A SECOND UPDATE REGARDING ONGOING EMERALD ASH BORER RESEARCH AT DOE FARM

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In our last update (if you haven't read it yet, please see: https://www.ci.durham.nh.us/sites/default/files/fileattachments/conservation_commission/page/19571/update-1_24june2020.pdf), we described the initial phases of our ongoing study at Doe Farm investigating the role of tree size and age on the suitability of green ash to the emerald ash borer and two species of parasitic wasps released to slow down the growth of populations of the pest. This update included an explanation of our first experimental treatment, wherein we placed eggs of emerald ash borer on some ash trees in our study at Doe Farm. Allowing emerald ash borer to colonize some trees in our study facilitates our understanding of how attack by an herbivore that feeds within the phloem of ash may change the composition of defensive chemicals that influence the success of immature emerald ash borer, referred to as larvae. After hatching from their eggs, young larvae are often highly susceptible to tree defenses. It is at this point that many larvae may be outright killed by the presence of certain defensive chemicals. Comparing the defensive chemicals present in artificially infested trees against control trees (*i.e.*, those that have nothing done to them other than sampling their phloem) will allow us to identify changes in the amounts of specific chemicals, or the presence or absence of other chemicals which may be responsible for killing the larvae of emerald ash borer.

Since the previous update on our research progress at Doe Farm, we applied the second treatment to ash trees in our study, the plant hormone methyl jasmonate. We timed this treatment to occur approximately two weeks later, coinciding with initiation of feeding by young larvae of emerald ash borer. Methyl jasmonate is a chemical produced by most, if not all plants after they are attacked by herbivores that chew on parts of the plant (as opposed to herbivores such as aphids or some stink bugs that insert their mouthparts into plant tissues and feed by sucking plant nutrients into their bodies). Numerous studies (including those on ash trees) have shown that application of methyl jasmonate to plants simulates attack by an herbivore, leading to the production of additional defensive chemicals. Thus our second treatment is what is known as a "positive control". The use of a positive control in our experiment will allow us to compare: 1) the composition of defensive chemicals in trees that we know have received the signal (methyl jasmonate) that they are under attack, 2) our emerald ash borer treatment, which should have a similar composition of defensive chemicals to our positive control, and 3) our control trees, which should have a composition of defensive chemicals similar to trees that have not been attacked by an herbivore. After the completion of our methyl jasmonate treatment, all eggs that were previously placed on trees were removed. These eggs will be examined at a later date to determine the number of eggs that hatched, allowing a more accurate measurement of mortality caused by tree defenses to the emerald ash borer. The Tyvek wrap that was placed on trees to protect the eggs (or control for the effect of Tyvek on trees) was also removed to limit its potential impacts on our study trees.

More recently, in the last week of July, we collected our set of post-treatment phloem samples from all of our experimental trees. At this point in time, ash trees in our experiment should have modified their composition of defensive chemicals in response to authentic (emerald ash borer egg treatment) or simulated (methyl jasmonate treatment) attack. Collecting these two sets of samples allows us to compare the composition of defensive chemicals from all trees at both their constitutive, non-induced levels (*i.e.*, the pre-treatment samples we collected from all trees in June, prior to application of treatments), as well as those in their induced state (post-treatment samples). Ultimately, these data should answer the question of how tree size and age affects the composition of defensive chemicals within ash trees, and how trees of different sizes or ages may be better or worse at defending themselves from emerald ash borer.

While we have completed the chemical defense part of our research, we still have additional, ongoing components of our study. In the beginning of September we will return to Doe Farm to release two species of parasitic wasps that are specialists on the emerald ash borer. This will begin the second part of our study, which evaluates the impacts of the size and age of our experimental trees on larvae of the emerald ash borer that can tolerate plant defenses. Our next update will explain this in detail.