

THE RESPONSE OF 12 CLONES
OF EASTERN WHITE PINE (Pinus strobus L.)
TO OZONE AND NITROGEN DIOXIDE/

by

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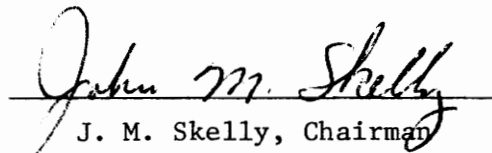
Thesis submitted to the Graduate Faculty of
the Virginia Polytechnic Institute and State
University in partial fulfillment of the
requirements for the degree of

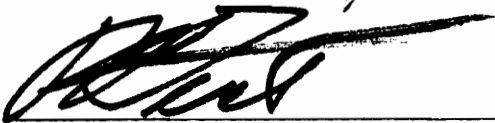
MASTER OF SCIENCE

in

Plant Pathology

APPROVED:


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November 1977

Blacksburg, Virginia

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ACKNOWLEDGMENTS

This research was sponsored and supported by the U. S. Department of the Army, Army Research Office, Research Triangle Park, North Carolina under grant No. DAAG 29-75-0043.

The author would like to thank the following individuals and organizations for their support throughout the course of this research:

Dr. F. W. Morthland, Army Research Office, for his consideration and cooperation in the approval and administration of this grant;

Dr. Prescott Ward, Director of the Bio-Medical Research Laboratory, Edgewood Arsenal, Aberdeen Proving Grounds, Maryland, for his encouragement and help in initiating support for this work;

Dr. J. M. Skelly for his thoughtful guidance and sincere encouragement through every stage of this research, from the initial design to the preparation of this thesis;

Dr. P. P. Feret for his very knowledgeable advice and guidance over the past two years, especially during the preparation of this thesis;

Dr. H. B. Couch for his council in the design stage of this research;

The Radford Army Ammunition Plant and Hercules Incorporated for their cooperation, especially Lt. Col. R. E. Snyder, Col. R. B. Henry, Col. J. R. C. Roop, and Mr. D. H. Maybury;

Mr. Lance Kress and Mr. Stan Long for their many hours of help and knowledgeable advice over the last two years;

Dr. Marvin Lentner and Ms. Jill Stewart for their very able help in the statistical design and analysis of the data presented in this thesis;

And finally, to the one person who, without her love, understanding, and encouragement none of this would have been possible, my wife Molly.

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INTRODUCTION

As the United States begins its third century of development as a nation, many new conflicts are going to arise with regard to the quality of the environment and the subsequent quality of life. With the advent of the automobile and the rapid industrialization during the early twentieth century, people began to recognize that there were environmental debts to be paid for technological progress. Many problems were identified during this period and through inventiveness and desire, man was able to solve most of them. Man's perception of his improved life style calls for more fuel to drive his automobiles and heat his homes and businesses, more electricity to power the numerous convenience appliances, better housing, a better selection of goods at the market, and, of course, an optimistic outlook for the future. In short, man relies on a technology which is wholly dependent on energy, the production of which either directly or indirectly results in some form of air pollution. Historically man has not reacted to a problem situation until he was directly affected or severely threatened. The first National Air Pollution Symposium held in the U. S. was in California in 1949 and the next year the first U. S. Technical Conference on Air Pollution was held in Washington, D. C. (71). These were convened in response to the appearance of the Los Angeles smog problem in the 1940's and other specific air pollution episodes that had taken place in the U. S. and elsewhere since 1930 (71). In 1952 London, England experienced a major air pollution disaster resulting in the passage of the Clean Air Act in that country some four years later.

Similarly, it was not until 1955 that the Public Health Service of the Department of Health, Education, and Welfare (the forerunner of the Environmental Protection Agency) was tasked with providing federal support for air pollution research, training, and technical assistance in the U. S. (71). The timing of increased public awareness concerning air pollution problems indicated that due to the industrialization that had occurred since the turn of the century, the air pollutant load in the atmosphere had probably reached a critical point by about 1940. Since 1940 this trend has not only continued but in most categories related to air pollutant emission (production of electrical power, numbers of vehicles, etc.), it has increased (Table 1).

Table 1 shows that while the population growth rate of the U. S. has slowed since 1940, the growth rate of the manufacturing industries as measured by the added value of manufactured goods and electrical energy generation, as measured by kilowatt hours produced has increased 7.0 and 13.0 fold respectively over the period 1900-1940. It should be pointed out that the indices used for these two industries do not take into account: (i) inflation in the case of value added to manufactured goods measured in dollars and (ii) increased efficiency in the case of the production of electricity. Nonetheless, in both cases the trend is certainly apparent even though the magnitude of each industries' contribution to air pollution is most probably smaller than stated due to the above mentioned reasons. During the period 1940-1974, the increase in the numbers of powered vehicle registrations showed only a 3.8 fold increase since the 1906-1940

Table 1. Increase of various parameters related to air pollution emission in the U. S. and Virginia for the periods 1900-1940 and 1940-1975.^a

| PERIOD | POPULATION | | AUTOMOBILE REGISTRATIONS | | VALUE ADDED TO MFG. GOODS | | ELECTRIC POWER PRODUCED BY PUBLIC UTILITIES USING FUELS | |
|-----------------------------|---------------------|-------|---|---------|---|--------|---|--------|
| | U.S. | VA. | U.S. | VA. | U.S. | VA. | U.S. | VA. |
| 1900-1940 | 11.74x ^b | 1.44x | 226.26x | 608.14x | 5.27x | 7.63x | 4.01x | N/A |
| 1940-1975 | 1.61x | 1.88x | 3.83x | 5.44x | 12.26x | 13.08x | 17.03x | 19.77x |
| Major pollutants associated | Various | | NO _x , O ₃ , PAN ^c | | SO ₂ , various acid mists, and others ^d | | SO ₂ , NO _x | |

^aSee Table 1, Appendix, for raw data, references, and explanations of parameters.

^bNumber represents multiplicative increase over base year.

^cPAN = peroxyacetyl nitrate.

NO_x = oxides of nitrogen.

O₃ = ozone

^dsulfur dioxide

period. However that figure is brought into perspective when it is shown that as of 1975, 133,727,000 vehicles were registered in the U.S. as compared to only 34,894,000 vehicles in 1941 (Appendix: Table 1). Motor vehicles are known to be the major source of both oxides of nitrogen (NO_x) and hydrocarbons (71). These two compounds are the primary components of the photolytic cycle that under optimum conditions leads to the build up of high ozone (O_3) concentrations.

In Virginia the growth rate in all four categories as shown in Table 1 during the period 1940-1975 was greater than for the U. S. In addition, the population growth rate of the state, unlike that for the U. S., increased over that of the 1900-1940 period. Approximately 90% of Virginia's population resides east of a line drawn from Washington, D.C. south through Richmond to the North Carolina border (12). Thus the Piedmont and mountain areas of the state should be relatively free of air pollution; however, that is not the case. Skelly et al. (66) reported in 1976 that the hourly average O_3 concentration had increased two to three fold in the mountainous section of the state since 1962 when Berry (7) reported base line data for O_3 concentrations in the nearby mountains of North Carolina and West Virginia. In addition it was shown that periods of elevated O_3 concentrations were associated with winds out of the northeast while comparatively low O_3 concentrations were associated with winds from the south to southwest. It was also shown that sensitive and intermediately sensitively clones of eastern white pine (*Pinus strobus* L.) known for its variable sensitivity to air pollution was significantly damaged during the periods of high O_3 levels while tolerant clones were injured to a lesser degree.

This data demonstrated that air pollutants (or their precursors) can be transported over long distances and cause significant damage at a site far removed from the source.

To enable researchers and government agencies to assess the exact impact of pollutants in rural areas of the country, monitoring sites must be established in these areas to accurately define the components of the polluted air mass. The establishment of a monitoring site capable of continually monitoring and recording three pollutants (O_3 , NO_x , and sulfur dioxide) is relatively expensive. As more precision is required in defining the constituents of ambient air, the costs increase accordingly. A monitoring site designed for the measurement of five different air pollutants and basic meteorological data (temperature, relative humidity, wind speed and direction) has been estimated to cost approximately 5 times more than the aforementioned instrumentation. Obviously the establishment of a number of these sites would be an extremely costly operation. Several researchers (5, 56) have suggested the use of various plant species of known pollutant sensitivity as a comparatively economical method to monitor air pollutants. A bio-indicator system should be able to detect the presence of a given pollutant and with the use of more refined clonal material, be able to estimate the pollutant concentration responsible for a given level of plant damage. Annual plants have been used as air pollution indicator plants. However, because they are annuals they must be replanted each year, thereby requiring new planting material. Even though the seed is certified as to its genotypic quality, a plant species that would not have to be replaced each year would have the added advantage of allowing

the researcher to compare results from year to year on the identical plant, thus adding a higher degree of accuracy that should aid further to the repeatability of important results. Eastern white pine has been proposed as a replacement for annual plants in an air pollution bio-indicator system.

The Radford Army Ammunition Plant (RAAP) located six kilometers (km) north of Radford, Virginia is one of the largest industrial complexes in the southwestern section of the state. The facility was constructed at the initiation of World War II and produced powder and ammunition beginning in April 1941. After the war the RAAP was closed down until 1949 when the production of powder was begun on a limited scale in response to the Korean War. Since then the facility has not been shut down. Production reached a third high point in 1969 in response to the Vietnam conflict. The production of nitrogenous based ammunitions led to the emission of various air pollutants, primary of which is oxides of nitrogen (NO_x) with nitrogen dioxide (NO_2) being the most biologically important constituent. The RAAP produces its own electricity and steam through coal fired generators which emit significant quantities of SO_2 in the effluent. Ozone is also known to exist in the ambient air at the RAAP. Thus three of the major phytotoxic air pollutants (O_3 , SO_2 , NO_2) are present to varying degrees in the ambient atmosphere at this facility. The first air pollution abatement program at the RAAP was funded by the Department of the Army in fiscal year 1971. By 1975 funding for all pollution abatement programs at the facility had reached \$112,400,000 (88). In addition to

the emphasis placed on pollution abatement, the Department of the Army also showed an interest in the effects of the pollutant regime at the RAAP on the surrounding vegetation by actively supporting research programs designed to elucidate these effects. As data accumulated on the correlations between pollutants and vegetation damage, RAAP administrators were interested in biologically demonstrating that the tremendous capital expenditures for pollution abatement programs were paying dividends with regard to reduced plant damage. Eastern white pine was chosen as a primary indicator species for the RAAP because: i) it is indigenous to the area and found abundantly within the installation and (ii) it has been shown in previous studies (58, 72) to exhibit the strongest correlation between average annual increment growth and RAAP production rates.

The purpose of this study was to begin the development of an air pollution bioindicator system for the RAAP using clonal material from indigenous eastern white pines.

The specific objectives were: (i) to determine the relative sensitivity of 12 selected clones to O_3 and NO_2 both singly and in combination and (ii) to develop efficient propagation and maintenance techniques suitable for the production of a large number of eastern white pine ramets for use in a pollutant sensitivity screening program.

LITERATURE REVIEW

Eastern White Pine and White Pine Needle Blight

Eastern white pine (Pinus strobus L.) is the largest of the coniferous species found in its range. This five needled (five needles per fascicle) pine at maturity usually ranges from 24.4 to 30.5 meters (m) in height and 0.6 to 1.1 m in diameter at breast height (DBH). The largest tree on record measured 67.1 m in height and 1.8 m DBH (31).

Eastern white pine is a major component of four forest types and associated with 14 others (87). It is found primarily in northeastern North America covering approximately seven million acres from the western Great Lakes, east into New England and Nova Scotia, and then south along the Appalachian Mountains into Georgia. Throughout this range the mean July temperature is 17° to 22° C (62°-72° F) and there is normally an excess of moisture in all seasons. Eastern white pine grows well on a variety of sites ranging from moist bottomlands to drier rocky ridgetops (86). Today, some of the larger stands are found in the southern Appalachians, while throughout its range the species is more often typified by an old field tree. Prior to the late 1800's the more extensive stands of eastern white pine were found in the Northeastern United States where it was a large and valuable timber species (93).

Eastern white pine is known to have three serious enemies in its natural habitat. Two were readily diagnosed, i.e. the white pine weevil (Pissodes strobi Peck) in 1817 (55) and white pine blister rust caused by Cronartium ribicola Fisher in 1905 (59). Air pollution,

however, now known to be a third important problem for eastern white pine was not diagnosed until the 1960's. This disorder was first referred to as white pine needle blight or white pine blight.

White pine blight was reported for the first time in 1905 in both Concord, New Hampshire (18) and northern Ontario, Canada (28). The reddish brown color of the current year's foliage was the predominant symptom (18). The "blight" was reported in nearly all the white pine regions of New England by the spring of 1907. Trees of all sizes were affected. They were not found in groups within a stand but rather as single individuals spread throughout the stand. Spaulding (68) reported on the presence of the disease in New York and New England in 1907 and 1908. The symptoms were virtually identical to those described by Dana (18) in New Hampshire but Spaulding added that they were unlike those of any other disease he had observed. The initial symptoms were observed each year about July 1 as reddish brown needle tips which faded to a dull brownish gray by September or October. Of the symptomatic trees tagged in one stand during the summer of 1907, only half were symptomatic in 1908. Drought was ruled out as a possible cause because of the fact that in the study area, the year 1908 was drier than that of 1907. The cause of the disorder remained unknown and for the next 14 years nothing of importance was reported concerning white pine needle blight. However, in 1922, Faull (28) working in Canada reported that, "the injury was not a winter killing of the foliage, an enzymatic disease, or due to fungi or insects, nor could it be induced in healthy branches by fusing them at freshly abraded spots with diseased branches

of affected trees". Thus from this report it seems unlikely that the disease was virus transmitted as will be born out by future researches.

In 1944 Swingle (73) working in Ohio described a chlorotic dwarf condition of eastern white pine. This chronic disease had been observed since 1936 in plantations throughout Ohio, and by 1944 symptomatic white pine were reported in Indiana and West Virginia. Analysis of foliage also indicated that the disease occurred in Kentucky, Virginia, Delaware, Pennsylvania, Michigan, New York, and several of the New England states. Swingle recognized that the condition was similar in some respects to the white pine blight as described by Spaulding in 1904 (68). The chlorotic dwarf condition was characterized by stunted tops and roots and by greenish-yellow foliage. By mid to late summer only the current year's growth remained on the tree. Swingle stated that the cause of this problem was most often associated with drought, natural competition and suppression, nutrient deficiencies, and poor planting practices resulting in balling of the roots. In addition to these causes, researchers had looked to a possible physiological-genetical complex, fungus root rot, feeding by sucking insects, lack of mycorrhizae, and viruses among others to explain the condition. However, no evidence had been presented that would substantiate any of these factors. The research on the problem was to continue with major emphasis to be in the area of viral pathogens and physiological disorders.

Five years later, Toole (81) working in Asheville, North Carolina described white pine blight in the southwestern United States. The

first reports of the problem in this area were received in 1943 from several areas in the southern Appalachians. By 1949, white pine blight was reported in four counties in Maryland, 11 in Virginia, 14 in North Carolina, one in West Virginia, two in Kentucky, and four in Georgia. Two stages of the blight were described: one was associated with reddening and eventual dieback of the current year's needles three to four weeks after emergence (coinciding with Spaulding's description) and the second was a more advanced stage where only a slight dieback was associated with chlorosis and dwarfing of the tree which were the predominant symptoms (coinciding with Swingle's description). Toole surmised that the two symptom complexes were caused by the same unknown factors. Needle fungi were discounted while the significance of: (i) root aphids as either the primary cause or as vectors for a virus, and (ii) root rot fungi had yet to be determined.

Baldwin (1) working in New Hampshire during 1954 reported virtually the identical symptoms of needle blight of eastern white pine as did his predecessors. However, like Spaulding and others he associated the disease with drought injury.

The true nature of the white pine needle blight causal agent began to emerge in 1960, when Linzon published a report that described the problem as it occurred in a four year old stand of naturally seeded white pine (43). The saplings were tagged and examined almost daily throughout the growing seasons of 1957 and 1958. The new growth of what turned out to be blighted and healthy saplings was compared and measured each week. Injury was first observed in the semimature

tissue of the needles on July 1, 1957. The semimature tissue was about four weeks old at this time and located about two-thirds of the way from the base of the needle. Faint, pinkish spots appeared on the stomata bearing surface and developed into orange-red (necrotic) bands which spread distially to the older needle tissue within a few days. When another outbreak of the disease was observed it always affected the semimature tissue located at the same position relative to the base of the fascicle. The symptom development was identical to the initial outbreak. Of the 600 trees in the study only 19% exhibited varying degrees of the blight symptoms. The occurrences of blight were always associated with one to two days of rainy weather followed by a continuous period of bright sunshine. These findings led Linzon to conclude: (i) that local weather conditions played a role in the development of the foliar symptoms on the "susceptible" trees and (ii) eastern white pine varied in their degree of "susceptibility" to the blight due to a genetically controlled and therefore heritable trait.

In order to prove that the occurrence of white pine needle blight was genetically controlled and that the disease was not communicable, Linzon conducted a field grafting project in 1959 (45). A total of 80 grafts were made in two plantations of eastern white pine at the Petawawa Forest Experiment Station, Chalk River, Ontario. Four combinations of grafts were made: (i) healthy scions to healthy stock branches, (ii) healthy scion to needle blighted stock branches, (iii) needle blighted scion to healthy stock branches, and (iv) needle blighted scion to needle blighted stock branches. The results showed

no transmission of the needle blighted condition from the blighted to the healthy partner and secondly that the needle blighted scion initiated symptom development in the same sequence as the donor tree. This further illustrated the genetic control involved in the disease syndrome.

As researchers concentrated on the etiology of white pine needle blight, attention was focused on several conditions that were similar in appearance to the originally described disorder. In a paper written in 1960, Hepting and Berry (35) working in Asheville, North Carolina compared five needle blight syndromes as found in the southeast. Two were caused by fungi, Bifusella linearis (Pk.) Hoehn and Scirrhia acicola (Dearn) Sigg. (causing brown spot) while the exact nature of the remaining three was not entirely clear although air pollutants were thought to be involved. The symptomatology of emergence tipburn, as these authors termed the malady, equated almost exactly to those of the white pine needle blight (35), while post-emergence acute tipburn and post-emergence chronic tipburn were somewhat different. The presence of the fruiting bodies of the two needle blight fungi and the fact that only one year old needles showed symptoms immediately separated these two from the other three tipburns. Emergence tipburn was distinguished from the post-emergence acute and chronic tipburns by the fact that in the case of emergence tipburn, only the current year's growth was affected while all needles were affected in the latter two cases. In addition, the demarcation between the reddish-brown necrosis and healthy tissue on a given needle was sharp, while in post-emergence

chronic tipburn there was no sharp interface but rather a gradation from tipburn to mottle to healthy tissue along the length of the needle. This condition was thought to be caused by various unidentified stack gases. The study area was in a moderately developed industrial area where high concentrations of various gases were known to exist. Post-emergence acute tipburn was usually associated with industrial processes where chlorine gas was a primary effluent. This condition also developed after experimental fumigations with sulfur dioxide (SO_2) in concentrations well above those ever recorded in areas of white pine blight in Tennessee.

Berry first attributed white pine needle blight or emergence tipburn as he called it to O_3 in 1961 (3). Four experiments were designed and conducted in which all of the evidence pointed to increased concentrations of O_3 as the causal agent. After isolating from the roots of healthy and diseased trees, no recognized root pathogens from the 27 genera of fungi found were identified. In a second experiment, a total of 44 needle blighted seedlings from Marlinton, West Virginia were used in a transplanting experiment. Groups of blighted seedlings were subjected to the following transplant treatments: (i) moved 225 miles south into an area where the disease had not been observed in recent years; (ii) moved 20 miles south (in the same general area of West Virginia); (iii) dug up and immediately replanted in the same hole; and (iv) check seedlings were left undisturbed. The results showed that while most of the seedlings that were moved out of the immediate area were blighted the first year, none showed symptoms the second year.

Sensitive check seedlings were blighted during both seasons. Thus, the emergence tipburn phenomenon was associated with a specific area in West Virginia. In a third experiment, Berry confirmed Linzon's grafting work (45) using all possible combinations of blighted and healthy scion and rootstock. It is interesting to note that these two researchers working almost simultaneously (Berry beginning in 1958 and Linzon in 1957) were reaching the same conclusions independently. The most conclusive experiment Berry conducted, however, was the artificial fumigation of ramets of both sensitive and tolerant clones of eastern white pine. Fumigations of 10 parts per hundred million (pphm) O_3 for two hours produced typical white pine needle blight/emergence tipburn symptoms on the sensitive ramets while none were observed on the tolerant ramets. Berry described the symptoms as closely following Linzon's (44) description of needle blight symptom development. Pink spots appeared on both stomata bearing surfaces some distance back from the needle tip 48 hours after the fumigation. The pink spots coalesced into a band that was gradually followed by necrosis of the distal portion of the needle which changed from pinkish yellow to a brownish red; the necrotic area was sharply distinct from the healthy portion.

Researchers became more specific in the following years in terms of research objectives. Histological examination of blighted white pine needles by Linzon (46, 48), and more definitive artificial fumigations of the species with O_3 and other pollutants by several investigators, began to illustrate the many faceted phenomenon of air pollution damage.

In 1962 Linzon (46) first illustrated certain morphological characteristics by which semimature tissue of eastern white pine needles could be identified. This was an especially important finding because this was the tissue that was apparently injured initially in the white pine needle blight syndrome. The occurrence of needle blight symptoms in the semimature tissue coincided with the initiation of the Casparian strip (suberization) on the radial and transverse walls of the endodermal cells of the needles. This stage in the maturation process of needle tissue occurred three to four weeks after emergence of that tissue from the basal meristem in the fascicle. Five years later Linzon (48) published a more comprehensive study on the histology of what he now calls semimature tissue needle blight (SNB) of eastern white pine. The mesophyll parenchyma cells, usually those adjacent to the substomatal cavity, were the first to be affected. These cells would initially appear disorganized, then discolored probably due to the oxidation of the phenolic compounds, and finally collapsed. This condition then spread distally and laterally toward the stele in the mesophyll parenchyma cells. This phenomenon usually occurred initially on only one stomata bearing surface; spread to the second stomata bearing surface and finally to the adaxial or non stomata bearing surfaces. The mesophyll cells adjacent to the stele were affected first in the adaxial surface while the cells adjacent to the hypodermis were last. After the completion of a band in the affected tissue, the necrosis spread distally into the mature tissue of the needle. There the mesophyll was the first tissue to be blighted, followed by the "dermal",

and finally by the stelar tissue (xylem/phloem). This sequence of tissue degradation held true for all SNB specimens analyzed.

In addition to describing the sequence of tissue necrosis associated with SNB, Linzon also indicated that there was a difference in the developmental needle morphology of sensitive and tolerant clones. Needles of sensitive clones were found to have: (i) faster cellular maturation; (ii) a more rapid and earlier development of a thick suberized layer (the Casparian strip) on the endodermal cell wall; (iii) less overlapping of the transverse endodermal cell walls in the longitudinal direction, and (iv) less volume in the intercellular air spaces in the mesophyll region.

As Linzon's investigations became increasingly more detailed, he was able to distinguish between the symptomology of SNB, SO_2 and O_3 damage on eastern white pine (47, 49). (Linzon stated that the SNB syndrome as observed by him was not caused by O_3 .) This work was initiated in 1960 but was not reported until 1966 and 1967. The study areas were in the forests around the ore smelters at Sudbury, Ontario where SO_2 was the major air pollutant, and in the Patawawa Forest Experiment Station where semimature tissue needle blight symptoms had been observed and where O_3 was known to be present. The ore smelters have been in operation since 1888 in the Sudbury area and by 1945, 6000 tons of SO_2 were being emitted into the atmosphere daily. This is contrasted to the Chalk River area which was remote to the smelters and thereby comparatively pollution free. However, in this area white pines had been exhibiting symptoms of both SNB and O_3 damage. Visual

symptoms were recorded throughout the growing seasons and tissue samples were obtained at intervals for microscopic analysis in order to fully document the differences in these three disorders.

The differences between SNB and SO_2 were described first by Linzon (47). These differences were: (i) SO_2 caused damage to any age needle tissue at any time of the year when concentrations were high enough while SNB symptoms were observed only on the current year's needles during a nine week period of the growing season from late June to mid-August; (ii) SO_2 injury usually involved the entire length of the needle while SNB only affected the semimature tissue and then necrosis moved acropetally; (iii) necrosis associated with SO_2 injury was initially grayish-green or whitish over the injured area progressing through various color changes to reddish brown, while the color of SNB necrosis was orange-red; (iv) usually all needles were injured in a given fascicle by SO_2 while with SNB there may be totally green unaffected needles present in a fascicle; (v) SO_2 injury occurred on numerous conifer and hardwood species while SNB affected only eastern white pine; and finally (vi) SO_2 injury was observed on a large proportion of the eastern white pine population while SNB symptoms were observed on only a smaller proportion of the population.

A second publication (49) was devoted to describing the differences between O_3 damage and SNB symptoms on eastern white pine. For this work clones of eastern white pine known to be sensitive and tolerant to the unidentified agent causing SNB were fumigated with 40 to 60 ppm O_3 from 2 to 12 hours. The injured tissue was then analyzed microscopically. This analysis showed that there were differences between

SNB and damage caused by artificial fumigations with O_3 . The differences were (i) O_3 damage occurred on trees both sensitive and tolerant to SNB; (ii) the initiation of SNB was in the semimature tissue where the suberization of the endodermal cell wall had just begun, while O_3 damage was initiated "simultaneously over the injured area of the needle" the proximal end of which extended into immature tissue (lacking suberization); (iii) with SNB the mesophyll cells in one stomatal surface of the needle collapsed first, followed by those in the second stomatal bearing surface, and finally by those in the adaxial, non-stomatal surface, while with O_3 damage, mesophyll cell collapse began in any of the three surfaces; (iv) the mesophyll cells in the proximal portion of the necrotic area collapsed diagonally from the stele to the hypodermis or vice versa in SNB. In addition, this occurred without the immediate distortion of the transfusion cells in the turgid stele. In the case of O_3 damage however, the collapse of the mesophyll cells in this same relative area occurred in a straight line while the transfusion cells may be distorted and the stele shrunken before the mesophyll collapse even began; (v) the initial symptoms of SNB occurred as pink spots on the abaxial surface followed by an orange-red band encompassing the needle which then spread distally taking several days to reach the needle tip. Within hours after the O_3 fumigations the injured area of the needles appeared to be collapsed and pale green in color that progressed to bronze, pale-orange, and orange-brown within a week; (vi) after completion of the symptom development in SNB there was no green banding in the necrotic area while this

banding symptom was observed in needles damaged by O_3 ; (vii) with semi-mature needle blight entirely green needles were often found in injured fascicles while all needles in a given fascicle were injured by the O_3 fumigations.

Berry's work with ramets of both sensitive and tolerant clones of eastern white pine was continuing during this time in Asheville. In the summer of 1961, Berry (4) conducted a field experiment using 50 ramets from the same sensitive parent tree (ortet). Twelve ramets were placed in each of three chambers while the remaining 14 were placed in an open plot adjacent to the chambers. The study area was in an area of West Virginia known for its occurrence of emergence tip-burn. The chambers were constructed with polyethylene film and fitted with either: (i) a filter dusted with MnO_2 (MnO_2 is known to be a catalyst for the decomposition of O_3); (ii) one dusted with MnO_2 plus activated carbon; or (iii) no filter. Ambient air was drawn in through the filtered/non-filtered opening, over the plants and exhausted via electronic motors. Ozone was monitored at the field site with a Mast Ozone Meter while relative concentrations within the chambers were obtained through comparing the degree of cracking of rubber strips. This experiment was initiated on June 6 and terminated on July 6, 1961. The results showed that the symptoms developed twice (June 27 and July 3) during the course of the season. Both were associated with elevated O_3 concentrations for the 48 hour period prior to the development of symptoms as follows: June 27 - greater than 4.5 pphm O_3 was monitored for eight hours which included a maximum of 5.0 pphm. Only

light symptoms were observed. July 3 - greater than 4.5 pphm O_3 was monitored for 31 hours which included a high of 6.5 pphm for almost five hours. Moderate symptoms were observed after this period. At the termination of the experiment 16% of the ramets in the MnO_2 plus carbon filtered chamber were injured, 75% in the non-filtered, 91% in the MnO_2 filtered, and 100% of the ramets in the open plot exhibited symptoms. Ramets from this same sensitive clone plus those from a field tolerant clone were also exposed to an artificial fumigation of O_3 at 6.5 pphm for four hours. The sensitive clone was the only one injured. The typical emergence tipburn symptoms observed were very similar to those observed on the ramets in the field experiment and also to those found on the ortet. The combined results demonstrated the cause and effect relationship between O_3 and emergence tipburn as observed in West Virginia.

As the cause of emergence tipburn was being established in the eastern United States by Berry (3, 4), investigations into the chlorotic decline or "x" disease of ponderosa pine (P. ponderosa Laws.) in the western United States were also productive. In 1962 Miller et al. (54) fumigated branches of ponderosa pine with 40 to 50 pphm O_3 for nine hours per day for periods of 9 and 18 days respectively. Ponderosa pine normally holds four years of healthy needles, however, on trees showing severe symptoms of chlorotic decline only one to two year old needles were maintained and they were exhibiting an intense chlorotic mottle. Symptoms observed on the O_3 treated needles were found to be similar although more severe to those of chlorotic decline. The

chlorotic mottle and needle abscission was more intense in every needle age except for the current year's needles as contrasted to the control. One of the conclusions of this work was that like eastern white pine, ponderosa pine exhibited varying degrees of sensitivity to O_3 which was apparently the primary phytotoxic constituent of the Los Angeles smog.

Of the three types of white pine blights thought to be caused by air pollutants described by Hepting and Barry in 1961 (35), the cause/causes of two of them (emergence tipburn and post emergence acute tipburn) had been determined. The cause of post emergence chronic tipburn (PECT) was the topic of a paper by Berry in 1964 (6). The symptoms of PECT that distinguished it from emergence tipburn of eastern white pines located in an industrialized area of eastern Tennessee were as follows: (i) premature shedding of older needles; (ii) yellowing and then browning of the distal parts of the current needles, gradually changing from green at the base with no sharp demarcation between; (iii) often stippling and mottling of foliage; and (iv) the gradual onset of discoloration at any time of the year. Research on the causes of the two diseases had been conducted concurrently. Due to the proximity of the affected trees to a large industrial complex, it was thought that a combination of stack gases and O_3 might be the cause of PECT. The possibility that SO_2 and/or fluoride was involved in the problem was investigated through foliage analysis. Excessively high concentrations of neither of these two pollutants were found in the foliage of sensitive ramets. Therefore, since no other air pollutants were known to be in the area the cause of PECT remained unknown.

As Berry's work with eastern pine progressed, it became increasingly evident to him that there were certain physical/genetical traits of the species that would make it ideal for use as an air pollution bio-indicator (5). Eastern white pine had been found to be sensitive to various species of air pollution and in varying degrees among genotypes (clones). In addition, it was a perennial plant and therefore could be used throughout several years in contrast to tobacco and alfalfa which are annuals. Only a small number of clones of eastern white pine have exhibited sensitivity during the winter months. Also, clones had been identified that were specific in their sensitivity to air pollutants. Therefore, through the use of a number of clones exhibiting varying degrees of sensitivity to specific pollutants and pollutant combinations, the establishment of an air pollution bioindicator system seemed feasible.

While research was begun on white pine needle blight by Berry and Linzon in the late 1950's, work was also being initiated in Ohio in 1959 by Dochinger to determine the cause of chlorotic dwarf (20, 21, 25). Chlorotic dwarf was a second physiogenic disease of eastern white pine that was first described by Swingle (73) in 1944. As was the case with white pine needle blight, eastern white pine showed a wide range of symptom expression with regard to the chlorotic dwarf syndrome. The most sensitive trees held only the current years needles which were stunted and exhibited severe chlorotic mottle while the great majority of trees appeared healthy. Grafting work was begun in 1959 using diseased and healthy material and after a six year study period results

were published in 1965 (20). In that study the chlorotic dwarf disease was not transmitted to healthy scion or rootstock through unions with diseased material. This confirmed the findings of both Berry (3) and Linzon (45) in their work with white pine needle blight. In addition it illustrated that white pine had a high degree of genetic variation within the natural population and that like white pine needle blight, chlorotic dwarf was most probably caused by some type of air pollutant. In a second publication in 1965 (21) and a lengthier one in 1968 Dochinger et al. (22) stated that chlorotic dwarf was caused by the harmful action of "gaseous dispersoids" on the foliage of "susceptible" individuals. Trees exhibiting typical chlorotic dwarf symptoms and those observed to be healthy were enclosed in chambers fitted either with or without charcoal filters. Within two years the needle mottling and the premature loss of needles on sensitive trees growing in charcoal filtered environments had ceased. In addition, the new shoot growth and needle length was significantly larger than that of the checks. Dochinger (22) stated that the only feasible control method was through a breeding program that would identify and propagate individuals tolerant to air pollution.

Evidence indicating that the cause of chlorotic dwarf was an O_3/SO_2 synergism was first reported by Dochinger in 1969 (23) and in a more comprehensive report in 1970 (25). In these reports it was stated that O_3 and SO_2 , either independently in high concentrations, or in combination at concentrations where neither one singly would cause visible damage, were responsible for the initial symptoms of chlorotic dwarf.

When any phytotoxic concentrations occurred over an extended period of time, the net result was a reduced chlorophyll concentration in the needles and early abscission of the older needles. The loss of the photosynthetic potential in the current years needles, i.e., the loss of chlorophyll, in successive years was believed to be the primary cause of the stunted growth. Ambient concentrations of O_3 and SO_2 in an Ohio pine plantation were each greater than 5 pphm on 16% of the days between June and September 1967. The maximum hourly average concentrations of O_3 and SO_2 were 21 pphm and 7.8 pphm, respectively, while the highest combination monitored was 12 pphm O_3 plus 7.8 pphm SO_2 . Artificial fumigations of both sensitive and tolerant clones of eastern white pine were conducted using 10 pphm of each pollutant both singly and in combination. Three percent of the needles showed symptoms with O_3 alone, four percent with SO_2 alone, while 16% were injured with the combination.

Soon after Berry and Linzon began their work on white pine needle blight, a third researcher initiated additional work on the problem. Costonis began to intensively investigate the occurrence of white pine needle blight in New York during 1963 (13). Observations of symptom development and measurement of O_3 concentrations were made in a 10 to 15 year old natural white pine stand in central New York from 1963 to 1967 and 1966 to 1967 respectively. In addition, four and five year old seedlings representing sensitive, intermediate, and tolerant symptom expression classes were selected for artificial O_3 fumigations during 1966 and 1967. The symptoms observed on the sensitive trees,

whether in field observations or in the artificial O_3 fumigations, were consistent. Small silver flecks were first observed on the stomatal surfaces within 0.5 to 1.0 mm of the tips of new needles that were 1 to 3 mm long. These flecks could coalesce to form yellow macroscopic lesions which upon microscopic analysis revealed that individual mesophyll cells nearest the stomata collapsed first, followed by those radiating from that point. Symptoms developed rapidly, requiring only six to ten hours to progress from the fleck stage into yellow lesions. In severe cases the condition was observed to intensify to include necrotic banding. Microscopic analysis of the necrotic tissue indicated that the stelar tissue and endodermis were initially unaffected but soon (12 to 24 hours in acute exposures) the tissues were collapsed and yellowish-brown in color. In the needles of the most sensitive trees, the necrotic band spread distally taking from one to four weeks to reach the needle tip. This condition represented the classic white pine needle blight symptom.

In the field portion of Costonis' study (13), a significant degree of variation was observed in injury between trees, branches on the same tree, fascicles on the same branch, and even between individual needles in a given fascicle. The five needles comprising a fascicle may not all be injured to the same degree after O_3 fumigations. In addition, there may be several different types of symptoms observed on the needles that were damaged. When recorded O_3 concentrations in the field were compared to actual injury observed on the field trees, it was found that new lesions developed within 24 to 48 hours after increases in O_3

concentrations occurred. Polyethylene bags were used to protect various branches of field trees before budbreak in 1967 to illustrate the effect the ambient atmosphere had on symptom development and needle retention. No symptoms characteristic of O_3 injury were found on bagged branches while neighboring trees showed typical O_3 injury symptoms. In addition, by July 6, 83% of the one year old needles remained on the bagged branches while only 15% remained on the unbagged branches. These findings were substantiated when the needles retained on field trees were compared to those on potted trees grown in a charcoal filtered air chamber. In this study, concluded in September, the field trees retained 31% of their one year old needles while the trees growing in the filtered environment retained 74%. Artificial O_3 fumigations of eastern white pine showed that injury occurred on sensitive individuals at 3 pphm O_3 for 48 hours or 7 pphm for four hours. Also, the prime age for needle sensitivity to O_3 was determined to be six to eight weeks after emergence from the fascicle sheath.

Several of the field trees that were utilized in the previous study were exhibiting symptoms when O_3 concentrations were found to be at or below normal. This led Costonis to investigate the possibility that some phytotoxic air pollutant other than O_3 was causing the damage (15). The trees that were showing symptoms were close to power-plants and therefore SO_2 was believed to be the pollutant in question. This hypothesis was probably in part based on the findings of Drummond and Wood (26) reported three years earlier. In that study it was found that within two years after the height of a smoke stack was increased

at a fossil fuel burning electrical generation plant, eastern white pine trees near the plant showed a detectable increase in both height and diameter growth. Also, as the distance from the stack increased the growth rates decreased. Although SO_2 was not mentioned as a possible cause of this phenomenon, it was a generally accepted fact that this gas was highly phytotoxic.

With the knowledge that pollutants other than O_3 were causing injury to eastern white pine, Costonis designed a study (15) using only ramets sensitive to air pollution as observed in the previously discussed field work (13). Ten current year fascicles on randomly selected branches were marked with artists oil paint. One branch on each ramet was used as a control in each fumigation by placing a polyethylene bag over a particular branch. These bags were known to be impenetrable by either O_3 or SO_2 . In all cases the new growth was three to five weeks old at the time of fumigation. Branches were subjected to four different concentrations of SO_2 ranging from 5 to 25 pphm and only one concentration of O_3 (30 pphm). Several fumigations were conducted using tissue from the same individual ramet. To do this some of those branches bagged initially during the first fumigation were uncovered and fumigated in subsequent fumigations while branches bagged during both fumigations were used as the control. The results showed that SO_2 caused injury to eastern white pine at all concentrations between 5 pphm for one hour and 25 pphm for two hours. The exposure period appeared to be the predominant factor in determining injury severity. This was illustrated in fumigations with 5 pphm SO_2

for one, two, and three hours. In this period, all fascicles were injured in each exposure period but the lesion severity at the three hour exposure was more than twice that observed at one hour with the two hour fumigation being intermediate. This same relationship was not observed by manipulating the pollutant concentration.

In the same paper (15) Costonis contrasted the differences between O_3 and SO_2 injury. The differences in lesion development between the two pollutants were most evident during the first 24 hours after fumigation. Sulfur dioxide induced a slight yellowing and collapse of the affected mesophyll cells on the stomatal surface, and within four to six hours after the fumigation the lesion appeared to be resin soaked. Costonis noted that semimature tissue as described by Linzon (46) was the only tissue initially affected. Necrosis spread distally from the semimature tissue often taking only five days to reach the tip of the needle. Usually all five needles in a fascicle were affected equally. Lesion development in tissue fumigated with O_3 initially appeared as silvery flecks on the stomatal surfaces. This symptom was not restricted to semimature tissue but was also observed on both immature and mature tissue to a lesser degree. Tip necrosis normally developed within one to two weeks and occurred on one to two needles in any given fascicle. After 72 hours the two symptom types (caused by SO_2 vs. O_3) became less distinct making a determination of the causal agent impossible.

Costonis (15) continued with a discussion of Linzon's SNB syndrome as observed in Ontario, Canada (47, 49). It was pointed out that

Linzon did not monitor for SO_2 in remote areas where only typical SNB symptoms were observed. In addition, the SNB sensitive ramets were never artificially fumigated with SO_2 to enable an actual comparison of symptom types. Costonis observed injury on sensitive eastern white pines at 5 pphm SO_2 for one hour. Evidence was presented by Costonis that concentrations of as much as 5 pphm SO_2 had been reported for brief periods in what was believed to be a remote area of Ontario during one investigation undertaken in 1961. Therefore, Costonis concluded that SNB may be caused by either SO_2 alone, or in combination with O_3 which is known to be present in the atmosphere in low concentrations.

Menser and Heggestad (51) observed a relationship between O_3/SO_2 and weather fleck of tobacco in 1966. Ozone was first reported to be the cause of weather fleck by Heggestad and Middleton in 1959 (30). However, during the early 1960's typical weather fleck symptoms were appearing on sensitive tobacco cultivars when the O_3 concentrations were 2 pphm or below (51). Thus there had to be another cause for the symptoms observed when O_3 concentrations were low. Menser and Heggestad's paper in 1966 reported that when sensitive tobacco cultivars were fumigated with either 3 pphm O_3 or 24 pphm SO_2 for either two or four hours no weather fleck symptoms developed. However, when the two pollutants were used in combination at the same concentrations and fumigation durations, typical weather fleck symptoms developed. These findings coupled with Costonis' own results (15) indicated that his hypothesis concerning the etiology of SNB as

discussed above was probably true. In the same year that the hypothesis was proposed (1970), direct experimental evidence supporting it was published. Jaeger and Banfield (40) working at the University of Massachusetts reported that air pollution sensitive ramets of eastern white pine sustained greater foliar damage from 5 pphm SO_2 plus 5 pphm O_3 than from either of the two pollutants singly at the same concentrations. This, and later work by Costonis and others, was strongly supportive of the O_3/SO_2 SNB relationship which would soon be widely accepted. (See Appendix: Table 2 for a comparison of white pine needle blight symptomology as described by Berry, Linzon, Dochinger and Costonis).

In 1971 Costonis (16) presented evidence from field grown eastern white pine trees and ramets of known sensitivity to support his earlier claim (15) that low concentrations of SO_2 could be phytotoxic to sensitive genotypes. This work was conducted near Asheville, North Carolina where 40 pollution resistant, 40 intermediate, and 39 highly sensitive trees in a 10 year old plantation were evaluated monthly for symptom development during the growing season (May 20 - August 30). Three weeks after budbreak, ramets of known pollutant sensitivity (the same genotypes as were used previously (15)) were removed from a charcoal filtered greenhouse and planted adjacent to the plantation under study. The injury observed on the ramets generally reflected that observed on the field trees. Although the causal pollutant cannot be easily determined from visible symptoms, Costonis implicated SO_2 . In addition, ambient concentrations of both O_3 and SO_2 were being monitored

throughout the growing season in the study area. Analysis of this data showed that symptom development was associated with increase concentrations of SO_2 and not O_3 . Average concentrations of 6-10 pphm SO_2 for as little as four hours were recorded on 26% of the days during the primary period of sensitivity (when tissue age was 6-8 weeks old). During this same period, O_3 concentrations reached a maximum of 4 pphm only twice while maintaining a daily average of between 1.0 and 1.9 pphm.

During the late 1960's and early 1970's, researchers began to investigate the physiologic effects of pollutants on coniferous species. In 1971, Botkin et al. (10) showed that the net photosynthesis of three randomly selected symptomless eastern white pine trees was reduced at O_3 concentrations of 50-100 pphm. This reduction was not correlated with visible injury of the treated seedlings. One year later Botkin et al. (11) refined these results by screening five trees (four additional trees plus one used the previous year). In both studies the net photosynthesis was calculated by determining the difference in carbon dioxide (CO_2) concentration of the air stream prior to and after contact with the foliage. The O_3 concentrations used ranged from 20 to 100 pphm with durations from 5 to 15 hours. Three response classes were identified in the five trees studied: sensitive (three), intermediate (one), and resistant (one). Sensitive trees had their net photosynthetic rate reduced to near zero (100% reduction) within a few hours of O_3 exposure. The intermediately sensitive tree had its photosynthetic rate reduced by approximately 50% and the resistant

tree showed no response to O_3 exposures. Visual symptoms were observed on all but one tree, however, the timing varied from 24 hours to 16 days after exposure. The appearance of visible symptoms was not correlated with net photosynthesis suppression which was observed during each fumigation. This phenomenon occurred because of: (i) the variation in the age of the tissue at the time of fumigation; (ii) the variation in the O_3 concentrations and exposure time during the fumigation, and (iii) the natural genetic variation within the species. Botkin noted that eastern white pine exhibited a considerable variation in the net photosynthesis response to O_3 exposure which was compatible with the results of Costonis (14) relative to visible injury. However, Botkin also stated that: "In spite of the variation among trees with respect to O_3 induced photosynthetic suppression and visible symptom expression, branches on individual trees reacted similarly."

Berry's research at Asheville on eastern white pine had been continuing since his initial work during the early 1960's (3, 35). During this period, part of his work involved the screening of various coniferous species for their relative sensitivity to air pollutants. In 1971 seedlings of red (P. resinosa Ait.), jack (P. banksiana Lamb.), and white pine were fumigated with two concentrations (25 and 50 pphm) of both SO_2 and O_3 for two hours (8). Seed for the study was obtained from a commercial distributor and the young seedlings were fumigated at either three, five, or seven weeks of age. No difference was observed in symptom expression on the seedlings at the various age. Ozone was consistently found to be more phytotoxic than SO_2 even

though the visible symptoms caused by the two pollutants were indistinguishable. Jack pines was found to be the most sensitive of the three species with white pine intermediate followed closely by red pine.

In 1973 Berry (9) reported on the differential sensitivity of eastern white pine to three different air pollutant regimes (oxidant- O_3 , NO_x etc., SO_2 , and fluoride). The oxidant regime was found adjacent to a large metropolitan area, the SO_2 regime near a fossil fuel burning electrical power plant, and the fluoride regime near a fertilizer plant. The object of the investigation was to determine whether various individuals (genotypes) of eastern white pine would respond to the different air pollutant regimes in a characteristic fashion, thereby enabling those genotypes to be used as a bioindicator of the particular air pollution regime. A secondary benefit of the study was the identification of clones that were resistant to all the pollution regimes. A total of 2400 2-0 seedlings were obtained from forest tree nurseries in North Carolina and planted in one gallon buckets. These seedlings were divided into four groups of 600 each and establishment of the plots began one year later. Each group was exposed in all three areas, remaining in a given area one year. The results identified 64 trees (4.5% of the total) that were tolerant to all three regimes. The fluoride regime was the most phytotoxic (1465 seedlings injured) with the majority of the injury occurring during the summer months (1008 seedlings injured). The SO_2 regime was intermediate (875 seedlings injured) followed closely by the oxidant regime (710 seedlings injured). The most important

conclusion of this study however, was the fact that eastern white pine exhibited a differential sensitivity to the various pollutant regimes and therefore would be suitable for use in an air pollution bioindicator system.

Costonis' most recent contribution to the elucidation of air pollution effects to eastern white pine was published in 1973 (17). In this work four sensitive clones and one resistant clone were used. The sensitive clones were identified during previous work (13, 15) and were known to be more sensitive to SO_2 than to O_3 . The five ramets were subjected to five treatments: (i) O_3 - 5pphm, (ii) SO_2 - 5 pphm, (iii) SO_2 - 5 + O_3 - 5 pphm, (iv) O_3 - 5 pphm followed 0.5 hours later by SO_2 - 5 pphm followed 24 hours later by SO_2 - 5 pphm + O_3 - 5 pphm, and (v) charcoal filtered control. All treatments lasted for two hours. The injury caused by treatment iv was the most phytotoxic followed by treatment ii and then iii. No injury was observed on the new needles of ramets fumigated with O_3 alone, treatment i, or on the control ramets, treatment v. The mean lesion severity for treatment iv eight days after the fumigation was 100% while that for ii was approximately 80% and iii was approximately 50%. The injury that was observed on the emerging needles in treatment iv was not distinguishable until 72 hours post fumigation. At that time the distal 6-10 mm of the needles, whose mean length was 20 mm, turned grayish-green. When viewed microscopically (96X), the injured tissue appeared silver with numerous internal resinous areas. The semimature tissue as described by Linzon (46) did not show selective injury as was the case when either of the two

pollutants were used singly. In immature tissue, silver to rusty-brown lesions were also observed. After one week the distal portions of the needles became bronze, soon turning a conspicuous orange-brown. These results indicated that the four known sensitive clones of eastern white pine could be injured by concentration of SO_2 and O_3 well below those previously reported. In addition, discontinuous fumigations of O_3 , SO_2 , and then a combination of the two pollutants, was significantly more phytotoxic than either of the two singly or in combination. The discontinuous fumigation sequence probably represents that which would be more commonly found in the field.

In 1973 Houston (38), working in Wisconsin, reported on sensitivity screenings he conducted on 30 clones of eastern white pine. Fifteen sensitive and 15 tolerant ortets were selected in the field based on typical air pollution symptoms. One year old ramets were subjected to either 5, 10, 30 and 60 pphm O_3 ; 2.5, 5, 15, and 45 pphm SO_2 ; and 2.5 pphm SO_2 plus 5 pphm O_3 . The tissue age at the time of fumigation was three to four weeks. The fumigations lasted for six hours. The new needles were evaluated based on visual injury and growth differences calculated by subtracting the length of the needles prior to fumigation from the length measured four weeks later. The tolerant and sensitive clones reacted distinctly in each treatment. Since the O_3 injury observed was not always consistent between the two classes at the lower concentration, it was determined that O_3 was not the primary factor in the field syndrome as observed in Wisconsin. Sulfur dioxide and SO_2 plus O_3 on the other hand, were effective in consistently identifying

the two classes. Very sensitive clones were injured by the 2.5 pphm SO_2 treatment while concentrations of 5 to 15 pphm were required to damage half of the tolerant clones. The tolerant clones coincide with data Costonis published in 1970 (15). The tolerant clones responded in a graded manner to the increased SO_2 concentrations (a positive correlation) while sensitive clones were damaged equally in all fumigations. There was no effect on the needle growth difference in the O_3 fumigations since there was no clear significant deviation from that of the controls. However, in the SO_2 fumigated ramets, the needle growth differences were significantly different from those of the controls. There was also a noticeable difference between the sensitive and tolerant classes with the sensitive clones consistently showing reduced growth. Another important finding was that even when there was no visible damage to the tolerant clones, there was reduced growth, indicating injury at the physiological level.

Photochemistry of NO_2 and O_3

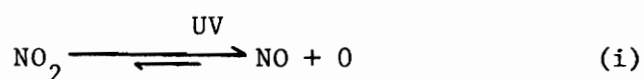
The phenomenon of photochemically generated air pollutants was first investigated in Los Angeles, California during the late 1940's and early 1950's (32, 52). What would be known today as typical oxidant air pollution symptoms were reported on several leafy crop species in 1944 (32). These symptoms occurred one to three days after the smog dissipated and were unlike any known to occur due to SO_2 , hydrogen fluoride (HF), or any other known air pollutant. However, because the close correlation with the presence of smog, the symptoms were believed to be caused by some unidentified air pollutant.

Thus began a research effort aimed at the elucidation of the type and concentrations of air pollutants found in the Los Angeles smog that produced these unusual symptoms.

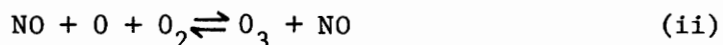
In the intervening 38 years, a tremendous quantity of research has been conducted on this problem. In 1953 researchers were surprised to learn that the Los Angeles smog consisted, at least in part, of oxidizing agents rather than the more commonly known reducing agents, e.g., SO_2 (33). Ozone was found to be the cause of the cracking in rubber tires (oxidation of the rubber) in the Los Angeles area. The severity of the cracking was positively correlated with the concentration of O_3 present in the atmosphere. At that time however, O_3 was known to be formed only by electrical discharges (lightening or electrical arching) or through the photolysis of O_2 molecules by ultraviolet (UV) light. Neither of these two methods could account for the build-up of concentrations that would cause the type or severity of damage that had been observed on rubber products. In addition, it was later determined that the symptoms observed on leafy crop species were not entirely characteristic of O_3 damage. Therefore, there must have been other unidentified constituents of the polluted air masses, some of which were responsible for this damage. The most important compounds that were later identified included oxides of nitrogen (primarily NO and NO_2), hydrocarbons, and a family of peroxyacetyl nitrates (primarily peroxyacetyl nitrate-PAN). In 1953 Hagen-Smit et al. (33) discovered that when synthetic mixtures of hydrocarbons and NO_2 in O_2 were irradiated with artificial sunlight, O_3 was produced. This focused

the attention of numerous researchers on the fact that high concentrations of oxidant air pollutants could be produced through the photochemical reaction of the constituents of urban smog.

The O_3 precursors found in polluted air masses were known to be NO_2 and nonmethane hydrocarbons hereafter referred to as hydrocarbons (32). Leighton (42) later calculated the half life of NO_2 in sunlight to be approximately two minutes. The products of this photolysis were determined to be NO and an atom of oxygen as follows (70):



The free oxygen atom produced readily reacts with one of the numerous O_2 molecules present and O_3 is formed as follows:



However the O_3 molecule readily oxidizes the NO molecule and the original reactant is formed as follows:



This reaction has been shown to reach a dynamic equilibrium or steady state within a very few minutes, and the only continuing process is the absorption of light and its conversion into heat (70). Thus this reaction would not allow for an appreciable concentration of O_3 to build up (Leighton (42) estimated 1 pphm) in an atmosphere free of hydrocarbons. Therefore there must have been a competing reaction that would inhibit reaction (iii) above. This was postulated because O_3 concentrations of 30 to 50 pphm had been recorded in polluted atmospheres.

Significant concentrations of hydrocarbons have been reported to occur in polluted atmospheres due to the combustion of fossil fuels (33, 88). The hydrocarbon molecules interfere with reaction iii) above by reacting with the NO molecule formed by the photolysis of NO_2 . This would allow O_3 concentrations to increase because the equilibrium has been shifted to the left whereby O_3 would no longer be broken down by its reaction with NO. Stephens (70) has shown that there is a direct relationship between the ratio of NO_2 to NO and O_3 concentrations, e.g., a 30:1 ratio will yield an O_3 concentration of 30 pphm. Knowing the reactivity of the gases involved, the maximum O_3 concentration that would theoretically be formed in this system would be 100 pphm. In addition to elevated concentrations of O_3 being formed when the NO_2 to NO ratio is large, concentrations of PAN have also been shown to increase.

Ozone has also been shown to be produced by natural constituents of non polluted rural air masses. Ripperton et al. (61) irradiated: (i) untreated ambient air and (ii) mixtures of filtered ambient air α -pinene, and NO_2 or formic acid (CH_2O) and produced O_3 in all cases. Ozone concentrations were all less than 11 pphm in the synthetic mixtures and less than 3 pphm in the experiments conducted with ambient air. The high O_3 concentrations were achieved using unusually rich mixtures and probably represent maximum potential concentrations for these mixtures whereas the low O_3 concentrations probably equate to background levels. Rasmussen (60) reported that the principle hydrocarbon emissions from pine and hardwood species were α -pinene and

isoprene. In addition, the natural sources of hydrocarbons are responsible for a 6.2 fold increase in the level of hydrocarbon emissions as compared to that of anthropogenic sources. However, as was the case with the NO_x emissions, the natural sources of hydrocarbons are more evenly distributed thereby reducing the concentrations in any one area. Ripperton's work illustrated that there is a potential for O_3 production in the unpolluted ambient atmosphere and gave further evidence that oxidant air pollutants are photochemically generated.

The Effects of Nitrogen Dioxide on Vegetation

Nitrogen dioxide (NO_2) and nitric oxide (NO) rank first and second respectively as most important phytotoxic air pollutants of the six oxides of nitrogen (NO_x) known to exist in the atmosphere (88) Microorganisms produce 10 times the amount of NO_x primarily in the form of NO emitted by anthropogenic sources. However, because the NO produced by microorganisms is spread throughout the world, their relative contribution to the overall NO_x air pollution problem has been considered to be small. This is in contrast to the anthropogenic sources which are usually concentrated in a relatively small area and account for virtually all of the problems associated with this group of air pollutants. Robinson and Robins (62) reported an estimated ratio of 15 to 1 on natural NO emissions vs. anthropogenic NO_2 emissions. High temperature combustion particularly of fossil fuels, is known to be the source of a large percentage of the NO_x emissions. During high temperature combustion the primary product is NO, however, traces of

NO_2 are also formed (a normal NO to NO_2 ratio has been reported to be 9:1 (75)). High concentrations of these two gases are associated with coal burning electrical generating plants and industrial facilities manufacturing nitrogenous compounds including fertilizers, munitions and nitric acid (89). Nitric oxide is readily converted to NO_2 which in high concentrations is known to be brown in color. This has been reported to be the only visible phytotoxic air pollutant. Nitrogen dioxide is known to be a primary component of the photolytic cycle which produces high concentrations of O_3 (see page 39).

Nitrogen dioxide was first shown to be a constituent of the phytotoxic Los Angeles smog by Haggen-Smit in 1952 (32). Hydrocarbons from gasoline vapors did not cause typical smog type injury to five agronomic crop species during a five hour exposure. However, when 40 pphm NO_2 were added to the gasoline vapors and the mixture irradiated with sunlight, typical smog type symptoms were observed. These included a metallic sheen, silvering, or bronzing on leafy vegetables and speckled necrosis on grasses. As was the case in fumigations with only gasoline vapors, NO_2 alone at 40 pphm did not cause injury. This work initiated detailed investigations into the NO_2 /hydrocarbon photolytic cycle with particular emphasis being placed on the identification of the phytotoxic products of this now classic reaction process. It was generally accepted at this time, based on Haggen-Smit's work, that NO_2 was not present in the atmosphere in concentrations high enough to be phytotoxic by itself (33). A maximum NO_2 concentration of 30 pphm was reported in California during 1958 (52). However, by 1965 the maximum NO_2 concentration reported in California was 393 pphm while normal ambient

concentrations generally were well below 50 pphm (74). This meant that sensitive and some intermediate species could be injured by NO₂ episode. These conclusions were confirmed by the research of others working on the problem during the next 35 years. During this period, research on the effects of NO₂ on vegetation was mainly confined to agronomic crop, horticultural crop, ornamental and weed species. Only three studies involving the fumigation of forest/shade tree species with NO₂ have been reported to date.

In 1967 Van Haut and Strattman (90) working in Germany published the first report where forest/shade tree species were fumigated with NO₂ (in this case mixtures of NO + NO₂). In that study 60 plant species from a wide range of families including 10 coniferous and 15 hardwood tree species were fumigated with 1:1 mixtures of NO and NO₂. Pollutant concentrations ranged from 250 to 1000 pphm NO + NO₂ for durations of four to eight hours. The rapidly growing leaves were determined to be more sensitive than either the immature or mature leaves. The initial symptoms caused by acute fumigations were gray green to slightly brownish spots on the leaves which later became necrotic. However, no mention of the specific dose required to produce these symptoms was provided. The injury observed was similar to that caused by SO₂, however, the NO/NO₂ mixtures were 1.2 to 5.0 times less toxic than SO₂ to the plant species tested.

In 1974 Hill et al. (37) studied the effects of SO₂ and SO₂ + NO₂ on 87 species native to the cold desert areas of Utah and New Mexico. This area is characterized by valleys ranging from 4500 to 6500 feet

above mean sea level (MSL) surrounded by mountains ranging from 7000 to 10,000 feet MSL. A mobile fumigation chamber was utilized for the study in which plants were fumigated in situ for period of two hours. Pollutant concentrations ranged from 50 to 1100 pphm SO₂ and 10 to 500 pphm NO₂. The NO₂ concentrations averaged 33% of the SO₂ concentrations as this is the ratio of pollutants found in the plumes down wind of coal-fired power plants. Of the 87 species studied 13 were tree species (9 coniferous and 4 hardwoods). No difference was found between the SO₂ and SO₂ + NO₂ treatments therefore all treatment results were combined. Of the tree species tested only narrowleaf cottonwood (Populus angustifolia James and P.) was found to be sensitive. Damage on this species ranged from 2% of the total leaf area exhibiting necrosis at 200 pphm to 20% at 1000 pphm SO₂. Pinyon pine (Pinus edulis Engelm.), ponderosa pine (P. ponderosa Laws.), Rocky Mountain juniper (Juniperus scopulorum Sarg.) and Utah juniper (J. osteosperma Torr.) were found to be relatively resistant to SO₂ and SO₂ + NO₂ fumigations as concentrations of 1000 pphm SO₂ were required to cause visible injury.

In a more recent study published in 1977, Kress and Skelly (41), working in Virginia, fumigated seedlings of tolerant and sensitive lines of both loblolly pine (P. taeda L.) and American sycamore (Platanus occidentalis L.) with SO₂, NO₂ and O₃ and mixtures of these three pollutants. The pollutant concentrations used in all the treatments were 14 pphm SO₂, 10 pphm NO₂, and 5 pphm O₃. The exposures lasted six hours per day for 28 consecutive days. In all cases the fumigations

were initiated when the pine seedlings were one week old and the sycamore seedlings two weeks old. The seedlings were rated for visual injury prior to the exposure, at the termination of the exposure, and two weeks later. In addition, several height measurements were taken on both species in order to determine the effect of the pollutants on the growth rates of the lines. Analysis of the foliar injury data for the sensitive line of loblolly pine showed that in the one treatment where NO_2 was used alone there was no significant injury. When NO_2 and O_3 were combined, the resultant foliar injury was significantly different from the control but not significantly different from the O_3 treatment. The SO_2 treatment was the only single pollutant fumigation that produced significant foliar injury. When all three pollutants were combined, the resultant foliar injury was significantly different from the control and the O_3 treatment, however, it was not significantly different from the $\text{O}_3 + \text{SO}_2$ treatment. Significant growth reductions of 26% and 21% were only observed in the $\text{O}_3 + \text{SO}_2$ and the $\text{SO}_2 + \text{NO}_2 + \text{O}_3$ treatments respectively. Analysis of the foliar injury data for the tolerant line of loblolly pine also showed that there was no visible effect in the NO_2 treatment. Even when O_3 and NO_2 were combined, the foliar injury was not significantly different from that of the O_3 treatment. However in the $\text{O}_3 + \text{NO}_2$ treatment, the severity of the injury on the tolerant line was approximately 12 times less than that observed on the sensitive line. When all three pollutants were combined, the resultant foliar injury was significantly different from that of the O_3 treatment, however, it was not significantly different from that of the $\text{O}_3 + \text{SO}_2$ treatment. In the three pollutant combination treatment,

the tolerant line was only two times less severely injured than the sensitive line. The only significant reduction in height growth of the tolerant line was in the $O_3 + SO_2$ treatment and the three pollutant combination treatment. These differences were observed at the termination of the 28 day exposure period. The data on the sycamore showed that foliar injury was not a reliable indicator of sensitivity under these experimental conditions as no treatment was consistently significantly different from any other. In the treatment with all three pollutants, a 45% and 35% growth reduction was observed on the sensitive and tolerant lines respectively. This was especially important because no significant foliar symptoms were observed in either case. Significant growth reductions were also observed in the $O_3 + SO_2$ treatment where 34% and 17% growth reductions were observed on the sensitive and tolerant lines respectively. Again, no significant foliar symptoms were observed.

Since Hagggen-Smit (32) identified NO_2 as a constituent of the Los Angeles smog in 1952, numerous research projects have been conducted to determine the effects of the pollutant on vegetation. Three studies involving forest/shade tree species were discussed above. However, far more work has been conducted using other plant species and the following will be a brief overview of the findings of the more pertinent studies.

In 1958 Middleton et al. (53) working in California reported that pinto bean (Phaseolus vulgaris, L. var Pinto) showed damage after eight hour artificial fumigations with 300 to 400 pphm NO_2 . These symptoms were indistinguishable from those of SO_2 , however, three to four times

the amount of NO_2 was required to cause similar damage. It should be noted that in this study, Middleton found that the threshold for O_3 damage to the same species was 25 pphm. The O_3 symptoms observed were minute speckling and chlorosis of the upper leaf surface. Middleton concluded that O_3 was 12 times as phytotoxic as NO_2 .

Taylor et al. (74) investigated the effects of both short and long term NO_2 fumigations. The results of the short term fumigations showed that concentrations of 230 pphm NO_2 for eight hours were required to cause leaf injury on tobacco (Nicotina glutinosa L.) while a concentration of 1000 pphm was required for four hours to cause leaf injury on bean. These concentrations were ten times higher than those of O_3 which caused the same type of lesions. The results of the long term (10 to 22 day) NO_2 exposures on pinto beans and Pearson improved tomato (Lycopersicon esculentum Mill) showed significant reductions in growth as measured by both fresh and dry weights. In the fumigations with pinto beans, the NO_2 concentration was maintained at 30-40 pphm while in the fumigations with tomato the concentrations ranged from 15 to 57 pphm. These results were confirmed by Spierings (69) working in the Netherlands with tomato variety moneymaker.

Thompson et al. (77) working in California in 1970 reported results of a long term NO_2 fumigation experiment on naval orange trees (Citris sp.). It was concluded that at some concentration below 25 pphm, NO_2 was toxic to citrus trees. However, because these were continuous fumigations and NO_2 concentrations averaged only about 5 pphm through the entire growing season, NO_2 was not believed to have a significant

effect on citrus orchards under normal ambient conditions in California. When the effects of O_3 on naval orange production were investigated (79), it was found that the ambient O_3 concentrations were causing a significant yield reduction. However, the remaining oxidant portion of the photochemical smog (NO_x , PAN, and others) in California was responsible for the greater percentage of the loss.

The idea that NO_2 can cause invisible (physiologic) injury to vegetation was further investigated by Hill et al. (36) working in Utah. The apparent photosynthesis rate was determined by measuring the rate at which carbon dioxide (CO_2) was used by oats (Avena sativa L. var Park) and alfalfa (Medicago sativa L. var Ranger) under varying pollutant concentrations. The threshold concentration for growth reduction of the two species by NO_2 was estimated to be approximately 60 pphm for two hours. In a second study conducted by the research group in Utah, White et al. (92) observed a synergistic effect on the apparent photosynthesis of alfalfa by SO_2 and NO_2 . When alfalfa was subjected to two hour fumigations with 25 pphm of either pollutant, the apparent photosynthesis rate was reduced two to three percent by SO_2 , while no effect was observed with NO_2 . The use of both pollutants in combination, however, induced a 9 to 15 percent inhibition.

Tingey et al. (80) working in North Carolina also investigated the effects of NO_2 and SO_2 on vegetation. Six crop species were utilized in the study which was designed to determine at what concentration the two pollutants were phytotoxic and to describe the visual symptoms that developed. No visual symptoms were observed on any of the species at NO_2 concentrations below 200 pphm for four hours. The threshold for

injury with the SO_2 was 50 pphm for four hours. In pollutant combination fumigations injury was initially observed on four of the six species when the concentration of each pollutant reached 10 pphm or greater. On the remaining two species injury was observed at concentrations of 5 pphm for each pollutant. As was the case with the results of White et al. (92), a synergistic effect was observed at a fairly low pollutant concentration and as the concentrations increased above the level where maximum injury was observed, the degree of synergism decreased.

The Radford Army Ammunition Plant

Investigations were begun by Skelly et al. (65) at the RAAP in 1970 to evaluate the extent of air pollution damage to a three year old stand of eastern white pine (64). The pine seedlings were growing on a hillside adjacent to the main source of NO_x and by 1971, 95% of the seedlings had died (Figure 1: WP3). All of the remaining seedlings, some of which measured less than 12 inches in height, exhibited acute symptoms. The needles were short and tufted with severe tip burn which was delineated from healthy tissue by an area of orange-colored pigmentation. As would be expected, all seedlings were not affected to the same degree but the overall performance of these eastern white pines was significantly below the expected.

Larger eastern white pines within the RAAP also exhibited reduced growth (58, 65, 72). In one 13 year old stand of planted eastern white pine located 300 meters northeast of the main NO_x source, a 50 tree circular plot was established (Figure 1: WP1). The concentrations of both NO_2 and SO_2 were monitored during the study. The maximum one hour

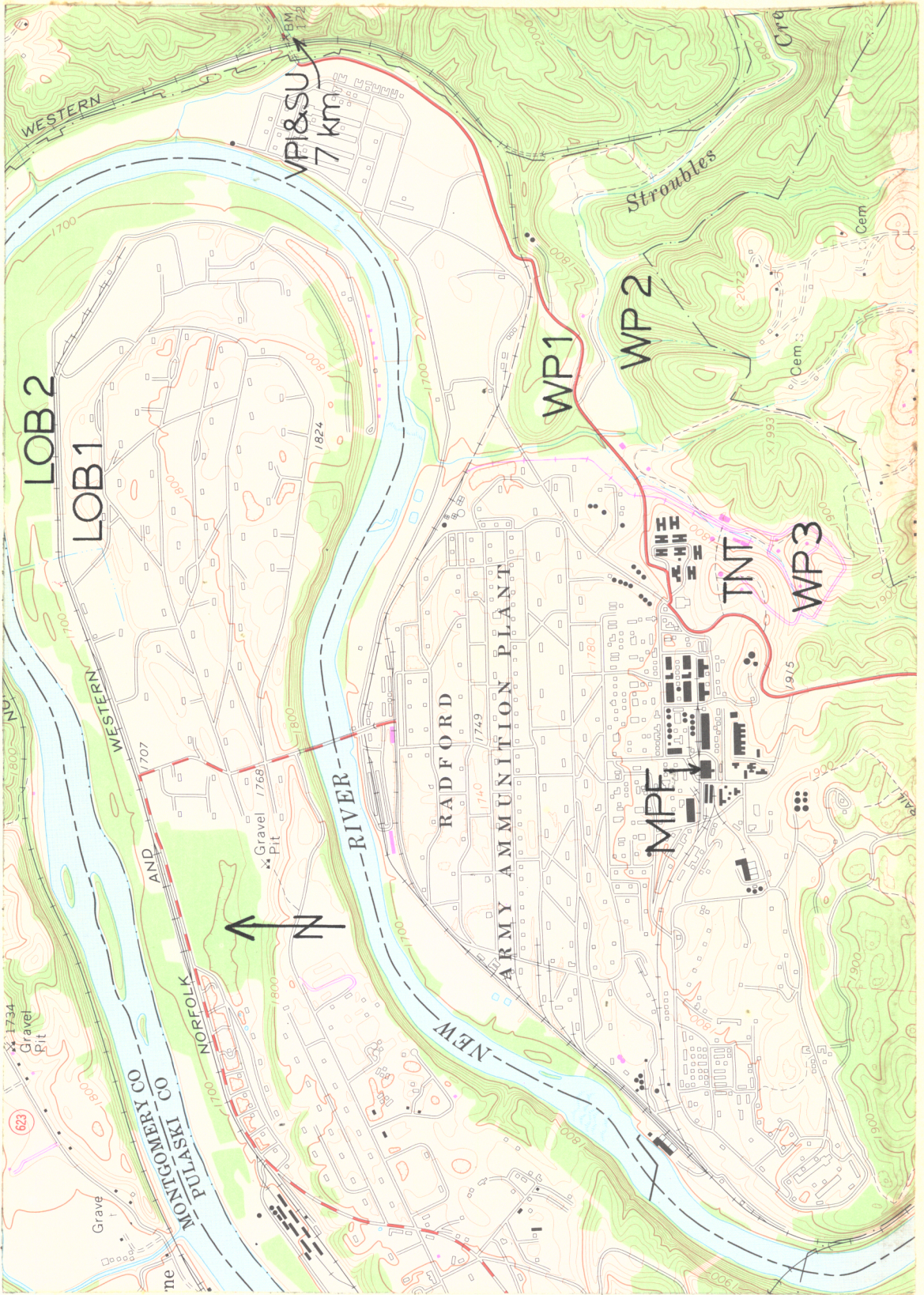
Figure 1. Topographic map of the Radford Army Ammunition Plant (RAAP) Radford, Virginia, showing research plots and facilities.

Map Legend:

- LOB 1 - A stand of 18 year old loblolly pine: loblolly pine stand #1
- LOB 2 - A stand of 21 year old loblolly pine: loblolly pine stand #2
- WP 1 - A stand of 15 year old eastern white pine: white pine stand #1
- WP 2 - A stand of 37-142 year old eastern white pine: white pine stand #2
- WP 3 - A three acre plot of eastern white pine planted in 1968:
white pine stand #3
- MPF - Main power facility
- TNT - Trinitrotoluene (TNT) production area

Scale: 1: 24,000

Contour Interval: 6.1 m (20 feet)



concentrations recorded at a site 600 meters from the main pollution sources at the RAAP were 58.5 pphm and 69.0 pphm NO₂, SO₂ respectively. Based on the degree of symptom expression of the trees within the 13 year old stand, the trees were broken down into three distinct severity classes. Six percent of the trees exhibited typical chlorotic dwarf symptoms, i.e., tufted short needles, only one year old needles present, and stunted terminal growth (tree height of less than five feet). The intermediate trees making up 74% of the total were taller, only one and two year old needles were present, and the symptom expression varied from necrotic tipburn to chlorotic mottle. Twenty percent of the trees appeared healthy having an average height of 18.8 feet. The three year old needles on these trees were sometimes present. Other white pines were found as far as two miles downwind from the RAAP that had developed varying degrees of chlorotic mottle. These trees were usually standing among healthy individuals indicating again, the fact that eastern white pines sensitivity to air pollution is genetically controlled. Other coniferous species were also evaluated for visual symptoms during the course of the study and found to be comparatively tolerant of the air pollution regime found at the RAAP.

In 1974 Stone and Skelly (72) reported that the production rate at the RAAP for the period 1941 through 1971 had an inverse relationship to the average annual increment growth of both eastern white pine and tulip popular (Liriodendron tulipifera L.). This inverse relationship was determined through the use of linear regression analysis. The analysis showed that when variables such as annual and seasonal rainfall

were held constant, the annual growth rate was significantly reduced in the two species.

Phillips (58), also working at the RAAP from 1973 to 1975, conducted two studies designed to elucidate the effects of the air pollution regime at the RAAP on the growth of several forest tree species. In the first study seedlings of yellow poplar, sycamore (Platanus occidentalis L.), red oak (Quercus rubra L.), green ash (Fraxinus pennsylvanica Marsh), sweet gum (Liquidambar styraciflua L.) and Virginia pine (P. virginiana Mill.) were each placed in (i) a chamber receiving charcoal filtered air, (ii) a chamber receiving non filtered air, and (iii) an open plot at three locations within the grounds of the RAAP. Data on terminal elongation and senescence was collected from June to October 1973. Green ash, red oak, and Virginia pine seedlings maintained in the filtered air chamber exhibited significantly greater terminal elongation as compared to those grown in the open plot. Of the six species tested only red oak and Virginia pine exhibited significantly greater terminal elongation on seedlings maintained in the charcoal filtered chamber as compared to those in the non-filtered chamber. Analysis of the senescence data showed that those deciduous species maintained in the non-filtered chamber and open plot were consistently in later stages of senescence than those grown in the charcoal filtered chamber.

Phillips' second study (58) involved the analysis of radial increment growth data on three tree species to determine if correlations existed between that and the production rate at the RAAP. Multiple linear regression analysis was utilized where the dependent variable

was annual radial increment growth and the independent variables were annual production levels, annual and seasonal rainfall, and tree age. The three species utilized in the study were eastern white pine, loblolly pine, and sycamore. Two of the three loblolly pine stands and one of the two eastern white pine stands analyzed, demonstrated a very significant (at the 0.01% level) inverse relationship between growth and production levels. Multiple linear regression analysis was used to predict the theoretical growth loss of these three stands as compared to similar stands growing in a pollution free environment. It was determined that the two loblolly pine stands (Figure 1: LOB 1 and 2) had each undergone a 45% reduction in diameter growth as compared to a 10% reduction in the white pine stand.

Phillips noted that the crowns of the individual eastern white pines in eastern white pine stand number 2 (Figure 1: WP2) which was the stand used in the above analysis, exhibited a variety of crown symptoms. These symptoms ranged from crowns having needles with necrotic tipburn to those having healthy needles. The crowns were then visually rated according to the degree of symptom expression as follows:

- 1 = more than 25% of the needles exhibiting necrotic tipburn;
- 2 = not more than 25% of the needles exhibiting necrotic tipburn;
- 3 = the needles exhibited chlorosis but not necrotic tipburn and the overall crown was thin due to stunted growth or defoliation;
- 4 = healthy, symptomless.

The multiple linear regression analysis was run again on this stand after classifying the individuals by crown symptom expression. The

results showed that even when the trees in each of the four classes were run together as a class, the inverse correlation between growth and production remained highly significant. Thus, the growth rate of the overall stand was being depressed at approximately an equal rate (10%), however, there was variability in the degree of symptom expression of the crowns of the trees sampled.

MATERIALS AND METHODS

Eastern white pine stand number 2 (Figure 1: WP2) was chosen as a source of plant material for use in the development of an air pollution bioindicator system at the RAAP because of the variation in symptom expression as described by Phillips (58). The white pines in this stand were components of a natural, uneven aged, mixed stand of conifers and hardwoods growing on a sharply sloping northeast exposure, 1.6 km east of the main power facility.

Vegetative Propagation

The establishment of an air pollution bioindicator system requires the fumigation of a large number of individuals from several genotypes. In order to propagate genetically identical individuals from each of the selected eastern white pines, grafting was used. Scion was cut from the crowns of three ortets in each of the four sensitivity classes as described by Phillips (58). The pole pruner used in this work had a maximum manageable height of 45 feet and thus restricted the selection of ortets. Due to the size of the trees in the study area, most cuttings came from the lower one-third of the live crown; in fact, some were from the lowest branches in the crown. Ortets were selected which had average growth forms and a live crown which would be accessible based on the restrictions discussed above. Scions were collected at two intervals, February 29 to March 5, and April 8 to 10, 1976. The material was placed in plastic bags with moistened towels and stored in a cold room at 5° C until grafted. It should be noted that the buds of the scion taken in March were tight, exhibiting no

indications of eminent bud break, while the buds on the scion collected in April had begun to swell and in some cases initial candle extension was eminent.

Two year old eastern white pine seedlings were obtained from the Virginia Division of Forestry. These were potted in 32 fluid ounce plastic containers with a 2:2:1 by volume ratio of Webbite (an expanded shale product of the Webster Brick Company, Roanoke, Virginia), vermiculite, and peat. Plantings were begun in November 1975 and extended through April 1976 in order to replace rootstock mortality. The newly potted pine seedlings were grown in a greenhouse with only natural sunlight. Rootstock was not used in the grafting program unless the terminal bud was actively growing. The rootstock that was potted early in the program had significant candle extension when grafting began in late March while some of those potted later had only the initiation of candle extension when they were grafted.

Grafting began in late March and ended in mid May 1976. This phase of the program was conducted in three stages in order to facilitate a workable fumigation schedule. The three stages were as follows: (i) March 2 to 11; (ii) March 30 to April 6, and (iii) May 4 to 11. The first two groups were grafted with scions collected in March while the last group was grafted with scion collected in April. In each group, at least 66 grafts were made for each of the 12 clones for a total of at least 792 ramets per grafting period (Table 3). The side veneer or side tongue grafting method was used (29). Care was taken to accurately align at least one side of the cambium of the smaller diameter scion

with one side of the cambium of the rootstock. The point of union was wrapped with a strip of rubber and sealed with an asphalt emulsion type grafting compound. The date of grafting was recorded for each ramet. The ramets were then put under a mist system in a greenhouse with charcoal filtered air for three to four weeks. The ramets were visually inspected each five to seven days to accurately determine the correct date of the initiation of shoot elongation.

Fumigation Procedures

Ramets were selected for treatment based on the age and condition of the new growth. Eight weeks after bud break was selected as the average age at which the ramets would be fumigated. A second criterion used to select ramets for fumigation was based on the condition of the current years growth. If the needle length was unusually short or the overall growth form was abnormal, a ramet was not utilized. Five different ramets from each of the 12 clones were selected for each treatment (a total of 60 ramets) from a group of even aged individuals. As ramets from the youngest group of a given clone were exhausted ramets from the next older group were selected and so on until the treatments were complete.

Treatments were conducted as follows: (i) O_3 - 10 pphm, (ii) O_3 - 30 pphm, (iii) NO_2 - 10 pphm, (iv) NO_2 - 30 pphm, (v) O_3 - 10 pphm + NO_2 - 10 pphm, (vi) O_3 - 10 pphm + NO_2 - 30 pphm, and (vii) no pollutant. Each treatment was usually repeated the next day, the one exception being the no pollutant treatment which had 21 days separating the two treatments (Table 2). Thus there was a total of 14

TABLE 2. Eastern white pine ramet fumigation schedule for 1976.

| Treatments | DATE | | | | | | | | | | | | | | |
|------------------|------|----|----|----|----|----|----|----|----|---|------|----|----|----|----|
| | JUNE | | | | | | | | | | JULY | | | | |
| | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 1 | 2 | .. | 15 | 16 | 17 |
| O_3-10^a | | | | | | | | | | | | | X | X | |
| O_3-30 | X | X | | | | | | | | | | | | | |
| NO_2-10 | | | | | | X | X | | | | | | | | |
| NO_2-30 | | | X | X | | | | | | | | | | | |
| $O_3-10+NO_2-10$ | | | | | | | | | | | X | X | | | |
| $O_3-10+NO_2-30$ | | | | | | | | X | X | | | | | | |
| No Pollutant | | | | | X | | | | | | | | | | X |

^aAll pollutant concentrations are in pphm.

treatments, requiring a total of 840 ramets. In the no pollutant treatment, the ramets were placed in the chamber and the treatment conducted identically to the other six, except no pollutants were injected into the chamber.

Eleven consecutive treatments (including one no pollutant treatment) were conducted between June 22 and July 2, 1976 while the remaining three were conducted on 15, 16, and 17 July 1976. All treatments lasted six hours beginning between 0800 and 1000 hours. During the 14 treatments, the relative humidity ranged from 50 to 83% and was measured by a Belfort Hygrothermograph (Belfort Instrument Company, Baltimore, Maryland) which was calibrated with a wet bulb/dry bulb sling psychrometer. The temperature during the fumigations ranged from 21 to 28° C. The ramets were maintained in a greenhouse drawing charcoal filtered air both prior to and after the fumigation. During the course of this study temperatures in the greenhouse ranged from above 20° C to above 40° C while the relative humidity ranged from 95% during the night to 25% during the day. All fumigations were conducted in a specially modified version (55) of the circular open top field chamber designed by Heagle et al. (34).

The modified open top field chamber measured 3.1 meters (m) in diameter and 1.1 m in height with a bottom area of 7.3 square meters (Figure 2). Air was pulled through a charcoal filter which was sealed into a filter/fan assembly box by a 5/8 horsepower fan motor and blown into the double plenum of the side walls made of polyvinyl chloride plastic (PVC). The air reached the inside of the chamber through evenly spaced holes on the inside plenum. The top and bottom of the



Figure 2-(A-D). The modified open top chamber used for indoor fumigations at the Air Pollution Research Laboratory, VPI & SU, Blacksburg, Virginia.

A) Overhead view of the chamber. 1 = intake port; 2 = entry port into the double plenum of the chamber; 3 = high pressure sodium lights; 4 = expanded double plenum; 5 = exhaust port.

B) Filter/fan box assembly. 1 = fan box 2 = filter box.

C) Top view of the chamber during fumigation. 1 = door seal; 2 = hygrothermograph.

D) Pollutant monitoring instrumentation. 1 = 15 point sample system; 2 = recorders; 3 = Ozone analyzer; 4 = NO, NO₂, NO_x analyzer; 5 = Calibration system; 6 = total sulfur analyzer; 7 = sample system stream selector; 8 = ozone generator; 9 = hydrogen generator (fuel source for total sulfur analyzer).

chamber were covered with PVC plastic. A rug pad was used to protect the bottom panel from abrasions. Air was exhausted from the system through a 40.6 centimeter (cm) diameter port which was cut in the top panel and fitted with a 1.8 m long section of fabricated PVC plastic tubing. The filter/fan motor assembly box was housed outside the laboratory in an adjacent insulated shed. They were aligned in series parallel to the wall and connected at each end to the laboratory with 45.7 cm diameter sheet metal elbow sections. Air was then drawn from the laboratory through the charcoal filters and blown into the chamber. However, because the fumigations were conducted in the summer, the laboratory was air conditioned, thus, the relative humidity of the air in the room was below 50%. To supplement the relative humidity in the fumigation chamber, a 45.7 cm diameter PVC plastic tube was fabricated and one end was connected to the exit port of an Alpine cooler (water drip system) while the other was connected to the intake port of the filter/fan motor assembly box inside the laboratory. This moisture laden air was passed through the charcoal filters where some of the moisture was removed and blown into the chamber by the fan. Even though some of the moisture was removed by the charcoal filters, once they became moisture saturated, moisture remained in the air to maintain a sufficient relative humidity in the fumigation chamber (>55%). The air flow inside the chamber was about 1100 liters/second or approximately eight complete air changes per minute.

Lighting for the fumigation chamber was provided by two high pressure sodium lights (Hubble Lighting Company, Christiansburg,

Virginia). The lights were suspended 1.6 m above the top of the plants. These lights provided from 18,300 lux directly under the lights to 10,800 lux near the edge of the chamber. Pollutants were injected into the system between the fan motor housing and the double plenum. This allowed ample time for the pollutants to be equally distributed in the charcoal filtered air and blown over the plants inside the chamber. Pollutant mixing and distribution was tested by measuring O_3 concentrations at four different locations inside the chamber which was found to have a range of only 1.5 ppm.

During the fumigations, O_3 was generated as needed with an electric arc type ozone generator Model C2P-6C (PCI Ozone Corporation, West Caldwell, New Jersey). The O_3 produced was then introduced directly into the fumigation chamber. The O_3 concentration was monitored during the fumigations using a Bendix chemiluminescent Ozone Monitor model 8002 (Bendix Corporation, Lewisburg, West Virginia) and regulated by direct control of the voltage output reostat on the O_3 generator. The Bendix Ozone Monitor was calibrated prior to each fumigation using the 1% neutral buffered KI method (39). Nitrogen dioxide was introduced into the fumigation chamber via a flow meter from a bottle of 0.44% NO_2 in nitrogen. The NO_2 was monitored with a Bendix NO- NO_2 - NO_x Monitor model 8101-B (Bendix Corporation, Lewisburg, West Virginia) and concentrations controlled by adjusting the flow meter. The NO- NO_2 - NO_x Monitor was calibrated using a tank of span gas whose concentration had been determined by the Analytical Quality Control Section of the Environmental Protection Agency, Research Triangle Park, North Carolina.

Evaluation of Ramets

All ramets were evaluated for visual symptoms immediately prior to fumigation (to detect any pre-fumigation mimicking symptoms) and then two, seven, and 14 days after fumigation. The rating system used was as follows:

- 0 = no symptoms;
- 1 = chlorotic spot;
- 2 = chlorotic mottle;
- 3 = chlorotic band;
- 4 = chlorotic tip;
- 5 = necrotic spot;
- 6 = necrotic band;
- 7 = necrotic tip burn.

All of the fascicles on the new growth were evaluated and if one needle of a fascicle exhibited a symptom, the entire fascicle was rated as such. In a case where more than one symptom was observed on the five needles of a fascicle, that fascicle received the most severe rating of all the symptoms observed. The total number of fascicles present on the new growth of a terminal leader (candle) was counted. The mean length of five of the new fascicles was measured immediately prior to fumigation as well as 11 weeks later. Even though the fascicles were not marked, the five fascicles around the base of the new bud/candle were always the ones measured. In addition, the mean length of five of the one year old needles was also measured using the same criterion wherever possible. The length of the new emerging candle at the time of fumigation

was measured for all ramets. The evaluation system was as follows: 1 = no extension; 2 = candle extension 0 - 0.6 cm long; 3 = candle extension 0.6 - 1.3 cm long; and 4 = candle length > 1.3 cm long. In addition, the age of the ramets at the time of fumigation in weeks since budbreak was recorded.

Statistical Analysis

A system severity index (SSI) was calculated for each ramet per evaluation period in the following manner: (i) the number of fascicles exhibiting a given symptom was converted to a percent using the total number of new fascicles on the emerging candle as the denominator; (ii) the percent value was multiplied by the numerical value of the symptom type giving a weighted percent value; and (iii) the weighted percent values were then totalled across the seven possible symptoms types giving an SSI for the ramet. In cases where injury was observed prior to treatment, that SSI was subtracted from the SSI's of the remaining three evaluation periods in order to correct for the error. In addition, the mean severity index (MSSI) of the control treatment for a given class-clone was also subtracted from the individual SSI's of ramets in the other six treatments further correcting for non pollutant effects. In all cases 0.5 was added to each SSI so that zeroes were not present in any data set. The square root of the corrected SSI was then calculated and used in the subsequent analyses.

The SSI was calculated for each of the class/clone/treatment combinations by evaluation period and analyzed by analysis of variance (ANOVA) using the standard F test as performed by the Statistical

Analysis System (SAS). To test for specific differences between clones within a given treatment, the following linear model was used:

$$Y_{ij} = \mu + D_i + C_j + (DC)_{ij} + E_{ij} \quad (i)$$

where:

Y = SSI (i = day, j = clone),

μ = overall mean,

D_i = the effect of the i -th day (each treatment was repeated thus requiring two separate days),

C_j = the effect of the j -th clone,

$(DC)_{ij}$ = the component for the day by clone interaction,

E_{ij} = residual component.

The results of these ANOVA tests were then used for the Duncan new multiple range tests (27) which showed which clones were significantly different in each treatment at the .05 level. A significant F test was not required as a prerequisite for the Duncan's new multiple range test (27).

The impact of the treatments used in this study on the growth of the 12 clones of eastern white pine was determined through analysis of covariance. The needle lengths of the ramets at 11 weeks post treatment were adjusted for their needle lengths prior to treatment through analysis of covariance. Significant differences based on adjusted mean needle lengths were determined for both ramets as averaged over all seven treatments, and treatments as averaged over all 12 clones, using the Duncan's new multiple range test (27).

Multiple linear regression analysis was also utilized to determine which of the following independent variables, if any, can be used to predict the dependent variable SSI, of a given clone: X_1 = age of the tissue at fumigation, X_2 = length of the one year old needles, X_3 = length of the needles prior to fumigation, X_4 = length of the needles 11 weeks after fumigation, X_5 = the candle form, and X_6 = the class designation of the clone. The analysis was run on data from the O_3 -30 pphm and the O_3 -10 pphm + NO_2 -30 pphm fumigations. The multiple regression model used was:

$$Y = B_0 + B_1X_1 + B_2X_2 + \dots + B_6X_6$$

where the dependent variable Y was regressed on the independent variables $X_1 - X_6$ as listed above. Estimates of the population parameters B_0 and $B_1 - B_6$ were determined from the sample data as previously discussed.

RESULTS

Vegetative Propagation

As shown in Table 3, 71.2% of all of the eastern white pine grafts made in the spring of 1976 were viable six to eight months later when put in the cold frame for the winter. Of the three grafting periods (March 2 to 11, March 30 to April 6, and May 6 to 11), the March 30 to April 6 period had the highest survival rate with 90.4% vs. 65.9% for the third period and 56.7% for the first period. In some clones, ramet survival varied among the three periods, e.g., clone I-3 exhibited a low survival rate in the first period (53.0%) followed by very high rates in the second and third periods (97.0% and 100.0% respectively), while clone IV-3 showed a low rate in the third period (43.9%) and a high rate in the first and second (84.8% and 89.4% respectively). Of the 12 clones utilized in the study, I-3 and II-3 had the highest percent overall survival with 83.3% and 82.1% respectively while I-1 had the lowest with only 45.8%.

Description of Symptoms

The most diagnostically important symptom associated with air pollution injury observed through the course of this study was chlorotic mottle (Figure 3). Even though necrotic tipburn was the most prevalent symptom observed through the two and seven day evaluation periods, it was not characteristic of the pollution doses used (4, 38, 46). At least a portion of the necrotic tipburn observed during the course of the study was believed to have been the result of high temperatures in the charcoal filtered greenhouse. Chlorotic mottle was usually observed

Table 3. Results of the 1976 eastern white pine grafting program in % of viable ramets by class-clone and grafting period.

| Class/Clone | Number of Viable Ramets/Number Grafted (%) | | | | | | TOTAL |
|--------------------|--|------------------|----------|----------|---------|-----------|--------|
| | Grafting Periods | | | | | | |
| | March 2-11 | March 30-April 6 | May 6-11 | May 6-11 | 93/203 | 132/200 | |
| ^b I - 1 | 18/66 | 59/71 | 16/66 | (83.1) | (24.2) | 93/203 | (45.8) |
| | 33/66 | 66/68 | 33/66 | (97.1) | (50.0) | 132/200 | (66.0) |
| | 35/66 | 64/66 | 66/66 | (97.0) | (100.0) | 165/198 | (83.3) |
| II - 1 | 31/66 | 56/66 | 44/66 | (84.8) | (66.7) | 131/198 | (66.2) |
| | 37/66 | 63/66 | 53/66 | (95.5) | (80.3) | 153/198 | (77.3) |
| | 48/66 | 64/69 | 53/66 | (92.8) | (80.3) | 165/201 | (82.1) |
| III - 1 | 34/66 | 59/66 | 55/66 | (89.4) | (83.3) | 148/198 | (74.7) |
| | 48/66 | 68/70 | 28/66 | (97.1) | (42.4) | 144/202 | (71.3) |
| | 52/66 | 62/69 | 35/66 | (89.9) | (53.0) | 149/201 | (74.1) |
| IV - 1 | 30/66 | 57/66 | 55/66 | (86.4) | (83.3) | 142/198 | (71.7) |
| | 27/66 | 56/68 | 55/66 | (82.4) | (83.3) | 138/200 | (69.0) |
| | 56/66 | 59/66 | 29/66 | (89.4) | (43.9) | 144/198 | (72.7) |
| TOTAL | 449/792 | 733/811 | 522/792 | (90.4) | (65.9) | 1704/2392 | (71.2) |

^aDetermined by the number of ramets put into cold-frame in October 1976.

^bClass I - > 25% of the needles exhibiting necrotic tipburn, Class II - < 25% of the needles exhibiting necrotic tipburn, Class III - needles chlorotic but not necrotic, Class IV - needles healthy.

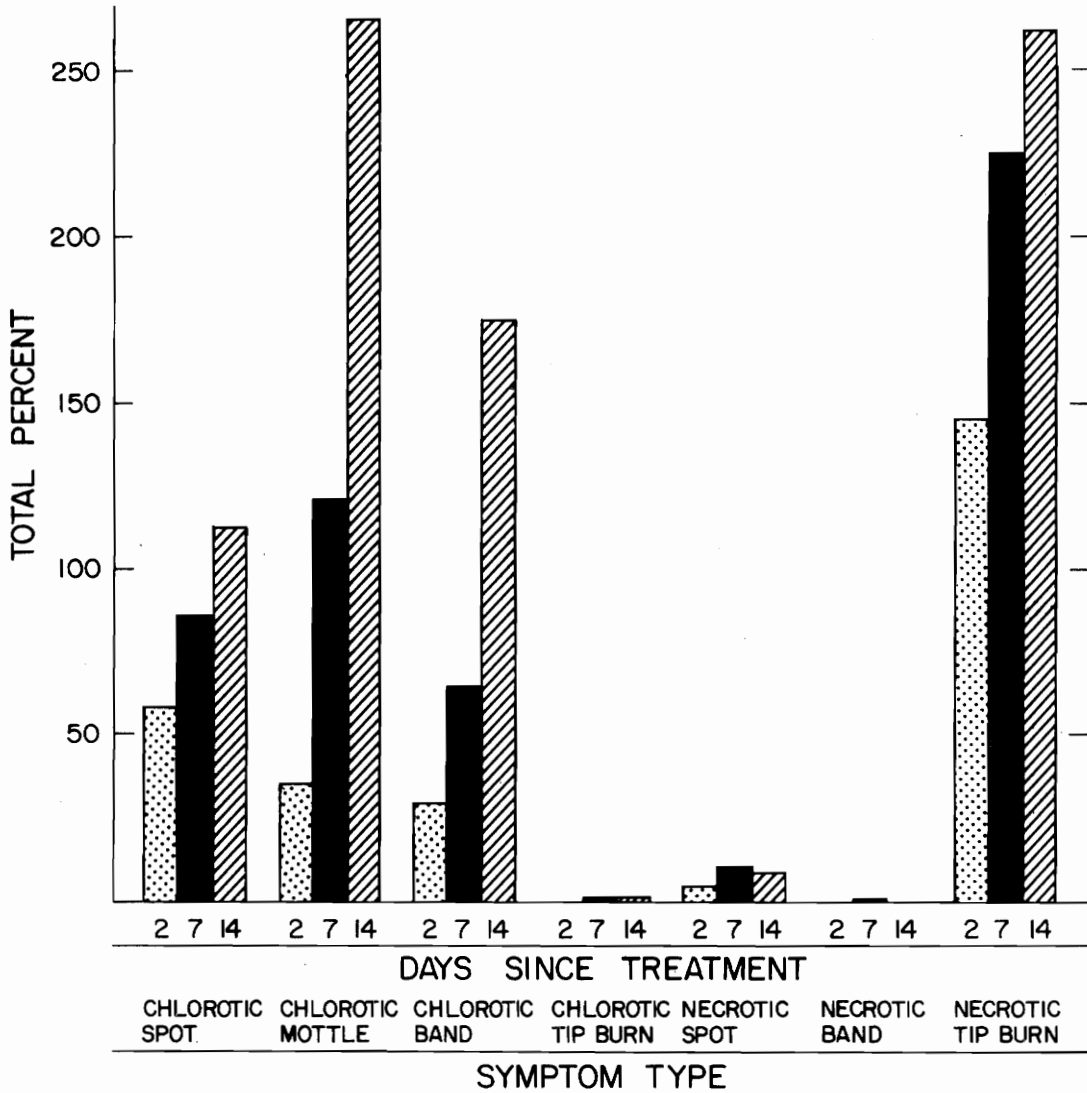


FIGURE 3. TOTAL PERCENTAGE OF FASCICLES INJURED BY SYMPTOM TYPE ON 12 CLONES OF EASTERN WHITE PINE SUMMED OVER ALL SEVEN TREATMENTS USED IN THE STUDY.

to develop from an adaxial aggregation of pink lesions, however only infrequently did it tend to develop from a chlorotic spot. Chlorotic symptoms (spot, mottle, band, and tip) were normally first observed on the adaxial needle surfaces. In severe cases, the chlorotic mottle developed into a chlorotic band which was visible on the abaxial needle surface and then progressed acropetally into a necrotic tipburn. Quite often symptoms developed from a chlorotic spot or mottle to a chlorotic band which appeared at a point roughly one-half to three-quarters of the distance up the length of the needle allowing the terminal end (tip) to remain green. However, when chlorotic band was the first symptom observed, it tended to progress to a necrotic tipburn within five to seven days.

Necrotic tipburn was the second most prevalent symptom observed at the 14 day evaluation period (Figure 3). Of the seven treatments, four (O_3 -10 + NO_2 -10, O_3 -10 + NO_2 -30, NO_2 -10 and the no pollutant) were responsible for a large percentage of necrotic tipburn observed (40-72 total percent of the fascicles exhibiting the symptom) while the remaining three (NO_2 -30, O_3 -10, and O_3 -30) were responsible for relatively little (2 to 18 total percent) (Table 4). When the percent injury by symptom type was analyzed by treatment, it was noted that none of the clones from the three treatments, where relatively little necrotic tipburn was observed, exhibited more than 8% of the fascicles with necrotic tipburn (Appendix: Tables 5, 6, 9 respectively). This is contrasted to the other four treatments where the maximum percentage of fascicles observed with necrotic burn was 46% in clone I-1 from the O_3 -10 + NO_2 -10 treatment (Appendix: Table 7). In three of these four

Table 4. Total percentage of fascicles injured in all 12 clones of eastern white pine by symptom type and evaluation period for each of the seven treatments

| Treatments (Conc. in PPHM) | Total Percent Injury by Symptom Type and Evaluation Period (Days Post Treatment) | | | | | | | | | | | | | | Total | Rank | | | | | | | | | | | | |
|--|---|----|------------------|----|----------------|-----|---------------|----|---------------|---|---------------|----|-------------------|----|-------|------|----|---|-----|-----|-----|-----|-----|-----|-----|---|---|---|
| | Chlorotic Spot | | Chlorotic Mottle | | Chlorotic Band | | Chlorotic Tip | | Necrotic Spot | | Necrotic Band | | Necrotic Tip-Burn | | | | | | | | | | | | | | | |
| | 2 | 7 | 14 | 2 | 7 | 14 | 2 | 7 | 14 | 2 | 7 | 14 | 2 | 7 | | | 14 | 2 | 7 | 14 | | | | | | | | |
| 0 ₃ -10 | 4 ^a | 6 | 3 | 1 | 20 | 26 | 0 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 5 | 33 | 36 | 7 | 6 | 7 | | |
| 0 ₃ -30 | 10 | 31 | 31 | 2 | 47 | 111 | 5 | 9 | 17 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 15 | 16 | 20 | 104 | 176 | 5 | 2 | 1 | |
| NO ₂ -10 | 16 | 6 | 18 | 9 | 4 | 11 | 0 | 11 | 50 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 6 | 34 | 43 | 31 | 57 | 123 | 4 | 4 | 4 |
| NO ₂ -30 | 10 | 9 | 8 | 5 | 6 | 17 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 6 | 18 | 18 | 25 | 44 | 6 | 7 | 6 |
| 0 ₃ -10+NO ₂ -10 | 7 | 8 | 17 | 4 | 15 | 24 | 3 | 20 | 48 | 0 | 0 | 1 | 3 | 6 | 6 | 0 | 1 | 0 | 61 | 69 | 72 | 78 | 119 | 168 | 1 | 1 | 2 | |
| 0 ₃ -10+NO ₂ -30 | 1 | 12 | 15 | 2 | 13 | 38 | 14 | 6 | 30 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 27 | 43 | 57 | 44 | 75 | 137 | 2 | 3 | 3 | |
| No Pollutant | 8 | 10 | 15 | 4 | 17 | 26 | 12 | 3 | 7 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 29 | 37 | 40 | 44 | 57 | 88 | 2 | 4 | 5 | |
| TOTAL | 56 | 82 | 107 | 27 | 112 | 253 | 25 | 58 | 157 | 0 | 1 | 1 | 5 | 11 | 9 | 0 | 1 | 0 | 127 | 205 | 248 | 240 | 470 | 772 | | | | |

^aThe maximum possible injury in any given block would be 1200 (100% x each of 12 clones)

treatments a relatively large percentage of chlorotic banding was also observed (the one exception was the no pollutant treatment - Appendix: Table 3 - with a total of only 7% chlorotic banding). This was in contrast to the other three treatments where necrotic tipburn was not observed; two of the three did not have a large percentage of chlorotic banding observed (the one exception was the O_3 -30 treatment which was responsible for a total of 17% chlorotic banding in all 12 clones as compared to 5% and 0% in the other two treatments - Table 4). From these data it appeared that when necrotic tipburn comprised a relatively large proportion of the total observed injury, it was usually associated with chlorotic banding.

The chlorotic banding symptom developed in one of two ways. Chlorotic spots/mottle was first observed on the adaxial surface of one of the five needles in a given fascicle and then, within five to seven days, progressed to a chlorotic band which encompassed the entire fascicle at a point one-half to three-quarters the distance up from the base of the fascicle. At this time the chlorosis was visible on both abaxial and adaxial needle surfaces. The second pattern of chlorotic band development was more severe. Usually within two days after the treatment, a definite chlorotic band would be observed as described above. Within five days the band would turn necrotic and the entire fascicle would be rated as having necrotic tipburn.

Symptoms which were believed to have been caused by high temperatures in the greenhouse were first observed on ramets from the NO_2 -10 treatment at the two day evaluation on the evening of June 30, 1976.

These symptoms were observed primarily on clone I-1 and sparingly on clones I-2 and IV-3. No symptoms were observed on these ramets at their prefumigation evaluation which was completed by 0800 hours of June 28. During the pretreatment evaluations of the ramets of the remaining treatments, identical symptoms were observed primarily on clones I-1, I-2, II-3, and IV-3; to a lesser degree on clones II-1 and II-2; and only sparingly on clones III-2 and III-3. The symptoms observed in all cases were dark tan to brown lesions usually appearing as bands or necrotic tipburn. These lesions appeared somewhat as a water soaked area of collapsed cells which was more pronounced on the abaxial surface. Because of the difficulty in distinguishing between pollutant injury and "heat injury" especially in the advanced stages of development, no attempt was made to differentiate the two symptom types after the pre-treatment evaluations.

The severity of incidence associated with injury in a given treatment was usually light. Only 11% of the clone-treatment combinations exhibited injury on more than 25% of the needle fascicles. The percent of needle fascicles injured by symptom type in each of the 12 clones is presented by treatment in the Appendix: Tables 3-9.

The high total percentage of fascicles injured in the 12 clones tested was due to the O_3 -30 (hereafter all pollutant concentrations are assumed to be in pphm) treatment while the O_3 -10 + NO_2 -10 treatment ranked second (Table 4). The maximum percentage of fascicles injured observed during the course of the study was on clone I-1 from the O_3 -10 + NO_2 -10 treatment (Appendix: Table 7) where a total of 65%

of the fascicles of this clone exhibited some type of symptom: chlorotic spot - 4%, chlorotic band - 14%, chlorotic tip - 1%, and necrotic tipburn - 46%. This was in contrast to two clones (usually III-1 and IV-2) that were not injured except in the O_3 -30 treatment where all 12 clones were injured to some degree. The specific visual symptoms associated with each of the seven treatments as compiled from all 12 clones were as follows (see Table 4 for the injury incidence ranking of the various treatments):

(i) O_3 -10: Very sparse chlorotic spotting was the prominent symptom observed after two days. This appeared to progress into chlorotic mottle after seven days which increased only slightly after 14 days.

(ii) O_3 -30: The symptom development and progression was very similar to that of the O_3 -10 treatment except that this treatment generally produced five times the amount of injury observed in the O_3 -10 treatment. A second difference between the two O_3 alone treatments was that in the O_3 -30 treatment there was an eight fold increase in the necrotic tipburn.

(iii) NO_2 -10: Symptom development was similar to that of the O_3 -10 treatment, however, larger proportions of chlorotic mottle and necrotic tipburn were observed at the two day evaluation period. After seven days, necrotic tipburn accounted for more than 50% of the observed injury. At the 14 day evaluation, necrotic tipburn and chlorotic banding were the dominant symptoms followed by chlorotic spot and chlorotic mottle.

(iv) NO_2 -30: Chlorotic spot was the primary symptom associated with this treatment at the two day evaluation. Symptom development became more general at the seven day evaluation with chlorotic spot, chlorotic mottle, and necrotic tipburn accounting for the majority of the injury. After 14 days, necrotic tipburn and chlorotic mottle dominated followed by chlorotic spot.

(v) O_3 -10 + NO_2 -10: Necrotic tipburn was the dominant symptom observed in all three evaluation periods. After 14 days, chlorotic banding was the second most prevalent symptom followed by chlorotic mottle, and finally chlorotic spot. In this treatment the incidence of the necrotic tipburn as observed initially did not increase significantly while the increased injury rating in the later two evaluation periods was due to the other three mentioned symptom types.

(vi) O_3 -10 + NO_2 -30: Symptom development and progression generally paralleled that as observed in the O_3 -10 + NO_2 -10 treatment.

(vii) No pollutant: Symptom development and progression generally paralleled that as observed in the O_3 -10 + NO_2 -10 treatment although fewer symptoms were observed in the control treatment.

Statistical Analysis of the Symptom Severity Index Data

In order to determine significant differences between any of the clones in a given treatment through Duncan's new multiple range test, an ANOVA (F test) was conducted first. The model used in these analyses (model 1, page 66) contained only two main factors: day and clone. The results of the ANOVA tests are shown in Table 5 for each of the seven treatments used in the study.

Analysis of the ANOVA test results for each of the seven treatments (Table 5) at the two day evaluation showed significant (.05 or .01 level) clone effects for five of the seven treatments (the O_3 -30 and no pollutant treatments were the only two not showing significance). At the seven day evaluation three of the seven treatments showed significant clone effects (NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30). By the 14 day evaluation four of the treatments showed significant clone effects (the same three as above plus the O_3 -30 treatment). The only significant day effects were for the NO_2 -30 and O_3 -10 + NO_2 -30 treatments and these were consistent through all four evaluations. Significant day by clone interactions were found in three treatments at the two day evaluation (NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30). At the seven day evaluation only the NO_2 -10 treatment showed the significant day by clone interaction and by the 14 day evaluation the NO_2 -10 treatment had been joined by the no pollutant treatment.

Because of the significant interaction components as stated above, care must be taken in the interpretation of the significant main effects involved in those interactions. At the two day evaluation the significance of the clonal effects in the NO_2 -30 and O_3 -10 treatments was a stronger indicator of significantly different clonal responses than that in the NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30 treatments. Following the same interpretative process at the seven day evaluation, the significance of the clonal effects in the O_3 -10 + NO_2 -30 and O_3 -10 + NO_2 -10 treatments was stronger than in the NO_2 -10 treatment.

Table 5. F values for clone effects in each of the seven treatments used in this study.

| Treatment (Conc. in ppm) | F value by Day (D), Clone (C) and DxDC Evaluation Period in Days Since Treatment | | | | | | | | | | | | | | |
|--|---|--------|--------|--------|--------|-------|--------|--------|-------|---|---|-----|---|---|-----|
| | 2 | | 7 | | 14 | | | | | | | | | | |
| | D | C | DxC | D | C | DxC | D | C | DxC | D | C | DxC | D | C | DxC |
| O ₃ -30 | 3.59 | 1.05 | 1.19 | 0.73 | 1.26 | 0.32 | 0.00 | 2.28* | 0.35 | | | | | | |
| NO ₂ -30 | 0.75 | 5.77** | 0.25 | 1.08 | 1.29 | 0.76 | 0.24 | 1.18 | 0.59 | | | | | | |
| No pollutant | 1.00 | 1.00 | 1.00 | 1.66 | 1.81 | 1.88 | 1.92 | 1.89 | 2.04* | | | | | | |
| NO ₂ -10 | 4.38* | 2.23* | 2.32* | 5.44* | 3.64** | 2.07* | 8.80** | 3.56** | 2.09* | | | | | | |
| O ₃ -10+NO ₂ -30 | 7.24** | 7.65** | 2.81** | 7.32** | 5.44** | 1.09 | 9.41** | 5.08** | 1.75 | | | | | | |
| O ₃ -10+NO ₂ -10 | 1.01 | 6.23** | 3.47** | 0.24 | 3.31** | 1.21 | 0.37 | 3.60** | 1.19 | | | | | | |
| O ₃ -10 | 0.50 | 3.04** | 0.58 | 0.01 | 1.75 | 0.89 | 0.00 | 1.18 | 1.73 | | | | | | |

* = Significance at the .05 level
 ** = Significance at the .01 level
 NOTE: The model for the above ANOVA tests was as follows:

$$Y_{ij} = U + D_i + C_j + (DC)_{ij} + E_{ij}$$
 where
 Y_{ij} = symptom severity index of a given ramet;
 U = overall mean;
 D_i = the effect of the i-th day (i = 1,2);
 C_j = the effect of the j-th clone (j = 1, ..., 12);
 $(DC)_{ij}$ = the component for the day by clone interaction;
 E_{ij} = residual component.

Similarly, at the 14 day evaluation the significance of the clonal effects in the O_3 -30, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -10 treatments was stronger than in the NO_2 -10 treatment.

Clonal Response

As shown in Table 6, clone I-1 was ranked number one or two (1 = most sensitive) in six of the seven treatments. This clone however, was not significantly injured in the O_3 -30 treatment as measured by the MSSSI. As shown in Table 7, clone I-1 had sustained no measurable injury in the O_3 -30 treatment by the 14 day evaluation and was significantly different from only two other clones (See Appendix: Table 10 for the results at the seven day evaluation). Clone I-2 ranked one or two in five of the seven treatments falling to number four in the NO_2 -30 treatment and number three in the O_3 -10 + NO_2 -30 treatment. However after 14 days post treatment in the NO_2 -10 treatment, no clone reacted significantly different (all significance levels are at the .05 level) from any other, while in the O_3 -10 + NO_2 -30 treatment clone I-2 was significantly different only from clones I-1 and IV-3 (Table 7). Clones III-1 and IV-2, and to a lesser degree clones III-3 and IV-1, were consistently tolerant to the pollution doses used in this study. Clone III-1 was injured only in the O_3 -30 treatment where it was significantly different only from the clone having the highest MSSSI for that treatment - clone III-2. Injury was observed on clone IV-2 in only three treatments: O_3 -30, O_3 -10 + NO_2 -30, and the no pollutant. In all three cases clone IV-2 was not significantly different from clone III-1. Likewise clones III-3 and IV-1 were

Table 6. Ranking of the 12 clones of eastern white pine based on the mean symptom severity index^a of the 14 day post treatment evaluation for each of the seven treatments.

| Rank in decreasing MSSI | Clones of Eastern White Pine | | | | | | |
|-------------------------|------------------------------|-------------------------------|---------------------|---------------------|-------------------------------|-----------------|------------------------|
| | Treatments (Conc. in PPHM) | | | | | | |
| | 0-30 O ₃ | 0-10 + NO ₂ -30 | NO ₂ -10 | NO ₂ -30 | 0-10 + NO ₂ -10 | No Pollutant | 0-10 O ₃ |
| 1 | III-2 | I-1 | I-1 | I-1 | I-2 | I-1 | I-2 |
| 2 | I-2 | IV-3 | I-2 | II-2 | I-1 | I-2 | I-1 |
| 3 | IV-2 | I-2 | IV-3 | I-3 | III-2 | II-2 | II-1 |
| 4 | IV-1 | III-2 | I-3 | I-2 | I-3 | IV-2 | III-2 |
| 5 | IV-3 | I-3 | III-2 | II-3 | IV-3 | II-1 | I-3 |
| 6 | II-1 | II-1 | II-2 | IV-3 | II-3 | I-3 | II-2 |
| 7 | III-3 | IV-2 | IV-1 | II-1 | II-1 | III-2 | III-3 |
| 8 | I-3 | II-2 | II-3 | III-2 | II-2 | IV-3 | IV-1 |
| 9 | II-2 | III-3 | II-1 | III-1 | III-1 | II-3 | II-3 |
| 10 | III-1 | II-3 | III-1 | III-3 | III-3 | III-1 | III-1 |
| 11 | II-3 | III-1 | III-3 | IV-1 | IV-1 | III-3 | IV-2 |
| 12 | I-1 | IV-1 | IV-2 | IV-2 | IV-2 | IV-1 | IV-3 |

^aMean symptom severity index = MSSI.

^bMSSI of clones below this line \leq 25.0.

^cMSSI of clones below this line = 0.

Table 7. Duncan's new multiple range test on the mean symptom severity indices for treatments by clones at the 14 day evaluation period.

| Treatments (Conc. In PPHM) | Mean Symptom Severity Index at the 14 Day Evaluation Period ^a | | | | | | | | | | | | TOTAL |
|-------------------------------|--|---------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|--------|
| | Class/Clone | | | | | | | | | | | | |
| | I-1 | I-2 | I-3 | II-1 | II-2 | II-3 | III-1 | III-2 | III-3 | IV-1 | IV-2 | IV-3 | |
| No Pollutant | 74.1a | 12.0b | 2.0b | 2.5b | 9.0b | 0.4b | 0.0b | 1.2b | 0.0b | 0.0b | 3.7b | 0.8b | 105.7 |
| NO ₂ -10* | 157.8a | 102.0ab | 13.4c | 0.5c | 12.4c | 1.1c | 0.0c | 13.1c | 0.0c | 5.0c | 0.0c | 64.4bc | 369.7 |
| NO ₂ -30* | 52.1a | 12.2a | 37.4a | 3.8a | 43.1a | 12.1a | 0.0a | 0.8a | 0.0a | 0.0a | 0.0a | 8.8a | 170.3 |
| O ₂ -10 | 6.6ab | 11.3a | 1.7b | 3.2ab | 1.5b | 1.1b | 0.0b | 2.1ab | 1.4b | 1.3b | 0.0b | 0.0b | 30.2 |
| O ₃ -10+* | 79.0ab | 93.6a | 67.0ab | 3.9c | 0.7c | 29.0bc | 0.0c | 76.0ab | 0.0c | 0.0c | 0.0c | 41.5abc | 390.7 |
| NO ₂ -10 | | | | | | | | | | | | | |
| O ₃ +10+ | 208.7a | 68.8bc | 26.5c | 22.6c | 6.9c | 0.6c | 0.0c | 51.5bc | 4.8c | 0.0c | 12.3c | 119.3b | 522.0 |
| NO ₂ -30 | | | | | | | | | | | | | |
| O ₃ -30* | 0.0c | 58.2ab | 17.5bc | 20.9bc | 16.9bc | 16.2bc | 16.9bc | 91.9a | 17.7bc | 40.0bc | 46.5abc | 28.0bc | 370.7 |
| | | | | | | | | | | | | | 1959.3 |

^aMeans with the same letter in a given row are not significantly different.

*Significant clonal effects as determined through ANOVA (Table 5).

injured only in three treatments and at no time were they significantly different from clone III-1.

The remaining clones I-3, II-1, II-2, II-3, III-2 and IV-3 were intermediate in their response to the pollutant doses used in this study. Clone III-2 would probably rank as one of the most sensitive of the intermediate group. In the O_3 -30 treatment this clone ranked first as shown in Table 6 and was significantly different from the remaining 11 clones in that treatment as shown in Table 7. However, in the NO_2 -30 treatment, clone III-2 ranked eighth, third in the O_3 -10 + NO_2 -10 treatment, fourth in the O_3 -10 + NO_2 -30 treatment, etc. As shown by clone III-2, the intermediate clones were not consistent in their response to the pollutant doses. A second characteristic of the intermediate clones was the fact that the variability of symptom expression was great, for in each treatment there was a large number of clones that were not significantly different. These six intermediate clones comprised the majority of this group.

The visual response of the individual 12 clones in each of the seven treatments also tended to be somewhat different. At 14 days post treatment, the major visual differences for each of the 12 clones were as follows:

Clone I-1: The O_3 -10 + NO_2 -30 and NO_2 -10 treatments were the most phytotoxic. In five of the seven treatments (NO_2 -10, NO_2 -30, O_3 -10 + NO_2 -10, O_3 -10 + NO_2 -30, and no pollutant) chlorotic banding and necrotic tipburn usually were the dominant symptom types observed. Necrotic tipburn usually was the more dominant of the two. The two

O_3 alone treatments, however, did not follow this pattern. As stated previously, this clone was unexpectedly tolerant of the O_3 -30 treatment where only 4% of the needle fascicles exhibited any injury which included chlorotic spot, mottle, and band. In the O_3 -10 treatment, 23% of the needle fascicles exhibited injury with chlorotic mottle dominating over chlorotic band (19% vs. 4%).

Clone I-2: the NO_2 -10 and O_3 -10 + NO_2 -10 treatments were the most phytotoxic. In five of the seven treatments (O_3 -10, NO_3 -10, O_3 -10 + NO_2 -10, O_3 -10 + NO_2 -30 and no pollutant), the incidence of necrotic tipburn ranged from 12% of the needle fascicles in the NO_2 -10 treatment to 4% in both the O_3 -30 and O_3 -10 + NO_2 -30 treatments. In all five of these treatments either chlorotic band or chlorotic mottle was also associated in at least equal incidence. In the remaining two treatments O_3 -10 and NO_2 -30, chlorotic mottle was the dominant symptom type.

Clone I-3: The two pollutant combination treatments and the two NO_2 alone treatments were responsible for the most injury with this clone. Necrotic tipburn was observed in three of these treatments: O_3 -10 + NO_2 -10, NO_2 -10, and NO_2 -30. In the former two, chlorotic spot, mottle, and band were also present while no other symptoms were observed in the latter. In the remaining three treatments very light amounts of chlorotic spot and mottle were observed.

Clone II-1: The O_3 -30 and O_3 -10 + NO_2 -30 treatments were the most phytotoxic. In the former, chlorotic mottle was the only important symptom, while in the latter, chlorotic spot and chlorotic band

dominated. In the other five treatments very light amounts of the three aforementioned symptoms were observed.

Clone II-2: In five of the seven treatments (O_3 -30, O_3 -10, NO_2 -30, NO_2 -10, and no pollutant), chlorotic mottle was the dominant symptom in some cases only slightly more prevalent than chlorotic spot. Necrotic tipburn was observed in only the NO_2 -30 treatment and thereby was the most phytotoxic.

Clone II-3: A very slight amount of necrotic tipburn was observed in five of the seven treatments and accounted for the dominant symptom in these treatments. In the remaining two treatments, O_3 -10 + NO_2 -30 and O_3 -30, only the latter accounted for any appreciable injury which was in the form of chlorotic mottle. The three most phytotoxic treatments were: O_3 -10 + NO_2 -10, O_3 -30, and NO_2 -30 while the no pollutant accounted for the least injury.

Clone III-1: Injury was observed in only the O_3 -30 treatment. Chlorotic spot was the dominant symptom followed by slight chlorotic mottle and banding.

Clone III-2: Three treatments (O_3 -30, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30) were responsible for the majority of the injury. In each case, necrotic tipburn was observed although to a limited extent while the majority of the injury was reported as either chlorotic mottle or banding. Chlorotic mottle dominated only in the O_3 -10 + NO_2 -10 treatment. Very sparse necrotic tipburn was also observed in the no pollutant treatment. Chlorotic banding was also observed in the NO_2 -10 treatment while very little injury was observed in the other two treatments.

Clone III-3: Three treatments (O_3 -30, O_3 -10 + NO_2 -30, and O_3 -10) were responsible for all of the injury observed. Only in the O_3 -30 treatment was any appreciable injury consisting of chlorotic mottle observed.

Clone IV-1: The O_3 -30 treatment was responsible for the great majority of the injury which consisted of sparse necrotic tipburn and chlorotic mottle at the ratio of 1:2.

Clone IV-2: The O_3 -30 treatment was again responsible for the great majority of the injury which consisted primarily of chlorotic mottle. Necrotic tipburn accounted for all the injury observed in the two treatments: O_3 -10 + NO_2 -30 and no pollutant.

Clone IV-3: In the three treatments where large amounts of injury were observed (O_3 -10 + NO_2 -10, O_3 -10 + NO_2 -30, and NO_2 -10), necrotic tipburn and/or chlorotic banding accounted for the majority. In the O_3 -30 treatment light necrotic tipburn was observed however, chlorotic spot and mottle dominated.

Needle Growth Impact

The seven treatments used in this study had an obvious effect on the needle growth of the sample population of eastern white pine clones tested as shown in Table 8. The analytical technique used to demonstrate this effect was analysis of covariance where the needle lengths in mm at 11 weeks post treatment were adjusted for the pretreatment lengths. This enabled ramets to be compared even though their initial needle lengths were not equal. It should be noted however, that the needle growth analysis suffered from the fact that the days of

Table 8. Analysis of covariance table for the mean needle length of eastern white pine at 11 weeks post-treatment adjusted for the pretreatment mean needle length.

| Source | df | ss | F |
|---------------------|------|------------|--------------------|
| Model | 169 | 821,474.31 | 108.8** |
| Clones | 11 | 100,607.18 | 27.71** |
| Trt ^a | 6 | 29,053.15 | 14.67** |
| Clones X Trt | 66 | 25,355.86 | 1.16 ^{ns} |
| Error a | 85 | 28,053.34 | |
| NL Pre ^b | 1 | 200,976.53 | |
| Residual | 3182 | 142,187.09 | |
| Total | 3351 | 963,661.40 | |

^aTrt = treatment

^bNL Pre = needle length pretreatment (covariate)

** = Significance at the .01 level

ns = not significant

NOTE:

1) The model used in this analysis was:

$$Y_{ijk} = \mu + C_i + T_j + (CT)_{ij} + D_k(CT)_{ij} + B(X_{ijk} - \bar{X}) + E_{ij}$$

where:

Y = mean needle length post treatment (i = clone, j = treatment, k = day);

μ = overall mean;

C_i = effect of the i-th clone (i = 1, ..., 12);

T_j = effect of the j-th treatment (j = 1, ..., 7);

$(CT)_{ij}$ = component for the interaction of clones and treatments;

$D_k(CT)_{ij}$ = component for day nested within the clone x treatment interaction (Error a);

$B(X_{ijk} - \bar{X})$ = covariate component (NL Pre);

E_{ijk} = residual component.

2) The variables clone, trt, and clone x trt were tested against Error a which was the day within clone x trt interaction component.

treatment were confounded with treatments, and was therefore useful only in a limited context.

Both the clone and treatment effects were highly significant (.01 level) while the interaction effect was nonsignificant. The O_3 -30 treatment appeared to stimulate the needle growth of the ramets (Table 9) although there was not a significant difference between the mean needle lengths of the 12 clones in this treatment and that of the no pollutant and O_3 -10 treatments (66.0 mm vs 64.1 and 63.9 mm respectively). The greatest negative needle growth impact was observed in the NO_2 -10, O_3 -10 + NO_2 -10 and O_3 -10 + NO_2 -30 treatments where the mean needle lengths (58.2, 58.7 and 58.7 mm respectively) were not significantly different from each other. However, these three treatments were significantly less than that of the remaining four treatments, including the no pollutant.

In Table 10, the mean needle lengths of the 12 clones averaged over the seven treatments are compared. Three of the four tolerant clones (III-1, IV-2, and III-3), as identified by MSSSI analysis presented previously, had consistently longer needle growth while the two sensitive clones (I-1 and I-2) exhibited significantly reduced needle growth. Clone IV-1 had the longest needles of the 12 clones tested having a mean needle length of 75.2 mm which was significantly longer than that of any other clone. Clones III-1 and IV-2 ranked second and third with 67.6 and 65.5 mm, respectively. Clone III-3 was the only clone previously ranked as tolerant that was not among the leaders in mean needle length, ranking seventh with 61.2 mm. Clones I-1 and I-2 produced the shortest needles of the 12 clones

Table 9. Mean needle length of all 12 clones of eastern white pine at 11 weeks post treatment by treatment.^a

| Treatments (Conc. in PPHM) | Mean Needle Length (mm) |
|--|----------------------------|
| O ₃ - 30 | 66.0 a* |
| No pollutant | 64.1 ab |
| O ₃ - 10 | 63.9 ab |
| NO ₂ - 30 | 62.6 b |
| O ₃ -10 + NO ₂ -10 | 58.7 c |
| O ₃ -10 + NO ₂ -30 | 58.7 c |
| NO ₂ - 10 | 58.2 c |

^aThe mean needle lengths 11 weeks post treatment were adjusted for the mean needle length at pretreatment through analysis of covariance.

*Means with the same letter are not significantly different at the 0.5 level.

Table 10. Mean needle length of each of the 12 clones of eastern white pine at 11 weeks post^a treatment averaged over all seven treatments.

| Class-Clone | Mean Needle Length (mm) |
|-------------|----------------------------|
| IV-1 | 75.2 a* |
| III-1 | 67.6 bc |
| IV-2 | 65.5 bcde |
| IV-3 | 64.4 cdef |
| I-3 | 63.9 def |
| II-3 | 63.0 efg |
| III-3 | 61.2 fgh |
| II-1 | 59.9 ghi |
| II-2 | 58.3 hij |
| III-2 | 56.9 ij |
| I-1 | 54.9 j |
| I-2 | 50.2 k |

^aThe mean needle length 11 weeks post treatment were adjusted for the mean needle length at pretreatment through analysis of covariance.

*Means with the same letter are not significantly different.

tested and were significantly different from each other having mean needle lengths of only 54.9 and 50.2 mm, respectively. Clone I-1 was not significantly different from the two clones (III-2 and II-2) immediately above. Clone III-2 was ranked as one of the more sensitive clones of the intermediate group as defined by MSSSI analysis and its ranking 10 of 12 in mean needle length would tend to substantiate previous ranking.

The clones previously ranked as intermediate in response to the pollutants used in the study remained in that category through needle growth analysis. The six clones had mean needle lengths ranging from 64.4 mm (Clone IV-3) to 56.9 mm (Clone III-2) as shown in Table 10.

Regression Analysis

Regression analysis was run on two treatments, O_3 -10 and O_3 -10 + NO_2 -30, initially using six independent variables: i) age, ii) candle type, iii) crown symptom severity class, iv) length of the one year old needles, v) length of the current year needles prior to treatment, and vi) length of the current year needles six weeks post treatment in an attempt to predict the SSI - the independent variable. The independent variable of age was eliminated from the equation for the O_3 -30 treatment as all of the ramets in this treatment were of equal age. However, as shown in Tables 11 and 12, the two equations were only able to explain 9.9% and 24.6%, respectively, of the variation (R^2 value) in the SSI's observed. Therefore, since these independent variables were unable to explain at least 50% of the variation, no further analysis were attempted.

Table 11. Analysis of variance table for the regression analysis on six independent variables as they affect the symptom severity index for ramets from the O_3 -10 pphm + NO_2 -30 pphm treatment.

| Source | df | ss | MS | F | R ² |
|----------------------|-----|-----------|----------|--------|----------------|
| Regression | 6 | 352722.0 | 58787.0 | 6.23** | 0.249 |
| NL1 ^a | 1 | 188611.8 | 188611.8 | 20.0** | |
| Age | 1 | 73518.4 | 73518.4 | 7.8** | |
| Candle ^b | 1 | 2469.5 | 2469.4 | 0.3 | |
| NL Pre ^c | 1 | 45203.9 | 45203.9 | 4.8* | |
| NL Post ^d | 1 | 41332.4 | 41332.4 | 4.4* | |
| Class ^e | 1 | 1586.1 | 1586.1 | 0.2 | |
| Residual | 113 | 1066085.8 | 9434.4 | | |
| Total | 119 | 1418807.9 | | | |

^aLength of the one year old needles

^bCandle type

^cLength of the current year's needles prior to fumigation

^dLength of the current year's needles six weeks post fumigation

^eSymptom severity class of the ramets: I, II, III or IV

*Significant at the .05 level

**Significant at the .01 level

Table 12. Analysis of variance table for the regression analysis on five independent variables as they affect the symptom severity index for ramets from the O_3 -30 pphm treatment.

| Source | df | SS | MS | F | R ² |
|----------------------|-----|----------|---------|-------|----------------|
| Regression | 5 | 33623.3 | 6724.7 | 2.49* | 0.099 |
| NL1 ^a | 1 | 1650.7 | 1650.7 | 0.61 | |
| Candle ^b | 1 | 689.3 | 689.3 | 0.26 | |
| NL Pre ^c | 1 | 18012.5 | 18012.5 | 6.68* | |
| NL Post ^d | 1 | 11363.2 | 11363.2 | 4.22* | |
| Class ^e | 1 | 1907.6 | 1907.6 | 0.71 | |
| Residual | 113 | 304621.3 | 2695.8 | | |
| Total | 118 | 338244.6 | | | |

^aLength of the one year old needles

^bCandle type

^cLength of the current year's needles prior to fumigation

^dLength of the current year's needles six weeks post fumigation

^eSymptom severity class of the ramets: I, II, III, or IV

*Significant at the .05 level

DISCUSSION

The specific objectives of this study were: i) to determine the relative sensitivity of the 12 clones of eastern white pine from white pine stand #2 at the RAAP to O_3 and NO_2 , both singly and in combination, and ii) to develop efficient propagation and maintenance techniques suitable for the production of a large number of eastern white pine ramets for use in a pollutant sensitivity screening program. Both of these objectives were achieved.

The side veneer grafting technique utilizing proper post grafting environmental conditions resulted in a maximum survival rate of 90.4%. The sensitivity rankings of the 12 clones were confirmed using two independent rating systems: i) visual symptoms as measured by the symptom severity index (SSI) and ii) growth impact as measured by needle elongation. During the course of the study, what was believed to be heat injury was observed on ramets from the NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30 treatments affected the results somewhat. Because the ramets of all treatments were not subject to the high temperatures at the same time relative to treatment, comparisons between treatments were severely biased. However, comparisons between clones within treatments were possible.

Vegetative Propagation

Vegetative propagation (grafting or rooting) is the only method available for the production of large quantities of homogeneous plant material. Since rooting is known to be an inefficient method for the

propagation of eastern white pine (93), grafting was the logical choice for the production of large numbers of eastern white pine. However, the survival rate of the ramets in a grafting program is dependent on a number of factors including the time of the year the scion is collected, the age of the scion, the grafting technique used, and the post grafting environmental conditions in which the new grafts are maintained (29). As shown in Table 3, there was a large difference in the survival rate of the ramets grafted over the three periods. Of the three periods, the second had a substantially higher survival rate than either the first or the third periods (90.4% vs 56.7% and 65.9%, respectively). The scions used in the first two grafting periods were collected March 1-5, 1976 when the new buds were still tight. However, the scions for the third period were collected five weeks later when the buds had begun to swell. The substantial increase in the survival rate observed in the second grafting period (March 30 to April 6) over the first was probably due to a refinement of grafting technique. During the first grafting period good grafting techniques were not followed. In the subsequent two periods only three people were involved in the grafting. By this time considerable expertise had been acquired and close attention was paid to the maintenance of proper grafting techniques. The scions for the third grafting period were collected as ortets were initiating growth. The terminal buds of these new grafts were therefore ready to begin candle extension as soon as conditions were again favorable. This rapid candle extension, initially utilizing the stored energy

in the bud, required that the graft union be functioning at an earlier stage than those ramets in the previous two grafting periods. Thus environmental conditions had to be ideal for sufficient callus tissue to form in time to insure normal growth of the ramet. This advanced stage of scion development, as compared to those used previously, may have been responsible for some of the reduced ramet survival rate in the third grafting period.

The survival of newly grafted material is dependent on the formation of adequate quantities of callus tissue between the scion and rootstock. The rootstock accounts for the majority of the callus tissue produced. The callus tissue is composed of thin walled, turgid parenchyma cells which are subject to easy dehydration and death if optimum conditions for the growth of callus tissue are not maintained (29). These cells are heat and moisture labile whereby any fluctuation from optimum environmental conditions results in the death of the callus tissue and ultimately, grafting failure. For optimum callus tissue growth, the temperature must range from 13.8° C to 32.0° C depending on the species (29). In apple (Malus sp.), death of this tissue is known to occur at temperatures greater than 40.0° C. In addition, a high relative humidity must be maintained throughout the healing (callusing) process. Wax covered wrappings aid in this process but the first two weeks after grafting is the most critical period (29). Newly grafted material, therefore, should be placed in a mist system through this critical period as the presence of a water film over the grafting union greatly aids in reducing dessication.

On May 22 and 23, 12 days after the end of the third grafting period (May 6-11), temperatures in the greenhouse reached at least 35.5° C and 36.0° C, respectively (Figure 4). On May 31 and June 1 the ramets were not under the mist system and temperatures were again excessive in the greenhouse, reaching at least 37.7° C and 34.4° C, respectively. In both of these two day periods, the temperatures were above that for optimum callus tissue growth and approaching the threshold for tissue death as reported for apple. Although the exact timing of the ramet necrosis was not recorded, it was noted that the great majority of the necrosis occurred when the candles of the ramets had elongated to two to three inches. This was in contrast to the first two grafting periods where ramet necrosis was observed before the candles ever began to elongate. Ramet necrosis associated with elongated candles was probably a result of the two periods of high temperature in the greenhouse, and if these could have been avoided, the survival rate of the third grafting period would most probably have been comparable to that of the second.

The overall ramet survival rate (71.2%) achieved during the course of this study was comparable to what had been reported by Linzon (45), Berry (3, 6), and Dochinger (20). However, all of these researchers utilized field grafting techniques using needle blighted and healthy eastern white pine. Linzon (45) reported an overall survival rate of 56.3% after one growing season based on a total of 80 grafts (64 grafts were made with the bottle graft technique and 16 with the crown grafting technique). In one series of 16 grafts using the bottle graft technique, a survival rate of 87.5% was achieved. In this group the

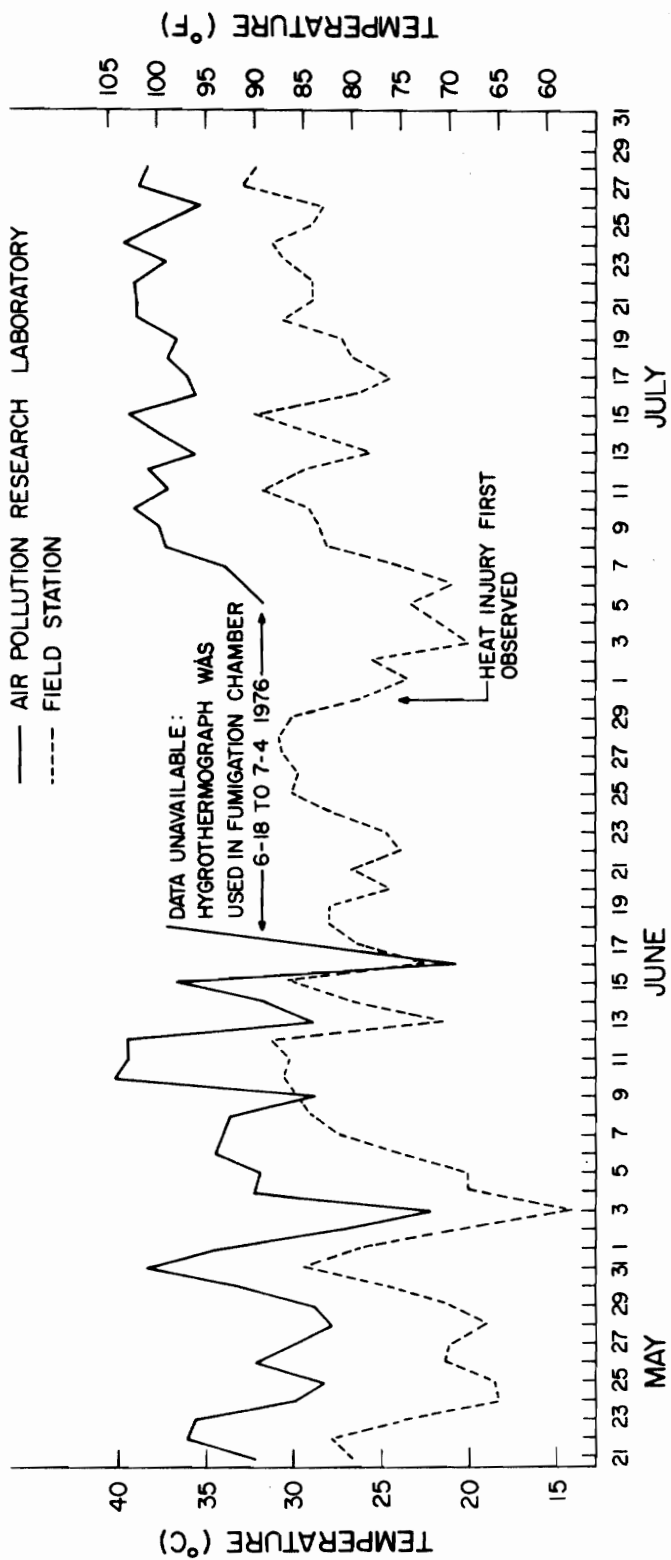


FIGURE 4. MAXIMUM DAILY TEMPERATURES IN THE CHARCOAL FILTERED GREENHOUSE AT VPI&SU AIR POLLUTION RESEARCH LABORATORY, BLACKSBURG, VIRGINIA AND A FIELD STATION LOCATED 2.2 km WEST OF THE LABORATORY FOR THE PERIOD MAY 21 TO JULY 28, 1976.

scion and field stock had just begun "active top growth" when the grafts were made and after two years 75.0% remained viable. All of the mortality in this case was accounted for by grafts where needle blighted scion was used. Similar results were obtained using the cleft crown grafting technique with a sample size of only four. Berry utilized the side cleft grafting technique, and in two studies, reported a 63.6% (6) and 62.9% (3) survival rate based on 55 and 70 total grafts, respectively. In these two studies, higher graft mortality was associated with both needle blighted scion and stock, whereas Linzon (45) reported reduced ramet survival rates predominantly with needle blight scion. Dochinger and Seliskar (20) reported the results of a grafting program where a total of 220 grafts involving healthy and chlorotic dwarf eastern white pines were made. After one growing season a 75.0% overall survival rate was achieved. However, unlike the results of Linzon and Berry, Dochinger and Seliskar reported that the highest survival rate was observed in the group where chlorotic dwarf was used as both scion and stock (90.0%), followed closely by the group using healthy scion and chlorotic dwarf stock.

When comparing the results obtained by these three researchers to those of the study being reported here, several similarities appear. Generally speaking, a reduced survival appeared to be related to the use of needle blighted scion (Class I and II in this study). Grafting should be undertaken during the spring of the year, preferably just prior to the initiation of terminal growth by the scion and when the stock material is actively growing. The results obtained in this study showed that the survival rate of eastern white pine grafts can

be high. This can be achieved if the conditions under which newly grafted ramets are maintained for the two weeks immediately after grafting are optimum for the growth of the callus tissue (high relative humidity and moderate temperatures). This was illustrated by the results of the second grafting period (Table 3) where the overall ramet survival rate was high (90.4%) even for sensitive clones.

Symptom Development and Pollutant Related Injury

Based on results obtained by other investigators (13, 25, 35, 38, 75, 80, 90), the injury observed during the course of this study was excessive. At the onset it was expected that the O_3 -30 treatment would be the most phytotoxic. The NO_2 -10 and the NO_2 -30 treatments were expected to have little if any effect on eastern white pine based on research conducted by Van Haut (90), Tingey et al. (80), Middleton (53), and others (36, 74, 77). Of these studies only one (90) even dealt with coniferous species and eastern white pine was not among the species tested in that study. Tingey et al. (80) reported the lowest phytotoxic concentration of NO_2 . At least 200 pphm NO_2 for four hours was required to cause even light chlorosis on only one of the six crop species used in the study. In addition, an $NO_2 + SO_2$ synergism was demonstrated at concentrations as low as 5 pphm of each pollutant for four hours. White et al. (92), investigating the effect of $NO_2 + SO_2$ on the apparent photosynthesis of alfalfa, also reported an $NO_2 + SO_2$ synergistic effect. At 15 pphm of each pollutant for up to four hours, a 7% reduction in apparent photosynthesis was observed while no effect was observed with the two pollutants singly. Therefore, based on these

results, NO_2 when in low concentrations and in combination with other pollutants (at least SO_2 and probably others), had a demonstrated capability to elicit a synergistic response both physically and physiologically within some sensitive plant species.

At the initiation of this study, the sensitive clones of eastern white pine were expected to be sensitive to $\text{O}_3 + \text{NO}_2$ fumigations although the overall injury was expected to be light (chlorosis but no necrosis). Several clones were significantly injured in both the single pollutant NO_2 fumigations and the combination ($\text{O}_3 + \text{NO}_2$) fumigations. However, in all four cases, necrotic tipburn accounted for from 35% to 43% of the total injury observed in these treatments (Table 4). Thus even though the overall response was generally as expected, the severity of the injury was greater than expected considering the pollutant concentrations utilized (10 and 30 pphm of both O_3 and NO_2).

Chlorotic mottle has been widely recognized as an important air pollutant symptom on eastern white pine since 1905 (9, 18) and it was also observed in this study. The symptom was consistently associated with injury on the clones used in this study, and was the most prevalent in the one treatment where the highest percentage of fascicles were injured - O_3 -30. Therefore, chlorotic mottle was the best diagnostic indicator of pollutant injury observed.

The general symptoms observed in the O_3 -30 treatment (page 75) were similar to those reported by Linzon (44) and Costonis (13), and very close to what was expected. As shown in Table 4, this treatment was the only one where no necrotic tipburn was observed at the two day evaluation, and only 1% and 2% at the subsequent seven and 14 day

evaluations, respectively. Chlorotic mottle was the most prevalent symptom observed in this treatment. The O_3 -30 treatment also produced expected results although more necrotic tipburn was expected especially on the more sensitive clones.

The results of the NO_2 -10 and NO_2 -30 treatments were exactly opposite of what was expected, as the NO_2 -10 treatment caused more injury than did the NO_2 -30 treatment (MSSI's of 369.7 versus 170.3 respectively - Table 8). However, in the NO_2 -10 treatment, the majority of the injury observed was necrotic tipburn which was associated with chlorotic banding. This was in contrast to the NO_2 -30 treatment where the degree of necrotic tipburn equated to that of the chlorotic mottle, while no chlorotic banding was observed in this treatment after 14 days. In those treatments where necrotic tipburn was associated with chlorotic banding, pretreatment injury due to high temperatures was also observed. The timing of the onset of the pretreatment symptoms affected only certain treatments as was shown in the analysis of the ANOVA results. The injury observed in the NO_2 -10 and NO_2 -30 treatments relative to each other tend to indicate the presence of an external effect.

The same phenomenon occurred in the two pollutant combination treatments as in the two NO_2 treatments: the theoretically less phytotoxic treatment (O_3 -10 + NO_2 -10) of the two, was responsible for more injury when measured by total percent of fascicle injured (Table 4). As was the case above, the O_3 -10 + NO_2 -10 treatment produced relatively more necrotic tipburn and chlorotic band than the O_3 -10 + NO_2 + 30 treatment, again implicating "heat injury" via association. However,

the injury produced by these two treatments ranked first and second (1 = most severe injury) out of seven at the two day evaluation, and second and third at the 14 day evaluation based on the total percent fascicles injured (Table 4). (See page 107 for additional discussion)

Necrotic tipburn has been reported by numerous researchers as a characteristic symptom of O_3/SO_2 injury on sensitive genotypes of eastern white pine but never to the degree observed in this study. As stated previously, necrotic tipburn was the most prominent symptom type observed during the two and seven day evaluation periods (Figure 3) accounting for 52.9% and 43.6%, respectively, of the total injury observed (Table 4). As shown in Appendix: Tables 3-9, necrotic tipburn was also present in clones from classes III and IV (tolerant) treatments in both the lower pollutant concentration and no pollutant treatments (NO_2 -10, O_3 -10, O_3 -10 + NO_2 -10, and no pollutant), e.g., in the no pollutant treatment (Appendix: Table 3) clones I-1 and I-2 exhibited 26% and 10% respectively necrotic tipburn while clones II-3, III-2, III-3, and IV-3 each exhibited 1%. In addition, there was no consistent gradation in the incidence of necrotic tipburn observed over the seven treatments used in that, as discussed previously, the theoretically more phytotoxic treatments did not have the higher percentage of injury.

Linzon (44) reported that some degree of necrotic tipburn was observed in 19% of the trees in a naturally seeded stand of eastern white pine in Canada. Baldwin (1) reported a 1.1% and 3.4% incidence of white pine needle blight in two plantations in New Hampshire. Houston (38) fumigated five different sensitive and tolerant clones

of eastern white pine in each of three different O_3 -concentrations (10, 30 and 60 pphm) for six hours. Overall, 30% of all of the clones fumigated exhibited some degree of injury consisting primarily of very light necrotic tipburn. Phillips (58), working in the same stand at the RAAP from which the ramets used in this study were obtained, rated 31% of the eastern white pine present as having some necrotic tipburn. In the study being reported here, necrotic tipburn was associated with all of the 12 clones (100%) tested at some time during the study. Thus the observance of necrotic tipburn in all 12 clones (three of which were field rated as being healthy/symptomless) used in this study may not be attributed solely to the pollutant doses used.

Chlorotic banding was described by earlier researchers (35, 44) as being intermediary in the development process whereby necrotic tipburn was the ultimate symptom. In this study, chlorotic banding was usually associated with necrotic tipburn and found primarily in those treatments where heat injury occurred. The O_3 -30 treatment was the one exception to this pattern where chlorotic banding was observed without at least equal quantities of necrotic tipburn. This may have been the only instance where chlorotic banding was solely the product of the pollutant dose used and thus would be consistent with the findings of previous investigators.

Statistical Analysis of Symptom Severity Index Data

The results of the ANOVA tests for each of the individual seven treatments (Table 5) correlated well with observed pretreatment

symptoms. In the three treatments where pretreatment symptoms were observed (NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30), significant day x clone interactions were found at some time during the three evaluations. Thus even though the results of the ANOVA tests did not indicate what the nature of the interaction was, they did correlate well with the treatments where pretreatment symptoms were observed.

At the 14 day evaluation period, four of the seven treatments (O_3 -10, NO_2 -10, O_3 -10 + NO_2 -10, and O_3 -10 + NO_2 -30) showed significant clone effects. However, the NO_2 -10 treatment also showed a significant day x clone interaction effect. Therefore care must be taken in the interpretation of the significant single factor effect (clone) which was present in the significant interaction (day x clone). The significant day effect in the NO_2 -10 and O_3 -10 + NO_2 -30 treatments was attributed to either changing environmental conditions within: i) the exposure chamber during treatment or ii) the greenhouse during the pre or post treatment periods to the extent that symptom expression was significantly affected.

Heat Injury

The developmental progression of symptoms observed on ramets of eastern white pine, closely paralleled that which had been reported previously (13, 35, 44) with regard to chlorotic mottle and to a lesser degree, necrotic tipburn. Necrotic tipburn was the primary indicator of what was thought to be heat injury as observed at the pretreatment evaluation. As discussed previously, there were certain subtle differences between pollutant caused necrotic tipburn and that believed to

have been caused by high temperatures. These differences were obvious primarily during the initial stages of symptom development. There were probably pre-necrotic heat related symptoms on the needles of the eastern white pine. However, no distinguishing characteristics were found that would enable the conclusive separation of those caused by pollutants and those caused by "heat" during the later stages of necrotic symptom development. In 1936 Shirley (63) reported results from tests with eastern white pine and three other coniferous species in a heat chamber. Needles of eastern white pine were killed (necrotic) after five hours exposure to 47.8° C in high relative humidity (85%). No mention was made of pre-necrotic symptoms and in fact, accurate symptom descriptions were totally lacking.

During the course of this study the temperatures within the greenhouse used to house the ramets before and after fumigation became extremely high (Figure 4). However, during the period of time when the fumigations were being conducted, the recording hygrothermograph normally stationed in the greenhouse was used inside the fumigation chamber. Therefore, greenhouse temperature data for that period was not available. By comparing maximum daily temperature recorded in the greenhouse with those recorded at a field station located 2.2 km west of the laboratory for the periods before and after the fumigation dates, an approximation of the maximum temperatures in the greenhouse for the period in question can be made. As shown in Figure 4, for the period June 10 to June 12, 1976, the maximum daily temperature at the field station ranged from 30.5° C to 31.5° C while the corresponding temperatures in the greenhouse ranged from 39.5° C to 40.25° C. This

same trend of an approximate temperature differential of 10°C generally held true in the period July 11 to July 28. Thus it can be assumed that the maximum daily temperatures in the greenhouse for the three day period of June 27, 28, and 29 ranged from at least 39°C to 40°C . These estimates are thought to be conservative for the following reasons: i) the maximum recording temperature of the hygrothermograph in question is 43.3°C and the temperatures in question are above this range; ii) the response time of the instrument was slow at temperatures above 38°C . This would indicate that during the time in question (July 27-29, 1976) the temperature in the greenhouse could have reached 45°C for short periods of time.

The first "heat injury" observed in this study was in the two day evaluation of the first fumigation of the NO_2 -10 treatment on the evening of June 30. Thus there were no symptoms observed on these ramets when they were evaluated on the morning of June 28. The onset of "heat injury" was therefore confined to this three day period -- June 27, 28, 29. On those three days, the two NO_2 -10 fumigations and the first fumigation of the O_3 -10 + NO_2 -30 treatment were being conducted (Table 2). The maximum temperatures in the greenhouse during that period were at least 40°C and probably higher. On June 30 the maximum temperature in the greenhouse fell to an estimated 36.5°C and July 1 was cooler yet (est. 33°C), while July 2 was intermediate (est. 35.5°C) between the two previous days (all greenhouse temperatures given for the period 6-18 to 7-4 - 1976 are estimates of the actual temperatures based on the rationale discussed above). Fumigations during the period June 27-29 were begun at 0830 which meant that the

ramets were back in the greenhouse by 1430 -- in time to be subjected to the hottest temperatures of the day. Of the three treatments conducted during the period June 27 to July 2 (NO_2 -10 on June 27, 28; O_3 -10 + NO_2 -30 on June 29, 30; and O_3 -10 + NO_2 -10 on July 1, 2), all three exhibited very high MSSI's at the 14 day evaluation period (Table 7) and ranked fourth, first and second, respectively, out of the seven treatments as measured by MSSI. When these same treatments were analyzed according to the total percent of fascicles injured (Table 4), the rankings were fourth, third and second, respectively. Thus, due to the method in which the MSSI was calculated, the rankings of the O_3 -10 + NO_2 -10 and O_3 -10 + NO_2 -30 treatments were reversed when compared to that of the total percent of fascicles injured data. Therefore even though there were more fascicles injured in the O_3 -10 + NO_2 -10 treatment, the severity of the injury was greater in the O_3 -10 + NO_2 -30 treatment. The majority (62.8%) of the total MSSI for the O_3 -10 + NO_2 -30 treatment was carried by only two clones: I-1 and IV-3 which had 30% and 20%, respectively, of their needle fascicles exhibiting necrotic tipburn.

From the above line of thought, it is believed that the increasing injury associated with the three treatments, NO_2 -10, O_3 -10 + NO_2 -30, and O_3 -10 + NO_2 -10, was due to an external effect, probably the high temperatures in the greenhouse. It appeared that the predisposition of the ramets in the O_3 -10 + NO_2 -10 and O_3 -10 + NO_2 -30 treatments during the June 27-29 hot period may have been responsible for the overall increased injury in those treatments when compared to that

of the other five treatments. In addition, the timing of the high temperatures probably had a further confounding effect on the ultimate visible injury because some ramets were exposed to the heat after treatment (O_3 -30, NO_2 -30 and one of the two no pollutant treatments), others were exposed during treatment (NO_2 , and one of the two O_3 -10 + NO_2 -30 treatments), while the remaining treatments were exposed prior to treatment. During the July 15-17 treatment period (O_3 -10 on July 15, 16 and no pollutant on July 17) the maximum temperatures in the greenhouse ranged from 39.5° C on July 15 to 36.0° C on July 17. These temperatures were also excessive, especially on July 15, however, there was not as much injury observed in the O_3 -10 treatment as there was in the previously discussed three treatments. Ramets for the July 15-17 treatments were the only ones used from the third grafting group. During the ramet selection process only those ramets relatively free of necrotic tipburn and chlorotic mottle were used. This selection criterion, therefore, selected for relative heat resistant ramets -- ones with sufficient callus tissue to insure "apparently normal" growth.

Clonal Response

Eastern white pine is known for its genetic heterogeneity (90) which causes a variation in pollutant response. The twelve clones of eastern white pine tested proved to have a diversity of responses to the pollutant doses used in this study. However, the overall injury was light. The highest MSSSI was only 208.7 out of a

maximum of 700 by clone I-1 in the O_3 -10 + NO_2 -30 treatment while the overall mean MSSI for all 74 clone-treatment combinations excluding the no pollutant treatment, was only 25.7. Thus, based on the findings from these 12 clones and under these experimental conditions, it appears that the eastern white pine genotypes that were very sensitive to O_3/NO_2 may have been removed from the ecosystem at the RAAP.

As was shown in Appendix: Table 9, the maximum percentage of needle fascicles exhibiting necrotic tipburn from the O_3 -30 treatment was 5% in clone III-2 while eight of the 12 clones exhibited no necrotic tipburn. When Houston (38) fumigated five tolerant and five sensitive clones of eastern white pine with the same O_3 dose as used in this study, none of the tolerant clones were injured while three of the five sensitive clones were. In the three injured clones at least 50% of the current year's needles were injured which in all cases consisted of necrotic tipburn. Thus in Houston's study, when O_3 sensitive clones were found, the injury was extensive and severe. If Houston's data was converted to the SSI system as used in this study, the SSI for each of the three injured clones would be 350 while the MSSI for that treatment would be 105. These figures are substantially above the values found for the most phytotoxic treatment used in this study and add further evidence to support the hypothesis that the eastern white pine genotypes that were very sensitive to O_3/NO_2 may have been removed from the RAAP ecosystem.

Clone I-1 was ranked either first or second (1 = most severe injury) in six of the seven treatments but ranked twelfth in the

O_3 -30 treatment. The two fumigations making up the O_3 -30 treatment were the first two conducted in the study (June 22, 23 - Table 2). The ramets used in these two fumigations were randomly selected from an even aged group of ramets from the second grafting period and as such, represent that group of ramets whose graft unions had probably most efficiently healed. Therefore, those ramets of clones I-1 used in the O_3 -30 treatment were vigorously growing and as such were possibly more tolerant of the O_3 than the ramets used in the remaining treatments. In addition, a contributing factor could be that this particular clone was rendered tolerant to that relatively high dose of O_3 because of its genotype. This may have acted either physiologically by slightly altering the reaction sites for O_3 molecules in the cell membranes, or physically by allowing the stomates to close upon stimulation by the high concentration of O_3 (82). In either case, the response was uniform and warrants further investigation.

Needle Growth Impact

Houston (38) reported a significant negative growth impact on the needle growth of eastern white pine by SO_2 and $SO_2 + O_3$ but not by O_3 alone. The SO_2 concentrations that achieved this were as low as 2.5 pphm for six hours, while the $SO_2 + O_3$ concentrations were 2.5 pphm and 5.0 pphm, respectively, also for six hours. In all cases the impact on the sensitive clones was greater than that on the tolerant lines. In both of Houston's two O_3 fumigations which were comparable to those used in the study reported here, the total needle elongation of

the tolerant clones was slightly greater (though nonsignificant) than that of the no pollutant, while there was a slight but nonsignificant reduction in the sensitive lines. These results were similar to those achieved in this study. As shown in Table 9, the O_3 at the higher concentration appeared to stimulate needle elongation; showing a 1.9 mm increase over that of the no pollutant (66.0 mm vs 64.1 mm). This difference may have been caused by either a true O_3 stimulation of the needle growth or by the fact, as discussed previously, that since the O_3 -30 fumigations were conducted first, the ramets in the two fumigations of that treatment represented a group whose graft unions had healed most efficiently and thus had initiated growth earlier. This appeared to be the best interpretation of the data in light of the fact that when the adjusted mean needle lengths for each treatment were compared, in order of fumigation as shown in Table 2, a definite decreasing trend was observed as follows: i) O_3 -30 - 66.0 mm, ii) NO_2 -30 - 62.6 mm, iii) no pollutant - 64.1 mm, iv) NO_2 -10 - 58.2 mm, v) O_3 -10 + NO_2 -30 - 58.7 mm, vi) O_3 -10 + NO_2 -10 - 58.7 mm, and vii) O_3 -10 - 63.9 mm. The treatments conducted from June 22 to July 2 all used ramets from the second grafting period, while the second no pollutant treatment and the two O_3 -10 fumigations were conducted using the first ramets from the third grafting period which, based on previously discussed information, should have been the fastest growing ramets of that group. Therefore, the mean needle lengths for the O_3 -10 treatment should have been, and were not consistent with, the decreasing mean needle lengths of ramets selected from the end of the second grafting group. The ramets that initiated

candle extension first and therefore had the more efficient graft unions, were treated first, because of the selection process. Thus a bias was injected into the needle growth data. Even though the final lengths were adjusted for the initial starting lengths through analysis of covariance, it was probably not possible to totally isolate and adjust for the total potential growth loss of a ramet that was selected for one of the last fumigations from the second grafting group. If controls could have been run for every fumigation, which would have been the ideal situation, this problem could have been totally defined. However, the controls as planned were inadequate to provide such data. Although "heat injury" also probably had an impact, the primary force responsible for affecting the mean needle length data appeared to have been the bias from the ramet selection process. The three treatments where the shortest needle lengths were found ($O_3-10 + NO_2-10$, $O_3-10 + NO_2-30$, and NO_2-10) happened to be conducted through the highest temperature periods. In addition, these treatments received the last ramets from the second grafting period which also contributed to their short needle lengths.

The above discussion notwithstanding, there was a strong relationship between the clonal needle length and the MSSSI rating: the two clones (I-1 and I-2), determined to be the most sensitive through MSSSI analysis, also proved to have the least needle growth. These were followed very closely by the most sensitive clone in the intermediate group (III-2) which had the third shortest needles (Table 10). This trend also held for the three most tolerant clones (III-1, IV-1, and IV-2) in that they had the best needle growth. The bias due to the

ramet selection method affected comparisons between treatments but because all 12 clones were present in each treatment, the comparison of clones within a given treatment, or over all seven treatments as shown in Table 10, was valid. Therefore, no matter what impact the "heat injury" had on the results obtained in this study, the ranking of the clones as to their relative sensitivity was valid.

Costonis (13), among others, has shown that a relationship exists between needle length and air pollutant sensitivity where the higher the clone's sensitivity, the shorter its needles. This trend was also observed in this study as discussed above. The adjusted mean needle lengths as presented in Table 10, were a gross indicator of relative growth. However, the effect of the various pollutant treatments would have to be compared, in order to determine the true meaning of these data. Because between treatment means cannot be compared in the study reported here, the data presented in Table 10 more accurately reflect relative phenotypic variation rather than any specific treatment induced effect.

Regression Analysis

In order to predict the symptom severity index (dependent variable) for ramets in a given fumigation, the degree of variation among the independent variables measured must be normally distributed. In addition, if the distribution of the dependent variable is not normal, then accurate prediction of that parameter is impossible. Such was the case in the regression analysis run in this study. The SSI's were biased due to the "heat injury" but also, to a larger

degree, the differential physiological conditions of the ramets selected first in the program as compared to those selected later. This later problem also contributed to the excessive variability in several of the independent variables, primarily the needle lengths as discussed above and the candle type. All of these factors contributed to the inability of the equation used, to explain a sufficient amount (at least 50%) of the variation in the dependent variable using the R^2 statistic.

In future studies using these same ramets, the growth differences due to the grafting inefficiencies will be minimized and the same regression equation should prove to be a better predictor of ramet injury. However, other independent variables that might have to be added to the equation to enable the highest possible precision in the prediction of the SSI would be: i) concentrations of various metabolites, ii) concentrations of biochemicals related to membrane permeability; i.e., steroids, iii) stomatal frequency and iv) physical characteristics of the stomata including the size of the stomatal opening and substomatal cavity.

Eastern White Pine as an Air Pollution Bioindicator

A tremendous amount of research has been conducted to elucidate the full extent of the air pollution sensitivity of eastern white pine (1, 2, 9, 11, 15, 25, 28, 38, 47, 73). It is widely known for its broad genetic variability (91) and several researchers have used this as the founding concept to utilize the species as a gross air pollution bioindicator (9, 16). Clones of eastern white pine have been identified by various researchers that are selectively sensitive to particular

pollutant regimes, including fluoride (9), SO₂ (16, 47, 47), O₃ (9, 16, 44), and combinations of O₃ and SO₂ (17, 38). The validity of Costonis' work with SO₂ (16, 17) was at one time debated because of his reports that certain clones of eastern white pine were injured by SO₂ fumigation of as low as 2.0 pphm for four hours. However, in the interim, those findings have been collaborated by Houston (38) and this author in an as yet unpublished work. The important point is that there still exist very sensitive clones in the natural gene pool that are selective for particular pollutants. In areas where any of the phytotoxic gases have been present for extended periods, the eastern white pine genotypes sensitive to that pollutant have been eliminated and as the dose increases more and more phenotypes (those originally classified as intermediates) will also be eliminated (26, 43). As a general rule, the eastern white pine population, throughout its range in the heretofore thought to be unpolluted rural areas, is being subjected to elevated concentrations of oxidant air pollutants. This has been brought about by the increasing production of O₃ precursors (NO_x), primarily by motor vehicle exhaust and then the long distance transport of these precursors into rural areas (66).

The effects of O₃ and SO₂ on eastern white pine have been demonstrated (17, 24, 38, 47). The natural gene pool has certainly been reduced to a significant degree by the actions of these two pollutants both singly and in combination in and around SO₂ point sources (26, 43). As the nation is forced to switch power sources from cleaner burning fuels, such as natural gas and oil to coal, this

problem will increase in scope unless adequate pollution control measures are applied within a reasonable, economically feasible time period.

The effects of NO_2 singly or in combination with O_3 and SO_2 on eastern white pine had not previously been investigated. Kress and Skelly (41) were first to demonstrate a very significant growth loss in both tolerant and sensitive lines of loblolly pine and sycamore due to $\text{NO}_2 + \text{O}_3 + \text{SO}_2$ fumigations. These results were achieved in continuous 28 day fumigations (six hours/day) with concentrations of these three pollutants below the national air quality standards. Thus, under conditions where the pollutants are present in the ambient atmosphere, growth loss can occur. Background O_3 concentrations appear to be increasing over time in rural areas (66) and with that, the NO_x concentrations. The threshold for injury to sensitive clones of eastern white pine by $\text{O}_3 + \text{NO}_2$ may be as low as 10 ppm for each pollutant when in combination, here in the eastern U.S. (but it is likely to be higher). It has already been reported that average daily NO_2 concentrations in California, as much as 30-50 ppm, have been recorded. In the western U.S. the relative humidity is normally quite low thereby reducing plant injury. The potential for high NO_x and O_3 concentrations here in the relatively humid eastern U.S., of half that reported in California, is not totally out of the question. If these concentrations were achieved here in the eastern U.S., the potential threat of tremendously increased incidences of plant injury would be realized. The potential for high SO_2 concentrations is associated with point sources and with current control techniques,

should be easily confined to restricted areas. NO_2 concentrations are known to be as omnipresent as the automobile and unless automobile pollution control standards are achieved, NO_x concentrations will increase directly with the numbers of automobiles. As shown in Table 1, the number of motor vehicles have increased 226.3 fold in the U.S. since 1940 and 1.6 fold since 1965. Without NO_x pollution controls, either through the use of control equipment or better designs for the internal combustion engine, the future with regard to oxidant pollution is not very bright. The potential for increased NO_x levels here in the eastern U.S. is real and represents a much greater threat to plant ecosystems than even SO_2 , if synergistic interactions are conclusively proven with O_3 and NO_2 .

CONCLUSIONS

- I - Two of the 12 clones of eastern white pine tested (I-1 and I-2) were rated as being sensitive in the treatments used in this study. Four clones (III-1, III-3, IV-1, and IV-2) were rated as being tolerant while the remaining six clones (I-3, II-1, II-2, II-3, III-2, and IV-3) were rated as intermediate.
- II - These sensitivity rankings are generally consistent with Phillips' (58) field classifications of the sensitivity of the respective ortets and therefore substantiates Phillips' findings.
- III - Because the overall injury to the 12 clones was light (maximum MSSI value of 208.7 out of 700), it appears that these clones were not very sensitive to O_3/NO_2 under the experimental conditions used in this study.
- IV - Eastern white pine can be vegetatively propagated using the side veneer grafting technique in sufficient numbers to support an air pollution sensitivity screening program. The postgrafting environmental conditions appeared to be the critical factor in the determination of the ramet survival rate. High relative humidity and moderate temperatures produced the highest ramet survival rate during the course of this study.

SUMMARY

The objectives of this study were: 1) to rank 12 clones of eastern white pine from the Radford Army Ammunition Plant (RAAP) as to their sensitivity to ozone (O_3) and nitrogen dioxide (NO_2) both singly and in combination, and 2) to develop efficient propagation techniques for eastern white pine. The ultimate goal, of which this study was the initial step, is to establish an air pollution bio-indicator system at the RAAP using eastern white pine as the bio-indicator species.

Scions were collected during March and early April 1976 from various eastern white pine ortets growing at the RAAP in an uneven aged stand of mixed conifers and hardwoods. The scions were placed in plastic bags with moistened paper towels and stored at 5° C until needed for grafting. Three ortets were selected from each of four symptom severity classes which were defined as follows: Class I = greater than 25% of the crown exhibiting necrotic tipburn, Class II = less than 25% of the crown exhibiting necrotic tipburn, Class III = crown chlorotic but not necrotic, and Class IV = crown healthy/symptomless. Grafting was conducted in three approximately five day periods from early March to early May using the side veneer grafting technique. Scions for the first two grafting periods was collected in early March while that for the third was collected in early April. In all cases 2-0 eastern white pine rootstock was utilized. Immediately after grafting the ramets were placed in a greenhouse drawing charcoal filtered air until treatment was begun, roughly

eight weeks after initiation of the current year's growth. Only ramets from the second and third grafting periods were utilized in the experiment.

The study consisted of seven treatments as follows: 1) O_3 -10 pphm, 2) O_3 -30 pphm, 3) NO_2 -10 pphm, 4) NO_2 -30 pphm, 5) O_3 -10 pphm + NO_2 -10 pphm, 6) O_3 -10 pphm + NO_2 -30 pphm, and 7) no pollutant. Each treatment was duplicated once. Five different ramets from each of the 12 clones were present in each treatment (total = 60 ramets/treatment). Each treatment lasted for six hours and the environmental conditions during the 14 treatments which were conducted during June and July 1976 averaged: 26° C, 64% RH and 16 K. lux. All treatments were conducted in a modified open top chamber located inside the laboratory. The circular aluminum frame chamber was 3.1 meters (m) in diameter, 1.1 m high, and covered with polyvinyl chloride plastic. All ramets were returned to the greenhouse immediately after treatment. Ramets were evaluated for visible injury prior to treatment and then two, seven, and 14 days thereafter. Statistical analysis was based on the symptom severity index (SSI) which was calculated for each ramet by treatment and evaluation period. In addition, growth impact was assessed by comparing mean needle lengths measured prior to treatment to those 11 weeks later. Other parameters measured were the average length of the one year old needles, the length of the current year's candle prior to treatment, and the number of fascicles on the current years growth.

The overall ramet survival rate was 71.2%. Of the three grafting periods, the highest survival rate was achieved in the second period

(90.4%) followed by that of the third (65.9%) and the first (56.7%) periods. The low survival rate in the first period was caused by a lack of grafting expertise while that of the third was probably caused by high temperatures in the greenhouse. The healing process in grafts requires the formation of a large quantity of parenchymatous callus tissue between the scion and rootstock. Several periods of temperatures above 35° C were recorded in the greenhouse. These temperatures may have been responsible for the death of the callus tissue and ultimately the ramet.

Because what was believed to be heat injury was observed on ramets from at least three treatments, and because of experimental design, comparisons between treatments for a given clone were not possible. Analysis of the SSI data for clones within each treatment identified differential clonal responses and allowed the 12 clones of eastern white pine to be ranked.

Only one clone consistently exhibited differential sensitivity to the two pollutants used in the study. Clone III-2 was observed to be the most sensitive of the 12 clones tested in the O₃-30 pphm treatment while it ranked intermediate to tolerant in all others. Other than this one exception, sensitive and tolerant clones were generally consistent in their respective ranking throughout the study. Clones I-1 and I-2 were ranked as sensitive in the study while clones III-3, IV-1, and to a lesser degree clones III-1 and IV-2 were ranked as tolerant. The remaining six clones (I-3, II-1, II-2, III-2, III-3, and IV-3) were ranked as intermediate in

sensitivity to O_3 and NO_2 . These rankings were confirmed by both visible injury as measured by the MSSSI and growth impact analysis as measured by mean needle length of the current years needles. In the latter, analysis of covariance was utilized to adjust the mean needle lengths at 11 weeks post treatment by the mean needle lengths prior to treatments and to test for significant differences.

Regression analysis was run in an attempt to predict the symptom severity indices of ramets in a given treatment. However, R^2 of less than 50% were obtained, indicating that the independent variables measured in this experiment were unable to explain a sufficient amount of the variation in the SSI to make an accurate prediction equation.

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APPENDIX

TABLE 1. CHRONOLOGICAL LISTING OF FOUR PARAMETERS RELATED TO AIR POLLUTION EMISSIONS FOR THE PERIOD 1900 TO 1970 IN THE U.S. AND VIRGINIA.

| YEAR | POPULATION IN 1,000's ^a | | VALUE ADDED TO MANUFACTURED GOODS IN MILLIONS OF DOLLARS ^{b,8} | | PRODUCT OF ELECTRIC POWER USING FUELS BY PUBLIC UTILITIES IN 1,000,000 KWH ^c | | YEAR | MOTOR VEHICLE REGISTRATIONS IN 1,000's ^d | |
|------|---------------------------------------|-------|---|-----------------------------|---|--------|-----------|---|-------|
| | U.S. | VA. | U.S. | VA. | U.S. | VA. | | U.S. | VA. |
| 1975 | 212,000 | 5,052 | 405,225 (1973) ^e | 6,179.0 (1972) ^e | 1,612,605 | 34,078 | 1975 | 133,727 | 3,172 |
| 70 | 204,879 | 4,648 | 300,228 | 4,921.5 | 1,282,254 | 27,838 | 1970 | 108,407 | 2,577 |
| 65 | 194,303 | N/A | 226,940 | 3,573.6 | 861,341 | 22,577 | 1965 | 90,358 | 2,059 |
| 60 | 180,671 | 3,964 | 163,999 | 2,433.0 | 603,342 | 17,068 | 1960 | 73,869 | 1,534 |
| 55 | 165,275 | N/A | 135,023 | 1,827.0 | 433,678 | 8,493 | 1955 | 62,689 | 1,327 |
| 50 | 151,684 | 3,319 | 89,750 | 1,326.5 | 233,115 | 5,536 | 1950 | 49,162 | 984 |
| 45 | 139,928 | N/A | N/A | 1,051.6 (1947) ^e | 142,431 | 3,056 | 1945 | 31,055 | 607 |
| 40 | 132,122 | 2,678 | 24,487 | 376.3 (1939) ^e | 94,700 | 1,724 | 1941 | 34,894 | 583 |
| 35 | 127,250 | N/A | 18,553 | 247.0 | 56,915 | N/A | 1931-1940 | 278,294 | 4,257 |
| 30 | 123,077 | 2,422 | 18,601 | 366.4 ^f | 59,922 | N/A | 1921-1930 | 119,199 | 3,003 |
| 25 | 115,829 | N/A | 25,668 | 274.8 | 39,653 | N/A | 1911-1920 | 38,809 | 434 |
| 20 | 106,461 | 2,309 | 23,842 | 228.2 ^f | 23,644 | N/A | 1906-1910 | 1,230 | 7 |
| 15 | 100,546 | N/A | 9,386 | 108.7 (1914) ^e | N/A | N/A | | | |
| 10 | 92,407 | 2,062 | 8,160 | 94.2 (1909) ^e | N/A | N/A | | | |
| 05 | 83,822 | N/A | 6,019 | 65.2 (1904) ^e | N/A | N/A | | | |
| 1900 | 76,094 | 1,854 | 4,647 | 49.3 | N/A | N/A | | | |

^aREFERENCES: U.S.: 1975 (85), 1900-1970 (83); VIRGINIA: 1975 (12), 1900-1970 (83).

^bREFERENCES: U.S.: 1973 (85), 1900-1970 (83), VIRGINIA: 1972 (84), 1900-1970 (50).

^cDATA WAS COMPILED FROM THE MONTHLY PUBLICATION OF THE U.S. FEDERAL POWER COMMISSION ENTITLED: PRODUCTION OF ELECTRICAL ENERGY IN THE U.S.

^dREFERENCES: U.S.: 1975 (85), 1906-1970 (83); DATA FOR VIRGINIA WAS COMPILED FROM THE ANNUAL BIENNIAL REPORTS OF THE COMMISSIONER OF THE DIVISION OF MOTOR VEHICLES, COMMONWEALTH OF VIRGINIA FOR THE YEARS 1955-1975.

^eWHERE DATA WAS NOT AVAILABLE FOR A GIVEN YEAR, THE MOST RECENT DATA IS PRESENTED AS SHOWN.

^fWHERE ACTUAL DATA WAS NOT AVAILABLE FOR A GIVEN YEAR, DATA PRESENTED REPRESENTS AN AVERAGE OF THE PREVIOUS AND SUBSEQUENT YEARS.

⁸VALUE ADDED BY MANUFACTURER: THIS FIGURE IS DERIVED BY SUBTRACTING THE COST OF THE MATERIALS, SUPPLIES, FUEL, PURCHASED ELECTRICITY, AND CONTRACT WORK FROM THE VALUE OF SHIPMENTS FOR PRODUCTS MANUFACTURED PLUS RECEIPTS FOR SERVICES RENDERED. THE RESULT OF THIS CALCULATION IS THEN ADJUSTED BY THE ADDITION OF VALUE ADDED BY MERCHANDISING OPERATIONS (THE DIFFERENCE BETWEEN THE SALES VALUE AND COST OF MERCHANDISE SOLD WITHOUT FURTHER MANUFACTURE PROCESSING OR ASSEMBLY) PLUS THE NET CHANGE IN FINISHED GOODS AND WORK-IN-PROCESS INVENTORIES BETWEEN THE BEGINNING AND END OF THE YEAR (50).

TABLE 2. A COMPARISON OF VARIOUS WHITE PINE NEEDLE BLIGHTS ACCORDING TO HEPTING/BERRY, LINZON, COSTONIS, AND DOCHINGER.^a

| RESEARCHER | HEPTING/BERRY | | | LINZON | | COSTONIS | DOCHINGER |
|-------------------------------------|--------------------------------|---|---|---|--------------------------------------|---------------------------------------|---------------------------------------|
| | WEST VIRGINIA | TENNESSEE, NORTH CAROLINA | ONTARIO, CANADA | NEW YORK | OHIO | | |
| DIAGNOSTIC TERMINOLOGY | BROWN SPOT BIFUSILLA BLIGHT | EMERGENCE TIPBURN POST-EMERGENCE CHRONIC TIPBURN | POST-EMERGENCE ACUTE TIPBURN | SEMIMATURE NEEDLE BLIGHT | ODONE DAMAGE | SULFUR DIOXIDE DAMAGE | CHLOROTIC DWARF |
| NEEDLES AFFECTED | CURRENT | CURRENT | ANY - USUALLY CURRENT | CURRENT | CURRENT | ANY | CURRENT |
| TIME OF OBVIOUS BLIGHTING | IN SPRING ON 1 YR. OLD NEEDLES | IN SPRING ON WHEN NEWLY EMERGING | ANY SEASON | LATE JUNE TO MID-AUGUST | ANY SEASON | ANY SEASON | EARLY SUMMER |
| MARGIN: HEALTHY VS. DISEASED TISSUE | SHARP | SHARP | INDEFINITE | SHARP | USUALLY SHARP | USUALLY SHARP | GRADUAL |
| FUNGAL FRUITING BODIES | LARGE BLACK | NONE | NONE | NONE | NONE | NONE | NONE |
| MOTTLING | NONE | NONE | NONE | COMMON | NONE | VERY SLIGHT ONLY IN CHRONIC EXPOSURES | COMMON |
| NEEDLE SHEDDING | SOMETIMES IN 1 YR. OLD NEEDLES | PROFUSE IN SPRING | PROFUSE IN 2ND YR. NEEDLES | PROFUSE IN 2ND YR. NEEDLES | PROFUSE IN 2ND YR. NEEDLES | PROFUSE IN 2ND YR. NEEDLES | PROFUSE IN 2ND YR. NEEDLES |
| ONSET | SUDDEN | GRADUAL | SUDDEN | SUDDEN | SUDDEN | SUDDEN | SUDDEN |
| COLOR OF LESIONS | BLACK STROMATA | CHLOROTIC PROGRESSING TO BROWN | REDDISH-BROWN TO BROWN TO GRAYISH | REDDISH-ORANGE-RED | PALE GREEN TO BRONZE TO ORANGE-BROWN | GRAY GREEN TO WHITE TO REDDISH-BROWN | SILVER TO YELLOW-PINK TO YELLOW-BROWN |
| CAUSE | BIFUSILLA LINEARIS | SCIRIBELLA ACICOLA | UNKNOWN - PROBABLY O ₃ AND SO ₂ | PROBABLY O ₃ AND SO ₂ | O ₃ | SO ₂ | O ₃ AND/OR SO ₂ |
| REMARKS | USUALLY STRIKES SINGLE TREES | RESIN DROP USUALLY FOUND ON LESION | WIDESPREAD PROBLEM IN EASTERN U.S. | GEN. CHLOROSIS > THAN EMERG. TIPBURN | ASSOC. WITH INDUS. AREAS IN TN. | ASSOC. WITH POINT SOURCES | TREES REACH 2 1/2 FT. TALL MAXIMUM |
| REFERENCES | 35 | 35, 67 | 4, 35 | 6, 34 | 15, 47, 49 | 45, 47, 49 | 13, 14, 15, 21, 25 |

^a CHART FORMAT TAKEN FROM HEPTING AND BERRY (35).

Table 3. The percent needle fascicles of each of 12 clones of eastern white pine injured in the no pollutant treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days post fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|---|---|--------|----|----|----|---------|---|---|---|----|----|----|----|---|---|---|----|
| | 2 days | | | | 7 days | | | | 14 days | | | | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 |
| I | 1 | 1 | | | | | 17 | | | | 1 | | | 24 | | 4 | 6 | | | 26 |
| | 2 | 1 | 2 | 1 | | 9 | | 7 | 3 | 1 | | | 10 | | 5 | 10 | 1 | | | 10 |
| | 3 | | | | | | | | | | | | | | 1 | 1 | | | | |
| TOTAL | 7 | 1 | 2 | 1 | | 26 | | 7 | 3 | 2 | | | 34 | | 6 | 15 | 7 | | | 36 |
| II | 1 | 1 | 3 | | | | | 1 | 3 | | | | | | 3 | 3 | | | | |
| | 2 | | | | | | | 1 | 1 | 1 | | | | | 4 | 7 | | | | |
| | 3 | | | | | 1 | | 1 | | | | | 1 | | 1 | | | | | 1 |
| TOTAL | 1 | 3 | | | | 1 | | 3 | 4 | 1 | | | 1 | | 8 | 10 | | | | 1 |
| III | 1 | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | 1 | | | | | | | 1 | | 1 | | | | | 1 |
| | 3 | | | | | 1 | | | | | | | 1 | | 1 | | | | | 1 |
| TOTAL | | | | | | 2 | | | | | | | 2 | | 2 | 1 | 1 | | | 2 |
| IV | 1 | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | | | | | | | | | | 1 |
| | 3 | | | | | | | | | | | | | | | | | | | |
| TOTAL | | | | | | | | | | | | | | | | | | | | 1 |
| GRAND TOTAL | 8 | 4 | 2 | 0 | 1 | 0 | 29 | 10 | 7 | 3 | 0 | 0 | 37 | 15 | 26 | 7 | 0 | 0 | 0 | 40 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 4. The percent needle fascicles of each of 12 clones of eastern white pine injured in the NO₂-10 ppm treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days Post fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|---|--------|---|---|---|---------|---|----|---|---|----|----|----|----|----|---|---|---|----|
| | 2 days | | | 7 days | | | | 14 days | | | | | | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | | | | | |
| I | 1 | 2 | 5 | | | | 3 | 1 | 1 | 4 | | | 22 | 1 | 1 | 10 | 24 | | | | |
| | 2 | 7 | 1 | | | | | 1 | 1 | 7 | | | 8 | 5 | 2 | 13 | 12 | | | | |
| | 3 | 1 | | | | | 1 | | | | | | | 5 | 2 | 1 | 1 | | | | |
| TOTAL | 10 | 6 | | | | | 3 | 3 | 1 | 11 | | | 30 | 11 | 5 | 24 | 37 | | | | |
| II | 1 | | | | | | | 1 | | | | | | 1 | | | | | | | |
| | 2 | 3 | 2 | | | | | 2 | 3 | | | | | 5 | 6 | | | | | | |
| | 3 | | | | | | | | | 2 | | | | 1 | | | 2 | | | | |
| TOTAL | 4 | 2 | | | | | 3 | 3 | 3 | 2 | | | | 7 | 6 | | 2 | | | | |
| III | 1 | | | | | | | | | | | | | | | | | | | | |
| | 2 | | 1 | | | | | | | | | | | | | 6 | | | | | |
| | 3 | 1 | | | | | | | | | | | | | | | | | | | |
| TOTAL | 1 | 1 | | | | | | | | | | | | | | 6 | | | | | |
| IV | 1 | | | | | | | | | | | | | | | | 1 | | | | |
| | 2 | | | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | 3 | | | | | | 4 | | | 19 | 4 | | | | |
| TOTAL | 1 | | | | | | 3 | | | | | | 4 | | | 20 | 4 | | | | |
| GRAND TOTAL | 16 | 9 | 0 | 0 | 0 | 0 | 6 | 6 | 4 | 11 | 0 | 2 | 0 | 34 | 18 | 11 | 50 | 0 | 1 | 0 | 43 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 5. The percent needle fascicles of each of 12 clones of eastern white pine injured in the NO₂-30 ppm treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days Post Fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|---|---|---|---|--------|---|---|---|---|---|---------|---|---|----|---|----|---|---|----|
| | 2 days | | | | | | 7 days | | | | | | 14 days | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| I | 1 | 2 | | | | | | 2 | 2 | 3 | | | | | 4 | | | 13 | | | |
| | 2 | 2 | | | | | | 2 | 2 | | | | | | 2 | 8 | | | | | |
| | 3 | | | | | | | | | | | | | | | | | | | | 7 |
| TOTAL | 4 | | | | | | | 2 | 2 | 3 | | | | | 4 | 2 | 8 | 13 | | | 13 |
| II | 1 | | | | | | | 1 | | | | | | | | | | | 1 | 1 | |
| | 2 | 5 | 4 | 1 | | | | 4 | 3 | | | | | | 1 | 1 | 6 | | | | 4 |
| | 3 | 1 | 1 | | 1 | | | 2 | 1 | 1 | | | | | 1 | 1 | 1 | | 1 | | 1 |
| TOTAL | 6 | 5 | 1 | 1 | 1 | | | 7 | 4 | 1 | | | | | 2 | 3 | 7 | | 1 | | 5 |
| III | 1 | | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | | | | | | 1 | | | | | |
| | 3 | | | | | | | | | | | | | | | | | | | | |
| TOTAL | | | | | | | | | | | | | | | | 1 | | | | | |
| IV | 1 | | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | | | | | | | | | | 2 | 2 | | | | |
| TOTAL | | | | | | | | | | | | | | | | 2 | 2 | | | | |
| GRAND TOTAL | 10 | 5 | 1 | 0 | 1 | 0 | 1 | 9 | 6 | 3 | 0 | 1 | 0 | 6 | 8 | 17 | 0 | 0 | 1 | 0 | 18 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 6. The percent needle fascicles of each of 12 clones of eastern white pine injured in the 0₃-10 pphm treatment by evaluation period and symptom type.

| | | % Needle Fascicles Injured by Evaluation Period (Days Post Fumigation) and Symptom Type | | | | | | | | | | | | | | | | | |
|-------------|----------------|--|---|---|--------|---|---|----|---------|---|---|----|---|---|----|---|---|---|---|
| | | 2 days | | | 7 days | | | | 14 days | | | | | | | | | | |
| Class/Clone | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | | | |
| I | 1 | 4 | | | | | | 17 | 5 | | | 19 | 4 | | | | | | |
| | 2 | | | | | | | 3 | 1 | | | 2 | 1 | | | | | | |
| | 3 | | | | | | | | | | | 1 | | | | | | | |
| TOTAL | 4 | | | | | | | 3 | 17 | 6 | | 22 | 5 | | | | | | |
| II | 1 | | | | | | | | | | | | | 1 | | | | | |
| | 2 | 1 | | | | | | 1 | 2 | | | 2 | 3 | | | | | | |
| | 3 | | | | | | | | | | | | | 1 | | | | | |
| TOTAL | 1 | | | | | | | 1 | 2 | | | 2 | 3 | 2 | | | | | |
| III | 1 | | | | | | | | | | | | | 1 | | | | | |
| | 2 | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | | 2 | | | | | 1 | | | | | | |
| TOTAL | | | | | | | | 2 | | | | | 1 | 1 | | | | | |
| IV | 1 | | | | | | | | | | | | | 1 | | | | | |
| | 2 | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | | | 1 | | | | | | | | | | |
| TOTAL | | | | | | | | | 1 | | | | | 1 | | | | | |
| GRAND TOTAL | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | 20 | 6 | 0 | 0 | 1 | 3 | 26 | 5 | 0 | 0 | 2 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 7. The percent needle fascicles of each of 12 clones of eastern white pine injured in the O_3 -10 pphm + NO_2 -10 pphm treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days Post Fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|---|---|---|----|--------|----|----|----|----|---|---------|----|----|----|----|---|----|----|----|
| | 2 days | | | | | | 7 days | | | | | | 14 days | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| I | 1 | 1 | 1 | 1 | 1 | 1 | 42 | 9 | 46 | 4 | 14 | 1 | 46 | 4 | 14 | 1 | 46 | 4 | 14 | 1 | 46 |
| 2 | 4 | 4 | 2 | 2 | 2 | 6 | 6 | 2 | 8 | 3 | 4 | 4 | 7 | 2 | 11 | 9 | 7 | 2 | 11 | 9 | 8 |
| 3 | 2 | 2 | 2 | 2 | 2 | 5 | 5 | 2 | 2 | 2 | 7 | 7 | 7 | 3 | 3 | 4 | 7 | 3 | 3 | 4 | 8 |
| TOTAL | 6 | 4 | 3 | 2 | 2 | 53 | 4 | 10 | 12 | 4 | 60 | 9 | 14 | 27 | 1 | 5 | 62 | 9 | 14 | 27 | 62 |
| II | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 1 | 1 |
| 2 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 1 | 1 | 1 | 1 | 1 | 5 | 1 | 1 | 1 | 5 | 1 | 1 | 1 | 6 |
| 3 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 2 | 1 | 1 | 1 | 1 | 6 | 6 | 6 | 1 | 6 | 6 | 6 | 1 | 7 |
| TOTAL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 2 | 3 | 1 | 1 | 2 | 3 | 1 | 1 |
| III | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 15 | 2 | 2 | 3 | 15 | 1 |
| 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| TOTAL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 1 | 2 | 3 | 15 | 1 | 2 | 3 | 15 | 1 |
| IV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| TOTAL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| GRAND TOTAL | 7 | 4 | 3 | 0 | 3 | 0 | 61 | 8 | 15 | 20 | 0 | 6 | 1 | 69 | 17 | 24 | 48 | 1 | 6 | 0 | 72 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 8. The percent needle fascicles of each of 12 clones of eastern white pine injured in the 0₃-10 pphm + NO₂-30 pphm treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days Post Fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|----|---|---|----|--------|----|----|---|---|---|---------|----|----|----|----|---|---|---|----|
| | 2 days | | | | | | 7 days | | | | | | 14 days | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| I | 1 | 1 | 1 | 8 | | | 18 | 1 | 1 | 2 | | | | 29 | | | 15 | | | | 30 |
| | 2 | 1 | 6 | | | 1 | 1 | 3 | 4 | | | | 3 | | 4 | 8 | 7 | | | | 4 |
| | 3 | | | | | | | | | | | | | | | 11 | | | | | |
| TOTAL | 1 | 2 | 14 | | | 19 | 1 | 4 | 6 | | | | 32 | 4 | 19 | 22 | | | | | 34 |
| II | 1 | | | | | | 6 | 3 | | | | | | | 6 | | 3 | | | | |
| | 2 | | | | | | 1 | | | | | | | | 1 | 2 | 2 | | | | |
| | 3 | | | | | | | | | | | | | | 1 | | | | | | |
| TOTAL | | | | | | | 7 | 3 | | | | | | | 8 | 2 | 5 | | | | |
| III | 1 | | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | 1 | 2 | 2 | | | | 1 | | 1 | 12 | 1 | | | | 3 |
| | 3 | | | | | | | 2 | | | | | | | 2 | 1 | | | | | |
| TOTAL | | | | | | | 1 | 4 | 2 | | | | 1 | | 3 | 13 | 1 | | | | 3 |
| IV | 1 | | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | | | | | | | | | | | | | | 3 |
| | 3 | | | | | | 7 | 4 | | 1 | | | 10 | | 4 | 2 | | | | | 17 |
| TOTAL | | | | | | | 7 | 4 | | 1 | | | 10 | | 4 | 2 | | | | | 20 |
| GRAND TOTAL | 1 | 2 | 14 | 0 | 0 | 0 | 27 | 12 | 13 | 6 | 0 | 1 | 0 | 43 | 15 | 38 | 30 | 0 | 0 | 0 | 57 |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, 7 = necrotic tipburn.

Table 9. The percent needle fascicles of each of 12 clones of eastern white pine injured in the 0₃-30 pphm treatment by evaluation period and symptom type.

| Class/Clone | % Needle Fascicles Injured by Evaluation Period (Days Post Fumigation) and Symptom Type | | | | | | | | | | | | | | | | | | | | |
|-------------|--|---|---|---|---|---|--------|----|---|---|---|---|---------|----|-----|----|---|---|---|----|---|
| | 2 days | | | | | | 7 days | | | | | | 14 days | | | | | | | | |
| | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| I | 1 | 5 | 1 | 2 | | | 2 | 2 | 2 | 2 | | | 1 | 2 | 1 | | | | | | |
| | 2 | 1 | | 1 | | | 5 | 1 | 6 | 1 | | 3 | 1 | 1 | 12 | 1 | | | | | 4 |
| | 3 | | | | | | 4 | 5 | | | | | 7 | 6 | | | | | | | |
| TOTAL | 6 | 1 | 3 | | | | 11 | 8 | 8 | 1 | | 3 | 9 | 9 | 13 | 1 | | | | | 4 |
| II | 1 | | | | | | 2 | 2 | | | | | 1 | 10 | | | | | | | |
| | 2 | 2 | 1 | | | | 1 | 5 | | | | | 2 | 13 | | | | | | | |
| | 3 | | | | | | 3 | 2 | | | | | 4 | 5 | | | | | | | |
| TOTAL | 2 | 2 | 1 | | | | 6 | 9 | | | | | 7 | 28 | | | | | | | |
| III | 1 | | | | | | 12 | 2 | | | | | 8 | 2 | 2 | | | | | | |
| | 2 | | 2 | | | | 1 | 10 | 1 | 1 | | 5 | 2 | 33 | 1 | | | | | | 5 |
| | 3 | 1 | | | | | 1 | 3 | | | | | 1 | 5 | | | | | | | |
| TOTAL | 2 | 2 | 2 | | | | 14 | 15 | 1 | 1 | | 5 | 11 | 40 | 3 | | | | | | 5 |
| IV | 1 | | | | | | | | 2 | | | 4 | | 8 | | | | | | | 4 |
| | 2 | | | | | | | 8 | | | | | 1 | 18 | 1 | | | | | | |
| | 3 | | | | | | | 5 | | | | 3 | 3 | 8 | | | | | | | 3 |
| TOTAL | | | | | | | | 15 | | | | 7 | 4 | 34 | 1 | | | | | | 7 |
| GRAND TOTAL | 10 | 2 | 5 | 0 | 0 | 0 | 31 | 47 | 9 | 1 | 1 | 0 | 15 | 31 | 111 | 17 | 0 | 1 | 0 | 16 | |

^a1 = chlorotic spot, 2 = chlorotic mottle, 3 = chlorotic band, 4 = chlorotic tip, 5 = necrotic spot, 6 = necrotic band, and 7 = necrotic tipburn.

VITA

Christopher Robin Nicholson was born in Hampton, Virginia on March 10, 1947. He attended Hampton High School from 1962 - 1965. He initiated studies at Virginia Polytechnic Institute and State University in 1965 and in June 1967 took a position with the Central Intelligence Agency under the co-operative education program. He graduated from V.P.I. & S.U. in June 1970 with a B.S. in Forestry Management and soon after took a staff position with the C.I.A. as an intelligence research specialist in aerial photography interpretation. In December 1974 he resigned his position with the C.I.A. in order to initiate studies in the Department of Plant Pathology and Physiology at V.P.I. & S.U. leading toward a Master of Science in Plant Pathology.

He is a member of the Gamma Sigma Delta, Phi Sigma, American Phytopathological Society (A.P.S.), and the Potomac Division of the A.P.S.

He married Molly Matson Price on September 19, 1970 and has one daughter Margaret Cabel, born August 25, 1977.

A handwritten signature in cursive script that reads "Chris R. Nicholson". The signature is written in black ink and is positioned in the lower right quadrant of the page.

THE RESPONSE OF 12 CLONES OF EASTERN
WHITE PINE (PINUS STROBUS L.) TO OZONE AND
NITROGEN DIOXIDE

by

Christopher Robin Nicholson

(ABSTRACT)

Grafts were made using 2-0 rootstock and scion from 12 ortets of eastern white pine (Pinus strobus L.) growing at the Radford Army Ammunition Plant (RAAP). The 12 ortets represented 4 symptom severity classes (3 ortets/class) ranging from trees with > 25% of their crowns exhibiting necrotic tipburn (Class I) to those with healthy crowns (Class IV). Grafts were made in spring 1976 and ramets were grown in a greenhouse drawing charcoal filtered air. Each treatment was performed twice, on separate days for a total of 10 ramets/clone/treatment. Five ramets/clone were used in each 6 hour treatment. The current years growth was 7-10 weeks old when treated. The treatments were as follows: 1) O₃-10 pphm, 2) O₃-30 pphm, 3) NO₂-10 pphm, 4) NO₂-30 pphm, 5) O₃-10+NO₂-10 pphm, 6) O₃-10+NO₂-30 pphm, 7) no pollutant. The fumigation chamber was a modified open-top field chamber located indoors. Environmental conditions during the 14 treatments averaged: 26 C, 64% RH, and 16 Klux. The ramets were evaluated prior to fumigation and then 2, 7, and 14 days thereafter for visible symptoms. The overall injury was generally light with only 11% of the clone treatment combinations exhibiting injury on > 25% of the needle fascicles. Clones I-1 and I-2 were the most

sensitive clones while clones III-1, IV-2 and to a lesser degree clones III-3 and IV-1 were tolerant. These results agree with field ratings of eastern white pine sensitivity at the RAAP and provide the first step in the development of an air pollution bioindicator system at the installation.