# Translocation in Phloem Necrosis-Diseased American Elm Seedlings

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ABSTRACT

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Seedlings of *Ulmus americana* in their second season of growth were exposed to $^{14}CO_2$ and allowed to transport assimilated materials for periods of 4 to 24 hr. At the end of the transport period, samples consisting of the treated leaf, serial stem segments, and the root system were extracted in boiling 80% ethanol; extracts were analyzed for $^{14}C$. Healthy-appearing leaves on seedlings previously graft inoculated with the agent of phloem necrosis (PN) exported assimilate less rapidly than did leaves on healthy trees. Also, transport of $^{14}C$ in stems of infected seedlings was impaired significantly prior to development of histopathologic symptoms in the secondary phloem. Translocation occurred, although slowly, in secondary phloem exhibiting histologic aberrations. These results indicate that callose deposition and sieve tube collapse, which are early symptoms of PN, are not solely responsible for the impairment of translocation in PN-diseased elms.

*Additional key words:* Plant-infecting mycoplasma.

As indicated by the name of the disease, phloem is selectively destroyed in American elms that phloem necrosis (PN) affects. Phloem symptoms include callose deposition on sieve plates and lateral sieve areas, and collapse of sieve elements and companion cells. Cambial hyperactivity produces abundant replacement phloem (10) containing sieve elements smaller than normal, which soon become necrotic. These symptoms develop in concert with phloem discoloration. Nondiscolored phloem from diseased plants appears normal (1,7).

Extensive necrosis of feeder roots (6) often precedes foliar symptoms, described as resembling those caused by nutritional deficiencies (13) or water stress (12). Phloem degeneration, reported to begin in the fine roots (6,7), is usually prominent in buttress roots and the basal parts of stems by the time foliar symptoms appear. Relationships among root necrosis, phloem degeneration, and foliar symptoms are unclear.

No previous study of phloem transport has been done in elms with PN. Further, although phytopathogenic mycoplasmas are phloem limited, few studies of translocation in mycoplasma-infected plants have been done (2,14). The objectives of this research were to learn (i) whether disturbances in translocation precede development of histopathologic symptoms in secondary phloem and (ii) whether replacement phloem is capable of translocating assimilated materials. We also hoped to gain insight into the relationship between necrosis of fine roots and other symptoms.

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## MATERIALS AND METHODS

**Plant material.**—Two-year-old greenhouse-grown seedlings of *Ulmus americana* L. selected for uniform size and vigor were used. During midsummer, when greenhouse temperatures often reached 40 C, trees were held in an air-conditioned chamber in the greenhouse (natural light) at 27 ± 2 C. During growth, trees were well watered and fertilized with dilute soluble fertilizer solution weekly. Trees were graft inoculated with bark patches obtained from diseased elms. Each plant received two patches (0.4 × 1.0 cm) about 15 cm above the soil line. Control trees received patches from healthy elms. Phloem discoloration was apparent 7–9 wk after inoculation. Experiments were performed with presymptomatic (4 wk after inoculation) and symptomatic (8–12 wk after inoculation) seedlings, using four plants per treatment. One exception was an experiment concerned with mechanical girdling in which two girdled and two control trees were used. Since only three trees could be exposed to $^{14}CO_2$ and sampled in any one day, the eight trees in each experiment were treated in random sequence during a 3-day period.

**$^{14}C$-assimilate labeling and translocation.**—A single, fully expanded leaf on each tree was sealed in a Plexiglas chamber with heat-filtered illumination of 9,000 lx. $CO_2$ was generated from a 0.25-ml solution of $NaH^{14}CO_3$ (25 $\mu$Ci, specific activity 0.11 mCi/mg) by addition of 1 ml of 3 N $H_2SO_4$. Air within the chamber was circulated with a peristaltic pump. Following a 25-min exposure to $^{14}CO_2$, 5 ml of 1 N KOH was injected into a dish within the chamber and air was circulated for an additional 5 min to allow absorption of excess $CO_2$. Exposures to $^{14}CO_2$ were always made between 0800 and 1100 hr.

After exposures, Plexiglas chambers were removed and the plants were placed in the greenhouse and allowed to

transport assimilated material for 4, 8, or 24 hr prior to sampling. Trees in 4- and 8-hr experiments received supplemental lighting (cool-white fluorescent lights) for the entire transport period. Trees in the 24-hr experiment received supplemental lighting for the first 8 hr.

At the end of the transport period, leaves were removed and the entire stem was cut into 20-cm lengths distally and proximally from the node of the treated leaf. Each tree was examined at this time and the severity of the PN symptoms recorded. Foliar symptoms had not yet developed. Phloem discoloration and root necrosis were rated as none, slight, moderate, or severe. Bark was separated from the xylem and the latter was discarded. The treated leaf and bark segments were extracted separately in 20 ml of boiling 80% ethanol for 30 min. The entire root system was similarly extracted in 100 ml. A 1-ml amount of each sample was added to 15 ml of scintillation fluid (4 g of Omnifluor [New England Nuclear NEF906], 667 ml of toluene, 333 ml of Triton X-100) and analyzed for radioactivity in a Beckman LS-230 liquid scintillation counter. Counting rates were adjusted for quenching using an external standard, and counts per minute were converted to disintegrations per minute. The amount of $^{14}C$ in each sample was expressed as a percentage of the total ehtanol-soluble $^{14}C$ extracted from the entire plant. Data were transformed to arcsin $\sqrt{\text{percentage}}$ for statistical analysis. Root accumulation and leaf retention data were analyzed using Student's *t*-test.

During preliminary experiments, the water potential of each tree was measured at the time of sampling using the pressure chamber technique (11). This was done to insure that water-stressed trees were not used. When it became apparent that none of the experimental trees were so stressed, the practice was discontinued.

**Histologic procedures.**—Samples for microscopic examination were obtained from each tree at the end of the transport period. A portion of the first leaf above the treated leaf and stem samples 10 and 100 cm proximal to the treated leaf were fixed in 10% acrolein in 0.08 M phosphate buffer (pH 7.0) at 4 C. Tissue samples were dehydrated, and half were embedded in Epon-Araldite and half were stored in *n*-butanol at −20 C for later use in callose and starch determinations. Plastic-embedded material was sectioned (0.5–2.0 μm) with glass knives, affixed to slides, and stained with toluidine blue O.

The extent of callose deposition on sieve plates and lateral sieve areas was determined by staining radial sections of fixed, rehydrated tissue with dilute aqueous resorcin blue (5). Starch was assessed qualitatively in similarly prepared material using an iodine-potassium iodide (IKI) stain (4).

Estimates were made of the cross-sectional area occupied by functional sieve tubes at the bases of two healthy stems and in comparably located discolored phloem of two diseased seedlings. The trees sampled were those used in the translocation experiments. The diseased trees had been inoculated 8–12 wk previously. First, the thickness of the conducting phloem and the diameter of the xylem cylinder were measured. The area occupied by the band of conducting phloem was then calculated. The frequency of sieve tubes within this tissue was then determined in cross sections at a magnification of 500 × (ten microscopic fields examined per tree). All sieve elements in which protoplasts were not collapsed were considered functional. The average cross-sectional area of an individual sieve tube was then estimated (30 measurements per tree). From these data the total value was obtained and corrected for the proportion of sieve plates in which the pores were occluded by callose.

**Starch in leaves.**—Leaf disks were cut from healthy, PN-affected, and girdled trees at the end of a 12-hr light period and following 18 hr of darkness. Disks were cleared in boiling 80% ethanol, stained in dilute IKI solution, examined microscopically, and scored for relative amounts of starch.

## RESULTS

**Symptom development.**—Symptoms in the graft-inoculated seedlings were similar to those described for naturally infected trees (12,13). Necrosis of feeder roots and discoloration of the most recently formed phloem of large roots and lower stem developed before foliar symptoms (epinasty, yellowing, premature defoliation). Phloem discoloration in which normally white tissue became yellow to tan was first noted 7–9 wk after inoculation, and first developed near the inoculum patch. This discolored phloem usually had a faint wintergreen odor. Root necrosis always developed concurrently with or following phloem discoloration.

**Translocation.**—Both healthy and diseased trees

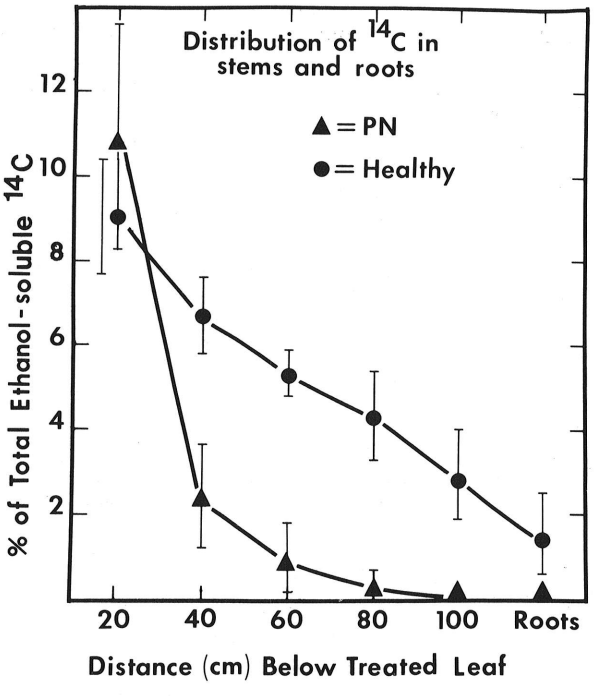


**Fig. 1.** Distribution of ethanol-soluble, radioactively labeled compounds within stems and roots of American elm seedlings, healthy and presymptomatically infected with the agent of phloem necrosis (PN), 4 hr after $^{14}CO_2$ exposure. Experiment was conducted 4 wk after inoculation. Bars indicate standard errors.

showed great variability in the fixation of $CO_2$. For this reason, data were standardized by expressing the amount of $^{14}C$-assimilate in each sample as a percentage of the total $^{14}C$ extracted from the plant. Based on total counts in ethanol extracts, $CO_2$ fixation was usually lower in diseased than in healthy plants, but these differences were not statistically significant. The average (± SE) ethanol-soluble radioactivity per plant across all experiments was $2.61 \pm 0.20 \times 10^6$ dpm.

In Experiment 1, conducted 4 wk after grafting, infected trees were presymptomatic, ie, had no foliar or histologic abnormalities. Figure 1 shows the distribution of $^{14}C$-assimilate in the stems and roots of healthy and inoculated elms 4 hr after $^{14}CO_2$ exposure. Labeled materials were translocated further in the controls than in presymptomatic infected trees. Significantly ($P = 0.05$) more labeled assimilate was retained in the treated leaves of the presymptomatic trees than in leaves of the controls. These results suggest that the disease causes an impairment of phloem transport before the development of any of the symptoms heretofore known.

Experiments 2, 3, and 4 were conducted with trees inoculated 8–12 wk previously. In these experiments, all diseased elms were in approximately the same stage of symptom development: phloem was discolored in the vicinity of the bark patches; root necrosis, if present, was slight to moderate; and foliar symptoms had not yet developed. Symptoms were assessed during dissection of seedlings after the transport period. Pressure bomb measurements indicated that no differences occurred at this stage between healthy and diseased trees with respect to xylem pressure potentials. In Experiments 2, 3, and 4, trees were allowed 4-, 8-, and 24-hr translocation periods, respectively, following $^{14}CO_2$ exposure.

In all experiments, healthy-appearing treated leaves of infected elms retained more $^{14}C$ assimilate than did the treated leaves of healthy elms (Fig. 2). Also, accumulation of labeled assimilate in roots was greater in healthy than in symptomatic elms (Fig. 3). This was true as early as 4 hr after $^{14}CO_2$ exposure, although little labeled material had yet reached the roots. All differences were significant at $P = 0.05$ except in the 8-hr experiment in which $P = 0.10$.

Treated leaves of symptomatic elms exported only 0.4% of their ethanol-soluble radioactivity in the 4 hr following $^{14}CO_2$ exposure, whereas healthy elms exported 10.1%. This delay in the onset of export was characteristic of symptomatic elms. Export at an appreciable rate began between 4 and 8 hr after $^{14}CO_2$ exposure. During that interval, the average export rate (percent of total EtOH-soluble $^{14}C$ lost from the treated leaf per hour) was 6.6% for infected elms compared with 11.7% for healthy elms.

Translocation rates varied considerably from one experiment to another. Control plants in the 4-hr experiment involving symptomatic trees exported 10.1% of their total ethanol-soluble radioactivity, but controls in the girdling experiment (see below) exported 25.4% and controls in the 4-hr experiment involving presymptomatic trees 30.5%. In the first case, shoot growth had ceased and terminal buds had set before the plants were exposed to $^{14}CO_2$. In the latter two cases, the plants were growing vigorously when treated. Expanding shoots are strong sinks for phloem assimilate, and this possibly accounted for the relatively high rates of export from leaves on elongating stems.

Since earlier studies (1,7) showed that sieve tube necrosis is extensive in the secondary phloem of PN-diseased elms and since the quantity of sieve tubes

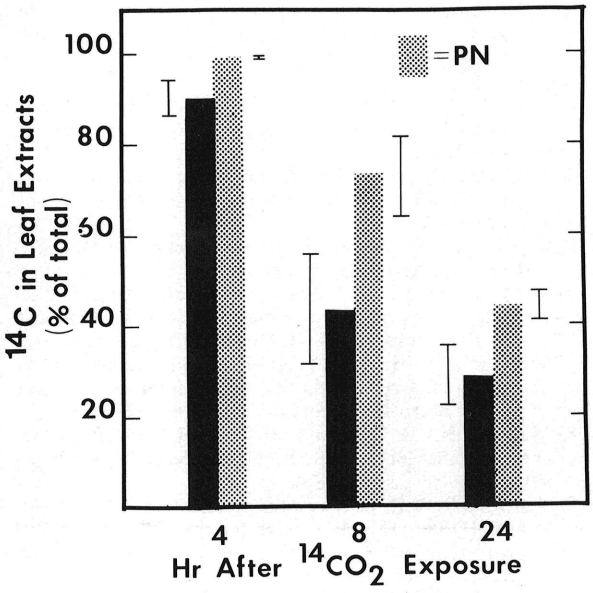


Fig. 2. Retention of $^{14}C$ assimilate within treated leaves of American elm seedlings, healthy and infected with the agent of phloem necrosis (PN), at 4, 8, and 24 hr after $^{14}CO_2$ exposure. One experiment, with plants inoculated 8–12 wk before exposure and all around same stage of symptom development, was performed per transport period. Each data point represents four plants. Standard errors are indicated.

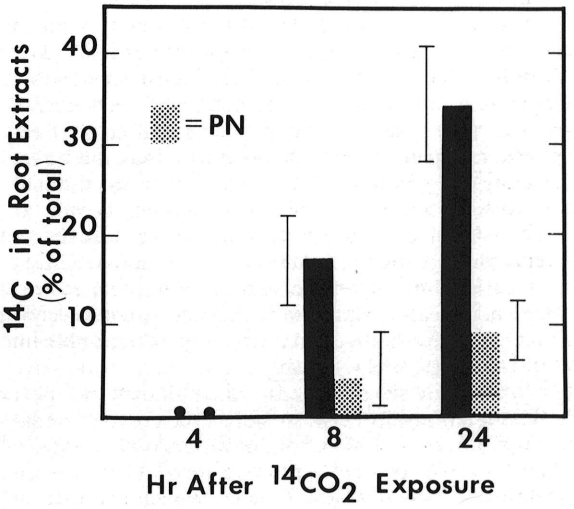


Fig. 3. Accumulation of $^{14}C$ assimilate within roots of American elm seedlings, healthy and infected with the agent of phloem necrosis (PN), at 4, 8, and 24 hr after $^{14}CO_2$ exposure. One experiment, with plants inoculated 8–12 wk before exposure and all around same stage of symptom development, was performed per transport period. Each data point represents four trees. Standard errors are indicated.

available for translocation might affect phloem transport, estimates were made of the cross-sectional area that sieve tubes occupied at the bases of healthy stems and in the discolored phloem of diseased stems between 8 and 12 wk after inoculation. The average area (± SE) that functional sieve tubes occupied in healthy elm seedlings was $10.55 \pm 0.75 \times 10^{-2}mm^2$; the value was $5.85 \pm 0.15 \times 10^{-2}mm^2$ in symptomatic stems. Thus, stems with discolored phloem contained only 56% as much functional sieve tube area as comparable healthy stems.

**Translocation in girdled trees.**—An experiment was conducted to test whether the 4-hr delay in export from leaves of symptomatic trees was due to a constricting influence of the decreased sieve tube area, which might cause an increase in the carbohydrate content of the leaves and stem. Constriction was achieved by girdling two healthy elms at 10 cm above the soil line. Exposed xylem was immediately wrapped with a grafting band. Exposure to $CO_2$ took place 14 days after girdling. The girdled and two healthy trees were exposed to $^{14}CO_2$ in the aforementioned manner and allowed to translocate for 4 hr before sampling. Treated leaves of the girdled trees retained 86.2% of the $^{14}C$ assimilate compared with 74.6% for control trees. This difference was significant ($P = 0.05$). Girdled trees contained significant ethanol-soluble radioactivity (at least five times greater than background) 70 cm below the treated leaf. Control trees had significant amounts of $^{14}C$ in the roots more than 100 cm below the treated leaf.

**Starch content of leaves.**—No differences were noted in the starch content of leaves of infected and healthy seedlings following a 12-hr period in the light. Immediately after an 18-hr dark period, however, leaf tissue from diseased and girdled trees contained more starch than leaf tissue from healthy elms.

## DISCUSSION

Our results are similar to those of Catlin et al (2), who examined phloem transport in pear trees infected with a mycoplasma-like organism associated with leaf curl, a form of pear decline. Leaves of curl-affected trees exported considerably less $^{14}C$ assimilate than did leaves of healthy trees. Inhibition of translocation was thought to be due to reduced export from leaves as well as blockage of phloem in the stem. Necrosis of phloem in the leaves was postulated as a factor contributing to reduced translocation, but anatomic observations were not made.

Phloem in healthy elm stems contained almost twice as much sieve tube cross-sectional area as discolored phloem in diseased trees. Clearly, heavy callose deposits on sieve plates and lateral sieve areas can impair phloem transport (8). The relationship between sieve tube cross-sectional area and phloem translocation, however, is not so clear. Geiger et al (3) reported a positive correlation between translocation rate and sieve tube cross-sectional area in sugar beet petioles. They believed that the sieve tube area available for conduction of assimilates limited the translocation rate. Wardlaw and Moncur (15), however, found that severing half of the vascular tissue in wheat peduncles had no effect on the dry weight accumulation of grains during the following 48 hr.

Two lines of evidence indicate that callose deposition and reduced sieve tube cross-sectional area are not solely responsible for the observed disturbances in phloem function in PN-diseased elms. First, phloem transport was found impaired 4 wk after inoculation, prior to the development of phloem discoloration. Based on previous observation (1), no histopathologic symptoms had developed at that time. Second, girdling, which drastically reduces the sieve tube cross-sectional area and would be expected to induce callose deposition distal to the girdle (9), did not affect translocation to the extent seen in symptomatic elms.

When this study was undertaken, we wondered whether the early feeder root necrosis in American elms was at least partially due to phloem malfunction. Since we observed a substantial presymptomatic impairment of phloem function, starvation indeed may play a role. Since some $^{14}C$ reached the roots of diseased trees in the 24 hr following $^{14}CO_2$ exposure, starvation is not likely to be solely responsible for feeder root necrosis. Also, since some assimilate reached the roots even in instances in which discolored phloem encircled the stem, replacement phloem apparently is capable of translocating assimilated materials, albeit inefficiently.

Although the data indicate that phloem function is impaired prior to the development of other symptoms in the secondary phloem, they provide no insight as to the mechanism by which translocation is affected.

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