PHLOEM-FEEDERS VERSUS PHLOEM SEALING MECHANISMS

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Phloem is one of the two main transport tissues in plants and is responsible for translocation of most of the organic nutrients (e.g., carbohydrates, amino acids, proteins) that are transported throughout the plant. Many insects, almost exclusively hemipterans, exploit phloem sap as their primary source of nutrition. Two of the major advantages of specializing on phloem sap are 1) the high nutritional content of phloem sap, and 2) phloem sieve tubes (the actual transport conduit in phloem) function as a pipeline with a continuous flow of phloem sap; thus once a sieve tube is "tapped" it can provide an almost inexhaustible supply of sap, allowing a phloem-specialist to feed from the same sieve tube for hours, or in the case of whitefly nymphs, an entire nymphal instar. However, just the same as animals have clotting and coagulation mechanisms to prevent loss of blood when their vascular system is damaged, plants have analogous mechanisms that seal damaged sieve tubes to prevent the loss of phloem sap. Consequently, in order to successfully exploit the pipeline properties of sieve tubes, all phloem-specialists need to have counter-measures that either prevent triggering the sealing response and/or to reverse the sealing response if it occurs. Phloem-specialists such as aphids and whiteflies inject considerable amounts of watery saliva into sieve tubes the function of which has been widely hypothesized to prevent or reverse the sieve tube sealing response. Recent evidence *in vitro* indicates that watery saliva of aphids is capable of reversing at least one of the sealing mechanisms in legume phloem: a protein plug that arises from a proteinaceous inclusion body, the forisome, that is characteristic of legume sieve tubes. This presentation describes the early stages of a 3-year project to determine the role of saliva or other counter-measures in overcoming the sieve tube sealing response using *in vivo* methods. Ingestion of phloem sap from sieve tubes by aphids and whiteflies is always preceded by a period of salivation into the sieve tube. This salivation period has frequently been postulated to be a response to reverse sieve tube sealing that presumably occurs when the sieve tube is pierced. This hypothesis was tested with the pea aphid, *Acyrthosiphon pisum*, feeding of faba bean, *Vicia faba*. The feeding site of the aphid was cryofixed shortly after penetration of the sieve tube near the beginning of the salivation phase. This fixed the tissue almost instantaneously and the stylets usually remained in the sieve tube; consequently, the specific sieve element (the cells making up the sieve tubes) that was pierced could be identified and the state of the forisome (either in its compact non-plugging phase or in its dispersed plugging phase) in that sieve element could be determined at this exact point in time using confocal microscopy. No evidence of forisome plugging of sieve tubes was detected. Pea aphids generally precede "phloem-phase" behavior (salivation into and ingestion of phloem sap from sieve tubes) by a repeating series of penetrations of the sieve element. In order to test the hypothesis that these "pre-phloem phase" penetrations resulted in forisome plugging that was eventually reversed by the time phloem phase began, the cryofixation technique was used to

instantaneously fix the phloem and stylets so that the state of the forisome could be examined during "pre-phloem phase" sieve tube penetrations. Again, no evidence of forisome plugging of sieve tubes was detected. Consequently, it appears that initial penetration of the sieve elements does not trigger a phloem-sealing response at least in the pea aphid - faba bean system, and that the function of the initial salivation into sieve elements immediately following penetration is something other than reversing a sealing response. These results also generate the question: why/how does stylet penetration of a sieve element not trigger a sealing response?