Developing Hemlocks Resistant to Hemlock Woolly Adelgid

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ALLIANCE FOR Saving Threatened Forests

Developing Hemlocks Resistant to Hemlock Woolly Adelgid

- History and Biology of HWA
- Importance of Understanding Host/Insect Interactions
- Review of Work on Host/Insect Interactions
    - Foliar Chemistry
    - Host/Water Relations
    - Feeding Behavior
        - Observations with Scanning Electron Microscopy (SEM)
    - Screening Studies
    - Genetics and Breeding
- Future Needs
    - Alliance for Saving Threatened Forests

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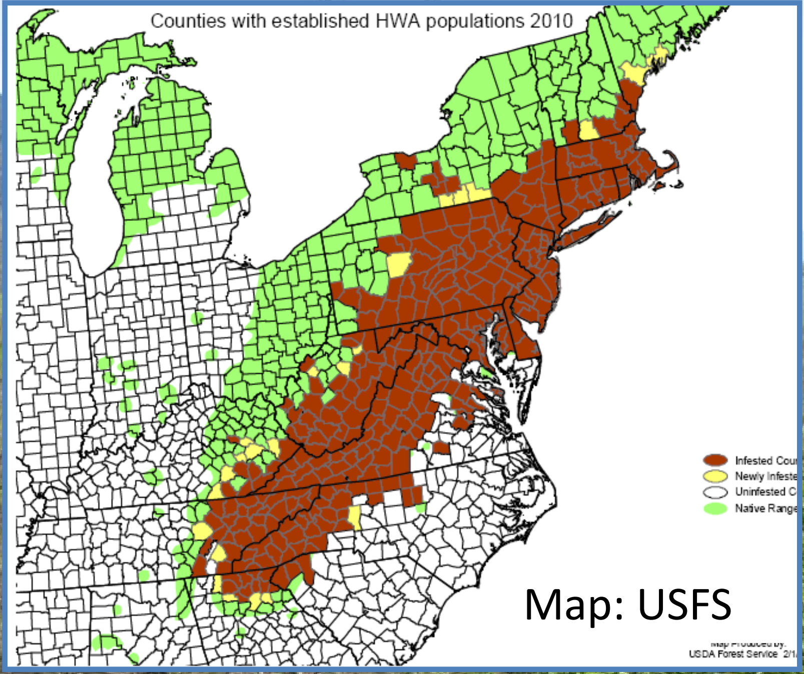
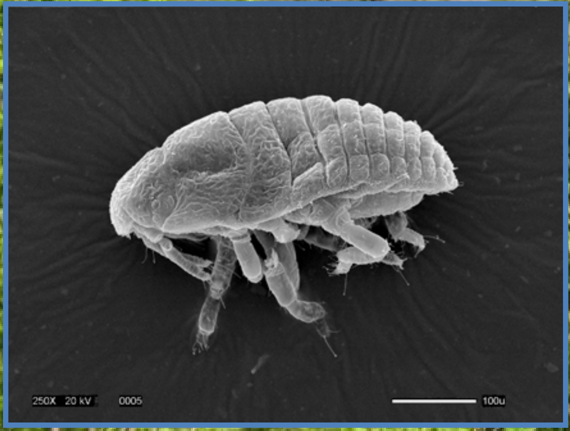
Hemlock Woolly Adelgid, *Adelges tsugae*

- Exotic pest from Japan
- Threatens eastern & Carolina hemlock populations
- Range now includes 18 eastern states
- Kills tree in as little as 4 years

Hemlock Woolly Adelgid, *Adelges tsugae*

- 80-90% mortality
- Native range: Asia, Pacific NW
- Variation in host resistance





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- History and Biology of HWA
- **<u>Importance of Understanding Host/Insect Interactions</u>**
- Review of Work on Host/Insect Interactions (including that at NCSU)
    - Observations with Scanning Electron Microscopy (SEM)
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Hemlock Resistance

Species	Range	HWA resistance
Eastern hemlock *T. canadensis*	Eastern U.S.	~~No resistance~~ Variable
Carolina hemlock *T. caroliniana*	Southern Appalachians	~~No resistance~~ Variable
Chinese hemlock *T. chinensis*	China	Very resistant
T. diversifolia	Japan	Resistant
T. dumosa	Himalayas	Moderate
T. forrestii	China	Moderate
T. heterophylla	Northwestern U.S.	Moderate
T. mertensiana	Northwestern U.S.	Moderate
T. sieboldii	Japan	Moderate

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Foliar Chemistry Summary

- High N & K may increase palatibility (Pontius et al. 2006)
- Foliar terpenoids related to alleged resistance; grouped EH & CH in one group & resistant hemlock in another (Lagalante & Montgomery 2003)
- High levels of isobornyl acetate found in EH & CH (despite phylogenetic relationships) (Lagalante et al. 2007)

Foliar Chemistry Summary

- Presence of α-hexacosanol in epicuticular wax may stimulate HWA feeding (Kaur 2008)
- High levels of α-humulene in resistant species; more abundant in needle cushion, especially during periods of feeding (Lagalante et al. 2007)

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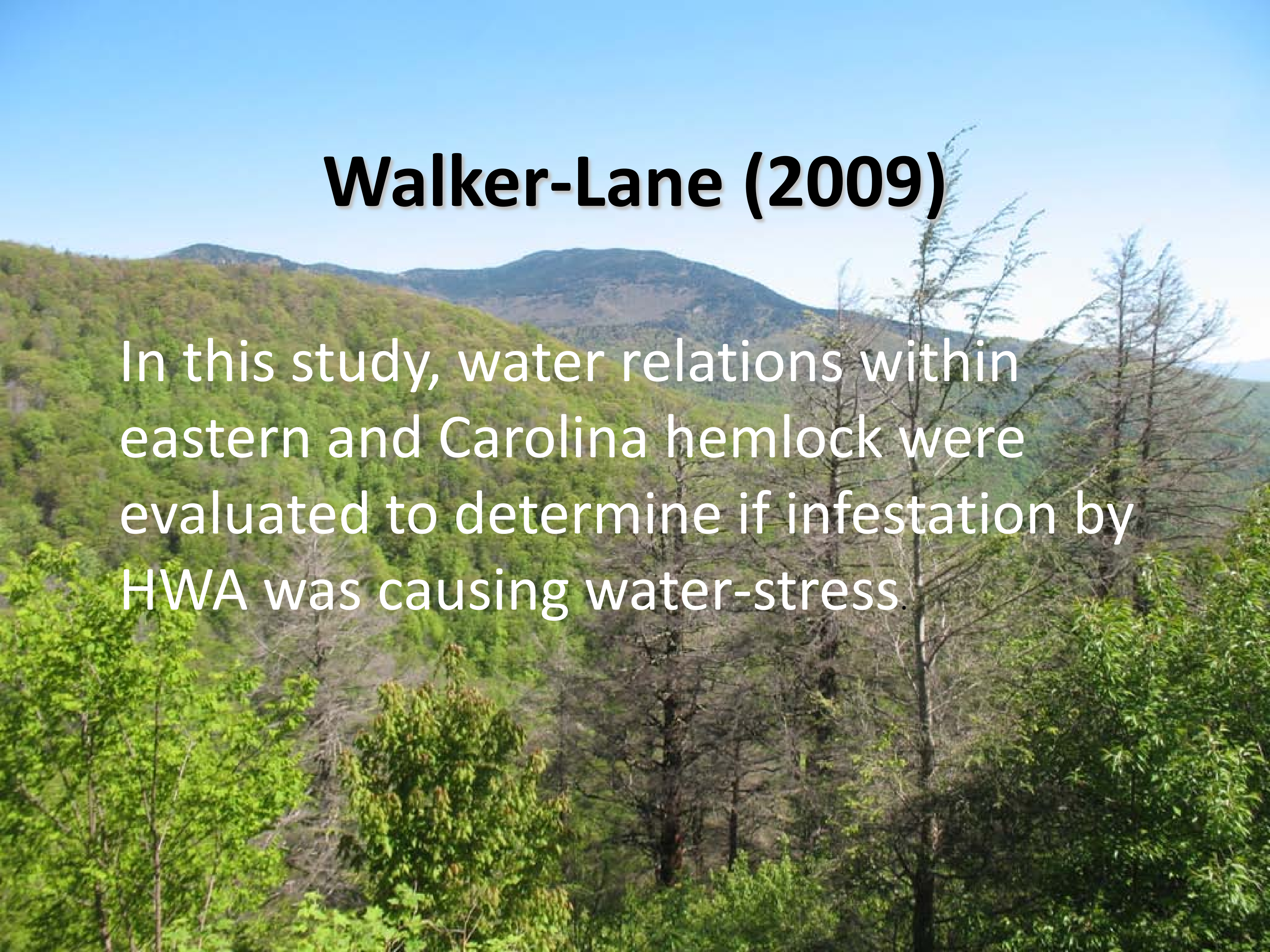
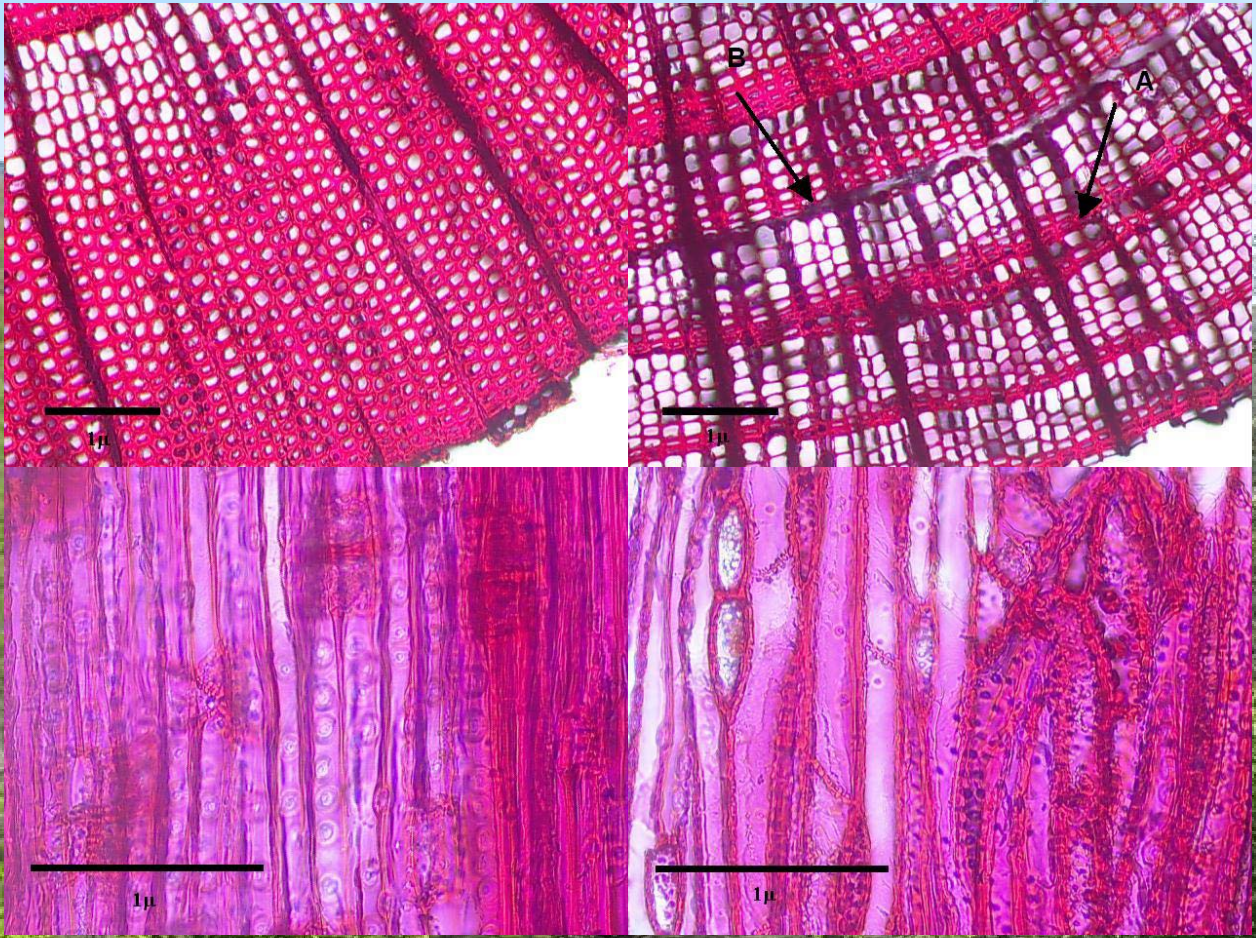


Walker-Lane (2009)

In this study, water relations within eastern and Carolina hemlock were evaluated to determine if infestation by HWA was causing water-stress.

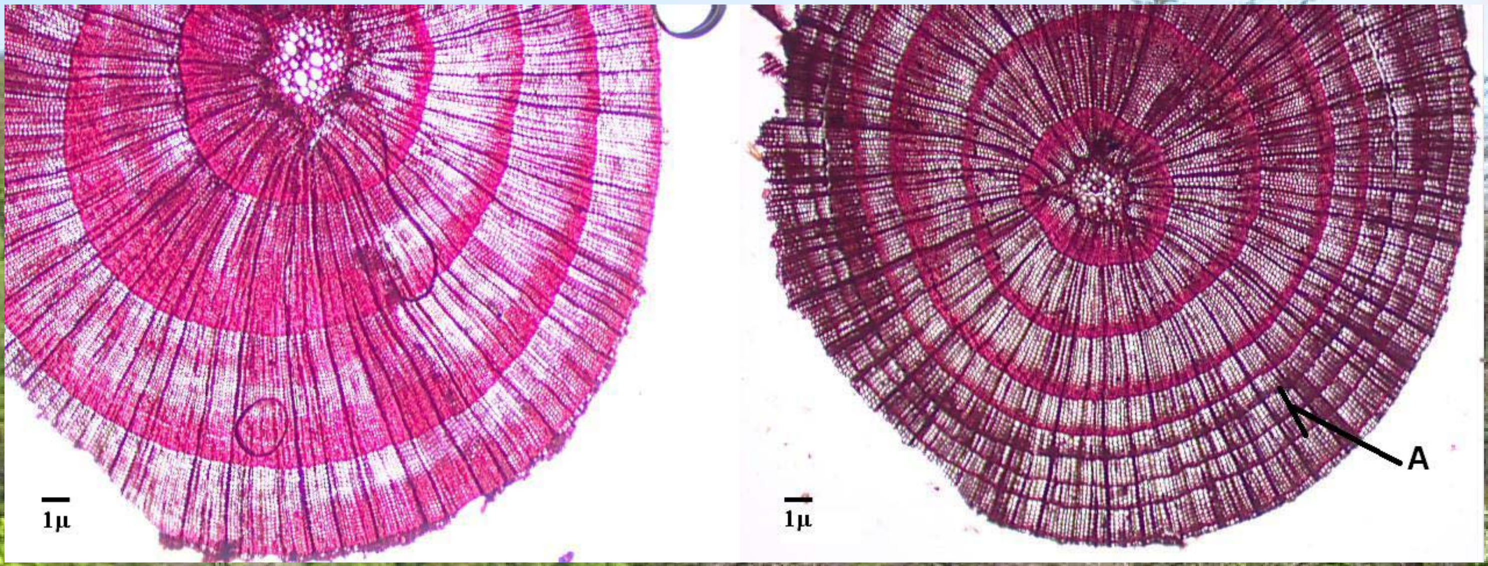
Fraser fir cross section showing rotholz and premature heartwood.

Wood Anatomy



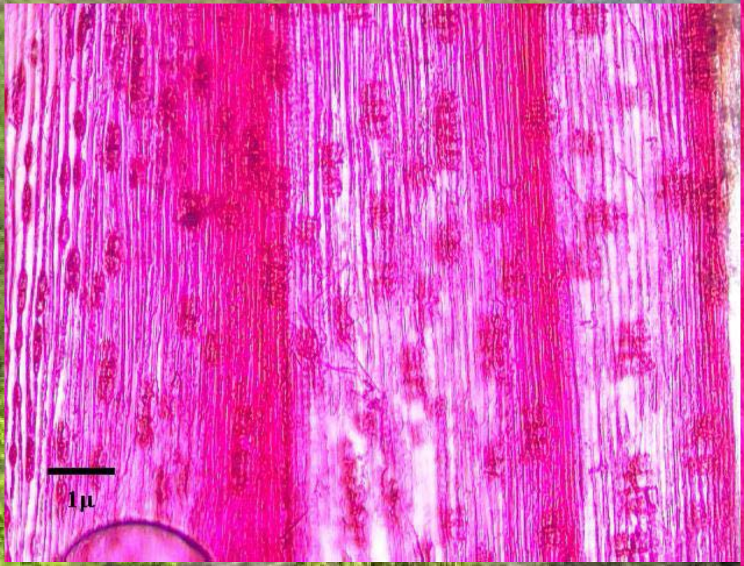
Clockwise from the top left: healthy hemlock cross-section, poor health hemlock cross-section (arrow A displays false-ring structure, arrow B displays increased longitudinal parenchyma), poor health hemlock tangential section, healthy hemlock tangential section.

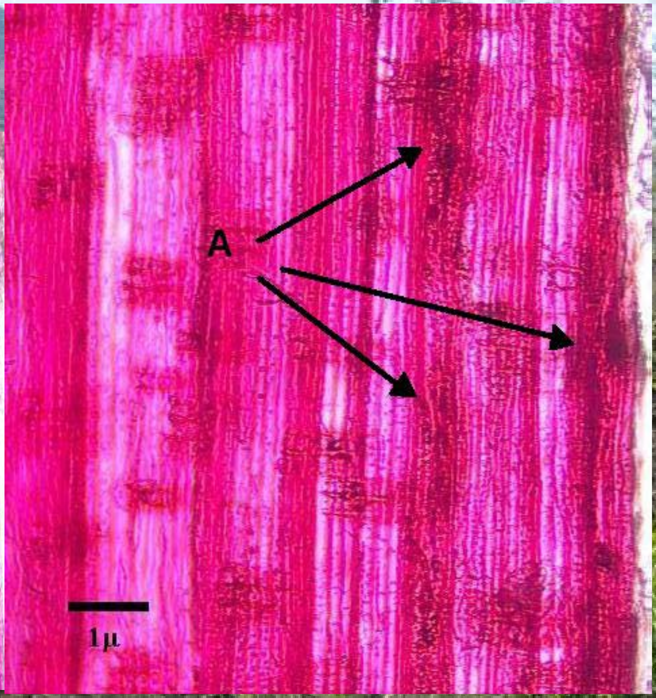
Wood Anatomy (4x)



From left to right: healthy hemlock cross-section, poor health hemlock cross-section (A indicates false-ring structures).

Wood Anatomy (10x)





From left to right: healthy hemlock tangential section, poor health hemlock tangential section (arrows indicate abnormal wood).

Wood anatomy of the branches provided evidence that infested hemlocks are experiencing abnormal wood production in the xylem. Confirmed by:

- Pre-dawn branch water potential measurements were more negative in infested hemlock than in non-infested trees

- Carbon isotope ratios of the branches were more positive for infested trees

- Means of stomatal conductance was lower in infested trees).

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Critical Factors in HWA Feeding

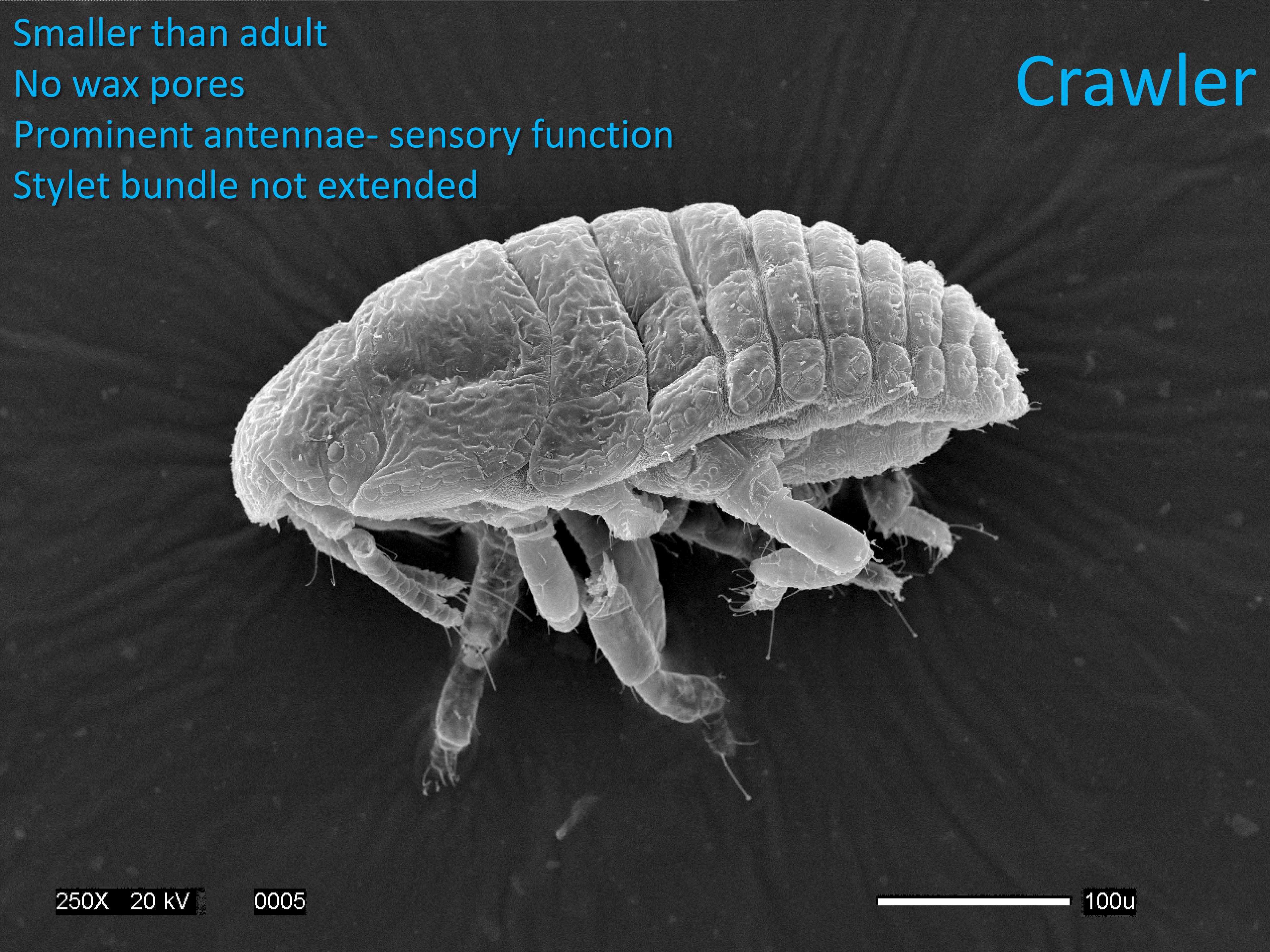
- Ability to penetrate host tissue with a very delicate stylet
- Ability to find a suitable feeding site within the host tissue for development and reproduction

Purpose of Using SEM for HWA

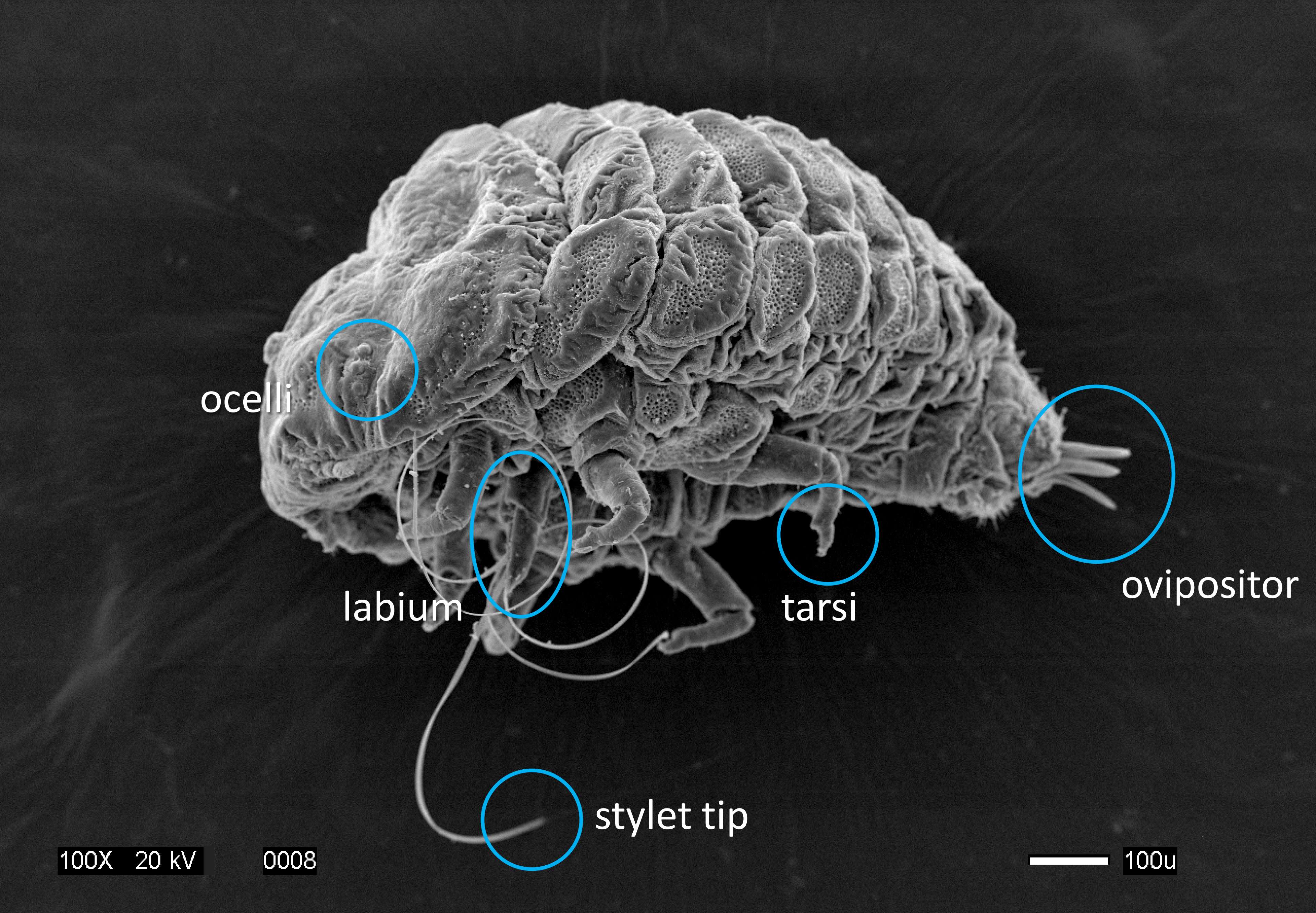
- Determine feeding sites & behavior
- Analyze & compare host surfaces
- Examine sheath material

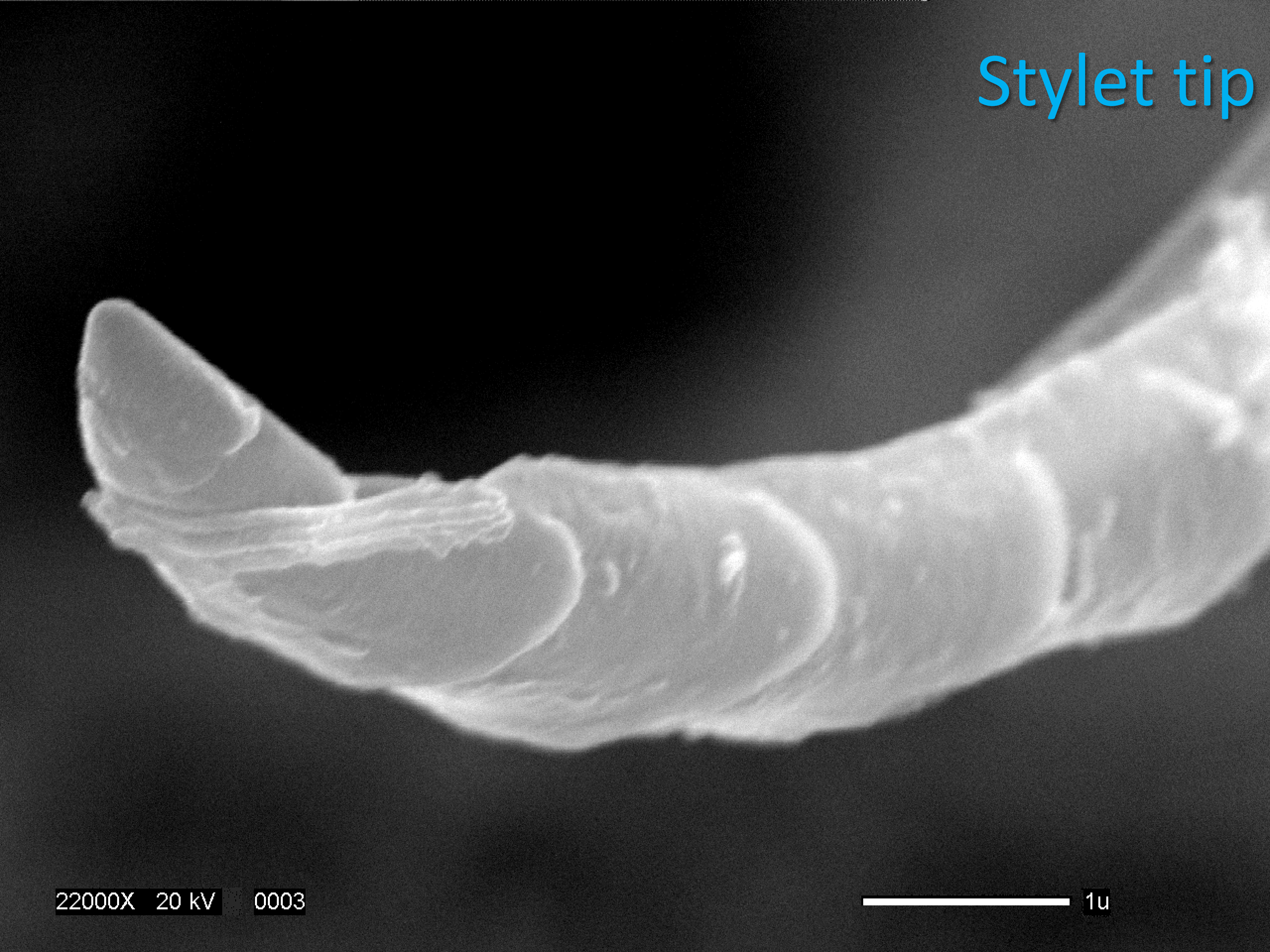
Crawler

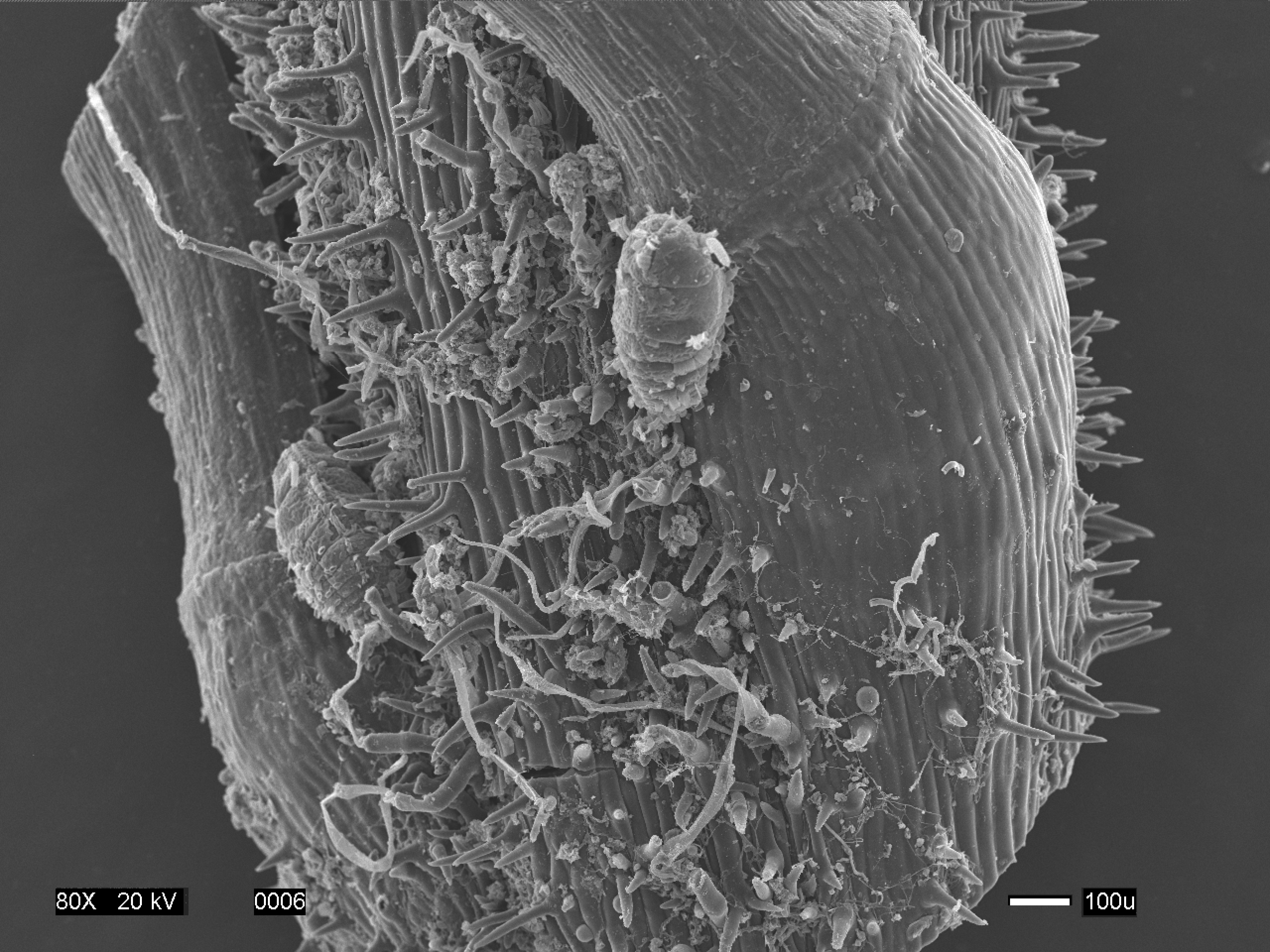
Smaller than adult
No wax pores
Prominent antennae- sensory function
Stylet bundle not extended

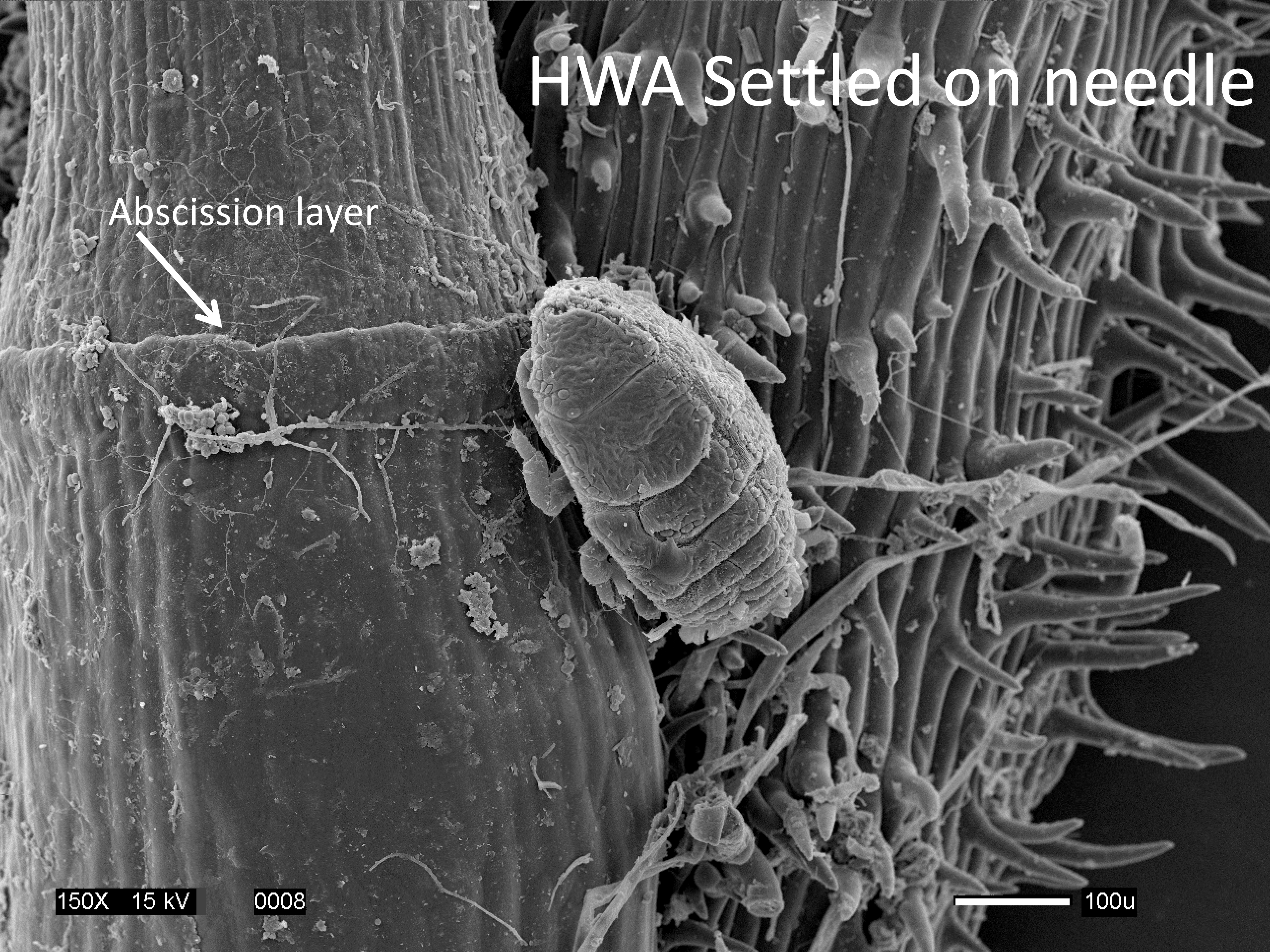


HWA Adult

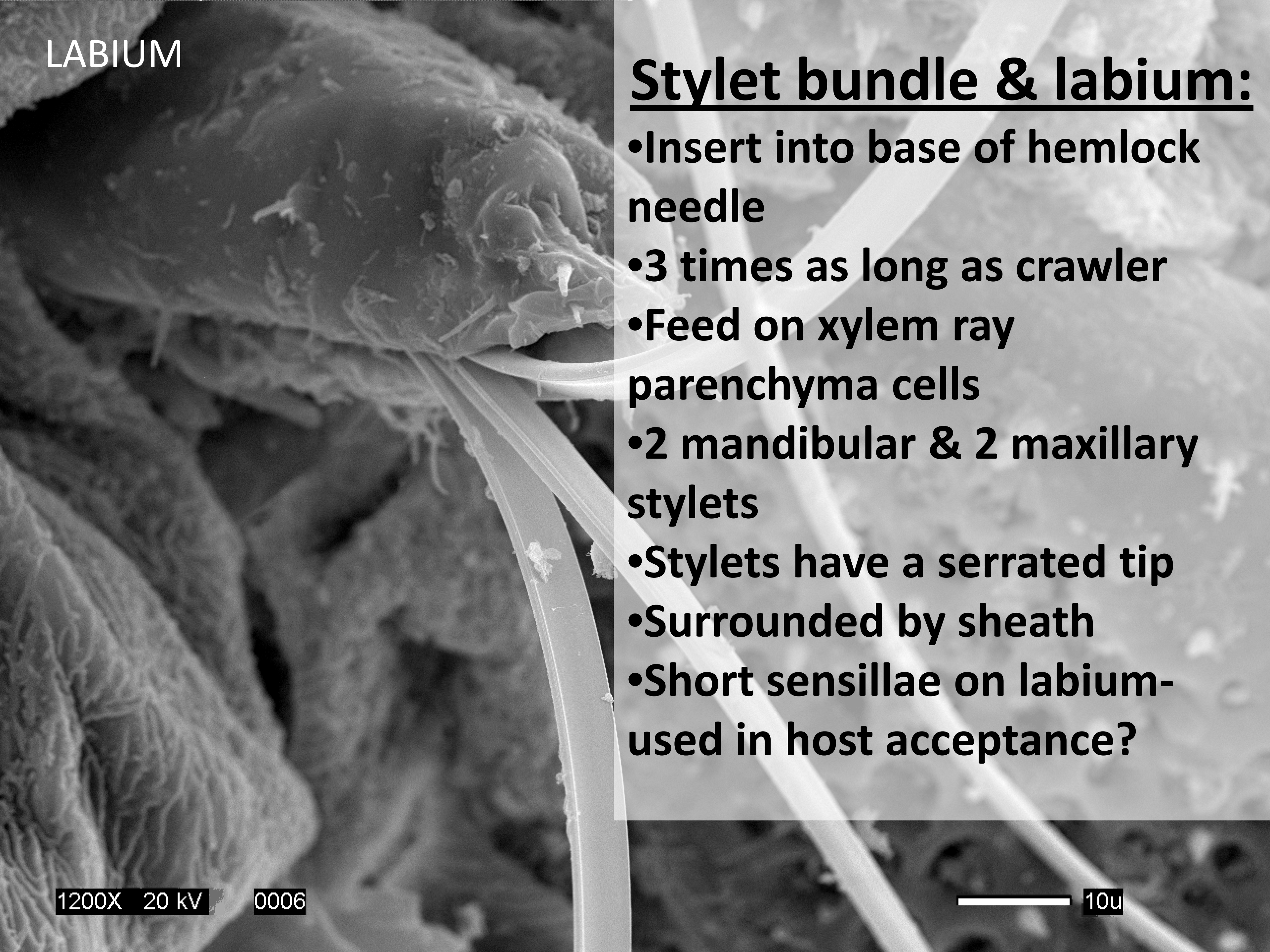

ocelli
labium
tarsi
ovipositor
stylet tip
100X 20 kV
0008
100u


Stylet tip
22000X 20 kV 0003
1u


80X 20 kV
0006
100u



HWA Settled on needle
Abscission layer
150X 15 kV
0008
100u



Stylet bundle & labium:

- Insert into base of hemlock needle
- 3 times as long as crawler
- Feed on xylem ray parenchyma cells
- 2 mandibular & 2 maxillary stylets
- Stylets have a serrated tip
- Surrounded by sheath
- Short sensillae on labium- used in host acceptance?

HOW DOES HWA SELECT ITS FEEDING SITE?

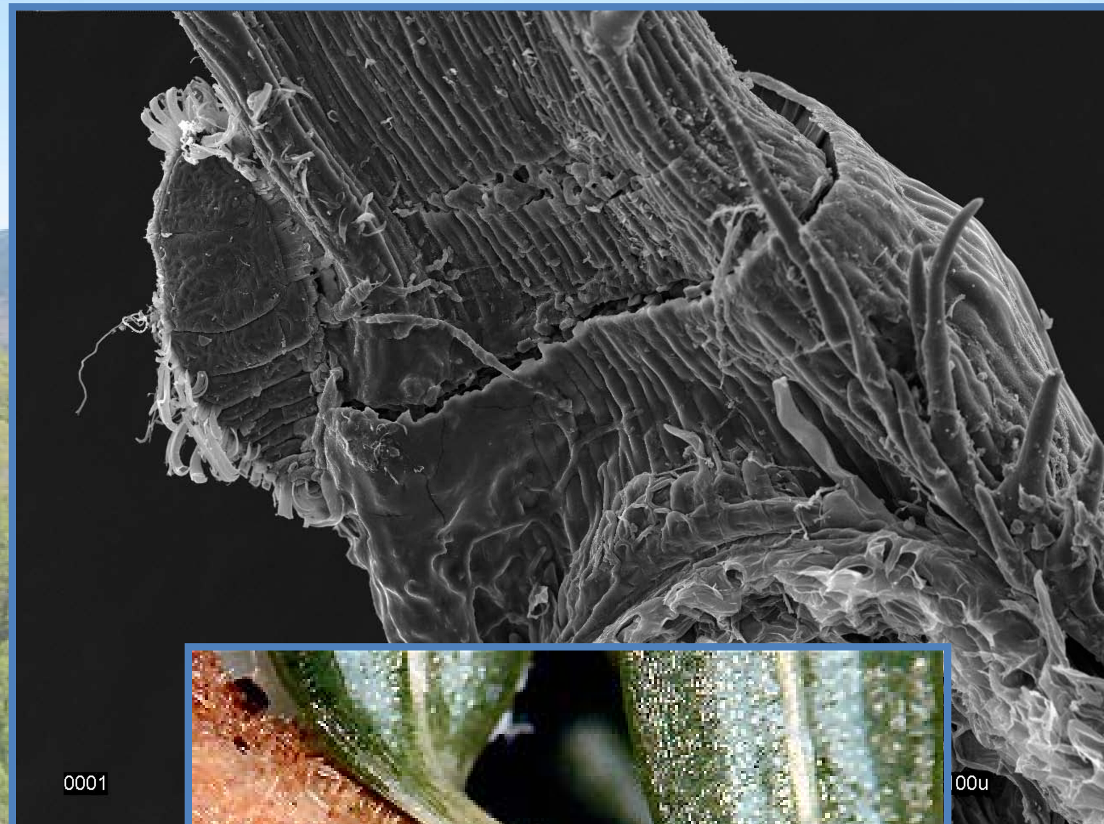
Sequence of steps in host selection[1]:

1) Habitat location

2) Host location

3) Host acceptance

4) Host use



[1] Bernays, E. A. and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. New York: Chapman & Hall. 312 pp.

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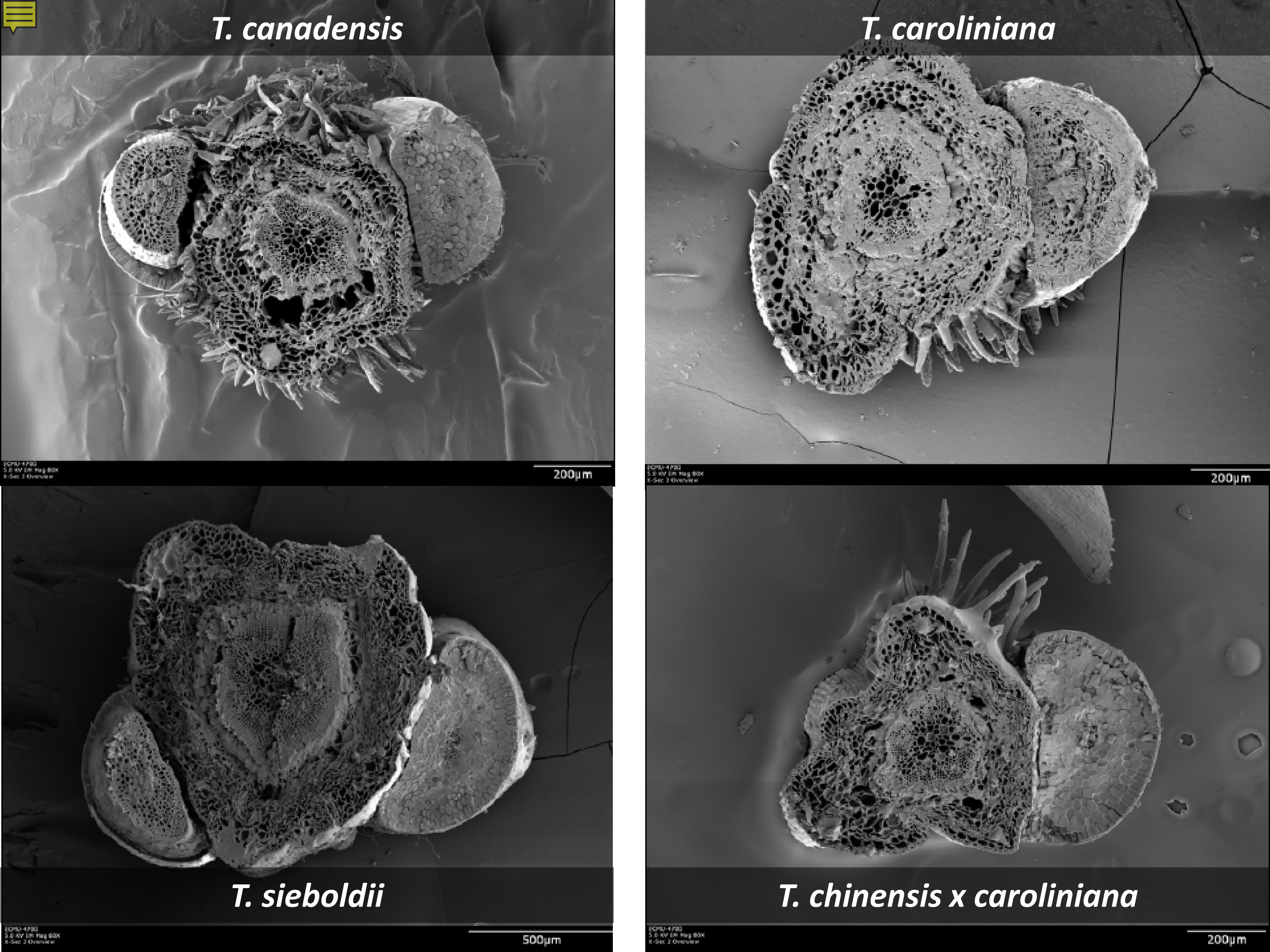
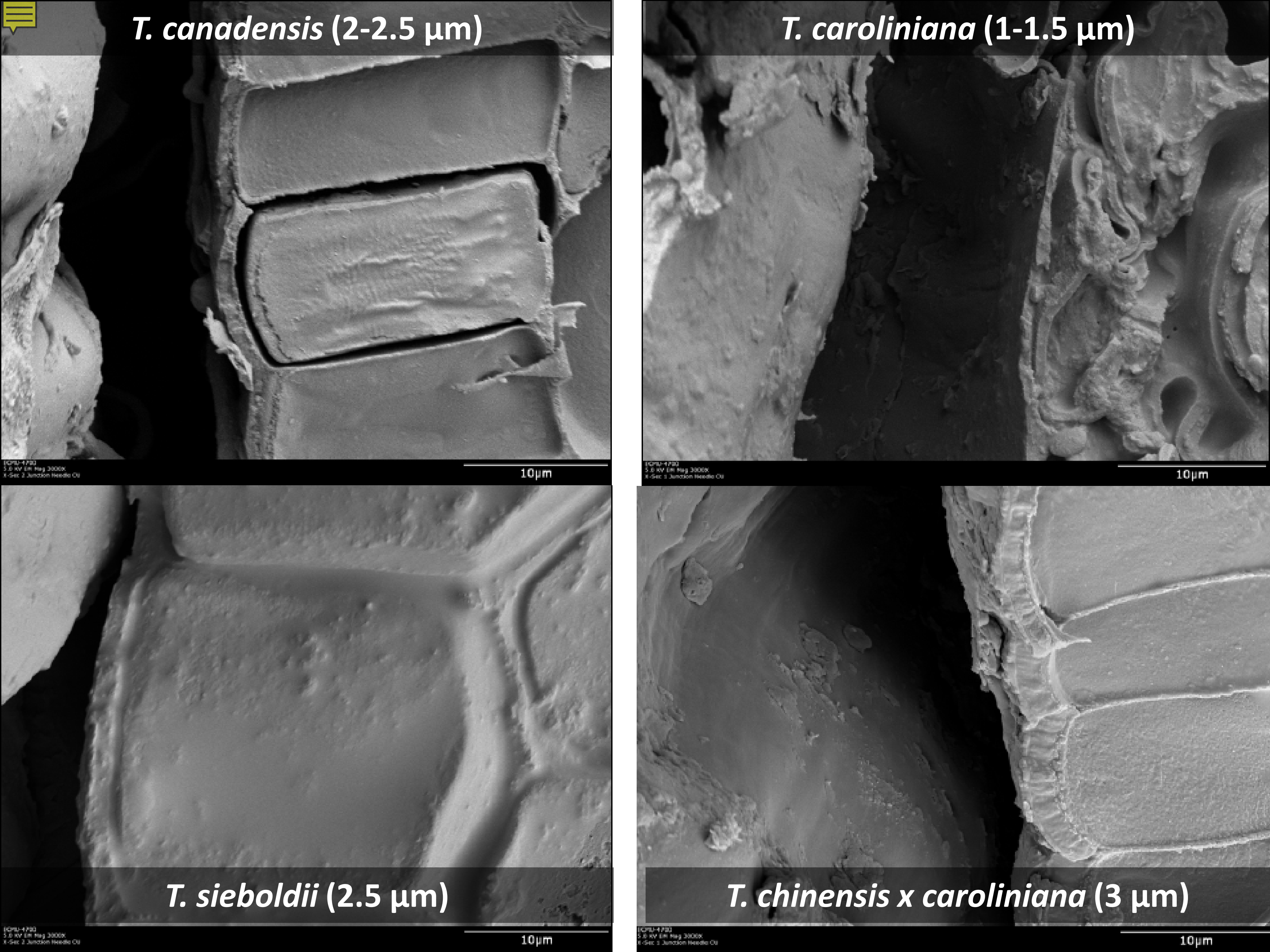
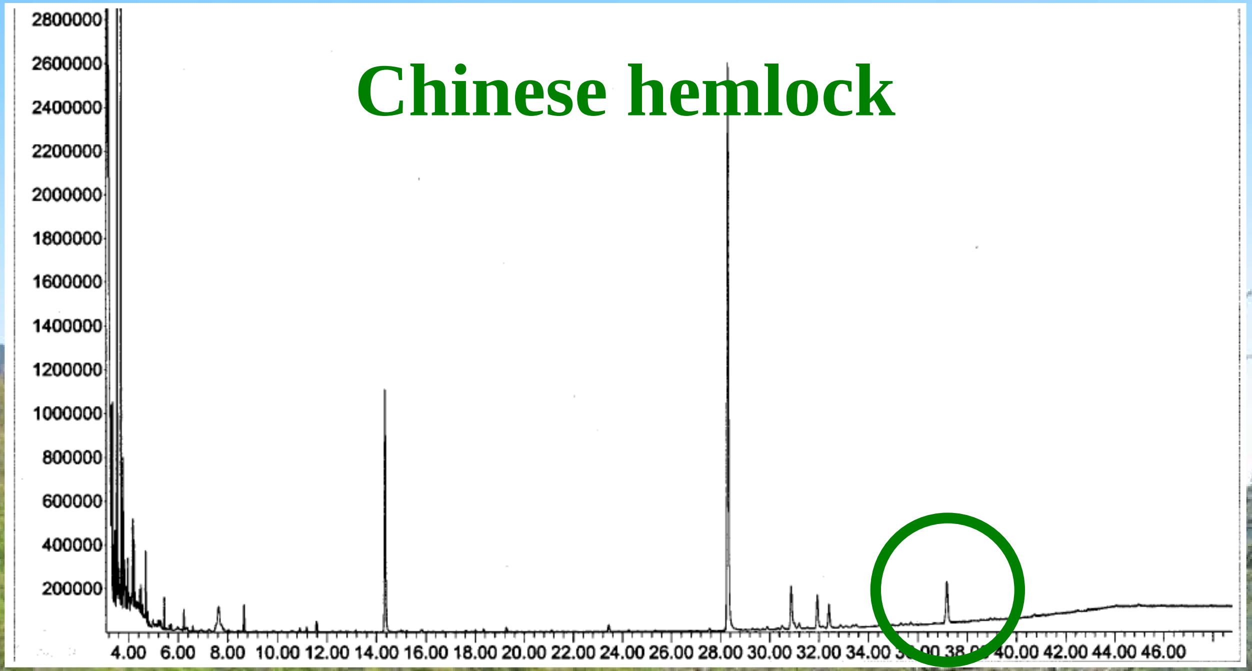
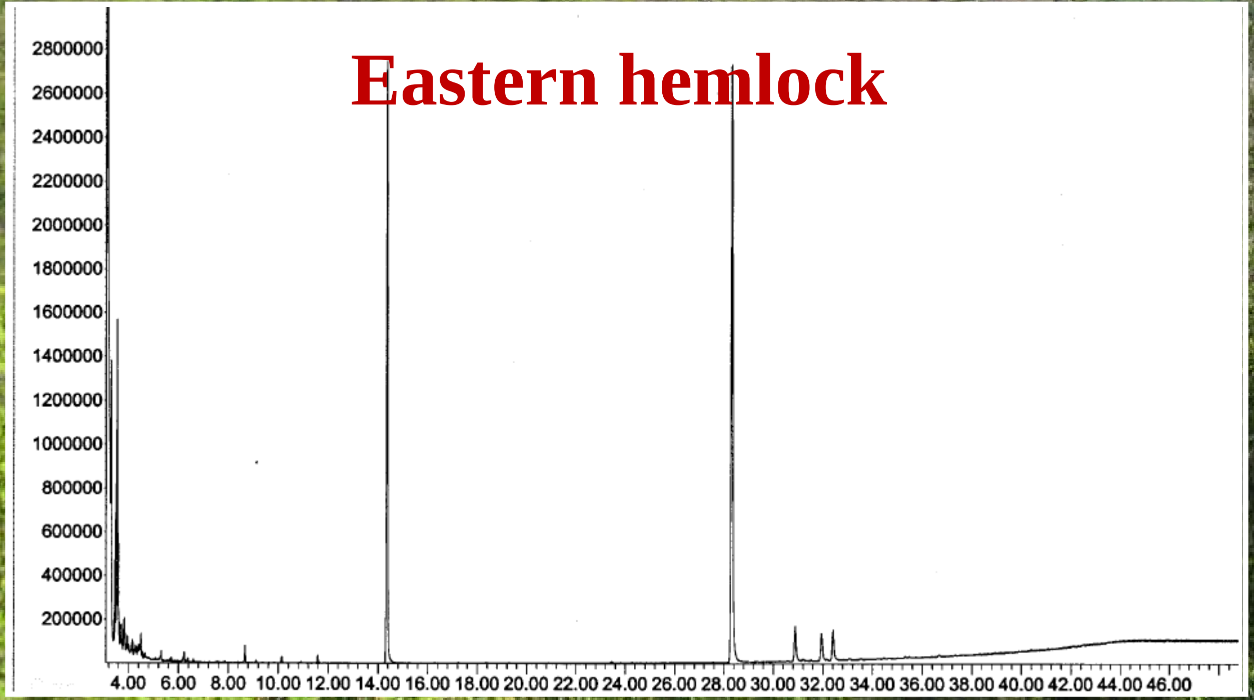

T. canadensis
200μm
T. caroliniana
200μm
T. sieboldii
500μm
T. chinensis x caroliniana
200μm


T. canadensis (2-2.5 μm)
10μm
T. caroliniana (1-1.5 μm)
10μm
T. sieboldii (2.5 μm)
10μm
T. chinensis x caroliniana (3 μm)
10μm

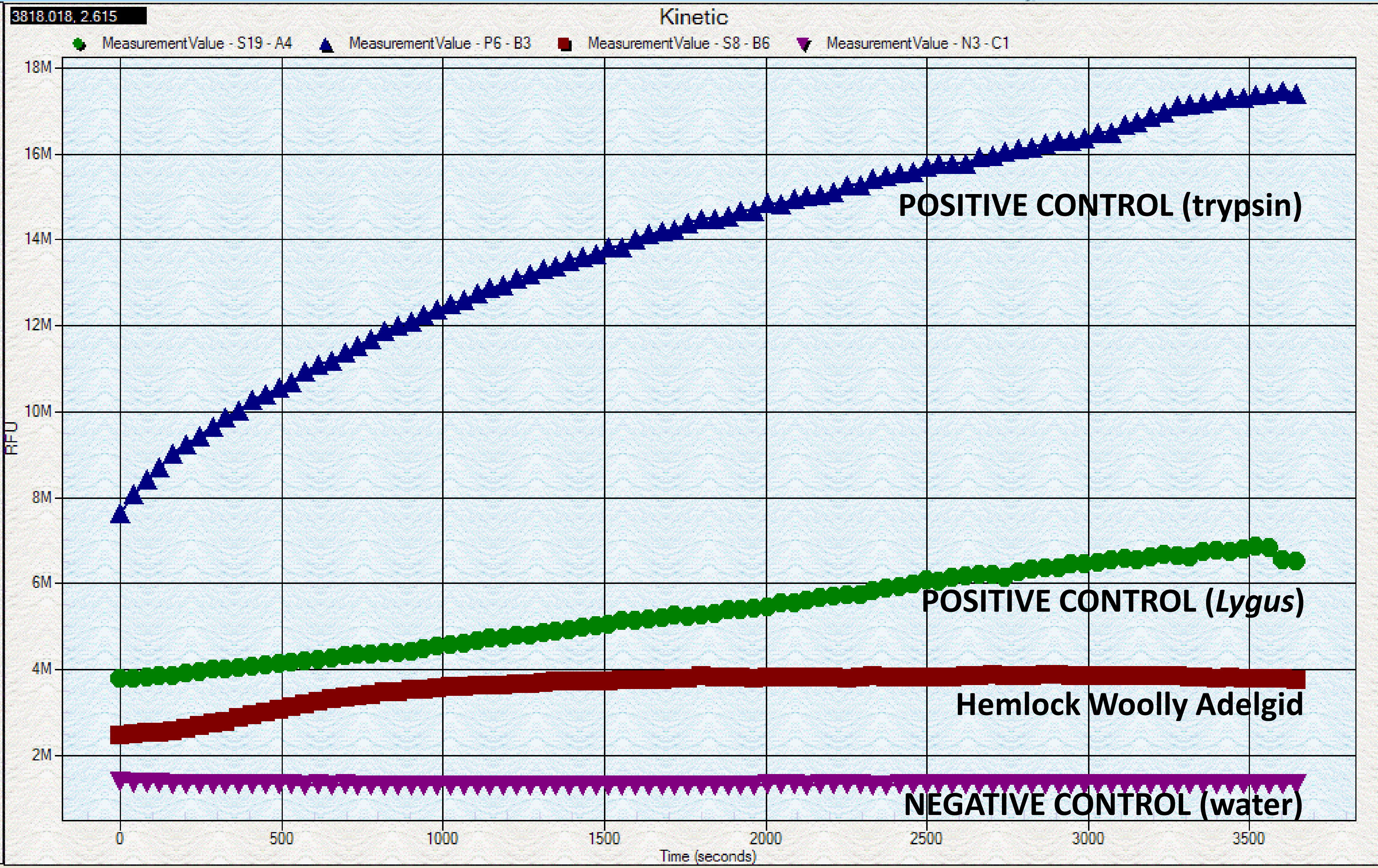
Epicuticular Wax Extraction

- 50 samples:
  - 7 hemlock species + 1 hybrid
- 3 g hemlock material/sample
  - Yields 2 -15 mg wax
- Dipped in 50 mL CH_2Cl_2
- Analyzed with GC/MS


Chinese hemlock
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00
40.00 42.00 44.00 46.00


Eastern hemlock
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00

Protease Activity of Hemlock Woolly Adelgid Whole-body Homogenate



Conclusions

- Trichomes likely do not deter feeding;
- Wax/cuticle thickness should be investigated further;
- Chemical components may be involved in resistance between hemlock species;
- Protease indicates digestion of proteins; do resistant hemlocks inhibit the activity of this and/or other enzymes?

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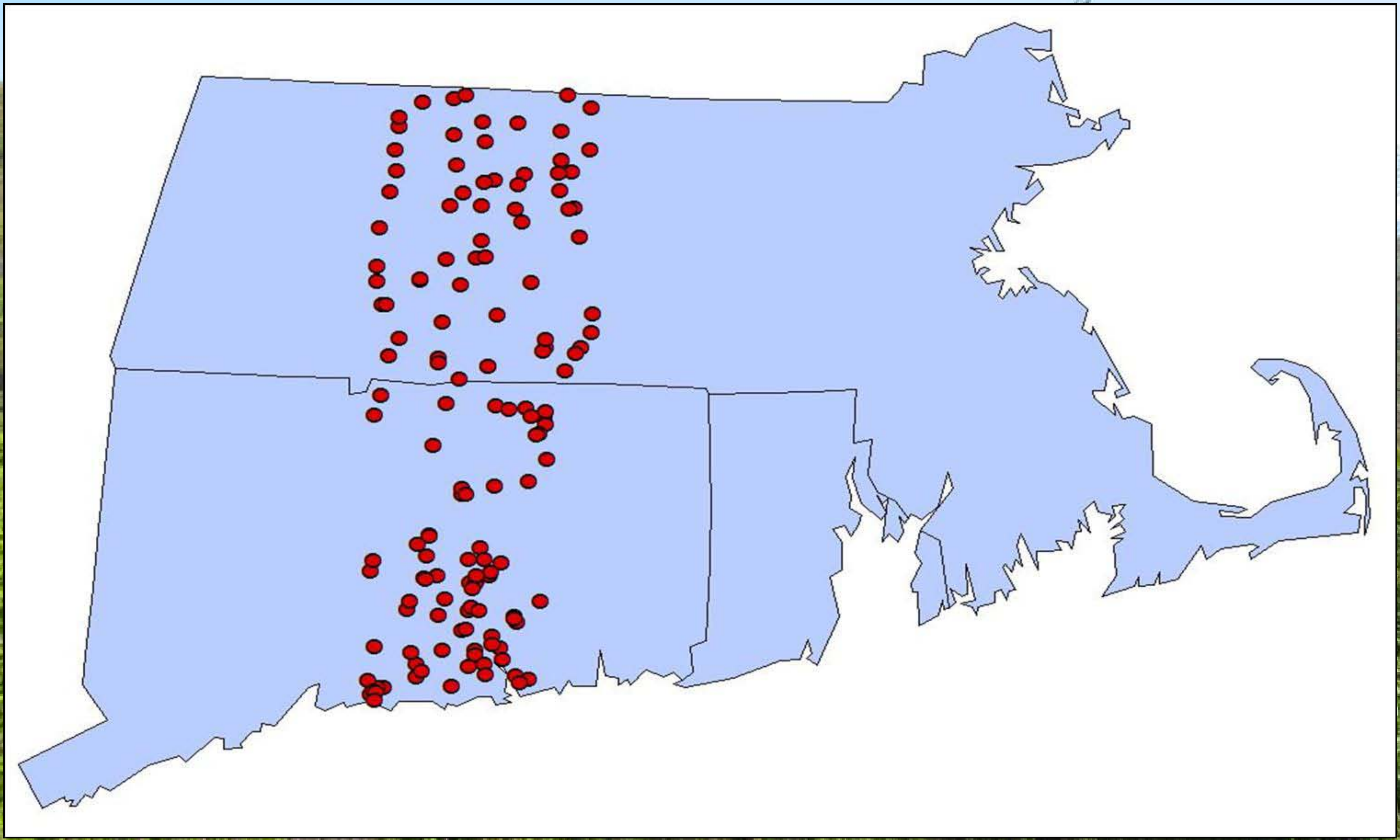
Are some eastern hemlocks resistant to HWA?

University of Rhode Island Team:
Todd Caswell
Richard Casagrande
Laura Ingwell
Brian Maynard
Evan Preisser
Laura Radville
Sara Gomez

Preisser et al. (2008) sampled 142 eastern hemlock stands in 2005



“Putatively resistant hemlock”

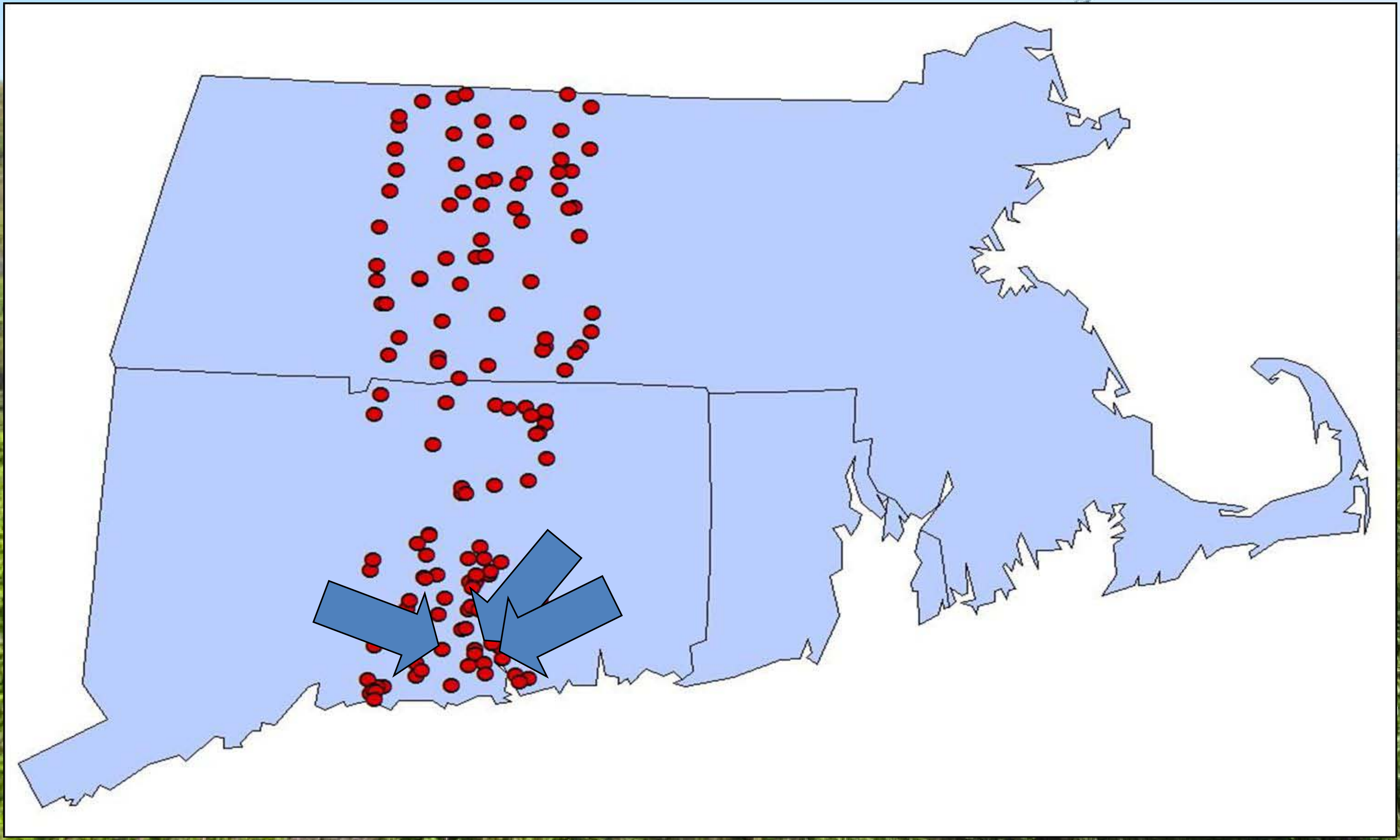
- In FOREST stands with ≥95% mortality
- Mature (>10 m) trees
- Appeared relatively healthy
- Largely free of HWA and elongate hemlock scale

INNATE OR ENVIRONMENTAL?

Evaluating hemlock resistance

1. Propagate cuttings
2. Grow under identical conditions
3. Infest with HWA under controlled conditions

CT sample sites



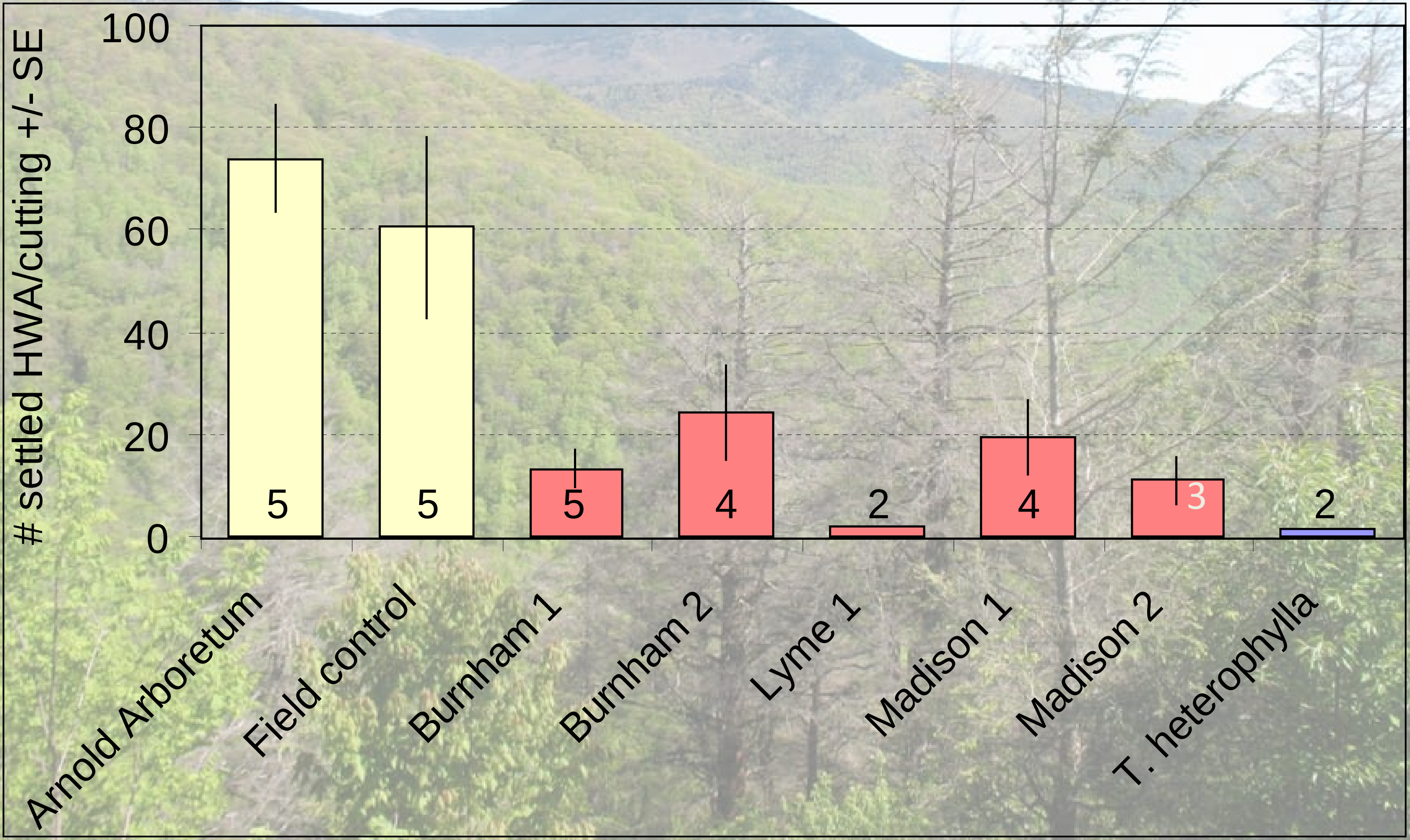
January 2006 cuttings

Rooted cutting in June

HWA infested

Infested plant

2006 HWA results



Mark Mayer's NJ "bulletproof stand"

2007 sample sites
100 cuttings each from

- 6 CT Trees
- 5 NJ Trees (Mark Mayer)
- 2 PA Trees (Scott Stitzer)
- 1 MD Tree (Dave Mausel)
- 4 Control Sites (MA, NY)

18 Trees (1800 cuttings)

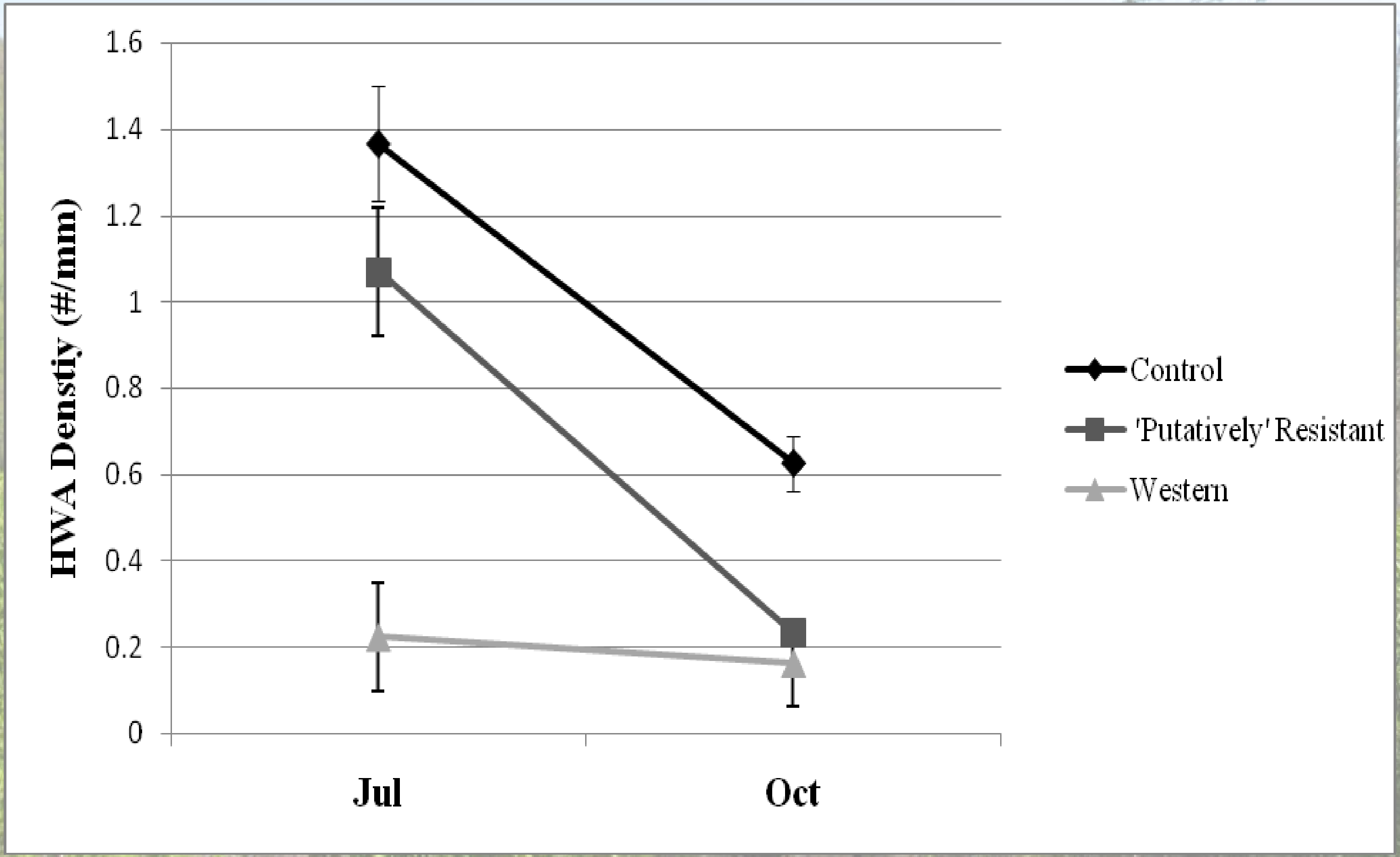
2007 rooted cuttings held for testing in spring 2009

- Allow additional time to recover from rooting and outgrow field environmental effects
- Provide 12 months of growth under standardized conditions
- Improve timing of plant growth and HWA inoculation

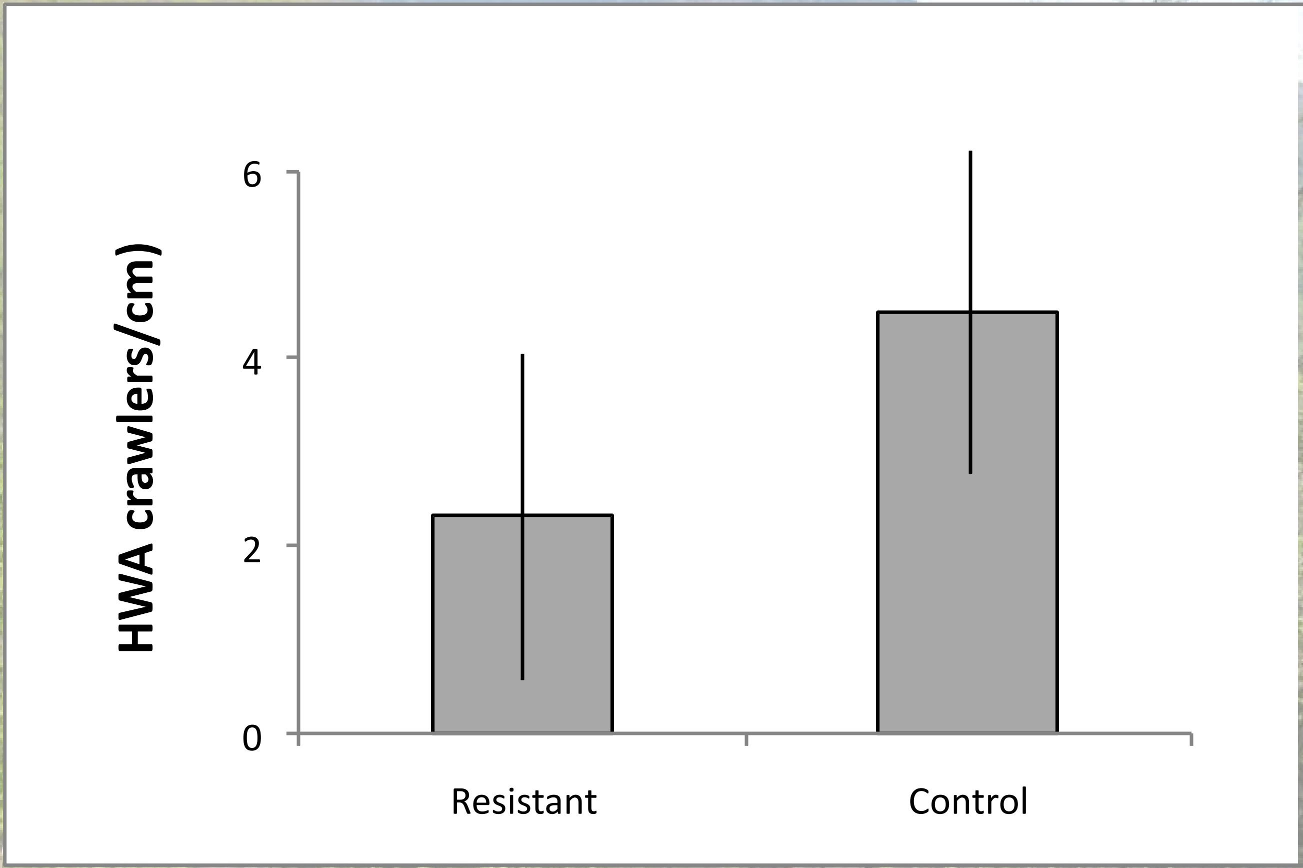
HWA infested tests – 2009

- Infest all rooted cuttings with HWA in early spring
- 3 groups: control, resistant, and cuttings from western hemlocks
- Assessed HWA settlement and survival

2009 HWA infestation results



2010 HWA infestation results



Jetton et al. (2008)

- Efforts to develop a bioassay screening protocol
- Infested 3 species with HWA (low & high)
- Quantified infestation rates & fecundity

Level of infestation

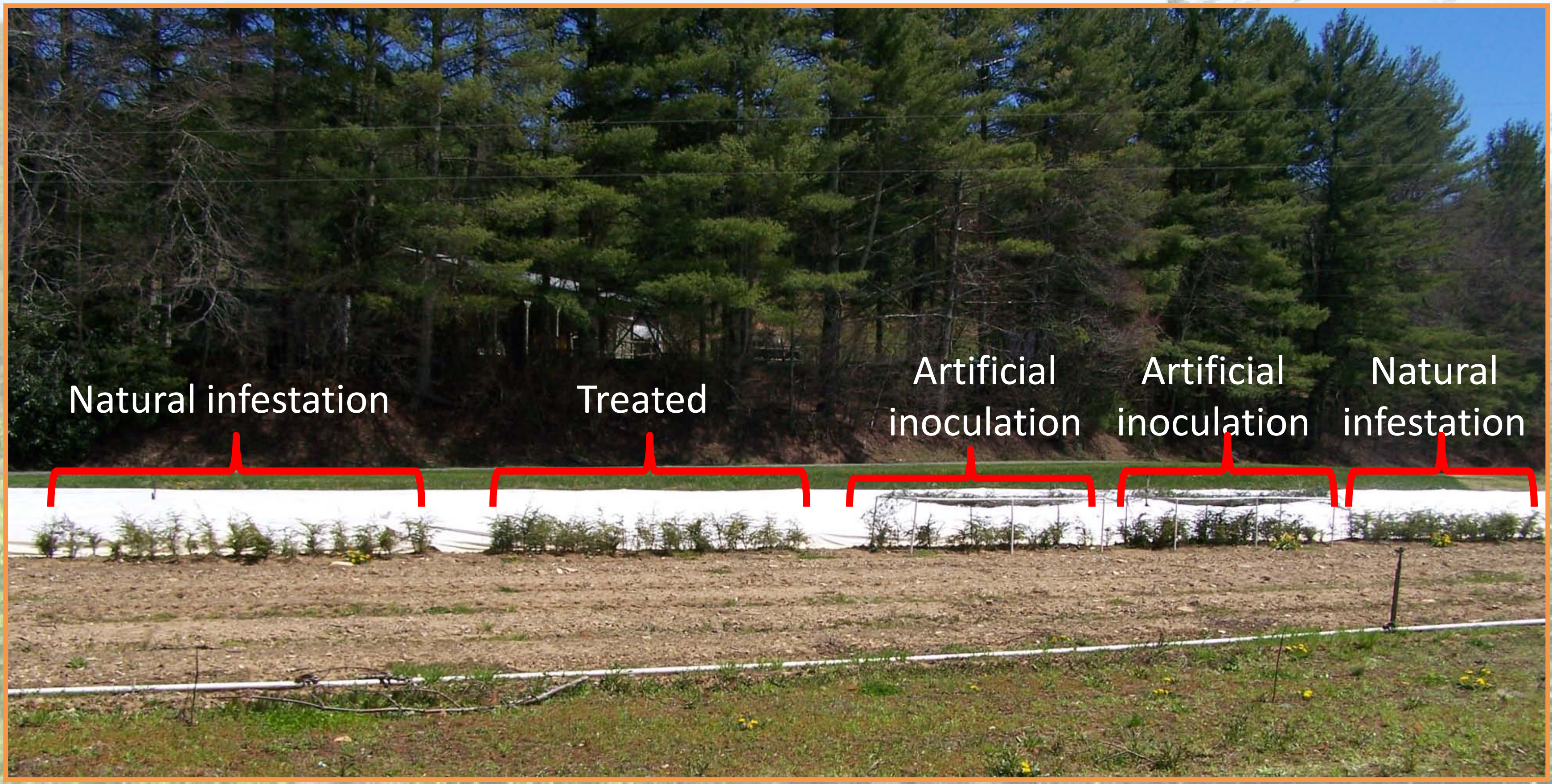
Hemlock species	HWA/cm of seedling height
Eastern hemlock	3.98 ± 0.41 a
Carolina hemlock	0.88 ± 0.41 b
Western hemlock	0.23 ± 0.41 b

Current Research at LRN

- Linville River Nursery
- Randomized block
- 3 different species
  - Eastern hemlock
  - Carolina hemlock
  - Western hemlock
- 3 different treatments
  - Artificially infested (2 reps)
  - Natural infestation (2 reps)
  - Chemically treated w/ Talstar (1 rep)

Talstar foliar spray, operated by Jerry Moody

Current Research at LRN



Infestation Rates

2008

Hemlock species	Infested	Uninfested	Infestation
Eastern hemlock	31	2	93.94%
Carolina hemlock	6	21	22.22%
Western hemlock	4	29	12.12%

2009
After thinning

Hemlock species	Infested	Uninfested	Infestation
Eastern hemlock	13	11	54.2%
Carolina hemlock	4	20	16.7%
Western hemlock	1	13	7.1%

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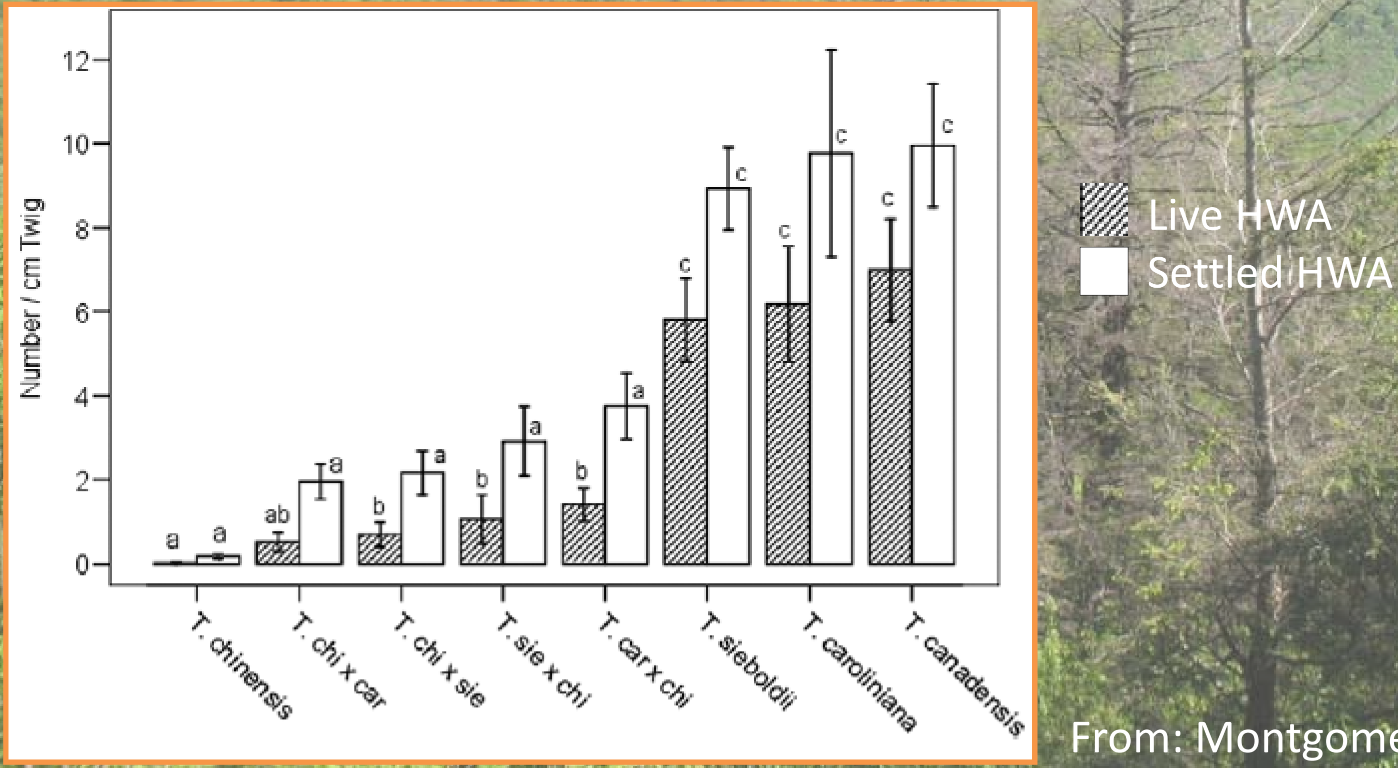
Breeding Programs

- U.S. National Arboretum
- Hybridizing Asian & eastern U.S. hemlocks
- EH crosses unsuccessful
- CH successfully crossed with *T. chinensis*
- Future work: Field trials

Montgomery et al. (2009)

- Infested: *T. canadensis*, *T. caroliniana*, *T. chinensis*, *T. dumosa*, *T. sieboldii*, *chinensis* x *caroliniana*, *chinensis* x *sieboldii*



From: Montgomery et al. 2009

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IMPLICATIONS FOR HOST RESISTANCE

- Selection of resistant individuals in native populations
- Screening for resistance within a breeding program

T. chinensis x caroliniana

Courtesy of: The American Chestnut Foundation

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Alliance for Saving Threatened Forests (ASTF)

- US Forest Service
- NC State University
- CAMCORE
- CSREES Southern Region IPM Center
- University of Rhode Island
- University of Vermont
- University of Kentucky

http://www.threatenedforests.org/index.cfm)

ASTF Goals:

- breeding for resistance, and
- understanding the biological basis for resistance

Modeled after the American Chestnut Foundation

What we know:

- Level of resistance in our native firs and hemlocks is low
- There are isolated cases where trees have survived for reasons that are not yet fully understood

What we need to do:

- Extensive testing of surviving trees, and trees from other parts of the world that are known to contain levels of resistance, needs to be conducted
- These trees need to be bred to one another and the offspring tested with challenges from the adelgid.
- Understand the biological basis for resistance for the targeted introduction of resistance genes into native trees.

First Step: Common Gardens

- Two in North Carolina
- One in Rhode Island
- Two in Kentucky

Ultimately:

- A breeding and selection approach will be employed to develop resistant planting stock and accelerate natural selection in restoring fir and hemlock
- A series of regional clonal seed orchards grafted from trees with verified resistance will be established to provide locally adapted resistant seed

If you wish to know more about

The Partnership for Saving Threatened Forests

go to our web site at:

http://www.threatenedforests.com/index.cfm

Acknowledgments

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USFS - Northern & Southern Research Stations
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Contributors:

NCSU Forest Entomology Lab
Valerie Knowlton, John MacKenzie (SEM lab)
Gary Bauchan (cryo-SEM; USDA, Beltsville, MD)

Hemlock samples:

NC State Parks
Morton Arboretum, Lisle, IL
National Arboretum, Beltsville, MD
Glenn Kohler (Washington state)

Questions??