ETHYLENE PRODUCTION BY STORED PINE SEEDLINGS AND ITS RELATION TO ROOT REGENERATION AND SURVIVAL[1/]

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Abstract: Amounts of ethylene emanating from different tissues and entire seedlings of Pinus taeda and P. elliottii varied with species, kind of tissue, and with storage time.

Loblolly, slash, and Virginia (P. virginiana) pine seedlings were stored under five different levels of ethylene for 16 and 30 days. Root regeneration potential (RRP), height growth, and survival of Virginia pine were not significantly affected by ethylene treatments. With loblolly pine seedlings, RRP was significantly greater when stored with an ethylene absorbent and a high ethylene concentration (5.0ppm) significantly reduced height growth. Survival and height growth of slash pine were significantly reduced when stored in naturally produced levels of ethylene.

Keywords: Pinus taeda, P. elliottii, P. virginiana, height growth, root regeneration potential (RRP).

INTRODUCTION

Ethylene is known to affect the behavior of fruits, flowers, and entire plants in storage. Ethylene present in concentrations as small as .01 ppm can enhance senescence of various plant parts and cause a general decrease in plant vigor (Abeles 1973). Much work has been done on the effects of ethylene on fruits, flowers, and horticultural plants in storage, but little information is available on the effects of ethylene on conifers. Some work has been conducted on Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), western hemlock (Tsuga heterophylla (Raf.)Sarg.), and loblolly pine (Pinus taeda L.) (Anonymous 1979, Barnett 1980, Graham and Linderman 1981, Johnson and Stumpff 1984). Barnett (1980) found that placing the ethylene absorbent Purafil ES[4/] in bags of loblolly pine held in storage for six weeks improved root regeneration potential (RRP) and survival over that of the controls. He concluded that ethylene may be partly responsible for the rapid deterioration of loblolly pine seedlings in storage.

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[4/]Purafil ES is the trade name for an absorbent composed of potassium permanganate absorbed on an aluminum medium.

The purpose of this study was to determine if ethylene present in the storage environment adversely affects subsequent field survival and performance of loblolly, slash (P. elliottii Engelm.), and Virginia (P. virginiana L.) pine seedlings. All are commercially valuable species which are regenerated primarily by planting bare-root seedlings. Specifically this study was conducted to:

1) determine the levels of ethylene emanated from roots, stems, and needles of stored loblolly and slash pine seedlings; and

2) determine the effects of different concentrations of ethylene present in the storage environment over time on subsequent seedling survival and performance in the field.

MATERIALS AND METHODS

Excised tissues

Nursery grown 1-0 loblolly and slash pine seedlings were obtained in the late winter of 1983 and stored in open-ended seedling bales at 3° C in a seedling cooler. Virginia pine was not available for this study. Samples of three seedlings per species were withdrawn after 1, 2, and 4 weeks in storage. Sample seedlings were thoroughly washed with water, immediately blotted with paper towels and portions of the root, stem, and needles were excised from each. The excised tissues were weighed, placed in 1-dram vials capped with teflon septa and were then incubated in a water bath at 30°C for 16 hours. Two hundred fifty microliters of head space were sampled and analyzed for ethylene using a Hewlett-Packard 5830A gas chromatograph equipped with a flame ionization detector (FID) and a 6-foot x 2mm ID glass column packed with 80/100 mesh activated alumina. Column, injection port, and detector temperatures were 70°, 125°, and 110°C, respectively. Carrier flow rate was 20 ml of nitrogen per minute. Identity of ethylene was verified by comparing the retention time with an ethylene standard coupled with peak enrichment. An external standard was used for quantification.

Entire seedlings

Loblolly, slash and Virginia pine seedlings were obtained from the Mississippi Forestry Commission. The seedlings were lifted the first week in January 1985 and roots dipped in a clay slurry. It had been previously determined by gas chromatography that no ethylene was produced by the clay slurry.

Seedlings of each species were separated into bundles of thirty and weighed. Each bundle was placed in a nylon bag and the bags heat-sealed. Average bag volume (3.25 liters) was determined by immersing the sealed bags in water and measuring the volume of displacement.

Seedlings were stored at 3°C in a seedling cooler. Two storage periods were used: 16 days and 30 days. Within these storage periods, seedlings were subjected to five ethylene treatment concentrations; (1) Purafil added, (2) naturally produced levels, (3) .05 ppm, (4) 0.5 ppm and (5) 5.0 ppm. These treatments were replicated three times. In treatment 1, seedlings were stored

with 24 g of an ethylene absorbent, Purafil, enclosed in the bags. Treatment 2 consisted of levels of ethylene naturally produced by seedlings. For treatments 3, 4, and 5, ethylene was injected into bags to bring them to chosen treatment levels.

During storage, ethylene levels within the bags were monitored by gas chromatography using a Hewlett-Packard 5880A gas chromatograph equipped with a FID and a six-foot x 2mm ID glass column packed with activated alumina. The GC was run isothermally at 110°C with flow rates of 40, 40, and 400 ml per minute for nitrogen, hydrogen, and air, respectively. Cochromatography with an ethylene standard was used to identify ethylene.

A maximum storage time of 30 days was chosen since most seedlings of these species are not held in storage that long. This 30 day period was uninterrupted with slash pine; however, with the other two species it was broken into 2 periods (16 plus 14 days) because an airtight container was used. When plants are enclosed in an airtight environment, oxygen can become limited causing undue stress. Reduced oxygen levels were an important consideration because ethylene production by plants requires oxygen. In order to prevent this from becoming a problem, bags were also monitored for detection of ethane which could indicate reduced oxygen tension around the seedlings (Ward et al. 1978). At the end of 16 days, ethane had reached detectable levels within the bags of loblolly and Virginia pine seedlings. Therefore, the bags of loblolly and Virginia pine seedlings being stored for 30 days were opened, aerated, Purafil packets replaced, the bags resealed, and ethylene reinjected.

At the end of the designated storage periods, 10 loblolly, slash, and Virginia pine seedlings per treatment were potted and placed in a greenhouse. At the end of eight weeks, survival, height growth, and RRP were determined. RRP was defined as the total number of new first order and second order roots produced by a seedling. Extension of any existing root was counted as a new root.

Three replications of 20 seedlings per treatment for each species were planted in the field and survival and height growth were measured.

Survival, height growth, and RRP data were analyzed using a 2 x 5 factorial, completely randomized design. Mean separation was done using least significant difference (LSD).

RESULTS AND DISCUSSION

Excised tissues

Levels of ethylene emanating from the different seedling parts varied with storage time (Table 1). Prior to storage (0 week), ethylene from loblolly and slash pine roots, stems, and needles was below detectable levels. Highest concentrations of ethylene in loblolly pine were detectable after 1 week in cold storage, followed by a progressive decline in all tissues with storage time. While the pattern of decline was similar for all tissues of loblolly pine, ethylene from slash pine stem tissue remained constant; the needles progressively declined and root tissues increased. Loblolly pine

Table 1. Emanated ethylene from different tissues of loblolly and slash pine seedlings sampled during storage.

Time in Storage	Levels of emanated ethylene (nl/g tissue) loblolly pine root	loblolly pine stem	loblolly pine needle	slash pine root	slash pine stem	slash pine needle
0 week	0	0		0	0	0
1 week	23.5±2.1	33.6±3.0	29.4±3.1	0	14.8±1.7	20.2±1.9
2 weeks	14.5±1.6	20.6±2.2	19.6±2.1	15.3±1.3	15.3±1.0	18.3±2.0
4 weeks	10.6±1.2	11.5±1.1	15.2±1.6	23.2±2.0	14.0±1.3	0

appears to produce more total ethylene than slash pine during the first 30 days of storage, but production was declining. Stumpff (1984) also found that ethylene decreased with storage time with loblolly seedlings. Ethylene production by excised tissues accurately reflected the trend in ethylene production by entire seedlings, suggesting that the ability of a seedling to produce ethylene during cold storage could be assessed conveniently using plant segments.

Entire seedlings

Ethylene levels measured for entire seedlings by treatment are shown in Table 2. They illustrate the difference between species in their capacity for production of ethylene with storage time.

In the natural treatment, loblolly seedlings produced much more ethylene during the first half of the storage period than the last half. This declining production of ethylene agrees with the findings of Stumpff (1984) and with the findings with excised tissues. With slash pine, the level of ethylene remained the same after 16 days and Virginia pine produced more ethylene the latter half of the period.

The addition of ethylene can inhibit or stimulate further ethylene production depending upon species, kind of tissue, concentration, and conditions of the experiment (Abeles 1973). The levels of inhibition or stimulation cannot be precisely stated for these species based on the results obtained with the techniques used. However, the 5.0 ppm treatment stimulated ethylene production in all species over the longer storage time (30 days). The .05 and 0.5 ppm treatments had lower levels of ethylene when compared with the natural treatment with one exception. This may be due to inhibition of ethylene production; however, more sophisticated research techniques would be necessary to ascertain this.

Table 2. Ethylene levels (ppm) in bags of loblolly, slash, and Virginia pine seedlings in storage.

Ethylene Treatments	Purafil Added		Natural		.05 PPM		.5 PPM		5.0 PPM	
Storage Periods	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days
Species										
Loblolly*	< .01	< .01	4.4	0.9	0.1	0.2	1.0	1.0	8.0	8.0
Slash	< .01	< .01	1.0	1.0	0.06	0.2	0.7	0.7	5.0	13.8
Virginia*	< .01	< .01	2.4	3.0	0.3	0.5	4.0	0.7	9.6	11.0

*Bags of seedlings in the 30 day treatments were opened and aerated at 16 days.

Root regeneration potential (RRP)

Root regeneration potential of slash and Virginia pine seedlings subjected to five different ethylene concentrations was not significantly different ($P < .05$). However, there is a trend of inhibition of lateral root formation for slash pine seedlings stored with Purafil and Virginia pine seedlings in the 0.5 ppm treatment (Table 3). Maximum reduction in vigor and inhibition of lateral root formation at 0.5 ppm were also found for Douglas-fir seedlings (Anonymous 1979, Graham and Linderman 1981). The slash pine seedlings obtained in 1985 did not appear to be first quality seedlings as evidence by a sparse root system and a high shoot to root ratio. The low RRP for slash pine seedlings stored with Purafil and higher RRP's for seedlings stored with ethylene added indicates that seedlings in poor physiological condition may require higher ethylene levels to stimulate lateral root formation. The addition of Purafil, which lowered bag concentrations to below 0.01 ppm in loblolly pine seedlings, significantly stimulated the formation of new first order and second order roots, i.e. RRP (Table 3). Barnett (1980) also found improved RRP when loblolly pine seedlings were stored with Purafil. Low levels of ethylene (.006 - .01 ppm) also stimulate lateral root formation in Douglas fir (Graham and Linderman 1981). Higher ethylene concentrations (5.0 ppm) were found to enhance lateral root formation in Douglas fir, ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws), white fir (*Abies concolor* (Gord. and Glend.) Lindl.) and barley seedlings (*Hordeum vulgare* L.) (Alvarez and Linderman 1983, Anonymous 1979, Crossett and Campbell 1975). In contrast to these findings, higher ethylene concentrations in the 5.0 ppm treatment did not appear to stimulate root formation in loblolly pine (Table 3). The root system of loblolly pine was more sensitive to different ethylene concentrations than slash and Virginia pine.

Table 3. Root regeneration of loblolly, Virginia, and slash pine seedlings grown for eight weeks in a greenhouse

Species	Loblolly			Virginia			Slash		
Ethylene Treatment	First Order Roots	Second Order Roots	Total number = RRP	First Order Root	Second Order Roots	Total number = RRP	First Order Roots	Second Order Roots	Total number = RRP
Purafil	*	*	*	NS	NS	NS	NS	NS	NS
Added	10.85a	52.65a	63.5a	8.3	52.0	60.35	1.5	30.2	31.7
Natural	10.35ab	43.10 bc	53.45 b	7.8	58.5	66.3	2.6	33.5	36.1
.05 ppm	5.6c	42.80bc	48.40 b	12.6	50.75	63.35	2.7	35.9	38.6
0.5 ppm	7.4bc	45.90ab	53.30 b	7.6	39.55	47.15	3.9	34.2	38.2
5.0 ppm	8.0 abc	36.13 c	44.15 b	7.8	46.0	53.8	3.0	34.7	37.8

* Means not followed by a common letter differ ($P < .05$) as determined by LSD
NS Not Significant

Shoot growth

There was no significant difference in height growth, eleven weeks after outplanting, of Virginia pine seedlings stored under different ethylene concentrations (Table 4). Greatest height growth occurred in loblolly pine seedlings stored in 0.5 ppm and 0.9 ppm (natural-30 day treatment) for 16 days. High ethylene concentrations significantly inhibited height growth of loblolly pine seedlings stored in the natural and 5.0 ppm treatments for 16 days and stimulated height growth of slash pine seedlings in the 5.0 ppm treatment of ethylene (Table 4). An ethylene concentration of 5.0 ppm in the storage environment was found to increase apical bud burst of ponderosa pine, Douglas fir, and white fir (Alvarez and Linderman 1983). Hinesley (1980) found that 17.5 ppm of ethylene in the storage atmosphere significantly reduced terminal shoot elongation in Fraser fir (Abies fraseri (Pursh) Poir.) seedlings and increased terminal bud abortions. Reduced height growth, terminal bud abortion, and needle senescence were more evident in slash pine stored in natural levels (1.0 ppm) of ethylene. Stimulation or inhibition of shoot elongation in response to ethylene is dependent upon ethylene concentration and species sensitivity.

Table 4. Height growth (cm) of loblolly, slash, and Virginia pine seedlings eleven weeks after planting.

Ethylene Treatments	Purafil Added		Natural		.05 PPM		.5 PPM		5.0 PPM		
Storage Periods	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	
Species											
Loblolly	7.7^{bc}	7.1^{c}	5.4^{d}	9.2^{a}	7.4^{c}	6.8^{c}	8.9^{ab}	6.8^{c}	5.4^{d}	7.3^{c}	*
Slash	3.4^{de}	3.9^{cd}	2.8^{e}	2.8^{e}	2.8^{e}	5.1^{ab}	3.2^{de}	4.4^{bc}	3.6^{cde}	5.5^{a}	*
Virginia	7.4	7.4	7.6	7.7	7.9	8.8	7.0	8.8	8.4	8.4	NS

*Means not followed by a common letter differ ($P < .05$) as determined by LSD
NS Not Significant

Secondary branch formation occurred on the upper third of loblolly pine seedlings stored in higher levels of ethylene (natural 16-day and 5.0 ppm treatments). This may be due to a response similar to that found in other studies. Ethrel, an ethylene releasing compound, has been reported to induce growth of basal and lateral buds of woody plants (Abeles 1973). Hillman and Yeang (1979) in their work with Phaseolus vulgaris L. found that ethylene released axillary buds indirectly by inhibiting active apical growth, leading to a redirection of nutrients and other growth factors.

An epinastic response of needles occurred in Virginia pine seedlings stored in higher ethylene concentrations (0.5-5.0 ppm treatments). This is a

characteristic ethylene response of some species noted in previous studies (Abeles 1973).

Field survival

There was no significant difference in survival of loblolly and Virginia pine seedlings stored in different ethylene concentrations (Table 5). Field survival of loblolly pine and western conifers were also unaffected by ethylene treatments in cold storage (Johnson and Stumpff 1984, Alvarez and Linderman 1983). Survival of slash pine seedlings was significantly reduced when stored in natural levels of ethylene, approximately 1.0 ppm, for 30 days. Reasons for this low survival are not readily apparent.

Table 5. Percent field survival of loblolly, slash, and Virginia pine seedlings sixteen weeks after outplanting

Ethylene treatment	Purafil		natural		.05 ppm		0.5 ppm		5.0 ppm	
Storage Period	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days	16 Days	30 Days
Loblolly	98	100	100	100	98	100	100	97	100	100
Slash	88a	88a	80a	62b	88a	93a	85a	97a	93a	92a*
Virginia	97	100	98	100	100	100	98	98	98	100

*Means not followed by a common letter differ ($P < .05$) as determined by LSD

SUMMARY AND CONCLUSION

Results demonstrate that ethylene is produced by all seedling parts of the species used in this study. Ethylene production by these plant parts varied with time in storage. Wide differences were found in the response of loblolly, slash, and Virginia pine seedlings to different concentrations of ethylene.

Ethylene present in the storage environment, regardless of concentration, appeared to have no effect on Virginia pine seedlings. This may be due to the fact that Virginia pine seedlings consistently produced high amounts of ethylene during storage which reduced their sensitivity to exogeneous ethylene. Storing loblolly pine with an ethylene absorbent improved root regeneration while higher ethylene levels inhibited height growth when seedlings were stored for 16 days. Survival and height growth was significantly reduced when slash pine seedlings were stored in naturally produced levels (1.0 ppm). Overall, field survival of the three species was not significantly affected by ethylene present in the storage environment, at the levels measured. Thus, ethylene in storage environments is not a significant factor affecting the survival and performance of loblolly, slash, or Virginia pine seedlings after outplanting.

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