

# Insertion Site Selection and Feeding of the Hemlock Woolly Adelgid: Implications for Host-Plant Resistance

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The hemlock woolly adelgid (HWA), *Adelges tsugae* (Hemiptera: Adelgidae), is an invasive forest pest that threatens the existence of eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*T. caroliniana* Engelm.) in the eastern United States. It is a small, aphid-like insect with piercing-sucking mouthparts that it uses to penetrate its host plant and feed off the xylem ray parenchyma cells (Young et al. 1995). The hemlock woolly adelgid is native to eastern Asia and northwestern North America where it feeds on, but does not kill, its host (Annand 1924, Havill et al. 2006). The mechanism of resistance and/or tolerance is unknown. The invasive population in the eastern United States was first detected near Richmond, Virginia in 1951 (Gouger 1971, Souto et al. 1996). The range of HWA in the east has since spread through the range of hemlocks, now affecting 18 states (USDA FS 2011). The mortality caused by HWA to eastern and Carolina hemlocks is tremendous. Approximately 80 to 90 percent of infested hemlocks native to the eastern United States have already vanished as a result of this exotic insect (Hale 2004, Townsend and Rieske-Kinney 2006). Moreover, the production of hemlocks for the ornamental industry, valued at \$34 million between Tennessee and North Carolina alone, has been virtually eliminated (Bentz et al. 2002).

Following initial infestation, a susceptible hemlock declines in health, evident by needle drop, bud abortion, and inhibition of new growth (McClure 1991). A healthy hemlock dies in as few as 4 years, but may survive beyond 10 (McClure 1987, 1991). In its native range, HWA is described as a minor pest and almost never kills its host (Bentz et al. 2002, Furniss and Carolin 1977, Keen 1938). For years, it was believed that eastern and Carolina hemlocks were entirely and exclusively susceptible to HWA. However, in the wake of widespread mortality, anecdotal evidence suggested surviving individuals or stands of eastern and Carolina hemlocks may be less susceptible. This has since been corroborated by research, and distinct populations have been selected and continue to be pursued as putatively resistant to HWA (Caswell et al. 2008, Ingwell and Preisser 2010, Kaur 2009, Oten et al. 2011). In addition, Carolina hemlock has been identified as less susceptible to HWA than eastern hemlock (Jetton et al. 2008, Oten 2011).

The purpose of this research is to investigate host-plant selection and host use of HWA to further understand mechanisms of resistance that occurs both inter- and intra-specifically. Understanding this may facilitate progress toward a resistant hemlock by identifying characteristics that can be identified among individuals in natural stands for selection and/or to screen within a breeding program.

The crawler stage of HWA is the first stage and thus the host-seeking stage of the insect. Crawlers lack wings and compound eyes; consequently, transportation between host trees is entirely passive. Dispersal occurs either by crawling to nearby branches, wind, phoresy on macro-vertebrates (e.g., birds, deer), or by human movement of infested stock (Butin et al. 2007, McClure 1990). Therefore, habitat and host location, the initial two steps in host-plant selection by phytophagous insects (Bernays and Chapman 1994), does not occur as part of the host selection behaviors of HWA. Using scanning electron microscopy (SEM), we studied the sensilla of HWA crawlers because it is this stage that must accurately determine feeding sites on hemlocks. The distal segment of the HWA labium possesses two fields of sensilla, including six long subapical sensilla trichodea and five shorter apical labial sensilla trichodea. Two apical sensilla are observed touching the stylet bundle in some

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images and are hypothesized to play a role in sensing the orientation and/or movement of the stylets during insertion site selection. In aphids, stylet penetration is initially based on substrate color and texture and chemoreception occurs after short probing of the host plant and internal gustation (Ibbotson and Kennedy 1959, Miles 1958, Powell et al. 2006). Given their biological similarities to aphids, we theorize that HWA use a similar host acceptance tool, and probes the plant briefly, sensing its host with internal contact chemoreceptors. However, this research should be extended to substantiate this conjecture. HWA tarsi are two-segmented and bear a claw on the distal end. A long pair of knobbed setae and four pairs of shorter, spatulate setae are observed. Such setae are present in other adelgids: a pair in the pine leaf adelgid (*Pineus pinifoliae*) and three per tarsus in the spruce gall adelgid (*Adelges lariciatus*). However, the authors of these studies did not speculate about the function of the setae (Cumming 1968, Underwood 1955). We suggest they may be involved in adhesion based on casual visual observations of surface contact during inverted walking and the morphological similarity to the adhesive tenant hairs of some springtails (Blottner and Eisenbeis 1984). In comparison to the sessile adults, antennae of the same generation crawlers are well-developed and elongated. The scape and pedicel each have a pair of trichoid sensilla, seated in a pit and lacking an observable pore at its tip, indicating they likely function as mechanoreceptors. The flagellum has a long terminal sensillum and four secondary sensilla at the distal end. A sensorium lays subapically on the posterior edge of the flagellum and is surrounded by a cuticular fringe that may aid in keeping foreign particles out. The sensorium is likely analogous to the primary rhinaria of chemoreceptory function in aphids and is referred to by Stoetzel (1998) in alate female Adelgidae.

Using whole-body homogenate of HWA, general protease enzyme activity was assayed using the EnzChek<sup>®</sup> protease assay kit (Invitrogen, Eugene, OR). Trypsin-like activity in HWA was present, although lower than in *Lygus lineolaris*, which was used as a positive control. The kinetic for HWA enzymatic activity reached its asymptote at 30 minutes, whereas the *L. lineolaris* comparison continued to increase after more than 1 hour (fig. 1).

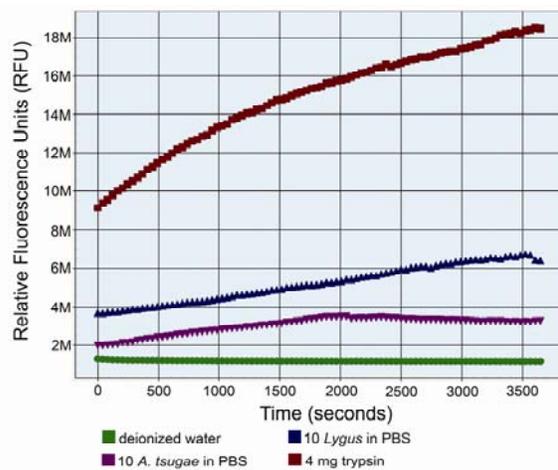


Figure 1—Enzyme kinetics of trypsin-like assay.

Although this enzyme could be present in the salivary gland complex, midgut, or hemolymph of the insect, its occurrence is nevertheless revealing of the feeding habits of HWA. Generally, the presence or absence of specific enzymes is indicative of a consumer's ability to digest plant or prey materials (Agusti and Cohen 2000; Baptist 1941; Cohen 1990, 1998). The presence of trypsin-like enzyme activity in HWA confirms the ability of HWA to digest protein rather than depending upon free amino acids as is the case for sap-feeding insects such as aphids. If used extra-orally by HWA (i.e., injected into their host plant) the enzyme may be used to digest structural proteins of the plant that are otherwise insoluble (Hori 1971). In addition, the presence of protease may have implications for host response and host-plant resistance. The wound-response pathway, which may be triggered by the feeding of herbivorous insects, is one of these responses that may induce protease inhibitor genes

(Dietrich et al. 1999). The systemic response of hemlock to HWA feeding, which is currently uncharacterized, implicates HWA feeding in inducing the systemic response (Radville et al. 2011, Walker-Lane 2009). The response could be triggered by mechanical wounding or physiological elicitors in the saliva. Future research should focus on this interaction to understand if HWA is triggering the wound-response pathway in hemlocks via mechanical or salivary wounding and the differences in the response between susceptible and resistant hemlocks.

Physical characteristics of hemlock surfaces were observed using low-temperature SEM to document differences and how they may relate to resistance. There were differences in trichome density, placement, and length on the species studied, but they did not relate to the degree of susceptibility to HWA and are likely not involved in host selection or resistance. Cuticle thicknesses were compared for various locations on the pulvinus: at the HWA stylet bundle insertion point, at the side of the needle where the HWA settles, and on the outside of the needle (fig. 2). We compared thicknesses to determine if cuticle was related to insertion site selection. Based on preliminary observations, there appears to be a slight trend. The cuticle thickness at the point on stylet insertion appears to be thinner than the other two locations on the pulvinus that were measured. Although this needs to be investigated more thoroughly, including a statistical analysis, this suggests that the specificity for stylet bundle insertion of HWA may be a result of accessibility to host tissues.

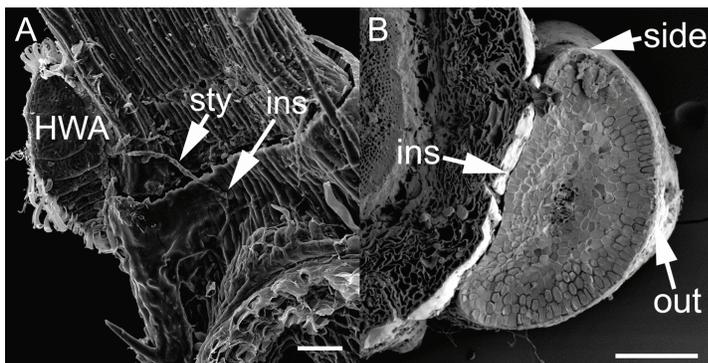


Figure 2—A) Settled hemlock woolly adelgid (HWA), showing adaxial side of the needle and pulvinus. HWA, hemlock woolly adelgid; ins, insertion point; sty, stylet bundle. Scale, 100  $\mu$ m. B) Locations measured on pulvini for cuticle thickness. ins, insertion point; out, outside. Scale, 200  $\mu$ m.

Understanding the interactions between HWA and naïve hemlocks in the eastern United States furthers our understanding of mechanisms involved in host-plant selection and resistance, aiding the progress towards a hemlock resistant to HWA. Ideally, a resistant hemlock will be a component of an integrated pest management program to control populations of HWA in the eastern United States, allowing ecosystem restoration and restoring the hemlock ornamental industry.

## Literature Cited

- Agusti, N.; Cohen, A.C. 2000. *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. *Journal of Entomological Science*. 35: 176–186.
- Annand, P.N. 1924. A new species of *Adelges* (Hemiptera: Phylloxeridae). *The Pan-Pacific Entomologist*. 1: 79–82.
- Baptist, B.A. 1941. The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. *Quarterly Journal of Microscopical Science*. 83: 91–139.
- Bentz, S.E.; Riedel, L.G.H.; Pooler, M.R.; Townsend, A.M. 2002. Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. *Journal of Arboriculture*. 28(4): 200–205.

- Bernays, E.A.; Chapman, R.F. 1994.** Host-plant selection by phytophagous insects. New York: Chapman and Hall. 312 p.
- Blottner, D.; Eisenbeis, G. 1984.** Ultrastructure of long tibiotarsal spatula-hairs in *Tomocerus flavescens* (Collembola: Tomoceridae). *Annales de la Société Royale Zoologique de Belgique*. 114: 51–57.
- Butin, E.; Preisser, E.; Elkinton, J. 2007.** Factors affecting settlement rate of the hemlock woolly adelgid, *Adelges tsugae*, on eastern hemlock, *Tsuga canadensis*. *Agricultural and Forest Entomology*. 9: 215–219.
- Caswell, T.; Casagrande, R.; Maynard, B.; Preisser, E. 2008.** Production and evaluation of eastern hemlocks potentially resistant to the hemlock woolly adelgid. In: Onken, B., Rheardon, R., eds. Fourth symposium on the hemlock woolly adelgid in the eastern United States. FHTET-2008-01. Morgantown, WV: U.S. Department of Agriculture, Forest Service, Forest Health Protection: 124–134.
- Cohen, A.C. 1990.** Feeding adaptations of some predaceous Hemiptera. *Annals of the Entomological Society of America*. 83(6): 1215–1223.
- Cohen, A.C. 1998.** Feeding biology of the silverleaf whitefly (Homoptera: Aleyrodidae). *Chinese Journal of Entomology*. 18: 65–82.
- Cumming, M.E.P. 1968.** The life history and morphology of *Adelges lariciatus* (Homoptera: Phylloxeridae). *Canadian Entomologist*. 100(2): 113–126.
- Dietrich, R.A.; Lawton, K.; Friedrich, L. [et al.]. 1999.** Induced plant defense responses: scientific and commercial development possibilities. In: Chadwick, D.J.; Goode, J.A., eds. *Insect-plant interactions and induced plant defence*. Chichester, England: Wiley: 205–216.
- Furniss, R.L.; Carolin, V.M. 1977.** *Western forest insects*. Miscellaneous Publication 1339. Washington, DC: U.S. Department of Agriculture, Forest Service, Government Printing Office.
- Gouger, R.J. 1971.** Control of *Adelges tsugae* on hemlock in Pennsylvania. *Scientific Tree Topics*. 3: 1–9.
- Hale, F.A. 2004.** *The hemlock woolly adelgid: a threat to hemlock in Tennessee*. SP503-G. Knoxville: The University of Tennessee, Agricultural Extension Service.
- Havill, N.P.; Montgomery, M.E.; Yu, G.; Shiyake, S.; Caccione, A. 2006.** Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America*. 99(2): 195–203.
- Hori, K. 1971.** Studies on the feeding habits of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) and the injury to its host plants. I. Histological observations of the injury. *Applied Entomology and Zoology*. 6: 84–90.
- Ibbotson, A.; Kennedy, J.S. 1959.** Interaction between walking and probing in *Aphis fabae*. *Journal of Experimental Biology*. 36: 377–390.
- Ingwell, L.L.; Preisser, E.L. 2010.** Using citizen science programs to identify host resistance in pest-invaded forests. *Conservation Biology*. 25(1): 182–188.
- Jetton, R.M.; Hain, F.P.; Dvorak, W.S.; Frampton, J. 2008.** Infestation rate of hemlock woolly adelgid (Hemiptera: Adelgidae) among three North American hemlock (*Tsuga*) species following artificial inoculation. *Journal of Entomological Science*. 43(4): 438–442.
- Kaur, N. 2009.** Developing artificial rearing techniques for hemlock woolly adelgid, *Adelges tsugae* and balsam woolly adelgid, *Adelges piceae*; artificial infestation and epicuticular wax study of Carolina hemlock, *Tsuga caroliniana*, provenances. Raleigh, NC: North Carolina State University. 118 p. M.S. thesis.
- Keen, F.P. 1938.** *Insect enemies of western forests*. Misc. Pub. 273. Washington, DC: U.S. Department of Agriculture, Forest Service.
- McClure, M.S. 1987.** Biology and control of hemlock woolly adelgid. Bull. 851. New Haven: Connecticut Agricultural Station.
- McClure, M.S. 1990.** Role of wind, birds, deer, and humans in dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology*. 19(1): 36–43.
- McClure, M.S. 1991.** Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology*. 20(1): 258–264.
- Miles, P.W. 1958.** Contact chemoreception in some Heteroptera, including chemoreception internal to the stylet food canal. *Journal of Insect Physiology*. 2(4): 338–347.

- Oten, K.L.F. 2011.** Host-plant selection by the hemlock woolly adelgid, *Adelges tsugae* Annand: sensory systems and feeding behavior in relation to physical and chemical host-plant characteristics. Raleigh, NC: North Carolina State University. 259 p. Ph.D. dissertation.
- Oten, K.L.F.; Walker-Lane, L.N.; Jetton, R.M. [et al.]. 2011.** Developing hemlocks resistant to the hemlock woolly adelgid. <http://www.threatenedforests.com/research/wp-content/uploads/2011/08/NAFIWC-2011.pdf>. (11 August 2011).
- Powell, G.; Tosh, C.R.; Hardie, J. 2006.** Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*. 51: 309–330.
- Radville, L.; Chaves, A.; Preisser, E.L. 2011.** Variation in plant defense against invasive herbivores: evidence for a hypersensitive response in eastern hemlocks (*Tsuga canadensis*). *Journal of Chemical Ecology*. 37: 592–597.
- Souto, D.; Luther, T.; Chianese, B. 1996.** Past and current status of HWA in eastern and Carolina hemlock stands. In: Salom, S.M.; Tignor, T.C.; Reardon, R.C., eds. *Proceedings of the first hemlock woolly adelgid review*. Morgantown, WV: U.S. Department of Agriculture, Forest Service: 9–15.
- Stoetzel, M.B. 1998.** Antennal and other characters useful in identification of the Aphidoidea (Homoptera). *Proceedings of the Entomological Society of Washington*. 100(3): 588–593.
- Townsend, L.; Rieske-Kinney, L. 2006.** Meeting the threat of the hemlock woolly adelgid. ENTFACT-452. Lexington: University of Kentucky. Cooperative Extension Service.
- Underwood, G.R. 1955.** The external morphology of the five forms of *Pineus pinifoliae* (Fitch) (Homoptera: Phylloxeridae). *Canadian Entomologist*. 87(5): 201–209.
- U.S. Department of Agriculture, Forest Service [USDA FS]. 2011.** Counties with established HWA populations 2010. <http://na.fs.fed.us/fhp/hwa/maps/2010.pdf>. (24 August 2011).
- Walker-Lane, L.N. 2009.** The effect of hemlock woolly adelgid infestation on water relations of Carolina and eastern hemlock. Raleigh, NC: North Carolina State University. 61 p. M.S. thesis.
- Young, R.F.; Shields, K.S.; Berlyn, G.P. 1995.** Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America*. 88(6): 827–835.