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Use of near-infrared spectroscopy as an indicator of emerald ash borer infestation in white ash stem tissue

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ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis*), is a non-native beetle responsible for widespread mortality of several North American ash species (*Fraxinus* sp.). Current non-destructive methods for early detection of EAB in specific trees are limited, which restricts the effectiveness of management efforts to contain this invasive threat. This study explored the application of near-infrared (NIR) spectroscopy as a novel method for detection of EAB in white ash (*Fraxinus americana*). Increment borers were used to non-destructively and efficiently collect increment cores from visibly healthy and EAB infested white ash trees for subsequent NIR analysis. White ash trees were sampled across sites of different land use categories (i.e., natural forests versus recreational areas) and different latitudinal regions in the eastern Lower Peninsula and in the western Upper Peninsula of Michigan. NIR diffuse reflectance spectra were collected from three stem tissue types (outer bark, phloem, and xylem) using a NIR spectrometer over the wavelength range of 1134–2190 nm. Spectral measurements were treated with a Savitzky–Golay smoothing filter, followed by a 1st and 2nd derivative transformation to increase spectral sensitivity. Near-infrared spectroscopy was successfully able to discriminate between EAB infested and visually healthy trees using step-wise linear discriminant analysis for each of the three stem tissue types (outer bark, phloem and xylem). The NIR spectra of xylem tissue transformed using a Savitzky–Golay smoothing filter with a 1st derivative transformation over the full NIR wavelength range (1134–2190 nm) had the highest classification accuracy (97%) for discriminating between healthy versus EAB infested trees. This study underscores potential for future research in developing NIR spectroscopy as a cost-effective method of early detection and monitoring of emerald ash borer and other stem-boring insects in support of current detection methods.

1. Introduction

Emerald ash borer (EAB), (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is a phloem feeding beetle native to Asia, discovered in 2002 in Detroit, Michigan, USA as the cause of widespread mortality of ash trees (*Fraxinus* spp.) (Haack et al., 2002; Cappaert et al., 2005; Poland and McCullough, 2006). It has been designated as one of the most economic and ecologically destructive non-native forest insect in the United States, and to date it has killed millions of ash trees throughout the eastern US and Canada (Herms and McCullough, 2014). EAB can negatively influence forest biogeochemistry (Flower et al., 2013a). One estimate of the economic cost of EAB in 25 eastern US States predicted a $10.7 billion dollar loss by 2019 (Kovacs et al., 2010; Aukema et al., 2011). While 16 endemic species of ash in North America are susceptible to EAB, white ash (*Fraxinus americana*) is considered to be one of the most common and taxonomically important species (Poland and McCullough, 2006; MacFarlane and Meyer, 2005).

Initial detection methods of EAB have relied on the visual signs and symptoms of infestation which tend to appear after populations have expanded in an area and significant dispersal has already occurred (Smitley et al., 2008; Flower et al., 2013b; Herms and McCullough, 2014). Research efforts are focused on developing new methods for detecting and monitoring the spread of EAB in order to mitigate damage (Pontius et al., 2008). Destructive examination of girdled trap trees is currently one of the most effective ground based detection methods for EAB, but is costly and labor intensive, and not always feasible in urban areas (Herms and McCullough, 2014). Purple prism traps coated with a sticky

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substance and baited with a volatile lure (e.g., (Z)-3-hexanol or Manuka oil) are also used for widespread detection of EAB in the United States (USDA APHIS PPQ, 2015). Satellite-based hyperspectral imaging has been used to monitor EAB-induced ash decline and infestation levels based on sampling of tree foliage (Pontius et al., 2008; Eastman et al., 2005; Zhang et al., 2014).

Near-infrared spectroscopy (NIR) (wavelength range 780–2500 nm) has been previously used primarily in the wood products industry for rapidly and non-destructively measuring the physical and chemical properties of wood and its decay (Axrup et al., 2000; So et al., 2004; Schimleck et al., 2002; Watanabe et al., 2012; Fackler and Schwanninger, 2012). Regarding forest health studies focused on detection and monitoring of forest insect pests, the majority have measured foliage samples (either field based or using hyperspectral imaging) (Pontius et al., 2005, 2008; Riggins et al., 2011). For instance, field-based NIR spectroscopy and satellite-based hyperspectral NIR spectroscopy have the potential to detect early stages of hemlock wooly adelgid induced hemlock decline (Pontius et al., 2005). Available spectrometers come in a wide selection of wavelength range options and models that cover wider portions of the electromagnetic spectrum (up to 2500 nm) are generally more expensive than lower range models, many of which only go up to 1700 nm wavelengths. A nondestructive, field based method for sampling trees using NIR spectroscopy involves the collection of increment cores, which preserves the trees for later research or product utilization (Roberts et al., 2004; Schimleck et al., 2002). Sampling trees at a height of 1.1–2.2 m has been shown to provide a good representation of the entire tree in NIR studies (Roberts et al., 2004). Wood properties are highly influenced by the amount of moisture in the sample, the effects of which can be minimized by oven drying all samples before collecting NIR measurements (Schwanninger et al., 2011).

Induced systemic plant resistance results when insect herbivory (or other forms of damage) stimulates a signal that is translocated from the damaged tissue to other parts of the plant, resulting in chemical or physical changes in tissues away from the damaged area (Ananthakrishnan, 2001; Leon et al., 2001; Eyles et al., 2010). Studies have shown that after girdling the bark and phloem region of a tree, ash trees become stressed and release volatile compounds that are highly attractive to adult EAB beetles, leading to the effectiveness of utilizing girdled trap trees for EAB detection (McCullough et al., 2009a, 2009b). Increases in volatile compounds, including mono terpenes and sesquiterpenes have been measured in the bark and phloem from girdled green ash (*Fraxinus pennsylvanica* Marsh.) compared to non-girdled trees (Crook and Mastro, 2010; Crook et al., 2008). EAB larvae can induce changes in levels of amino acids and total phenolics in infested trees (Chen et al., 2012). Differences between *Fraxinus* species have indicated that possible nutritional properties and hypothesized defensive mechanisms in the outer bark and phloem could influence EAB feeding (Hill et al., 2012). While limited studies have been conducted on changes in sapwood quality for girdled trees, physical and chemical responses in the sapwood below phloem-girdles have been identified (Taylor and Cooper, 2002; Parkin, 1938). For example, phloem girdling has shown to increase levels of phenolic compounds in the sapwood of red maple (*Acer rubrum*) below the girdle compared to above the girdle and in un-girdled trees (Taylor and Cooper, 2002). Also, parenchyma viability and moisture content has been shown to be reduced in the sapwood of girdled pines beneath a girdle (Taylor and Cooper, 2002). Despite the tendency of EAB to target the upper stem and crown in early stages of infestation, it is expected that systemic induced response mechanisms stimulated by phloem girdling resulting from larval feeding elicits a physical and/or chemical change throughout the entire tree (Ananthakrishnan, 2001; Leon et al., 2001; Taylor and Cooper, 2002; Eyles et al., 2010). While this current study does not directly measure the specific physical and chemical properties commonly associated with stressed or girdled trees, it is hypothesized that systemic changes in the bark, phloem and xylem chemistry and physical properties can be detected using NIR.

This project explores the potential application of ground based, near-infrared (NIR) spectroscopy for early detection of EAB by collecting spectral measurements of white ash bark, phloem and xylem tissue. The objective of this project is to investigate the ability of NIR spectroscopy to differentiate between white ash with known EAB infestation and non-symptomatic white ash throughout the state of Michigan. Since EAB is not confined to a small geographic range, it is important to address the potential usefulness of NIR spectroscopy in being applied across a broad geographical area with trees grown in different site conditions. To address this objective, the study will answer three questions: (1) Using qualitative analytical methods, can NIR spectroscopy differentiate between white ash tissues with known EAB infestation and non-symptomatic trees of the same species? (2) Do different spectral pretreatment methods influence the ability of stepwise linear discriminant analysis to classify samples into predetermined groups? (3) Does reducing the spectral range (i.e., full NIR range vs. lower NIR range (<1700 nm) and upper range (>1700 nm) change the accuracy in classification of EAB infested trees?

2. Methods

2.1. Study site and field sampling

Since the initial discovery near Detroit, EAB has spread throughout the entire Lower Peninsula and is currently moving west in the Upper Peninsula (MDARD, 2015). In the Upper Peninsula, EAB has not yet been discovered in seven of the western counties (which are designated as outside the EAB quarantine) and quarantine regulations prohibit transporting ash samples into these counties.

In the Lower Peninsula, sites with known EAB infestation were selected from three regions along a latitudinal gradient starting near Flint, Michigan and extending northward into the Huron National Forest area. Sampling took place in 2013 from June to early September (Fig. 1). These three regions (from North to South) are: EAB Region 1 (Huron National Forest: Iosco and Alcona counties), EAB Region 2 (Gladwin City and Arenac County), and EAB Region 4 (Lapeer County). An additional region, north of the Huron National Forest (Lower Peninsula: Cheboygan and Otsego counties), was selected as a control region, and is defined as having no visible signs or symptoms of EAB infestation at the time of site selection. Visual signs or symptoms of EAB include D-shaped adult exit holes, vertical bark splits, visible S-shaped larval galleries, woodpecker activity, and epicormic sprouting (Pontius et al., 2008; Smitley et al., 2008; Flower et al., 2013a, 2013b). The second year of field collection (June and July, 2014) took place in the Western Upper Peninsula in Ontonagon, Iron, Menominee, and Delta counties (Fig. 1). At the time of sampling, the counties were outside the quarantined areas with the exception of Delta County.

All sites sampled are characterized by the EPA Level III ecoregion (Northern lakes and Forests) which contains coniferous and northern hardwood forests growing on thick, nutrient-poor glacial soils (EPA, 2015). Soil in the Upper Peninsula control sites generally consisted of well drained sandy loams while the Lower Peninsula control region soils ranged from moderately to excessively drained sand/loamy sands (Natural Resources Conservation Service, 2015). The soils in the EAB regions were generally sandy loams.

In the three latitudinal regions in the Lower Peninsula of Michigan sampled in the summer of 2013, four plots per region were sampled, with three focal trees with known EAB infestation