Chairman: F. Thomas Ledig

SELECTION SYSTEM FOR EVALUATING RESISTANCE OF SCOTCH PINE SEEDLINGS TO OZONE AND SULFUR DIOXIDE[1]

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INTRODUCTION

A new objective of our forest genetics research is to develop methods for producing varieties of Scotch pine (*Pinus sylvestris* L.) resistant to ozone and sulfur dioxide (Gerhold and Palpant, 1968). Population growth and urbanization have caused increased activities in transportation, manufacturing, and generation of power. These industrial activities have released ever-increasing amounts of phytotoxic gases into the atmosphere, of which ozone and sulfur dioxide are two of the most important. Current trends indicate that air pollution problems will increase in the near future (Wood, 1968; Anon, 1971). Engineering devices for pollution abatement and legal requirements are not totally effective, and often result in compromises whereby pollution levels are reduced but not eliminated. Air quality standards that give adequate protection to people may still permit damage to the more sensitive plants, including certain trees (Heggestad, 1969). For these reasons tree varieties and clones with improved resistance to air pollutants would be especially useful for planting in urbanized and industrial areas. Recent evidence (Hill, 1971; Waggoner, 1971) indicates they may play a significant role in cleansing air, in addition to muffling noise and providing shade and beauty.

In this paper, we describe a rating system used to evaluate resistance of the foliage of nursery-grown Scotch pine seedlings to injuries caused by ozone and sulfur dioxide. Estimates of individual trees' resistance have two purposes: (1) for use in genetic analyses that provide information for evaluating different breeding systems, and (2) for use in selecting resistant trees that can be used further in breeding programs or for mass production of resistant clones or varieties.

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METHODS

The plant material consisted of four replications of 2-year-old Scotch pines in 177 full-sib or half-sib families derived from artificial matings within and among diverse populations from Europe and Asia. The material was adequate for estimating genetic variance components and for screening numerous individual trees for resistance. Genetic analyses and selected material will be reported at a later date.

The fumigation method consisted of inserting needle fascicles of nursery seedlings into clear plastic tubing placed between seedling rows, and subjecting these needles to acute dosages of ozone or sulfur dioxide (see cover picture). Portholes for needles were made by drilling 3/16-inch holes in the tubing and covering them with 5/16-inch discs of 30-gauge rubber sheeting held in place by vinyl plastic reinforcing rings (page-savers). One needle fascicle per tree was inserted through a hole in the disc made by a dissecting needle and held open by tweezers. The same trees were used on subsequent fumigations, only different needles on each tree were inserted. Using this method, a number of observations can be made on each tree over a season using different pollutants, concentrations, and durations without sacrificing the tree. A more detailed description of the tubing fumigation method has already been reported (Gerhold et al., in press).

Care must be exercised not to injure needle fascicles as they are inserted, and not to insert needles already showing any type of injury. Occasionally, gas escaping between needles and the rubber sealing disc injured a fete needles in the immediate area. Mechanical injury to needles sometimes resulted from bending during insertion or from being rubbed against the stiff rubber disc. Injured needles were picked and discarded regardless of cause.

To have an effective rating system, we had to find fumigant concentrations that enable optimum discrimination of phenotypic differences in resistance. Previous experiments showed that concentrations of 50 to 55 pphm (parts per hundred million volume) ozone were optimum. Two durations, six and twelve hours, at 50 pphm were tried. The twelve-hour exposure consisted of six-hour exposures on successive days. Ambient and within tubing temperatures, relative humidity, and fumigant concentration were recorded continuously during fumigation.

During sulfur dioxide fumigations, we used a concentration of 350 pphm sulfur dioxide for a duration of approximately four hours. Because visible symptoms due to sulfur dioxide develop on some needles during fumigation, variable durations were used in trying to obtain consistent injury levels. The fumigation was terminated when 50 percent of the inserted needles showed visible signs of injury.

After seven days, two observers picked the fumigated needle fascicles, planted them temporarily in moist sand, and evaluated them. The most severely injured surface of each exposed needle was evaluated as to the percentage of injury in five percent increments in each of three symptom classes. If observers did not agree within five percent, the needle was reexamined and reevaluated by each observer. The three injury classes were: (1) necrotic - tissue dead with a brown or reddish-brown color, (2) mottled - green and yellow or yellow-brown patches of tissue on needle surface, and (3) dark fluid bands - caused by accumulation of resin under needle epidermis (only appeared during late August and September ozone exposures). Ozone and sulfur dioxide mottled injury symptoms are visibly different. Sulfur dioxide mottling tends to be irregular patches of necrotic and chlorotic tissue amongst healthy tissue, whereas ozone mottling tends to consist of fairly regular circular or oval spots of necrotic and chlorotic tissue amongst healthy tissue. Percent injury in each symptom class for each needle was recorded for quantitative analysis. A typical needle fascicle and its evaluation are shown in figure 1.

The data were analyzed in two ways. First, the total percent of injured length of each needle was determined by addition of percent injury in all symptom classes. This gave two values of total injury for each tree evaluated per fumigation, one for each needle in the fascicle. Second, the weighted total injury on each needle was determined by addition of the necrotic and band classes plus one-half the mottled class. We assumed that the mottled class was an indication of less injury than the necrotic and band classes, and chose the weighting factor of 0.5 somewhat arbitrarily to represent the estimated proportion of healthy green tissue contained in it. Miller et al. (1969) have shown that photosynthesis of ponderosa pine is reduced substantially by O_3 in trees at low concentrations that cause no visible symptoms.

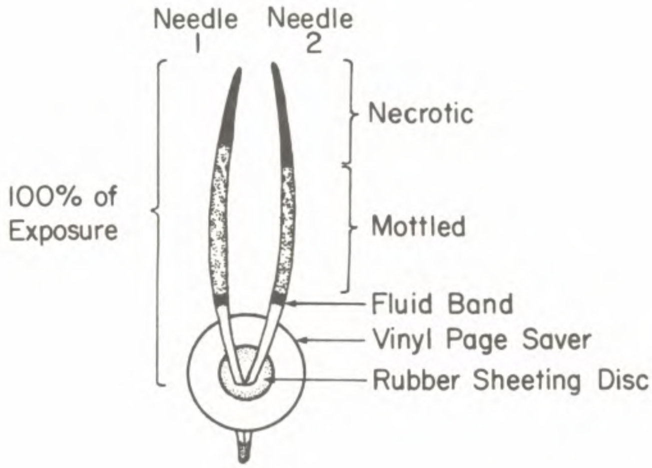
Analyses of variance and means were calculated using the total and weighted data for each fumigation, and combined analyses were calculated on fumigations using the same pollutants at different dates. Regression and correlation analyses were performed on data from fumigations that resulted in weighted injury means between 40 and 60 percent. Perfectly correlated scores of 0 or 100 were not used in computing correlations.

RESULTS AND DISCUSSION

Visible needle symptoms resulting from ozone and sulfur dioxide fumigations developed at different rates, but remained stable during a 5- to 10-day period after fumigation. Ozone needle injury was not apparent at the termination of fumigation. Symptoms gradually developed over a 3- to 5-day period. In contrast to the slower rate of ozone symptom development, some trees fumigated with sulfur dioxide developed visible needle injuries as early as hours after the fumigation started. Most trees developed visible sulfur dioxide symptoms during the three days after fumigation. The stability of symptoms for several days after they completed development was convenient for scheduling measurements.

Weighted injury scores were considered preferable to total injury scores because they take into consideration the presumed healthy tissue in the mottled symptom class. Weighted versus total injury scores were closely correlated and thus substantiate the use of weighted injury data (Figure 2).

A weighted experimental mean between 40 and 60 percent was considered optimum for discrimination of phenotypic differences. Beyond these limits, extreme scores of 100 or near 0 were excessive, resulting in skewed distributions, underestimation of error terms, and loss of ability to discriminate real phenotypic differences (Figures 3 and 4). Distributions that resulted from 6- and 12-hour ozone fumigations with similar weighted mean injuries resembled each other closely (Figure 5). Although 6- and 12-hour experimental means both varied considerably, it appeared that there was no difference in results and no need to extend acute dosages beyond 6 hours.



	Total Injury		Weighted Injury	
	Needle 1	Needle 2	Needle 1	Needle 2
Necrotic	25	30	25	30
Mottled	45	40	23	20
Fluid Bands	5	5	5	5
Total	75	75	53	55

Figure 1.--Evaluation of a typical fumigated scotch pine needle fascicle.

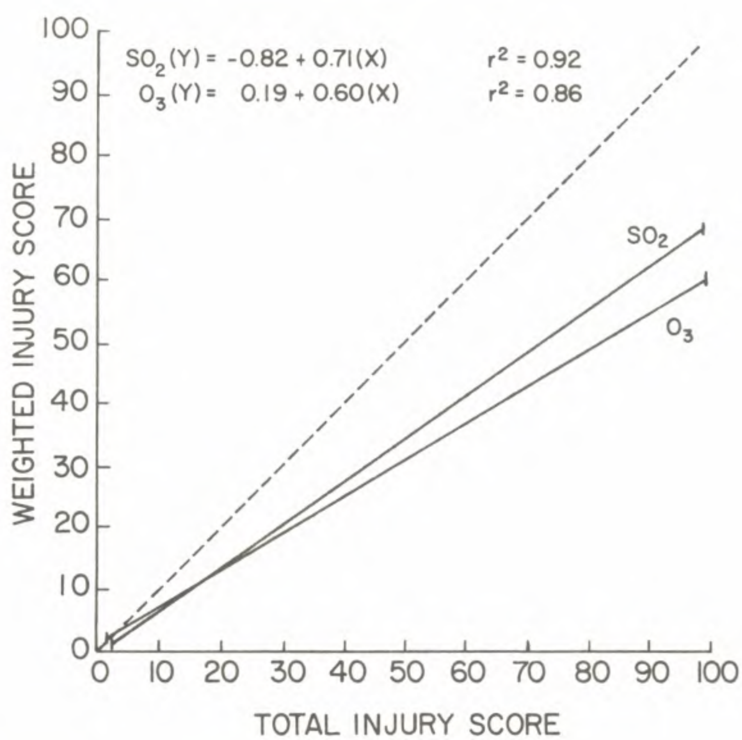


Figure 2.--Weighted versus total injury scores (Omitting X = 100, Y = 100, and X = 0, Y = 0).

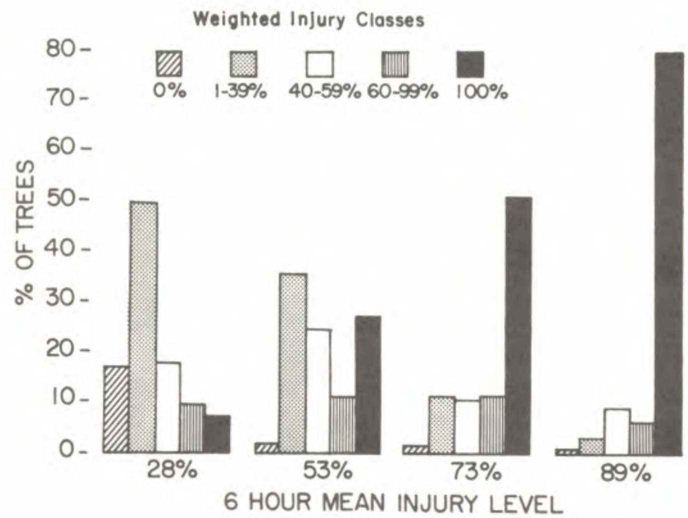


Figure 3.--Distribution of O_3 injury scores changes with injury level.

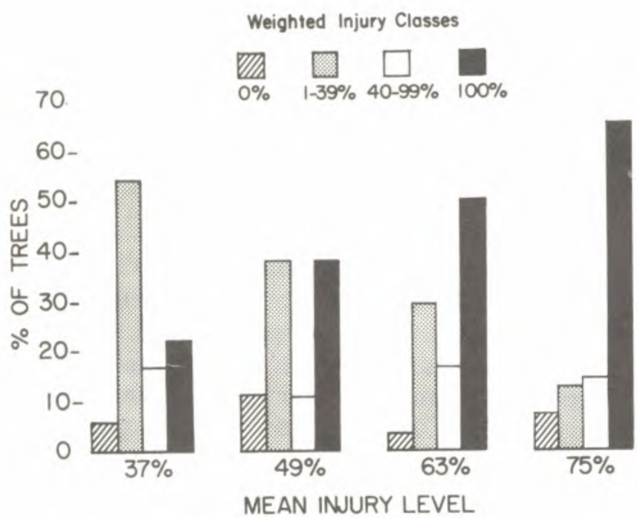


Figure 4.--Distribution of SO_2 injury scores changes with injury level.

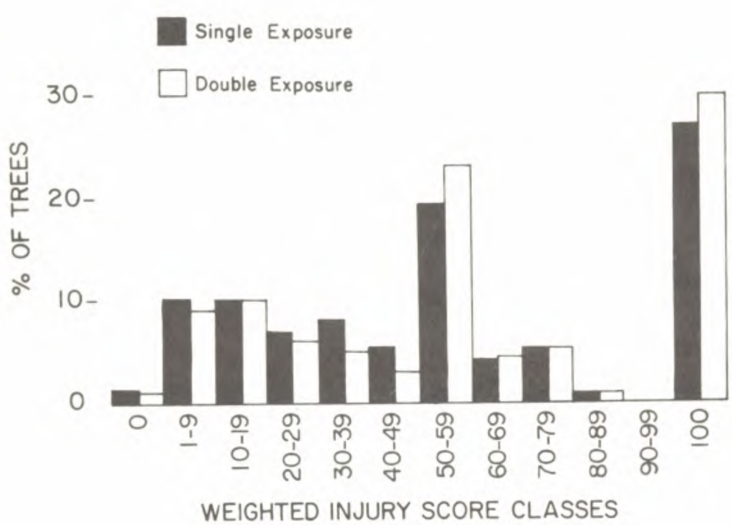


Figure 5.--Distribution of O_3 injury scores resulting from single vs. double 6 hour exposures.

Analyses of variance indicated there was little variability between needles of the same fascicle, moderate variability among needles of the same tree exposed to several fumigations, and large variability among different trees. Severity of needle injury caused by ozone or sulfur dioxide ranged from no visible injury to total necrosis of the inserted portion of the needle. Part of the variability between needles from the same fascicle may be due to damage during insertion. Needles that had been bent until creased sustained less injury. Because undamaged needles from the same fascicle show very little variability, the more severely injured needle could be used in analyses as an indication of a tree's resistance or susceptibility.

A portion of the variability among fumigations can be attributed to environmental differences prior to, during, and after fumigations. Temperature, light, relative humidity, and soil moisture are presumed to be the main sources. In general, the response of plants of all kinds to ozone is dependent on environmental factors (Ting and Dugger, 1968; Heck, 1968). Factors that favor open stomates and photosynthesis, such as bright sunlight, high relative humidity, and optimum temperature also favor sulfur dioxide assimilation. Plants are more responsive during morning and forenoon than during the afternoon. Plants are more sensitive when there is a high relative humidity as well as adequate soil moisture. Thus plants are subject to greater hazard on clear days following an adequate rain (Zimmerman and Crocker, 1934; Thomas et al., 1943; and van Haut and Stratmann, 1970). Davis (1970) found environmental conditions such as temperature, light, and relative humidity influenced the response of Virginia pine seedlings to ozone; the amount of injury was inversely related to exposure temperature and directly related to relative humidity.

More precise control of environmental conditions among fumigations probably could be achieved by irrigation of the nursery beds before fumigation to bring soil moisture to field capacity and by using a more sophisticated apparatus to control relative humidity and temperature in the tubes. However, it is still more realistic to test phenotypes under a variety of environmental conditions in order to get a more comprehensive evaluation of the phenotype. Therefore, the preferable means of holding injury levels within the desired range of 40-60 percent is by adjusting fumigation concentration of ozone or duration of sulfur dioxide. Means near 40 are preferable for estimation of variance components because extreme scores of 0 and 100 are not in excess. Means near 60 are preferable for selecting resistant phenotypes in order to minimize the number of trees in the undamaged class. A more precise method of predicting ideal concentrations and durations still needs to be developed.

The fumigation system does allow for uniform conditions within the 150-foot-long fumigation chamber that are representative of environments in which resistance would have value. Temperature generally was 5° to 8°F higher, and relative humidity 0 to 10 percent higher in the fumigation tubing compared to ambient conditions. There was very little decrease in concentration of ozone or severity of injury along the fumigation tubing and practically none for sulfur dioxide (Figure 6).

Reduction in unwanted variation within fumigations might be achieved by taking into account the position of the fascicle on the leader and the percentage of needle actually inserted and fumigated. It is not always possible to insert fascicles from similar positions on the leader or the

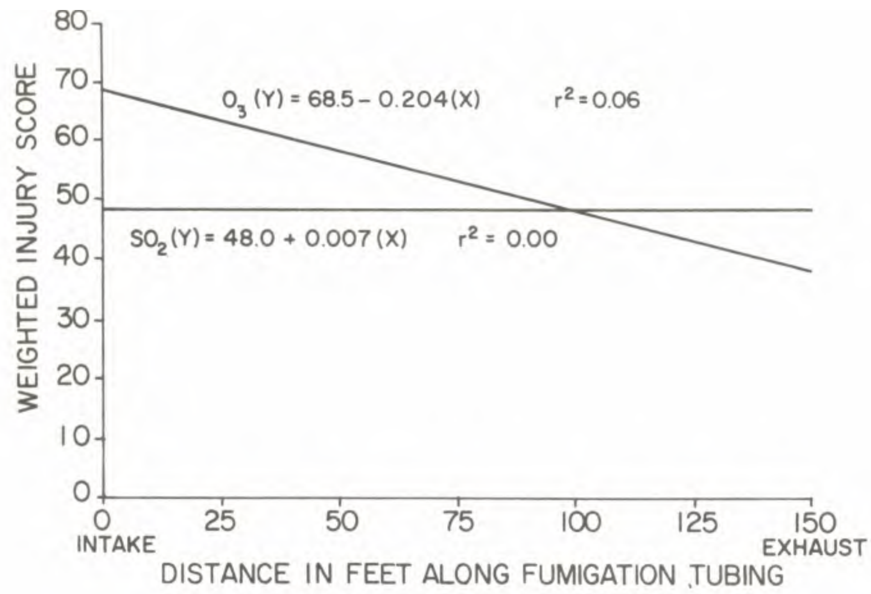


Figure 6.--Injury levels of scotch pine remain nearly uniform along fumigation tube.

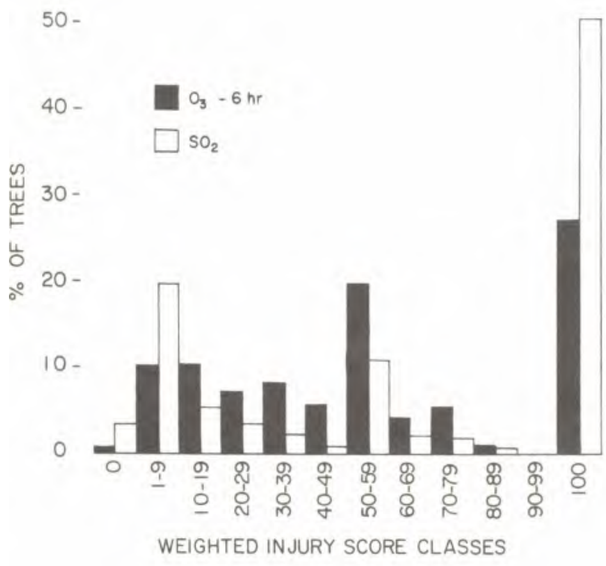


Figure 7.--Distribution of O_3 and SO_2 injury scores resulting from optimum fumigation conditions.

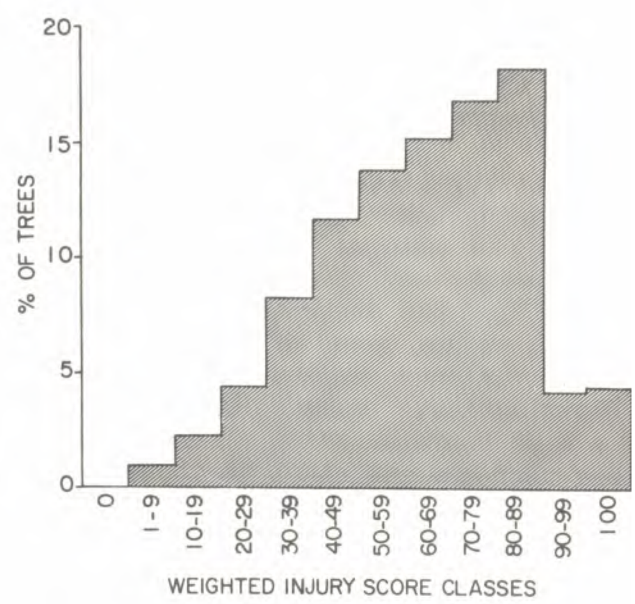


Figure 8.--Distribution of mean O_3 injury scores resulting from 4 fumigations on 708 trees.

same percentage of needle into the tube because of the fixed position of the tubing in relation to differential tree heights and positions in the nursery bed. Better control of needle position and insertion percent could be achieved with potted plants, but extra hours would be required for potting and positioning the seedlings. Resulting data would be representative of a potted environment rather than the more natural nursery environment.

Histograms showing frequency of weighted injury scores by needle injury classes for ozone or sulfur dioxide fumigations that gave data within the optimum range showed that data were multi-modal in nature (Figure 7). Multi-modal distributions were caused partly by scale effects, attributable to environmental factors and dosages that result in excessive scores of 100 or near O. The bimodal distribution of intermediate scores could possibly be the result of the proportion of needle exposed, reversible and irreversible injury reactions, or distinctive major gene effects. Rich (1964) proposed four mechanisms that could confer resistance to O_3: high sugar levels, high levels of natural antioxidants, increased suberin content of mesophyll cell walls, and closing of stomata in response to O_3. Börtitz and Vogl (1969) found that net assimilation rates of SO_2 resistant clones drop later and recover earlier than in susceptible clones. Weighting of injury scores was not the underlying cause of the distribution of injury score classes being multi-modal because weighted and total scores produced very similar distributions. The precise cause of the multi-modal distribution needs to be clarified.

It may be possible to improve the needle injury classification system by accounting for the portion of needle exposed and the position of the needle fascicle on the leader and by scoring needles at different times following exposure. It has been observed that young needles may recover from the chlorotic injury from sulfur dioxide fumigations, so that some time after exposure only the necrotic tip is seen (van Haut and Stratmann, 1970). Repeating evaluations may detect any reversible or irreversible injury reactions. Irreversible injury reactions may result from needles being injured to a certain degree from which they cannot recover and they either remain stable or proceed to a more severe injury class.

A better distribution of data for quantitative analysis may be achieved by averaging the scores after repeated exposures. A histogram showing frequency of mean weighted injury scores by needle injury classes for four ozone fumigations revealed that the data approached being normally distributed (Figure 8). The distribution consists of scores from fumigations having weighted injury means of 28.1, 34.8, 53.5, 58.0, 72.8, 80.5, 88.4, and 89.4. Genetic variance components could be estimated and trees selected from such a distribution. Trees selected and components estimated would be indicative of a wide representation of environments in which resistance would be of value. Trees that are resistant to these rather high acute dosages could be expected to show little injury in polluted regions (Heggestad, 1969; Guderian and Stratmann, 1968).

Correlations of weighted injury data indicate that at least two fumigations per tree are needed to obtain a good evaluation of the resistance or susceptibility of a tree. Correlation of individual tree scores after 6-hour ozone fumigations increased from 0.44 to 0.85-0.86 when two fumigations per tree were considered. Likewise, correlation of sulfur dioxide fumigations increased from 0.31 to 0.80-0.81 when two fumigations per tree were considered (Table 1).

Table 1.--Correlation of 2-year height and mean injury scores for individual and combined ozone and and sulfur dioxide fumigations.

	Ht.	SO_2	SO_2 E*	SO_2 L*	O_3	O_3 6hr-E*	O_3 6hr-L*	O_3 12hr-E*	O_3 12hr-L*	O_3 6hr*	Needles
SO_2	-0.17										4
SO_2-E	-0.09	0.81									2
SO_2-L	-0.18	0.80	0.31								2
O_3	-0.17	0.30	0.21	0.28							8
O_3-6hr-E	-0.13	0.28	0.24	0.22	0.72						2
O_3-6hr-L	-0.11	0.30	0.19	0.29	0.73	0.44					2
O_3-12hr-E	-0.17	0.11	0.09	0.09	0.58	0.26	0.22				2
O_3-12hr-L	-0.05	0.06	0.01	0.09	0.51	0.01	0.15	0.14			2
O_3-6hr	-0.13	0.34	0.25	0.29	0.85	0.86	0.82	0.29	0.09		4
O_3-12hr	-0.13	0.11	0.06	0.12	0.71	0.16	0.24	0.68	0.82	0.23	4
Needles	---	4	2	2	8	2	2	2	2	4	

E* = July or early August fumigation.

L* = Late August or early September fumigation.

6hr & 12hr = Fumigation duration.

The fumigation and selection system allows for a large number of trees to be evaluated for resistance. Three men can fumigate, evaluate, and record information on 700 to 800 trees per week. The main limiting factors are the times necessary to insert and evaluate the needles. A good technician can insert or evaluate 25 to 30 needle fascicles per hour under ideal conditions.

SUMMARY

A selection system based on visible needle injuries has been developed and tested for evaluating the resistance of two-year-old nursery-grown Scotch pine seedlings to acute exposures of ozone and sulfur dioxide. The system enables optimum discrimination of phenotypic differences using a scale suitable for quantitative analyses. Data indicated that weighted injury means of experiments near 40 were optimum for estimating variance components and near 60 for selecting resistant phenotypes. Multi-modal distributions occurred after every exposure which may imply that a few genes may be responsible for resistance to ozone and sulfur dioxide. Differential reactions of needles or portions of needles within an internode may also be responsible for the multi-modal distributions. Exposure of two or more needle fascicles per tree on different dates is recommended for good evaluation of its resistance. In this way, genotypic reactions are tested under various environmental conditions that influence expression of resistance.

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