Running Heading:

Age Class Dependent Ethylene Production in Pine Needles

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ETHYLENE PRODUCTION BY DIFFERENT AGE CLASS PONDEROSA AND
JEFFERY PINE NEEDLES AS RELATED TO OZONE EXPOSURE AND
VISIBLE INJURY¹

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ABSTRACT

Ethylene production by different needle age classes was characterized using a mercuric perchlorate traps in natural populations of two ponderosa pine varieties (Pinus ponderosa var. arizonica [Engelm] Shaw and var. ponderosa Dougl. ex Laws.) and Jeffery pine (Pinus jeffreyi Grev. and Balf.). All ozone-exposed populations contained individuals which were symptomatic and asymptomatic with respect to visible ozone injury. Ethylene production of different needle age classes was also characterized in Pinus ponderosa var. ponderosa seedlings grown in open top ozone fumigation chambers. Older age class needles produce more ethylene than younger age class needles. Needles of both P. ponderosa var. ponderosa and P. jeffreyi exhibiting ozone injury in the field produced significantly ($p>0.05$) higher levels of ethylene than asymptomatic conspecifics. Seedlings exposed to highest treatment level of ozone in the fumigation study produced the highest levels of ethylene followed by fumigation with medium and low ozone concentrations and carbon filtered air. These data indicate that measurement of ethylene in conifer needles as a measure of stress needs to be calibrated for needle age class. It also suggests that the sensitivity of a tree to ozone injury may be regulated by the inherent ability of the individual to produce ethylene.

INTRODUCTION

Ethylene production in response to pollution exposure has been characterized (3, 4, 11), and has been employed as a means of quantifying a pollution induced stress response (10, 17, 18, 19, 20). Previous studies have been designed to observe ethylene production by the entire plant (17, 19) or as an average of different age classes of leaf tissues (10, 20). Thus, the contribution of total ethylene by the tissues of different age class was not apparent.

Older plant tissues usually exhibit an increase in the emission of ethylene compared to younger tissues on the same plant (1). It is logical to hypothesize that older leaves will produce greater quantities of ethylene than younger leaves on the same plant and as such, a composite sample of a large number of needle flushes may not be a sensitive indicator of pollution stress. Species that retain leaves for several years are ideal for an investigation of age class dependent ethylene production. Trees within the genus *Pinus* can retain needle leaves for several years (13). Both *Pinus ponderosa* Dougl. ex Laws. and *Pinus jeffreyi* Grev. and Balf. are known to be sensitive to ozone, exhibiting leaf mottle and accelerated needle loss symptomatic of ozone exposure (12). Therefore, these two species were selected to test the needle age dependent ethylene production hypothesis.

A new method of sampling needle tissues for ethylene production needed to be developed which would allow for accurate

measurement of ethylene under field conditions. Methods used in the past could not accurately account for the loss of ethylene across seals or caps. For example, Nelson et al. (14) indicated that a variety of rubber type seals lost a significant amount of ethylene due to diffusion across the seal during a 24h period. The technique employed in this study involved trapping the ethylene molecule in a solution of mercuric perchlorate (1). The trapped ethylene remains stable, without loss of gas, until released with the addition of sodium chloride. Large volumes of gas can be scrubbed of ethylene with this method. When released in a small volume, the concentration of the ethylene is increased in a greatly reduced head space. The system is field portable and not time dependant once the traps have been removed from the needle chamber, sealed and stored under refrigeration.

The focus of this study was the characterization of ethylene production by different age class pine needles from trees with different degrees of symptomatic visible ozone damage and from seedlings exposed to known concentrations of ozone grown in open top fumigation chambers. The production of ethylene by leaf tissue may be used as a screen for ozone sensitive individual trees within a wild population. The effect of carbon dioxide as a competitive inhibitor of ethylene action (1) and possible inhibitor of synthesis in leaf tissue was also tested.

METHODS AND MATERIALS

PLANT MATERIAL - Needle leaves representing different age classes were collected from mature trees in September 1989 from field locations at: 1) Inspiration Rock Lookout, elevation 2440 m, Santa Catalina Mountains, Pima County, Arizona, (P. ponderosa var. arizonica [Engelm.] Shaw), 2) near Giant Forest Grove, elevation 1920 m, (P. jeffreyi Grev. and Balf.) and, 3) near Crystal Cave, elevation 1524 m (P. ponderosa var. ponderosa Dougl. ex Laws.), Sequoia National Park, California. Needle leaves representing two different age classes of P. ponderosa var. ponderosa were also collected from three-year-old seedlings fumigated with ozone for 90 days on a pre-programmed 30 day diurnal target profile (5, 6). Four different ozone exposures characterized as high (80% over base ozone concentration), medium (60% over base ozone concentration), low (base ozone concentration) and charcoal-filtered air were used in the experiment (5, 6). The open top chambers were located at a U.S.D.A. Forest Service Research Facility, Kenworthy Ranger Station, Garner Valley, California at an elevation of 1250 m.

ETHYLENE SAMPLING - The feasibility of trapping ethylene released by needles and the effect of simultaneously trapping carbon dioxide in a closed system was tested first. A single tree (P. ponderosa var. arizonica) was sampled in the field to minimize variability. Two needle fascicles were removed from each of the

current years whorl of needles, one year old whorl of needles and three year old whorl of needles. The pairs of fascicles were placed in a 5X40 mm culture tube containing 0.25 mL distilled water. The culture tube containing the water and needles was then inserted into a 25X150 mm screw capped culture tube along with an ethylene and carbon dioxide trap. The ethylene trap consisted of a 5 mL screw cap vial containing 0.5 mL 0.25 M mercuric perchlorate in 2 N perchloric acid (1). The carbon dioxide trap consisted of a 10X25 mm shell vial containing 1.0 mL of a 40% potassium hydroxide (wt/vol) aqueous solution. A replicate set of containment chambers containing pairs of needles and ethylene traps was prepared without the carbon dioxide traps. Both tests were replicated six times.

The larger culture tubes containing the traps and needles were sealed within two minutes after needles were removed from the tree. They were maintained in the dark at 21°C for a 24h period. At the end of the incubation period, the tubes were opened, and the traps and needles removed. The ethylene traps were sealed using screw caps and stored at 4°C until they were analyzed. The needles were removed from the smaller culture tubes, blotted dry, and weighed to determine fresh weight. Dry weights were obtained by drying needles between sheets of Whatman #1 filter paper and dried to constant weight at 40°C.

Two groups of trees (*P. ponderosa* var. *arizonica*) from the Santa Catalina Mountains, Arizona, with and without ozone-induced chlorotic mottle, were tested for ethylene production. Each

group consisted of three trees and each tree was sampled six times. A similar procedure was employed in sampling P. ponderosa var. ponderosa and P. jeffreyi growing in Sequoia National Park, California. Each sample group contained eight trees and each tree was sampled three times. This procedure was replicated for the analysis of seedling needles grown in the open top chambers. Eighteen trees from each of the four fumigation treatments (total of eight chambers) were tested. A replicate analysis was conducted on seedlings in the fumigation chambers which exhibited premature needle loss under conditions of high and medium ozone exposure.

ETHYLENE ANALYSIS - The ethylene traps were removed from refrigeration and the solid screw caps were replaced with chromatography septa caps. Ethylene trapped in the mercuric perchlorate solution was released into the vial head space by injecting 0.5 mL 4 M NaCl into the sealed vial (1). Total head space gas samples (2.5 mL) were analyzed for ethylene content using gas chromatography (16).

Mercuric perchlorate solutions will gradually absorb ambient ethylene from the atmosphere during handling. Therefore, the initial amount of ethylene present in the solution must be determined to accurately calculate the amount of ethylene that has been trapped from the plant material. Five ethylene traps were treated similar to those used with needles. The difference being the absence of needles from the trapping chambers. These

blanks were maintained for the duration of the experiment. The amount of ethylene present in the blanks was averaged and subtracted from each sample in the replicate.

DATA ANALYSIS - An analysis of variance (ANOVA) and a multiple comparison between of means using the Student-Newman-Keuls multiple-range test was performed on individual ethylene data sets. A student T-test was performed to determine significance between the needle retention and needle loss groups tested from the open top ozone fumigation chambers.

RESULTS

Three year old needles emitted significantly ($p>0.05$) higher amounts of ethylene than current or one year old needles (Table 1). No significant differences were observed within age classes in the amount of ethylene produced by needles incubated with or without carbon dioxide traps (Table 1). Although the younger needles of symptomatic P. ponderosa var. arizonica tended to produce more ethylene than asymptomatic conspecifics, these differences were not significant (Table 2). Asymptomatic P. ponderosa var. ponderosa and P. jeffreyi from Sequoia National Park retained five and four years of needles respectively, whereas the symptomatic individuals of these two species only retained two years of needles (Table 3). Significantly ($p>0.05$) greater amounts of ethylene were produced by the symptomatic

needles compared to the asymptomatic conspecifics for both species. No significant differences were observed in the amount of ethylene produced by different needle age classes within either asymptomatic or symptomatic groups, although there was a trend toward increasing ethylene production with increasing needle age (Table 3).

Seedlings fumigated with the highest level of ozone produced significantly ($p > 0.05$) greater amounts of ethylene in both needle age classes compared with the low and medium fumigation levels (Table 4). Ethylene emitted by the medium ozone fumigation group was intermediate between the high and low fumigations (Table 4). There was also an observable trend toward increased ethylene production with increased needle age. This increase was significant in both the low and medium ozone exposure groups. Compared to current year needles, there was a 500% increase in ethylene emitted by one year old needles exposed to high concentrations of ozone (Table 4). Seedlings which loose older needles tended to produce more ethylene in the current year needles (Table 5). The observed difference is significant ($p > 0.03$) for seedlings exposed to medium ozone concentrations. The difference is not significant for the high ozone fumigation group (Table 5).

DISCUSSION

Stress induction of ethylene production in plants has been well documented (1). The relationship between the amount of stress and the production of ethylene has been implicated as a means of monitoring the level of stress in a plant. Tingey et al. (19) first suggested that the measure of stress ethylene could be a fast and sensitive method to measure ozone stress in plants. However, the induction of ethylene by numerous abiotic and biotic stresses, genotypic variations in ethylene production rates and tissue type and age dependant differential ethylene production may render this method invalid for monitoring the level of a specific stress (18). The need exists for a more extensive characterization of the production of ethylene by plant tissues before it can be used as a bioindicator of environmental stress. The data presented here attempt to further characterize a possible age dependant and genotypic dependent variation in the production of ethylene to a known ozone stress under controlled conditions and a monitored ozone stress (15) in association with other non-monitored stresses under field conditions.

A carbon dioxide trap was tested to determine the impact of a buildup of carbon dioxide on the release of ethylene from the leaf tissues. The data presented in Table 1 indicates that there is no effect of carbon dioxide on ethylene production under dark conditions. This finding is consistent with that reported by Kao and Yang (8). The data presented in Tables 1,2,3 and 4 support

the hypothesized increase in the amount of ethylene emitted by older needle tissues.

The age of the tissues is not the only factor which can regulate the rate of ethylene production. The physiological state of the tissue, whether actively growing or dormant, may determine the amount of ethylene produced. Jeffery pine, which produced the least ethylene of all trees sampled, grows at a site with the coolest autumn temperatures during sample collection. Therefore, Jeffery pines may be in a relatively more dormant state, producing less ethylene than the ponderosa pines.

The increase in ethylene emission from needles of symptomatic trees could be a response to increased injury and senescence of foliar tissues. However, the increase may also represent an inherent ability of the symptomatic trees to produce more ethylene than asymptomatic trees. Mehlhorn and Wellburn (11) suggested stress ethylene production can determine plant sensitivity to ozone and Telewski and Jaffe (16) have identified high and low ethylene producing trees within the same species (P. taeda L.). If symptomatic trees naturally produce more ethylene than the asymptomatic trees, the greater availability of ethylene would react with the ozone to yield greater quantities of highly reactive free radicals, initiating peroxidative events in the leaf tissue resulting in necrosis or mottle (4, 11). Other studies have implied a genetic component of ozone sensitivity for a number of tree species (2, 7, 9), however, these studies have not investigated the ability of the ozone sensitive trees to

produce stress ethylene. The relationship between the inherited ability of a tree to produce ethylene and the sensitivity of the tree to ozone as a genetic component of sensitivity merits further investigation.

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Table 1. Ethylene production by different age class needles from a single Pinus ponderosa var. arizonica with and without 40% (wt/vol) potassium hydroxide traps. Means (n=6) with dissimilar letters are significantly different at $p > 0.05$.

Needle age class (Years)	Ethylene (nM gDW ⁻¹ 24 h ⁻¹)	
	With CO ₂ Trap	Without CO ₂ Trap
3	29.5 A	24.2 AB
1	10.7 BC	6.3 C
0	6.5 C	6.6 C

Table 2. Ethylene production by different age class needles from a single Pinus ponderosa var. arizonica with and without symptomatic ozone-induced chlorotic mottle. Means (n=3) with dissimilar letters are significantly different at $p>0.05$.

Needle age class (Years)	Ethylene (nM gDW ⁻¹ 24 h ⁻¹)	
	Asymptomatic	Symptomatic
3	14.1 A	14.1 AB
1	7.4 BC	9.4 C
0	2.9 C	5.8 C

Table 3. Ethylene production by different age class needles from Pinus ponderosa var. ponderosa and Pinus jeffreyi with and without symptomatic ozone-induced chlorotic mottle. Means (n=7) within each species with dissimilar letters are significantly different at $p>0.05$.

Needle age class (years)	Ethylene (nM gDW ⁻¹ 24 h ⁻¹)			
	<u>Pinus ponderosa</u>		<u>Pinus jeffreyi</u>	
	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
4	8.3 BC	- -	- -	- -
3	6.1 BC	- -	3.3 BC	- -
2	6.6 BC	- -	2.8 BC	- -
1	4.3 C	32.6 A	2.0 C	6.7 A
0	2.8 C	21.4 AB	2.0 C	5.3 AB

Table 4. Ethylene production by different age class needles from Pinus ponderosa var. ponderosa exposed to different levels of ozone. Means (n=18) with dissimilar letters are significantly different at $p > 0.05$.

Needle age class (years)	Ethylene (nM gDW ⁻¹ 24 h ⁻¹)			
	CHARCOAL FILTERED	LOW	MEDIUM	HIGH
1	57.0 C	42.8 C	318.4 B	642.4 A
0	7.8 D	10.3 D	20.9 CD	125.9 B

Table 5. Ethylene production by the youngest needle age class from Pinus ponderosa var. ponderosa representing trees with two-year-old needles (W) and trees without 2-year-old needles (WO) when exposed to high or medium concentrations of ozone. n=18

Needle age class (years)	Ethylene (nM gDW ⁻¹ 24 h ⁻¹)			
	High Ozone Exposure		Medium Ozone Exposure	
	W	WO	W	WO
0	125.8	148.1 ^{ns}	20.9	36.5*

*Significant at p>0.03, ^{ns}Not significant.