Survivor Trees and EAB

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INTRODUCTION

Comprising 5% of New England's forests, the green and white species of ash trees (*Fraxinus americana* and *Fraxinus pennsylvanica,* respectively) are extremely vulnerable to the Emerald Ash Borer (*Agrilus planipennis*), an invasive beetle from East Asia. The Emerald Ash Borer (EAB) reproduces by laying eggs on the bark of ash trees, eggs which hatch into larvae, gnaw through the bark, and chew long, serpentine tunnels underneath in the phloem layer, cutting off the flow of water to the tree's leaves and severely weakening or even killing it. EAB exists in East Asia as a secondary predator of the Manchurian ash (*Fraxinus mandschurica)*, attacking trees weakened by diseases or age. However, in North America, the insect is able to act as a primary predator, attacking healthy trees and killing up to 98% of all ash trees. But the 2% that survive offer hope of repopulating American forests with resistant trees.

To study this resistance, we challenged branches from a survivor tree with EAB larvae, alongside branches from naïve (unattacked) trees, and epicormic branches (from the base of the trunk), from trees that had succumbed to infestation.

We hypothesized that the larvae would flourish in the previously attacked ash branches and do well in those from the naïve ash. The question was whether the survivors' branches would withstand this attack, and if its survival in the wild was due to inborn resistance rather than luck or circumstance.

Top image: EAB Adult, National Park service.
Bottom image: EAB Larva, https://commons.wikimedia.org/wiki/File:Eablarva.jpg

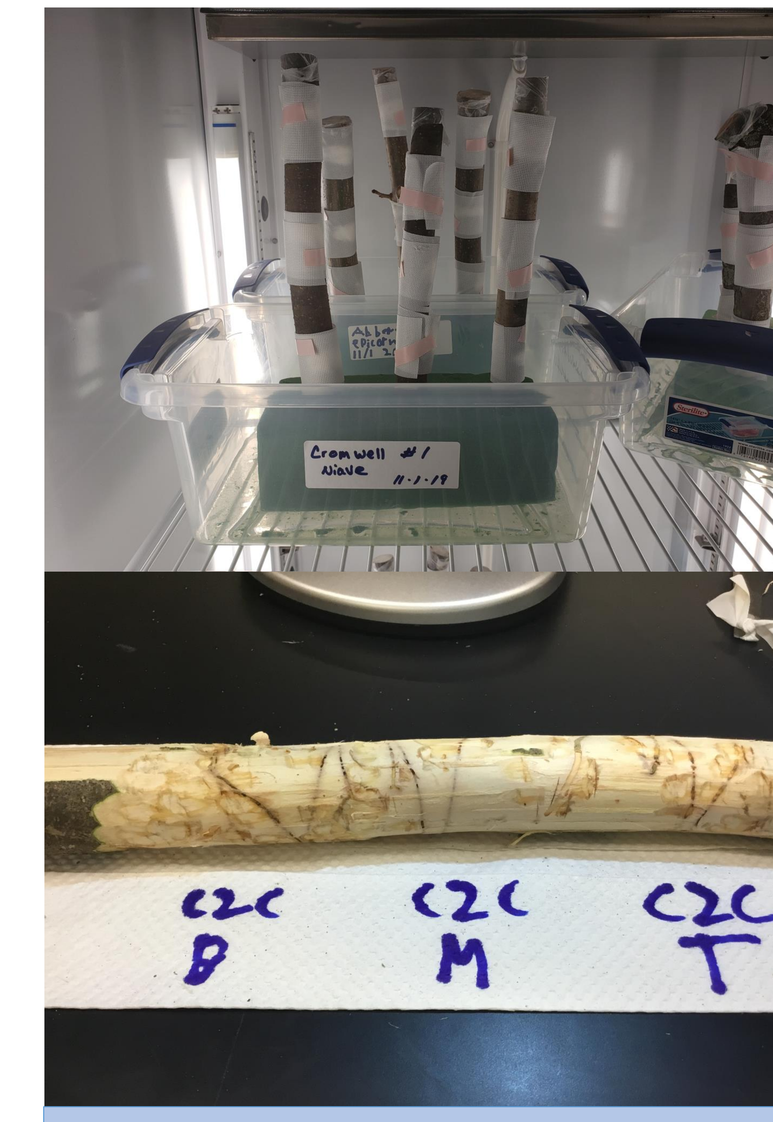
EXPERIMENT DESIGN

We searched Bethany, CT (New Haven county), an area heavily infested for the past 7 years, for survivor trees. Despite extensive searches in a variety of areas, no adult ashes were found alive. A small tree in a row of dead ashes was the only survivor located. A naïve tree from Cromwell and two epicormics in New Haven were also found.

In early November 2019, we took branch samples from each tree and cut them into foot-long segments. On each segment, EAB eggs on coffee filter paper were glued and secured with cotton batting and tape, evenly spaced across the top 3 quarters. The segments were embedded upright in blocks of wet floral foam that were put in incubation at 25 Celsius, and 50% humidity for 40 days, with 16 hours of light and 8 hours of dark every day. The water was regularly refilled and the eggs monitored.

At the end of the incubation period, the branches were removed from the foam blocks, and the bark was removed with scapels. The larvae were extracted with tweezers, placed in a labelled well plate, and refrigerated to perserve them for later micoscopic measurement and examination.

This experimental technique of is a combination of the one described in Abell et al. (2012) for the placement of eggs on the branches, and of Peterson (2014) for the use of wet floral foam in an incubator.

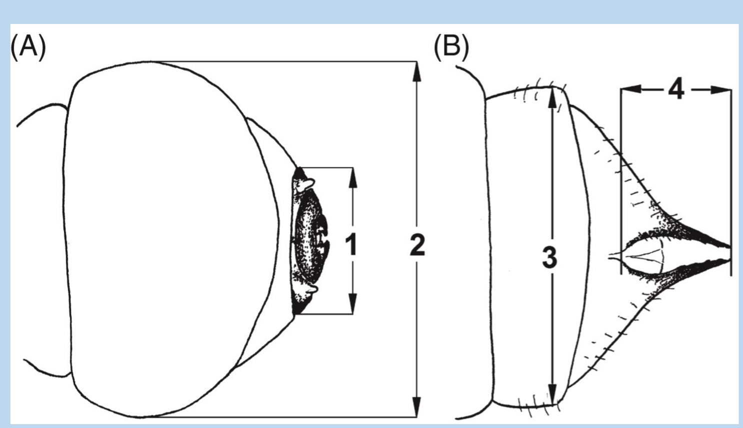


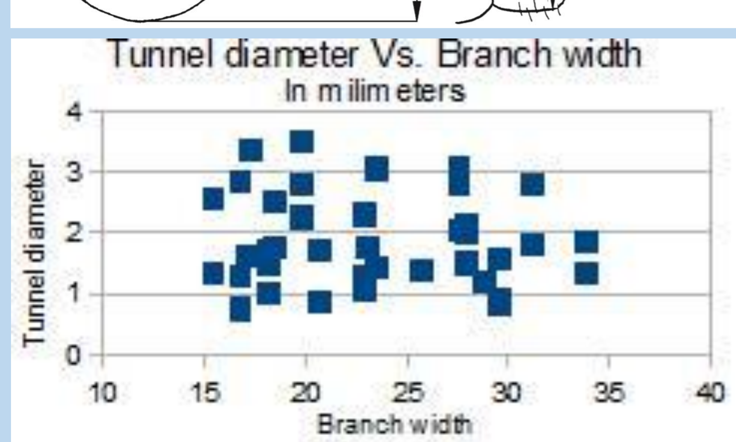
Top image: Experiment setup in incubator.
Bottom image: Debarked branch with larvae removed.

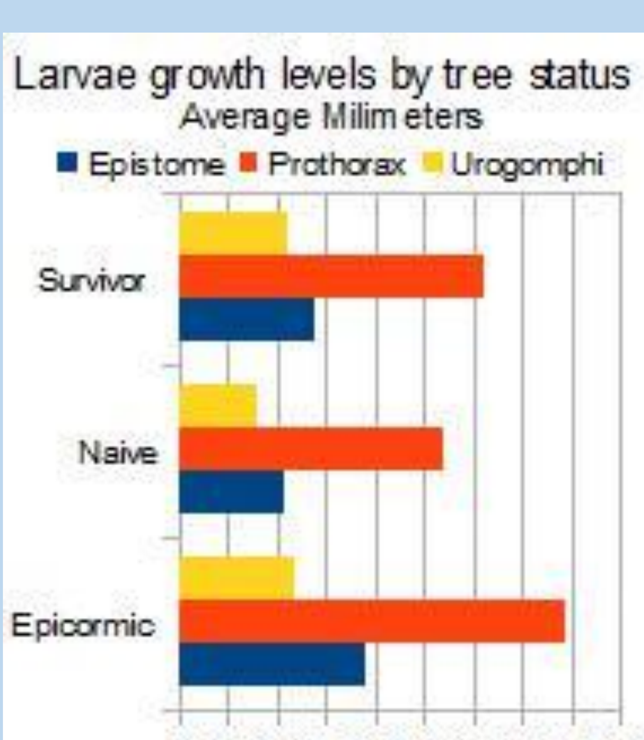
RESULTS

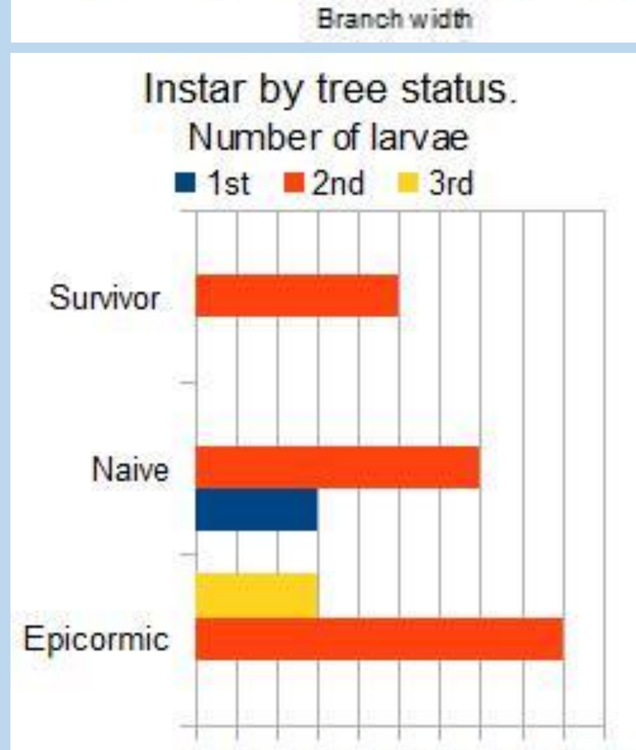
Important EAB Variables

- Epistome: Width of mouth (1)
- Prothorax: Width of head segment(2)
- Urogomphi: Length of tail parts(4)
- Instar: Growth stage reached by larvae.
- Callus: Darkening of larvae tunnel edges, rated 0-1. Indicates how much the tree responded to the larvae by increasing defensive compounds in its tissues.
- Frass: Excrement in tunnel, rated on a scale of 0 for a very light color to 1 for very dark color. Indicates how much of the defensive compounds were eaten and digested by the larvae.





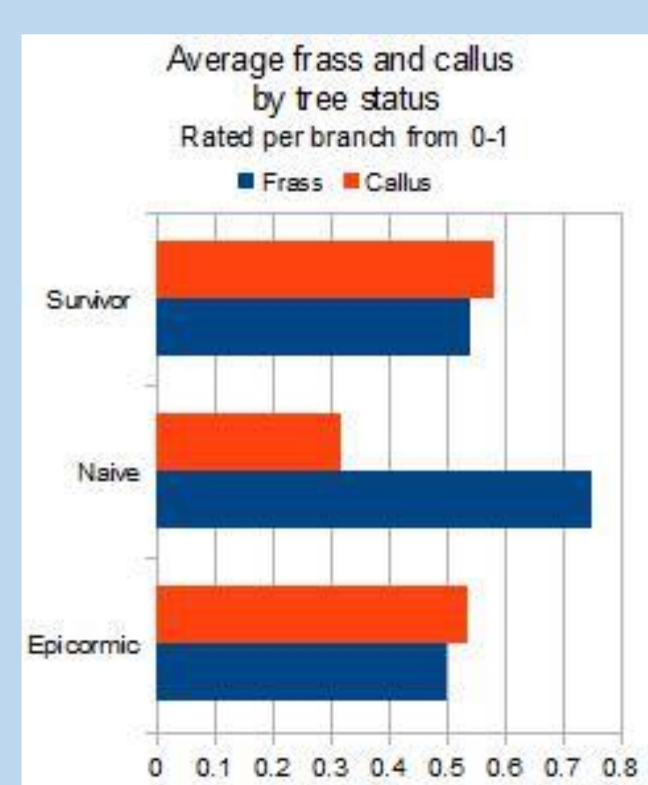


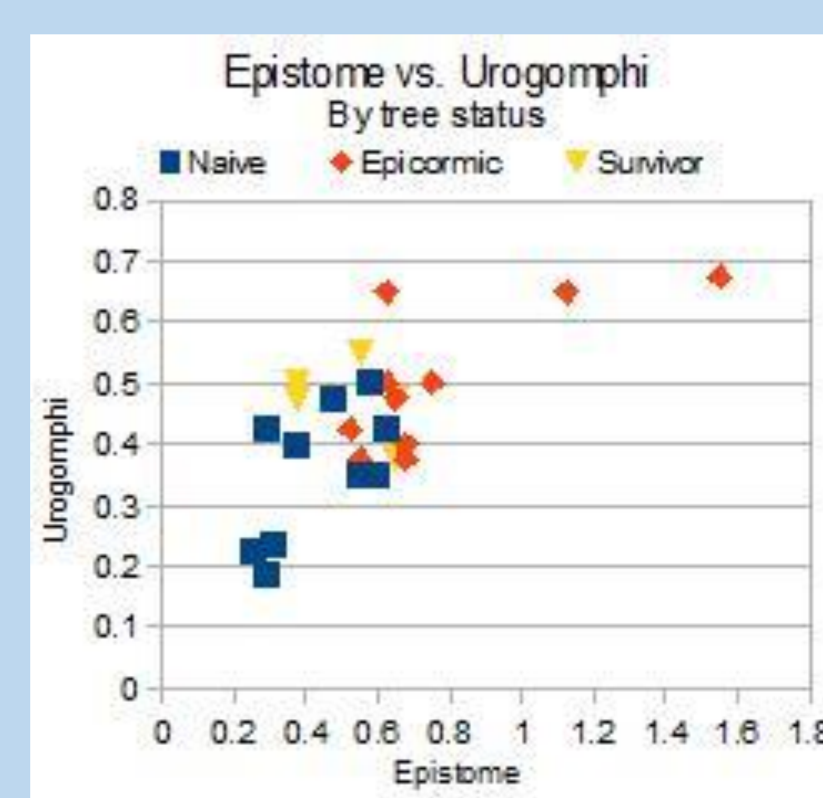


RESULTS CONT. & ANALYSIS

Analysis of the recorded data revealed the following:

- The branch diameter has little relation to tunnel width, implying that branch size is less responsible for larvae variation than the inherent qualities of the trees.
- As anticipated, the epicormic tree, either already weakened or naturally vulnerable to EAB, had the highest rates of larvae growth.
- Contrary to expectations, it was the naïve tree, not the survivor, whose larvae fared the poorest.
- The survivor was an intermediate between the naïve and epicormic in levels of larvae growth.
- The naïve tree had much higher levels of frass color and lower levels of callus color, implying that it was able to better resist the infestation.





CONCLUSION

The epicormic trees have, by far, the largest larvae size. The survivor tree, although more resistant, was less so than the naïve trees. The degree to which the naïve tree resisted the larvae is surprising. One possible explanation is that both the survivor and epicormic trees were weakened. But whether the survivor tree was truly resistant or partway through decline cannot be determined. The experiment, although not conclusive with a relatively small sample size (a total of 54 eggs were placed), provides clear enough correlation in its results, and paves the way for future experiments.

REFERENCES & CREDITS

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Photo Credits:
EAB Adult - https://www.nps.gov/piro/learn/nature/nonnativespecies.htm
EAB Larva - https://commons.wikimedia.org/wiki/File:Eablarva.jpg

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