An Innovative Method for Laboratory Rearing of Emerald Ash Borer Larvae

Beneficial Insect Introduction Research Activity Newark, DE 19713

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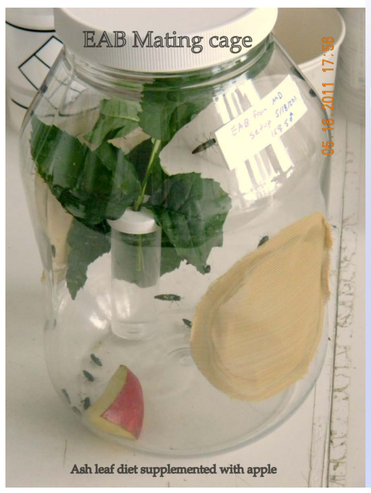
INTRODUCTION

Historically, Emerald ash borer (EAB) have been obtained by collecting infested *Fraxinus* spp. logs (Ash), storing the logs in a 2 C cooler, and removing the logs from cold storage to encourage adult EAB emergence. An even more labor intensive method involves manually excising EAB pre-pupae from infested trees and storing the pre-pupae at 24 C. After pupation the dark pupae develop into adult EAB in ~ 25 days (Bauer et. al 2004). Some EAB researchers have experimented with "artificial infestation", where a series of slits are cut into an ash log, EAB larvae are secured within the slits, and the EAB larvae are then allowed to develop within their natural host wood, or presented to parasitoids as need be (Ulyshen et. Al. 2010). While these methods have allowed researchers to produce EAB larvae and adults, they are resource intensive, require large investments of time, personnel, and entail considerable financial investments. Here we report on an alternative EAB rearing protocol which is considerably streamlined in comparison to present rearing strategies.

MATERIALS & METHODS

The Emerald ash borer (EAB) rearing protocol described here was developed at the Beneficial Insect Introduction Research Laboratory (BIIRL) in Newark, DE. The new rearing protocol is a vast improvement over previous methods.

EAB MATING



- 5 ♀/5♂ EAB are placed into 1 gallon ventilated mating arenas
- Diet consists of:
  - tropical ash (*Fraxinus uhdei*) foliage
  - red delicious apple slices
- Adult beetles allowed to mate 2 weeks
- After 2 weeks exposure ♀ should be at peak egg-laying ability

EAB EGG COLLECTION

- 5 ♀/2 to 3 ♂ EAB are placed into 32 oz. clear plastic oviposition arenas
- Tops covered with nylon screen material (mesh =1 mm^2) and unbleached coffee filter paper
  - Provides favorable oviposition sites
- Filter papers with eggs stored in large Petri-dish at 25°C with moist Kimwipe
- Eggs may only be stored ≤ 12 days

ABSTRACT

Presently there is no practical method enabling researchers to rear Emerald Ash Borer (EAB) in the laboratory. In order to obtain sufficient numbers of EAB specimens researchers have resorted to either collecting EAB from infested ash bolts or collecting pre-pupae by bark stripping. Both methods are time consuming, resource intensive and neither method guarantees a consistent supply of high quality EAB for experimental purposes.

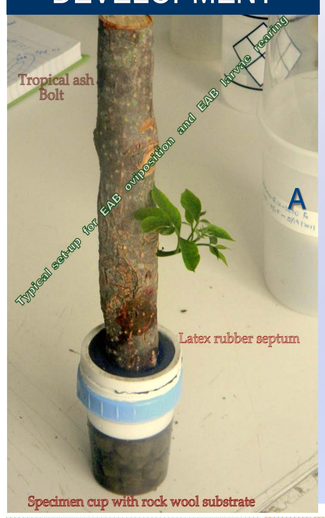
Here we present a protocol which allows us to rear an unlimited numbers of high quality EAB larvae in a laboratory environment.

MATERIALS & METHODS

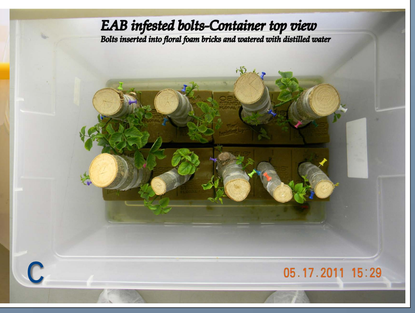
Egg Grafting

- Coffee filters with fertilized eggs are cut into sections containing individual or groups of eggs
- Filter paper sections with eggs are placed against a smooth surface on the log, and wrapped lightly in parafilm, taking care not to suffocate the egg(s).
- Number of eggs grafted to stick depends upon the stick size:
  - ≤1.5cm in diameter ~5 eggs
  - 1.5-2.5cm in diameter ~10 eggs
  - ≥2.5cm in diameter ~15 eggs.

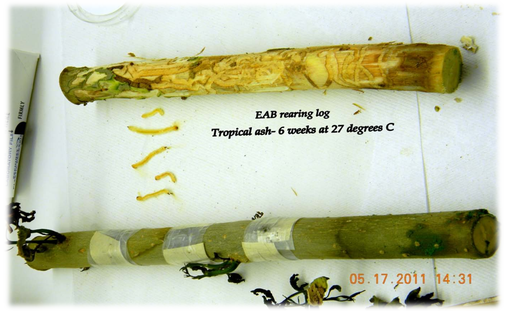
EAB DEVELOPMENT



- Prepared sticks are then place into a growth media. We currently use 3 media:
  - A- 105 mL specimen cup with rock wool
  - B- Oasis® Floral foam bricks
  - C. 32 oz cups with rock wool
- Logs are held for ~6 weeks at 27°C, RH ~65%. After which most larvae are 4th instar and ready to give to parasitoids
- Some logs are allowed to develop past 6 weeks. By ≥8wks larvae are in the J-shaped developmental stage. and can be given a chill treatment. Upon completion of chill treatment, the logs will be removed and held at 27C for adult emergence.



MATERIALS & METHODS



Final Product: High quality EAB larvae with out time consuming insertions!

DISCUSSION

The Emerald ash borer rearing method developed at BIIRL Newark is a leap forward from previous methods. To the best of our knowledge, no other laboratory is able to rear EAB larvae as easily and in the volumes that our method potentially permits us to produce. The results we have produced suggest several important extensions of this research. First, it should now be possible to produce EAB adults in the laboratory. While our research in this area is still ongoing, this would be an exciting development. Presently, the only source of adult EAB beetles is from infested wood harvested from the field. Not only is harvesting the wood expensive, requiring travel, personnel, material, and facility funds, the wood must be cold treated for several months requiring large fiscal investments in refrigeration equipment as well as a severe time cost. The ability to produce EAB adults in the laboratory represents a huge reduction in research costs, something of vital importance in the face of the current budget austerity measures the USDA now faces.

Secondly, this method should allow us to produce unlimited amounts of parasitoids for use in EAB biological control projects. In the past our efforts have always been hampered by two main factors: 1) availability of EAB larvae, and 2) the quality of EAB larvae. We have found that parasitism rates are extremely low when using poor quality larvae collected from the field (≤ 30%), making it very challenging to produce the needed numbers of parasitoids. Preliminary results using logs produced using the EAB rearing protocol suggest that the parasitism rates are much higher, often approaching 95%. Since the great majority of the parasitoids we utilize are obtained from oversea sources, the ability to maintain long term colonies from the original stock material is of vital importance; it costs a great deal of money to fund the travel necessary to obtain the proper parasitoids. It is our sincere hope that other organizations can benefit from this research and method development.

LITERATURE CITED

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