Alteration of Sugar Translocation in Aspen by Hypoxylon mammatum

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ABSTRACT

Hypoxylon mammatum causes sugars to be accumulated above and below cankers on aspen (*Populus tremuloides*). These accumulated sugars are found in the xylem but not the phloem, and in highest concentration in xylem most recently invaded by the fungus. Use of radioisotope-labeled glucose showed that the fungus is able to inhibit sugar translocation at a distance from parasitized tissue. Since the xylem beneath the canker is plugged, it serves as storage tissue for the accumulated sugar. The hypothesis is advanced that this accumulated sugar is utilized by the fungus to support its growth vertically in the xylem and also into the bark. Phytopathology 61:366-368.

Hypoxylon mammatum (Wahl.) Mill., a serious canker-causing pathogen of aspen (*Populus tremuloides* Michx.), kills its host by girdling the stem (2). Before this girdling action is completed, an increasing percentage of the stem circumference is unable to continue its translocational functions. The fate of the water and substrate, whose movements are affected, and their involvement in the development of hypoxylon canker infections remain unclear. The work reported here relates to the fate of this material.

MATERIALS AND METHODS.—To ascertain the distribution of free sugars in infected trees, three cankered quaking aspens were sampled at each of five different times (15 trees total) during 1968 and 1969, including all seasons of the year. The diam at breast height (dbh) of the trees averaged 7.6 cm, and the infections were estimated to be 1 to 3 years old. Samples consisted of 1-cm discs, approx 1 cm thick (4), removed serially along a vertical line through the center of each canker (Fig. 1). As each sample was removed, it was divided into bark and sapwood, after which the pieces were placed into separate vials where they were immediately dried for 24 hr at 80 C and weighed. The soluble sugars were extracted in 70% ethyl alcohol (added immediately after weighing) at 25 C. Aliquots of the extracts were assayed for total sugar content by the phenol-sulfuric acid method (3). Some of the sugars present also were identified by gas-liquid chromatography of their trimethylsilyl ethers. This was accomplished by removing acidic and basic compounds with ion-exchange resin, lyophilizing the neutral fraction, and preparing trimethylsilyl ethers of its components using Tri-Sil Z (Pierce Chemical Co.). An aliquot was analyzed using a Varian Aerograph Model 1200 gas chromatograph equipped with a flame ionization detector and a 1.8 m stainless steel column (3.18 mm outside diam) packed with 3% OV-1 on 60/80 mesh Chromosorb W. Immediately after injection, the temp of the column oven was programmed to rise from an initial 130 C to 300 C at 4 C/min. In this way, the sugars in the extract (mono- through tri-saccharides) could be separated during a 45-min run.

To study the effect of hypoxylon cankers on the movement of sugars in aspen, 10 infected quaking aspens plus two control trees, with an average dbh of 13.5 cm, were selected. The infected trees, which had been inoculated 33 months earlier, had well-developed cankers with average dimensions of 49 × 11 cm. None of the cankers had sexual stroma. Treatment involved drilling a 2-mm-diam hole approx 1 cm deep in each tree at a predetermined point adjacent to the canker (Fig. 2). Immediately after each hole was made, a 50 μliter solution containing a mixture of glucose-6-^{3}H and sucrose-U.L.-^{14}C with a specific activity of 66.7 μc ^{3}H and 6.7 μc ^{14}C/ml was introduced with a micropipet. One hr after treatment, discs (as before) were removed from 46 predetermined sampling points (23 within the cankered region) systematically arranged in and around the cankered portion of each tree. The discs were placed into separate scintillation vials, frozen with dry ice, and brought to the laboratory where the sugars were extracted as previously described. The ethyl alcohol was slowly evaporated, and the wood and bark discs were covered with 15 ml of dioxane containing 8.0 g butyl PBD [2-(4′-t-butylphenyl)-5-(4′-biphenyl)-1,3,4-oxdiazole] and 0.5 g PBBO [2-(4′-biphenyl)-6-phenyl-benzoxazole] per liter. Radioactivity was measured with a Beckman LS-150 liquid scintillation spectrometer. Count data were quantified to an error of less than 5% with the aid of an electronic computer (5).

RESULTS.—The sapwood of control trees was found to have an average of 35.1 ± 1.3 μg of free sugar per mg tissue, while the bark, with a higher level and somewhat more seasonal variation, averaged 139.2 ± 5.1 μg per mg tissue (Fig. 1). Considerable variation in the distribution of sugars in the cankered trees was noted. In the bark of these trees, there was a decrease in sugars in the cankered area, with the least amount occurring at the midpoint of the affected portion (22.1 ± 4.3 μg/mg). The xylem of the infected trees, however, had a considerable increase in free sugars, with the max accumulation (126.8 ± 12.7 μg/mg) located slightly above the canker and a second, somewhat smaller accumulation (89.4 ± 15.9 μg/mg) at the lower edge of the infected zone.

Gas chromatographic analysis of the neutral com-

pounds occurring in the bark and sapwood in the region of sugar accumulation above the canker indicated that the compounds in the sapwood were similar to those found both in the phloem of healthy trees and in the bark of the infected trees at some distance above the cankers. Compounds that could have resulted from enzymatic hydrolysis of the cellulose and hemicellulose in this region (cellobiose, glucose, galactose, mannose, arabinose, and xylose) did not appear to have increased in concn over that found in healthy sapwood. Since a number of the materials present were not identified, the gas chromatographic data were used only for comparative purposes.

When labeled glucose and sucrose were introduced into healthy trees, glucose was translocated in all directions, but primarily upward in a column directly above the point of application, while sucrose moved only upward (Fig. 2-D). The rapid upward movement of these sugars indicates that some of the material was carried upward in the xylem. Slower downward movement of labeled glucose shows the rate of phloem translocation to be about 15 to 20 cm/hr. The rate of movement of glucose in the phloem was greater in the trees sampled in the afternoon than in the morning. The downward movement of glucose was accompanied by lateral movement, so that labeled glucose was found in the entire circumference of the tree from the point of application to the lower limit of translocation.

When the labeled sugars were introduced near the canker in infected trees, there was a different translocational response. Sucrose did not move from the point of application; however, glucose was translocated when applied either above (Fig. 2-A), to the side (Fig. 2-B), or at the base (Fig. 2-C) of the canker. When application was made in the center of the canker, little or no movement of either sugar was detected. The absence of rapid upward movement of labeled sugars in the cankered trees indicates that in the affected area the xylem of these trees is nonfunctional, and in fact, tyloses have been observed in histological preparations from this region. Downward movement of glucose applied at the periphery of cankers may be due to phloem or fungal translocation, or to both. Glucose applied at the base of a canker moved downward at a rate almost identical with the downward movement in healthy trees, but the pattern of its movement was different. When applied at the side or base of a canker, glucose was also distributed in a narrow ring of tissue at the level of application. This ring was also apparent when label was applied at the top of a canker, but was much wider.

Discussion.—Accumulation of natural sugars was noted above the point of infection in the xylem of cankered trees, even though only 25% or less of their circumference was infected. No corresponding increase occurred in the sugar content in the bark of these trees.

The site of this large increase in sapwood sugar content above the visible edge of the canker was found to correspond closely with the location of the

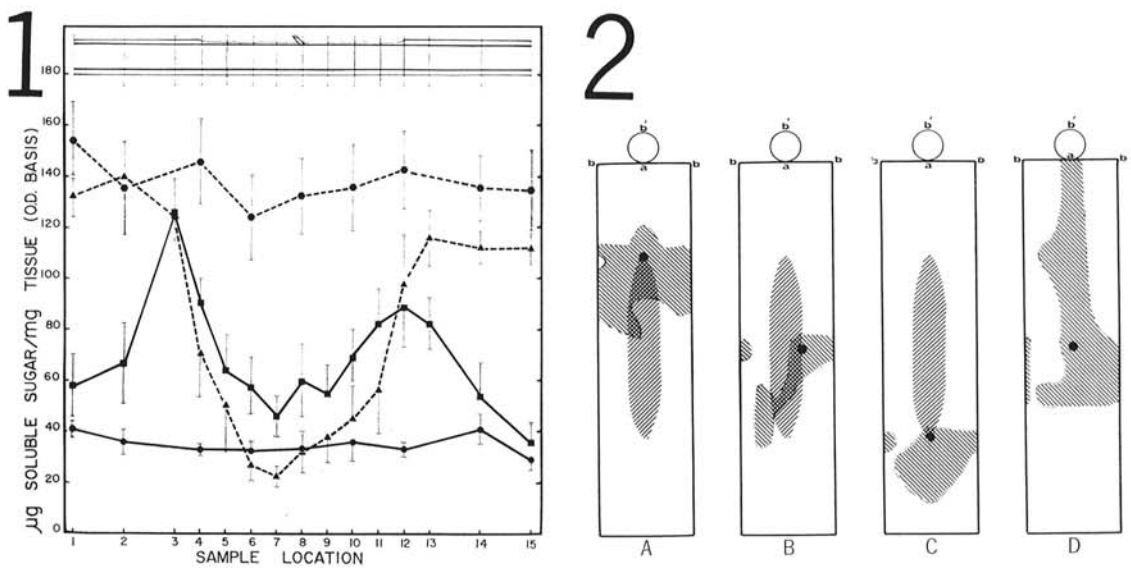

Fig. 1-2. **1)** Distribution of soluble sugar in bark and sapwood of cankered and healthy aspen trees (*Populus tremuloides*). Visible cankering (diagram at top) begins at sampling position 4 and ends at 12. Discolored sapwood extends upward to between positions 2 and 3 and downward to between positions 13 and 14. Curves represent sugar content in ●------●, healthy bark; ▲------▲, cankered bark; ●——●, healthy sapwood; ■——■, cankered sapwood. **2)** Movement of labeled glucose about *Hypoxylon mammatum* cankers. Each diagram represents a segment of an aspen (*P. tremuloides*) stem from which a cylinder of bark and sapwood (circle ab′) has been shown in a plane (bab). The canker is indicated by the lines slanting to the right, distribution of labeled glucose by lines slanting to the left, and the point of application by the solid circle. Cross-hatched areas indicate where label entered the necrotic tissue of the canker. **A)** Labeled glucose applied at the apex of the canker; **B)** labeled glucose applied at one side of the canker (label was applied at either the left or right side of a canker—this is a summary of movement of label from both application points); **C)** labeled glucose applied at the base of the canker; **D)** labeled glucose applied to healthy aspen trees.

advancing edge of the fungus mycelium which, too, is found in the xylem (7, Schipper, *unpublished data*). Since sugar accumulated several cm above areas showing visible phloem damage, it appears that the xylem most recently penetrated by the fungus may serve as a metabolic sink, enabling the fungus to utilize this sugar to support its continued advance in the xylem and its subsequent growth outward into the cambium and bark. Alternatively, the large increases in free sugar content above the canker may be attributed to phloem blockage in the cankered area and the resultant shunting of the translocates into the xylem ray parenchyma. The action of the pathogen on cell wall and cytoplasmic constituents also may contribute a small amount of free sugar to this accumulation.

Other unidentified compounds found by gas chromatography in healthy aspen bark also were found in the xylem of infected trees in the region where sugars accumulate above the canker. These same materials were not found in healthy aspen sapwood or in sapwood of infected trees above the region of sugar accumulation. It is not known what relationship, if any, these materials have with canker development.

In an attempt to identify the various types of translocation that take place in infected aspen, glucose and sucrose, both of which occur naturally in aspen, were chosen as the labeled sugars for study. Bagga (1) has reported that *H. mammatum* metabolizes glucose but not sucrose, and it was reasoned that since the fungus does not metabolize sucrose, it also might not translocate this material. If this were true, it would be possible to distinguish translocation which resulted from host activity from that which resulted from a combination of fungus and host activity. But this test was confounded because sucrose did not move from the point of introduction in cankered trees, and apparently moved only in the xylem of healthy trees. The movement of sucrose did, however, serve as a marker of water movement in the xylem.

The movement of labeled glucose around and through cankered areas may have been a result of fungal translocation, particularly where movement was directly across phloem and plugged xylem of the cankered area. This movement through the cankered zone indicates that hypoxylon infection does not pose a complete barrier to sugar translocation, a finding which agrees with the report of Hurbert et al. (8) regarding the translocation of ^{32}P in *Pinus monticola* that were infected with *Leptographium* sp. Their report concluded that ray parenchyma in the xylem permitted translocation of phosphorus past the lesion. Insufficient data were obtained in this study to determine the tissue in which the labeled glucose was moving.

The rapid movement of labeled sugar upward in the stem of the healthy tree suggests that some of the label was carried in the xylem. Since no such rapid upward movement of label occurred when the material was introduced adjacent to the canker, the xylem in the cankered area probably does not function in water transport as does healthy xylem. This is further suggested by the fact that tyloses frequently are found in vessels and tracheids in and above the cankered area (Schipper, *unpublished data*). The slower downward and lateral movement of label probably indicates phloem and mycelial transport.

The concn of labeled glucose in a narrow ring in cankered trees suggests that the fungus is able to inhibit sugar translocation far beyond the tissue it has actually invaded and accumulate this sugar in the xylem. The sugar so blocked may well be utilized by the fungus to support its growth into the bark, which contains toxic amounts of phenolic compounds (6). Additional research on this hypothesis is continuing.

LITERATURE CITED

1. Bagga, K. K. 1968. Physiology of Hypoxylon pruinatum and its pathogenesis on quaking aspen. Diss. Abstr. 29:1897-B.
2. Bier, J. E. 1940. Studies in forest pathology. III. Hypoxylon canker of aspen. Can. Dep. Agr. Publ. 691, Tech. Bull. 27, 40 p.
3. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
4. Guiher, J. K. 1969. Extraction of cambium samples from living trees. Forest Prod. J. 19(3):59-60.
5. Haissig, B. E., & A. L. Schipper. 1970. A computer system for use in quantifying liquid scintillation data. Anal. Chem. 42:1456-1458.
6. Hubbes, M. 1962. Inhibition of Hypoxylon pruinatum by pyrocatechol isolated from bark of aspen. Science 136:156.
7. Hubbes, M. 1964. New facts on host-parasite relationships in the hypoxylon canker of aspen. Can. J. Bot. 42:1489-1494.
8. Hurbert, E. E., W. K. Ferrell, & F. D. Johnson. 1955. Translocation past bark lesions of young western white pine. Plant Dis. Reptr. 39:500-503.