

Obtaining Xylem Fluid from *Gossypium hirsutum* and its uses in Studies on Vascular Pathogens

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The properties of xylem fluids from healthy cotton (*Gossypium hirsutum* L.) plants and those infected with *Verticillium albo-atrum* Reinke & Berthold (micro-sclerotial form) are important to studies of resistance. There are several methods for obtaining tracheal fluid. Gottlieb (5) centrifuged stem sections in his studies on *Fusarium* wilt of tomato, but the yield of fluid was low. Bennett et al. (2) preferred gas-displacement (vacuum) and liquid-displacement methods to centrifugation. Bollard (3), Chiarappa (4), and Kessler (7) obtained good yield of fluid from woody plant species with the vacuum method. When fluids were difficult to obtain, Gregory (6) used the water-displacement method in studies of red oak trees infected with *Ceratocystis fagacearum* (Bretz) Hunt. Wood (14) obtained relatively high yields of xylem fluid from tomato plants by the root-pressure method. Plants were decapitated above the soil line, and the stumps were fitted with pipettes to collect the fluid. Barker (1) collected fluid from banana xylem by inserting a capillary tube into a vessel and obtaining sap through a combination of capillary action, root pressure, and siphoning.

Plant species vary considerably in their ability to yield tracheal fluid. Although the vacuum and centrifuge methods resulted in yield of fluid from stem sections of greenhouse-grown cotton plants, the amount (0.01-0.1 ml) was inadequate for measurements of physicochemical properties, use as substrate for growing *V. albo-atrum*, and various other physiological studies.

The root-pressure technique yields adequate quantities of fluid from cotton plants. It is described in detail here as modified from the procedure used by Wood (14). Also listed are other uses of the method in the study of vascular plant pathogens.

Plants 6-16 weeks old growing in 4 or 6-inch pots in the greenhouse were decapitated 2.5-4 cm (higher for larger and older plants) above the soil line with a razor blade rinsed with 50% ethanol. Approximately 7 mm below the top of the stump, a ringing cut was made, followed by a vertical cut from the top of the stump to the ringing cut. The bark was peeled, exposing the woody xylem. The exposed xylem was washed thoroughly with 50% ethanol, and an alcohol-sterilized surgical-tubing adapter approximately 13 mm long was fitted over the exposed xylem (Fig. 1) and forced down to the ringing cut (not over the bark). A sterile (autoclaved) glass tube, 6-7 cm long, plugged at both ends with cotton, was inserted into the surgical-tubing adapter after first removing one of the plugs and wash-

ing that end with 50% ethanol. The tube was inserted to within 1-2 mm of the top of the stump (Fig. 1).

The above procedure was desirable for obtaining xylem fluid free of phloem contaminants and reducing contamination of the fluid with microorganisms. If one wishes to determine differences in gross yield of fluid between groups of plants (e.g., infected vs. healthy plants), it is not necessary to remove the bark and expose the xylem. Instead, the stump can be washed with alcohol, and the surgical tubing can be forced over the bark on the stump and the collecting tube attached as described.

Xylem fluid was forced into the collecting tube by root pressure. It was collected when the volume of fluid was within 5 mm of the cotton plug or when the peak volume for the day was reached. A 25 G needle of a sterile 2.5-ml serological syringe was pushed through the surgical tubing into the 1-2 mm gap between the top of the stump and the collecting tube and the fluid was withdrawn. Since the puncture caused by the needle was self-sealing, leakage developed only if samples were taken frequently from the same plant for a week or more. Use of the syringe to harvest fluids allowed collection without dismantling the apparatus, and permitted continuous sampling as long as the plant continued to yield.

Letting the collection tubes overflow favors contamination. Pots must be watered with care to prevent water from spilling over the apparatus and down into the collecting tube. Hence, subirrigation with watering saucers is desirable.

Xylem fluid was usually frozen at -20°C immediately after harvest. Storage bottles for each plant can be held at -20°C , and samples added daily.

The following observations were made over an 8-year period in which the root-pressure technique was used in various experiments involving over 400 plants. Yield per plant was greatest from 3 to 4-month-old plants. Some of the older plants gave as much as 2 ml/day for 14 days, although the average yield was 0.5 ml/day for 7 days. The percentage of plants that gave xylem fluid ranged from 10 to 80% in individual experiments. The variation was correlated with cloudy and sunny conditions. Yields were highest on bright days with greenhouse temperatures of 27°C or above, though exceptions occurred and good yields were obtained occasionally on cloudy days. When six low-yielding plants were moved from the greenhouse at below 27°C into a chamber at 38°C , yield was not enhanced in two tests. Warming of soil in pots during the daylight hours seemed to enhance yields, but other factors may have been involved.

The yield of fluid was cyclical. Fluid that moved into a collecting tube during the daylight hours often retreated into the stem in the late afternoon and at night. Even though the plants had been decapitated, the movement of sap through the xylem followed a pattern similar to the extension and drooping of leaves and petioles—extended during daylight but drooped in the late afternoon and evening, which in healthy plants is associated with reduced transpiration.

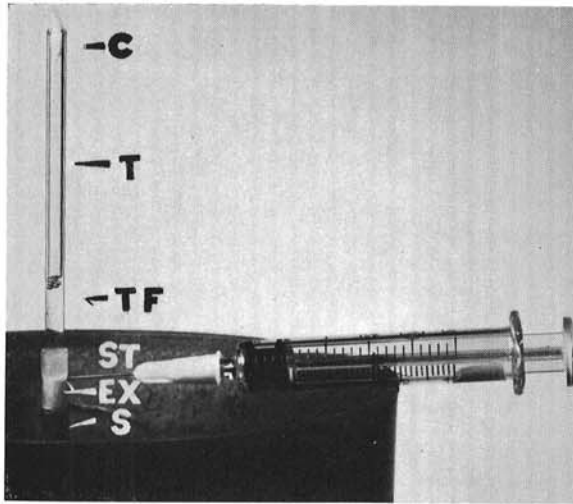


Fig. 1. Apparatus for obtaining xylem fluid from stems of cotton plants by root pressure. Cotton plants were decapitated, leaving a stump (S), and the bark was peeled, leaving the woody xylem exposed (EX). A surgical-tubing adapter (ST) was forced over the xylem, and a glass tube (T) plugged with cotton (C) was fitted into the surgical tubing. After tracheal fluid (TF) had moved into the collecting tube, it was withdrawn by a hypodermic syringe by inserting the point through the surgical tubing into the gap left between the stump and the collecting tube.

More plants yielded fluid, and in greater quantities, when cut close to the soil line, as described, rather than at midstem or higher.

Contamination of the apparatus with microorganisms, particularly bacteria, will reduce yields in studies that run for more than 4 days. Contamination can be reduced with aseptic procedures. If contamination is present in fluids, filtering is recommended.

I have studied the level and types of substrate available to *V. albo-atrum* in xylem vessels, some physicochemical properties, and the effects of fluids from healthy susceptible and resistant cotton varieties on conidial germination, growth of the pathogen, and conidial production (11). The method has been used to determine the numbers and rate of production of conidia in plants differing in susceptibility (12), the extent of vascular dysfunction by measuring differences in gross yield of fluid from plants with differing disease severity (8), and the formation of inhibitor in tolerant and resistant varieties infected with different strains of *V. albo-atrum* (9). Studies are in progress on the upward and downward movement of tracheal fluids in an attempt to explain bipolarity (13) of the pathogen in cotton plants. In cross-protection (10) experiments with different strains of *V. albo-atrum*, the fate of the strain from which cotton plants are protected could be determined by plating tracheal fluids, recovering

single conidia or colonies, and determining the ratio of occurrence of the strains. Systemic fungicides can be assayed directly from tracheal fluid, and rates of uptake by roots and concentration determined with relative ease. Systemic fungicides might be easier to detect in plants with this method, than with tissue-extraction or tissue-plating methods. Whether systemic fungicides reach the xylem of the stem after foliage application could be determined.

No doubt the method can also be used in other studies on the development and control of vascular plant pathogens of many different species. Applications would seem to be wider with relatively small plants, however, than with large ones.

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