GIBBERELLIN SYNTHESIS INHIBITOR AFFECTS ANNUAL XYLEM PRODUCTION AND VESSEL ELEMENT ANATOMY IN SOME TREES

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ABSTRACT

Red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.), sweetgum (*Liquidambar styraciflua* L.), and yellow poplar (*Liriodendron tulipifera* L.) were treated with paclobutrazol using the soil drench or soil injection method at a dose rate of 9.6 grams a.i. per tree. Five or six growing seasons after treatment, trees were harvested and cross-sections of the main stem were removed for analysis. Total tree height, diameter growth of the trunk, the width of annual rings of xylem, and the size and number of vessels in the earlywood were compared in paclobutrazol treated and untreated trees. Tree height was reduced in all four species, whereas diameter growth at 137 cm above ground-line (DBH) and annual ring width for five or six growing seasons were reduced by 33 and 36 percent, respectively, only in sweetgum and white oak trees treated with paclobutrazol. The cross-sectional area of individual vessel elements also was reduced by paclobutrazol treatment only in white oak and sweetgum. The number of vessels per unit area of xylem tissue was not affected by paclobutrazol in any of the four species.

INTRODUCTION

The growth regulator paclobutrazol (PBZ) used by arborists to reduce shoot growth has been shown to have additional benefits for trees and shrubs including improved resistance to drought stress, darker green leaves, protection against some fungal and bacterial pathogens, and enhanced development of fibrous roots (Chaney 2003; Chaney et al. 1996; Fletcher et al. 2000; Rademacher 2000). Cambial growth, as well as shoot growth, has also been shown to be reduced for up to three years after treatment in some tree species (Bai et al. 2004). The objective of this experiment was to determine total tree height, diameter growth of the trunk, the width of annual rings of xylem, and the size and number of vessels in the earlywood of several trees species five or six years after they were treated with the growth retardant paclobutrazol or left untreated.

MATERIALS AND METHODS

Experimental trees were located at Martell Experimental Forest Farm near the Purdue University campus in Tippecanoe County, Indiana. Red oak (*Quercus rubra* L.) and white oak (*Quercus alba* L.) ranging from 8-10 cm basal diameter were treated in April 1995 with paclobutrazol (PBZ) using the soil drench method at a dose rate of 9.6 grams a.i. per tree. The following April, sweetgum (*Liquidambar styraciflua* L.) and yellow poplar (*Liriodendron tulipifera* L.) (4-8 cm basal diameter) were treated with paclobutrazol using the soil injection method at the same dose rate. At the end of the growing season in 2000, three control and three treated trees of each species were randomly selected and harvested at ground level. Total tree

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height and diameter 137 cm above ground line (DBH) were measured before a 2.5 to 5.0 cm thick cross-section was removed approximately 137 cm from the base of each tree.

The stem disks were sanded smooth using a rotary sander to enable better viewing of the xylem rings at low magnification. Annual xylem ring widths were measured for the 1995-2000 growing seasons for the red and white oak and for the 1996-2000 growing seasons for the sweetgum and tuliptree with an Acu-Rite III digital readout system (Acu-Rite, Jamestown, New York). The samples were measured by placing them on the movable stage of the instrument and viewing the annual rings of xylem through a 10X magnifying glass with a cross-hair. As the stage was moved by a hand crank, the digital readout gave a measurement of ring width to the nearest 0.001 mm. Measurements were taken at four points at right angles on each sample.

To determine the cross-sectional area of vessels in the earlywood of the 2000 annual growth ring, a small section of wood was cut from each disk. The pieces were boiled for approximately one hour to soften the wood for sectioning. Twenty-um-thick cross-sections were made using an A.O. Spencer Sliding Microtome, model 860 (American Optical Corporation, Buffalo, New York). The microtome slices were stained with a 1% solution of safranin and mounted on glass slides. Ten vessel cells were randomly selected in the earlywood and two diameters of each cell at right-angles were measured at 20X magnification using a microscope with an ocular eyepiece. Cross-sectional area of the cells was determined using the equation Area = π x length 1 x length 2.

The number of vessel cells was also counted for each sample. Samples were viewed at 10X magnification and all the vessels in ten replications of an area of 1 mm^2 were counted.

Data were analyzed using analysis of variance and comparison of means with Tukey's Studentized Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Total tree height was reduced six years after treatment with paclobutrazol in white and red oak and five years after treatment in sweetgum and tuliptree (Table 1). Although no measurements of height were made at the time of treatment in 1995 or 1996, the height of the white oak and red oak were measured in 1991 for another study using the same trees and no difference in height was found within each species (Chaney and Byrnes 1993).

Table 1. Comparison of total height of trees untreated or treated with paclobutrazol.

Treatment	White Oak	Red Oak	Sweetgum	Tuliptree
	…………………………	m	…………………………	
Control	8.4 a*	8.9 a	9.9 a	11.1 a
PBZ-Treated	5.8 b	7.1 b	9.3 b	6.6 b

*Means in columns followed by the same letter are not statistically different ($p \leq 0.05$).

The width of the annual xylem ring increment was reduced in white oak and sweetgum beginning the year these trees were treated and continued to be reduced thoughout the study (Table 2). Annual accumulation of xylem was not affected in red oak until the fifth year after treatment, whereas tuliptree was not affected by treatment with paclobutrazol. This is the same pattern of response for the same tree species reported in an earlier study but over a shorter time period (Bai et al. 2004).

Table 2. Comparison of annual xylem ring increment (mm) of red oak and white oak for six years after treated with paclobutrazol (PBZ) and sweetgum and tuliptree five years after treatment with paclobutrazol.

Treatment	Species	Year					
	Red oak	1995	1996	1997	1998	1999	2000
Control		2.77 a*	2.13 a	2.09 a	2.69 a	2.74 a	3.33 a
PBZ-Treated		2.81 a	2.57 a	2.42 a	2.11 a	1.92 a	2.08 b
	White oak						
Control		3.04 a	3.08 a	2.08 a	2.79 a	1.51 a	2.00 a
PBZ-Treated		1.55 b	0.70 b	0.54 b	0.50 b	0.46 b	0.58 b
	Sweetgum						
Control			4.04 a	3.17 a	3.85 a	3.10 a	2.99 a
PBZ-Treated			3.29 b	2.39 a	1.55 b	1.68 b	1.34 b
	Tuliptree						
Control			2.89 a	2.95 a	2.34 a	2.60 a	3.71 a
PBZ-Treated			2.72 a	2.13 a	2.01 a	2.24 a	2.88 a

*Means in columns for each species followed by the same letter are not statistically different ($p \leq 0.05$).

The number of vessel elements per square millimeter of cross sectional area of the xylem did not varied between untreated and PBZ-treated trees for any of the four species investigated (Table 3). The number of vessels in white and red oak is small because these tree species are ring-porous and typically have fewer but larger vessels in the earltwood, whereas sweetgum and tuliptree are diffuse-porous in anatomical structure and have a large number of small vessels evenly distributed across annual rings of xylem.

Table 3. Number of vessel elements per mm^2 of cross sectional area.

Treatment	White Oak	Red Oak	Sweetgum	Tuliptree
	………………………	mm^2	…………………………..	
Control	9.52 a*	8.64 a	60.87 a	59.20 a
PBZ-Treated	10.10 a	9.46 a	59.67 a	54.19 a

*Means in columns followed by the same letter are not statistically different ($p \leq 0.05$).

Cross-sectional areas of vessels in white oak and sweetgum were significantly reduced in PBZ-treated trees compared to those in untreated trees, whereas the area of vessels in red oak and tuliptree was unaffected (Table 4).

The data reported here substantiate well established responses of reduction in height growth following treatment of trees with paclobutrazol (Fletcher et al. 2000; Rademacher 2000). Reductions in cambial growth in some tree species, but not others, have also been reported (Bai et al. 2004; Estabrooks 1993; Lehman et al. 1990), but the data reported here show that the effect of paclobutrazol can extent for at least 5-6

Table 4. Average cross-sectional area (mm^2) of individual vessels.

Treatment	White Oak	Red Oak	Sweetgum	Tuliptree
	………………………	mm^2	…………………………..	
Control	0.20 a*	0.16 a	0.0073 a	0.0086 a
PBZ-Treated	0.14 b	0.16 a	0.0033 b	0.0077 a

*Means in columns followed by the same letter are not statistically different ($p \leq 0.05$).

years after treatment of trees. In marked contrast to most published reports, Costa et al. 1995 showed an increase in trunk diameter of 'Blanquilla' pear trees when they were treated with paclobutrazol. The reduction in cross-sectional area of vessels found for white oak and sweetgum has not to our knowledge been reported before. Change in xylem anatomy could increase the resistance to water flow in transpiration and smaller vessels could reduce the potential for movement of disease organisms in xylem.

Generally, the larger springwood vessels of ring porous tree species are more conducive to rapid distribution of oak wilt (*Ceratocystis fagacearum*) conidia. In addition, tylose formation is much more extensive in large earlywood vessels as compared to smaller late vessels (Drake 1956; Nair 1964; Parmeter et al. 1954;). Larger vessels are also more susceptible to embolisms. Trees infected late in the summer or fall of the previous year often do not show wilt symptoms until the formation of earlywood begins the following year. This has been noted in numerous cases where trees inoculated on various dates late in the season, all show symptoms on nearly the same date the following year (Nair 1964; Skelly and Merrill 1968; Skelly and Wood 1974).

There have been several reports of growth regulators being used to alter host anatomy and to reduce susceptibility to oak wilt (Kuntz et al. 1968). Northern pin oaks treated with 2,3,6-trichlorophenyl acetic acid formed wood without xylem vessels, but these trees were able to conduct adequate water for growth and transpiration through xylem parenchyma, fibers, and tracheids. When trees infected with oak wilt (*C. fagacearum*) were treated with this compound, the pathogen and vascular plugging was confined to vessels formed prior to treatment (Geary and Kuntz 1962; Venn et al., 1968).

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