CONSTITUENT AND INDUCED TANNIN ACCUMULATIONS IN ROOTS OF LOBLOLLY PINES

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1. INTRODUCTION

Loblolly pine (*Pinus taeda* L) has become the most important source of wood fiber in the southern United States. This tree is an excellent competitor and recovers well from a variety of adverse conditions.[1,2] The value of loblolly pine as a plantation tree has encouraged many researchers to devote their careers to the study of this species. It is surprising then that the root system of loblolly pine has received so little attention! I felt that an anatomical study of loblolly pine roots would be valuable and chose to measure tannin abundance as a primary trait to evaluate root health at the microscopic level.

This histological study of tannin in pine roots follows extensive microscopy of pine cells in culture and in stem tissue begun in 1963.[3] Of particular interest throughout my studies is the manner in which tannins are contained in cellular organelles until tissue death or cell membrane rupture. These tannins are nonspecifically absorbed by cell walls and organelles. These processes are easily observed in loblolly pine roots that have a high turnover rate, especially cortical cells of mycorrhizal roots.

Nutritional tests with pine callus cells indicate that cells accumulate tannin within cell membranes of the endoplasmic reticulum.@ When nutrients such as nitrogen are altered, many callus cells fill with tannin. This seems also to occur in pine roots on certain sites. The hypothesis of this study was that two processes of tannin accumulation occur in root tissue. Constituent tannins accumulate in all root tissues, whereas the process controlling induced tannin is related to stress. Also, it appears that tannin has a close relationship to the deposition of cellulose and lignin in cell walls. After observing many thin sections, I focused on anatomical differences between constituent tannin accumulation in periderm cells and induced tannin in cells responding to stress. This paper describes the types of

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tannins in roots and quantifies their occurrence in plantation-grown loblolly pines.

2. MATERIALS AND METHODS

Observations of tannin anatomy were made of roots from 5- to 40-year-old loblolly pines in plantations. Ten trees were selected at random from each plantation. Each plantation was sampled from one to six times. A total of 2,619 roots were collected in Louisiana, 623 in Mississippi, and 1,221 in North Carolina.

After the observational study, tannin in roots from different levels of soil compaction and organic matter was quantified. The soil treatments have been described in detail.' Fourteen roots from 5 to 10 trees in 9 soil treatments were collected for a total of 1,017 roots.

An experiment to measure effects of severe stress on tannin accumulation used 1,447 roots from 68 trees that were progeny of control-pollinated parents. Treatments were felling, girdling, and severe pruning of crowns. Experimental trees were in their twelfth growing season and were located near the plantation's center. In all cases, roots were collected from a 25- by 25-cm area located lm from the stem of the pine. Roots were taken from a depth of 2 to 20cm and the roots were gently shaken to remove excess soil. The center 2 to 4cm of each root was excised and placed into formalin-acetic acid-alcohol (FAA) fixative for 14 days.' Fixed-root specimens were recut to 1 to 3mm, dehydrated in alcohol series, embedded in paraffin, and cut into 7 to 10 micron transverse sections. Sections from 12 to 18 roots were mounted on a slide. Nine slides were stained with a variety of schedules, including Papanicolaou's schedule, an acid-Schiff procedure, toluidine blue, ferric salt, safranin-aniline blue, acid fuchsin, Giemsa, Congo red, and Groett's methenamine.[9-11] Polarized light was used to differentiate tannins and primary cell walls from secondary cell walls, sclereid cells, and starch granules."

Root traits were defined as follows: *Cortex shedding:* Cortical cells are dead and either remain attached to the root or are released into the soil. The shed is invaded by many soil microbes. *Periderm forming:* This is the first stage of coating residual cortical cells with a protective layer of tannin and cellulose:lignin complex. This is constituent tannin. *Bark formation:* Bark cells encompass the root. *Degradation of starch:* Starch grains are in 50 percent of the cells. *Induced tannin:* Tannin-containing cells accumulate in cortex, rays, and inner xylem. These cells number from 10 to *over* 100. *Dead mycorrhizae:* Short roots that shed in cortical cell death. *New lateral roots:* Roots that originate in the xylem and phloem. ***Infection:*** Cambial or fiber cells infected. Invasion usually kills the tissues and the root. *Starch grains:* Number of plastids per cell with starch as viewed at a single focal length at 100 to 500 diameters.

For statistical analysis in soil compaction studies, trees within plots were used as replicates. This does not fit the rigid requirement of randomization. In the stress studies, analysis of variance was used to separate the means of the treatments. Regression analysis was used to approximate relationships between scored traits.

3. CONSTITUENT TANNINS

The constituent tannin component of the periderm appears immediately after the shed of the root cortex. The proportion of roots with this tannin was statistically different when soil bulk density was altered (table 1). As soil compaction decreased and organic matter increased, the synthesis of bark increased. The anatomy of tannin in periderm cells was similar for all soil treatments. This similarity was seen for both the tannin and cellulose and lignin containing cells in roots and stems of loblolly pines.

A typical loblolly pine root in secondary growth (fig. 1) uses starch (fig. 2) and produces periderm (bark) cells (figs. 3,4). Many layers accumulate to reduce the initial vulnerability that is created by the shedding of cortical cells. Roots that are 1 mm or shorter may not synthesize sufficient cellulose and lignin elements. Excess tannin cells temporarily accumulate (fig. 5). The incidence of periderm without tannin (fig. 6) occurred in less than one root per thousand.

Tannin in bark cells persisted in all stress treatments and well beyond cortical and cambial death (figs. 7,8). Five or more layers of cells with apparently normal tannin were seen in dead roots,

4. INDUCED TANNIN

Induced tannin accumulated in all parts of the root tissue. It was significantly higher (p = 0.001) in roots of loblolly pines that were lmm in diameter. For undisturbed trees, the proportion of roots with non-periderm tannin was 0.80, 0.37, and 0.05 for roots 0–1 mm, 1.1–10 mm, and llmm or greater in diameter, respectively The mean for proportion of tannin in 2,000 roots was 0.12. The proportion of roots with induced tannin was negatively correlated with numbers and size of starch grains in parenchyma cells (R = -0.56 and -0.50, respectively). All sectioned roots from undisturbed trees were normal using the criteria of stain affinity for nuclei and cambial elements.

To test the effects of mild stress on tannin accumulation, roots were collected from 6-year-old loblolly pines. Of the variety of soil treatments that were installed

Table 1. Effect of soil treatments on incidence of root processes

Trait	Severe compaction, O.M. removed	No compaction O.M. left	Probability significance
	Percentage of root sections		
Periderm/tannin present	52	28	0.002
Bark cells with tannin	43	70	0.008
Depletion of starch	29	6	0.008

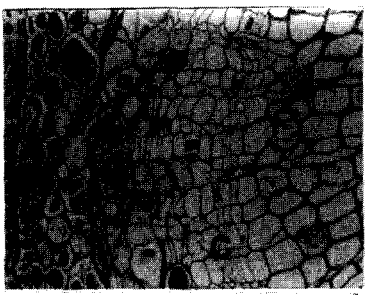

Figure 1. Typical transverse view of cells in a loblolly pine root. Note cambium (C) and starch grains (SG). The scale bar is 50 microns in all figures.

Figure 2. Stages of starch grain (SG) utilization. Depleted of starch (D).

Figure 3. Cellulose: lignin (CL) and tannin (T) components of the developing periderm of a root in secondary growth.

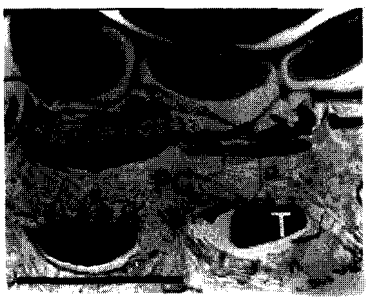

Figure 4. Completed periderm cells (PC) with constituent tannin (T).

Figure 5. Excess tannin (T) accumulating at the site of periderm formation.

Figure 6. Periderm cells (PC) that lack tannin. Note developing tannin elements (TE) in new periderm.

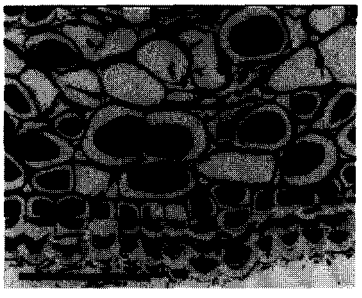

Figure 7. Intact periderm (P) in a root starved for 5 months.

Figure 8. Multilayered bark cells enclosing a dead root. Cortical cells (CC) lack structure.

in standard plots, only the severely compacted soil and soil with total aboveground biomass removed before planting showed differences. These rather extreme treatments did not affect tannin accumulation directly. However, periderm formation slowed. Induced tannin was low in all the root samples. Roots were healthy, and wounds were not common.

Roots were starved by felling or girdling stems. Starch was reduced in roots by removing 50 to 67 percent of the crown. Other roots were killed by prescribed burning.[13] After 4 or 5 months, roots from all felled trees began to accumulate more tannin than uncut controls. Roots from felling in the winter were statistically different after 5 months. Those from spring felling were statistically different after 4 months. The proportion of large roots with induced tannin for winter felling was 0.17 as compared to 0.54 for spring felling and 0.05 for the control. After 6 months, the mean for the large roots of trees felled in the winter was 0.83 (table 2). Tree values for proportion of roots within a sampling period ranged from 0.20 to 1.00, but they were not significantly different. However, ANOVA with month as the class showed month after felling to be significant ($P = 0.001$). Proportion of roots with tannin O-6 months after winter felling was not correlated to the number or condition of starch grains in roots (table 3). Observations of nuclei and cambial cells showed 61 percent of the roots were dead 6 months after winter felling. Since tannin was highest in roots 5 months after felling, this treatment period was used in sampling girdled or crown-pruned trees. The proportion of roots with tannin was 0.31 for girdled and 0.50 for severely pruned trees. Nuclei and cambial cells were normal in both treatments and not significantly different from the controls. Number and size of starch grains were significantly lower than controls and approximately twice as high as winter felling tree roots in the 5-month collection.

The number of cells with induced tannin in roots was highly variable. Roots from control trees and trees in soil treatments had low induced tannin. However, roots that were starved for at least 4 to 5 months or subjected to high heat contained high amounts of induced tannin. Examples of induced tannin

Table 2. Incidence of observed variables following winter felling of loblolly pines[a]

Month after felling	Starch use	Induced tannin	Cambium abnormal
	Proportion of roots with trait		
0	0.94	0.26	0.0092
1	0.95	0.53	0.0
3	0.96	0.53	0.024
4	0.78	0.38	0.027
5	0.30	0.63	0.384
6	0.0	0.83	0.611

[a] Traits were tabulated for 612 roots that were sectioned and stained.

Table 3. Relationship between starch and tannin after winter felling[a]

Month after fell	Number of roots	Starch grains vs tannin	Starch grain use vs tannin
		----------Pearson correlation coefficients----------	
0	109	-0.34	-0.02
1	130	-0.22	-0.21
3	126	-0.27	-0.19
4	112	-0.38	-0.34
5	117	-0.38	-0.35

[a] Roots from non-felled trees averaged -0.50 and -0.24 for the two comparisons.

accumulations are illustrated in figures 9-12. The tannin in figures 11 and 12 is accumulated in cortical-derived and modified thin-walled cells. As starvation progressed in stress treatments, cell structure disintegrated, and tannin bound to cell wall elements (fig. 13).

Fire was used to cause rapid death of roots. A thinned 40-year-old plantation was prescription-burned, and roots were collected at 2 to 20 cm depth after 30 days. Roots without lesions were pooled from 10 trees, fixed for light microscopy. Examination of 46 roots revealed 22 with dead nuclei and cambial elements. Their structure was normal, but induced tannin coated the cell walls of the cortex (fig. 14).

Figure 9. Emergence of a lateral root that is protected by tannin (T). Note shed (S) composed of dead cortex.

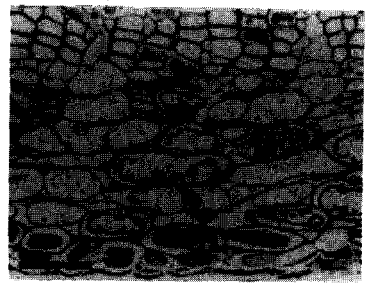

Figure 10. Granular tannin (GT) that is accumulating in the cortex of a root in nutritional stress.

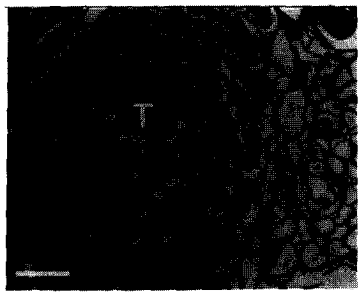

Figure 11. Tannin (T) accumulating in a wound of a root. Note the absence of a secondary wall matrix.

Figure 12. Tannin (T) being deposited to seal a severe wound affecting 40 percent of a pine root. Elements of tannin appear similar to those in figure 3.

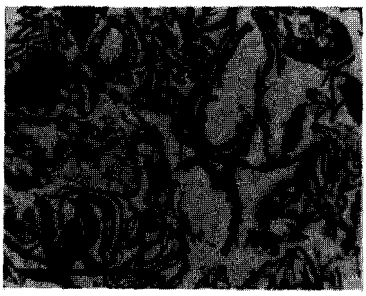

Figure 13. Appearance of cortex: cambial region of a root that died from starvation. Note tannin (T) has bound to dead cell organelles.

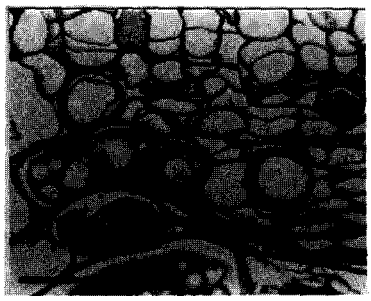

Figure 14. Pine root that has died from the heat of a burn. Much of the tissue morphology has been preserved.

Plastids were embedded in tannin, but their starch appeared to be intact. Walls of cambial cells were coated with tannin in some roots. Induced tannin that was contained within membrane-bound bodies in cells was released in most of the roots killed by high temperature. This pattern was quite different from the effects of drought on tannins reported by Pizzi and Cameron.[14]

5. CONCLUSIONS

The histological observations and analyses of deposition of tannin in loblolly pine roots confirm that two anatomical systems govern the accumulation. The first system is highly reliable since less than 0.1 percent of root periderms lacked tannin. Tannin in the periderm of roots often was deposited without the cellulose and lignin complex. Constituent tannin is apparently extremely durable in the periderm. Neither contact with soil microbes nor death of the underlying cortex generally changed the appearance of this tannin to a significant extent. Oxidations from orange to black deposits were seen in only a few roots. The formation of the tannin, cellulose, and lignin periderm appeared sensitive to soil conditions. The unfavorable silviculture treatments caused a significant delay in bark formation that led to an increased time of exposure for roots with shed bark. Roots less than 1 mm in diameter appeared to compensate for this via increased tannin excretion. Tissues from roots of trees that were felled, girdled, and severely pruned were ideal for determining the relationships between starch utilization and induced tannin accumulation. However, I did not find a statistically significant relationship. I repeatedly observed many starch grains in cells with tannin. Likewise, there was an absence of both starch and induced tannin in many roots. Cells in the process of tannin accumulation seemed to be using a source of carbon other than starch grains. Nuclei in such cells stained as though they were actively metabolizing.

Staining of tannin was not a necessary emphasis in this study.[15] Emphasis was placed on using stains to classify as functional the nucleus and other cell components. These staining schedules often enhanced the natural color of the tannins. The most interesting finding was the discovery that nuclei were active in cells that appeared filled with tannin (figs. 3,4). This verified my earlier report about stem tissues and further indicated that tannin is normally isolated from other organelles in the pine cell.[16] This feature appears of major importance in containing tannin in wounds without inhibiting adjacent cells.

Continuous generation and repair of the periderm and cortical tissues of roots are needed to overcome adverse effects by soil and microbial actions.[17,18] Sealing the many wounds from dying root hairs, mycorrhizae, and short roots is an important role for induced tannin. Roots are especially vulnerable to microbes during shedding of cortical cells. The induction of tannins causes the conversion of the root cortical cell into an elongated tannin body (figs. 3,4,5). The cellulose and lignin unit in the periderm (fig. 3) did not form in conjunction with induced tannin synthesis (see figs. 11 and 12 for mass of tannin cells). The induced tannin was in thin primary cell walls. The mass of tannin cells was generally disordered (fig. 11). The stress appeared to be reflected in lignin synthesis. Stress-induced phenylpropanoid metabolism as a precursor of tannin has been recently reviewed by Dixon and

Paiva.[19] Also, Stafford discussed relationships between tannins and lignins[20] in general. However, the relationship between lignin and tannin formation appears to be different for constituent and induced tannin. This is an exciting area for further histochemical research.

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