

Insertion Site Selection & Feeding by the Hemlock Woolly Adelgid: Implications for Host-Plant Resistance

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INTRODUCTION

The hemlock woolly adelgid (HWA; *Adelges tsugae* Annand) is an exotic insect causing widespread mortality of hemlocks in eastern North America. Introduced to the eastern U.S. in 1951, the range of HWA has spread to 18 eastern states (Fig. 1). In areas of infestation, 80-90% of hemlocks have vanished.

Of the nine hemlock species worldwide, the two in the eastern U.S. (*Tsuga canadensis* and *T. caroliniana*) are the only trees which succumb to the infestation

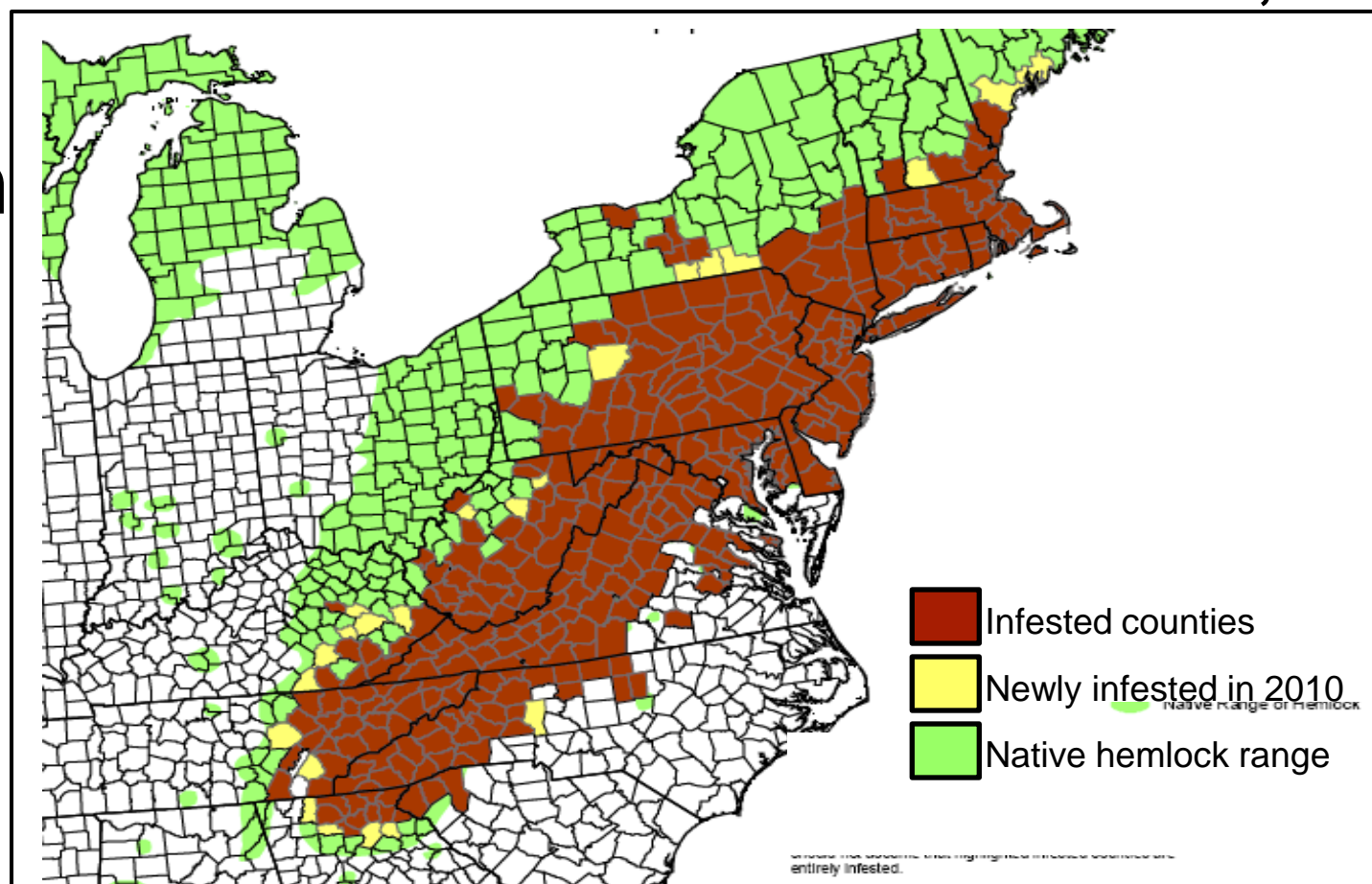


Figure 1. Range of HWA in the eastern U.S., 2010. Map: USFS

(Table 1). Although literature indicates they are wholly susceptible, both anecdotal evidence and research (Jetton et al. 2008, Kaur 2009) implies intraspecific variation. **Sources of such resistance are unknown.** In the native range of HWA, hemlock survival is attributed to: 1) a complex of natural enemies, 2) the scattered distribution of hemlocks, and 3) **host-plant resistance.**

The feeding behavior of HWA is poorly understood. They feed on ray parenchyma cells with an elongated stylet bundle comprised of four stylets. A gel-like sheath surrounds the stylets and plays many roles, such as protection against host defenses. Behaviorally, processes of host acceptance are unknown.

Table 1. Hemlock resistance to hemlock woolly adelgid.

Species	Native Range	Resistance to HWA
Eastern hemlock <i>Tsuga canadensis</i>	Eastern North America	Extent unknown
Carolina hemlock <i>T. caroliniana</i>	Southeastern North America	Extent unknown
Chinese hemlock <i>T. chinensis</i>	China	Highly resistant
Northern Japanese hemlock <i>T. diversifolia</i>	Northern Japan	Resistant
Southern Japanese hemlock <i>T. sieboldii</i>	Southern Japan	Moderate
Western hemlock <i>T. heterophylla</i>	Northwestern North America	Moderate
Mountain hemlock <i>T. mertensiana</i>	Northwestern North America	Moderate
<i>T. dumosa</i>	Himalayan Mountains	Moderate
<i>T. forrestii</i>	China	Moderate

OBJECTIVES

- 1) Investigate stylet bundle morphology and insertion behaviors
- 2) Examine morphology of host-plant surfaces of resistant and susceptible hemlocks
- 3) Determine chemical characteristics of foliar waxes in relation to host selection
- 4) Determine presence and function of digestive enzymes

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REFERENCES

Jetton, R. M., F. P. Hain, W. S. Dvorak, and J. Frampton. 2008. Infestation rate of hemlock woolly adelgid (Hemiptera: Adelgidae) among three North American hemlock (*Tsuga*) species following artificial inoculation. *J. Entomol. Sci.* 43: 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. M.S. Thesis. North Carolina State University, Raleigh.



HEMLOCK WOOLLY ADELGID FEEDING BIOLOGY

Samples were fixed with glutaraldehyde, dehydrated, and prepared for viewing with Scanning Electron Microscopy (SEM). HWA stylets consistently penetrated the adaxial side of needle below abscission layer; small setae on the labium will be investigated further as possible sensillae; sheath material was observed inside and outside hemlock; the mandibular stylet tip has serrations.

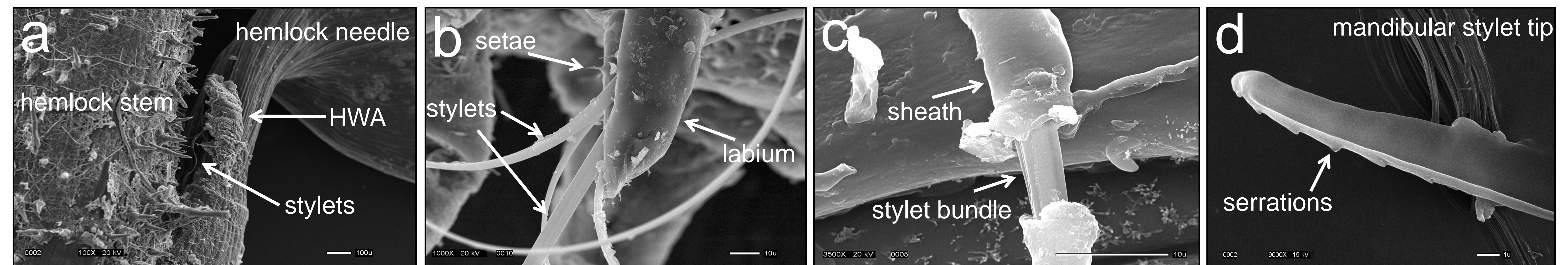


Figure 2. SEM images of a) HWA settled on eastern hemlock; b) mandibular & maxillary stylets stemming from labium; c) fracture in sheath, revealing stylet bundle enclosed; d) saw-like serrated tip of mandibular stylet.

MORPHOLOGY OF HOST SURFACES

Hemlock surfaces were studied with low temperature-SEM, a technique which preserves epicuticular waxes. Eastern hemlock is encircled in trichomes, and Southern Japanese hemlock (host species of the eastern U.S. HWA population) is void of trichomes, indicating trichomes likely do not deter HWA feeding. The resistant Chinese-Carolina hybrid has the thickest cuticle at stylet insertion point; Chinese hemlock cuticle will also be studied to determine if this characteristic deters feeding.

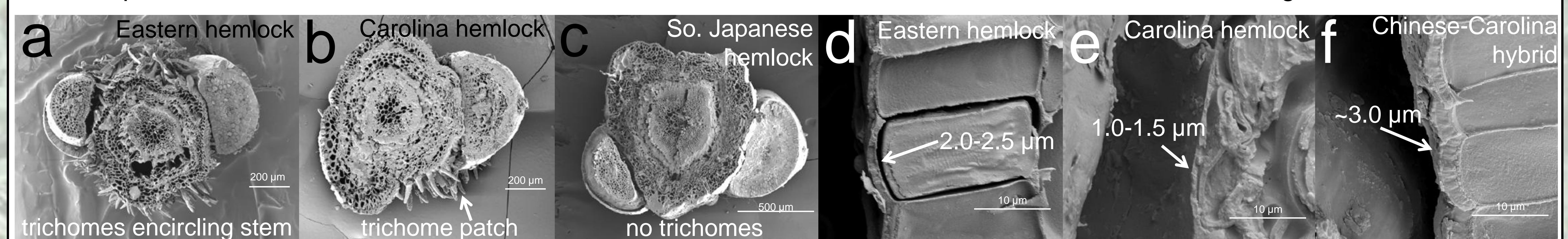


Figure 3. Trichome placement on cross-sections of various hemlock species (a-d); cuticle thickness at stylet insertion point (e-h).

CHEMICAL ANALYSIS OF WAXES

Foliar waxes were extracted with CH_2Cl_2 and analyzed with GC/MS. Preliminary results reveal an unidentified peak in Chinese hemlock; this may have implications for resistance.

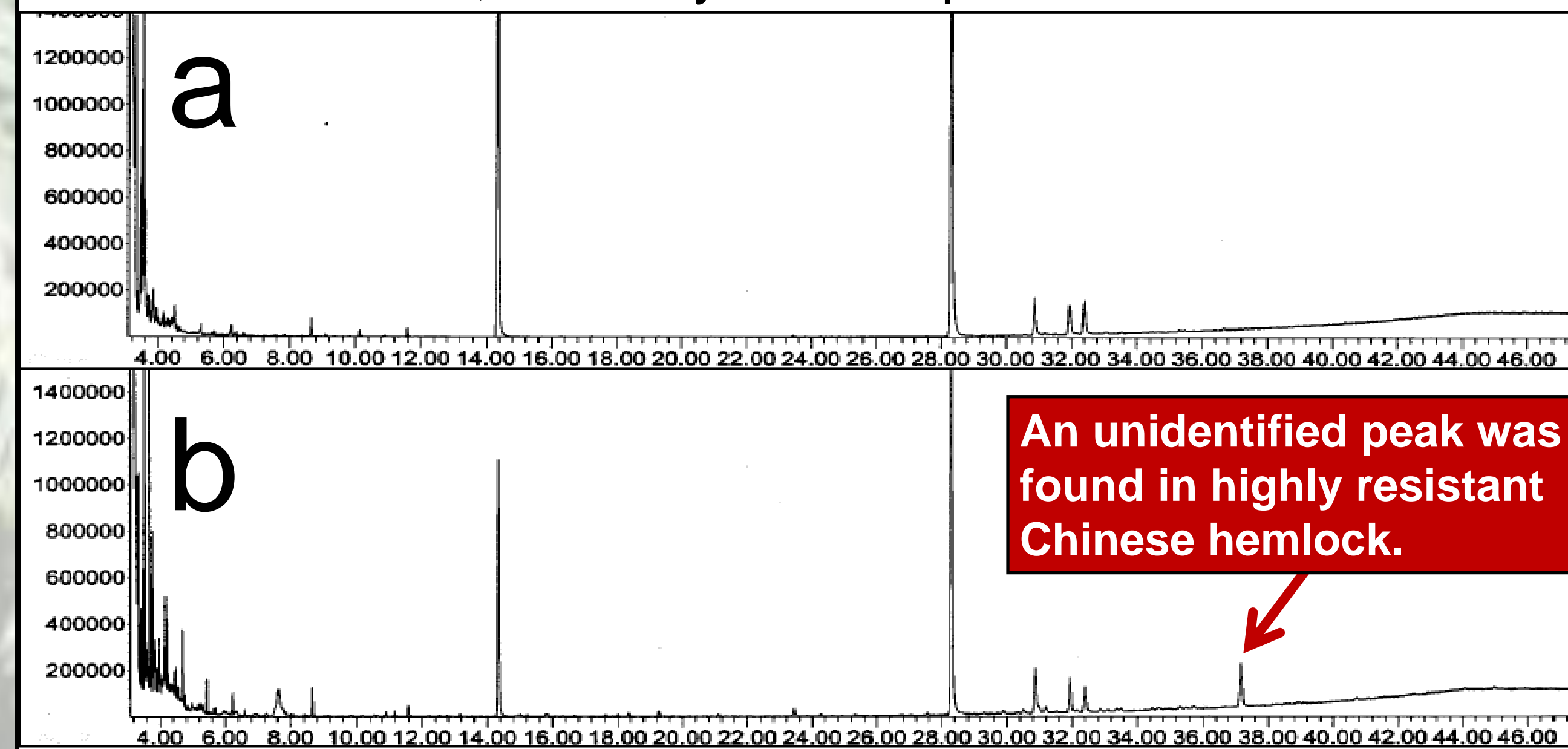


Figure 3. GC/MS profile of a) eastern, b) Chinese hemlock.

CONCLUSIONS

- Sheath material surrounds the stylets within & outside the tree.
- Sensillae on the labium (Fig. 2b), antennae, and tarsi will be examined for sensory function in future work. Understanding sensory reception will help determine cues for host selection.
- Trichomes likely do not deter HWA feeding.
- Cuticle thickness of the resistant hybrid may be a mechanism used to avoid HWA feeding. Future research will investigate all hemlock species, particularly highly resistant Chinese hemlock.
- Preliminary results indicate a chemical in the foliar waxes of Chinese hemlock may play a role in host selection. This work will be replicated and paired with behavioral studies.
- Protease (protein-digesting enzyme) was found in HWA. Future work will test for the presence of amylase (starch digesting enzyme) and investigate the ability of resistant trees to inhibit this enzyme activity as defense against HWA feeding.

DIGESTIVE ENZYMES

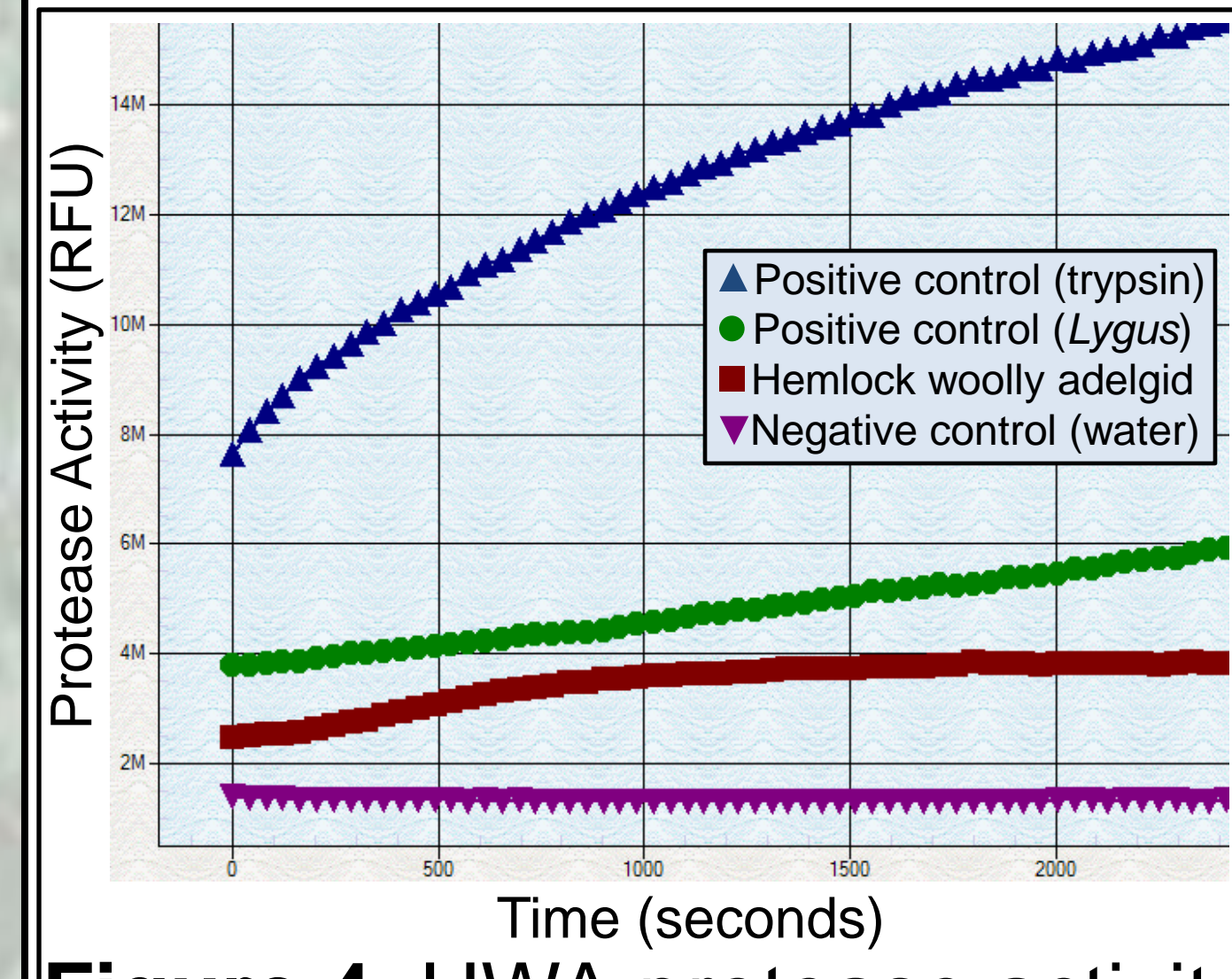


Figure 4. HWA protease activity

10 HWA were homogenized in 100 μ l phosphate-buffered saline, tested with EnzChek[®] protease assay kit, and fluorescence levels were measured. Protease was detected in HWA, indicating protein is being digested as food. Further work will discern its source: the gut or salivary glands.

SIGNIFICANCE

Understanding mechanisms of resistance in hemlocks will advance resistant breeding programs by helping us to:

- Select resistant individuals from native populations of hemlock
- Screen for resistance of breeding program progeny

With a resistant hemlock variety, we can restore vanished native stands and restore the once leading hemlock nursery industry.

