

Biological Control of Emerald Ash Borer: Bark sifting for *Oobius agrili*

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Special Acknowledgements

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Emerald Ash Borer (EAB)

- *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)
- Invasive phloem feeding beetle that attacks ash trees
 - Larval stage (yellow arrows) most damaging
- Discovered in U.S.A. in 2002
 - As of February 2015, EAB infestations found in 25 states (Bauer et al., 2015)



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EAB Infested Ash Trees

- Larval galleries cut off water and nutrients
 - Ash tree slowly starves
- MN has highest volume of ash trees in U.S. with ~ 1 billion trees (Minn Dept. of Ag)
 - Devastating economic and environmental impacts



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Healthy ash tree

Infested ash tree

Biological Control of EAB

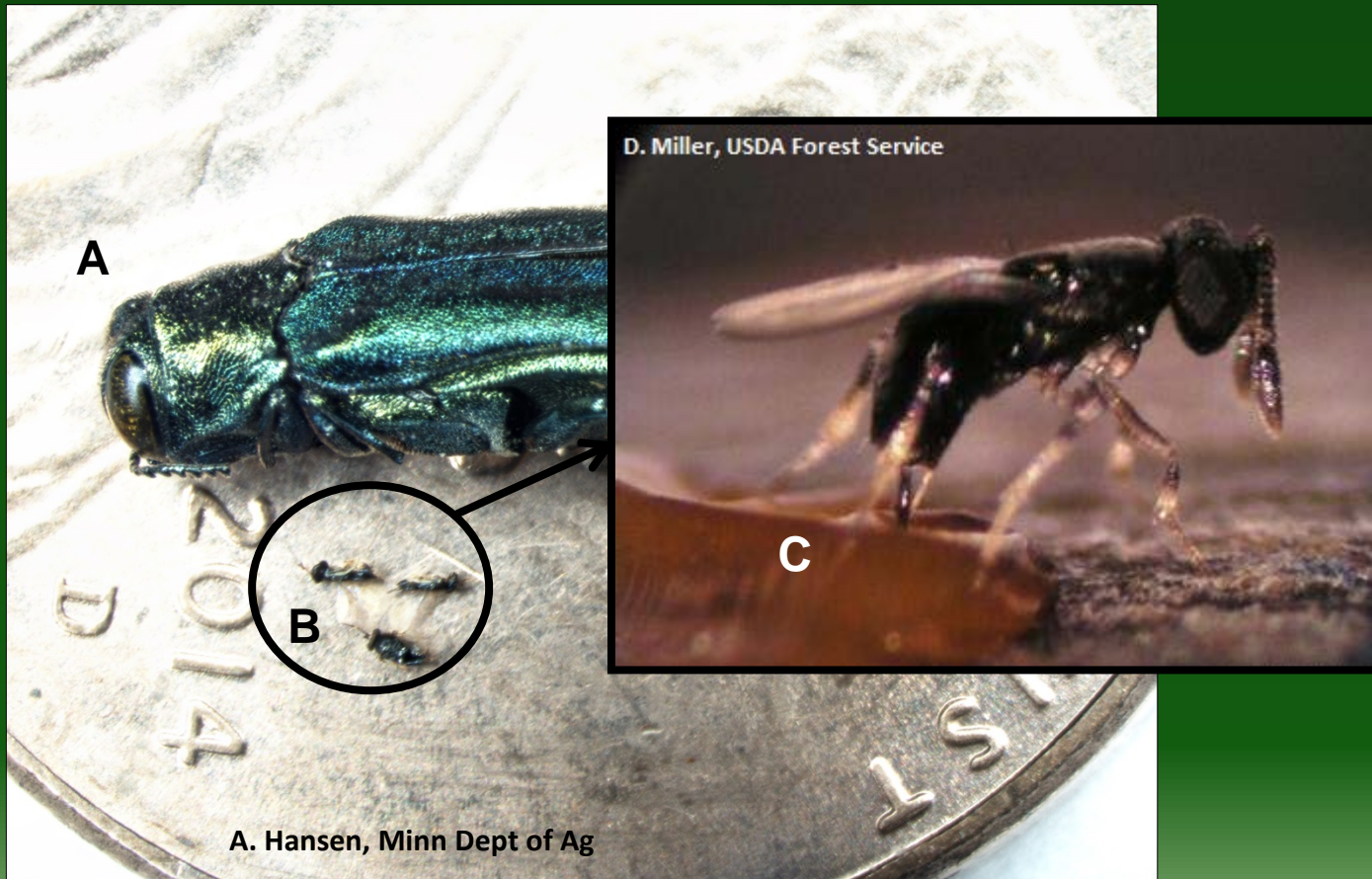
A- Emerald Ash Borer; B- *Oobius agrili*; C- *Tetrastichus planipennis*



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Biological Control Agents of EAB

A- Emerald Ash Borer; B- *Oobius agrili*; C- *Tetrastichus planipennis*



Oobius agrili (Hymenoptera: Encyrtidae)

- Solitary egg parasitoid found in China
 - Very small, stingless wasp
- Adult wasp lays eggs inside EAB egg
 - Developing wasp kills EAB larva before it hatches
- Released as an EAB biocontrol agent in U.S. since 2007 and Canada since 2015 (USDA APHIS, 2007)



J Duan, USDA ARS BIIR

Oobius agrili parasitizing EAB egg

Bark Sifting Protocol

- Evaluate field performance of *Oobius agrili*
 - Determine establishment and prevalence of released parasitoids
(Duan et al., 2012)
 - Labor intensive due to small size of EAB egg and parasitoid
(Duan et al., 2012)
- Bark sifting method developed in 2012 as more effective alternative to visual egg searches (Abell et al., 2014)

Collecting the Bark

- Collect bark in field, sift in lab
- Select live ash trees showing signs and symptoms of EAB attack
- Measure 10 x 50cm area on trunk. Shear off with draw knife
 - Sample from S, SW or W side of tree
- Plastic sheet set to collect sheared bark
 - Transfer bark to labeled paper bag
- Once sample has been collected and transferred to paper bag, let dry and store in cardboard box to avoid mold growing in the sample



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Preparing the Sample: Defense Against Static



- Static makes transferring fine debris and eggs difficult
 - Static can send eggs flying!
- Use anti-static dryer sheet to wipe surfaces of sieve and containers



Bark Sifting: Preparing the Sieve



USDA Standard Testing Sieve No. 14

- Place sheared bark into top half of sieve
 - Longer pieces of bark may need to be broken down
 - 2-3" pieces
- Avoid over-packing
 - Bark needs room to move during shaking
- Bark may need to be divided into subsamples
 - Label accordingly

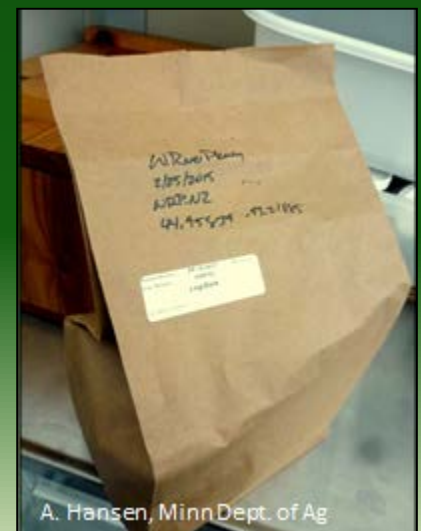
Preparing the Sample: Sieving

- Shake for 2 minutes
 - 15 seconds up and down
 - 15 seconds side to side
 - Repeat 3x



Transferring the Sifted Bark Sample

- Transfer fine, sifted bark debris into a white ceramic dish for sorting, and the remaining bark into a paper bag for storage
- (Optional) Record weight of debris and leftover bark
 - On spreadsheet



Bark Sifting: Preparing for Sorting

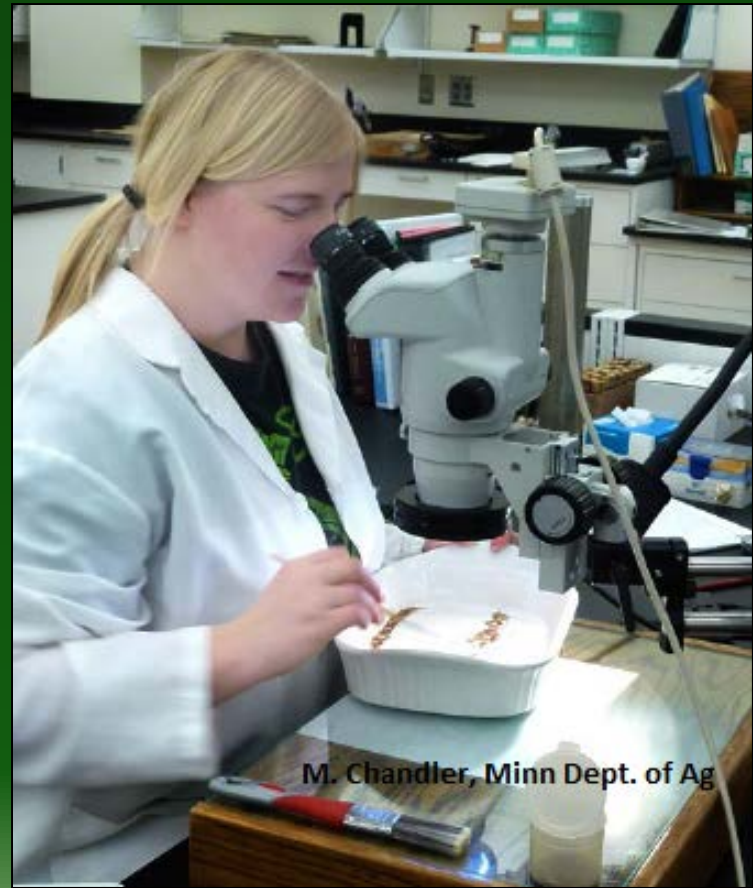


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- Useful tools:
 - Dissecting microscope
 - Ceramic dish
 - Ceramic is non-static
 - Permanent marker
 - Small petri dishes
 - Forceps (for larger insects)
 - Paintbrushes
 - 1" brush
 - moving large amounts of bark
 - Size 00 round brush
 - moving small amounts of bark
 - Size 0000 round brush
 - transferring eggs

Sorting Fine Bark Debris

- Sorting is done using a dissecting microscope due to small size of debris and EAB eggs



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Debris Sorting: Arranging the Bark



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Minimize concealing and missing eggs!

- Arrange debris in thin line along the dish
 - Be able to see entire width with one pass under scope
 - Dish should only have to move from left to right
- Ensure that debris is 1 layer thick
 - See individual particles with minimal manipulation

First Glance Under Scope

- The Bark:
 - Sample will primarily contain fine bark and lichen
 - Assortment of shapes and colors



- Size 00 paintbrush useful in moving small amounts of bark
- Size 0000 paintbrush useful in removing eggs/insects

Debris Sorting: What You'll See



- EAB eggs are ~1mm in length
- Distinct shape and amber color (usually)

EAB egg among debris

Debris Sorting: What to Collect



EAB egg among debris

- Collect all insects, eggs, and any fragments that appear to be eggs
- Transfer to labelled petri dish for further examination
 - If in doubt, collect it

Debris Sorting: Collecting

- Use fine paintbrush to transfer eggs
 - Size 0000
 - Using a paintbrush will help avoid damage to eggs
 - Moistened paintbrush may help facilitate removal

EAB egg in transfer



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Concealed EAB eggs



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Partially concealed EAB eggs can be difficult to spot. Sort carefully.

Concealed EAB eggs cont.

Partially concealed EAB eggs can be difficult to spot. Sort carefully.



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Debris Sorting

- When you've sorted the current line:



- Use 1" paintbrush to sweep debris to the bottom of the ceramic dish
 - Time efficient
 - Eliminates need for additional container
- Add new line of debris and repeat until all debris is sorted

What You'll See: EAB Eggs

- Adult EAB females lay eggs in bark crevices and between bark layers
- Eggs often adhere to flakes of bark
 - Collect egg on bark flake (to avoid damage, try to see signs/symptoms of parasitism w/o removal)



Non-parasitized EAB Eggs

Hatched

vs.

Unhatched

Top view

Bottom view



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Hatched eggs have larval exit hole on bottom where packed frass (larval waste) is visible

Bottom view

Top view



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No exit hole, no frass = unhatched egg

Hatched vs. Unhatched

Top view

Bottom view



Exit hole with packed frass circled in yellow

Bottom view

Top view



EAB eggs may not hatch for several reasons

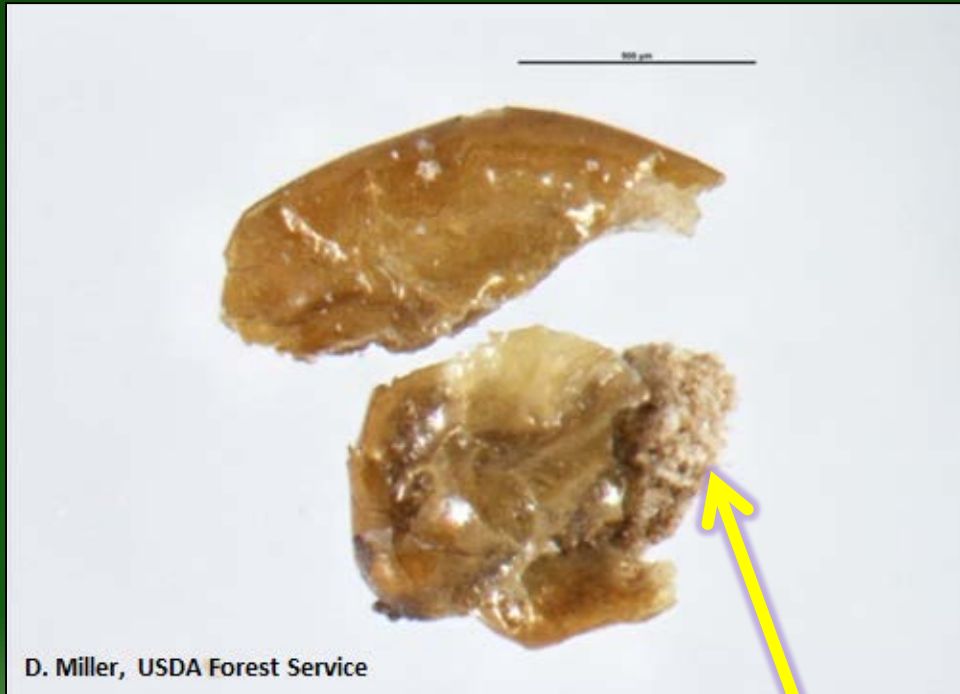
EAB Egg Fragments

- Not all eggs are recovered intact...



Examining EAB Egg Fragments

- Hatched or not?
 - Sometimes packed frass is visible. Fragments can be tricky



Visible packed frass

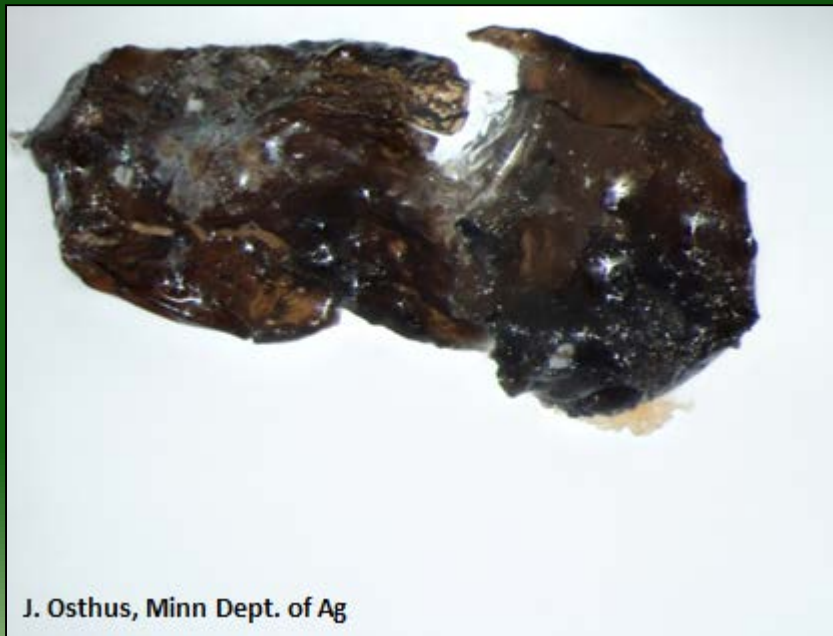
Parasitized EAB Eggs

- Before parasitism, EAB eggs have amber coloration
- Most EAB eggs turn dark after parasitism



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Healthy EAB egg, hatched



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Lab parasitized egg above

Parasitized EAB Eggs cont.

- Most EAB eggs turn dark after parasitism
- Parasitoid exit hole circled in yellow



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Healthy EAB egg, hatched



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Lab parasitized egg above

Other Parasitized EAB Eggs

- Not all EAB eggs turn black after parasitism...



Lab parasitized egg above

Look for dark areas inside eggs and/or adult parasitoid exit hole

Parasitized Egg That Did Not Change Color



Parasitoid
meconium (larval
waste) circled in
yellow

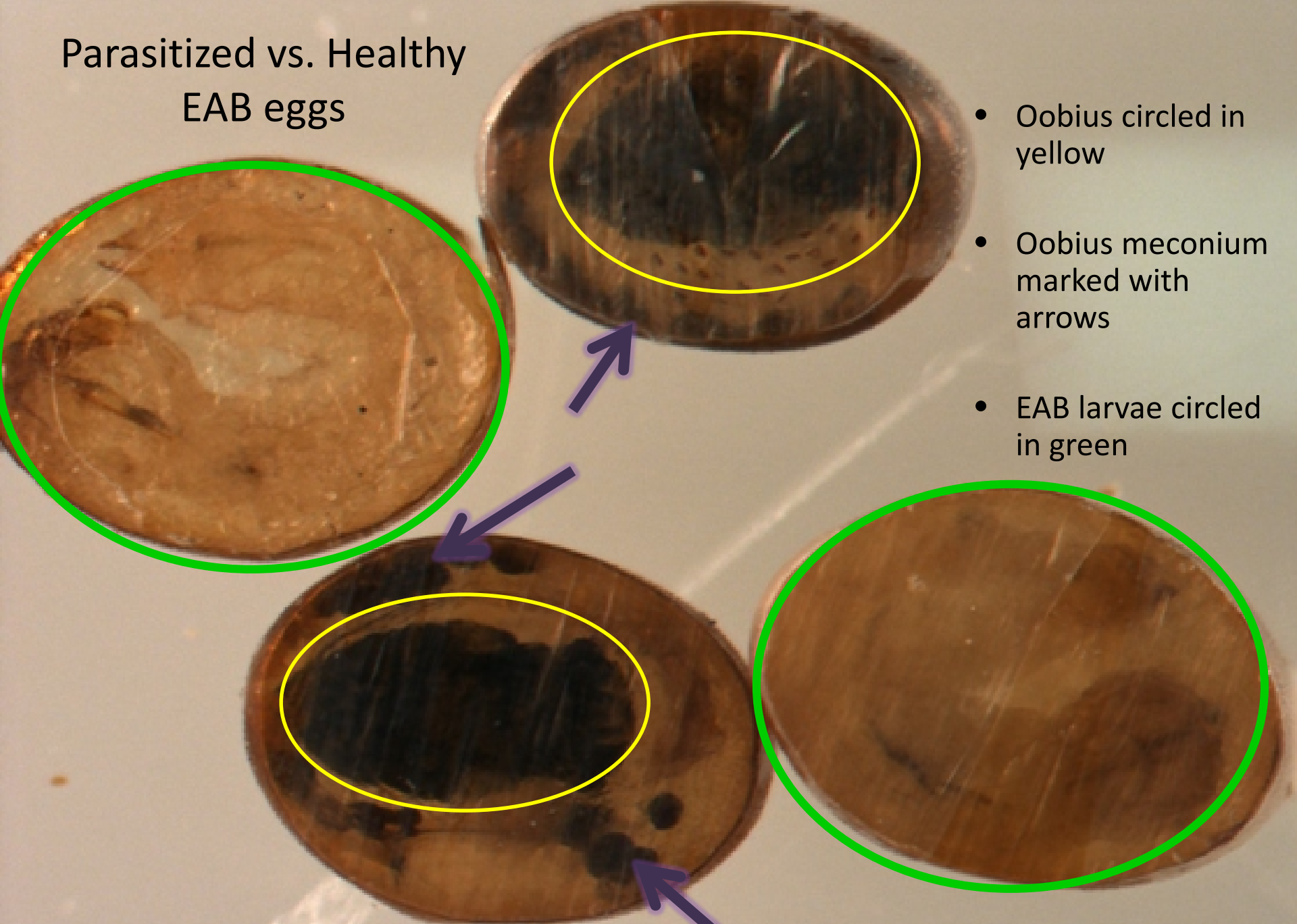
Round adult
parasitoid exit
hole circled in
green

Lab parasitized egg above

Which are Parasitized?



Parasitized vs. Healthy EAB eggs



- Oobius circled in yellow
- Oobius meconium marked with arrows
- EAB larvae circled in green

A Closer Look at Parasitized EAB Egg

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- Egg recovered from Great River Bluffs State Park, MN in February, 2015
- Most parasitized eggs recovered in the field are often **reddish brown** rather than solid black, as usually seen in the lab
- **Dark areas = suspect for parasitism**



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A Closer Look cont.

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- Parasitoid air tube (circled in blue)
- Broken chorion with visible meconium (arrow)

****No exit hole...*Oobius* still inside****



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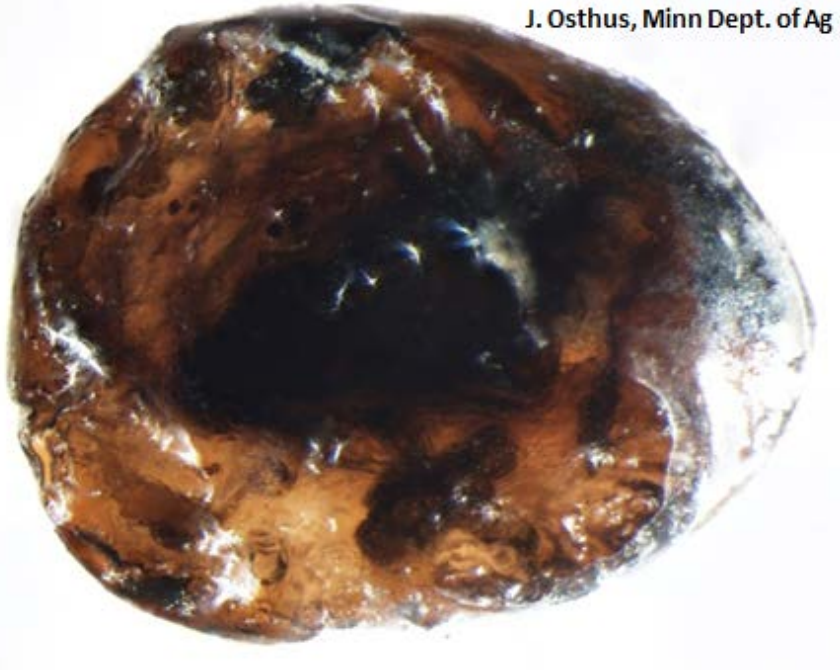


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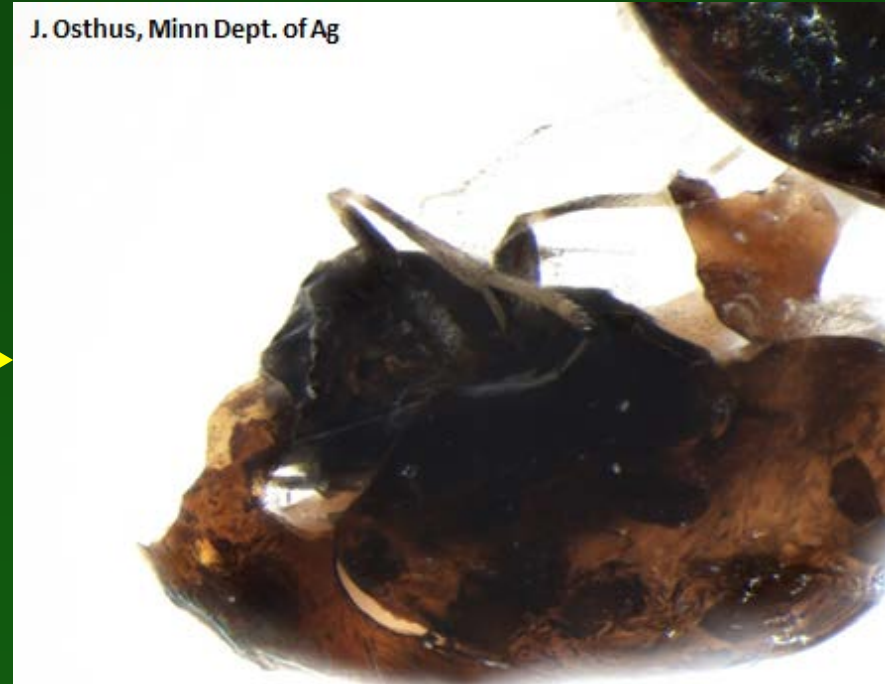
Dissecting Parasitized EAB Eggs

Dissecting eggs confirms *Oobius* presence and life stage

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- Parasitized egg recovered from Ft. Snelling State Park, MN.
- Dissection revealed adult *Oobius* inside

Dissecting Parasitized EAB Eggs Revealed

Dissecting eggs confirms *Oobius* presence and life stage

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- Recovered egg not completely black, but suspect for parasitism due to dark shadow (yellow circle)
- Dark shadow in center was adult *Oobius* (yellow arrow)

Dissecting Parasitized EAB Eggs cont.

- *Oobius* larvae dissected from parasitized EAB eggs (arrows)



H. Liu, Michigan State University and USDA Forest Service



D. Miller, USDA Forest Service



D. Miller, USDA Forest Service

EAB Eggs: Parasitized or Not??

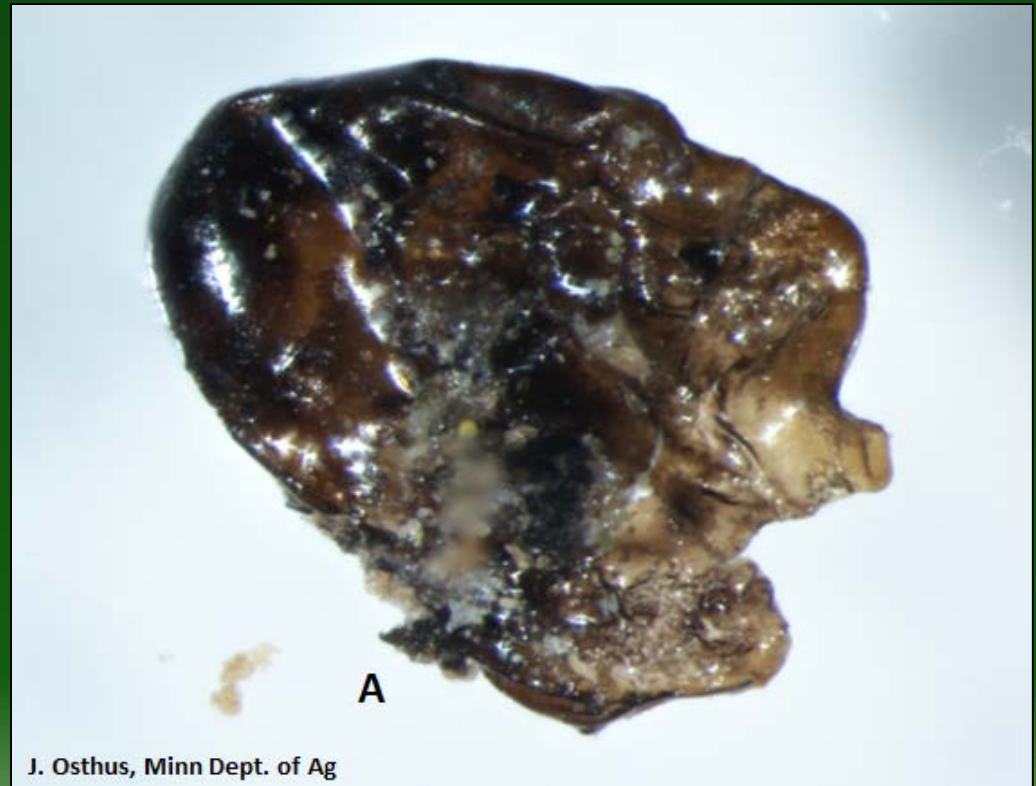
One of these 3 eggs from Great River Bluffs State Park, MN is NOT parasitized.

Which one?



Which Egg is NOT Parasitized?

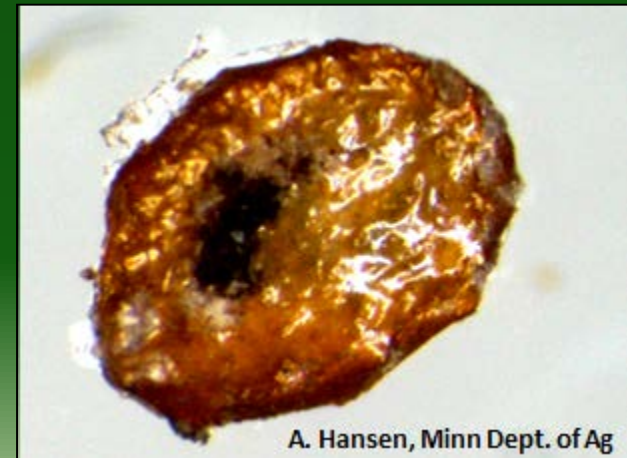
Egg A is NOT parasitized!



- Close inspection shows that Egg A is hatched
- Sometimes EAB frass becomes dark

EAB Eggs Suspect for Parasitism

Santa promises gifts, then delivers coal...more EAB eggs that are suspicious, but are NOT parasitized



EAB Eggs Suspect for Parasitism Revealed

These eggs are all hatched with dark frass...NOT parasitized!

– frass marked with arrows



Unusual EAB Eggs



Unknown organism chewed through EAB egg



EAB egg fragment with fungi



EAB eggs with faded coloration

****None of these eggs are parasitized**

EAB Egg Look-alike: Scale Insect



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- Scale can be easily confused for EAB eggs
- Similar size and color
- Distinguished by pointed end (scale mouthparts), wavy margin, segmented body

Scale insect even also contain a tiny black parasitoid, meconium, and/or round adult parasitoid exit hole



H. Liu

Scale vs. EAB Egg

Look for pointed end and other subtle differences to help distinguish

Fragments can be tricky



Scale vs. EAB Egg Revealed



Look for pointed end (yellow arrows) and other subtle differences to help distinguish scale from EAB eggs (black arrows)



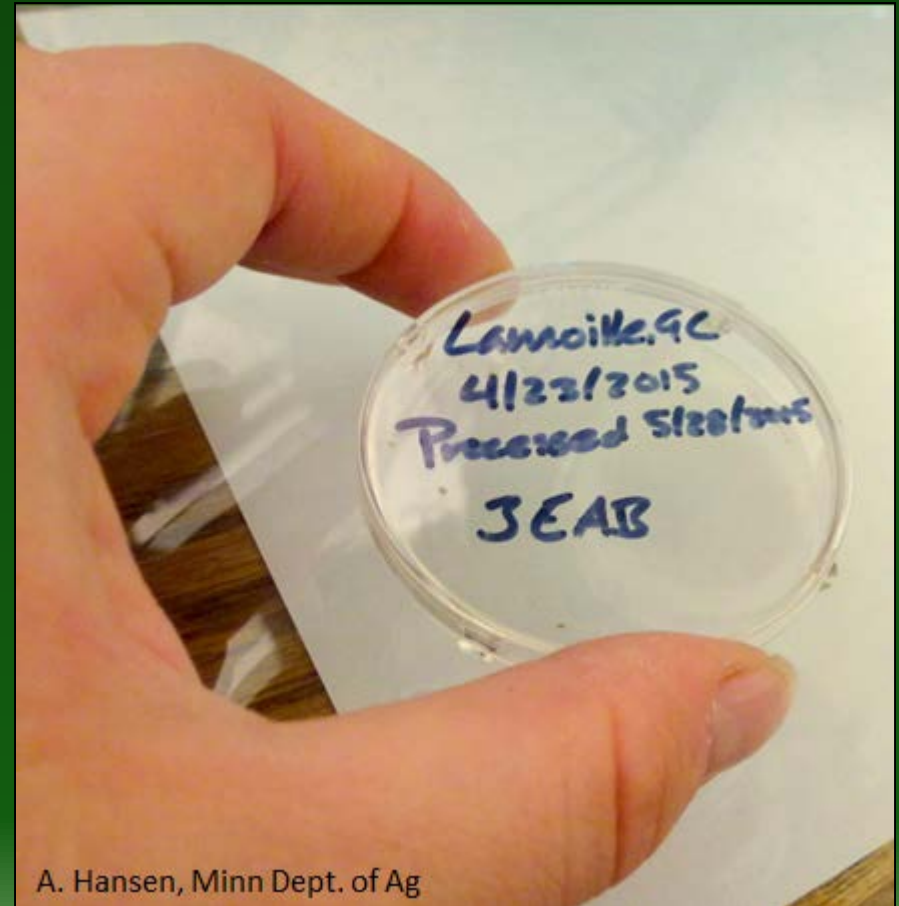
Misc.

- It is not unusual to find other eggs and arthropods in a debris sample. Collect what you find



Wrapping Up

- Transfer collected eggs, fragments and arthropods into labeled vial or petri dish with friction fit lid
- Return sorted debris into container
- Store bark debris and petri dish in designated lab location



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Where Are Parasitoids Being Released?

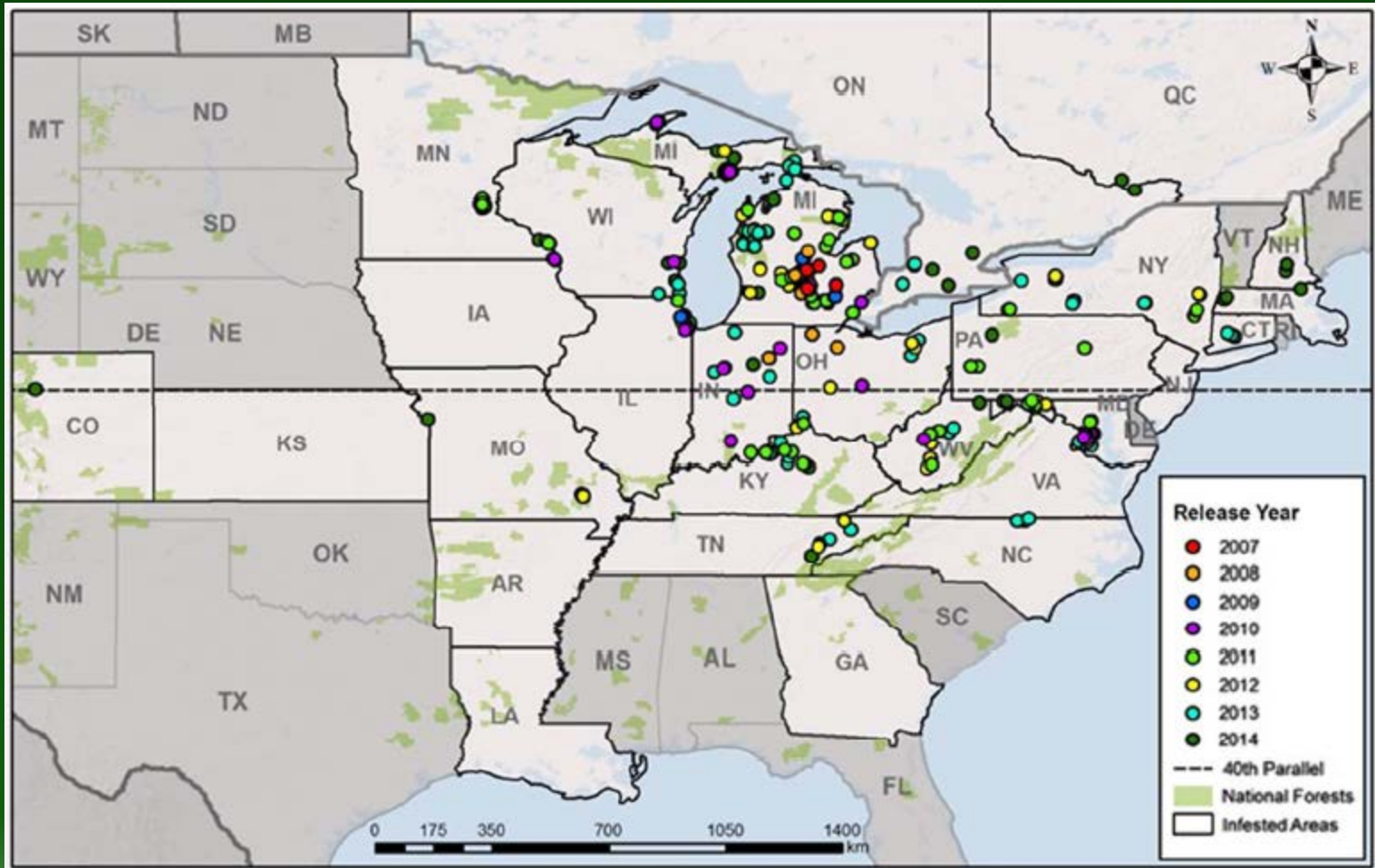


Fig. 1- Known distribution of emerald ash borer, *Agrilus planipennis*, in North America as of February 2015 and the locations of EAB biocontrol agents.. Map created by Applied Spatial Ecology and Technical Services, Department of Entomology, Michigan State University (East Lansing, Michigan, United States of America).

Acknowledgements

- Leah Bauer, USDA Forest Service
- Deborah Miller, USDA Forest Service
- Parasitoids were produced and supplied by the USDA EAB Parasitoid Rearing Facility in Brighton, MI



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