reduced needle dry weight in clone II-1 at all three concentration. In contrast, only 0.10 ppm and 0.20 ppm ozone exposures in clone III-2, and 0.20 ppm in clone IV-2 induced statistically significant biomass reduction (Table 4). The fumigation of 0.30 ppm sulfur dioxide significantly caused needle dry weight reduction in all three clones as well as 0.20 ppm in clone II-1 and 0.10 ppm in clone III-2. Under the same pollutant dosage, nitrogen dioxide only reduced needle biomass production in clone II-1 at 0.30 ppm exposure. No other significant biomass changes were observed at any of nitrogen dioxide test dosage in all test clones (Table 4).

Chlorophyll Content

Rresults of chlorophyll determination at the end of 50-day exposure period indicated that both chlorophyll a and chlorophyll b were reduced in plant tissues at all ozone and sulfur dioxide tested concentrations as well as in 0.20 and 0.30 ppm nitrogen dioxide exposures (Table 5). The reduction of chlorophyll content due to pollutant fumigation was more severe in sulfur dioxide and ozone exposures than in nitrogen dioxide exposures. Stimulatory effect of nitrogen dioxide on chlorophyll content was found in 0.10 ppm exposure. However, when the concentration of nitrogen dioxide was increased, a beneficial response was no longer observed and adverse effect became evident although the effect was not statistically sig-

nificant. Sulfur dioxide significantly decreased chlorophyll a and b concentrations in all three clones at 0.30 ppm concentration, in contrast, only clone II-1 and III-2 were affected by the same dosage of ozone (Table 5).

Net Photosynthesis Affected by Pollutant Fumigations

Since several pollutant treated clones exhibited reduction in needle length or biomass production without visible foliar symptoms (Figures 2-6; Tables 3,4), plant photosynthesis, the priciple assimilatory metabolism in vegetation, was monitored to determine the impact of pollutants on this process.

A. Effects of Ozone on Net Photosynthesis

In clone II-1 (sensitive clone), there was a reduction of net photosynthesis within the first hour after the beginning of ozone exposure. The magnitude of such reduction was proportional to ozone concentrations; 16-19% decrease for 0.30 ppm, 8-12% for 0.20 ppm, and 5-7% for 0.10 ppm (Figure 12A). Following the termination of ozone exposures, net photosynthesis recovered at various rates. In general, the higher the ozone concentration during fumigation, the longer the time needed to recover from ozone inhibited carbon dioxide assimilation. In none of the treated plants did net photosynthesis attain pre-treatment rates at one hour after the termination of ozonation.

Similar inhibition of net photosynthesis resulting from

ozone exposure was observed in clone III-2 (intermediate sensitive clone) and IV-2 (tolerant clone). However, the degree of reduction was much less than in clone II-1. The lowest rate of carbon dioxide uptake during four-hour ozone exposure was 92% of pre-treatment value at 0.10 ppm, 90% at 0.20 ppm, 88% at 0.30 ppm in clone III-2 (Figure 13A), and 98% at 0.10 ppm, 96% at 0.20 ppm, 93% at 0.30 ppm in clone IV-2 (Figure 14A), respectively.

B. Effects of Sulfur Dioxide on Net Photosynthesis

Daily exposure to sulfur dioxide decreased net photosynthesis in clone II-1, III-2, and IV-2. The maximum inhibition of carbon dioxide uptake during daily four-hour fumigation in clone II-1 was 7%, 13%, and 28% at 0.10, 0.20, and 0.30 ppm, respectively (Figure 15A). The corresponding figures were 3%, 11%, and 19% in clone III-2 (Figure 16A), and -2%, -2%, and -4% in clone IV-2 (Figure 17A). Net photosynthesis generally reached to its lowest point after the first two hours of fumigation and then maintained a constant rate during the remaining hours of daily fumigations. A recovery response of net photostnthesis was observed after pollutant exposures ended.

C. Effects of Nitrogen Dioxide on Net Photosynthesis

Four hour nitrogen dioxide exposures resulted in slight decrease of net photosynthesis in clone II-1 and III-2 (Figures 18A, 19A). The maximum reduction as obserbed at exposure

of 0.20 ppm and 0.30 ppm concentration was 8% and 9%, respectively in clone II-1, and 8% in clone III-2 for 0.30 ppm exposure. No reduction in the rate of net photosynthesis was observed in clone IV-2 at any nitrogen dioxide concentration.

Transpiration Affected by Pollutants

A. Effects of Ozone on Photosynthetic Transpiration

In clone II-1, there was a 1-2%, 3-4%, 4-8%, and 7-11% reduction of transpiration during four-hour ozone exposures at 0, 0.10, 0.20, and 0.30 ppm, respectively (Figure 12B). None of the test plant showed a complete stomatal closing due to ozone treatment. Recovery of partial inhibited stomata opening occurred after the termination of pollutant exposure (Figure 12B).

During the same four-hour ozone fumigation, the maximum reduction in photosynthetic transpiration was 2% of the pre-treatment value in control plants, 6% at 0.10 ppm, 9% at 0.20 ppm, 10% at 0.30 ppm in clone III-2 (Figure 13B) as compared to 0%, 2%, 4%, and 7% accordingly in clone IV-2 (Figure 14B). Recovery of transpiration was also noticed in clone III-2 and IV-2 at one hour after the end of ozone exposure.

B. Effects of Sulfur Dioxide on Photosynthetic Transpiration

Fumigation with sulfur dioxide increased photosynthetic transpiration in all three clones under test conditions (Figures 15B, 16B, 17B). The magnitude of transpiration increase

varied with clone and the concentration of sulfur dioxide.

Transpiration rate of clone II-1 was increased 7% at 0.30 ppm, 4% at 0.20 ppm, and 2% at 0.10 ppm after one hour of sulfur dioxide exposure (Figure 15B). Recovery of transpiration at 0.30 ppm exposure was found to be more dramatical than at the other two concentrations in this clone. However, none of tested concentrations resulted in full recovery of photosynthetic transpiration in clone II-1 at one hour after termination of pollutant exposure. Similar stimulatory effects of sulfur dioxide on transpiration was found in clone III-2 (Figure 16B), and clone IV-2 (Figure 17B). Such increased rates were 2%, 4%, and 5% in clone III-2 and 2%, 4%, and 4% in clone IV-2 for 0.10, 0.20, and 0.30 ppm sulfur dioxide, respectively.

C. Effects of Nitrogen Dioxide on Photosynthetic Transpiration

All nitrogen dioxide exposed plants exhibited a decrease of photosynthetic transpiration during four-hour exposure regardless of clone and pollutant concentration. The maximum depression observed was 4%, 7%, 9% in clone II-1, 4%, 10%, 8% in clone III-2, and 5%, 8%, 8% in clone IV-2 for 0.10, 0.20, and 0.30 ppm nitrogen dioxide, respectively (Figures 18B, 19B, 20B). Recovery of transpiration was noticed in every treatment after the termination of nitrogen dioxide exposure. Recovery rates varied with pollutant concentration and clone.

Dark Period Gas Exchanges Affected by Pollutant Exposures

In this study, the procedures for carbon dioxide and water vapor evolution measurements at night were the same as those in light environments. Plants were kept in darkness for four hours before dark respiration was measured. At each sampling day, they were exposed to designed pollutant concentration from hour 2300 EST to hour 0300 in the following day.

A. Effects of Ozone on Dark Gas Exchange Rates

Responses of dark respiration and transpiration to ozone exposure were presented in Figures 12C-D, 13C-D, and 14C-D for clone II-1, III-2, and IV-2, respectively.

There was an increase of dark respiration in clone II-1 at all test ozone concentrations. The highest rate occurred during a four-hour exposure was 103%, 106%, and 109% of pre-treatment value for 0.10, 0.20, and 0.30 ppm ozone, respectively. No recovery of dark respiration was noticed at one hour after the termination of dark period pollutant exposure. A simultaneous increase or decrease of water vapor evolution in clone II-1 was measured along with above dark respiration depending upon ozone concentrations (Figures 12C, D).

In clone III-2, similar increase of dark respiration and variable changes in transpiration were observed under the same ozone exposure. During four-hour exposure period, none of the hourly measurement of carbon dioxide and water vapor evolution exceeded 5% of pre-treatment value at 0.10 and 0.20

ppm exposure. It was very interesting to note that dark respiration of clone III-2 at 0.30 ppm ozone exposure continuously increased (4% higher) over the test period while simultaneous transpiration measurement decreased (5% lower) (Figures 13C, D).

In clone IV-2, the changes of these two gas exchanges due to ozone exposure within the same four-hour period were less than 5% of pre-treatment value at 0.10 and 0.20 ppm concentration. At 0.30 ppm concentration, ozonation resulted in an 7% decrease of dark respiration and 5% decrease of transpiration (Figures 14C, D).

B. Effects of Sulfur Dioxide on Dark Gas Exchange Rates

There were no significant changes of carbon dioxide and water vapor evolution due to sulfur dioxide exposure at 0.10, 0.20, and 0.30 ppm in clone II-1 (Figures 15C, D) , III-2 (Figures 16C, D), and IV-2 (Figures 17C, D) under dark condition except at the exposure of 0.30 ppm ozone on clone II-1. A continued increase (up to 9%) of carbon dioxide evolution was observed at the end of four hour fumigation in this clone.

C. Effects of Nitrogen Dioxide on Dark Gas Exchange Rates

Exposure of white pine to nitrogen dioxide at 0.10, 0.20, and 0.30 ppm for four hours under dark condition exerted no significant changes on dark respiration and water

vapor evolution on clone II-1 (Figures 14C, D), III-2 (Figures 19C, D), and IV-2 (Figures 20C, D).

Effects of Long-term Pollutant Exposures on Net Photosynthesis

During the 50-day-long experiment (four hours daily), net photosynthesis of test plants were measured at 10 days interval. At each sampling day, rates of photosynthesis were determined one hour prior to, hourly during, and one hour after daily four-hour pollutant exposure. There were six measurements obtained from each plant. The average of these six values was calculated and referred to the first day's pre-treatment value in order to relate the growth pattern of each white pine clone under the influence of pollutant exposures.

The relative patterns of net photosynthesis of sensitive, intermediate, and tolerant clones in pollutant-free environments were very similar throughout the experimental period (Figures 21-23); started at 100% in an arbitrary scale as pre-treatment value, the net photosynthesis steadily increased during the first 4-5 weeks of experiment to its peak. Peak photosynthesis rates varied with clones; 196%, 188%, and 210% being the highest rate observed in clone II-1, III-2, and IV-2 respectively. Following the net photosynthesis rate reached to its maximum, it either stabilized or gradually decreased during the remainder of the experiment.

A. Effects of Long-term Pollutant Exposures on Clone II-1 Net Photosynthesis

In clone II-1, net photosynthetic rates of 0.10 ppm ozone, and 0.10 and 0.20 ppm sulfur dioxide treated plants increased slightly to 130-140% of the pre-treatment value within the first 20 days of exposure and then leveled off for the rest of experiment period (Figure 21). The net photosynthesis of 0.20 ppm ozone treated plants increased in the first 10 days of exposure (to 120%) and then continuously decreased until the end of 50-day exposure (Figure 21). The most evident response of clone II-1 due to ozone and sulfur dioxide alone exposure was at 0.30 ppm; a significant decrease of net photosynthesis was observed in the later part of experiment. It was even lower than pre-treatment values. Resultes indicats that regardless of continuous needle elongation over time, net photosynthesis gradually declined in clone II-1 due to ozone or sulfur dioxide treatment (Figure 21).

The effects of nitrogen dioxide exposure on clone II-1 long-term net photosynthesis was not significantly distinguishable from controls at 0.10 ppm and 0.20 ppm exposure. In contrast, there was a suppression of net photostnthesis rate (20% as the maximum) due to 0.30 ppm nitrogen dioxide exposure without visible symptoms (Figure 21).

B. Effects of Long-term Pollutant Exposures on Clone III-2 Net Photosynthsis

In the clean air treated plants of clone III-2, the patterns of net photosynthesis over the 50-days were very much alike (Figure 22). Peaks of photosynthetic rates were observed on the 30-40th day after the initiation of fumigation and ranged from 170-188% of the pre-treatment value.

The maximum rate of photosynthesis monitored in the ozone treated plants was 158% of pre-treatment value on the 40th of the experiment in 0.10 ppm exposure, 136% on the 50th day in 0.20 ppm, and 152% on the 30th day in 0.30 ppm exposure, respectively. At the end of 50-day experiment, net photosynthesis was 154% of pre-treatment value in controls, 148% in 0.10 ppm, 136% in 0.20 ppm, and 126% in 0.30 ppm ozone exposure, respectively (Figure 22).

In sulfur dioxide exposures, net photosynthesis rate of clone III-2 was 116%, 153%, 174%, 173%, and 168% of pre-treatment value in pollutant-free treatment, 125%, 147%, 160%, 164%, and 152% in 0.10 ppm exposure; 129%, 143%, 151%, 146%, and 140% in 0.20 ppm exposure; 114%, 129%, 132%, 122%, and 114% in 0.30 ppm exposure at the 10th, 20th, 30th, 40th, and 50th day of fumigation, respectively (Figure 22).

In clone III-2, no significant stimulatory or inhibitory effect of net photosynthesis was found in the first 30 days of nitrogen dioxide fumigation. However, net photosynthesis rates of pollutant-treated plants declined in the later part

of long-term pollutant exposure. This was especially evident in the high nitrogen dioxide concentration treatments (Figure 22). At the end of 50-day experiment, photosynthesis was 181%, 174%, 154%, and 161% of pre-treatment value for 0.0 ppm, 0.10 ppm, 0.20 ppm, and 0.30 ppm nitrogen dioxide treated plant, respectively (Figure 22).

C. Effects of Long-term Pollutant Exposures on Clone IV-2 Net Photosynthesis

Net photosynthetic rate of clone IV-2 was little influenced by pollutant exposure when fumigated with the same dose of pollutants as clone II-1 and III-2 (Figures 21-23). The patterns of net photosynthesis throughout the entire test period in pollutant-free and pollutant-treated plants were very similar in clone IV-2 regardless of pollutant species and pollutant dosage. At certain stages of fumigation, net photosynthetic rates were even higher in pollutant-exposed plants than in controls. The lowest rate of photosynthesis was found on the 20-40th day after beginning of ozone exposure, while in sulfur dioxide exposure it was occurred on the 30-50th day. There was no distinguishable difference due to nitrogen dioxide exposures on net photosynthesis of clone IV-2 (Figure 23).

Effects of Long-term Pollutant Exposures on Dark Respiration

In clone II-1, the maximum dark respiration of clean air treated plants was found between the 10-30th day after the

beginning of exposure. These values ranged from 110% to 126% of the pre-treatment values (Figure 24). Similar patterns were also observed in clone III-2 (Figure 25), and clone IV-2 (Figure 26).

Dark respiration of clone II-1 was found to steadily decrease due to all test ozone concentrations over long-term exposures. Respiration was 87%, 71%, and 60% of first day's pre-treatment value for 0.10 ppm, 0.20 ppm, and 0.30 ppm, respectively at the end of 50-day exposure (Figure 24).

An early increase of dark respiration was found in clone II-1 resulting from 0.30 ppm sulfur dioxide exposure. This increase was followed by a dramatic decline after 10-20 days of exposure (Figure 24). The same type of response of an early increase followed by a steady decrease in dark respiration was also observed in clone II-1 induced by 0.30 ppm nitrogen dioxide exposure, although the rate of increase was not as great as in sulfur dioxide treatment.

There was no significant difference on dark respiration induced by 0.10 ppm sulfur dioxide, and 0.10 ppm and 0.20 ppm nitrogen dioxide exposure in clone II-1. All of these treatments exhibited nearly identical dark respiration patterns as that of control plants (Figure 24). It is very interesting to note that the patterns of dark respiration in clone II-1 during the long-term pollutant exposures was in the agreement with net photosynthesis measurements (Figures 21, 24).

In clone III-2, ozone and nitrogen dioxide exposures had

no significant effects on dark respiration. However, daily exposure of 0.20 and 0.30 ppm sulfur dioxide induced an early increase of dark respiration in clone III-2 (Figure 25).

During 50-day-long monitoring period, the dark respiration of pollutant-treated plants had similar patterns as those of control plants in clone IV-2 (Figure 26). However, there was a constant slight decrease rate of dark respiration in pollutant-treated plants as compared to controls, expecially at the high pollutant concentration exposures. Such decreases of dark respiration were not evident in the first 20 days of fumigation and became distinct at the end of long-term exposure (Figure 26).

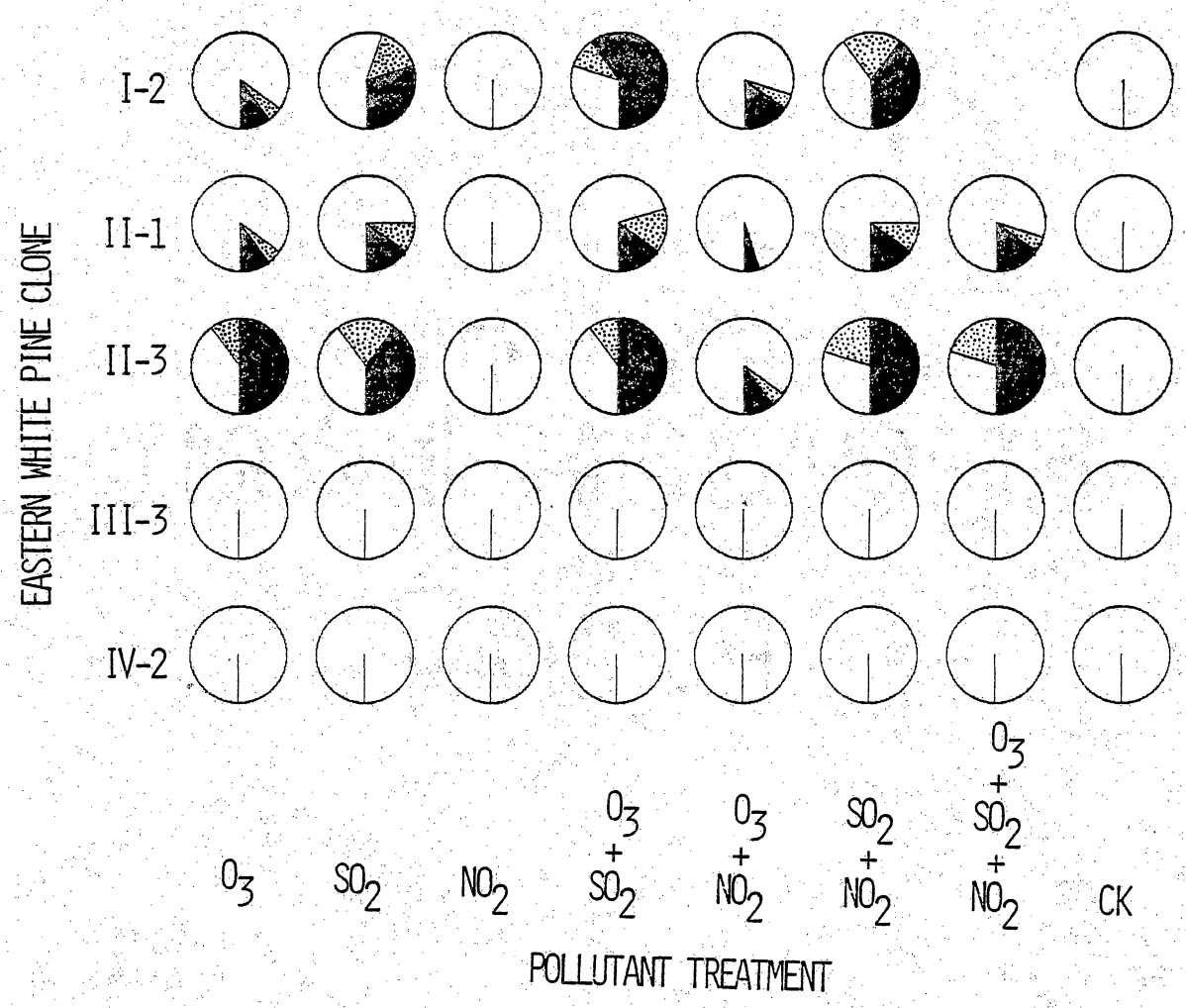


Fig. 2. Percent of chlorosis, mottling, and necrosis symptoms on current year eastern white pine needles after fumigated with 0.05 ppm of pollutants for 4 hours daily for 35 consecutive days. Total pie area is 100%. (chlorosis or mottling, necrosis, and unaffected.)

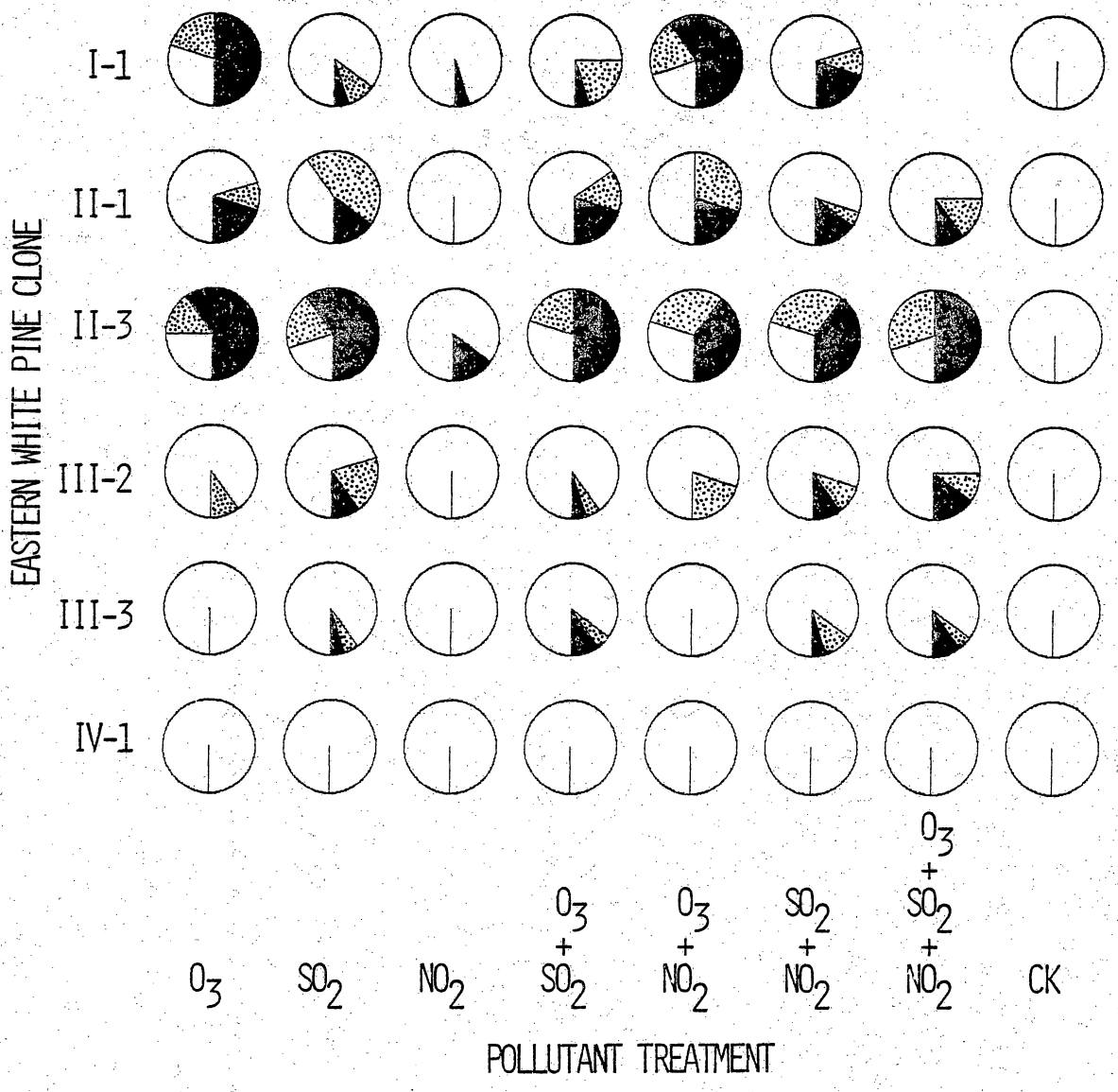


Fig. 3. Percent of chlorosis, mottling, and necrosis symptoms on current year eastern white pine needles after fumigated with 0.10 ppm of pollutants for 4 hours daily for 35 consecutive days. Total pie area is 100%. (chlorosis or mottling, necrosis, and unaffected.)

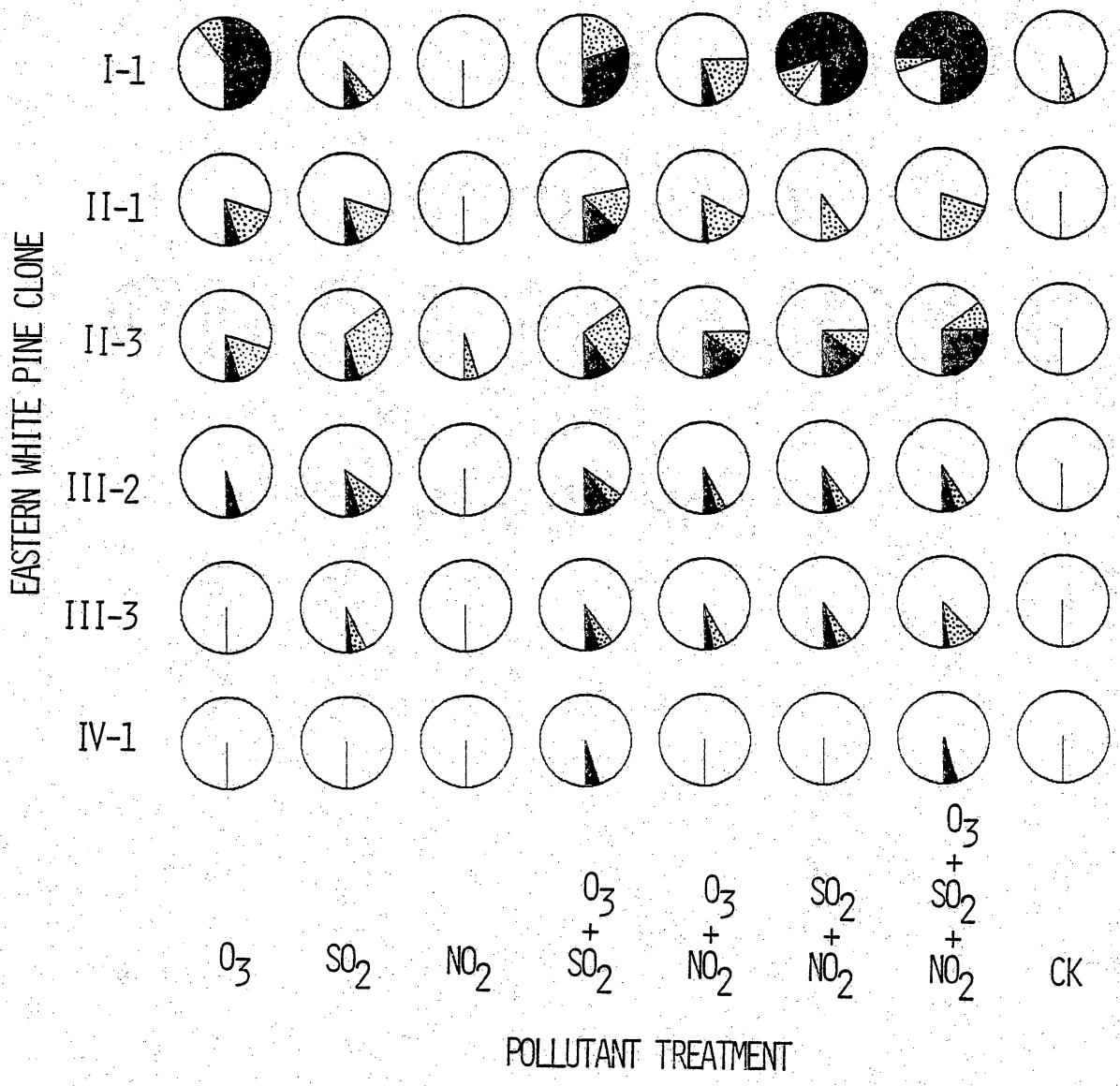


Fig. 4. Percent of chlorosis, mottling, and necrosis symptoms on current year eastern white pine needles after fumigated with 0.10 ppm of pollutants for 4 hours daily for 50 consecutive days. Total pie area is 100%. (▨ chlorosis or mottling, ■ necrosis, and □ unaffected.)

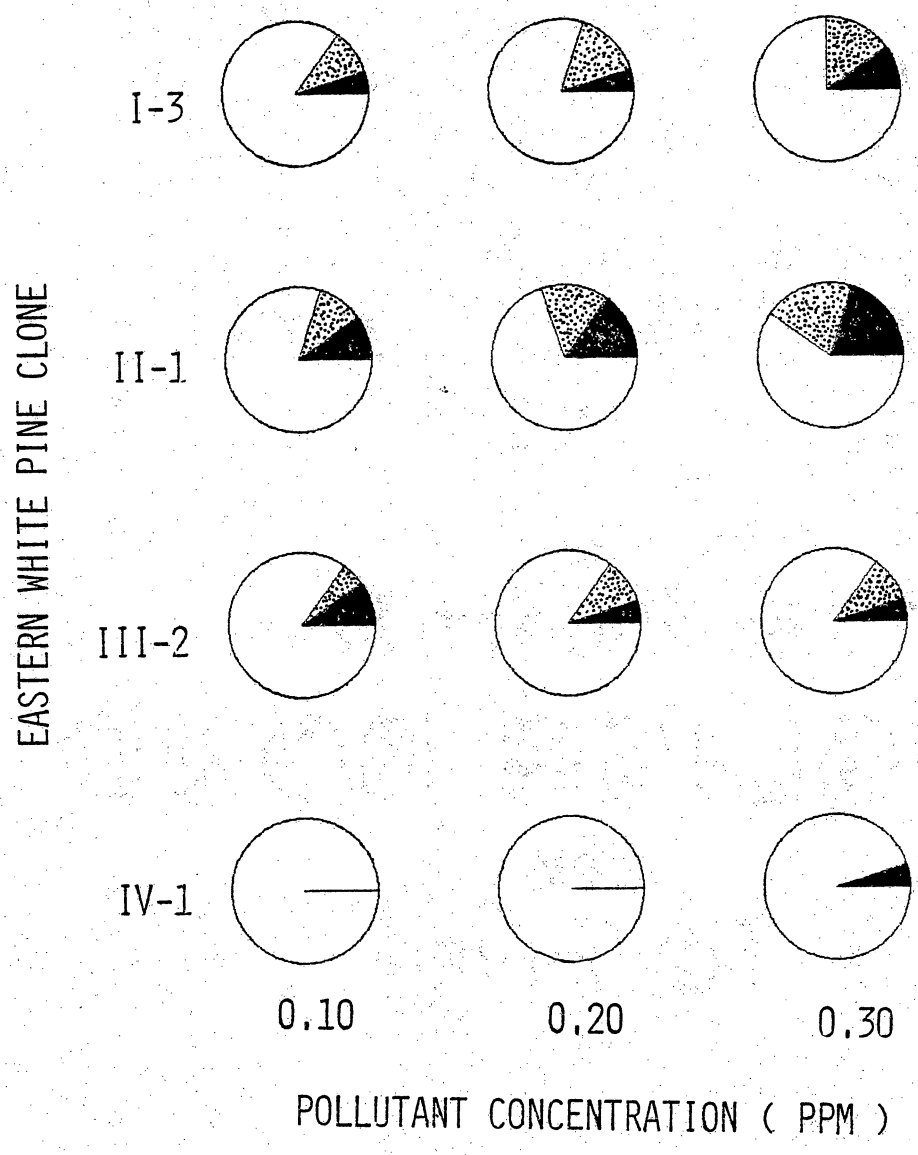


Fig. 5. Percent of chlorosis, mottling, and necrosis symptoms on current year eastern white pine needles after fumigated with 0.10, 0.20, and 0.30 ppm ozone for 4 hours daily for 35 consecutive days. Total pie area is 100%. (chlorosis or mottling, necrosis, and unaffected.)

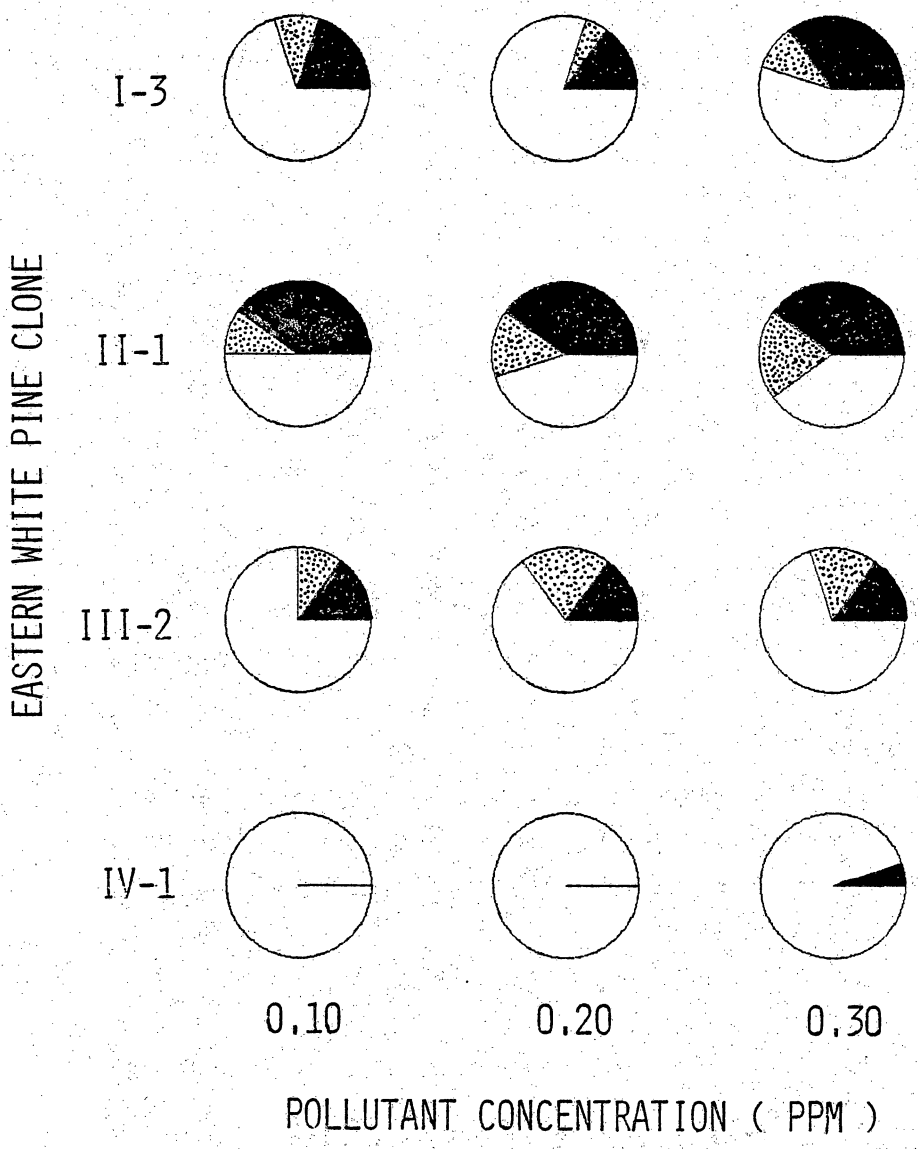


Fig. 6. Percent of chlorosis, mottling, and necrosis symptoms on current year eastern white pine needles after fumigated with 0.10, 0.20, and 0.30 ppm sulfur dioxide for 4 hours daily for 35 consecutive days. Total pie area is 100%. (chlorosis or mottling, necrosis, and unaffected.)

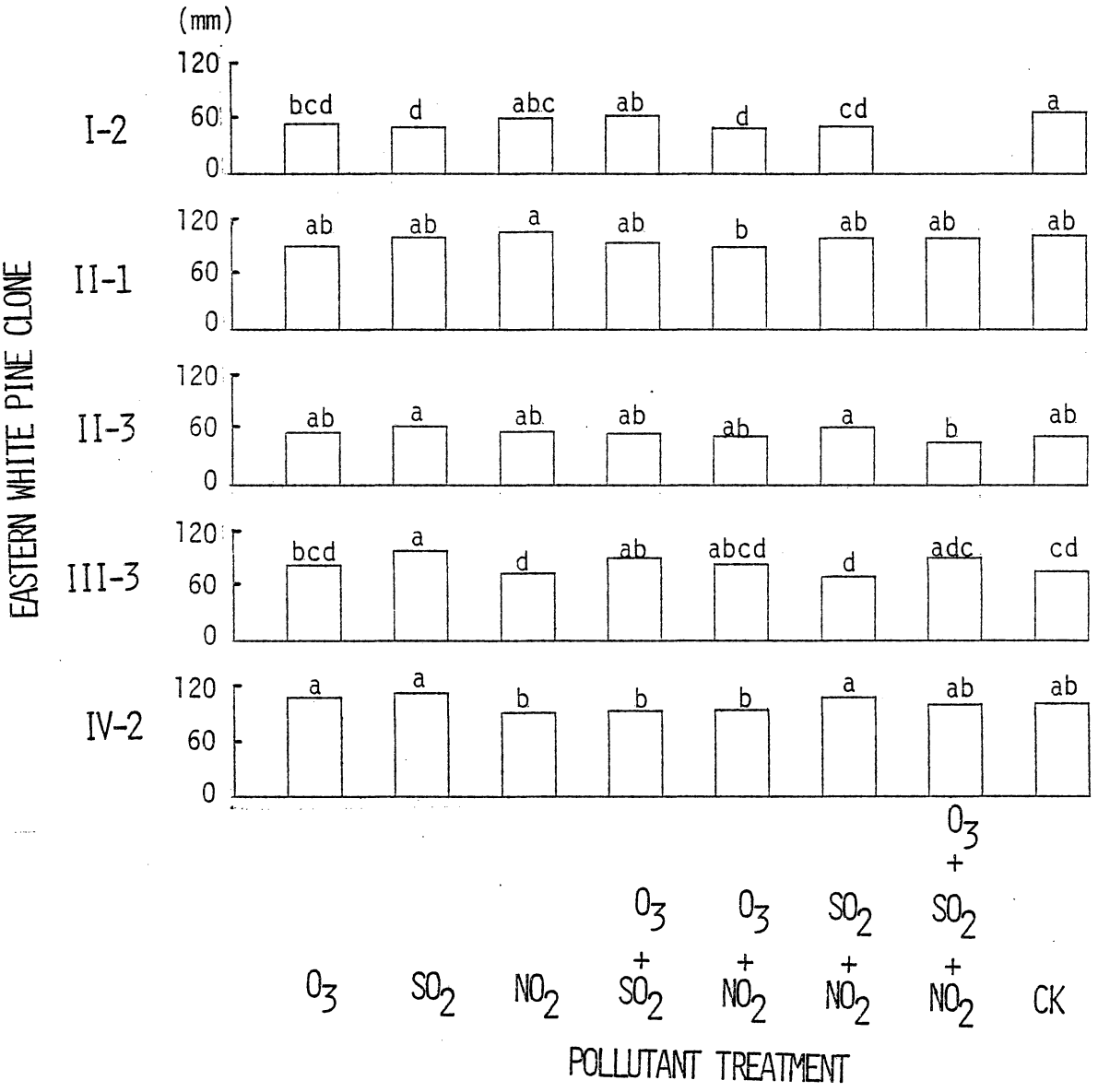


Fig. 7. Needle length of current year eastern white pine needles after exposed to 0.05 ppm pollutants for 4 hours daily for 35 consecutive days. Bars within each clone having the same letter are not significantly different at P=0.05 level according to Duncan's new multiple range test.

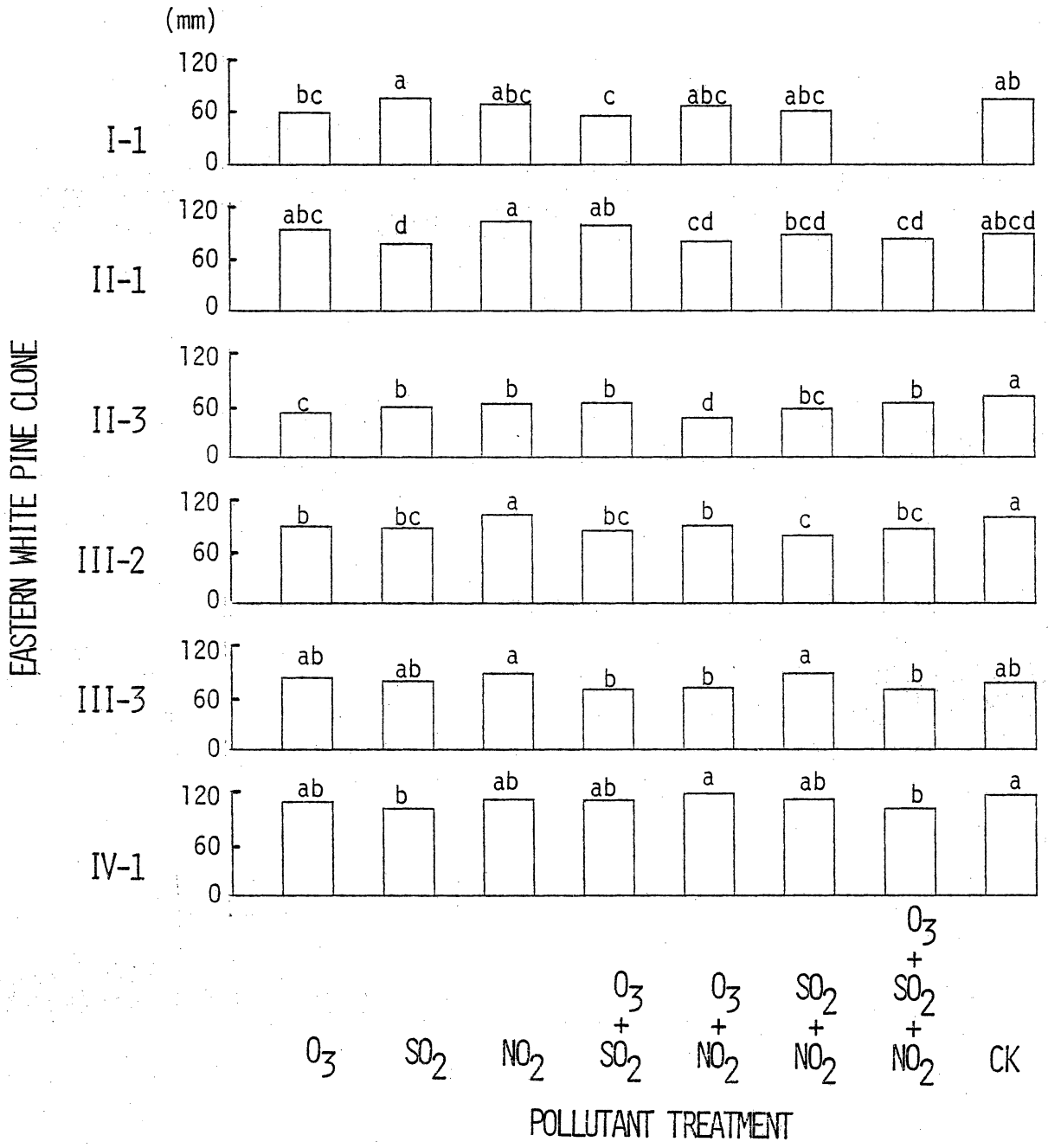


Fig. 8. Needle length of current year eastern white pine needles after exposed to 0.10 ppm pollutants for 4 hours daily for 35 consecutive days. Bars within each clone having the same letter are not significantly different at P=0.05 level according to Duncan's new multiple range test.

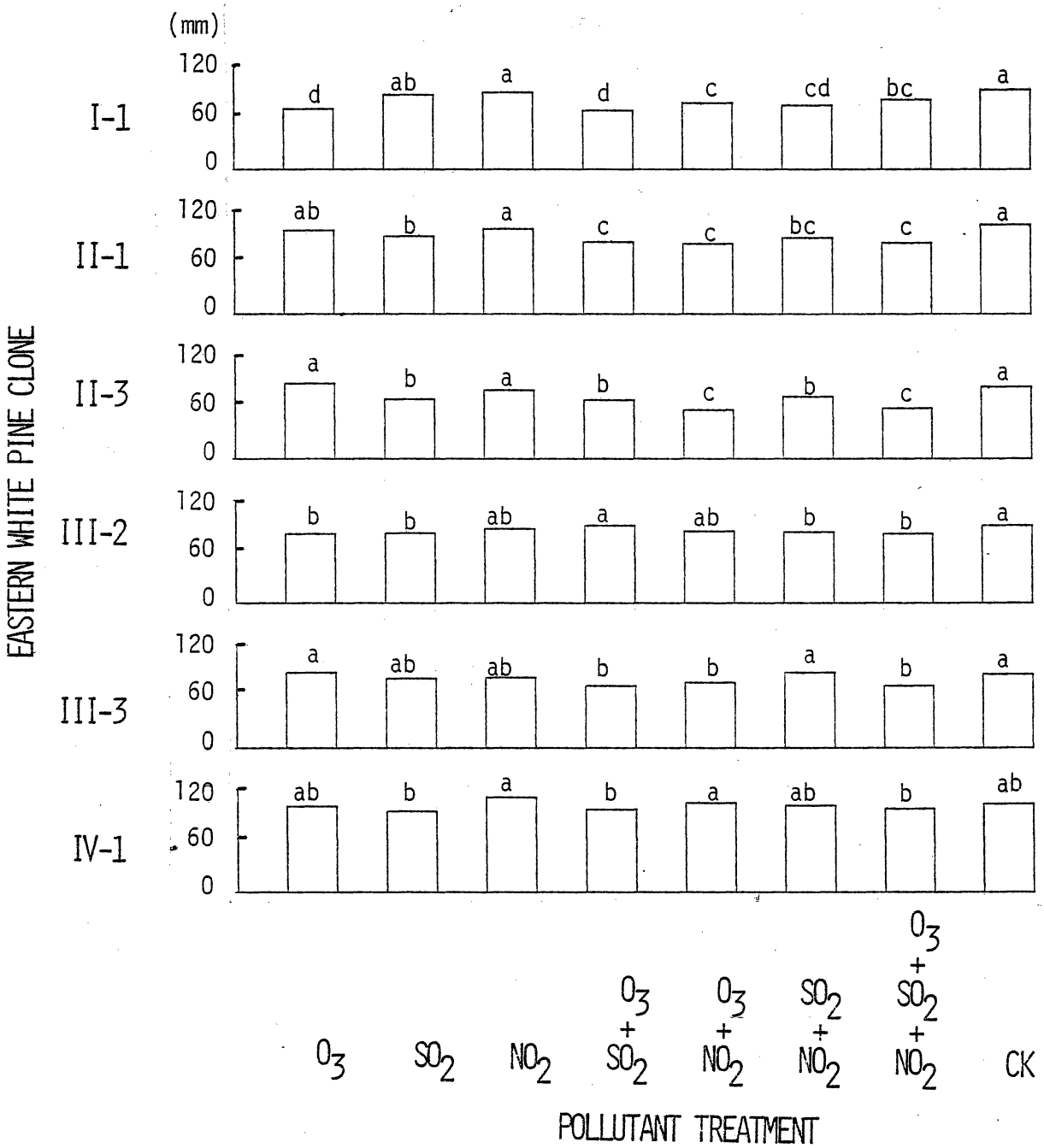


Fig. 9. Needle length of current year eastern white pine needles after exposed to 0.10 ppm pollutants for 4 hours daily for 50 consecutive days. Bars within each clone having the same letter are not significantly different at P=0.05 level according to Duncan's new multiple range test.

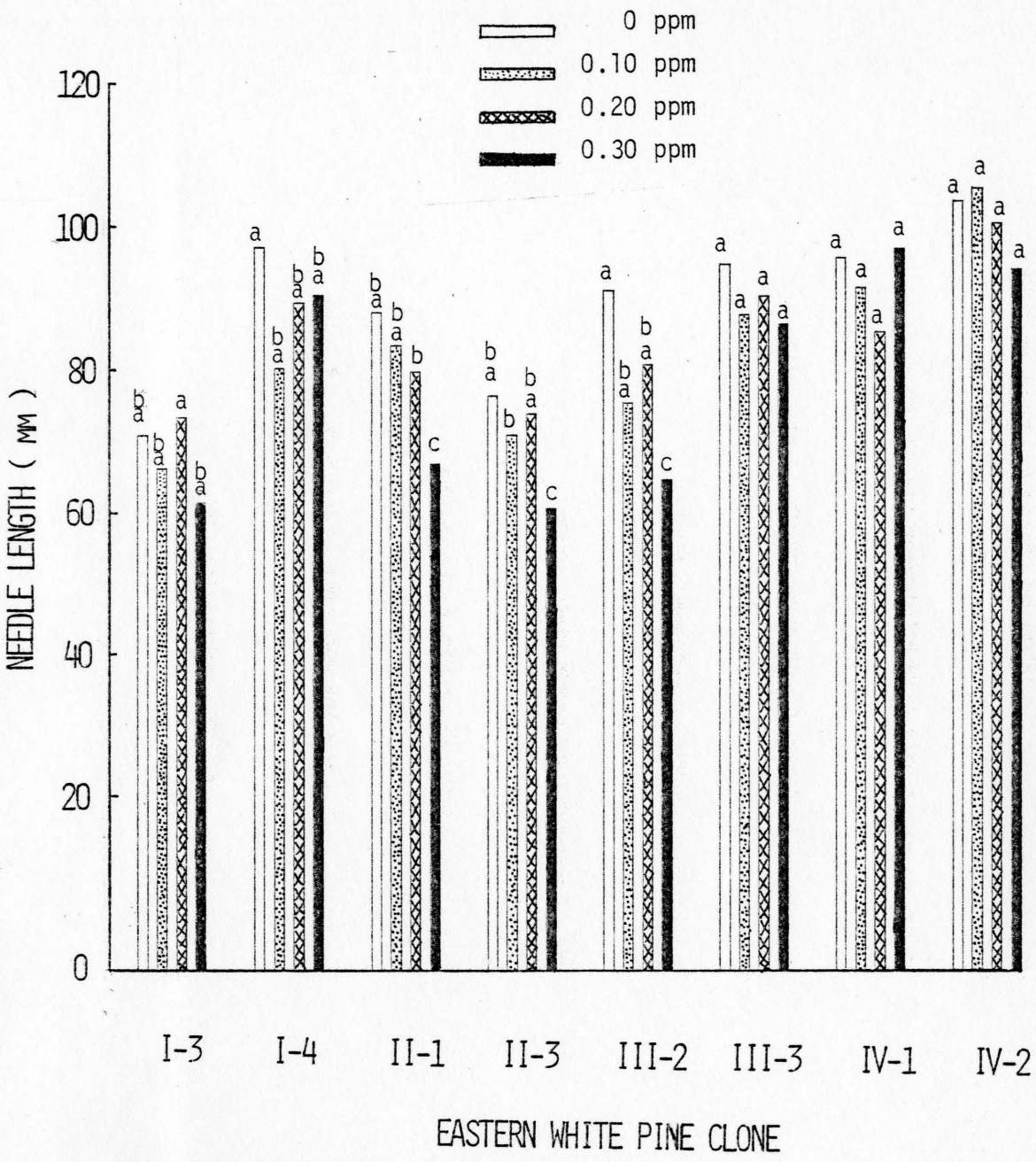


Fig. 10. Needle length of current year eastern white pine needles after exposed to 0.10, 0.20, and 0.30 ppm ozone for 4 hours daily for 35 consecutive days. Bars within each clone having the same letter are not significantly different at P=0.05 level according to Duncan's new multiple range test.

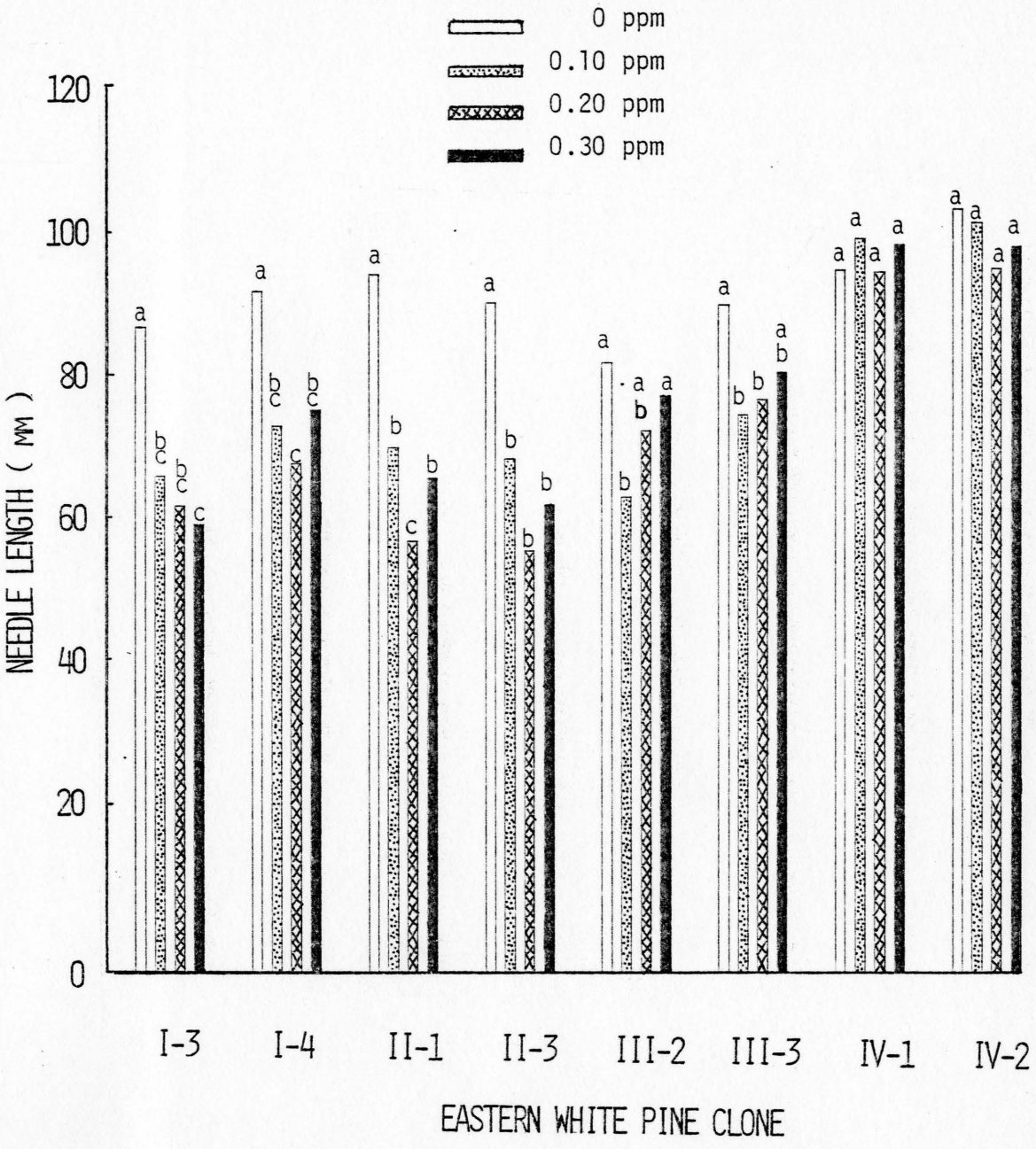


Fig. 11. Needle length of current year eastern white pine needles after exposed to 0.10, 0.20, and 0.30 ppm sulfur dioxide for 4 hours daily for 35 consecutive days. Bars within each clone having the same letter are not significantly differert at P=0.05 level according to Duncan's new multiple range test.

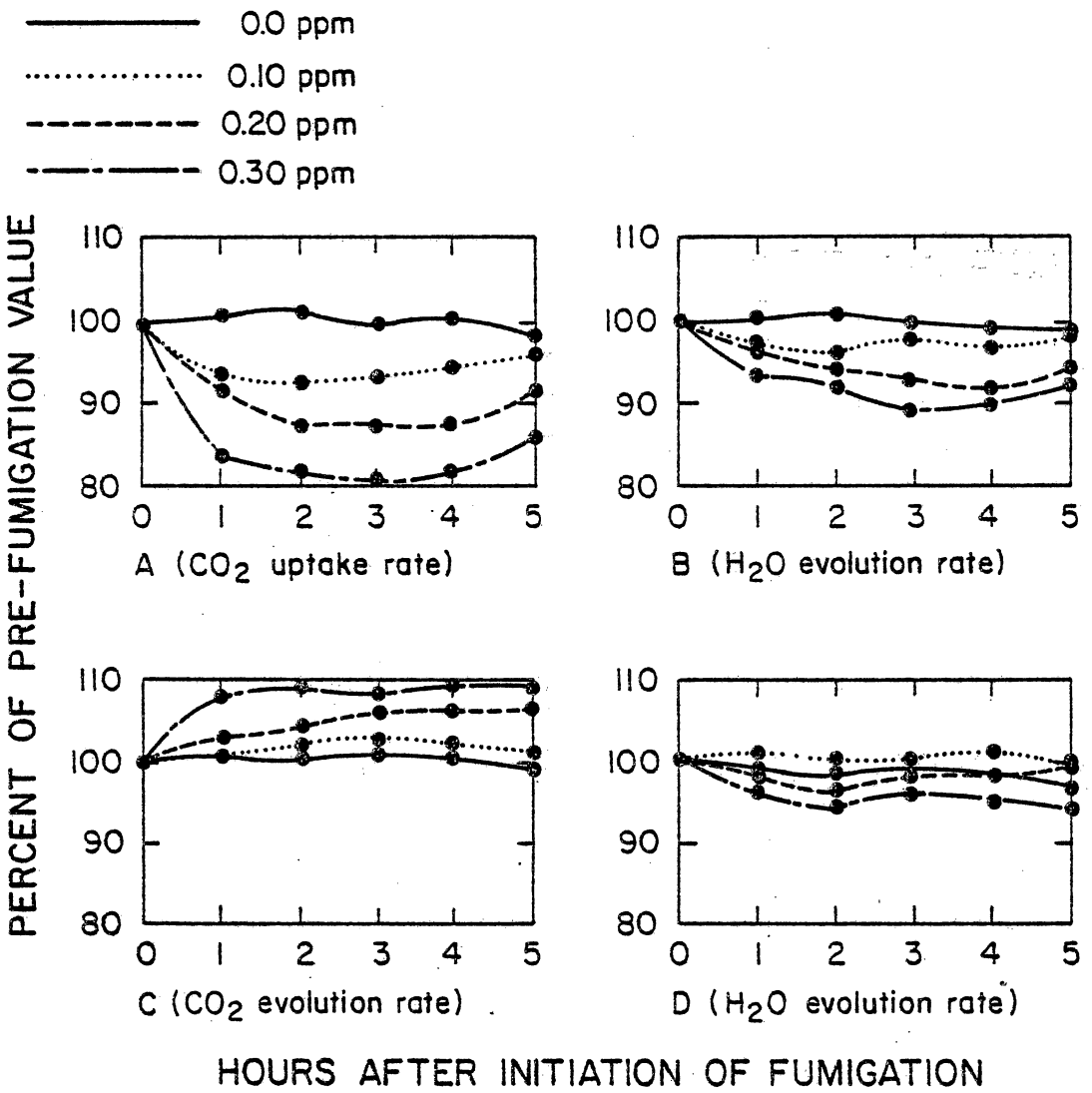


Fig. 12. Effect of ozone on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone II-1. Hour 5 represents the first hour after the termination of pollutant fumigation.

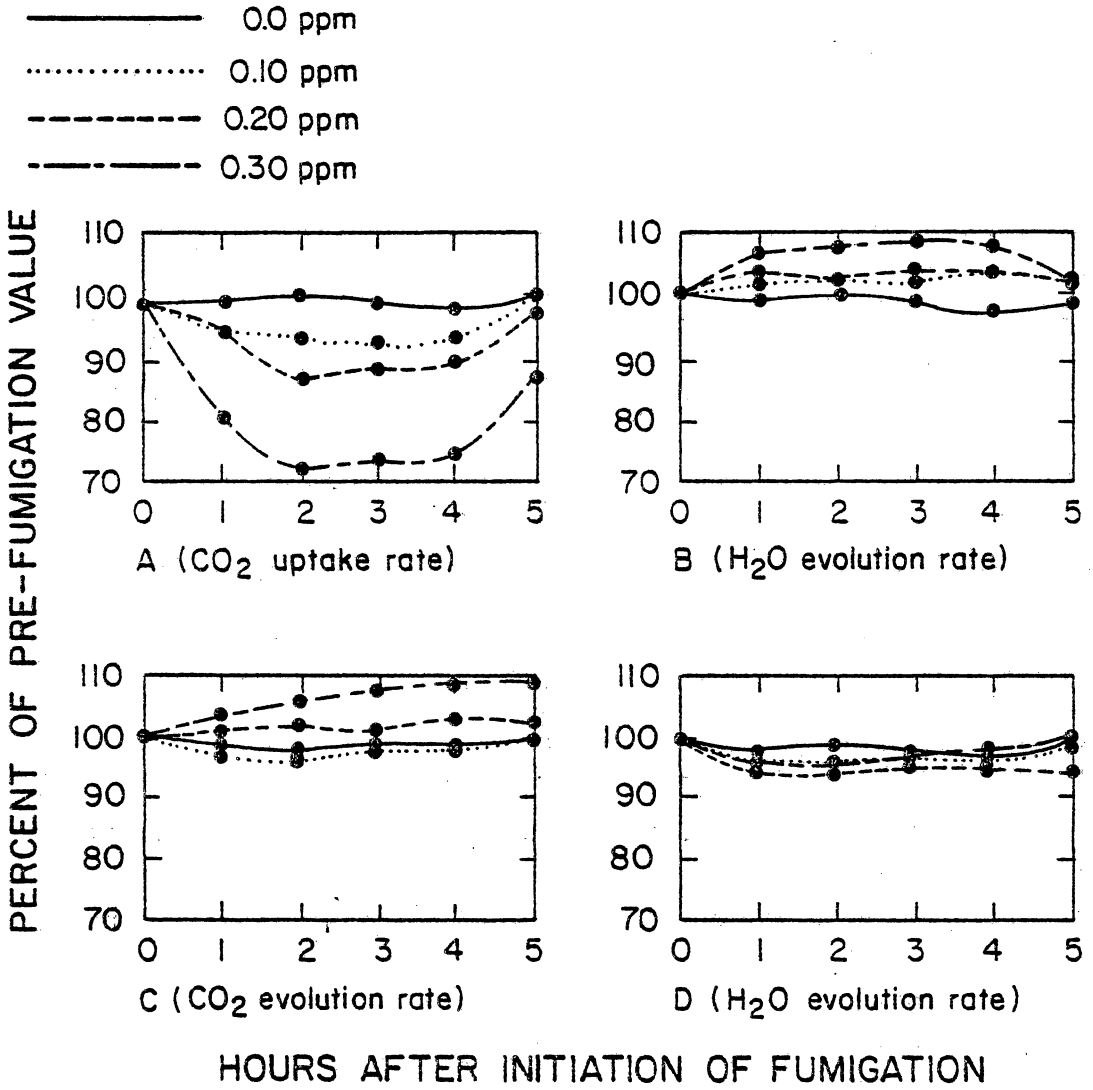


Fig. 13. Effect of ozone on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone III-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

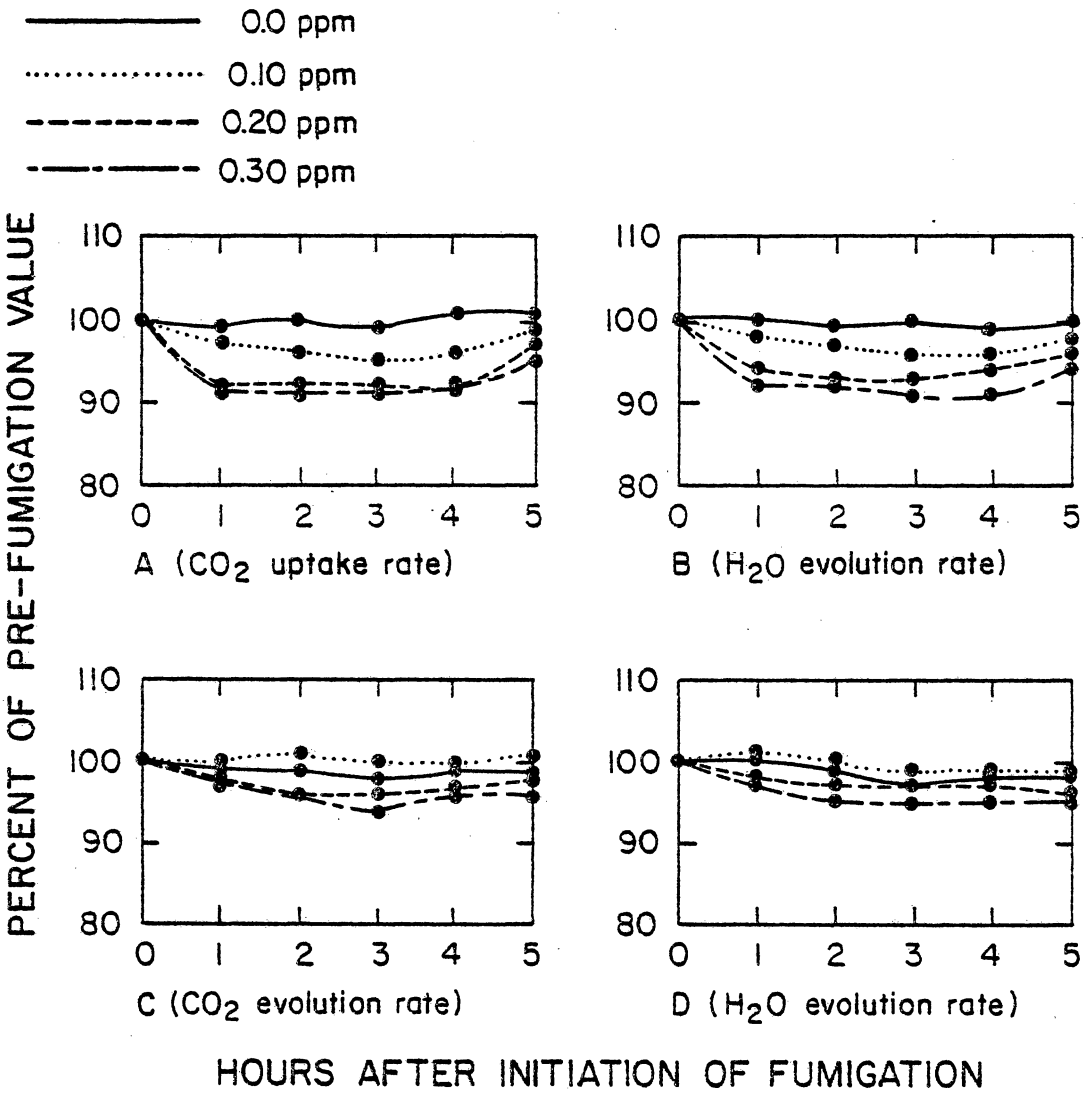


Fig. 14. Effect of ozone on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone IV-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

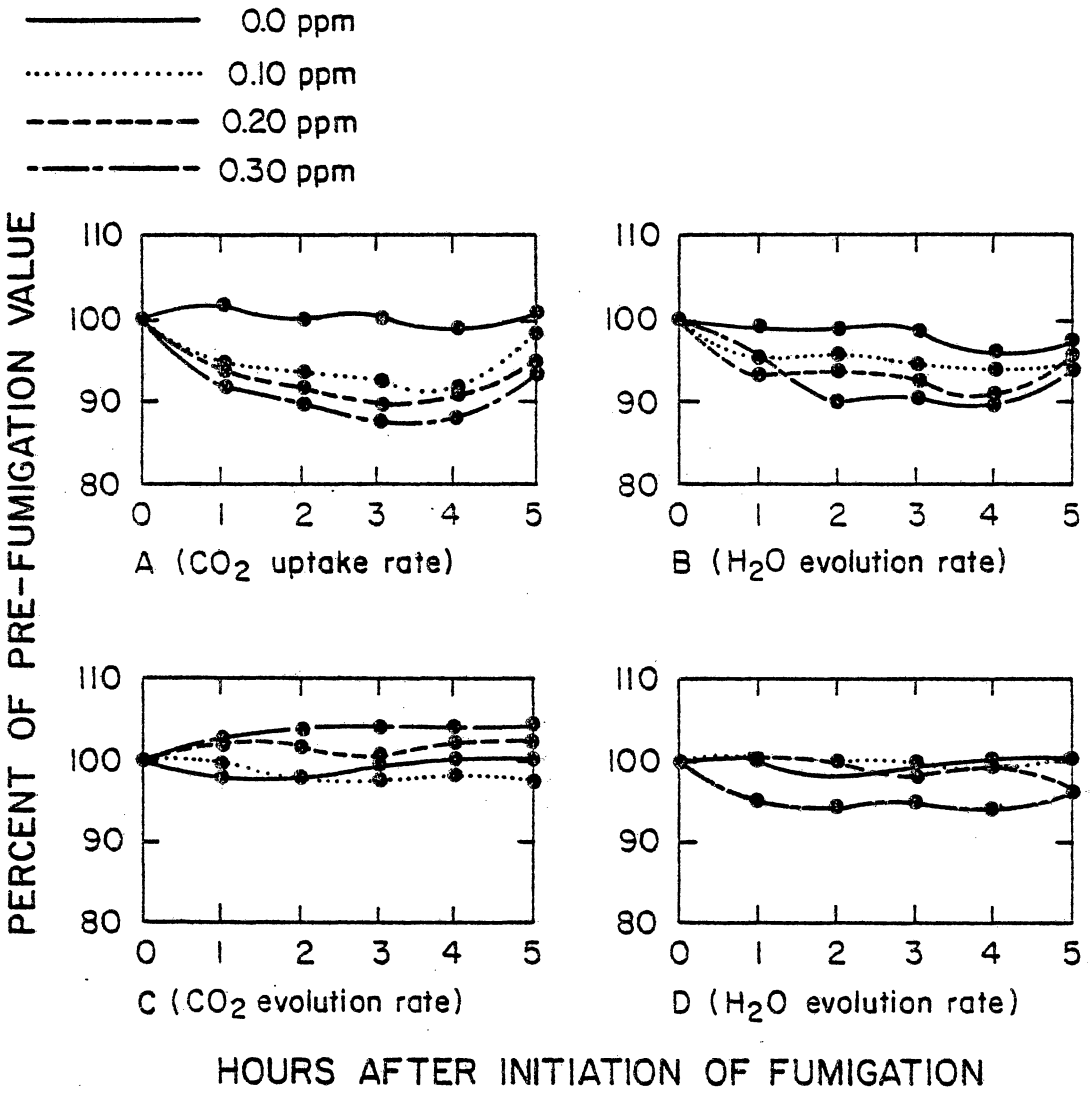


Fig. 15. Effect of sulfur dioxide on A) net photosynthesis , B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone II-1. Hour 5 represents the first hour after the termination of pollutant fumigation.

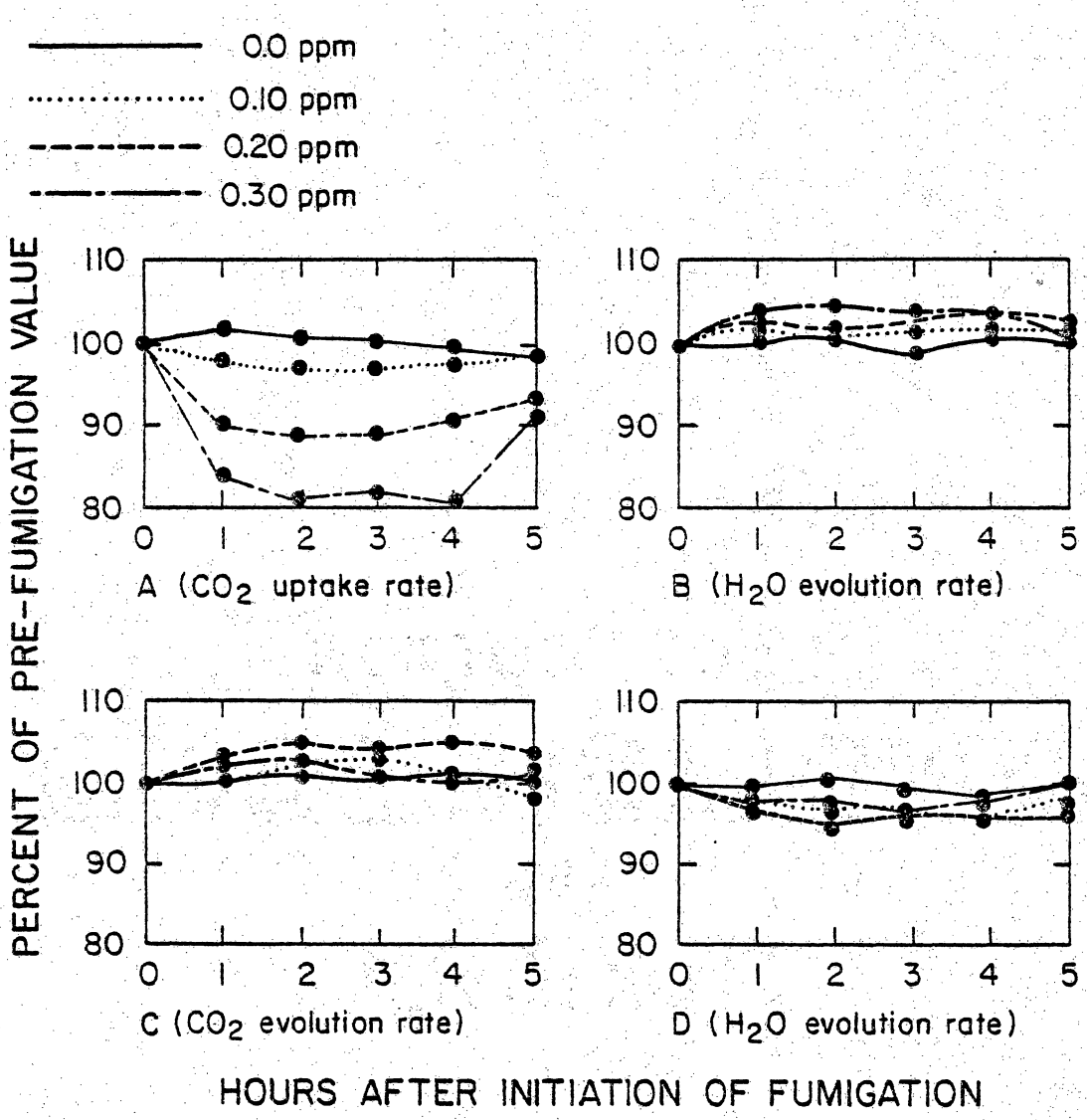


Fig. 16. Effect of sulfur dioxide on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone III-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

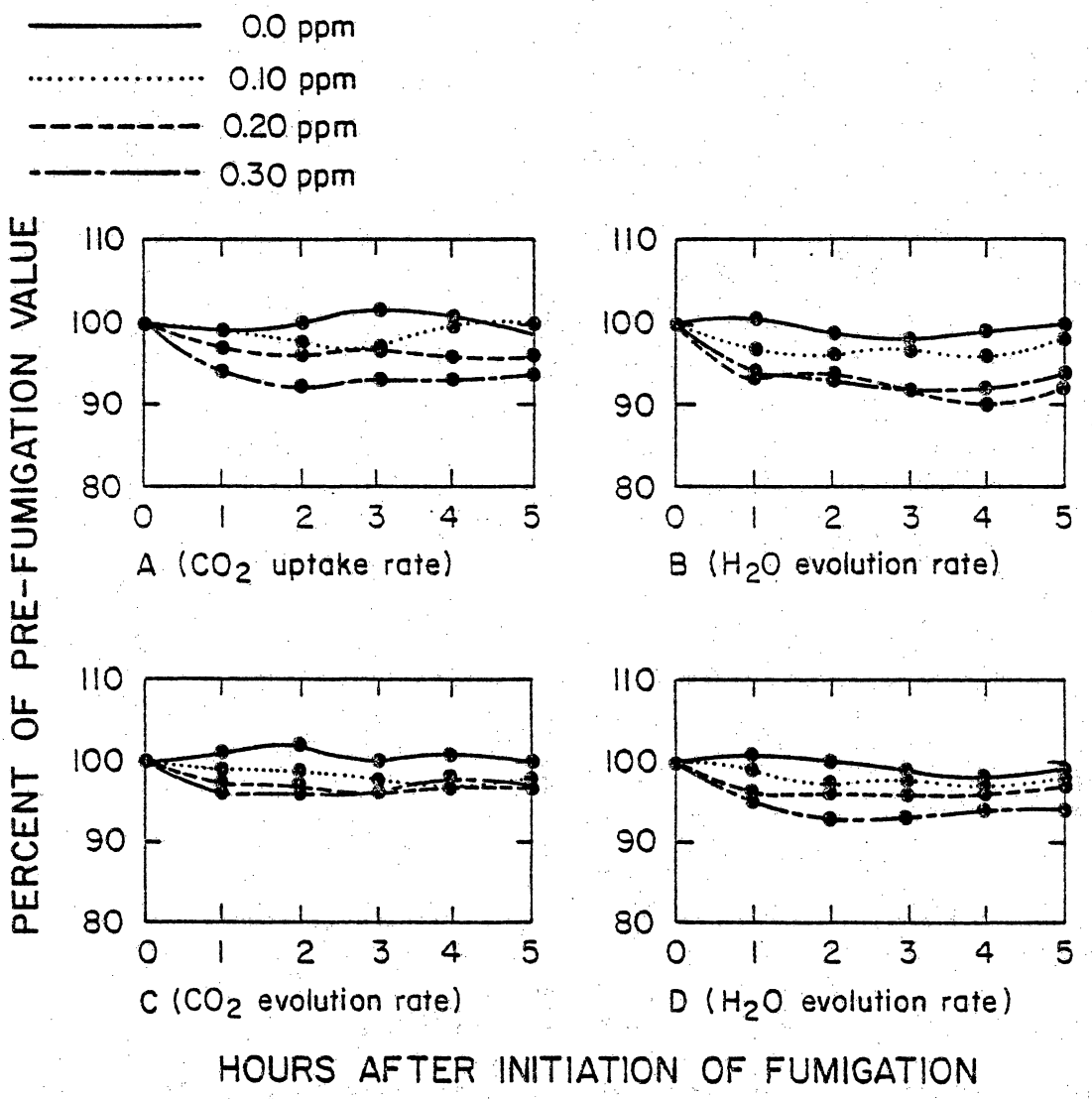


Fig. 17. Effect of sulfur dioxide on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone IV-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

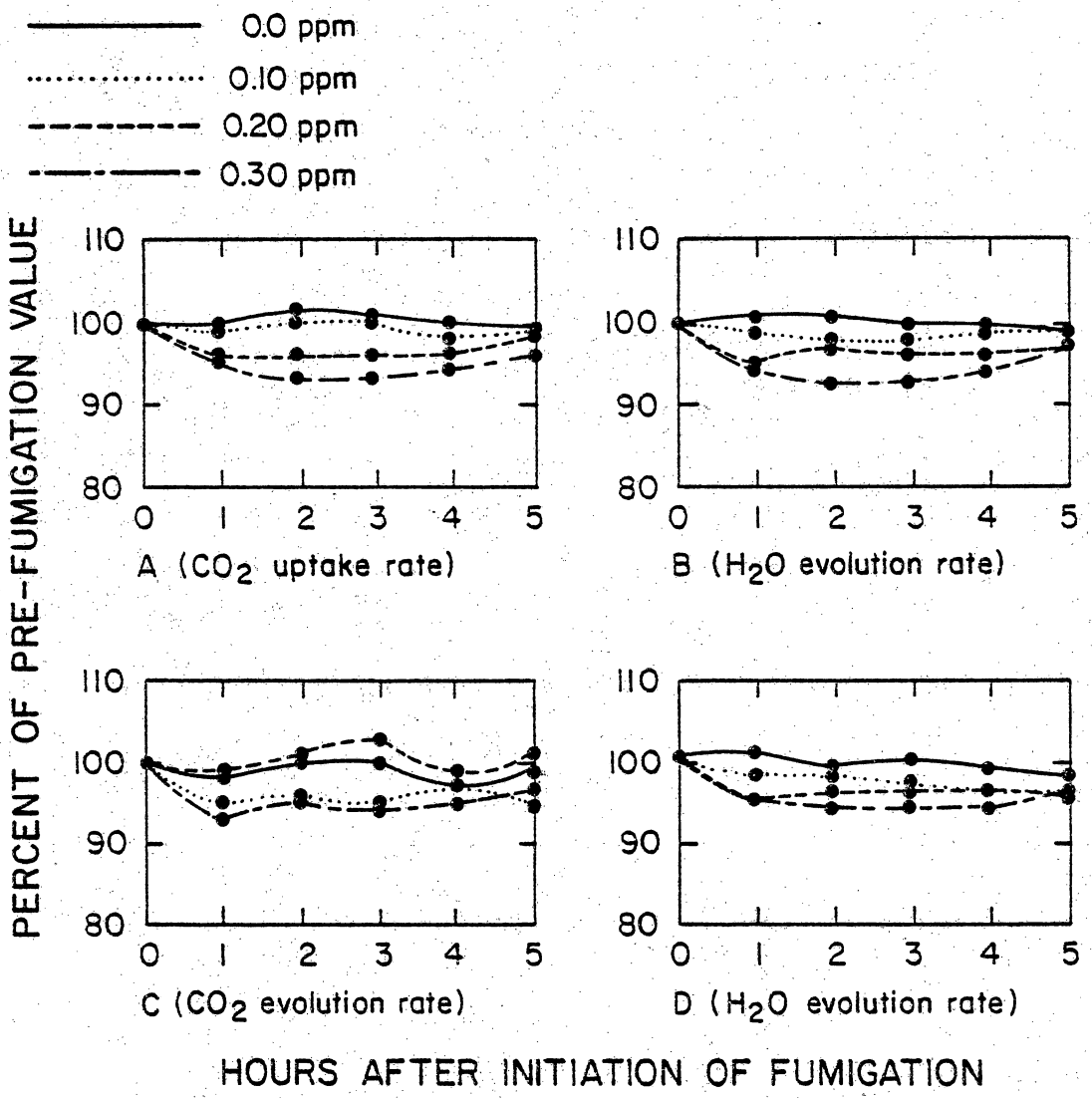


Fig. 18. Effect of nitrogen dioxide on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone II-1. Hour 5 represents the first hour after the termination of pollutant fumigation.

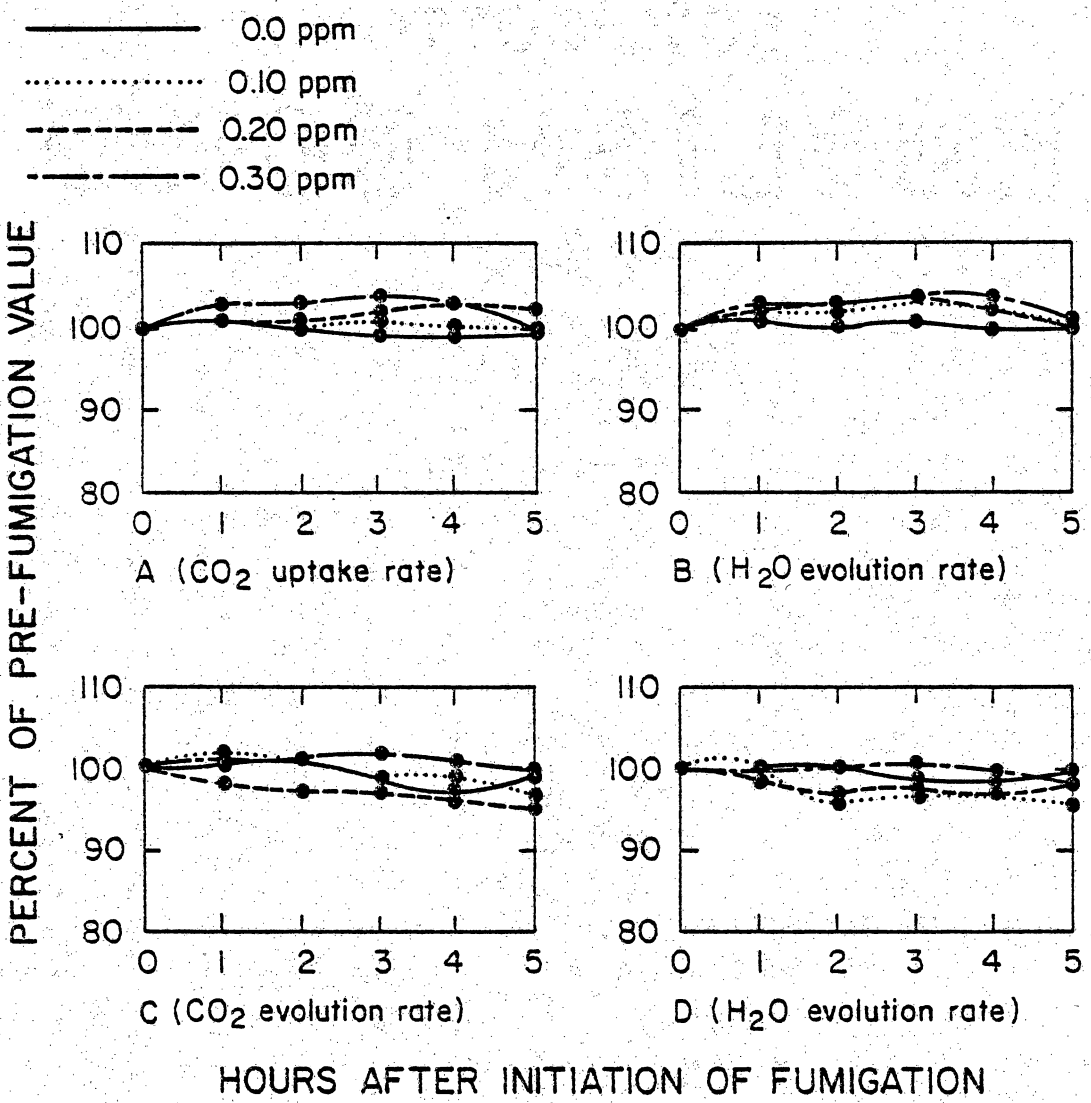


Fig. 19. Effect of nitrogen dioxide on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone III-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

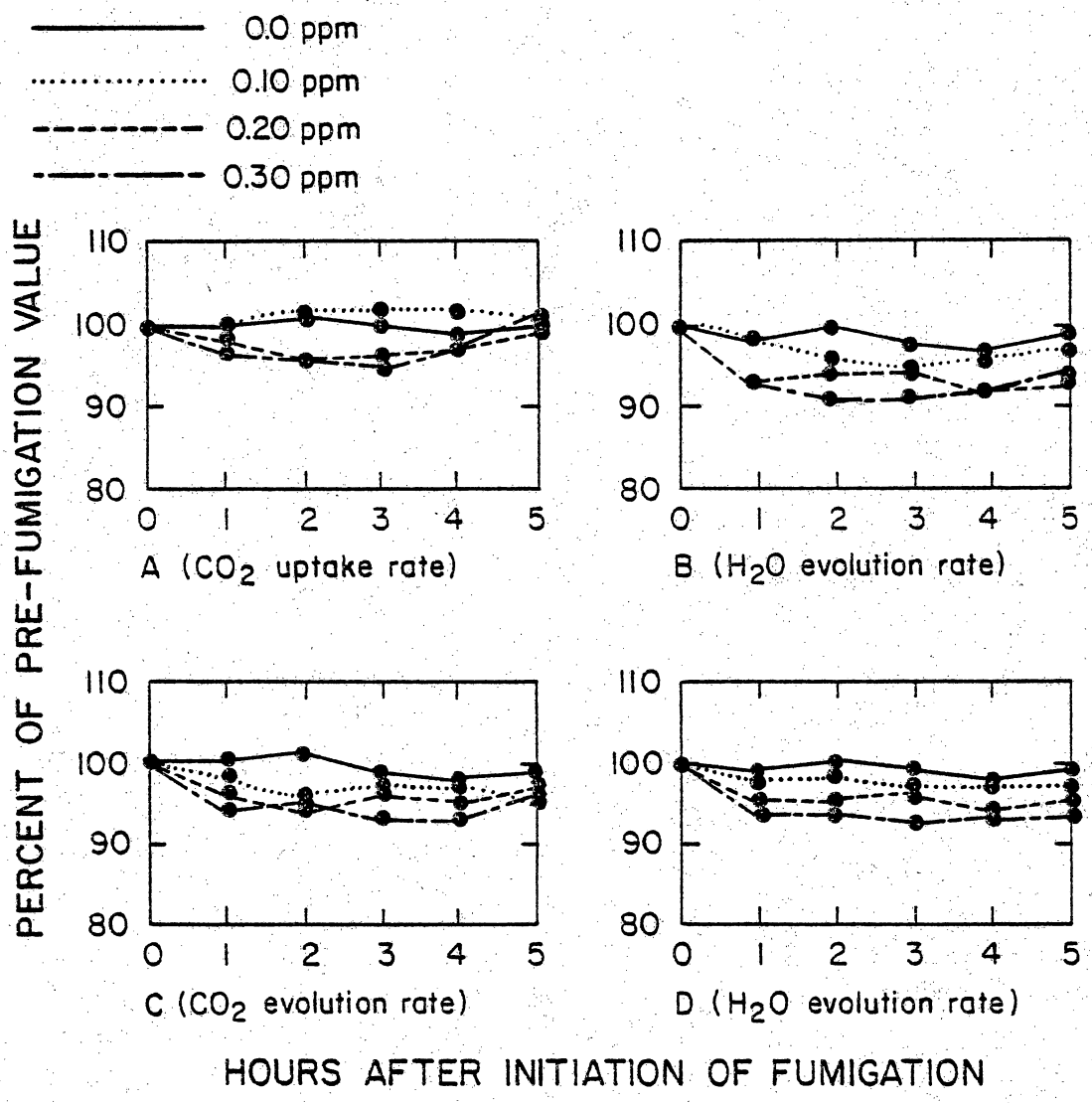


Fig. 20. Effect of nitrogen dioxide on A) net photosynthesis, B) photosynthetic transpiration, C) dark respiration, and D) dark respiratory transpiration of eastern white pine clone IV-2. Hour 5 represents the first hour after the termination of pollutant fumigation.

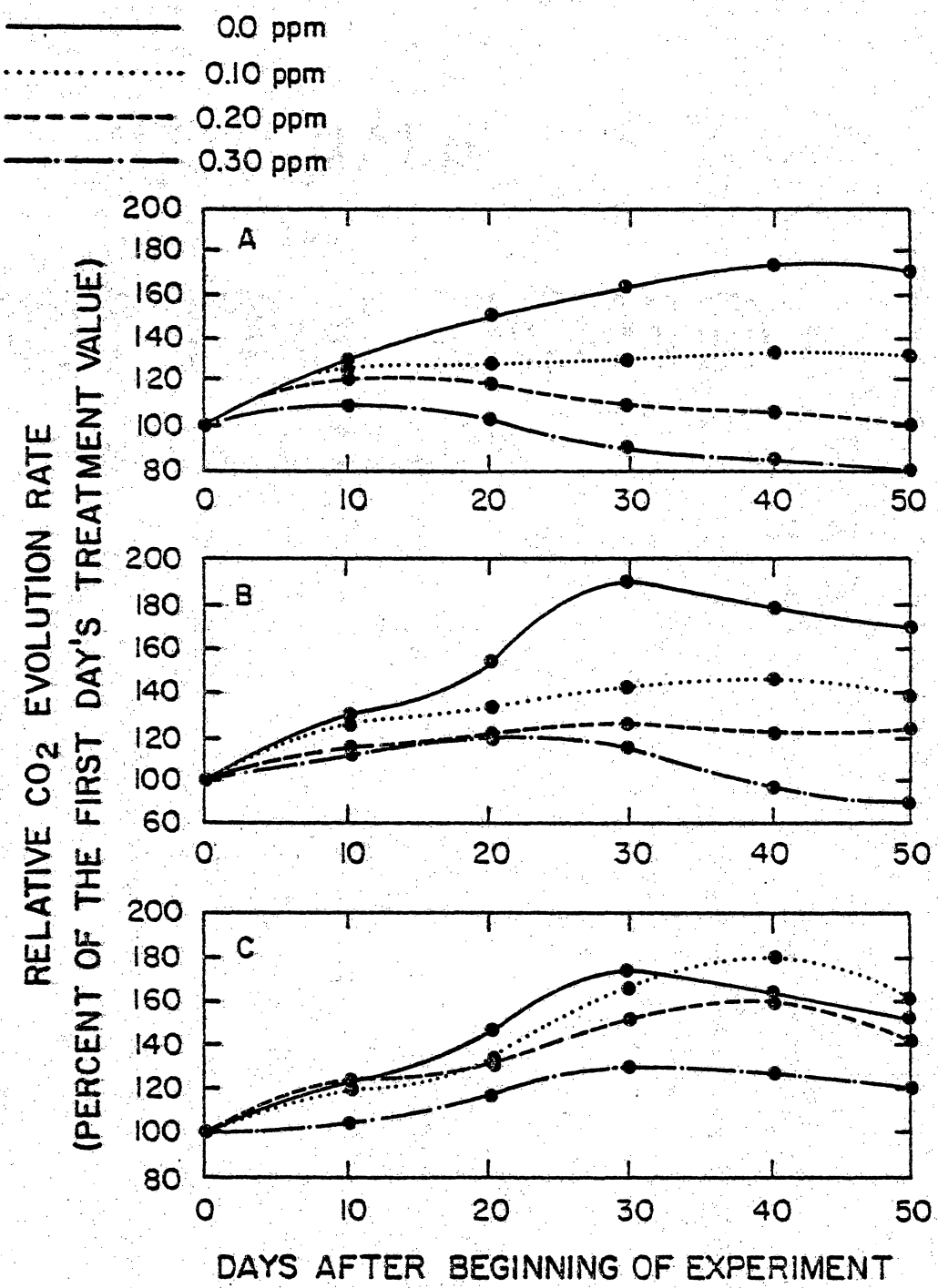


Fig. 21. Effect of daily 4-hour fumigation with ozone, sulfur dioxide, and nitrogen dioxide on relative photosynthesis rates of eastern white pine clone II-1 over 50 consecutive days.

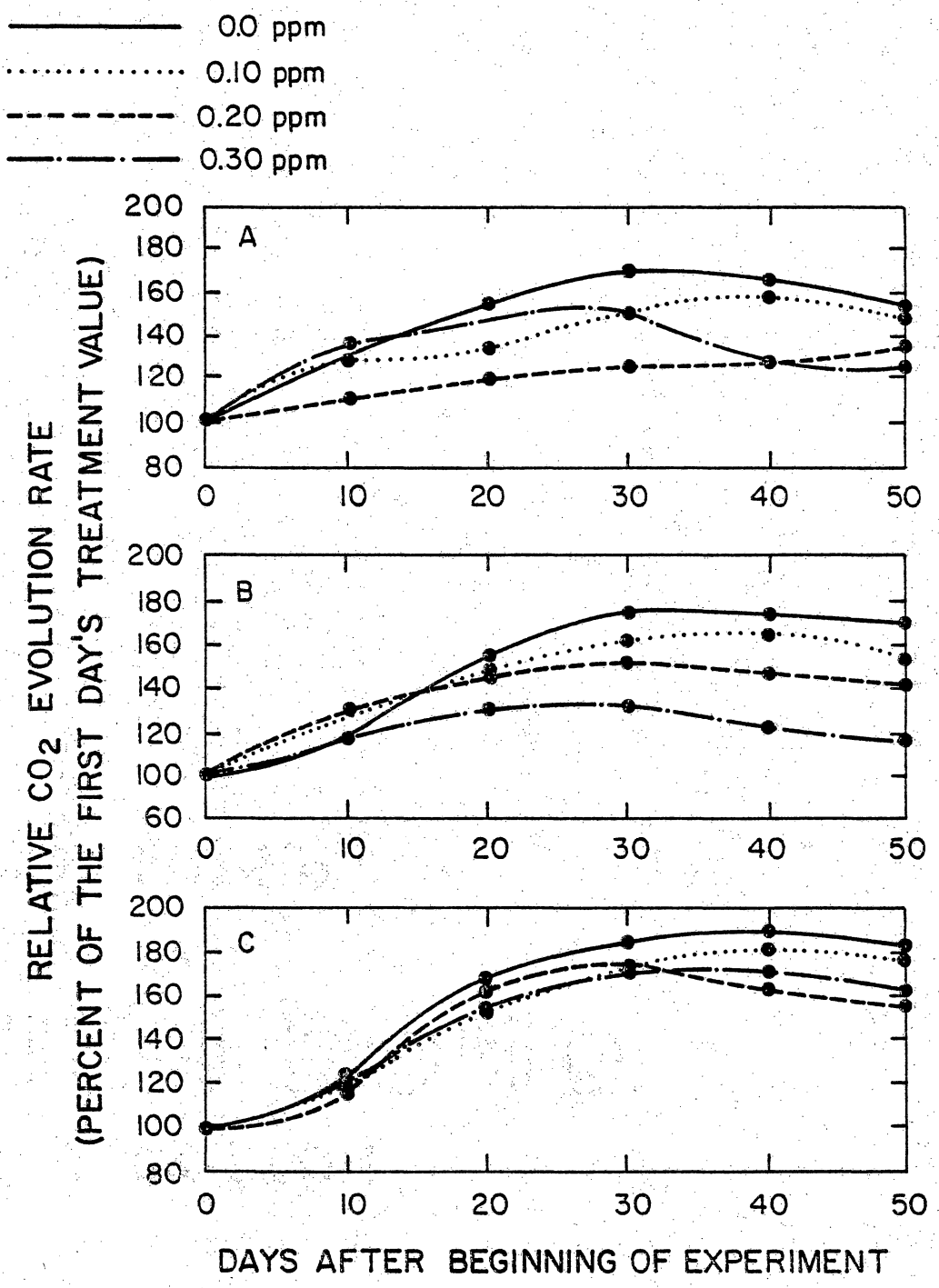


Fig. 22. Effect of daily 4-hour fumigation with ozone, sulfur dioxide, and nitrogen dioxide on relative photosynthesis rates of eastern white pine clone III-2 over 50 consecutive days.

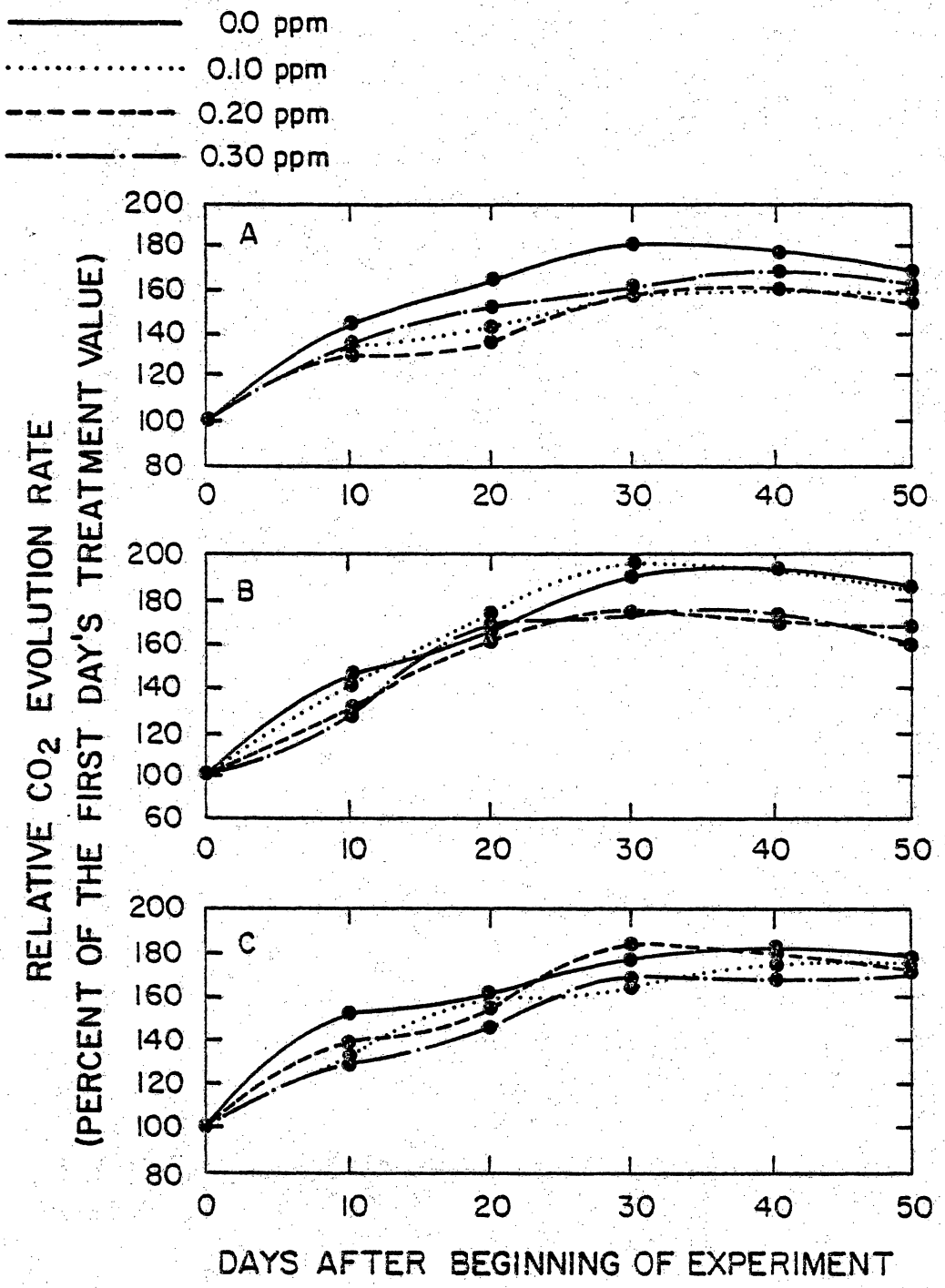


Fig. 23. Effect of daily 4-hour fumigation with ozone, sulfur dioxide , and nitrogen dioxide on relative photosynthesis rates of eastern white pine clone IV-2 over 50 consecutive days.

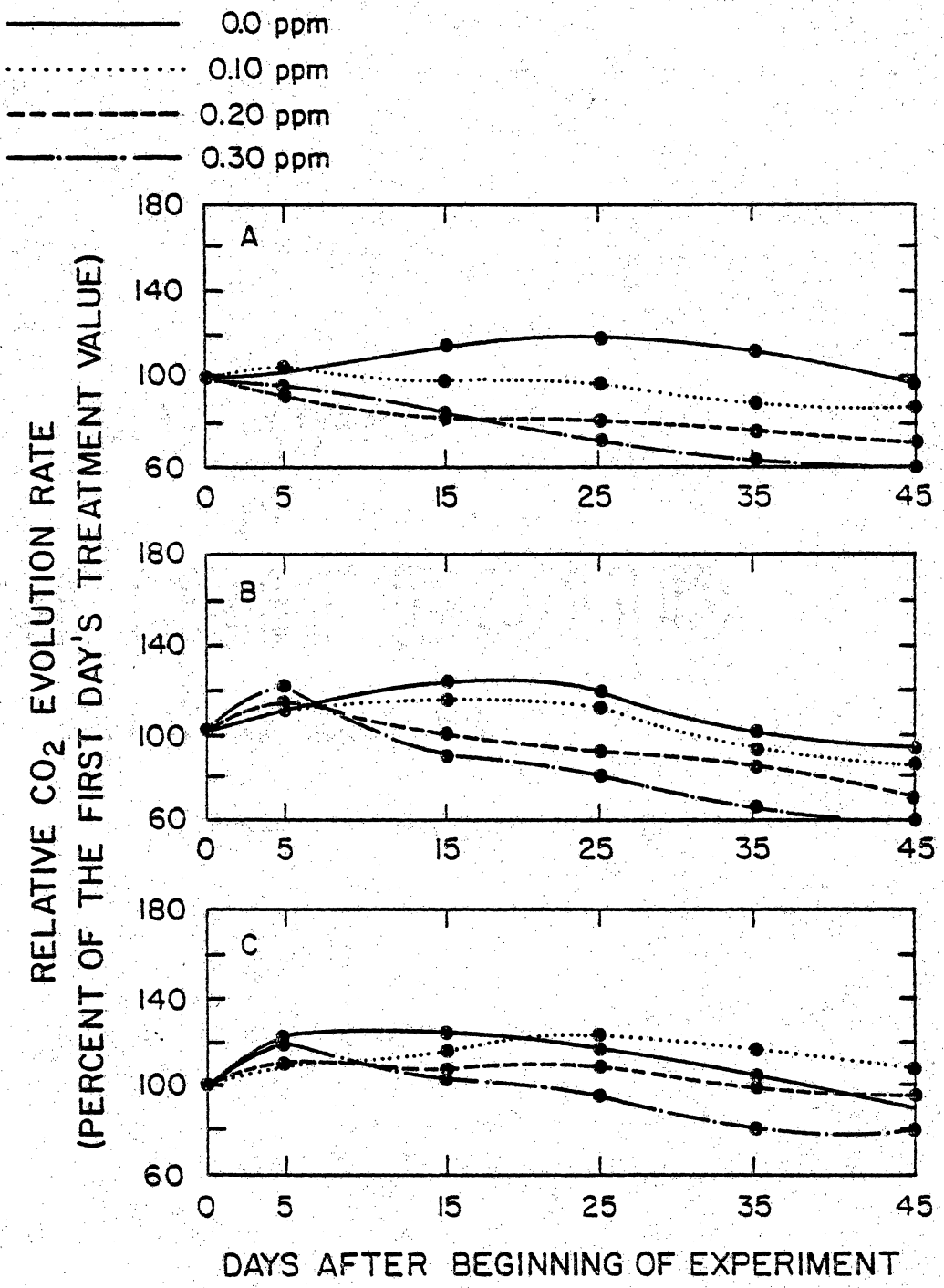


Fig. 24. Effect of daily 4-hour fumigation with ozone, sulfur dioxide, and nitrogen dioxide on relative dark respiration rates of eastern white pine clone II-1 over 50 consecutive days.

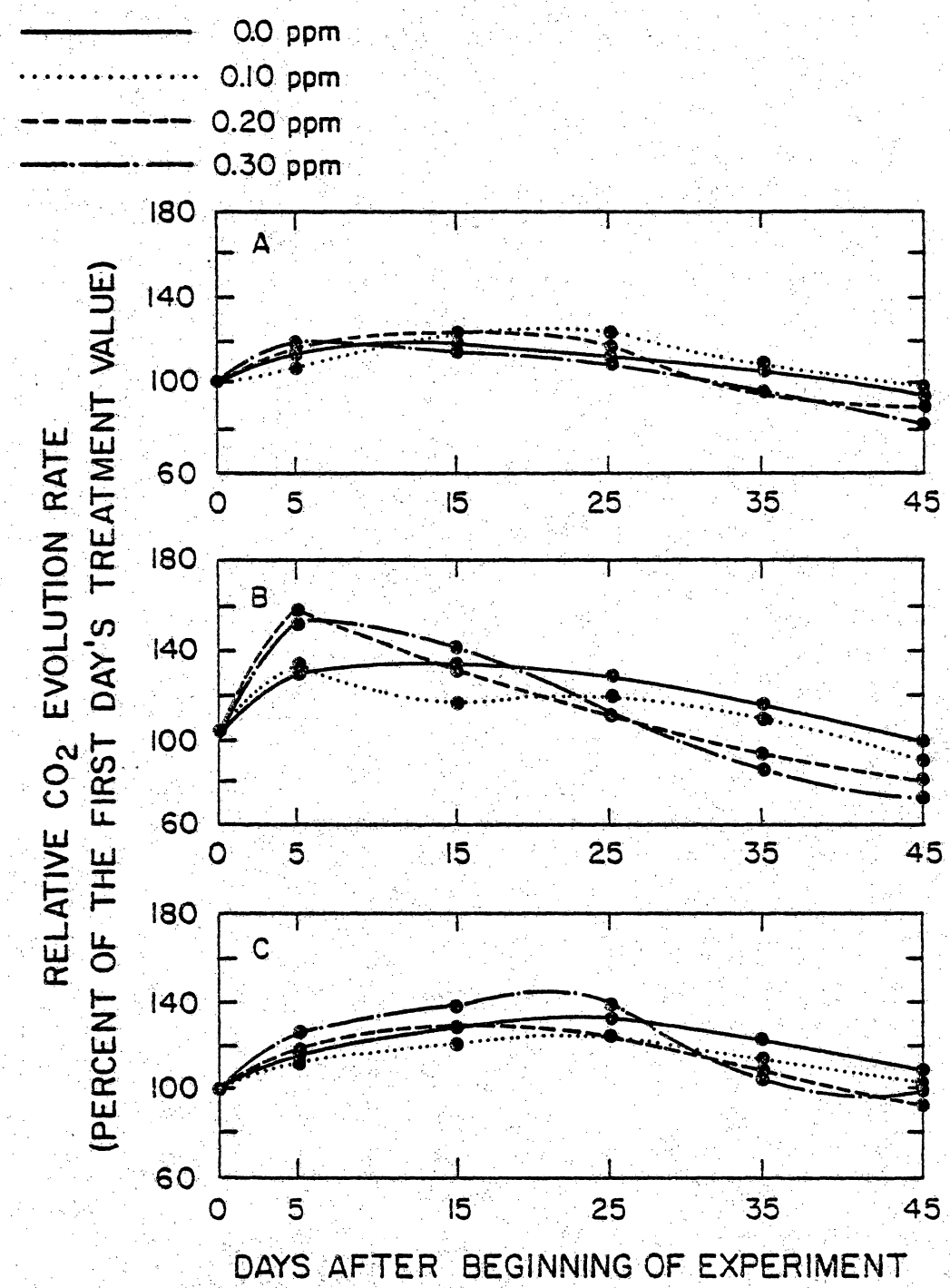


Fig. 25. Effect of daily 4-hour fumigation with ozone, sulfur dioxide, and nitrogen dioxide on relative dark respiration rates of eastern white pine clone III-2 over 50 consecutive days.

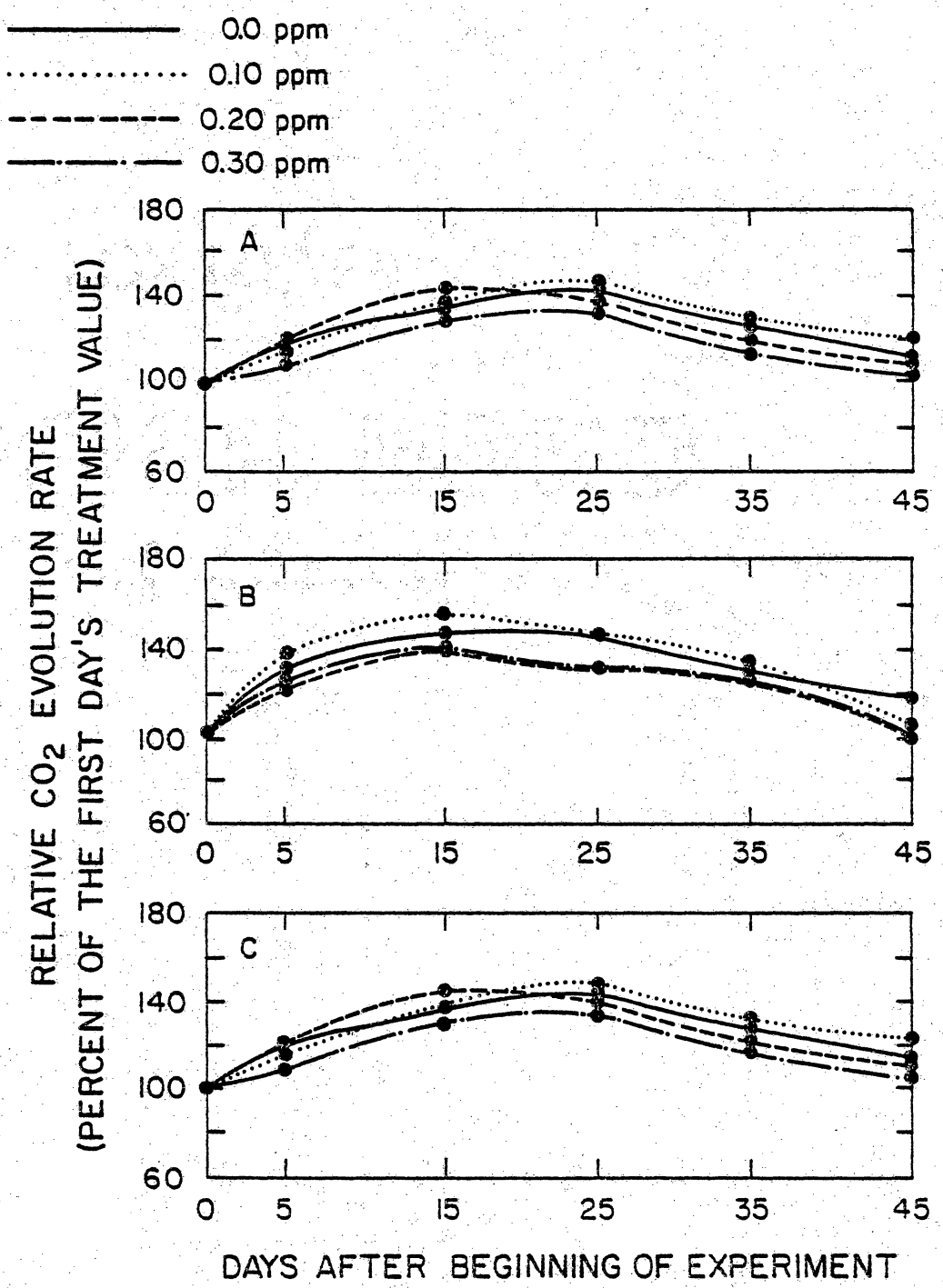


Fig. 26. Effect of daily 4-hour fumigation with ozone, sulfur dioxide, and nitrogen dioxide on relative dark respiration rates of eastern white pine clone IV-2 over 50 consecutive days.

Table 3. Average dry weight of eastern white pine fascicles after fumigated with 0.10 ppm ozone, sulfur dioxide, and/or nitrogen dioxide for 4 hours daily for 35 consecutive days.

Treatment	CLONE[1]		
	II-1	III-2	IV-2
	---- mg/mm fascicle ----		
O_3	375**	412**	573
SO_2	370**	476**	579
NO_2	507*	569	602
$O_3 + SO_2$	331**	478**	583
$O_3 + NO_2$	494*	531	616
$SO_2 + NO_2$	488*	529	572
$O_3 + SO_2 + NO_2$	422**	516*	610
Control	522	570	622

[1]Values represent mean of 20 observations with *5% and **1% significant difference from control treatment in each clone based on Duncan's new multiple range test.

Table 4. Average dry weight of eastern white pine fascicles after fumigated with 0.10, 0.20, and 0.30 ppm ozone, sulfur dioxide, or nitrogen dioxide for 4 hours daily for 50 consecutive days.

Treatment	Pollutant concentration	CLONE[1] II-1	III-2	IV-2
	--- ppm ---	---- mg/mm fascicle ----		
O_3	0.10	424**	454*	478
	0.20	430*	450*	442*
	0.30	381**	472	468
SO_2	0.10	445	449*	469
	0.20	437*	467	467
	0.30	402**	411**	440*
NO_2	0.10	458	474	483
	0.20	450	460	462
	0.30	441*	473	453
Control	0.00	450	478	474

[1]Values represent mean of 20 observations with *5% and **1% significant difference from control treatment in each clone based on Duncan's new multiple range test.

Table 5. Chlorophyll content of eastern white pines after exposed to ozone, sulfur dioxide, or nitrogen dioxide for 4 hours daily for 50 consecutive days.

Treatment	Pollutant concentration	CLONE[1] II-1 Chlorophyll a	II-1 Chlorophyll b	III-2 Chlorophyll a	III-2 Chlorophyll b	IV-2 Chlorophyll a	IV-2 Chlorophyll b
	--- ppm ---	--- ug/g needle dry weight ---					
O_3	0.10	94	43	97	41	94	39*
	0.20	80**	38	89	40	98	42
	0.30	76**	34*	78*	33**	95	43
SO_2	0.10	92	39	98	42	96	43
	0.20	81**	34*	86*	37*	90*	41
	0.30	64**	29**	73**	31**	88*	38*
NO_2	0.10	106	47	104	45	111	51
	0.20	94	40	97	43	101	45
	0.30	88*	39	91	41	94	42
Control	0.00	101	43	98	44	107	45

[1]Values represent mean of 4 observations with *5% and **1% significant difference from control treatment in each clone based on Duncan's new multiple range test.

Table 6. Rates of net photosynthesis and dark respiration of eastern white pines at the beginning of long-term pollutant fumigation.

Clone	Photosynthesis	Dark respiration
	--- mg CO_2/g needle dry weight/hr ---	
II-1	5.0	-2.0
III-2	4.1	-1.6
IV-2	4.5	-1.7

[1]Values represent mean of 9 observations.

DISCUSSION

Plant Culture Practice and Fumigation Facility

Various plant and environmental factors have been shown to have conspicious effects on plant sensitivity to pollutants (Heck, 1968; Heck and Dunning, 1968). In this study, all physical environmental factors such as temperature, relative humidity, irradiance, timing of daily fumigation, monitoring facilities, and plant growing media were maintained as uniform as possible among treatments. Vegetatively propagated clonal white pines of similar age were used in each set of fumigations in order to minimize any possible variation due to biological systems. Plant diurnal endogenous rhythms were suspected to occur during experimental periods (Botkin et al., 1972). By regular daily measurements of various plant responses at the same time within every 24-hour regime and the use of clonally materials, the possible variations due to such rhythms have been substantial reduced.

Simultaneous pollutant exposures in 12 identical Continuously Stirred Tank Reactors (CSTR) presented uniform tests to be statistically comparable. The dual-gas infrared analyzer, the function of minichamber, and the manifold sampling system greatly increased work capacity, accuracy, and efficiency in gas exchange measurements.

Through repeated use of the same branchlet of two-year-

old grafted white pine which was fitted perfectly into minichambers without significant overshadow or contact with the chamber walls , the intracrown variability of photosynthesis, transpiration, respiration, and chlorophyll content as observed with field grown trees (Wood, 1971) was thus not of consideration.

Based on the results of short-term, high dose pollutant fumigations, it is very easy to overstate or to project plant responses to pollutants which derived from sudden dramatic physiological changes. The long-term, low concentration fumigations certainly avoid this and provide adequate time for researchers to discern normal plant responses to air pollutants under the pollutant concentrations that often occur in the ambient air. This is especially important when the sensitive age of a given plant species is unknown (Davis and Wood, 1973b). For example, in this study, the effects of ozone, sulfur dioxide, and nitrogen dioxide at 0.10 and 0.20 ppm concentrations on clone II-1 photosynthesis were not distinguishable from controls in the early stages of pollutant exposure (Figure 21). However, at late stages of experiment, pollutant treatments manifested inhibitory effects on net photosynthesis at all ozone and sulfur dioxide concentrations, and at 0.30 ppm nitrogen dioxide exposure (Figure 21). The long-term monitoring of growth patterns of labelled branchlet is thus proven to be especially valuable in terms of pollution effects on plant metabolic

rates and its subsequent growth impact.

The type of response selected as criterion for plant sensitivity indexing to pollutants has been proven to be important in the determination of its results (Heagle, 1979; Ward, 1980). For example, Heagle (1979) studied the relative sensitvity rankings of four soybean cultivars to chronic and acute ozone exposures by using various injury and growth responses as monitoring criteria. He concluded that the rankings of soybean cultivars for sensitivity to ozone exposure was dependent on ozone dose as well as response selected as the measure of sensitivity. The ranking of cultivar obtained by exposure to chronic doses of ozone was often different from that obtained by exposure to acute doses. Rankings based on growth effects were usually different from those based on foliar injury. Similar results were observed in this study. Based on foliar symptom expressions, clone III-2 was more tolerant to ozone and sulfur dioxide than clone II-1 at 0.10 ppm concentration (Figures 3, 4), however, such ranking was less distinct as pollutant concentration increased (Figures 5, 6). According to needle length measurements, clone II-1 was ranked less sensitive to ozone exposure than clone III-2 (Figures 8, 9), however, the same sensitivity ranking was not observed in the gas exchange measurements (Figures 12, 13). These findings well demonstrated the risk and the limited value of plant sensitivity ratings which only relied on a single

plant response without taking the consideration of other responses.

In this study, several plant responses were measured during long-term pollutant exposures in order to project the pathogenesis and tolerance mechanisms in white pine pollutant-induced diseases. Measurements of photosynthetic, respiratory, and transpiratory rates would indicate stomatal behaviors which provide the information of pollutant uptake rates and plant assimilatory rates. Chlorophyll content and needle dry weight were also measured in order to assess direct impact of pollution exposures on plant growth.

Symptom Expressions

In 1979, conventional screening tests for identifying plant sensitivity to pollutants were carried out at pollutant concentrations lower than then National Ambient Air Quality Standard. Special attention was paid to clonal growth impact caused by single pollutant or various pollutant combination exposures.

Physical visible symptoms induced by ozone on current year's needles were chlorosis, pigmented mottling, necrotic banding and/or necrotic tip-burn (Figures 2, 3, 4, 5). They varied with pollutant dose, white pine clone, and stage of symptom development. Such observed ozone induced symptoms were very similar to other studies conducted in artificial fumigations (Berry and Hepting, 1964; Berry and Ripperton,

1963; Costonis and Sinclair, 1969).

The relationship between ozone dosage and symptom expression with a single linear positive relation was not observed either on clonal or sensitivity class basis (Figures 2, 3, 4, 5). Obviously, there was more than one variable (i.e. pollutant concentration) affecting the results of foliar injury in this study. The proportional increase rate of plant injured area was more rapid in lower concentration exposures (Figures 2, 3, 4) than in higher concentration (Figure 5).

Sulfur dioxide induced symptoms ranged from chlorosis, chlorotic mottling, pigmented mottling, necrotic banding to necrotic tip-burn (Figures 2, 3, 4, 6). They were undistinguishable from ozone-induced symptoms except in the early stage of symptom development or on the clone that was sensitive to sulfur dioxide but not to ozone.

The same dosage of sulfur dioxide caused more foliar injury on test clones than ozone (Figures 2-6) except in clone I-1 which was highly ozone sensitive. These results are in good agreement with those of Bennett and Hill (1973a, 1973b). In their study of pollutant penetration rates into a plant canopy, sulfur dioxide was found to be taken up 1.7 times faster than ozone in alfalfa canopy. Higher water solubility of sulfur dioxide than ozone at 20°C and its toxic property to plant at high concentrations were considered as major factors of sulfur dioxide in causing more sev-

ere injury to plant growth than ozone (Bennett and Hill, 1973a, 1973b). The greater injurious potential of sulfur dioxide than ozone on eastern white pine has been previous reported by Houston (1974) and Costonis (1970). However, Berry (1973) in his study of white pine seedling sensitivity to ozone and sulfur dioxide at 0.25 and 0.50 ppm concentrations reported a reverse result. Different gene pool of plant materials and plant age in Berry's materials are suspected at this moment as the cause of conflicting conclusions. In this study, the dose-response relationship between test sulfur dioxide concentrations and foliar symptom induction was like ozone exposures; no simple linear positive relationship could be concluded by using injured area as a variable to predict pollutant concentrations (Figures 2, 3, 4, 6).

Foliar injury induced by nitrogen dioxide in tested clones was not expected as compared to ozone or sulfur dioxide exposures. None of tested clones showed any pollutant induced symptoms by 0.05 ppm nitrogen dioxide at the end of four hours daily for 35 consecutive days exposure (Figure 2). Only two out of six clones showed visible symptom injury at the end of 0.10 ppm long-term fumigation (Figures 3, 4). The average foliar injury in these two clones was less than 10%. Such inability of nitrogen dioxide to induce foliar symptoms on white pine was consistant even following pollutant dosage being raised to 0.30 ppm for four hours

daily for 50 consecutive days. These results agree closely with those obtained by Bennett and Hill (1973a, 1973b) in their work with adverse effects of pollutants to plant growth. They found that at the same dosage of nitrogen dioxide, sulfur dioxide, and ozone, nitrogen dioxide was the least toxic to alfalfa photosynthesis.

All of the clonal materials with different pollutant sensitivity designation used in this study were classified based upon foliar symptom expression, needle length, and crown appearance of ortets in the field (Philipis et al., 1977a, 1977b). Evidently such plant sensitivity classification was only applied to ambient pollution in general without any specification to pollutants. Results of this study indicate that such white pine differential sensitivity ranking was not as obvious in nitrogen dioxide exposure as those in ozone or sulfur dioxide exposures. The tolerance of white pine clones to nitrogen dioxide was proven to be more common than to ozone and sulfur dioxide . Presented data also suggest that the test nitrogen dioxide dosages were not adequate to exert foliar injury on most of test clones if they were sensitive to nitrogen dioxide.

Early air pollution studies suggested that common nitrogen dioxide concentrations in the ambient air were unlikely to cause visible plant injury in field conditions (Hill et al., 1974), excluding cases of accidental nitrogen dioxide release from industries. Many of the confirmed

nitrogen dioxide induced foliar symptoms on plants required a threshold concentration higher than one ppm (Middleton et al., 1958; Taylor, 1968, 1973, 1975; Heck, 1964). However, recent studies have indicated that nitrogen oxides alone could become phytotoxic agents even at low concentrations. More importantly, nitrogen oxides could act more-than-additivelly with sulfur dioxide both in pphm concentration to affect plant growth (Horsman and Wellburn, 1975; White et al., 1974; MacLean et al., 1968; Thompson et al., 1970, 1971, 1972; Taylor and Eaton, 1966). In this study, the more than additive effects of nitrogen dioxide with sulfur dioxide on foliar injury were observed in clone I-1, III-2, and III-3 (Figure 4). A similar kind of more than additive effects between nitrogen dioxide and ozone were observed in clone I-1, II-1, and III-2 (Figure 3). However, it is important to point out here that such more than additive effects between nitrogen dioxide and other pollutants on foliar injury were not constantly observed throughout this study (Figures 2-4). For example, the effects of nitrogen dioxide + ozone on clone II-1 and II-3 foliar injury was less than additive (Figure 2).

There was a substantial variation in clonal visible foliar symptom expressions on white pine needles in terms of type of symptoms and total leaf area injured (Figures 2-6). They varied from experiment to experiment and from year to year. However, there was still a consistent significant

difference in symptom expressions among three different pollution sensitivity classes. Clones in sensitive class were consistently injured more frequently and more severely than those in intermediate clones or tolerant clones. Further tests demonstrated that the classification based upon field morphological characters generally matched well with physiological and yield response as measured by net photosynthesis and needle dry weight, respectively (Figures 21-23; Table 3). The only major contradiction was found in intermediate clones following nitrogen dioxide exposures (Figures 18, 19, 20).

There was a significant difference among clones in the timing of the appearance of the first visible symptom on needle surfaces in this study. Generally speaking, they appeared after a 12-56 hours of exposure at 0.05 ppm concentration and a 8-28 hours at 0.10 ppm concentration in sensitive clones. In intermediate clones, the appearance of chlorosis or necrotic symptoms on current year needles was week(s) later than those in sensitive clones and accompanied by less severe injury. In tolerant clone, none of the pollutant-induced symptoms was observed at the end of long-term fumigations (Figure 2). Such lag phase in symptom expression in tolerant clones may suggest that a higher internal pollutant threshold dosage is functioning in tolerant clones.

Literature data concerning the sensitivity of eastern

white pine to ozone, sulfur dioxide, or nitrogen dioxide were fragmentary and often contradictory. For example, Sinclair (1969) reported that sensitive white pine was injured after exposed to 0.06-0.08 ppm ozone for four hours or 0.02-0.04 ppm for 48 hours. Some other ozone related reports were 0.065 ppm for one hour (Berry, 1961), 0.05 ppm for one hour (Berry and Ripperton, 1963), 0.1 ppm for eight hours (Berry, 1971), 0.25 ppm for four hours (Davis and Wood, 1972), and one ppm for four hours (Botkin et al., 1971, 1972). In sulfur dioxide fumigations, Costonis (1970) reported 0.03 ppm for one hour was the threshold dosage for foliar injury. Houston induced sulfur dioxide injury on sensitive clones after exposed them to 0.025 ppm sulfur dioxide for six hours (Houston, 1974). Skelly et al. (1972) suspected one-hour highest nitrogen oxides concentration at 0.585 ppm combined with other pollutants was the cause of white pine disorder in the field. In this study, even under uniform culture practices and the use of clonal plant materials, pollutant sensitivity of different clones still exhibited a great degree of variation. These results clearly demonstrate that white pine pollutant sensitivity is genetically inherited and could vary greatly within the same species. When compared with the results of pollutant threshold dosage of other studies, the reasons for conflicting results could be due to one or more of the following factors: 1) the inability to duplicate the exact environmental conditions in

the experiments, 2) the different indexing criteria used as parameters to judge plant sensitivity, 3) the different pollutant exposure concentrations, durations, and timings of exposures, 4) the variations in plant materials, such as physiological age and culture practice, and 5) the different experimental designs, instrumentations and calibration procedures in pollutant fumigations.

Chlorophyll Content

Air pollution caused chlorosis, pigmented mottling, or necrosis in many plant species. Biochemically speaking, pigment change or discoloration is a process of chlorophyll degradation and/or formation of non-green pigments in plant cell such as anthocyanin, carotenes, or even phenolic compounds (Malhotra, 1976; Gowin and Goral, 1977; Swieboda, 1976; Puckett et al., 1973). In this study, chlorophyll content of white pine current year needles was decreased after long-term ozone and sulfur dioxide exposures. Different degrees of toxicity among the same dosage of sulfur dioxide, ozone, and nitrogen dioxide was observed in chlorophyll measurements. Sulfur dioxide was found to be the most toxic pollutant following by ozone. Under test conditions, nitrogen dioxide only significantly reduced chlorophyll a content of clone II-1 at 0.30 ppm exposure. The results are consistent with other studies in proving inhibitory effects of pollutants on chlorophylls (Malhotra, 1976; Suwannapinunt

and Kozlowski, 1980; Swieboda, 1976)

In light of other studies, such as Linzon (1971) stated that current year needle was the most important to tree's welfare among the three ages of needles on eastern white pine. Swieboda (1976) indicated that 80% of Scotch pine's needle chlorophyll was synthesized in the first year of plant growth. The adverse effects of air pollutants on plant chlorophyll contents as observed in this study (Table 5) could thus be at least partial contributed to the decrease of current year needles' photosynthesis (Figures 21, 22, 23).

Although the process of chlorophyll destruction is too slow to account for instant depression of net photosynthesis as observed in daily pollutant exposures (Figures 12-20), there is a general agreement in the literature indicating that such destruction of chlorophyll is considered as secondary plant process rather than primary metabolism like photosynthesis and transpiration. Substantial evidence exists which indicates that tissue discoloration and degradation of chlorophyll can only be observed in pollutant exposed plants after a substantial period of fumigation while the alteration of photosynthesis or transpiration is an instant plant response (Gowin and Goral, 1977; Ziegler, 1975; Hill and Bennett, 1970). However, it is difficult to illustrate the turning point of cellular metabolisms from primary to secondary process in a dynamic system, such as a pine needle in

this study.

Needle Elongation

Pollutant exposures were found to reduce needle elongation in this study (Figures 7-11). There was no simple direct correlation between plant pollutant sensitivity ranking and its needle length. Needles of sensitive clones may be shorter or longer than those of tolerant clones depending upon white pine clone and pollutant treatment. In other words, the length of needles among different sensitivity classes did not directly relate to their pollutant sensitivity. It is very interesting to note that under pollutant-free conditions, sensitive clones regained their growth in terms of needle length much more than those of tolerant clones as compared to pollutant-treated plants (Figure 12). Data suggest that the inhibitory effects of pollutant exposures on sensitive clones were more severe than those on tolerant clones.

Similar results of needle reduction due to pollutant exposures have been reported by several studies. Eckert and Houston (1980) fumigated sensitive and tolerant eastern white pine clones to 0.05 ppm sulfur dioxide for two hours and found that final needle lengths in the end of growing season were reduced. Although differences between control and fumigated needles were not statistically significant, all fumigated needles were shorter with the greatest reduc-

tion occurring in sensitive clones. Coyne and Bingham (1980) investigated the responses of ponderosa pine to ozone exposure in the field and reported that needles from the more injured trees tended to be shorter, smaller in girth, and less dense than needles of less injured trees. Correlation coefficients for specific leaf weight was 0.52; needle length 0.38; and needle width 0.55. All of these parameters were statistically significant at P=0.01 level although they were not particularly large.

The growth of needle in each clone is believed to be controlled by individual genetic constituents. Any given environmental factor (includes air pollution) or set of factors are suspected to exert their influence on needle elongation only to certain limits which would not override the gene control (Karnosky, 1977). The length of needles is thus considered not a reliable indexing factor to reflect test white pine's sensitivity to pollutants.

Pollutant Combinations

In the atmosphere, many kinds of pollutants can coexist in different combinations at various ratios. Very rarely can a pollutant exist alone in the entire air mass. It is thus possible that those pollutants in a mixture may influence each other chemically or physically in several ways and subsequently made the plant responses to pollutant combinations different from those induced by single pollutant expo-

sure. The observed more than additive, additive, and less than additive effects of pollutant combination on symptom expressions (Figures 2-4), needle length (Figures 7-9), and needle dry weight (Table 3) could have resulted from such interactions of pollutants in the ambient air or within plant tissues. The effects of pollutant combinations as compared with single pollutant exposures were depending upon pollutant treatment, white pine clone and plant response measured. The effects of pollutant combinations can be changed along with the changes of pollutant concentrations. For example, the more than additive effects of pollutant combination on foliar symptom expression were most evident at low concentration treatments (Figure 2) and becoming less pronounced as pollutant concentration increased (Figures 3, 4). Similar complexity of pollutant combinations on plant reaction has been well documented in the literature (Kress, 1978; Tingey and Reinert, 1975; Tingey et al., 1971a, 1971b, 1973a, 1973b). The actual plant response mechanisms to prolonged pollutant combination exposures are probably more complex than we can comprehend and beyond the scope of this study. No further plant physiological or biochemical response was measured on pollutant combinations in this study.

Plant Metabolisms and Stomatal Movements

Since the types of visible symptom and the patterns of lesion development induced by ozone, sulfur dioxide, and/or

nitrogen dioxide on white pine needles were so similar that quantitative and qualitative identification of causal pollutant by plant foliar symptoms were not possible (Figures 2-6). Furthermore, many pollutant exposures have reduced plant growth without the presence of visible injury (Tables 3, 4). It was thus necessary to determine plant physiological or biochemical response to pollutants in order to understand the mechanisms of pollutant injury and the causes of clonal differential sensitivities to pollutants. In other words, hidden injury of pollutants on plant growth could be illustrated by such measurements as depression of photosynthesis, stimulation of respiration, and low availability of metabolites which subsequently lead to the reduction of growth and yield, or overall unthrifty plant appearances without actual lesions on plant surfaces. The understanding of such relationship between pollutant exposures at sublethal concentrations and its adverse effects on fundamental plant metabolisms certainly would also aid immeasurably in the establishment of ambient air quality standards. Among many plant reactions, stomatal movements and assimilatory metabolisms were of major interest in this study. Since stomata are the principle entry of carbon dioxide and gaseous pollutants into plant tissues. Stomatal movements play key roles in pollutant uptakes and plant photosynthesis which directly relate to plant growth and yield.

The control plants in this study exhibited a steady

rate of photosynthesis and transpiration during daily six-hour gas exchange measurements (Figures 12-20). This indicates that the mid-day photosynthesis depression did not occur under test conditions. Sensitive clone in pollutant-free environments regained its photosynthesis efficiency and performed as well as intermediate or tolerant clone (Table 6). However, when exposed to pollutants, the inhibitory effects due to the same dosage of pollutant were much severe in sensitive clone than in intermediate and tolerant clone (Figures 21-23). Presented data support the view that the impacts of long-term pollutant exposures on white pine growth can be clearly reflected in the decrease of photosynthesis and the increase of respiration during daily gas exchange measurements (Figures 21-26).

A. Ozone Effects

Ozones was found to induce net photosynthesis (Figures 12A, 13A, 14A) and simultaneously decrease transpiration (Figures 12B, 13B, 14B) during four-hour day time exposure in clone II-1, III-2, and IV-2. The magnitudes of effects were proportional to ozone concentrations in each clone with the sensitive clone being suppressed the most. Data suggest that the induced adverse effects on carbon dioxide fixation may contribute to the partial closure of stomata. The closing response of stomata would then further reduce the total functional stomatal pore area on a unit leaf surface basis

and restrict the enflux of water vapor and the influx of carbon dioxide through the stomatal pores.

The other supporting evidence of such ozone injury on white pine gas exchanges was based on the observations that net photosynthesis appeared to be decreased more rapidly than transpiration (Figures 12A, 12B). This indicates that there is a rapid adverse effect on photosynthesis due to ozone exposure and a subsequent slow closing of stomatal aperture. Nevertheless, at present time, it is still unknown if the partial closing of stomata when exposed to ozone was due to passive inhibitory effect or active protective mechanism after carbon dioxide fixation process has been interfered with, or both. The observed response of gas exchanges during pollutant exposure may be a compromise between the optimum carbon dioxide uptake from ambient air for assimilation and the prevention of excessive water loss from opened stomata under test environmental conditions.

The disproportional reductions between net photosynthesis and transpiration by pollutant exposures as found in this study has been observed and theorized by several researchers. McLaughlin et al. (1979) and Coyne and Bingham (1980) listed two reasons for a lack of proportionality between water vapor evolution on transpiration and carbon dioxide uptake on assimilation found in pollutant exposures: 1) the temperature dependencies of these two gas exchange processes are different, and 2) transpiration is linearly

related to water-vapor concentration in leaf intercellular spaces but assimilatory rates follow a saturation curve with respect to intercellular carbon dioxide concentration.

Since the observed magnitudes of reduction in stomatal closing could not fully account for the reductions in carbon dioxide assimilation, the internal physiological and/or biochemical inhibition of carbon dioxide fixation due to ozone treatment is thus suspected under test conditions. The inhibitions due to the same ozone dosage was found to be much less in tolerant clone than in sensitive clone (Figures 12, 14). These data suggest that the internal threshold dosage for carbon dioxide assimilation inhibition is much higher in tolerant clone than in sensitive clone.

Ozone has been reported to reduce photosynthesis of several tree species by other studies. Miller et al. (1969) found that photosynthesis of ponderosa pine was reduced by 10, 70, and 80% at cumulative ozone dose of approximately 40, 80, and 120 ppm-hour, respectively. Meanwhile, photosynthesis has been illustrated to be more sensitive to ozone injury than stomatal conductance in field ponderosa pine study (Coyne and Bingham, 1980). Dugger et al. (1962) reported that stomata were not the primary controlling factors from ozone injury to pinto bean, instead, they testified that the physiological age of bean plants along with internal biochemical reactions should account for alleviating pollutant injury in their study. All of these findings

proved the similar results of ozone adverse effects on photosynthesis and photosynthetic transpiration as this study and are in a good agreement with presented data.

B. Sulfur Dioxide Effects

In sulfur dioxide exposures, a reduction of net photosynthesis was found in sensitive clone and intermediate clone at all three tested concentrations (Figures 15A, 16A). Such reduction was accompanied with a stimulatory effect on transpiration (Figures 15B, 16B). However, in the tolerant clone the increase of transpiration was observed along with a small increase of net photosynthesis (Figures 17A, 17B).

Based upon these observations, sulfur dioxide fumigation under test conditions (26-32°C, 60-70% R. H., and 21,000- 28,000 lux) seemed to stimulate the opening of stomatal aperture while also inhibite carbon dioxide fixation. By comparion of these two gas exchange measurements, the actual degree of sulfur dioxide inhibitory effect on net photosynthesis could be higher than figures given primarily due to a coincidental wider opened stomata.

Recovery of net photosynthesis and transpiration after the termination of pollutant exposures were found in all three clones at all test sulfur dioxide concentrations. The recovery rates varied with clone and sulfur dioxide concentration. There is an indication that the greater the depression, the longer the time which will be needed to

recover (Figures 15B, 16B, 17B). Meanwhile, a quicker recovery of transpiration than net photosynthesis was obsreved in treated plants (Figures 15, 16, 17). These results suggest that sulfur dioxide exposures exert more inhibitory effects on carbon dioxide fixation than on stomatal movement.

Similar results of sulfur dioxide adverse effects on plant photosyntehsis and transpiration have been reported by numerous studies. Ziegler (1972, 1973, 1975) suggested that sulfur dioxide could act by competing with carbon dioxide or bicarbonate for the binding sites on ribulose-1,5-diphosphate-carboxylase. The theory of competitive inhibition of sulfur dioxide on carbon dioxide fixation was later confirmed by the results from an in vivo study that showed that the depressed photosynthesis was fully recovered to the normal rates when returning to clean air after being exposed to low sulfur dioxide concentration (McLaughlin et al., 1979). The response of recovery from inhibited photosynthesis after the termination of pollutant exposures also indicated a reversible inhibition of carbon dioxide fixation process in which pollutant dosage was below the limits of permanent injury threshold (Tingey, 1974).

In other studies, Unsworth et al. (1972) showed that there was a brief and small increase in bean net photosynthesis when exposed to sulfur dioxide which was accompanied by stomatal opening. But within about 30 minutes, net

photosynthesis began to decrease and typically reached to minimum level after one to two hour of exposure. The magnitude of photosynthesis decrease in their study was about 20% of pre-treatment value. McLaughlin et al. (1979) observed that transpiration of kidney beans was less sensitive than photosynthesis to short-term (three hour) exposure of sulfur dioxide. They suggested that stomatal closure was not a major factor in the responses observed. All of these studies along with present data suggest that carbon dioxide fixation process may be the primary site of sulfur dioxide inhibitory reaction following by the movements of stomatal aperture.

There was a more pronounced adverse effect of sulfur dioxide exposure than ozone on net photosynthesis of clone III-2. This indicates that physiologically clone III-2 is more sensitive to sulfur dioxide than ozone exposure. Similar results of plant differential sensitivity to pollutants have been reported by Berry (1973).

C. Nitrogen Dioxide Effects

All of test nitrogen dioxide exposures slightly reduced net photosynthesis and transpiration under test conditions. There was a trend showing that the observed decreases of net photosynthesis were parallel to the magnitudes of transpiration reduction in all clones at all test concentrations (Figures 18, 19, 20). Results indicate that a nitrogen

dioxide threshold dosage higher than tested (0.30 ppm for four hours) would be needed to bring about any significant change in photosynthesis or transpiration. Data also indicate that the toxicity of nitrogen dioxide is much less than ozone or sulfur dioxide to white pine carbon dioxide fixation processes and the injury threshold of various pollutants on white pine photosynthesis are not the same.

These results of different modes of actions by various pollutants closely agree with the findings of Bennett and Hill (1974). They studied the effects of six major air pollutant on barley and oat canopies through two-hour exposures with equal pollutant concentration. The transpiration rate measurements showed that ozone increased leaf resistances to gas transfer in proportion to the amount of suppression on gas exchanges induced. However, in nitrogen dioxide exposure, transpiration rates were not significantly depressed while great reduction in carbon dioxide uptake was observed. The nitrogen dioxide appeared to inhibit carbon dioxide uptake rates by affecting the biochemical reaction of carbon dioxide fixation. In the investigations of sulfur dioxide exposures, they observed a tendency of stomatal closure along with the depressed net photosynthesis rate. They suggested that sulfur dioxide inhibited carbon dioxide uptake rates more by affecting biochemical fixation of carbon dioxide in the leaf than by impending gas transfer by inducing stomatal closure.

Effects of Long-term Pollutant Exposures on Dark Respiration

In dark period ozone exposures, plant dark respiration and water vapor evolution were not significantly affected in all three clones (Figures 12C, 12D, 13C, 13D, 14C, 14D) except in sensitive clone at 0.30 ppm exposure where dark respiration showed an 9% increase over four-hour exposure period while its transpiration was hold relatively constant (Figures 12C, 12D). Since such increased dark respiration did not recover after the termination of pollutant treatment, it is suspected that the increase of carbon dioxide evolution is not induced by dark period ozone exposure. It is suggested that the increase of dark respiration is due to the stimulated repairing or synthetic processes that prevailed in pre-clinical injured cells.

There is some supporting evidence in the studies of pollutant induced plant diseases for this rationale. First, cellular repair processes require many activated metabolic processes to provide bioligical energy in order to fend off the temporary injury or seal off the permanent injury caused by stress conditions (McLaughlin and Shriner, 1980). For example, activities of peroxidase, polyphenol oxidase, phenolase, and other oxidative metabolisms have been reported several-fold higher in pollutant exposed plants (Howell, 1974). Secondly, adenosine triphosphate (ATP) is known as an acid-liable phosphate (Pell and Brennan, 1973). The ATP

is a very vulnerable target in the event of lowing cellular pH value or destruction of cell buffering capacity that coincidently are fairly common in pollutant exposure cases. Furthermore, ozone has been reported to cause uncoupling of oxidative phosphorylation which in turn increased cellular respiration (Lee, 1967, 1968; Pell and Brennan, 1973). All of these biological repair processes, breakdown of ATP molecule, and uncoupling of oxidative phosphorylation would consequently increase the rate of respiration in pollutant treated plants as observed in this study.

Similar increases of respiration to recuperate from temporarily pollutant-induced injury are also suspected to occur in other pollutant treatments during or after day-time exposures although they had not been monitored in the dark respiration measurements. The time span between termination of day-time exposures and the beginning of dark period measurements was four hours. This four-hour period might be long enough for tolerant clones at test concentrations and sensitive clones at lower pollutant concentrations to resume their normal respiration rate when dark period exposure was begun.

In dark period sulfur dioxide exposures, the only significant changes observed was a continued increase of carbon dioxide evolution in sensitive clone at 0.30 ppm exposure regardless of the timing of administration of pollutant. As in ozone exposures, stimulated metabolisms for injury repair

or ATP production are suspected.

In the event of lesion formation before necrosis development in pollutant induced plant diseases, plants not only lose assimilatory units for carbon dioxide fixation but are also forced to invest some photosynthates to restore injured area. If plant succeeded, it could recover from such stress conditions without major loss in plant vigor and subsequently resume its biomass production. If plant lost in homeostatic repair processes, they not only suffered in terms of biomass production but also wasted valuable assimilates during repairing processes. Loss of photosynthate in repairing respiration without any visible symptoms could end up with hidden injury of diseased conditions in which substantial amount of energy could be lost, particularly in the prolonged pollutant exposure. Such sublethal concentration of pollutant can finally cause a zero growth in plants (Tables 3, 4).

Based upon presented data, it is evident that there was an increase in dark respiration temporarily before physical symptoms were noticed on the current year needles. However, needle dark respiration started to steadily decline and was even lower than pre-treatment values after the appearances of visible symptoms on needle surfaces (Figures 24-26). In long-term dark respiration measurement, data also indicate that as needles aged or pollutant dosage accumulated, the effects of pollutant exposures on sensitive clone's dark

respiration diverged from indistinguishable patterns as at the early stage of exposures to significant difference among treatments at the end of experiment. Meanwhile the responses of tolerant clones induced by different pollutant treatments still remained undistinguishable (Figures 24-26). Presented results tended to coincide with the theory that a higher dosage of pollutant is needed to induce the same degree of injury in tolerant plant than in sensitive plant.

By comparing the clonal responses of sulfur dioxide exposures at daytime and nighttime, it is concluded that the stimulative effect of sulfur dioxide on white pine stomata opening is a light-dependent reaction.

Effects of Long-term Pollutant Exposures on Net Photosynthesis

Apart from instant effects of pollutant exposure on gas exchanges obtained from hourly measurement (Figures 12-20), cummulative effect of pollutant exposures on disturbed photosynthesis was later manifested in biomass production (Tables 3, 4). The rates of photosynthesis did not fully recover from pollutant-induced depression at one hour after the termination of exposures. In general, the higher concentration of pollutants as well as the more sensitive of clone, usually a longer time is needed to recover. This suggest that there is a more severe injury on photosynthesis and its subsequent photosynthate production in sensitive

clones than in tolerant clones. Such continued depression of assimilatory metabolisms would mean less photosynthate prodution per leaf surface per time interval and is later confirmed by the significant reduction of plant vigor, yield, and plant productivity (Tables 3, 4). Meanwhile, photosynthate is essential for the repair of pollutant injured cellular constituents (McLaughlin and Shriner, 1980; Mann et al., 1980), a prolonged inhibition of net photosynthesis due to pollutant exposures would result in a decrease of photosynthesis efficiency and a reduction of available photosynthate (Taylor 1977; Vins and Mrkva, 1973).

Throughout the experiment, premature defoliation was observed freguently in sensitive clones but not in tolerant clones. This confirmed the field observations that sensitive trees only have current year needles remaining following low dose of pollutant exposures (Berry, 1973; Houston, 1974).

From a bioproduction view point, premature defoliation of pine needles is the complete loss of assimilatory apparatus which subsequently result in biomass reduction (Tables 3, 4). In the cases of symptomatic tissues, the rates of plant metabolisms are believed to be largely reduced in needles displaying chronic or acute injury as compared with asymptomatic tissues (Figures 21-23). With early manifestation of foliar symptoms (which is a sign of chlorophyll destruction) and premature defoliation in sensitive clones as

observed in these long-term exposures, growth reduction in these diseased plants could be explained by either less efficiency or inability of needles to supply the amount of photosynthate which is directly needed in cell repair and plant growth.

Similar chronic effects of ozone and sulfur dioxide on biomas production in intermediate and tolerant clones were also observed in long-term net photosynthesis measurements (Figures 22, 23). Decreased net gain of assimilatory metabolites due to pollutant exposures without visible injury is thus suspected even in the tolerant clone (Tables 3, 4).

Coyne and Bingham (1980) demonstrated that prolonged suppression of photosynthetic rates and premature loss of autotrophic tissue due to ozone exposures could result in reduced carbon accumulation per tree accompanied by reductions in the biomass and surface area per needle, the specific leaf weight, and the number of annual needle whorls retained. These factors contributed to the steady loss of tree vigor in their study, weakening trees to the point of vulnerability to pathogenic organisms such as root rotting fungi and bark beetles.

Similar results of pollution effects on white pine have been found at RAAP, Blue Ridge Parkway in Virginia area, and other places. Trees sensitive to pollutants have been reported to be more vulnerable to other biotic and abiotic stresses (Skelly, 1977, 1980; Lackner and Alexander 1980;

James et al., 1980; Cobb et al., 1968).

Closing Remarks

The differential plant sensitivity to pollutants have been largely attributed to different genetic constituents which expressed as different anatomical structures, physiological reactions, and/or biochemical functions (Levitt, 1972; Bingham and Coyne, 1980). Trimble (1980) examined the same white pine clones as used in this study and found that stoma numbers per needle surface area in ozone sensitive clones were not significantly different from those of tolerant clones. The anatomical structures thus play no roles in white pine differential pollutant sensitivity in this study. The avoidance tolerance as defined by Levitt (1972) was not the case of eastern white pine tolerance to pollutants since none of the white pine clones studied showed the response of complete stomatal closure when exposed to pollutants. However, the observed partial stomatal closing in eastern white pine induced by pollutants is to a certain degree of importance in the exclusion of pollutants from further entry into plant tissues. Other internal biochemical factors such as cellular sensitivity to pollutants and the integrity of cellular buffering capacity may be also involved in actual tolerance mechanisms by which differential sensitivities to air pollutants resulted. In other words, the observed clonal differential sensitivities to

pollutants are likely to be quantitatively rather than qualitatively expressed. The existance of different biochemical tolerance mechanisms which function after pollutants have entered into leaf tissues would reasonably be suspected.

The data presented indicate that the dose of pollutant is certainly important in the determination of the severity of metabolic dysfunction and the rate of these responses. When sensitive clones are exposed to the same dose of pollutants, rapid reaction of physio-biochemical changes are affected as compared with a slower reactions or no influence on intermediate and tolerant clones.

It is believed that a plant population can be differentiated through natural selection from its population gene pool to fit into air pollution stress ever since they encountered with this increasing environmental stress (Houston and Stairs, 1973; Karnosky, 1977; Treshow, 1968; Sinclair, 1969). However, the tolerance to a given pollutant does not mean a general tolerance to any other gases. It is evident from the present investigation that a general clonal sensitivity to all of the pollutants was not observed. For example, clone II-1 was sensitive to ozone and sulfur dioxide while not sensitive to nitrogen dioxide, clone III-2 was more sensitive to sulfur dioxide than ozone. Such results also indicate that different pollutant has different modes of injury action.

The differential sensitivities of white pine clones to gaseous pollutants investigated may involve one or more of the following mechanisms : 1) different stomata response in terms of controlling pollutant entry into plant tissues (Figures 12-20), 2) different sensitivity of cellular constituents to pollutants (Table 5), 3) different clonal internal biochemical threshold dosage to each pollutant (Figures 12-20), 4) different assimilation or repair efficiency among clones (Tables 3, 4, 6), and 5) varied recovery rate from temporary pollutant induced injury. From the results reported here, white pine clonal differential sensitivities to pollutants are concluded as different stomatal responses as well as different internal physiological reactions to pollutant treatments. All of these differences are attributed to the mechanisms controlled by clonal genetic constituents.

SUMMARY

A total of 10 clones of eastern white pine (Pinus strobus L.) with three different pollution sensitivity classes (sensitive, intermediate, and tolerant) were used in photosynthesis, transpiration, and dark respiration measurements during ozone, sulfur dioxide, and nitrogen dioxide exposures. In addition to gas exchange measurements, needle growth, visible symptom expression, chlorophyll content, and needle dry weight of current year needles were also determined during long-term single pollutant or pollutant combination fumigations to discern the progress of pathogenesis of pollutant-induced diseases and the mechanisms of clonal differential pollution sensitivity. Extensive efforts were conducted to systematically analyze, relate, and interpret the disease syndrome. The age of clonal materials was uniform and all plants were grown within a charcoal-filtered air supplied greenhouse. Plants from various clones were then subjected to identical environmental conditions during and after daily four-hour pollutant fumigations except using various concentrations of ozone, sulfur dioxide, and nitrogen dioxide. The primary goal of this study was to determine the clonal plant responses to long-term, low-dose pollutant exposures and to investigate the reliability of various plant reactions in terms of pollutant sensitivity predic-

tion.

Genetically controlled plant responses to pollutants were found in all clones under the test conditions with various degrees of repeatability. Clonal sensitivity to ozone, sulfur dioxide, and nitrogen dioxide was varied individally; a general sensitivity to all of the pollutants was not observed.

There seemed to be no general response patterns among clones to pollutant fumigations in terms of total injured area, types of symptoms, and needle elongation. These parameters varied from year to year. Foliar symptom expression was only categorically differentiated among classes but was confused by clonal plant response within the same class. Needle length was found having the greatest variation. At the end of long-term exposures, needle length was not significantly different among sensitive, intermediate, and tolerant classes nor among pollutant treatments.

Good agreement was observed among some of measured plant responses with respect to clonal pollutant sensitivity; tolerant plants usually accompanied with less inhibition of net photosynthesis, higher chlorophyll content per needle dry weight basis, and higher needle dry weight per fascicle length as compared to those of sensitive clones.

During daytime exposures, pollutant sensitive clone exhibited greater instant inhibition of net photosynthesis due to ozone and sulfur dioxide fumigation than those of

intermediate and tolerant clones. In all three clones, nitrogen dioxide at test concentrations did not significantly affect carbon dioxide uptake. In terms of stomatal reactions, data suggest that ozone and nitrogen dioxide inhibited stomatal opening during pollutant exposures, sulfur dioxide was stimulatory. During dark period pollutant exposures, no significant stomatal changes were observed in any of the clones. In pollutant treated plants, the rates of net photosynthesis of sensitive and intermediate clones declined significantly during long-term exposures. Early stimulation of dark respiration was observed in sensitive clones followed by a dramatic decrease during the late stages of long-term exposures.

Data demonstrate that the responses of white pine gas exchange and other photosynthetic apparatus to ozone, sulfur dioxide, or nitrogen dioxide exposures are more proportional to pollutant concentrations than physical symptom expressions or needle length measurements.

It is concluded that visible injury and needle elongation are less superior than gas exchange measurements, chlorophyll content, or needle dry weight in providing a reliable indexing parameter for pollution sensitivity prediction.

Data suggeste that plant physiological responses such as gas exchange rates or chlorophyll content can be used as a quick screening method to determine plant sensitivity to pollutants in tree improvement programs, especially in situ-

ations where pollutant dosage is too low to induce any visible symptom on foliage thereby preventing the growers from knowing that existing ambient air caused adverse effects.

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VARIATION IN THE PHYSIOLOGICAL PROCESSES OF EASTERN WHITE PINE (PINUS STROBUS L.) DIFFERING IN SENSITIVITY TO OZONE, SULFUR DIOXIDE, AND NITROGEN DIOXIDE

BY

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(ABSTRACT)

Ten clones of eastern white pine (Pinus strobus L.) representing three different pollutant sensitivity classes (i.e. sensitive, intermediate, and tolerant) were exposed to ozone, sulfur dioxide, and nitrogen dioxide singly, and in combinations at various concentrations. Visible symptom expression, needle length, and needle dry weight of current year needles were determined weekly during long-term pollutant exposures. One clone of each sensitive class was selected to study the effects of long-term exposures with ozone, sulfur dioxide, or nitrogen dioxide on photosynthesis, transpiration, and dark respiration.

Genetically controlled plant responses to pollutant exposures were found in all clones with various degrees of repeatability. A general plant sensitivity to all of the pollutants was not observed in test clones. Foliar symptom expression was only categorically differentiated among sensitivity classes but was not distinguishable in clonal response

within the same class. At the end of long-term exposures, needle length was not significantly different among sensitive, intermediate, and tolerant classes nor among pollutant treatments. Good agreement was found among white pine gas exchange rates, needle dry weight, and chlorophyll content with respect to clonal sensitivity. Sensitive clone exhibited the greatest reduction in net photosynthesis due to ozone and sulfur dioxide exposures followed by intermediate and tolerant clones. Early stimulation of dark respiration was induced by ozone and sulfur dioxide exposures in sensitive clone followed by a dramatic decrease at late stages of long-term experiment. Nitrogen dioxide at test concentrations did not significantly reduce net photosynthesis, transpiration, and dark respiration rates. There was a correlation between clonal needle dry weight, chlorophyll content, and degree of its pollutant injury. Different modes of injury actions by different pollutants are proposed based upon presented data.

Results support the concept that the ranking of plant sensitivity to pollutants could be varied with plant response chosen as indexing criterion. Presented data suggest that the adverse effects of pollutant exposures on white pine growth are primary due to inhibition of net photosynthesis, less chlorophyll content, and high respiration rate. Visible injury and needle length are concluded to be less superior than net photosynthesis, transpiration, dark respiration,

chlorophyll content, and needle dry weight measurement in providing reliable indexing parameter for white pine pollution sensitivity prediction.