

SUPPORT FOR THE SALIVATION-EGESTION HYPOTHESIS FOR *XYLELLA FASTIDIOSA* INOCULATION: SALIVARY GLUCANASE IS INJECTED INTO XYLEM DURING VECTOR FEEDING

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The salivation-egestion hypothesis for the inoculation mechanism of *Xylella fastidiosa* (Xf) proposes that saliva secreted into plants is taken up into the vector's precibarium. There, saliva loosens the Xf bacterial biofilm by enzymatically degrading the β -1, 4 glucans that form the chemical backbone of the exopolysaccharide matrix binding bacteria to the insect's cuticle. Bacteria-laden fluid is then egested into xylem vessels. The present study purified and tested enzymatic activities of all carbohydrases in the saliva of glassy-winged sharpshooter (GWSS), the primary economic vector of Xf. The most active salivary fraction was a type of cellulase, β -1,4 glucanase. Subsequently, over 2,000 pairs of GWSS salivary glands were dissected, extracted, and the glucanase purified, then used to produce a polyclonal antibody for immunohistochemical staining of GWSS salivary sheaths. Grapevine petioles were probed by sharpshooters recorded via electrical penetration graph (EPG), to determine when insects' stylets had reached xylem via production of an X wave. Grapevine tissues were excised, fixed, embedded in paraffin, sectioned, and examined using confocal scanning laser microscopy (CSLM). Results showed that red-stained glucanase-containing saliva co-localized (and therefore was secreted simultaneously) with sheath saliva for most of the probe. However, the narrowest branch(es) at the tip(s) of the salivary sheath were composed only of glucanase-containing saliva, which was also clearly injected into the terminal xylem cell. Glucanase-containing saliva formed a ring that lined the interior of the xylem vessel and penetrated into its cell wall. In addition, salivary lining of the xylem cell was found in adjoining sections, pulled over 200 μ m downstream from the point of entry and connected to a salivary deposit in the middle of the xylem cell. Glucanase-containing saliva was also injected into non-xylem cells, where it accumulated in loose, flocculent masses. Results support the egestion-salivation hypothesis by showing that bacteria carried in glucanase saliva could be injected into xylem.

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