NATIONAL
OAK WILT
SYMPOSIUM

JUNE 4-7, 2007 • AUSTIN, TEXAS

The Proceedings of the 2nd National Oak Wilt Symposium

Edited by:

Ronald F. Billings
David N. Appel

Sponsored by

International Society of Arboriculture – Texas Chapter

Cooperators

Texas Forest Service
Texas AgriLife Extension Service
The Nature Conservancy of Texas
Lady Bird Johnson Wildflower Center
USDA Forest Service, Forest Health Protection

2009

INJECTION, INFUSION, AND SYSTEMIC MOVEMENT IN TREES

Terry A. Tattar
Professor Emeritus, Department of Microbiology
University of Massachusetts
Amherst, MA 01003
Email: tatattar@embarqmail.com

ABSTRACT

Management of oak wilt often includes the use of systemic fungicides delivered by tree injection. Classic theory of sap movement being limited only to upward movement doesn't explain the efficacy of trunk-injected fungicides, whose site of action is in the root system. Movement of the xylem-mobile dyes, acid fuchsin, and saffranin O, after lower trunk/root flare injection, was found to occur both upward into the xylem of stems, twigs, and leaves, and downward into the xylem of woody roots, at most times of year. Similar patterns of movement of xylem-mobile dyes were observed on the following species tested: American chestnut, black birch, eastern hemlock, eastern white pine, red maple, red oak, weeping willow, white ash, and white birch. Downward movement of dye into root systems involved all ages of xylem tissues present within a root while upward movement was confined to the most recently formed xylem growth ring.

Key words: *Ceratocystis fagacearum*, dyes, fungicides, oak wilt

Trunk injection of systemic fungicides is often part of the management plan for the control of oak wilt (Appel 2007). Triazole fungicides delivered by trunk injection have been found to be effective in suppression of the oak wilt pathogen, *Ceratocystis fagacearum* (Wilson and Forse 1997, Wilson 2005). The ability of all systemic chemicals to suppress pathogens or control insect pests is dependent upon their systemic movement within the tree after injection. For systemic chemicals to move in the tree's vascular system is therefore dependent on the movement of sap within the tree.

The movement of sap from the roots to the top of tall trees has fascinated both scientists and others who wondered how a tree works. It is hypothesized that it is the loss of water (evaporation) from the leaves that causes a tension, or "pull", on many tiny water columns within wood (Campbell, Reece and Mitchell 1999). Since water is also cohesive, these combined forces can pull the water in a tree upward sometimes over 300 feet (100 meters) from the roots (Zimmerman 1983). This explanation for upward sap movement is known as the "cohesion-tension theory" and is widely accepted by tree scientists (Salisbury and Ross 1992). Since no corresponding theory has been proposed to explain the possibility of downward sap movement, it has often been concluded that sap flow only occurred in the upward direction.

However, experimental field data from those who studied sap movement in plants and vascular diseases of trees have reported evidence of downward movement for over 250 years (Banfield 1941). Many of these researchers used dyes or spore suspensions to track the downward movement of sap in trees. Banfield's studies on American elms demonstrated that both upward and downward movement occurred from injection points on the elm trees at equal speed. Extensive studies of sap movement by Greenidge (1958) using a sap mobile dye on a wide variety of trees, including American elm, balsam poplar, balsam fir, American beech, yellow birch, ironwood, sugar maple, white spruce, and white ash, supported the evidence of downward sap movement found earlier by Banfield (1941) and others.

Further evidence of downward movement of injected chemicals came form microinjection studies with the antibiotic oxytetracycline, which has been used to relieve symptoms of numerous bacterial diseases of trees, including bacterial leaf scorch (Kostka, Tattar and Sherald 1985), peach X-disease (Cooley, Tattar and Schieffer 1992), and lethal yellows of coconut palm (*Cocos nucifera*) (McCoy 1983). High populations of systemic bacteria within the root system have been associated with diseases caused by systemic bacteria (Sinclair, Lyons and Johnson 1987, Cha and Tattar 1991, Blanchard and Tattar 1997). However, it has puzzled scientists how a systemic chemotherapeutant, such as oxytetracycline, or a systemic fungicide, such as propiconazole, could be effective if xylem movement of injected materials only occurred in the upward direction. Preliminary studies by Tattar and Tattar (1999) presented evidence for downward movement in the xylem of trees following trunk injection with the use of xylem-mobile dyes.

The objectives of this study were (1) to determine the direction and magnitude of movement of trunk-injected materials within the xylem of trees using xylem-mobile dyes and (2) to determine how time of year of injection influences dye movement.

MATERIALS AND METHODS

The trees used in these studies were growing in the Shade Tree Laboratory Nursery in Hadley, MA and in the Cadwell Memorial Forest in Pelham, MA. Both these research facilities are part of the University of Massachusetts at Amherst. The trees ranged in size from 2 inches (5 cm) to 10 inches (25 cm) in stem diameter at 4.5 feet (1.4 meters) above ground. The following species were injected: red maple, *Acer rubrum*, eastern white pine, *Pinus strobus*, red oak, *Quercus rubra*, eastern hemlock, *Tsuga canadensis*, white birch, *Betula alba*, black birch, *B. lenta*, American chestnut, *Castanea dentata*, white ash, *Franxinus americana*, and weeping willow, *Salix babylonica*.

Tree injection wounds were made with a battery-powered drill (800 rpm) using an 11/64 inch (6 mm) high speed steel drill bit. Injection holes were made in the lower trunk and root flare areas and hole depths were between 1/4 inch (6 mm) and 1/2 inch (12 mm). In one study conducted during the 1997 summer season, however, injection wounds were made at 4.5 feet (1.4 meters) above ground to American chestnut and red oak trees. An unpressurized glass reservoir container, with an exit port at the bottom of the container, was filled with 25 to 50 ml of dye solution. The reservoir delivery system was attached via plastic (Tygon) tubing to a hollow plastic tube which was inserted into the injection wound, immediately after a drill-hole injection was made. The following xylem-mobile dyes at 2% w/v were each used during these experiments: acid fuchsin, gentian violet, and safranin O.

Trees were injected with the test dye solutions in late spring during leaf expansion through mid fall after leaf drop. Experiments were conducted over an 8-year period from 1998 through 2005. Dye injection studies were started either from 0800 to 1000, or from 1400 to 1600. In most experiments, injectors were left in the tree for 24 hours. Trees were harvested immediately after injector removal. In some experiments, dyes were injected in the morning, the experiments were terminated approximately 6 hours after injection, and trees were harvested in the afternoon. While in other experiments, trees were injected in the afternoon and harvested the next morning, approximately 16 hours after injection. Soil temperature was measured at 5 cm (2 inches) below ground, using a soil thermometer, at the starting time of each injection.

On most trees 10 cm (4 inches) and smaller in diameter, the woody roots were severed with a root ax and/or hand saw and the entire tree was examined. Soil was removed from roots by washing and the bark was peeled from the woody roots and stem. In some larger trees the root flare was exposed by removal of soil and only the large roots were cut with a chainsaw, approximately 20 to 50 cm (8 to 20 inches) from the trunk. All stem and root sections were photographed as soon as possible after the bark was removed.

Dye movement in both upward and downward directions in the xylem was assessed by visual examination of the leaves and by estimating the amount of xylem tissue stained by the injected dye after the bark was removed. We were usually able to follow patterns of dye movement throughout the test trees from the leaves to the roots.

RESULTS

The first studies were conducted in the fall during and after onset of leaf coloration and continued after leaf drop of deciduous trees. Dye patterns, regardless of species, were always bimodal, with some dye movement upward into the stem and downward into the roots from the injection sites at the root flare. Dye movement in the initial studies was approximately split between upward movement and downward movement. Later fall studies displayed progressively greater downward dye movement as soil temperatures declined from approximately 15°C (60°F) to 5°C (40°F). After complete natural leaf fall, dye movement was primarily downward until experiments were terminated in early November. These dye patterns were consistent with all the species studied, in both deciduous hardwoods and conifers. In addition, the dye patterns were also similar regardless of the dye solution used. Acid fuchsin and saffranin O were most easily observed.

Studies were also conducted during leaf expansion in late spring and continued into the summer when full leaf size of deciduous trees was attained. Our initial results were similar to early fall studies, with dye movement evenly split between upward and downward directions. Experiments conducted during summer were remarkably similar to those of late spring, but even with a progressive increase in upward movement, we always noted substantial downward movement. During moisture limiting soil conditions, downward movement was found to increase. Cross sections of roots revealed dye movement into several years of xylem tissue while stem cross sections of the same trees revealed dye confined only to springwood vessels of the current growth ring.

In an attempt to determine the speed of downward movement or upward movement, dye reservoirs were left on trees for fewer than 24 hours. However, even when dye reservoirs were in place for only 6 hours during day experiments and 16 hours during night experiments, bimodal movement was found. We noted on several occasions that, after downward movement into the roots, the injected dye would then reverse direction in the roots and progress upward on the opposite side of the stem.

In a study of the effect of the height of injection on systemic dye movement, American chestnut and red oak were injected at 4.5 feet (1.4 meters) above ground. Most of the acid fuchsin dye moved upward into the branches and foliage and only small amounts of dye moved downward, compared with similar trees that were injected at the root flare on the same dates and times.

DISCUSSION

Downward movement within xylem can be explained by the normal condition of the functioning xylem elements, which are under negative pressure or tension, and is consistent with the cohesion-tension theory of xylem movement. A break in the xylem elements, due to an injection wound, would allow movement of the injected solution in either upward and/or downward directions according to the forces within the xylem elements at the time of injection.

The results of this study agree with those of Banfield (1941), Greenidge (1955), and others who reported downward movement of dyes and fungal spores in the xylem of many tree species. The findings of the current study, based on dye delivery by trunk injection, combined with those of earlier researchers, can help to explain how materials injected into the sap stream at the root flare can have efficacy in the root systems of trees. This information is especially useful in explaining the control of root problems achieved using trunk injection of antibiotics, fungicides, insecticides, and micronutrients during the growing season with active leaf transpiration. For example, these findings may help to explain why trunk injection was found to be effective in the treatment of pathogens that are primarily transmitted through the root system, such as oak wilt (caused by *Ceratocystis fagacearum*), since the early 1990s (Osterbauer and French 1992, Appel 1994).

Osterbauer and French (1992) reported that location of injection sites on the root flare may have resulted in movement of the propiconizole into the root system since they could not detect the fungicide above a height of 3.0 meters. Although these researchers did not conduct any propiconizole assays of root tissues, results obtained in this study would support their conjecture of downward movement of the injected fungicide. One may also conclude from the results of the current study that downward movement of injected systemic chemicals is favored by placing injection sites in the root-flare zone.

Multi-year xylem sap distribution in roots would appear to explain vascular disease control beyond one growing season achieved using injectable fungicides, such as that reported by Osterbauer and French (1992) with propiconizole. Dye movement was found across the entire cross section of root xylem following lower trunk injection . It appears that portions of trunk-injected materials are transported downward into the roots and are then transported upward in the sapstream in the following season or seasons. This theory could also account for the efficacy of fall-injected materials in the following spring.

LITERATURE CITED

Appel, D.N. 1994. Identification and control of oak wilt in Texas. Journal of Arboriculture 20: 250-257.

Appel, D.N. 2007. The latest in oak wilt management. Tree Care Industry 18(3): 8-16.

Banfield, W.M. 1941. Distribution by the sap stream of spores of three fungi that induce vascular wilt diseases of elm. Journal of Agricultural Research 62: 637-681.

Blanchard, R.O., and T.A. Tattar. 1997. Vascular bacterial diseases, Pp. 223-239. *In*: Field and Laboratory Guide to Tree Pathology. Academic Press, San Diego, CA.

Campbell, N.A., J.B. Reece, and L.G Mitchell. 1999. Transport in plants, Pp. 695-713. *In*: Biology, 5th Ed., Benjamin/Cummings, Menlo Park, CA.

Cha, B., and T.A. Tattar. 1991. Symptom development of ash yellows and fluctuation of mycoplasma-like organism population in white ash (*Fraxinus americana* L.). Journal of Arboriculture 15: 323-343.

Cooley, D, T.A. Tattar, and J. Schieffer. 1992. Treatment of X-disease using oxytetracycline microinjection capsules. Horticultural Science 27: 235-237.

Greenidge, K.N.H. 1958. Rates and patterns of moisture movement in trees, Pp. 19-41. *In*: K.V. Thimann (ed.), The Physiology of Trees. Ronald Press, New York, NY. 678 pp.

Kostka, S., T.A. Tattar, and J.L. Sherald. 1985. Suppression of bacterial leaf scorch symptoms through oxytetracycline microinjection. Journal of Arboriculture 11: 54-60.

McCoy, R.E. (ed.). 1983. Lethal yellowing of palms. Institute of Food and Agricultural Science, University of Florida Bulletin No. 834. 100 pp.

Osterbauer, N.K., and D.W. French. 1992. Propiconazole as a treatment for oak wilt in *Quercus rubra* and *Q. ellipsoidalis*. Journal of Arboriculture 18: 221-226.

Salisbury, F.B., and C.W. Ross. 1992. Plant physiology. 4th Ed., Wadsworth Publishing Company, Belmont, CA. 747 pp.

Sinclair, W.A., H.H. Lyon, and W.T. Johnson. 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, NY. 574 pp.

Tattar, T.A., and S.J. Tattar. 1999. Evidence for the downward movement in the xylem of trees. Journal of Arboriculture 25: 325-332.

Wilson, A.D., and L.B. Forse. 1997. Sensitivity of Texas strains of *Ceratocystis fagacearum* to triazole fungicides. Mycologia 89: 468-480.

Wilson, A.D. 2005. Recent advances in the control of oak wilt in the United States. Plant Pathology Journal 4: 177-191.

Zimmerman, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag, New York, NY. 143 pp.