

## Extraction of Fluid from Healthy and Dutch-Elm-Diseased Elm Branches using Hydraulic Compression

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Based on part of a M.S. Thesis submitted to the University of Massachusetts. Paper No. 1005, Massachusetts Agricultural Experiment Station. This research was supported in part by Hatch project 110 (Cooperative Regional Research project NE-25). Appreciation for guidance in this research is expressed to Dr. Mark S. Mount and Dr. Trevor Robinson.

### ABSTRACT

Extracts were obtained from Dutch-elm-diseased and healthy elm branches using hydraulic compression. Phenol oxidation was inhibited by using low temp, N<sub>2</sub> atmospheres, and sodium metabisulphite during extraction. Polygalacturonase and phosphatidase activity were detected in both diseased and healthy tissue extracts, polygalacturonase being greater in the diseased tissue extracts.

Phytopathology 64:1265-1267

Additional key words: *Ulmus americana*, Dutch elm disease.

Obtaining fluid from woody tissue can be an obstacle in physiological studies of tree diseases. A common method is the use of negative air pressure to collect sap from excised branches (5). This offers access mainly to tracheal sap and yields relatively low volume for analysis. In some studies it is desirable to obtain fluid from other than tracheal cells, and to recover large volumes with as little change in original composition as possible. Elgersma (4), investigating cellulase and pectic enzyme activity in the Dutch elm disease, obtained wood extracts by grinding new annual ring tissue in a Braun homogenizer and subjecting the fluid to cold acetone extraction. The precipitate was resuspended in 0.2 M NaCl and analyzed. He found some evidence of enzyme activity in both diseased and healthy elm extracts, but his results were too inconsistent to report. Many investigators have found cellulase and pectic enzyme activity in cultures of *Ceratocystis ulmi*, but none has reliably extracted it from diseased tissue. An attempt here was made to design a method of extracting fluid from woody tissue which would: (i) give access to non-vessel fluid, (ii) yield significant volumes, and (iii) enable the recovery of enzyme activity which may be present in the healthy or diseased tissue.

An apparatus was designed which enabled the compression of woody tissue using hydraulic pressure and which allowed collection of expressed fluid in an ice-cooled reservoir (Fig. 1). The plunger, cylinder, and drain plug were machined from stainless steel in order to avoid

fluid contact with reactive metal surfaces. The restraining jacket was fashioned from thick-walled steel tubing which allowed the safe application of large forces (up to 213.5 kN was tested). The plug at the base contained a groove (canal) around its middle into which fluid could flow from vertical slots in the upper portion of the plug. The canal was tapped with a right angle tunnel through the plug base and was connected to a reservoir with plastic tubing. A gasket was placed between the base and cylinder to prevent escape of expressed fluid. The press was placed within a restraining apparatus, at the base of which was fastened a hand-operated hydraulic ram (Hein-Warner) capable of 71.2 kN force.

Ten branches of approximately equal diam (1.0 - 1.5 cm) were selected from three six-yr-old clonal American elm trees (6) and inoculated in early June with a spore suspension of *Ceratocystis ulmi*. Branches were excised at the first sign of wilt, stripped of leaves and bark, cut into 10-cm sections, rinsed in distilled water, and placed in plastic bags. The bags were flushed with nitrogen to reduce phenol oxidation, sealed tightly and stored at 5 C for no more than 24 h. Ten branches of similar diam were selected as controls from healthy trees of the same clone, and were treated in the same manner.

Immediately prior to the extraction procedure, each single branch sample was removed from the cold chamber, clipped into short (0.5 - 1.0 cm) sections and dropped into the press chamber, which had been previously chilled to 5 C, and through which there flowed a steady stream of N<sub>2</sub> gas. When the complete sample (average wt 42 g) had been placed in the chamber, the plunger was slipped in, the N<sub>2</sub> disconnected, and the force applied. As soon as the fluid began draining, further force was applied slowly; about 30 min was required for the complete compression of each sample.

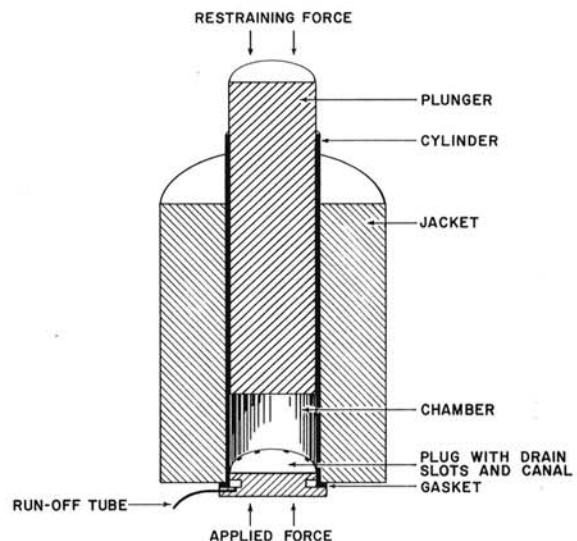


Fig. 1. Cut-away diagram of compression apparatus designed for fluid extraction from elm wood tissue. Machined by Owen Hødder, Argyle, New York. Chamber (cylinder) dimensions: 20.3 × 4.3 cm. Jacket dimensions: 14.6 (long) × 2.4 cm (wall thickness).

The fluid was collected in an ice-cooled reservoir containing 0.05 ml of 1.0 M sodium metabisulphite to inhibit phenol oxidation (1). The extract was centrifuged (10,000 g), dialyzed against distilled water for 12 h at 5 C, and lyophilized for storage at -20 C.

To further remove impurities, each lyophilized sample was resuspended in 1.0 ml distilled H<sub>2</sub>O, applied to a 0.7 × 8.7 cm DEAE Cellulose column at 5 C, and eluted in 1.0 ml fractions with distilled water at approximately one drop per 15 s. Preliminary studies showed that the protein peak was eluted in fractions 4 through 7 (9). These fractions were combined for each sample and subjected to analysis for polygalacturonase (PG) activity by measuring the loss in viscosity of a 1.2% solution of sodium polypectate at 30 C and pH 5.0, using size 300, Fenske-Ostwald viscometers (2).

The volume of fluid expressed from each sample varied from 3 to 25 ml depending upon the size and condition of the branch; the average was about 10 ml. At no time during the extraction or analysis procedures was discoloration caused by phenol oxidation apparent in the fluid extracts. Comparisons with extracts taken without the use of N<sub>2</sub> or metabisulphite showed a marked contrast in color.

PG activity was detected in all ten of the diseased tissue extracts, and only insignificantly in healthy extracts. After 1.0 h reaction time, the average percent viscosity loss for the 10 diseased tissue extracts was 4.6, while that of healthy tissue was 0.6. The average relative activity for the diseased extracts was 1.73, where one unit equals 1,000 divided by the time in minutes required for a 50% loss in viscosity. Qualitative analysis using the cup plate assay (3) also revealed the presence of phosphatidase in both diseased and healthy elm branch extracts.

The method of hydraulic compression of woody tissue described here allows the recovery of significant volumes of extract which, due to the high pressures, may be composed of ruptured living cell contents as well as tracheal sap. Although PG previously has been sought, but never reliably documented, in Dutch-elm-diseased tissue, its recovery in this case could be a result of the

method of extraction. *C. ulmi* may produce PG primarily while invading parenchyma, where this enzyme would aid cell-wall penetration and host colonization. Direct penetration by *C. ulmi* has been shown (7) and most of the hyphal growth is now reported to occur within parenchymal cells (8). PG produced during these activities would be difficult to recover without access to nonvessel cells. The use of N<sub>2</sub> gas, sodium metabisulphite, and low temp during the extraction, and the clarification of the crude extract before analysis may also contribute significantly to the success in recovering enzyme activity, by limiting any possible harmful effect of oxidized phenols, and by reducing interference of other unknown plant products found in the crude preparation.

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