Identification of a Biomarker Gene for *Fraxinus* spp.

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Abstract

At present, North American ash species (genus *Fraxinus*) are under attack by Emerald Ash Borer (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae; EAB), an aggressive, invasive insect native to southeastern Asia, whereas native Asian ash species are comparatively resistant to this phloem-feeding insect. To date, little research has been done to determine the basis for susceptibility of North American ash species to EAB. It is possible that Asian species are resistant because of metabolites they produce. One objective of my research is to compare metabolite profiles of North American and Asian ash species in order to better understand Asian ash species resistance. Insect feeding will be simulated by application of methyl jasmonate (MJ), a method often used in other insect pest-tree host systems. Following treatment, tissue samples will be harvested for metabolic profiling via various biochemical techniques. However, we do not know how much of a delay there is between imposition of the treatment and the plant's response, or if the molecule imparting resistance is distributed systemically. In order to ensure that we are sampling the right tissues at the right time, a biomarker has been developed to determine when the defense response pathway is up-regulated. This will allow us to predict when and where a response to simulated insect feeding is occurring. In a related transcriptome sequencing project, we identified a gene that was up-regulated in green ash (*F. pennsylvanica*) in response to feeding by EAB larvae. We have shown that this gene is also responsive to MJ treatment. Future work will integrate molecular and biochemical methods to predict candidate genes that may be involved in imparting resistance to EAB.

Introduction

We know very little about the biochemical and molecular biological effects of EAB on *Fraxinus*, which presents challenges for defining experimental parameters for research meant to expand our knowledge in these areas. While we know physiologically how EAB kills ash trees (the wood-boring larvae effectively girdle a tree by feeding on its phloem), we know nothing about the effects of this herbivory on the defense pathways of *Fraxinus*. For example, how quickly does a tree respond? Also, are the effects local or systemic, induced or constitutive, direct or indirect? Experiments designed to answer these questions need to be designed to ensure that our sampling occurs within an appropriate timeframe, and that we sample proper tissues.

A molecule that indicates a particular biological state is generally termed a *biomarker*. For instance, in medicine, a biomarker may indicate presence of a disease. For my dissertation research, a biomarker will be necessary for identifying when defenses against insect feeding are activated in *Fraxinus*.

Methods

Last summer, we planted green ash trees in two cages at Huntington Nursery (Huntington, IN). In one cage, two green ash trees were exposed to EAB adults. Control (uninfested) trees were planted in the other cage. This past fall, we completed a project in which the transcriptomes of these infested and control trees were sequenced. Analysis of the sequencing results showed several families of genes that were up-regulated in green ash in response to insect feeding. Any of these genes have the potential to serve as a biomarker. We are initially focusing on a gene whose expression increased nearly 10-fold in the infested tree as compared to the uninfested tree.

A time-course study was recently completed in which a MJ or control solution was applied as a root drench to greenhouse-grown green ash trees. Five tissues were harvested from each tree over 11 different time points. Analysis by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was completed in order to quantify expression of the gene of interest throughout tissue types and time points. If this gene shows a strong response to MJ treatment as well as insect feeding, it will be an ideal candidate for development as a biomarker in *Fraxinus*.

Results

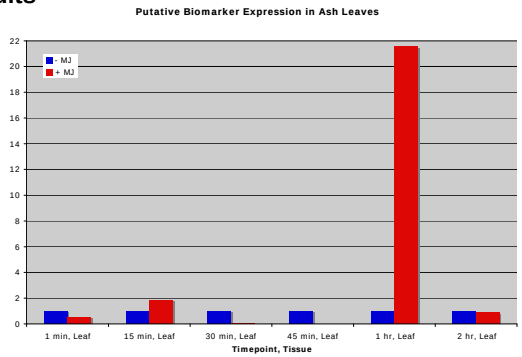

Figure 1. Relative expression of the gene of interest in distal leaves of green ash over several time points following MJ root-drench treatment. Note that expression appears to peak at one hour post-treatment.

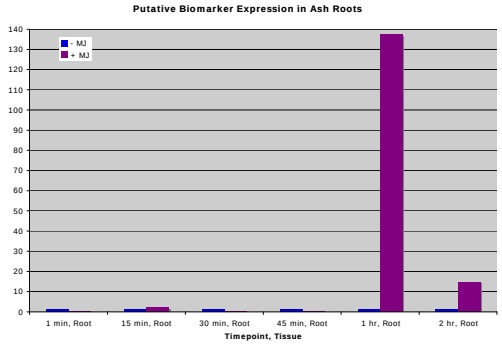

Figure 2. Relative expression of the gene of interest in roots of green ash over several time points following MJ root-drench treatment. Again, expression appears to peak at one hour post-treatment.

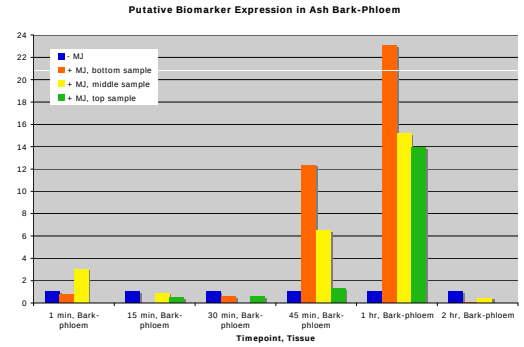

Figure 3. Relative expression of the gene of interest at three locations of bark-phloem samples of green ash over several time points following MJ root-drench treatment. Note that expression appears to gradually increase in all bark-phloem locations during the first hour following treatment. Expression seems to increase more rapidly during the first hour in the lower bark-phloem samples than in either the middle or upper bark-phloem samples. In all cases, expression appears to peak at one hour post-treatment.

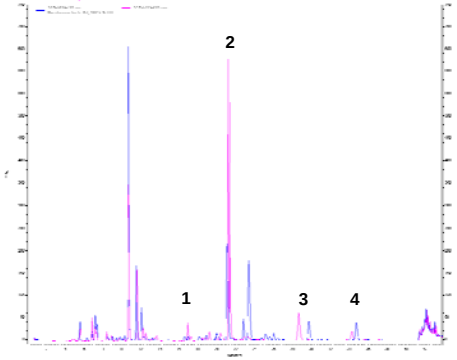

Figure 4. High performance liquid chromatography-UV(HPLC-UV) trace for methanol extracts of bark-phloem samples from **MJ-treated** and **control** Manchurian ash (*F. mandschurica*) trees. Samples were harvested one hour post-treatment, as informed by our biomarker expression study. HPLC is a separation technique that allows for isolation of metabolic compounds. In this case, a UV-VIS photodiode array detector was used to visualize those compounds (represented by individual peaks in the resulting trace) at 280 nm. In the MJ-treated sample relative to the control sample, note that peaks 1 and 2 are much more intense, and that peaks 3 and 4 are shifted. Manchurian ash are resistant to EAB. These four compounds may potentially be involved in the EAB resistance mechanism of Manchurian ash, since these changes appear to be induced by MJ treatment (and therefore, are likely to be induced by insect feeding).

Conclusions

Relative expression is nearly 140 times greater in MJ-treated root tissue than in the corresponding tissue of the control (Fig. 2); this is the highest relative expression rate detected across all tissues for the one-hour time point, which is not surprising since MJ treatment was applied via root drench. In all tissues, putative biomarker expression appears to peak at one hour post-treatment (Figs. 1-3). Additional time points will be analyzed to study relative expression of the gene of interest between 2 and 48 hours post-treatment. **This gene is an ideal candidate for development as a biomarker in *Fraxinus* because it shows a strong response to both MJ treatment and insect feeding.** Future greenhouse studies using MJ treatment as a surrogate for actual insect feeding on ash trees will employ the one-hour post-treatment time point as the appropriate tissue sampling interval. Analysis of metabolites present in EAB-resistant Manchurian ash trees harvested one-hour post-MJ treatment indicates four possible compounds of interest (Fig. 4). Identification of these metabolites may help elucidate the EAB resistance mechanism in Manchurian ash, which will ultimately be informative in understanding why North American ash trees have no innate resistance to this insect.