Alterations in Growth and Water-Transport Processes in Fusiform Rust Galls of Pine, Determined by Magnetic Resonance Microscopy

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ABSTRACT

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Galls on 10-mo- to 2-yr-old slash and loblolly pine seedlings inoculated with *Cronartium quercuum* f. sp. *fusiforme* were compared with healthy stems by magnetic resonance microscopy (MRM). After transpiration, high-resolution images (35–58 μm) of excised stem segments were acquired. MRM images showed a greater signal in the xylem of healthy stems than in galls, suggesting differing wood/water interactions. In 10-mo-old galled seedlings, the cambium and phloem were contiguous between healthy and galled regions. Water-transport disruption occurred in the xylem at the interface between galled and healthy regions, but in the center of the galls, secondary xylem appeared water-filled. At 2 yr of age, water transport was observed throughout the secondary xylem of a healthy stem but was completely impeded through the gall of a declining seedling. In contrast, a bright ring of conductive secondary xylem surrounded a dark, nonconductive center in the gall of a vigorous, 2-yr-old seedling. This study shows changes in anatomy and functional physiology in vivo with respect to water relations in fusiform rust galls on pine that are detectable by MRM.

Additional keywords: magnetic resonance imaging, MRI, plants.

Fusiform rust is one of the most prevalent and economically damaging diseases of pines in the southern United States. Both loblolly (*Pinus taeda* L.) and slash pines (*P. elliottii* Engelm. var. *elliottii*) are frequently infected, producing both stem and branch galls. Losses result not only from reduced wood marketability and tree mortality, but also from growth reductions and stem and branch breakage, resulting in reduced yield and wood quality (1). Although significant research has focused on the identification of resistant host families, mechanisms of resistance and tolerance have not been identified. In addition, the pathogen has been reported to have significant genetic variation, even between single-urediniospore isolates (2).

The pathogen, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow, is a macrocyclic heteroecious rust fungus, alternating between oaks and pines. In active galls of pine, vegetative mycelium is restricted to the ray parenchyma and cambial layers (5–7). Standard histological methods have shown seedlings of loblolly and slash pine with fusiform rust form both stem and branch galls displaying severe cellular dysplasia, hypertrophy, and alterations in cellular organization (4,6). Lack of cambium and xylem fibers in the most swollen regions of some stem-girdling galls has been reported, with significant necrosis observed in the constricted stem region below the gall (20).

Although this disease has been the subject of intensive research focusing on anatomical alterations and identification of resistant germ plasm, specific mechanisms mediating gall formation and the effect of galls on tree-water relations are not understood. Little is known about how trees develop strategies for tolerance and continued growth despite the development of stem and/or branch galls.

New techniques such as high-resolution magnetic resonance imaging (MRI) or magnetic resonance microscopy (MRM) offer the potential for detailed, nondestructive examination of plant tissues (10,12). In MRM, image contrast depends not only on water distribution within the tissue, but also on physiological functions mediated by degrees of water binding, reflected in proton relaxation times (the rate at which ^{1}H nuclei return to equilibrium after excitation from an externally applied pulse). Like classical histopathology with light microscopy, varied strategies for image acquisition with MRM can provide both detailed anatomical and functional information. Image contrast can be varied significantly, depending on the acquisition strategy used and can be adjusted to highlight water-binding, -distribution, -diffusion, and -transport patterns (10).

The primary objective of these studies was to determine the alterations in water-transport processes caused by changes in stem morphology with the development of fusiform rust galls. MRM was used to provide information on tissue organization and water transport throughout galls formed on stems of young loblolly and slash pines.

MATERIALS AND METHODS

Seeds of half-sib seedlots of slash and loblolly pine were germinated in a mix of sand/peat (1:1) and maintained in the greenhouse at 20–30 C, with 18 h of light. At 6–9 wk of age, seedlings were artificially inoculated by the concentrated basidiospore spray system (13) with basidiospores of *C. q. fusiforme* collected from oak. Individual seedlings 12 and 18 mo from seeding that displayed a range of rust symptoms, from no apparent gall formation to well-developed galls, were selected for MRI.

Immediately prior to MRI, stems were excised at the root collar and placed in either tubes filled with water or tubes containing a 1:200 dilution of an MR-contrast agent (tradename Magnavist,

Berlex Laboratories, Wayne, NJ). Shoots were illuminated to stimulate transpirational uptake. This reduced possible differences in water content between stems, which might have arisen from differences in the water content of the potting mix.

The degree of water binding, or freedom of molecular precession by the ^1H nuclei in the water, determines the rates of both spin-lattice (longitudinal; T1) and spin-spin (transverse; T2) relaxation. Reductions in T1 relaxation time have been positively correlated to overall plant water potential and negatively correlated with stem water content (18). Excision of shoots followed by uptake of unlimited amounts of water, therefore, minimized transpirationally related water-potential and -limitation effects. This water-uptake step has allowed acquisition of images with contrasts dependent only on wood/water binding and on wood water content not limited by water availability within the potting media.

Paramagnetic ions, such as Gd, Cu, and Fe, dissolved in water also decrease both T1 and T2 relaxation times and can be used to change image contrasts within the areas into which they have been introduced. Application of specific, defined dilutions of an MR-contrast agent such as Magnavist (Gd containing) causes increased signal intensity in water-filled stem regions into which Magnavist has been introduced with the transpirational stream (J. S. MacFall, *unpublished data*). Application of the contrast agent, therefore, allows definitive tracking of the water-transport paths up through the stem.

After a period of active transpiration (12–14 h) during which at least 1 ml had been taken up, segments of stem (2–3 cm in length) with and without galls were excised from the shoots. Pieces of stem were wrapped in plastic foam to prevent movement and desiccation, placed in a custom-built solenoid radio frequency coil tuned to 400 MHz. In some experiments, a healthy stem and a gall were imaged simultaneously. A reference tube, 3×10^{-3}M $CuSO_4/D_2O$ (1:3), was placed in the field of view in all image sets.

Images were acquired on replicate stems. For loblolly pines, four healthy stems and two with galls with water were imaged. For slash pines, two healthy stems and three with galls from 12-mo-old trees and two healthy stems and two with galls from 18-mo-old trees were imaged. Three healthy stems and five with galls from 12-mo-old slash pines with Magnavist also were imaged.

Images were acquired on a General Electric 9T Omega imaging system (General Electric Medical Systems, Freemont, CA). After the acquisition of locator images, three-dimensional image sets were acquired. The field of view ranged from 9 to 15 mm, depending on specimen size. Images were acquired with a simple spin echo pulse sequence with a repetition time of 200 milliseconds (ms) and an echo time of 7.5 ms. The number of slices in the three-dimensional volume ranged from 64 to 256, depending on specimen size and scanner availability. Individual two-dimensional image slices were 256×256 pixels, giving a digital resolution of 35–58 μm.

Images were reconstructed and viewed off-line on a Silicon Graphics IRIX (Silicon Graphics, Mountain View, CA) or Sun workstation. Reconstruction software was written in-house. Three-dimensional rendering software was a commercial product (VoxelView ULTRA, Vital Images, Fairfield, IA). Three-dimensional image-viewing software allowed slices to be viewed both individually and as fully rendered three-dimensional data sets, allowing the three-dimensional volume to be "sliced" and viewed through any arbitrarily chosen slice-plane.

T1 relaxation times were measured on one gall and one healthy stem of slash pine. The method of partial saturation was used, wherein six consecutive images were acquired with repetition times varying from 100 to 3,200 ms (11). An iterative three-parameter fit for T1 relaxation, water content, and flip angle was used for relaxation-time calculations.

Two-year-old seedlings of slash pine also were examined for water distribution and binding within a healthy stem and stems/taproots with galls formed at the root collar. Seeds from a bulk collection were germinated and inoculated with basidiospores by the forced air protocol (8) at 6–8 wk of age. After gall development and 2 yr of growth, three trees were selected for imaging: one that had failed to form a gall (healthy stem), one tree with a gall but no other above-ground symptoms, and one that had just begun to exhibit symptoms of decline. Trees were wrapped carefully in moist paper and plastic and shipped overnight from the USDA Forest Service, Gulfport, MS, to Duke University, Durham, NC, for the imaging experiments. Root collar segments, approximately 15 cm long with both stem and taproot portions, were cut from each tree immediately prior to imaging. Attached lateral roots were severed about 1 cm from the taproot. Stem/taproot pieces were wrapped in plastic to minimize desiccation. Images were acquired in a General Electric 2T Omega imaging system. A tube containing a $CuSO_4/D_2O$ reference solution was placed immediately adjacent to the stem segment. The field of view ranged from 40 to 45 mm (depending on the specimen size), providing digital resolutions of 156–175 μm. Repetition times were 200 ms, and echo times were 6 ms. Three-dimensional volumes of 256 slices were acquired. Data sets were reconstructed, viewed, and analyzed off-line as described above. The stem segments were cut into 0.6-mm-thick slices. The wood slices were visually compared to the corresponding MRM images.

RESULTS

Significant differences in stem anatomy and patterns of water distribution and binding were observed between galls and healthy stems, both in young and in 2-yr-old seedlings. In slash and loblolly pine seedlings, similar changes in anatomy and water binding/distribution were observed (Fig. 1). Specific tissues could be differentiated in MRM images of both galls and healthy stems, but alterations in tissue organization could be seen clearly with gall formation.

Within the healthy stems and stems below the gall, the secondary xylem, phloem, cortical parenchyma, and epidermis were clearly distinguishable in the images. Concentric rings of bright and dark could be seen within the secondary xylem (Fig. 2). This pattern has been consistently observed in stems and taproots of pine (12) and apparently is representative of regions of water transport

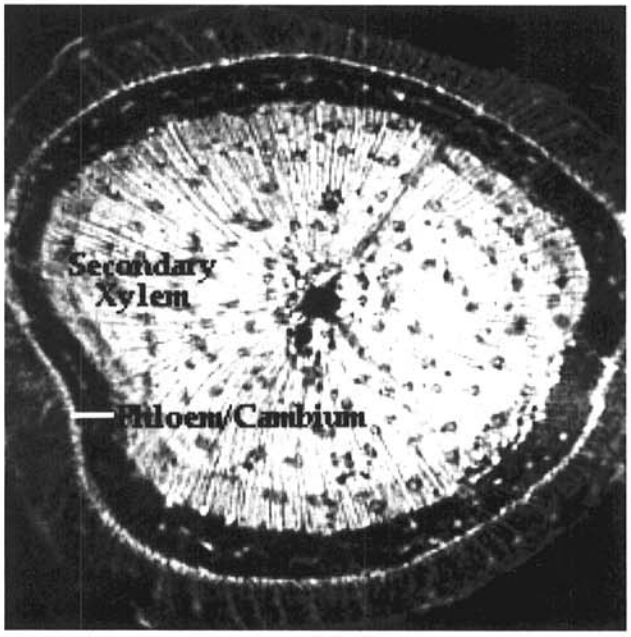

Fig. 1. High-resolution image of a cross section through a fusiform rust stem gall of loblolly pine. The tissue outside the phloem appears striated, and there are rays within the secondary xylem. The alternating pattern of bright and dark concentric rings interior to the phloem is typical of both healthy and galled stems/taproots of pine. The bright inner xylem ring is functional in longitudinal water transport. The reference tube diameter was 1.12 mm. The seedling took up a magnetic resonance (MR)-contrast agent (Magnavist) in the transpirational stream prior to the imaging experiments, increasing the signal intensity of regions functioning in longitudinal water transport.

up the stem (the bright zone) and regions not participating in longitudinal transport (the dark zone).

Identification of the regions of transpirational water transport was confirmed by examining stems that had Magnavist introduced into the transpirational stream. Previous studies (J. S. MacFall, *unpublished data*) have shown that applying a 1:200 dilution of this MR-contrast agent to water available for plant uptake will increase signal intensity (in images acquired with short repetition times) within stem regions that are functioning in transpirational water transport. The observed increase in signal in both galls and healthy stems after application of Magnavist confirms that water is transported through both galls and healthy stems. Signal increases observed in the cross-sectional views were limited mainly to the bright rings of secondary xylem, confirming that these are the regions of stem that are active in longitudinal water transport. This also confirms that the water-distribution patterns within the xylem seen in the images acquired of nontranspiring stem segments are indicative of water-transport patterns.

Changes in the anatomy of galled stems, as seen in the MRM images, were striking at the interface between healthy stem regions and regions with galls (Figs. 2 and 3). In the transition zone between healthy stem and a gall, the cambium and phloem became wider, with a proliferation of cortical parenchyma and secondary xylem. Progressing further up the stem, ray parenchyma and secondary phloem that were not seen in healthy stems formed and appeared to push out the cortical parenchyma. This new tissue was unique to galls, giving a striated appearance to regions outside the phloem ring.

Additional changes in anatomy were observed interior to the cambium of galls and within the region of transition between healthy stem and gall. Disruption in the secondary xylem was observed, as seen by the darkened, speckled areas in the region of transition. Traveling up the stem, this area appears as a dark, speckled band sweeping inward toward the gall center. It is likely that with the rapid proliferation of tracheids formed with gall development longitudinal connections were not fully formed, so end-to-end transport of water was impeded.

Within the center of the well-developed gall, little disruption in transport was apparent in the secondary xylem. This is likely indicative of lateral transport within the secondary xylem, filling with water tracheids that are not directly contiguous with xylem tissue lower in the stem. The secondary xylem also appears more striated compared to healthy tissue, due to proliferation of ray parenchyma within the gall. Similar ray enlargement in both loblolly and slash pines has been observed with light microscopy (4,6).

Alternating rings of bright and dark secondary xylem were distinguished in both galls and healthy stems. As discussed previously, the bright rings were regions of secondary xylem participating in water transport up the stem. When water without Magnavist was taken up by the plants prior to imaging, less signal intensity was measured from the bright secondary xylem rings in galls than from healthy stems (Table 1; Fig. 4). This trend was observed with both young loblolly and slash pine seedlings.

In 2 yr-old seedlings, MRM images of the galls through root collars showed differing patterns of water distribution compared

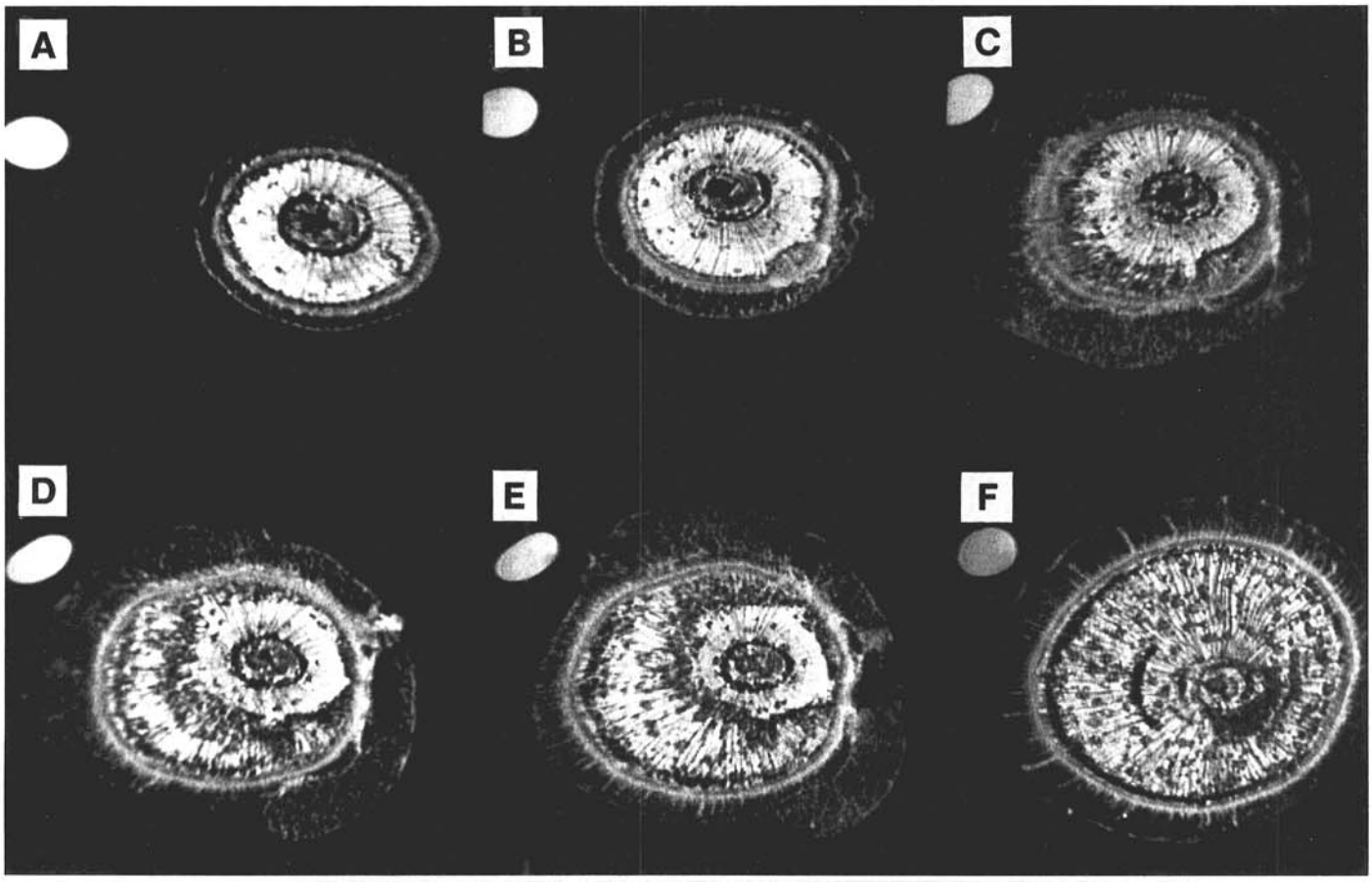

Fig. 2. Cross-sectional views of a 9-mo-old seedling at varied positions through a fusiform rust stem gall of pine. The reference tube (bright circle in upper-left corner) was 1.12 mm in diameter. **A,** Healthy stem section below gall. **B,** Beginning of the transition region between the healthy and galled stem. The phloem ring swelled and parenchyma external to the phloem proliferated. **C,** Lower region of the gall, with rapid proliferation of parenchyma outside the phloem, initiation of the secondary phloem, proliferation of secondary xylem, and disruption of water transport as seen by the dark regions within the secondary xylem. **D,** Section through gall further up the stem. The parenchyma external to the phloem appears pushed out and is replaced by parenchyma and secondary phloem with a striated appearance. The region also shows further proliferation of the secondary xyelm, with transport disruption. **E,** Cross section near gall center. The disorganized parenchyma has been nearly replaced by the "striated" tissue, and there is less disruption of water transport. **F,** Cross section of gall center. There is a change in the appearance of the secondary xylem compared to the healthy stem in **A** and in tissue organization external to the cambium and phloem. There is little disruption of longitudinal water transport in the gall center.

both to each other and to the healthy stem/taproot (Fig. 5). Images of the healthy stem/taproot showed the typical concentric pattern (going inward) of thin epidermis or suberized cells, cortical parenchyma, phloem/cambium (thin bright ring), bright ring of secondary xylem with resin ducts (dark spots), dark ring of apparently nonconducting xylem, and bright center of water-conducting secondary and primary xylem. In one of the image slices, vascular connections to lateral roots were clearly visible (Fig. 5A).

Images of the gall from the unwilted, galled slash pine showed low signal intensity in the center of the gall, suggesting comparatively low water content (Fig. 5B). Images of woody pine stems and taproots of other seedlings acquired with pulse-sequence parameters tailored to enhance observation of water-distribution patterns have shown that similar dark regions of pine stems have low water content (J. S. MacFall, *unpublished data*). Direct visual examination of the wood slice removed from the gall showed the presence of heartwood not found in the healthy stem. A wedge-shaped dark region was seen also in the images, corresponding to a slightly darkened region in the wood slice. No discoloration or other aberration was apparent on the surface of the stem or taproot. The dark wedge-shaped region seen in the images extended several millimeters down the length of the stem into the taproot and likely resulted from invasion by an unidentified wood-rotting fungus.

The 2-yr-old seedling that had just begun to decline showed significant alterations in water distribution within the secondary xylem and phloem (Fig. 5C). The dark center (heartwood) and outer bright ring (conductive secondary xylem), as were observed within the gall on the vigorous seedling, were not observed in this specimen. Instead, the secondary xylem uniformly gave a low signal, indicating low water content and/or very tight binding of the water to the wood. Darkened sectors within the xylem could be seen in the cross-sectional MR images, also suggesting invasion by other fungi. Correlative, slightly darkened discolorations could be seen within the wood slices. Again, no discoloration or other aberration was observed on the surface of either the stem or taproot.

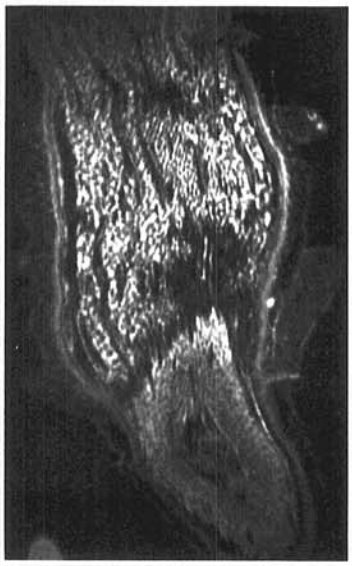

Fig. 3. Views at varied positions through a fusiform rust stem gall in a 9-mo-old seedling of slash pine. The same gall was viewed in cross section in Figure 2. Three-dimensional acquisitions such as this allow repeated study of the same specimen and "slices" through any orientation.

TABLE 1. Normalized signal of conducting secondary xylem in healthy and galled seedlings[a]

	Stems	
Treatments	Healthy	Galled
Water uptake		
Loblolly pines	0.67 ± 0.01 (4)[b]	0.47 ± 0 (2)
Slash pines		
12 mo	0.6 ± 0.04 (2)	0.33 ± 0.02 (3)
18 mo	0.38 ± 0.13 (2)	0.17 ± 0 (2)
Magnavist uptake		
Slash pine	0.74 ± 0.01 (3)	0.73 ± 0.16 (5)

[a]Normalized signal intensity was calculated from the signal intensity measured for the secondary xylem/signal intensity of the $CuSO_4/D_2O$ reference.
[b]Numbers in parentheses are the numbers of seedlings examined.

DISCUSSION

The observations made from MRM images of fusiform rust galls reported here are important in that they clearly show changes in tree anatomy and water relations associated with infection by *C. q. fusiforme* and subsequent gall development. Anatomical changes included the proliferation of cortical parenchyma that was then pushed out and replaced with enlarged phloem rays. The secondary xylem was seen clearly in both galls and healthy stems, but in the galls, it appeared more striated due to the proliferation of ray parenchyma between the conducting tracheids. Similar changes in anatomy have been described with standard histological techniques (5,6,19).

A surprisingly high degree of organization was observed in both tapered and stem-girdling galls in slash and loblolly pines at 12 mo. This is in contrast to previously reported histological observations describing stem-girdling galls in slash pine (20), wherein significant cellular disorganization was observed in the cortex, cambium, and xylem. The formation of numerous wound-callus cells, thought to interfere with normal translocation and to contribute to the mortality associated with this gall type, was reported. Similar disruption in transport might have been observed in our experiments had we followed the gall formation over an extended period of time.

The imaging experiments showed differences in the patterns of water distribution/transport between galls of 12- and 24-mo-old seedlings. In young stems, disruption in anatomy and water distribution was primarily at the transition zone between healthy and gall tissues. Little disruption in anatomy was observed in the center of the gall, demonstrating compensating patterns of flow from the regional disruption in the transition area. Similar compensating paths for the ascent of sap have been observed by others after stem wounding (9). In the 2-yr-old seedlings, however, the center of the gall was not conducting water, in contrast

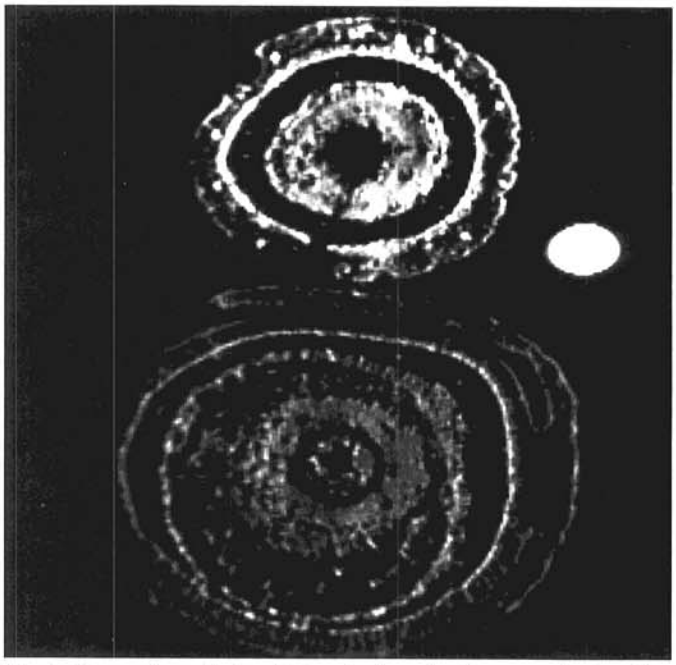

Fig. 4. Comparison of signal intensity in a healthy stem (top) and a fusiform rust stem gall (bottom) of pine. The diameter of the reference tube (bright circle in left center) was 1.12 mm.

to the stem from the healthy tree of the same age. The wood slice indicated the premature formation of heartwood, generally not considered to participate in water transport.

The 2-yr-old seedling that had just begun to decline showed significant disruption of water transport compared to the healthy tree and the gall on the tree without other symptoms of distress. There was little signal from the secondary xylem, suggesting low water content and reduced water transport. The onset of decline (wilt) observed in the foliage immediately prior to tree harvest confirms that the seedling was experiencing reduced water movement up the stem.

In the declining 2-yr-old galled seedling, variations in brightness with discrete boundaries were observed within the xylem. Corresponding discolorations within the wood slices showed the darkened sectors within the MR images also were likely to be from additional fungal infections. The extensiveness of the darkened sectors throughout the cross section suggests greater susceptibility to infection by secondary pathogens with gall formation in this seedling, contributing further to reduced water transport.

The differences in patterns of water transport through the galls of the declining and the nondeclining seedlings suggest differing responses by the seedlings to infection and gall development. Water distribution within the galls also appeared different than in the healthy stem, with the formation of heartwood in the gall from the nondeclining seedling. A bright ring of conducting secondary xylem was clearly visible both within this gall and within the healthy stem. On the other hand, the declining seedling did not appear to have formed heartwood and was experiencing difficulties with water transport, partially due to secondary infections and tissue changes. These differences strongly suggest differing mechanisms for maintaining water transport in the two galled seedlings.

In addition to disruption in xylem transport in the declining seedling, the phloem did not appear contiguous around the stem cross section within the MR image three-dimensional data set (Fig. 5C). Within the cross-sectional view, the bright ring that is usually associated with functional phloem could not be distinguished. This suggests disruption in phloem transport as well. The onset of decline occurred the day the seedling was sampled for imaging, with no prior symptoms of distress observed. The seedling had not previously exhibited symptoms of distress in reduced growth or decline.

Within the 12- and 18-mo-old seedlings, the phloem of galls did not appear to be affected by disease development. The longitudinal views through the three-dimensional MRM stem volumes of the young seedlings show that the phloem was contiguous through the gall (Fig. 3). Only in the declining 2-yr-old seedling was phloem disruption observed.

In addition to alterations in water distribution and anatomy, the difference in signal intensity of secondary xylem between galls and healthy stem tissues suggests differences in water binding to the wood. When water without Magnavist was taken up by seedlings, images of the excised stems from seedlings with galls showed less signal in the secondary xylem than from healthy seedlings.

In MRI experiments, signal intensity depends on several factors. The relationship between image brightness and MR characteristics can be described (3) as

$$S(^1\mathrm{H}) = N(^1\mathrm{H})\,(1 - e^{-\mathrm{TR}/\mathrm{T1}})\,(e^{-\mathrm{TE}/\mathrm{T2}}),$$

wherein $S(^1\mathrm{H})$ = signal intensity, $N(^1\mathrm{H})$ = water content, TR = time of acquisition repetition (milliseconds), T1 = spin-lattice relaxation time, TE = time of echo for a spin-echo acquisition (milliseconds), and T2 = spin-spin relaxation time.

Water uptake by transpiration through stems severed at the root collar was not reduced through galled stems. Equal or greater amounts of water were taken up by stems of seedlings with galls than by healthy seedlings. This observation implies that the water content of the galls was not less than the water content of the healthy stems, because rates of transpiration were not reduced with gall formation.

Reduction in signal, therefore, was likely due to a difference in water binding between galls and healthy stems, resulting in a longer T1 for the water within the galls. The equation shows that if water content and T2 times are equal, when images are acquired with a rapid repetition time (as was used in our imaging experiments) samples with a long T1 will give comparatively less signal than samples with a short T1. Spin-lattice relaxation times reflect the degree of mobility of the $^1\mathrm{H}$ within the water molecules. If the water is strongly interacting with its environment, the xylem cell walls in these experiments, the T1 will be shorter than if the water is less tightly bound. Preliminary measurements of the T1 of one gall and one healthy stem indicated that indeed the water within the gall had a longer T1 (1,539 ms) than did water within the healthy stem (1,040 ms), which resulted in less signal detected from water in the gall.

Additionally, the Magnavist-uptake studies suggest that the T1 for the gall differed from the T1 for the stem from the healthy seedling. Magnavist contains the paramagnetic ion gadolinium, which in low concentrations has the dominant effect of decreasing T1. Addition of Magnavist to the water taken up in transpiration effectively decreased the T1 in both the gall and healthy stems, making the T1 of water within the tissue more dependent on the contrast agent than on the interactions with the plant tissue. Addition of the contrast agent, therefore, effectively equalized

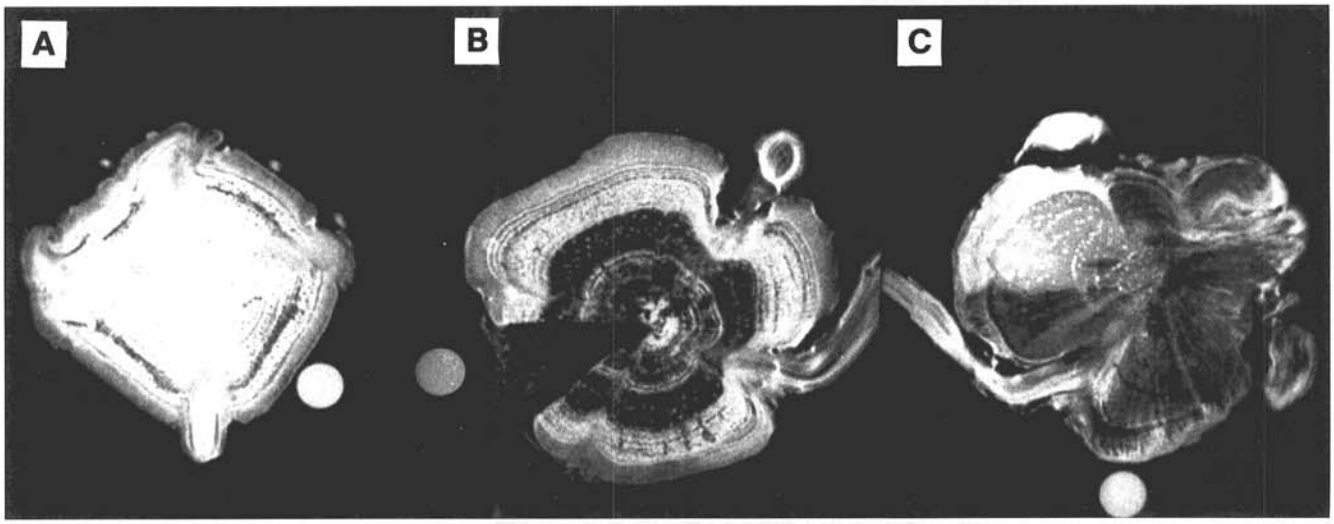

Fig. 5. Cross sections through root collar of 2-yr-old slash pines **A,** Healthy pine. There are normal vascular traces. **B,** Pine with fusiform rust gall at the root collar but no other apparent symptoms. The stem center is dark, indicating that the region was not transporting water. **C,** Pine with fusiform rust gall at the root collar, just beginning to wilt. The xylem appears darker, indicating reduced water transport.

the T1 of the galled and healthy stems. If the T1, T2, and water content of the galled and healthy stems were the same, the measured normalized signal intensity also would be equal, as was observed with the application of Magnavist (Table 1).

If the T1 of the gall differs from the T1 of the healthy stem, this implies that the interaction of the secondary xylem in the gall tissue with water in the transpirational stream is different than in healthy tissue. This hypothesis is reasonable given reported differences in tannins, phenols, specific gravity, cellulose, lignin, and cytokinin content between galls and unaffected stems (14–17). As the proportion of free water versus bound water (water in close association with the cell walls) increases, so does the T1 relaxation time. The longer T1 relaxation time of the water within the gall suggests, therefore, that the tissue within the gall is more hydrophobic than healthy tissue. This has many implications in discussions of lignin biosynthesis, xylem formation and development, stem development, and water-transport properties.

The observations made here suggest that there are alterations in the stem-tissue chemistry that are reflected by changes in tissue/water interactions with fusiform rust gall development. The changes in tissue chemistry occur within the first year of growth. The preliminary studies on 2-yr-old seedlings also show different patterns of water distribution between seedlings with galls and stems of healthy seedlings that appear to be associated with the ability to maintain transport of water through gall tissues.

Both the anatomy and water relations of the pine stem are altered by the presence of galls, and these changes can be studied successfully with MRI techniques. Future studies will provide additional information regarding relationships between the wood chemistry and the plant functional physiology as seedlings age and galls develop. Potentially, plant mechanisms for reducing disruption in physiological processes such as water transport with gall development can be identified and may be associated with genetic traits for tolerance to disease.

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