EVIDENCE FOR THE DOWNWARD MOVEMENT OF MATERIALS INJECTED INTO TREES
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Abstract. Movement of the xylem-mobile dyes (acid fuchsin, saffrannin O, and gentian violet) after lower trunk or 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 when soil temperatures equaled or exceeded 5°C (40°F). 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. Evidence is presented of initial downward dye movement followed by later upward movement within a 12-hour period. Our results suggest that xylem-mobile materials injected into trees may be distributed into stem, leaf and root tissues under most growing conditions.

Key Words. Systemic movement; fertilizer.

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 the loss of water (evaporation) from the leaves causes a tension, or “pull,” on many tiny water columns within wood (Campbell et al. 1999). Because water is also cohesive, these combined forces can pull the water in a tree upward sometimes over 100 m (300 ft) 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). Because no corresponding theory has been proposed to explain the possibility of downward sap movement, it has often been concluded that sap flow occurred in the upward direction only.

The objectives of this study were to determine 1) the direction of movement of injected materials in the xylem of trees using xylem-mobile dyes and 2) 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, Massachusetts, and in the Cadwell Memorial Forest in Pelham, Massachusetts. Both these research facilities are part of the University of Massachusetts at Amherst. The trees ranged in size from 5 cm (2 in.) to 25 cm (10 in.) in stem diameter at 1.4 m (4.5 ft) aboveground. 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 (*Fraxinus americana*), and weeping willow (*Salix babylonica*).

Tree injection wounds were made with a battery-powered drill (800 rpm), using an 6 mm (11/64-in.) high-speed steel drill bit. Injection holes were made in the lower trunk and root flare areas, and hole depths were between 6 and 12 mm (1/4 and 1/2 in.) In one study conducted during the 1997 summer season, however, injection wounds were made at 1.4 m (4.5 ft) above ground to American chestnut and red oak trees. Initial experiments were conducted using Mauget microinjection capsules filled with approximately 10 mL (0.34 oz) of xylem-mobile dye solutions, pressurized to 1 atmosphere, and attached to plastic feeder tubes, which were placed into injection wounds. Later experiments used an unpressurized reservoir container filled with 25 to 50 mL (0.85 to 1.7 oz) of dye solution that was attached via plastic (Tygon) tubing to a feeder tube and inserted into the injection wound. 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 a 3-year period from 1996 through 1998. Dye injection studies were started either from 0800 to 1000 hours, or from 1400 to 1600 hours. 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. In other experiments, trees were injected in the afternoon and harvested the next morning, approximately 16 hours after injection. In all experiments, trees were harvested at the starting time of each injection.

On most trees 10 cm (4 in.) and smaller in diameter, the woody roots were severed with a root ax and/or handsaw, 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, with only the large roots being cut with a chainsaw, approximately 20 to 50 cm (8 to 20 in.) from the trunk. All stem and root sections were photographed was soon as possible after the bark was removed.

Dye movement in both the upward and downward directions in the xylem was assessed by visually estimating the amount of xylem tissue stained by the injected dye after the bark was removed. In initial studies with Mauget capsules, the capsules would empty within a few hours; however, dye patterns were difficult to visualize and photograph. Once a larger dye reservoir was employed, we were able to follow patterns of dye movement throughout the test trees.

**RESULTS**

Our 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 studies displayed progressively greater downward dye movement as soil temperatures lowered from approximately 15°C (60°F) to 5°C (40°F). After complete defoliation, dye movement was primarily downward when experiments were terminated in early November. These dye patterns were consistent with all species studied, in both deciduous hardwoods and conifers (Figure 1*). In addition, the dye patterns were also similar regardless of the dye solution used. Acid fuchsin and saffranin O were most easily visualized.

Studies were 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 (Figure 2). During moisture limiting conditions, downward movement was found to increase. Cross-sections of roots revealed dye movement into several

*Figures for this article begin on page 328. Colorized versions of the figures in this manuscript can be viewed on Dr. Tattar's University of Massachusetts faculty Web site at http://www.bio.umass.edu/micro/tattar.html
years of xylem tissue (Figure 3), while stem cross-
sections of the same trees revealed dye confined only
to springwood vessels of the current growth incre-
ment (Figure 4).

In an attempt to determine the speed of down-
ward 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 up-
ward on the opposite side of the stem (Figure 5).

In the study on American chestnut and red oak
with injection wounds made on stems 1.4 m (4.5 ft)
aboveground, most of the acid fuchsin dye moved
upward into the branches and foliage. In these ex-
periments, only small amounts of dye moved down-
ward compared with similar trees that were injected
on the same dates at the root flare.

**DISCUSSION**

Downward movement within xylem can be explained
by the normal condition of the functioning xylem ele-
ments, 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 or downward di-
rections, or both, according to the forces within the
xylem elements at the time of injection.

Our results agree with those of Banfield, Greenidge
and others who reported downward movement of in-
jected dyes and fungal spores in the xylem of many tree
species. These recent findings, combined with those of
earlier researchers, can help explain how materials in-
jected 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 prob-
lems achieved using trunk injection of antibiotics, fun-
gicides, insecticides, and micronutrients during the
growing season with active leaf transpiration. For ex-
ample, these findings may help explain how trunk in-
jection can be effective in the treatment of pathogens
that are primarily transmitted through the root system,
such as in oak wilt (Osterbauer and French 1992;
Appel 1994), as well as provide control of the systemic
bacteria discussed earlier.

Osterbauer and French (1992) reported that loca-
tion of injection sites on the root flare may have
resulted in movement of the propiconizole into the
root system because they could not detect the fungic-
cide above a height of 3 m (10 ft). Although these
researchers did not conduct any propiconizole as-
says of root tissues, our results would support their
conjecture of downward movement of the injected
fungicide.

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. Our studies found dye
movement across the entire cross-section of root xy-
lem following lower trunk injection. It appears that
portions of trunk-injected materials are transported
downward into the roots and are then transported up-
ward in the sapstream in the following season or sea-
sons. This theory could also account for the efficacy of
fall-injected materials in the following spring.

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Resume. On a découvert que le mouvement des colorants mobiles du xyleme – acide fuchsin, safranin O et bleu de méthylène –, après leur injection dans la partie inférieure du tronc ou le pied de l'arbre, se produit à la fois vers le haut dans le xyleme des branches, des tiges et des feuilles et vers le bas dans le xyleme des racines ligneuses, et ce pratiquement toute l'année lorsque la température du sol est égale ou supérieure à 5°C. Des patrons similaires de mouvement du xyleme ont été observés sur l'ensemble des espèces suivantes qui ont été testées: châtaignier d'Amérique, bouleau gris, pruche du Canada, pin blanc de l'Est, érable rouge, chêne rouge, saule pleureur, frêne d'Amérique et bouleau à papier. Le mouvement vers le bas des colorants vers le système racinaire impliquait tous les tissus du xyleme présents dans la racine alors que le mouvement vers le haut était confiné au xyleme de l'anneau de croissance le plus récent. La preuve du mouvement initial vers le bas du colorant suivi par après de son mouvement vers le haut à l'intérieur d'une période de 12 heures est présentée. Nos résultats suggèrent que les composés injectés dans les arbres peuvent être distribués dans les branches, les feuilles et les tissus racinaires sous la plupart des conditions de croissance.


Resumen. Se encontró que el movimiento de tinturas móviles del xilema, ácido fúcsico, O safranín y violeta de genciana, después de inyecciones en la porción baja de la raíz, ocurre tanto hacia arriba como dentro del xilema de los troncos, brotes y hojas y hacia abajo dentro del xilema de las raíces leñosas, la mayor parte del año, cuando las temperaturas del suelo igualaron o excedieron 40F (5C). Patrones similares de movimiento del xilema fueron observados en todas las siguientes especies probadas: Castaño americano, Abedul negro, Abedul del Canadá, Pino blanco del Canadá, Arce de Virginia, Encino de Virginia, Sauce llorón, Fresno blanco Americano y Abedul Americano. El movimiento descendente del tinte dentro de los sistemas de raíces envolvió todos los tejidos del xilema presentes dentro de una raíz mientras el movimiento ascendente del xilema estuvo confinado en la mayoría de los anillos recientemente formados. Se presenta evidencia de un movimiento inicial descendente del tinte, seguido por un movimiento posterior ascendente en un periodo de 12 horas. Nuestros resultados sugieren que los materiales inyectados dentro de los árboles pueden ser distribuidos dentro del tronco, hojas y tejidos de las raíces, bajo la mayoría de las condiciones de crecimiento.
Figure 1. Downward movement of 2% acid fuchsin dye into roots of eastern hemlock (a) and yellow birch (b) 24 hours after trunk injection in October. Bark has been removed above and below injection sites. Solid arrows indicate dye position, and hollow arrows indicate site of injection wounds.
Figure 2. Downward movement of 2% acid fuchsin dye 24 hours following trunk injection of an American chestnut in July (a). Close-up, white plastic feeder tube indicates position of injection wound (b).
Figure 3. Cross-section of buttress of American chestnut 24 hours after trunk injection of 2% acid fuchsin dye in July. Note inward movement of dye across several years of xylem tissue.

Figure 4. Cross-section of American chestnut stem at 90 cm (3 ft) above ground 24 hours following trunk injection of 2% acid fuchsin dye in July. Note that dye is confined to large springwood vessels in the current year of xylem.
Figure 5. Upward movement of 2% acid fuchsin dye (solid arrows) after initial downward dye movement from an injection wound on the opposite side of the trunk on an American chestnut in July. Note position of plastic feeder tube with flag attached (hollow arrow) indicating position of injection wound.