Flatheaded Borer Ecology and Management Strategies in Nursery and Landscape

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Committee Page

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Dedication

This thesis is dedicated to my parents, Mr. Megharaj Gautam and Mrs. Kabita Gautam, who have been my role models throughout my life. Your belief in me and your constant support have been my academic journey's foundation. I am grateful for the sacrifices you have made and for always being there for me.

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Abstract

Red maple is a popular ornamental shade tree well known for its stunning autumn foliage and fast growth rate. It is a popular choice for both residential and commercial landscapes, parks, and public places across the United States. However, flatheaded borer species in the genus *Chrysobothris* (Coleoptera: Buprestidae) are some of the most damaging insects in red maple production, with infestations being most common on stressed and newly transplanted trees. In nurseries, 'Brandywine' red maples subjected to herbicide stress have been observed to experience flatheaded borer attacks, while the Freeman maple hybrid ‘Autumn Blaze’ was found resistant to flatheaded borers. There is little information available about the susceptibility of other red maple cultivars. To address this gap in knowledge, the primary objective of this study was to determine the physiological and biochemical changes in three red maple cultivars (‘Brandywine’, ‘Sun Valley’, and ‘October Glory’) and Freeman maple hybrid ‘Autumn Blaze’ following the application of two commonly used herbicides, Scythe (2.3%) and Roundup (0.8%). This study found that red maple response varied depending on the cultivar and treatment, with changes observed in physiological, and biochemical traits. A second objective of this study was to optimize purple sticky traps, previously determined to be effective for capturing flatheaded borers. To improve traps, optimal volatile lure type, trap type, and deployment height were determined. Sticky purple pole traps were more effective than Lindgren funnel traps for capturing *Chrysobothris*; and thus, had more potential for use in monitoring or possible management of infestations in nursery trees.

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Chapter 1: Introduction

The green industry is one of the most important contributors to the U.S. economy and to individual states and regions (Hall et al., 2020). The nursery sector, with a total of 6458 nurseries in operation, contributes around $4.5 billion to the national economy annually in the United States. Tennessee alone contributes $119 million revenue annually from 211 nurseries (USDA-NASS, 2019). Maple (*Acer*), belonging to the family Sapindaceae, is the most common and widely distributed deciduous street tree in eastern North America and Europe (Elmendorf et al., 2005). Maples are one of the most often grown ornamental trees in residential and commercial landscapes, parks, and public places in the United States (Frank et al., 2013). Maple accounts for more than 34% of trees grown in US nurseries (USDA, 2009). According to USDA-NASS, (2019), the red maple sales value in the United States was estimated to be $75 million, with $7 million from Tennessee. Red maple appeals to homeowners, landscaping experts, and landscape designers because of its aesthetic and horticultural features (Frank et al., 2013). The most appealing aspect of maple trees in urban environments is their magnificent fall color, ranging from bright yellows and oranges to deep reds, depending on the species and cultivar (Burns, 1990; Frank et al., 2013). In addition to being ornamental, some *Acer* species. are used for commercial products like furniture, lumber, sugar, and maple syrup or different medicinal properties (Hovanet et al., 2015; Xuanming et al., 2013; Yaltirik, 1970).

Herbicides are biologically active compounds that have become a mainstay in modern agriculture for the control of weeds (Cortés-Iza & Rodríguez, 2018). Globally, approximately 2 million tons of pesticides are used with 47.5% being herbicides, 29.5% being insecticides, 17.5% being fungicides, and the remaining 5.5% being classified as other types of pesticides (De et al., 2014). The United States makes up a substantial portion, estimated at between 16-18%, of the world's total expenditure on pesticides (Atwood & Paisley-Jones, 2017). Although using herbicides to boost agricultural yield is the goal, excessive use of herbicide with sensitive crops may disturb normal biochemical and physiological processes and put the main crop at risk (Caverzan et al., 2019). The extensive use of herbicides can also lead to the development of herbicide-resistant weed biotypes (Walsh & Powles, 2007), resulting in even higher herbicide rates to control problem weeds. The biological activity of any pesticide is usually not limited to the target organism but also extends to non-target organisms. Herbicides can have an impact on crop plants in addition to target weeds, either directly by causing phytotoxicity or indirectly through their effects on other species, which can have both negative and positive consequences (Katan & Eshel, 1973).

The use of herbicides to control weeds is quite common in nursery production and urban landscapes. Incidental spray drift or residual contamination may damage trees and make them vulnerable to different insect pests and disease. The effects of herbicide in ornamental trees can be diverse and detrimental for their growth and development. The morphological, physiological, and biochemical changes that plants make in response to abiotic stress either reduce their exposure to stress, limit damage, or aid in the recovery of damaged systems (Potters et al., 2007). In plants, the production of reactive oxygen species (ROS), is a result of various phytotoxic factors including herbicides (Choudhury & Kumar Panda, 2004). Superoxide anions (O_2^-), hydroxyl radicals (-OH), and singlet oxygen are ROS metabolic biproducts that regulate plant growth and development under ambient environmental condition (Maurya, 2020).

However, when a plant is subjected to any kind of biotic or abiotic stress, production of these reactive oxygen species increases. To combat these damaging molecules, plant cells develop non-enzymatic and enzymatic antioxidant mechanisms for ROS detoxification. If ROS generation exceeds the ability of antioxidant systems to scavenge them, it can cause oxidative stress and, eventual cell death (Chaki et al., 2020). Plants with higher antioxidant levels are reported to have greater resistance to oxidative damage. The enzymatic components in plants consisting of catalase (CAT), superoxide dismutase (SOD), glutathione reductase, and non-enzymatic compounds like phenolics, flavonoids, carotenoids, and proline can reduce the negative effects of ROS and provide tolerance or resistance to various environmental stressors (Hasanuzzaman et al., 2020; Kumar et al., 2017; Sachdev et al., 2021). Phenolics protect plants against insects by acting as both physical barriers and chemical deterrents. As cell wall-bound phenolics, lignin, suberin, and cuticular-associated phenolics, they can form a physical barrier in plant cell walls, cuticles, and other plant organs. Furthermore, stored phenolic compounds can behave as herbivore antifeedants or insecticides (Walling, 2000).

Maple trees are prone to a wide range of insect pests. There are over 81 arthropod pests that attack maple trees, and they can cause significant damage (Johnson & Lyon, 1976). Insect injury severity and potential damage varies depending upon the landscape site, maple species, cultivar, and weather (Seagraves et al., 2013). Flatheaded borers (FHB), especially in the genus *Chrysobothris,* are key pests of maple production in Tennessee because larval damage can kill or ruin the tree quality. A report by Oliver et al. (2010) and Potter et al. (1988) stated that FHB can cause up to 50% losses of unprotected red maples in commercial nursery production. The larvae of FHB feed beneath the outer bark, damaging the inner bark (phloem), cambium, and outer sapwood and making irregular tunnels that are partially packed with sawdust-like frass (Potter et al., 1988). The FHB larva will tunnel deeper into the wood during the fall and winter to pupate. The cryptic damage under the bark makes identifying, an infestation challenging until the larva grows large enough to produce visible injury on the trunk surface or visible branch dieback (Oliver et al., 2010).

The information currently available regarding the susceptibility of specific maple cultivars to FHB is confounded by the fact that nursery trees are usually planted in blocks of the same cultivar. Comparisons of different cultivars for borer susceptibility within the same planting have not yet been undertaken. Additionally, no mechanisms for resistance to FHB have thus far been identified. Among the popular maple cultivars, the Freeman maple hybrid 'Autumn Blaze' (*Acer rubrum* × *Acer saccharinum*), appears to have some resistance to flatheaded borers (Seagraves et al., 2013). Whether this putative resistance might be attributed to the faster growth rate of the tree, better drought tolerance, or other physiological factors is unclear. Previous studies have shown some differences in attacks by FHB on different maple cultivars (Seagraves et al., 2012). However, no study has yet attempted to correlate cultivar characteristics with borer resistance. A preliminary study in 2021 demonstrated that FHB were attracted to herbicide-stressed 'Brandywine' red maple trees (Perkovich et al., 2022).

Many species of wood-boring beetles use color as a visual cue to locate their host trees and potential mates (Chénier & Philogene, 1989; Ikeda et al., 1980; Phillips et al., 1988). The Buprestidae beetles are attracted to specific colors or color patterns that may be associated with wood types used for oviposition or with mate location. Different types of traps using a combination of different colors, sex pheromone, host plant volatiles including sticky traps, prism traps, and lure traps, are used for surveying and monitoring metallic woodboring beetles like emerald ash borer (*Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae) (EAB) in North America (Petrice & Haack, 2015). The purple traps designed for catching EAB have been observed to capture other insects, particularly *Chrysobothris* species like flatheaded apple tree borer, *Chrysobothris femorata* (Olivier) (Coleoptera: Buprestidae) (Petrice et al., 2013; Rutledge, 2020). In addition to the EAB, other metallic woodboring beetle species (Coleoptera: Buprestidae) have been documented to cause damage to nursery production and landscape plantings, including the Pacific flatheaded borer (*Chrysobothris mali* Horn), and the bronze birch borer (*Agrilus anxius* Gory) (Mizell et al., 2009). There is a need for similar efforts to control these other species of economically and ecologically injurious Buprestidae, including *Chrysobothris femorata* spp. (Imrei et al., 2020; Perkovich et al., 2022). The efforts could involve the development of traps to understand beetle ecology and to protect trees from damage, as well as other methods like cover crop plantings (Dawadi et al., 2019), insecticides(Addesso et al., 2020), or use of non-susceptible cultivars in plantings (Seagraves et al. 2013).

Thus, the overall goal of this research was to protect the maple tree through sustainable approaches. To achieve this, we had two different objectives. Our first objective was to evaluate how three red maple cultivars and the hybrid ‘Autumn Blaze respond to herbicide stress to identify potential characteristics of FHB resistant ‘Autumn Blaze’ that may differ from the susceptible red maple cultivars. The second objective was to optimize purple traps using an effective volatile lure, purple trap types, and height deployment evaluations for increasing *Chrysobothris* trap capture.

Chapter 2: Review of Literature

Maple and Maple Pest Management

Maple (*Acer*), belonging to the family Sapindaceae, is the most common and widely distributed deciduous street tree in eastern North America and Europe (Elmendorf et al., 2005). Maple is also one of the most often grown ornamental trees in residential and commercial landscapes, parks, and public places in the United States (Frank et al., 2013). Maple accounts for more than 34% of trees grown in US nurseries (USDA, 2009). Maple appeals to homeowners, landscaping experts, and landscape designers because of its aesthetic and horticultural features (Frank et al., 2013). There are about 128 species of maple worldwide under the genus *Acer*. Most maple species are large shade trees, such as red maple (*Acer rubrum* L.), silver maple (*Acer saccharinum* L.), and Norway maple (*Acer platanoides* L.) (Frank et al., 2013; USDA, 1998). These species are preferred over other shade tree genera because they grow larger, commonly above 30 m tall, and generate dense shade (Frank et al., 2013). The trees produce green, yellow, orange, or red colored flowers. Some maples are the source of pollination for bees, and some are used as bonsai. Maples are very prolific in seed production; the distinctive maple fruit containing the seed is called a samara. Their seed varies in size and color from small green to large orange, with thicker seed pods depending on the species. Different maple cultivars can be propagated by using asexual reproduction like cutting, grafting and budding to obtain desirable characteristics (Phillips et al., 2003).

In addition to being ornamental, *Acer* spp. are used for commercial products like furniture and lumber (Yaltirik, 1970). Maples are the source of maple syrup which can be collected from many species, but is mainly harvested from plantations of sugar maple *(Acer* *saccharum)* (Ball, 2007). Maples possess different medicinal properties and have been used for treatment of cardiovascular disease and cancer (Hovanet et al., 2015; Xuanming et al., 2013). Various types of extracts and chemicals obtained from the genus *Acer* have shown pleiotropic health benefits, including as antioxidant, anticancer, antibacterial, anti-inflammatory, antidiabetic, and hepatoprotective effects, in addition to encouraging osteoblast development (Bi et al., 2016).

Red maple (*Acer rubrum*), also known as swamp maple, water maple or soft maple is the most abundant and widely distributed native tree in eastern North America (Hutnik & Yawney, 1961). Red maples have wide tolerance to climate conditions – tolerating temperatures as low as -40º C, as well as hot and dry conditions (Dansereau, 1957; Walters & Yawney, 1990). Red maples can grow in a hardiness zone of 4-9 (Gilman et al., 2006). Red maples are found in a wide range of soil types, texture, pH and elevation, and can grow in sandy to clay soils, well drained to poorly drained, and can tolerate compacted soil (Abrams, 1998). Red maple is a fast-growing tree reaching heights of 18 to 23 m (60 to 75 ft). Maples have an average longevity of 80 to 100 years, with some individuals reaching 200 years (Abrams, 1998). Red maple grows well in full to partial sun. The tree produces small red to yellow clusters of flowers during winter to spring. Maple is often grown as a shade tree for landscapes because of its pleasing form and attractive fall foliage (Stevens, 1999).

In addition to its ornamental features, red maple is used for commercial products such as furniture, lumber, sugar, maple syrup and also possess different medicinal properties (Hovanet et al., 2015; Xuanming et al., 2013; Yaltirik, 1970). Red maple has over 40 different cultivars or hybrid (Fulcher & White, 2012), including ‘Autumn Blaze’, ‘Brandywine’, ‘Sun Valley’, ‘October Glory’, ‘Franksred’ (also often called ‘Red Sunset’), ‘Autumn Red’, etc. The most appealing aspect of these trees in urban environments is their magnificent fall color, ranging from bright yellows and oranges to deep reds, depending on the species and cultivar (Burns, 1990; Frank et al., 2013).

Maple trees are prone to a wide range of insect pests. There are over 81 arthropod pests that attack maple trees, and they can cause significant damage (Johnson & Lyon, 1976). Insect injury severity depends upon the landscape site, maple species, cultivar, and weather (Seagraves et al., 2013). Particularly in the southeastern US, common insect pests of maple include flatheaded appletree borer (*Chrysobothris femorata* [Olivier]) (Coleoptera: Buprestidae), ambrosia beetles (e.g., *Xylosandrus crassiusculus* [Motschulsky]) (Coleoptera: Curculionidae), maple and two-spotted spider mites (*Oligonychus aceris* [Shimer] and *Tetranychus urticae* Koch) (Trombidiformes: Tetranychidae), maple shoot borer (*Proteoteras aesculana* Riley) (Lepidoptera: Tortricidae), maple leaftier (*Episimus tyrius* Heinrich) (Lepidoptera: Tortricidae), potato leafhopper (*Empoasca fabae* [Harris]) (Hemiptera: Cicadellidae), and various species of armored and soft scales (Hemiptera) (Frank et al., 2013).

Flatheaded apple tree borer (FAB) is a common woodboring beetle distributed throughout the continental United States (Brooks, 1919). Adult *C. femorata* are bullet-shaped, 7.6 –15.2 mm long, with several irregular brassy spots on their elytra. The abdomen beneath the elytra is metallic greenish blue, with a metallic bronze ventral surface. Larvae are cream-colored, with bell-shaped abdominal segments and a flattened, sclerotized thoracic region (Wellso & Manley, 2007). Adults usually complete one generation per year, but there are reports of larvae requiring multiple years especially in northern regions (Brooks, 1919). In late spring and summer, adult females deposit eggs on the sunny-side of the tree. Fenton (1942) reported a pre-oviposition period when females feed on tree bark. Eggs require at least a week to hatch and potentially up to 15-20 days (Beddes et al., 2014; Blalock & Oliver, 2014; Brooks, 1919; Hansen et al., 2009). Females are reported to lay up to 100 eggs and prefer bark cracks and wounds to oviposit (Brooks, 1919; Fenton, 1942). Rough bark and crevices can facilitate FHB oviposition. About 7 days after oviposition, the eggs hatch, and larvae chew through the bottom of the egg directly into the host tree, avoiding desiccation and predation (Hansen et al., 2009). Larvae are reported to enter trees after about a 15–20 days egg developmental period, and entry into the phloem may not be successful if the tree is vigorous and there is sufficient sap flow to prevent the larva from entering (Brooks, 1919). The larvae feed beneath the bark, causing damage to the cambium, phloem, and outer sapwood and creating irregular tunnels filled with sawdust-like frass. The fully developed larvae overwinter in the sapwood or heartwood, pupating in the spring (Potter et al., 1988). Adults emerge by cutting distinctive D-shaped exit holes in bark (Frank et al., 2013).

Chrysobothris femorata prefers stressed, dying, or dead trees but also can attack freshly cut timber before it has dried (Swiecki & Bernhardt, 2006). The larval stage causes the most severe injury by feeding beneath the bark in the cambium layer disrupting the production of new vascular tissue, and disrupting the flow of water and nutrients in the vascular system (Potter et al. 1988, Coyle et al. 2005, Oliver et al. 2010). Logs and wood products are commonly damaged by larvae that tunnel into sapwood and heartwood. Larval tunneling over time can weaken the tree trunk leading to future trunk breakage, as well as diminished growth or even mortality (Solomon, 1980). Within one season, a single larva can girdle a young tree. Tree losses in unprotected red maples grown in commercial nursery production systems can be up to 50% (Potter et al. 1988, Oliver et al. 2010). Early damage symptoms are difficult to detect due to the small size of the larva. However, as the larva grows, sunken bark, bark splitting, and frass may become visible later in the fall or following spring (Blalock and Oliver 2014). The most obvious sign of a FAB attack is the wide, meandering galleries under the bark filled with tightly packed frass (Potter et al., 1988). The damaging nature of the borer varies by tree age. In older trees, feeding occurs within the thick inner bark, causing circular wounds confined to the sunny area. In young trees, they mainly feed from sapwood , and the wound appears elongated, often encircling and killing the tree (Brooks, 1919). Although FHB-affected trees do not always die, the damage to the trunk caused by larval tunneling degrades the economic quality of the nursery tree and can reduce the lifespan of a surviving tree.

The optimal management strategy for flatheaded borers is prevention. Once the borer infests the tree, it is more challenging to control with surface trunk sprays. However, systemic insecticides can still be used to control larvae that are inside the tree. The best way to prevent borer attack is to reduce tree stress, supply ample nutrients, meet the water requirements of the tree, and plant trees in locations favorable to the species and cultivar (Addesso et al., 2022). Newly transplanted trees and older trees that are already weakened or stressed trees due to various environmental factors are highly susceptible to borer attack because the root system is limited or weakened, resulting in lower sap flow or poor quality xylem (Brooks, 1919). Insecticide research has found some neonicotinoid insecticides like imidacloprid provide long residual activity against *Chrysobothris* borers (Oliver et al., 2010; Addesso et al., 2020). Trunk spray treatments like chlorpyrifos or bifenthrin were less effective, but more research is needed to determine if low control was due to low product efficacy or suboptimal application timing. According to (Dawadi et al., 2019), winter cover crops can be an alternative to insecticides, as they reduce the FHB attacks by physical blockage of oviposition sites and/or subsequent microclimate changes.

Natural enemies of *C. femorata* include hymenopteran and dipteran parasitoids and clerid beetles (Krombein et al., 1979). These predators and parasitoids can reduce borer populations under natural conditions, but their role in ornamental nurseries and landscapes are unknown (Fenton, 1942a). There was evidence of some parasitic wasps associated with FAB larvae from the trunks of damaged red maple trees in middle Tennessee. These Hymenoptera species included *Eusandalum* spp. (Eupelmidae), *Labena grallator* (Say) (Ichneumonidae), and *Phasgonophora sulcata* (Westwood) (Chalcididae) (Gonzalez, 2021).

Herbicide Stress on Tree

Plants are exposed to a wide range of biotic and abiotic stresses during their life stages, which can negatively impact their growth, development, and productivity. Biotic stresses are caused by living organisms such as insects, pests, pathogens, and weeds. In contrast, abiotic stresses are caused by non-living factors such as temperature extremes, drought, flooding, salinity, pesticides, and heavy metals (Anjum et al., 2014; Mittler, 2006). Herbicides are biologically active compounds that have become a mainstay in modern agricultural methods for the control of weed species (Cortés-Iza & Rodríguez, 2018). The use of herbicides to control undesirable vegetation has had a tremendous impact on the crop productivity however, excessive use may disturb normal biochemical and physiological processes and put crops at risk (Caverzan et al., 2019), as well as can lead to the development of herbicide-resistant weeds (Walsh &

Powles, 2007). The damage may extend from slight reduction in growth rate to total mortality depending on rate, type, method and time of application (Nelson & Haissig, 1986). The biological activity of any pesticide may not be limited to the target organism but can also extend to non-target organisms. Herbicides can have an impact on crop plants in addition to target weeds, either directly by causing phytotoxicity or indirectly through their effects on other species, which can have both negative and positive consequences (Katan & Eshel, 1973). About 80–90% of the pesticides applied to the environment volatilize within few days of application (Majewski, 2019). Herbicide volatilization from treated plants or soil surfaces can cause severe damage to adjacent non-targeted plants (Straathof, 1986). Herbicides inhibit biochemical and physiological processes in plants. Herbicide mode of action typically inhibit cell division, photosynthesis or amino acid production or mimic the natural plant growth hormones resulting in plant death (Ross & Childs, 1996).

The effects of different pesticides on the plant cell leads to the formation of reactive oxygen species (ROS) like superoxide anion (O2 -), hydrogen peroxide (H_2O_2), hydroxyl radicals (-OH), and singlet oxygen which are cytotoxic to the plants. Reactive oxygen species are by-products of aerobic metabolism constantly produced in the chloroplast, mitochondria, and peroxisomes (Apel and Hirt, 2004). Under normal circumstances, ROS are scavenged by various antioxidant defense systems (Foyer & Noctor, 2005). Different environmental stressors such as temperature extremes, drought, flooding, salinity, herbicides, and heavy metals induce an overproduction of ROS, which leads to oxidative cellular damage (Mittler, 2002). The accumulated ROS affect the plant by oxidizing membrane lipids and proteins, inhibiting enzyme activity, damaging DNA and RNA, and even leading to cell death (Sandalio et al., 2009).

Glyphosate (N-(phosphonomethyl) glycine), commercialized in 1974, is the most widely used herbicide in the world. It is a broad-spectrum systemic herbicide that is widely used in agriculture, landscaping and nurseries to control weeds (Duke et al., 2012). It works by inhibiting an enzyme, 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), involved in the synthesis of aromatic amino acids and phenolics in plants (Duke, 1988; Myers et al., 2016; Velini et al., 2008). Aromatic amino acids are responsible for synthesizing several compounds that are essential to growth regulation, wood quality, allelopathy, resistance to pests, and diseases (Velini et al., 2008). As a consequence of the inhibition of EPSPS plants may die or have reduced growth (Velini et al., 2010). In nurseries, glyphosate is commonly used to control weeds in containerized plant production areas or between rows of field-grown plants. Glyphosate systemic action allows it to effectively control both annual and perennial weeds, and it is relatively safe to use around desirable plants when applied according to label directions (Palai, 2010). Scythe (pelargonic acid) also known as nonanoic acid, is a contact broad-spectrum foliar applied herbicide to control annual and perennial weeds. Scythe is a fatty acid that occurs naturally in many plants (Webber III & Shrefler, 2007), and it is considered a low toxicity herbicide with low residual activity (Malkomes, 2006). Scythe disrupts the plant cell membranes, which leads to desiccation of the plants (Copping & Duke, 2007). Scythe damage appears shortly after application.

Response of Plant to Stress

Different environmental stressors trigger plant responses from alteration of gene expression and cellular metabolism to changes in their growth and productivity (Anjum et al., 2011). The morphological, physiological, and biochemical changes that plants make in response to abiotic stress either reduce their exposure to stress, limit damage, or aid in the recovery of damaged systems (Potters et al., 2007). Plants have a mechanism to respond to stress events, regardless of the type of stress they are experiencing. Examples of plant stress responses included slowed growth, lower photosynthetic rate, lower nutrient uptake or changes to their hormonal balance like, producing more abscisic acid and often less cytokinin (Chapin et al., 1988; Chapin III, 1980; Chapin III et al., 1990; Parsons, 1968). Additionally, accumulation of organic acids and specific osmolytes and, changes in carbohydrates metabolism are common plant responses to various environmental stressors (Bartwal et al., 2013). In plants, most stress conditions do not elicit specific responses; but rather, generic responses like production of excessive ROS (Apel and Hirt, 2004). To combat these damaging molecules, plants have developed both enzymatic and non-enzymatic antioxidants that play a significant role in ROS signaling and protection of the cell from oxidative injury (Sharma et al., 2012; Vranova et al., 2002).

Similarly, in response to stress, plants may increase the production of various secondary metabolites, which help the plants to defend against stress and pathogens. The secondary metabolites in plants do not fundamentally contribute to the maintenance of a plant's life processes but are crucial for the plant to interact with its environment to adapt and defend itself (Bartwal et al., 2013). Plant secondary metabolites are divided into three different groups: terpenes, phenolics and nitrogen-containing compounds. Terpenes act as plant growth regulators, defense against insects, pathogens, attracting compounds for pollinators, and can influence the growth and development of neighboring plants directly or indirectly. Phenolic compounds such as tannins, flavonoids, and phenylpropanoids, are associated with defense against herbivores.

Nitrogen containing secondary metabolites include alkaloids, which act as potential inhibitors of ROS (Bartwal et al., 2013; Blokhina et al., 2003; Pagare et al., 2015). Some plants also use secondary metabolites as signals for communication with symbiotic microbes, parasitoids of herbivores, as well as to attract pollinators and seed dispersers (Wink, 2003). Secondary metabolite production and accumulation varies between plant species or even within the same plant species grown under various environmental circumstances (Jaakola & Hohtola, 2010). Numerous stresses impair plant growth by altering shape and increasing levels of phenolic pigments, antioxidant activity, electrolyte leakage, flavonoids, proline, and tocopherols (Brzezinska et al., 2006). The detrimental effects of ROS are prevented by the presence of lipid soluble antioxidants (tocopherol, carotenoids), water soluble reductants (glutathione and ascorbate) and antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase present in plant cells (Desikan et al., 2004).

Trap Optimization for Buprestidae Beetles

Woody plants are attacked by a wide range of arthropod pests, which include spider mites, broad mites, scales, mealybugs, whiteflies, aphids, lace bugs, grasshoppers, sawflies, leaf miners, gall makers, leaf-feeding beetles, woodboring beetles, caterpillars slugs, and snails (Mizell et al., 2009). The Buprestidae family, commonly known as jewel beetles or metallic woodboring beetles, are known for their stunning and colorful appearance (Evans & Bellamy, 2000). In total, there are about 15,000 known species of jewel beetles, making them one of the most diverse families in the order of Coleoptera (Bellamy, 2002). The family is further divided into 13 subfamilies, 50 tribes, and more than 500 genera and sub-genera, showcasing the remarkable breadth of diversity within this group (Bellamy, 1985). The beetles are generally polyphagous and use a variety of woody deciduous plants as reproductive hosts (Burke, 1919). The larval stage causes damage by feeding and tunneling beneath the outer bark of tree trunks and branches (Beutel & Leschen, 2016). Trees are more prone to borer attacks when they are weakened by various factors such as drought, soil compaction, mechanical wounds, or transplant stress (Evans et al., 2004; Mizell et al., 2009). Borer control is challenging once larvae have entered the tree. Therefore, borer management primarily relies on preventative measures (Evans et al., 2004). Different types of traps (e.g., sticky, plastic prism or Lindgren funnel) using a combination of colors, sex pheromones, or host plant volatile lures, are used for surveying and monitoring buprestid beetles like emerald ash borer (*Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae) (EAB) in North America (Petrice & Haack, 2015). In addition to the EAB, several other buprestid species-have been documented to cause damage to nursery production and landscape plantings. For instance, the flatheaded appletree borer (*Chrysobothris femorata,* [Olivier]), the pacific flatheaded borer (*Chrysobothris mali,* Horn), the twolined chestnut borer(*Agrilus bilineatus,* Weber) and the bronze birch borer (*Agrilus anxius,* Gory) (Mizell et al., 2009). There is a need for similar efforts to control these other buprestid species including flatheaded apple tree borer, which could include the development of effective traps (Imrei et al., 2020; Perkovich et al., 2022).

Sticky traps are generally inexpensive and straightforward to use, and their effectiveness is improved by an increased knowledge in insect chemical ecology and the availability of synthesized semiochemical lures (Allison et al. 2004; Suckling, 2015; Hanks and Millar, 2016; Millar and Hanks, 2017). Baited traps are the most widely used strategy among the tools now available for the early identification of pest species (Augustin et al., 2012). Many woodboring beetles use color as a visual cue to locate hosts and potential mates (Francese et al., 2005; Imrei et al., 2020; Petrice et al., 2013). A purple sticky trap that simulates the shape of a young tree might be a useful and accessible instrument for tracking the flight activity of buprestid species near nursery sites (Oliver et al. 2004). In a trapping experiment with Buprestidae (Oliver et al., 2002), colors in the violet range of the electromagnetic spectrum were more attractive to at least 31 species of buprestids, including several *Agrilus* spp. Green or purple colored three-sided prism traps covered with insect glue or purple Lindgren multi-funnel traps have been reported to be effective in collecting buprestids like EAB and gold spotted oak borer (*Agrilus auroguttatus* Schaefer) (Coleman et al., 2014; Francese et al., 2008). Allison and Redak (2017) reported male EAB were more attracted to green traps, whereas female EAB were slightly more attracted to purple traps. Francese et al. (2005) and Rutledge et al. (2020) reported that *Chrysobothris* spp. (Coleoptera: Buprestidae) strongly preferred purple prism traps over green multi-funnel traps. In a study conducted by Skvarla and Dowling (2017), green multi-funnel traps were the preferred choice of *Agrilus* species, while *Dicerca* species showed a preference for purple multi-funnel traps. Similarly, *Chrysobothris* species were also found to have a preference for purple traps (Francese et al. 2005). Response of some buprestid species varies by the shape and color of the trap. A purple *Agrilus* silhouette trap attached to a white background captured a higher percentage of *Chrysobothris* male and female in comparison to a green *Agrilus* silhouette trap in a trapping experiment (Petrice et al., 2013). Pole-shaped traps mimicking the trunks of trees captured more *Chrysobothris* spp. in Tennessee (Perkovich et al., 2022). Multi-funnel traps were designed more recently in response to surveyor and researcher needs for a reusable, more user-friendly (i.e., non-sticky) trap (Francese et al., 2011). In comparison to the typical purple prism traps, both purple and green multi-funnel traps had comparable or superior trap effectiveness for EAB trapping. Larger multi-funnel traps (12 and 16 units) collected more EAB than the shorter multi-funnel traps (4 and 8 unit) in optimization of multi-funnel traps for EAB (Francese et al., 2013). The height at which traps are deployed is another important variable that affects trap capture (Graham et al., 2012; Ulyshen and Sheehan, 2019; Vance et al., 2003; Wong & Hanks, 2016), as certain species of wood boring beetles are most abundant in the tree canopy (Liebhold et al., 2017; Maguire et al., 2014), while others prefers the understory, such as those developing in roots, fallen trees, or the lower bole of standing trees (Dodds, 2014).

Many woodboring beetles including EAB uses host tree volatiles as cues for finding hosts (Chénier & Philogene, 1989; Ikeda et al., 1980; Phillips et al., 1988). Some male *Agrilus* species were found to be land on or next to dead female specimens of their own species and other species pinned to foliage (Domingue et al., 2013). Previous research showed that the release of (Z)-3-hexenol from leaves has varying effects on male and female EAB, as reported by Crook and Mastro (2010). Green leaf volatiles (GLV) including (Z)-3-hexenol, (Z)-3-hexenyl acetate, and (E)-2-hexenal from ash have been studied as EAB attractants. One compound, (Z)-3-hexenol, was a promising GLV attractant that increased trap catch of EAB on purple traps (De Groot et al., 2008), as well as male EAB attraction to green traps (Silk & Ryall, 2015).

The purple traps designed for catching EAB have been observed to capture other buprestids, particularly *Chrysobothris* species like *Chrysobothris femorata* (Petrice et al., 2013). These findings suggest that *Chrysobothris* beetles may respond to similar environmental cues as EAB. However, it is crucial to conduct further evaluations of trapping methods to optimize trap captures for *Chrysobothris* while minimizing bycatch of non-target buprestids.

Chapter 3: Methodology

Physiological and Biochemical Responses of Red Maple Cultivars and Hybrid Maples to Herbicide Stress

Experimental Design and Plant Materials for Container Experiment

The study aimed to examine the physiological and biochemical changes in red maple cultivars and hybrid maples after herbicide treatment. The experiment was conducted on red maple trees, (*Acer rubrum)* 'October Glory' (OG), 'Brandywine' (BW), and 'Sun Valley'(SV), and one (*Acer* x*freemanii*) Freeman maple hybrid 'Autumn Blaze'(AB) grown from rooted cuttings in number 3 containers (~11.4- liter volume). Fully opened foliage of trees were treated in mid-May with a contact Scythe® Herbicide (pelargonic acid 2.3%; Gowan Co, Yuma, AZ) or a systemic herbicide, Roundup (Glyphosate 0.8%; Monsanto, St. Louis, MO) for 5 seconds with an average tank mix volume of 143 ml per tree directed at on one side of the canopy. Initial tree height was measured using a meter stick, and trunk diameter was measured above 15 cm from the ground level using a vernier caliper (Mitutoyo Corporation, Kawasaki, Japan). Plant height and trunk diameter measurement were done at the beginning and 37 days after treatment.

Leaf tissues for the additional morphological and biochemical experimental procedures were collected from Roundup, Scythe and Control treatment trees at 7 days after treatment (DAT) with herbicides using fully expanded leaves collected from the untreated side of the canopy of each tree. Roundup-treated trees were dead after two weeks. Therefore, leaf tissue was collected from Control and Scythe-treated trees only on day 28 DAT. The harvested leaf tissue samples were either oven-dried at 65ºC for 48 hr or frozen at –80ºC for further assays. Experiments were conducted at the Otis L. Floyd Nursery Research Center, McMinnville, TN with 30 replicates for each cultivar and 10 replicates for each treatment. Treatments were applied in a 3 × 4 factorial design.

Leaf Chlorophyll Content Using a SPAD Chlorophyll Meter

A SPAD chlorophyll meter (Spectrum Technologies, Plainfield, IL) is a widely used, non-destructive, and rapid method for the indirect measurement of chlorophyll content in plant leaves. The SPAD value is proportional to the amount of chlorophyll in the leaves, and a higher SPAD value indicates a higher chlorophyll content. A non-destructive chlorophyll measurement in the leaves under field conditions was taken by using SPAD meter at 7 and 28 DAT. Readings were taken of each plant from three randomly selected untreated undamaged, fully expanded leaves. The obtained SPAD value from three leaves of each plant was averaged to estimate the measured chlorophyll value of that plant.

Photosynthetic Pigments of Leaf Tissue

Photosynthetic pigments, such as chlorophyll a (chla), chlorophyll b (chlb), and carotenoids, play a vital role in photosynthesis and production of antioxidants in plant cells. The impact of herbicide treatment on photosynthetic capacity of the plant and its ability to produce energy through photosynthesis was assessed by measuring the concentrations of chla, chlb, and changes in antioxidant capabilities via total carotenoids. The levels of these pigments were measured in laboratory conditions from frozen leaf samples. In the assay, three 6 mm leaf disks were punched from the portion of the leaf that did not contain a central petiole vein, kept in an Eppendorf tube with 1.6 mm beads, and then extracted using 1 ml of pure methanol. The mixture was homogenized using a Storm 24 bullet blender (Quasar Instruments, Colorado Springs, CO.), incubated at 4ºC for 24 h, and was centrifuged (Corning LSE High Speed Microcentrifuge, Tewksbury, MA) the next day at 1,800 g force for 15 minutes. The absorbance of the supernatant was recorded using a UV-Vis microplate spectrophotometer (BioTek, Winooski, VT) at 470 nm, 665 nm, and 652 nm to measure total carotenoids, chla, and chlb, respectively. The concentration for chla, chlb and total carotenoids was calculated using the following equations given for pure methanol solvent (Lichtenthaler & Buschmann, 2001), where C_a refers to chla, C_b refers to chlb, $C_{(x+c)}$ refers to total carotenoids, and A is a constant based on the solvent correction reading at the denoted wavelength:

$$\text{Eq. 1. } C_a\ (\mu g/ml) = 16.72\ A_{665} - 9.16\ A_{652}$$

$$\text{Eq. 2. } C_b\ (\mu g/ml) = 34.09\ A_{652} - 15.28\ A_{665}$$

$$\text{Eq. 3. } C_{(x+c)}\ (\mu g/ml) = (1000\ A_{470} - 1.63\ C_a - 104.96 C_b)/221$$

Electrolyte Leakage of Leaf Tissue

Electrolyte leakage is a commonly used method to assess the degree of membrane stability or damage in plant tissues. Electrolyte leakage was measured to determine the impact of herbicide treatment on plant ability to maintain membrane stability, which is important for normal cellular function and overall plant health. To measure electrolyte leakage, two 15 mm leaf disks including veins were taken from fresh leaf samples on the day of harvesting. These disks were placed in 20ml of deionized water in a test tube and kept at 4°C for 24 hours (Tongo et al., 2021). After 24 hours of cold storage, the resulting solution was tested for an initial electrical conductance (C1) baseline for the sample using Thermo Scientific Orion Star A215 Benchtop pH/ electrical conductivity meter. Then, the samples in solution were autoclaved at 121°C for 20 minutes to release all the electrolytes. After samples had cooled to 25°C, a final electrical conductivity (C2) measurement was re-taken for each sample. The resulting electrolyte leakage was calculated as a percentage using the formula:

Eq. 4. (C1/C2) * 100.

Phenolics and Tannins of Leaf Tissue

Polyphenols, such as phenolics, tannins, and flavonoids are a diverse group of phytochemicals that play an important role in plant metabolism and defense mechanisms. The extraction and analysis of polyphenols from maple cultivars can provide valuable information about the effects of herbicides on the accumulation of these compounds in the plant and the role they play in the plant's response to stress. Plant phytochemicals were extracted from foliar tissues that had not been in direct contact with herbicide treatments. The leaf samples (including veins, but not petioles) were first ground into a fine powder using a 2 mm mesh on a Mill 4 Wiley Mill (Mill 4, Thomas Scientific, Swedesboro, NJ). The total phenolic concentration was further analyzed using a modified Prussian blue assay (Hagerman, 2002). One ml of 70% acetone was added to 100 mg of the finely powdered sample and incubated for 30 minutes at 4°C. The extracted samples were centrifuged for 10 minutes at 2500 × g-force. The supernatant was then diluted 1:8 using 70% acetone. Further, 0.02 M $FeCl_3$ and 0.016 M $K_3Fe(CN)_6$ were added to the samples and incubated for 15 minutes at room temperature. The absorbance of the reaction was recorded at 700 nm using a UV-Vis spectrophotometer (BioTek, Winooski, VT). The results were expressed as gallic acid equivalents (GAE) as gallic acid is the most frequently found phenolic compound in the plants.

Total tannin concentration was quantified using a Radial Diffusion Assay (Hagerman, 2002) with some modification. To prepare 7–8 agar plates, 1 gram of agarose was added to 100 ml of buffer solution (buffer: 0.05 M acetate containing 60 µM ascorbic acid; pH 5). The mixture was heated with continuous stirring at a temperature of 80–100 ºC until the agarose dissolved completely. The beaker containing a clear hot solution was placed into warm water kept above a heater, set to a temperature of 45°C. The agarose solution was allowed to cool to 45°C. When the solution had reached 45°C, 0.10 g of Bovine Serum Albumin (BSA) (EMD Millipore Corp., Burlington, MA) was added with continuous stirring to dissolve it. The solution with dissolved BSA was poured into the petri dishes using a 10 ml beaker without allowing the solution to cool. The 10 ml solution was equally distributed to the surface of each dish and each dish was sealed with parafilm. The plates were stored at 4°C for up to several days before use.

Tannins were extracted from oven-dried leaf samples using 1ml 70% acetone in 100 mg finely powdered samples and incubated for 30 minutes at 4°C. The extracted samples were centrifuged for 10 minutes at 2500 × g-force. The plates containing solidified BSA agarose were punched using a 4 mm diameter cork borer to form 6-8 evenly spaced wells per plate. The supernatant was applied to a well by repetitively dispensing 8 µl aliquot samples a total of five times without letting the wells become completely dry between successive aliquots additions to be added. Each well contained a 40 µl sample in total. After applying the samples, the plates were sealed with parafilm and stored in an incubator at (25ºC) for 4 days. The tannin in the sample binds to the BSA in the agar as it diffuses outward from the well, causing the agar to turn a milky white color and forming a visible ring. The amount of tannin in the sample was estimated by calculating the area of the ring that formed on the plate. The results were expressed as tannic acid equivalents.

Flavonoids of Leaf Tissue

Total flavonoid concentration was determined using a method described by (Woisky & Salatino, 2015). The extraction was performed by adding 5 ml of 96% ACS grade ethanol to 100 mg of dried plant tissue. The tube containing the mixture was tightly covered with a cap and shaken with a Thermolyne 50800 Rotomix Lab Shaker (Thermolyne Scientific, Dubuque, IA) in a dark room at room temperature for 5 days. The resulting supernatant was diluted 1:2 with pure ethanol and 0.5 ml supernatant was collected and mixed with 0.5 ml of $AlCl_3$ ethanol solution. The solution was made by adding 0.5 g of pure aluminum chloride to 25 ml of 96% ACS grade ethanol. The mixture was incubated for 1 hour at room temperature and the absorbance of the supernatant (300 µl) was measured at 420 nm using a UV-Vis spectrophotometer. The results were expressed as quercetin equivalents (QE) as quercetin is a representative of large class of flavonoids found in the plants.

Total Non-Structural Carbohydrates (TNC) of Leaf Tissue

The total non-structural carbohydrates include sugars and starch stored in the tree and serve as energy sources for growth and defense against environmental stressors. Comparing TNC levels in different cultivars with and without herbicide stress can provide information on the relative ability of each cultivar to respond to stress. A protocol by Dubois et al. (1956), revised by (Newell et al., 2002) was followed to extract non-structural carbohydrates from foliar tissue. This method extracted 10 mg dry sample using a 1.5 ml 80% solution of aqueous ethanol wash. The microcentrifuge tube containing the mixture was placed in a VWR water bath shaker (Sheldon manufacturing Inc. Cornelius, OR) at 27 °C at 50 rpm for about 12-18 hours (overnight) to allow TNC extraction. The microcentrifuge tubes were then centrifuged at 8,300 g-force for 10 minutes to pelletize the solids. The supernatant was then transferred into the scintillation vials, without pouring out any solids. The same steps were repeated twice as before by adding 1.5 ml 80% ethanol solution to the same microcentrifuge tube. The total amount of ethanol in the sample was 4.5 ml and the supernatant was diluted with de-ionized water to bring the volumes up to 10 ml.

After the removal of sugar with several washes of ethanol, the remaining pellet was placed in 0.7 ml of 1.1% HCl for starch extraction. The mixture was placed in a water bath at 100°C for 45 min. One ml of de-ionized water was added to the mixture and placed in a centrifuge machine for 10 min at 8,300 g-force. The same steps were repeated by adding 1.5 ml de-ionized water to the same microcentrifuge tube. The supernatant was transferred to the scintillation vials and volumed up to 10 ml by using de-ionized water. Following the extraction of NSC from the sample, a phenol-sulfuric acid solvent was used which creates a colorimetric reaction for sugar and starch separately (Fournier, 2001; Tomlinson et al., 2013). A 100 µl quantity of freshly made 5% phenol in water and 1 ml of concentrated H_2SO_4 was added to 100 µl of the supernatant. The sample was then briefly vortexed for several seconds and incubated at 25ºC for 2 hours. The sample was then read on a UV-Vis spectrophotometer at 490 nm independently for sugar and starch. Total non-structural carbohydrates were calculated by adding percentage of sugar and starch. The results were expressed as glucose equivalents.

Total Antioxidant Capacity of Leaf Tissue

The total antioxidant capacity (TAC) in plants is a measure of the total ability of a plant to resist oxidative stress and to neutralize free radicals. The TAC measurement reflects the combined activity of all antioxidants (enzymatic and non-enzymatic) in a plant sample and provides information about the plant's overall antioxidant defense mechanism. The sample antioxidant capacity in maple cultivars was calculated from only a 7-d sample based on the Total Antioxidant Capacity Assay Kit (Abnova, Walnut, CA). A 100 mg sample of fresh tissue was cut into smaller pieces, kept in an Eppendorf tube with 1.6 mm beads, and stored at –80ºC before the assay procedure. The frozen tissue was thawed before extraction. The tissue was homogenized in 1 ml of ice-cold phosphate buffer (PBS) using a bullet blender for 12–15 minutes. The homogenate was incubated for 10 minutes on ice, and then centrifuged at 10,000 ×g-force for 12 minutes to obtain the supernatant. The supernatant was transferred to a new microcentrifuge tube and centrifuged again for 2 minutes at the same speed, and then vials were stored at –80ºC until supernatant volumes were used for the assay. To perform the TAC assay, the sample was diluted in a 1:15 ratio with cold PBS buffer by mixing 1 part of the sample with 15 parts of the diluent. Two µl of sample was added to each well in a plate. The volume of the sample was adjusted to 100 µl by adding 98 µl of distilled water to each well. Then, 100 µl of Cu^{2+} working solution was added to each well containing the sample. The plate was then incubated at room temperature for 90 minutes and absorbance was recorded at 570 nm using the UV-Vis spectrophotometer. The sample antioxidant capacity was calculated as:

Eq. 5. $TAC = (Ts/Sv) * D$

where,

Ts = TAC amount in the sample well calculated from the standard curve (nmol)

Sv = sample volume added in the sample wells (µl)

D = sample dilution factor

Leaf Enzymatic Antioxidant Activity

The activity of two plant enzymes: catalase (CAT) and superoxide dismutase (SOD), in response to herbicide treatment can provide information on the plant's ability to defend against oxidative stress and how these enzymes may impact the plant's overall health and growth. The antioxidant activity of these enzymes was evaluated at 7 DAT. The samples were prepared as described for total antioxidant extraction. The CAT and SOD activities were determined using colorimetric kits (Catalase Colorimetric Activity Kit and Superoxide Dismutase Activity Kit from Invitrogen, Thermo Fisher Scientific, Waltham, MA). For enzyme CAT, 25 µl of hydrogen peroxide reagent was added to each well containing 100 µl of sample. The sample was then incubated for 30 minutes at room temperature. After incubation, 25 µl of substrate and 25 µl of 1X HRP solution was added into each well in a quick succession using multichannel micropipette. The sample was again incubated for 15 minutes at room temperature before reading the absorbance at 560 nm with a UV-vis spectrophotometer. The concentration was calculated by using the equation from standard curve and expressed as U/ml CAT. For SOD, 50 µl of 1X substrate was added to 10 µl of each sample and the absorbance was recorded at 450 nm immediately. After obtaining the base line absorbance the chromogenic detection reagent (25 µl of 1X xanthine oxidase) was added to each sample, and samples were incubated for 20 minutes at room temperature before reading the sample again at 450 nm. The absorbance values obtained for each sample were then corrected by subtracting the baseline absorbance values from the corresponding absorbance values measured for each sample. The concentration was calculated from the equation obtained standard curve and unit was expressed as U/ml SOD.

The assay procedure involved measuring the activity of the enzymes by monitoring changes in color intensity, which was proportional to the enzyme concentration. This information was used to evaluate the role of enzymatic antioxidants in red maple cultivars under herbicide stress and to assess any changes in the activity of these enzymes in response to stress treatments.

Mineral Nutrient

The mineral nutrient analysis was performed on leaf and bark samples from four maple cultivars (AB, BW, OG, and SV) to evaluate the nutrient uptake and utilization in response to herbicide treatment. The analysis was performed from the leaf samples collected at 28 DAT. To determine the mineral nutrient concentration in the leaf samples, frozen (–80°C) leaf samples were oven dried at 60°C for 60 hours before sending them for analysis. Bark samples were collected from each tree 40 cm above the base of the trunk. The bark samples were carefully removed and placed in paper bags to prevent moisture loss. The samples were then sent to WARD Laboratories, Inc, (Kearney, NE) for analysis. The levels of various mineral nutrients, including macro-nutrients (e.g., nitrogen, phosphorus, potassium, carbon, calcium, magnesium, sulfur) and micro-nutrients (e.g., iron, zinc, manganese, boron, molybdenum) were quantified.

Twig Water Potential

Twig water potential was measured on 37 DAT by using Scholender Water Pressure Chamber (Model 600 Classic Pressure Chamber 40 Bar, PMS Instrument, Albany, OR). Healthy, newly re-flushed foliage samples were randomly selected from each cultivar to measure the water potential on a sunny day between 1300 to 1500 h. The plant twig was placed in the chamber and pressure was applied gradually until sap was released from the twigs. The pressure reading was recorded in unit of bars.

Field Study for Borer Infestation

The field study was conducted at Pleasant Cove Nursery in McMinnville, Tennessee, over a period of 10 months, from mid-April to January in the first year of the study. Three cultivars of red maple trees ('October Glory', 'Brandywine', and 'Sun Valley'), and one Freeman maple hybrid 'Autumn Blaze' were transplanted in the field in mid-April 2022. There were 50 replicates for each cultivar, with half of each cultivar being treated with herbicide (Scythe 2.5%) in mid-May. The trees were arranged in a completely randomized design. Before herbicide treatment, the trees were fertilized using a #2 Harrell’s spoon with a 12-g size using Plant Food 15-15-15 NPK (Tennessee Farmers’ Cooperative, Lavergne, TN) to ensure healthy growth. A post-emergent herbicide was sprayed to kill existing grass and weeds following field preparation. Staking, mowing, and pruning of the trees were performed as per the tree requirements. The trees were measured for growth parameters, including height and trunk diameter at the beginning and end of the season. The trees were checked for natural borer infestation in early November and late January. The infested trees were cut down and held under laboratory conditions to rear the adult beetles needed for species determination.

Data Analysis

To analyze the response of red maple cultivars to stress, two-way analysis of variance (ANOVA) was used in R software. Tukey's Honestly Significant Difference (HSD) test was used for mean multiple comparisons to determine a significant differences $(P \leq 0.05)$ among specific pairs of means. The experiment was a 3 × 4 factorial design with three herbicide treatments, four plant cultivars, and 10 replicates per treatment group for the container plants. In addition, four cultivars were also compared within the treatment groups separately in R software. The contrast comparison was performed between hybrid and red maples by sub-setting all red maples cultivars in R software to determine their baseline differences. To analyze borer infestations, a Chi-Square test was performed on percentage of attacks with the assumption that total hit rate will be divided equally among the four cultivars if no difference in beetle preference between the cultivars exist, which implies that each cultivar would have equal percentage of borer attack (i.e. 25% borer attack rate). All the graphs were generated in MS-Excel and R software using the *ggplot2* package.

Optimization of Traps for *Chrysobothris* spp. Through Evaluation of Shape, Height, and Effective Lure Components

To develop improved monitoring tools for *Chrysobothris*, experiments were performed during the summers of 2021 and 2022. In the first year, four tests were conducted at four different locations in middle Tennessee, including Circle J Nursery (35.822º N, – 85.81ºW), Hidden Valley Nursery (35.468º N, –85.851ºW), Rock Island State Park (35.810º N, –85.641ºW), and Moore Nursery (35.583º N, –85.713ºW). The objectives of these tests were to evaluate the effectiveness of modified purple panel traps in attracting buprestid borers, and to determine if any modifications could enhance trap capture rates compared to the standard purple panel trap. Before trap setup, the sites were sprayed with herbicides (Glyphosate) to maintain a clean area around the test traps. The traps were set up in mid-May and checked biweekly until the first week of September. There were four replications for each treatment arranged in a randomized completed block design. Beetles were removed from traps and placed on folded wax paper prior to cleaning off the glue. To preserve the collected beetles prior to processing, specimens were immediately stored in a freezer at –20ºC after collection. Beetles were then cleaned by shaking them in a Histo-Clear II (National Diagnostics, Atlanta, GA) solution for 2 days and stored in a microcentrifuge tube with molecular ethanol for further identification and preservation. The borer genus and species were identified using a microscope and taxonomic keys to discriminate characters used for differentiation based on their morphological features. Beetles in the genus *Chrysobothris* and others were identified to species if possible. Trap treatments were compared at buprestid genus and species levels.

Volatiles Attractant Tests 2021

In the first year of our study (2021), two experiments were conducted to determine the effectiveness of different volatile lures as attractants for buprestid beetles. In the first experiment, purple sticky panel traps (~20 × 28 cm) (AgBio, Inc., Westminster, CO) were tested. The traps were baited with volatile lure treatment either ultra-high release (UHR) ethanol (1 g/day) (Chemtica Internacional S.A., Heredia, Costa Rica) or benzaldehyde (10 mg/day) (Chemtica Internacional S.A., Heredia, Costa Rica). The third treatment was control traps which represent plain sticky panel traps without any volatiles lure. The second experiment utilized purple sticky panel traps with same dimension as first experiment made from corrugated plastic sheets and baited with either limonene (lab-fabricated lure, 15 ml Falcon tube, 5 ml limonene, 2 mm opening) or benzaldehyde. The first experiment was conducted at Circle J Nursery, McMinnville, Tennessee, while the second experiment was conducted at Rock Island Park, Rock Island, Tennessee. To capture the beetles, we attached a transparent film (Acco Brands, Inc., Lincolnshire, IL) to the traps. TAD hand applied insect trapping adhesive glue (TAD, Adair, OK) was applied to one side of the transparency film and attached over the surface of the purple panel traps using clip blinders with the glued side facing outwards. The transparency film was replaced during every trap check. The height of each trap was 132 cm, with a spacing of 5 m between treatments and 10 m between replicates. The lures were replaced every two months for ethanol, every two weeks for limonene, and monthly for benzaldehyde.

Traps with Reflecting Particles 2021

This experiment was conducted in Hidden Valley Nursery, Viola, TN. Purple sticky panel traps were coated with ultra-violet (UV) spray (Montana UV-Effect Luminescent Varnish Spray, Germany), painted with infra-red (IR) reflecting particles, or a combination of both. To create these traps, a rectangular section of purple corrugated plastic (Chloroplast, Vanceburg, KY) measuring 5.1 × 10.2 cm was cut from the middle of a full transparent film measuring 20.3 × 27.9 cm. The full film was then attached to the panel traps, with only the cut section being exposed. This exposed section was sprayed with the desired treatment of UV and/or IR coat, depending on the treatment group. For the combination treatment, a diagonal line was made to divide the 5.1 × 10.2-cm section into two parts, with each half part being sprayed with UV or half with IR particles (Figure 1). Traps were suspended on hook-ended, steel poles at a height of 132 cm above the ground. Treatments were spaced at 5 m intervals with at least 10 meters between replicates in a randomized complete block design.

Trap Type and Orientation 2021

For the final experiment of the first year, a trap study was conducted at Moore Nursery, Irving College, TN. The objective of the experiment was to determine the trapping efficacy of two different types of traps: sticky purple panel traps (~20 × 28 cm) and handmade triangle-folded coroplast purple pole traps measuring 121.92 cm (48 inches) height and 7.62 cm (3 inches) width each side (Figure 2), as well as compare the trap orientation: vertical vs horizontal.

There were three treatments in the experiment, including (1) purple panel traps facing the woodlots in a vertical direction, (2) purple panel traps facing skywards in a horizontal direction, and (3) purple pole traps. The pole traps were wrapped with transparent plastic that had been thinly coated with TAD hand applied insect trapping adhesive glue. Plastic adhesive was scraped to collect beetles during every check. Additionally, we placed a plastic sheet at the base of the poles to collect any fallen beetles and to protect the trap from touching the ground.

During the second year of the experiment, three tests were conducted in the summer of 2022, at two locations in middle Tennessee: forest areas near the Nursery Research Center, (McMinnville, TN) and Moore Nursery (Irving College, TN). Before trap deployed, the sites were sprayed with post-emergence herbicide to maintain a clean area of up to 2 meters around the test traps. In the first test, purple pole traps were used and baited with volatiles identified from herbicide-stressed maple trees (Perkovich et al., 2022). The second test involved the setup of two different types of traps in a trap array i.e. purple sticky pole vs purple Lindgren funnel, while in the third test, purple Lindgren funnel traps were used at different heights: ground level (~5 m) and in the tree canopy (~10 m) above ground level. The field traps were set up in late April and collected bi-weekly from May through August. The collected beetles from the traps were cleaned and identified, as previously mentioned in the experiment.

Volatile Attractant Test 2022

For this study, handmade triangle folded coroplast purple pole traps were used (referred to throughout as pole traps). The traps were made using purple corrugated plastic coroplast sheets. To make individual traps, the sheets measuring 121.92 cm (48 inches) height and 22.86 cm (9 inches) width were cut at surface level into three equal sections allowing us to fold the strips along their length. The sheets strips measuring ~ 7.62 cm (3 inches) were folded longitudinally, and zip tied along the cut sides to form a triangle pole that closely resembles the young tree trunk. The traps were baited with volatile plant chemicals identified from herbicide-stressed maple trees, including (Z)-3-hexenyl acetate, (Z)-3-hexanol, and a combination of both. A total of 21 traps were used, with eight traps coated with TAD hand applied insect trapping adhesive glue from each side and six baited with a volatile lure (100 µl) (Figure 3). Two glued but unbaited traps were used as Controls, 2 poles had (Z)-3-hexanol lures, and 2 poles had (Z)-3-hexenyl acetate lures, while the remaining 13 traps were unglued and unbaited, serving as visual cues to attract the beetles. The traps were checked biweekly, and the lure was changed every month from each trap. Traps were rotated clockwise during each check, and they were arranged in a 10 × 22 m plot with a 3-meter spacing between traps. Additionally, we placed a piece of the white corrugated plastic sheet at the base of the poles to collect any falling beetles and to protect the trap from touching the ground. Traps were supported with a metal trap rod.

Trap Type

To evaluate the effectiveness of different trap types for capturing *Chrysobothris* beetles, two different trap types (four purple Lindgren funnel traps and four purple pole traps) were arranged in a 2 × 6 m plot array. Paired traps were spaced at 2 m intervals and arranged in a randomized complete block design (RCBD) (Figure 4). Pole traps were compared against Lindgren funnel traps because funnel traps are cleaner to handle. All pole traps were glued, and for the purple Lindgren funnel traps, a collector cup was screwed onto the bottom of the funnel trap. The collector cup was half-filled with 50% purple non-ethanol environment friendly antifreeze (SPLASH Product Inc., Saint Paul, MN) to kill and assist in preserving the captured beetles. The solution was changed during each trap check to ensure the effectiveness of the trapping method. There were four replicates per treatment arranged in a completely randomized design.

Trap Location

To improve understanding of beetle flight activity at ground and canopy levels near the forest border purple Lindgren funnel traps were utilized. The traps were suspended using a rope in the tree canopy, and at ground level using trap rods. The canopies traps were positioned at a height of ~10 meters, while the lower traps were set at a height of 1.5 meters above ground level. Four replicates were performed per treatment.

Data Analysis

To determine the significant difference in total captured buprestids among different genera a non-parametric Kruskal-Wallis test was done in R software for both 2021 and 2022 data. The effect of treatment and genus independently on total buprestid capture was performed for all the experiment. Furthermore, to focus on the specific genus, we created a subset of that genus using subset function and Kruskal-Wallis test was performed on that subset. A post-hoc analysis was conducted using the Dunn's test using FSA package to identify pairwise differences between the treatment and genera on total count at P value ≤ 0.05.

Chapter 4: Findings

Physiological and Biochemical Responses of Red Maple Cultivars and Hybrid to Herbicide Stress

Growth Measurement of Container Grown Plants

The effect of herbicide treatment on four maple cultivars (AB, BW, OG, SV) was determined for height and diameter growth parameters at the end of the experiment. Trees exposed to the Roundup treatment were excluded from the growth data due to the death within two weeks of all plants exposed to this herbicide; only measurements made on trees exposed to the Scythe treatment were used to analyze the data. There was a significant negative effect of herbicides on height growth (F = 4.55, df = (1, 72), P = 0.0364) and diameter growth (F = 24.24, df = (1, 72), $P \leq 0.001$) (Table 1). The height and trunk diameter increase in herbicide (Scythe) treated trees was significantly lower compared to the increase in Control trees. However, no interaction was observed between cultivar and herbicide treatment on tree height increase (F = 1.13, df = (3, 72), P = 0.3419) and trunk diameter increase (F = 0.31, df = (3, 72), P = 0.8219) (Table 1). The hybrid cultivar AB showed significantly higher height increase compared to only one red maple cultivar (SV) when measurement was taken at 28 DAT (F = 3.58, df = (3, 72), P = 0.018). Red maple BW demonstrated a higher trunk diameter increase than only red maple cultivar OG (F = 2.93, df = (3, 72), P = 0.0392). Additionally, while comparing the cultivars separately within the treatment group in the Control trees, a hybrid (AB) cultivar had significantly greater height increase than only one red maple cultivar SV by 40% (F = 3.06, df = (3, 36), P = 0.041) whereas, no significant difference was observed between the cultivars in terms of trunk diameter increment (F = 1.40, df = (3, 36), P = 0.258) (Table 4). After treatment with Scythe, there was no significant difference between the cultivars in terms of height increase (F = 1.38, df = (3, 36), *P* = 0.264) and trunk diameter increase (F = 2.05, df = (3, 36), *P* = 0.125) (Table 4).

Contrast comparisons between all red maples (BW, OG, SV) versus the hybrid (AB) within the Control treatment showed that the hybrid grew faster compared to red maples in terms of height (F = 8.98, df = (1, 38), $P \leq 0.001$) (Figure 16A), whereas no difference was observed for trunk diameter increment (F=0.40, df = (1, 38), *P* = 0.531). The contrast comparison of cultivars within the herbicide groups showed no significant difference between the hybrid and red maple cultivars in terms of both height increment (F = 0.74, df = (1, 38), *P* = 0.396) and trunk diameter increment (F = 0.22, df = (1, 38), *P* = 0.639).

Leaf Chlorophyll Content Using SPAD

The effect of herbicide (Scythe and Roundup) treatment on non-destructive chlorophyll content of three red maple cultivars, SV, BW, OG (red maples), and AB (a hybrid) was evaluated using the SPAD value as a measure of chlorophyll content on 7 and day 28 DAT. The results showed no interaction between the herbicide treatment and the cultivar, indicating that the effect of the herbicide treatment on the SPAD values was not different among the cultivars on both day 7 (F = 2.05, df = (6, 108), *P* = 0.065) or 28 DAT (F = 0.73, df = (3, 72), *P* = 0.540) (Table 1). However, the non-destructive chlorophyll content was significantly affected by the herbicide treatment, with a significant decline in SPAD value observed in Scythe and Roundup herbicide treated trees compared to Control on 7 DAT (F = 33.38, df = (2, 108), $P \leq 0.001$) (Table 1 and Figure 6A). On 28 DAT, Roundup treatment was not included due to the death of all plants exposed to the herbicide treatment. The Scythe-treated trees showed a significant decrease in

SPAD values compared to the Control ($F = 26.45$, df = (1, 72), $P \leq 0.001$) (Table 1 and Figure 6B). Among the cultivars, red maple cultivar SV showed significantly higher SPAD value compared to other cultivars (BW, OG, AB) on 7 DAT ($F = 12.70$, df = (3, 72), $P \leq 0.001$). On 28 DAT, SV showed significantly higher SPAD value compared to hybrid AB and BW ($F = 7.83$, df = (3, 72), $P \leq 0.01$) (Table 1).

Additionally, when the cultivars were analyzed separately within the treatment groups, the result also demonstrated a significant effect of the cultivars on SPAD value, with the cultivar SV having a significantly higher SPAD value compared to other cultivars (AB, BW, OG) in both Control ($F = 8.82$, df = (3, 36), $P \leq 0.001$) and herbicide-treated (Roundup and Scythe) conditions on 7 DAT ($F = 6.04$, df = (3, 76), $P \leq 0.001$) (Table 2). On 28 DAT, SV still had a significantly higher chlorophyll content compared to other cultivars. In Control conditions, SV had a SPAD value significantly higher than AB and BW ($F = 4.49$, df = (3, 36), $P \leq 0.01$) (Table 5). While in the Scythe treated condition, SV had a significantly higher SPAD value than AB but differences were not detected in SV values compared to OG and BW ($F = 4.15$, df = (3, 36), $P = 0.0127$). These results suggest that the herbicide treatment affects the SPAD value for each cultivar, with cultivar SV being less affected by the herbicide treatment compared to the other cultivars.

Furthermore, the contrast comparison between the hybrid (AB) and all red maples (SV, OG, and BW) revealed that Control red maple cultivars and Control hybrid (AB) did not have different chlorophyll content on 7 DAT ($F = 1.77$, df = (1, 38), $P = 0.191$) (Figure 15) or 28 DAT ($F = 3.61$, df = (1, 38), $P = 0.065$). However, when the trees were treated with herbicide, SPAD value declined in herbicide-treated hybrid AB by 6.8% on 7 DAT ($F = 7.27$, df = (1, 78), $P =$

0.01) and 11.73% on 28 DAT (F = 8.10, df = (1, 38), $P \leq 0.01$) compared to herbicide treated red maples (Figure 15A). Additionally, the results suggest that herbicide treatment had a negative impact on SPAD value, especially for the hybrid cultivar.

Photosynthetic Pigments of Leaf Tissue

The effects of herbicide treatment and cultivar were evaluated on photosynthetic pigments chlorophyll a (chla), chlorophyll b (chlb), and total carotenoids on day 7 and day 28 DAT. The herbicide treatment significantly reduced photosynthetic pigments as evidenced by reduction in chla, chlb, and total carotenoids. On 7 DAT, chla was significantly affected by the treatment (F = 21.00, df = (2, 108), $P \leq 0.001$) and cultivar (F = 8.60, df = (3,108), $P \leq 0.001$) with significant differences observed between the treated and untreated groups (Table 1). Compared to the Control group, trees exposed to herbicide treatments (Scythe and Roundup) showed a significantly lower chla (Figure 7A). Among the cultivars SV showed significantly higher chla than only cultivar BW. Additionally, when all cultivars were compared separately within the treatment groups, a cultivar SV exhibited higher chla compared to AB and BW in Control conditions, but no differences were detected for chla values for OG (F = 6.03, df = (3, 36), $P \leq 0.01$) (Table 2). In herbicide-treated conditions, the SV cultivar still had higher chla compared to BW (F = 4.42, df = (3, 76), $P \leq 0.01$). No significant interaction between treatment and cultivar was observed on 7 DAT (F = 2.03, df = (6, 108), $P = 0.067$) (Table 1). On 28 DAT, chla was significantly affected by treatment (F = 5.77, df = (3, 72), $P = 0.018$), cultivar (F = 3.02, df = (3, 72), $P = 0.035$) and interaction between treatment and cultivar (F = 3.52, df = (3, 72), $P = 0.019$) (Table 1). The herbicide (Scythe) treated cultivar SV showed significantly lower chla than Control trees (Figure 7B). The cultivar effects showed cultivar SV had significantly higher chla than a hybrid AB. Furthermore, when comparing chla within-untreated and within-herbicide-treated trees, the SV cultivar had higher chla than the rest of the cultivars except for OG in Control conditions (F = 5.61, df = (3, 36), $P \leq 0.01$), but in treated conditions, no significant differences was observed between the cultivars (F = 0.61, df = (3, 36), $P = 0.619$) (Table 5).

On 7 DAT, chlb was significantly affected by the treatment (F = 10.71, df = (2, 108), $P \leq 0.001$) and cultivar (F = 6.44, df = (3, 108), $P \leq 0.001$), with the lower chlb in herbicide (Scythe and Roundup), treated trees compared to Control (Table 1 and Figure 8A). The effects of cultivar on chlb demonstrated that SV had higher chlb than cultivar BW and a hybrid AB. Similarly, comparison of cultivars within the treatment groups demonstrated that SV had higher chlb than the hybrid (AB) in Control trees (F = 3.27, df = (3, 36), $P = 0.0322$) and SV exposed to herbicides still had higher chlb content than hybrid (AB) and BW (F = 3.96, df = (3, 76), $P = 0.011$) (Table 2). On 28 DAT, only the treatment effect was observed in chlb content, with lower chlb content observed in Scythe treated trees than Control (F = 5.79, df = (1, 72), $P = 0.0187$) (Table1 and Figure 8B). Within Control trees cultivar SV showed significantly higher chlb content than a hybrid (AB) and red maple (BW) (F = 4.00, df = (3, 36), $P = 0.014$), while in herbicide treated conditions there was no significant differences between the cultivars (F = 0.13, df = (3, 36), $P = 0.943$) (Table 5). No significant interaction between treatment and cultivar was observed on both day 7 (F = 0.97, df = (6, 108), $P = 0.452$) and on day 28 (F = 2.69, df = (3, 72), $P \geq 0.05$) (Table 1).

Similarly, on 7 DAT, total carotenoids were significantly affected by both herbicide treatment (F = 9.17, df = (2, 108), $P \leq 0.001$) and cultivar (F = 11.20, df = (3, 108), $P \leq 0.001$), with significantly lower carotenoids in Roundup treated trees compared to Control (Table 1). Among the cultivars, SV showed significantly higher carotenoids than a hybrid AB and red maple cultivar BW. Moreover, when comparing cultivars within treatment groups, Control SV trees were found to have significantly higher carotenoid levels than OG, AB, and BW trees ($F = 11.41$, $df = (3, 36)$, $P \leq 0.001$). In herbicide treated trees, SV still showed significantly higher carotenoids level than cultivar BW ($F = 4.28$, $df = (3, 76)$, $P \leq 0.01$) (Table 2). However, a significant interaction occurred between treatment and cultivar ($F = 3.50$, $df = (6, 108)$, $P \leq 0.01$) in which lower carotenoid values were observed in the Roundup treated red maple (SV) compared to Control (Figure 9A). On 28 DAT, total carotenoids were affected by treatment ($F = 11.62$, $df = (1, 72)$, $P \leq 0.01$), cultivar ($F = 4.38$, $df = (3,72)$ $P \leq 0.01$) and interaction between treatment and cultivar ($F = 4.01$, $df = (3, 72)$, $P = 0.010$), with lower carotenoids values observed in Scythe treated red maple (SV) compared to the Control (Table 1 and Figure 9B). The cultivar effects on carotenoids indicated that cultivar SV had significantly higher carotenoids compared to hybrid and another red maple BW. Additionally, while comparing cultivars within the treatment groups SV showed significantly higher carotenoids compared to hybrid and another red maple BW ($F = 6.90$, $df = (3, 36)$ $P \leq 0.001$), while in herbicide treated trees there were no significant difference between the cultivars ($F = 1.89$, $df = (3, 76)$, $P = 0.148$) (Table 5). These results suggest that the herbicide treatment has a significant effect on carotenoid content and this effect is specific to the cultivar.

The contrast comparison of Control red maple cultivars (BW, OG, SV) versus Control hybrid (AB) did not indicate any significant differences except for chlb on 7 DAT ($F = 6.98$, $df = (3, 38)$, $P = 0.011$). The Control red maples recorded significantly higher chlb content than the

Control hybrid. However, no difference was observed among the trees treated with herbicides (Roundup or Scythe) between hybrid and red maple cultivars (F = 1.99, df = (3, 78), $P = 0.162$) (Figure 15B). The present study result is in line with Percival (2017), who reported degradation of chla, chlb, and carotenoids under herbicide (glyphosate) treatment with the significant difference among the maple cultivars. These findings also suggest that the observed decrease in chla in the ‘SV’ cultivar could be linked to the reduction in total carotenoid content, which is known to protect chlorophyll from photo-oxidative damage (Singh, 1996). The specific susceptibility of the cultivar ‘SV’ to herbicide-induced changes in pigment content may be attributed to inherent differences in its genetic makeup, and physiological and biochemical responses to herbicide stress.

Electrolyte Leakage of Leaf Tissue

The present study did not detect significant effects of herbicide treatment, cultivar effect or interaction for electrolyte leakage on 7 or 28 DAT (Table 1). The contrast comparison of hybrid versus all red maples did not show significant differences among the cultivars on electrolyte leakage in untreated and treated conditions at 7 or 28 DAT.

Phenolics, Flavonoids, and Tannin of Leaf Tissue

Herbicide treatment significantly impacted the concentration of polyphenols such as total phenolics, tannins, and flavonoids in maple cultivars. The concentration of phenolic compound was significantly affected by treatment (F = 17.09, df = (2, 108), $P \leq 0.001$), cultivar (F = 22.39, df = (3,108), $P \leq 0.001$), and their interaction (F = 3.72, df = (6, 108), $P \leq 0.01$) on 7 DAT (Table 1). Roundup treated trees showed significantly higher phenolics than Control, while there was no effect detected for Scythe treatment on phenolic compounds. The cultivar effects on phenolic compound demonstrated that cultivar SV had higher phenolic compounds than the hybrid (AB), and two red maple cultivars (OG and BW) on 7 DAT. Similarly, comparing the phenolic compound among the cultivars within the treatment groups showed cultivar SV having higher phenolic compounds than the hybrid (AB) within untreated trees (F = 18.96, df = (3, 36), $P \leq 0.001$), whereas after exposure to herbicide, cultivar SV showed significantly higher phenolic compound than hybrid (AB) and BW (F = 7.91, df = (3, 76), $P \leq 0.001$) (Table 3). A significant interaction between herbicide treatment and cultivar exhibited an increase of phenolic concentration in Roundup treated hybrid (AB) compared to Control on 7 DAT (F = 3.72, df = 6, 108), $P \leq 0.01$) (Table 1 and Figure 10A). In contrast comparison, red maple showed significantly higher phenolic concentration than hybrid within Control (F = 49.59, df = (1, 38), $P \leq 0.001$) and herbicide (Scythe and Roundup) trees (F = 8.33, df = (1, 78), $P \leq 0.01$) on day 7 (Figure 15C).

On 28 DAT, the phenolic concentration was affected by treatment (F = 12.29, df = (3, 72) $P \leq 0.001$) and cultivar (F = 12.29, df = (3, 72), $P \leq 0.001$) (Table 1). Scythe treated trees showed higher phenolics compared to Control trees (Figure 10). Among the red maples, cultivar SV showed significantly higher phenolics compared to hybrid and other red maples (OG and BW). Similarly, within untreated trees, phenolic concentration was higher in SV compared to BW (F = 8.29, df = (3, 36), $P \leq 0.001$); whereas, within herbicide treated trees (F = 5.53, df = (3, 36), $P \leq 0.001$), SV showed significantly higher phenolic concentration than BW and OG (Table 6). In contrast comparison of red maples versus the hybrid, there was no significant difference between the hybrid and red maple cultivars within Control (F = 0.00, df = (1, 38), $P = 0.988$) and within the Scythe treated groups ($F = 0.32$, df = 1, 38), $P = 0.576$) for phenolic content on 28 DAT.

There were significant effects of herbicide treatment on tannin concentration in maple cultivars on 7 DAT ($F = 27.22$, df = (3, 60), $P \leq 0.001$) and on 28 DAT ($F = 18.26$, df = (1, 40), $P \leq 0.001$) (Table 1). On 7 DAT, Roundup treated trees showed significantly higher tannin than Control. No difference was observed in the trees exposed to Scythe treatment (Figure 11A). Among the cultivars, the red maple cultivar SV showed significantly higher tannin concentration than the hybrid (AB), and two red maple cultivars (BW and OG) ($F = 7.16$, df = (3, 60) $P \leq 0.001$). In addition, comparing the tannin concentration among the cultivars within the treatment groups, no significant difference was observed within the Control trees ($F = 0.57$, df = (3, 20), $P = 0.642$). However, within herbicide treated trees, a significant difference was observed, with SV having higher tannin concentration than AB, BW and OG on 7 DAT ($F = 6.86$, df = (3, 44), $P = \leq 0.001$) (Table 3).

On 28 DAT, Scythe treated trees showed significantly higher tannin concentration compared to Control trees (Figure 11B). The cultivar effects on tannin concentration revealed that a cultivar SV had higher tannin concentration than the hybrid (AB) and other red maples (BW, and OG) ($F = 12.67$, df = (3, 20), $P \leq 0.001$). A similar 7 DAT pattern was observed at 28 DAT for cultivar effects on tannin concentration within the treatment groups. Under Control conditions, no significant difference in tannin was observed between cultivars ($F = 2.95$, df = (3, 20), $P = 0.057$). However, within herbicide Scythe-treated trees, SV had a significantly higher tannin concentration than hybrid (AB) and other red maples (BW, OG) ($F = 10.58$, df = (3,20), $P \leq 0.001$) (Table 6). In contrast comparison between cultivars (red maples vs. hybrid), there were no significant differences between a hybrid and red maples for tannin concentration within the Control and herbicide groups on 7 DAT ($F_{control}$ = 0.00, df = (1, 22), P = 0.96; $F_{herbicide}$ = 0.50 df = (1, 46), P = 0.485) or 28 DAT ($F_{control}$ = 0.94, df = (1, 22), P = 0.34, $F_{herbicide}$= 0.03, df = (1, 22), P = 0.874).

Herbicide treatments (Scythe and Roundup) significantly affected flavonoid concentrations. There was a significant decline in flavonoid concentration on herbicide treated trees compared to Control (F = 44.87, df = (2, 107), $P \leq 0.001$) (Table 1). No significant difference was observed among the cultivars for flavonoid concentrations on 7 DAT (F = 2.80, df = (3, 107), $P \geq 0.05$). The significant interaction between cultivar and treatment showed lower flavonoid concentration in Roundup treated hybrid and OG cultivar compared to Control on 7 DAT (F = 3.84, df = (6, 107), $P \leq 0.01$) (Table 1 and Figure 12A). Similarly, a comparison of cultivars within the treatment groups did not detect a difference in flavonoid concentration between cultivars within Control trees (F = 0.92, df = (3, 36), P = 0.441); whereas, within herbicide treated trees, hybrid (AB) showed significantly lower flavonoid concentration than BW and SV (F = 8.45, df = (3, 75), $P \leq 0.001$) on 7 DAT (Table 3).

On 28 DAT, Scythe-treated trees had a decrease in flavonoid concentration compared to Control trees (F = 20.98, df = (1, 72), $P \leq 0.001$) (Table 1 and Figure 12B). Among the cultivars, hybrid (AB) showed significantly lower flavonoid content than SV and BW (F = 7.05, df = (3, 72), $P \leq 0.001$). Furthermore, when comparing the flavonoid concentrations of different cultivars within the treatment groups, it was observed that SV and BW had higher flavonoid levels than OG and the hybrid cultivar within the Control trees (F = 8.85, df = (3, 36), $P \leq 0.001$). However, no differences were detected among the herbicide-treated trees (F = 1.71, df = (3, 36), P = 0.183)

(Table 6). Additionally, red maple cultivars demonstrated higher flavonoid content than hybrid cultivar within Control trees only on 28 DAT (F = 11.51, df = (1, 38), $P \leq 0.01$) and within herbicide treated trees on 7 DAT (F = 9.19, df = (1, 77), $P \leq 0.01$) and 28 DAT (F = 4.84, df = (1, 38), $P = 0.0339$) in the contrast comparison of cultivars (red maples versus hybrid) (Figure 16D).

Overall, herbicide-treated trees had higher concentrations of phenolics and tannins; whereas, flavonoid concentrations were higher in Control trees. The study results indicate that exposure to herbicide can have differential effects on different classes of phenolic compounds in different plant cultivars. The observed increase in phenolic concentrations in only Roundup treated hybrid cultivar 'AB' indicates these compounds may be upregulated in response to herbicide damage, potentially as a mechanism of defense against stress (Smirnoff, 2008). The upregulation of phenolics is consistent with previous studies that found increased phenolic compound in response to various environmental stressors (Koch et al., 2008; Muller et al., 1987). A study conducted by Perkovich et al. (2022) reported an increase in tannin concentrations in defoliated and herbicide treated red maples cultivar (BW) while studying different stress responses in red maples. However, the observed decrease in flavonoid concentration in a hybrid cultivar (AB) and red maple cultivar (OG) following Roundup treatment indicates that these polyphenols are more sensitive to herbicide exposure. Ali & Honermeier (2016) also reported that the application of haloxyfop resulted in higher levels of caffeoylquinic acids, while the highest levels of flavonoids were observed in Untreated trees at the first harvest of artichoke leaves. The reduction in flavonoid synthesis observed in some herbicide-exposed plants may be attributed to the inhibition of the enzyme 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase

(Lydon & Duke, 1989). Studies have shown that herbicides such as alachlor and glyphosate can inhibit the enzyme responsible for the synthesis of all cinnamate derivatives, the intermediates in flavonoid biosynthesis pathways. As a result, the reduction of cinnamate derivatives can lead to a decreased production of flavonoids in higher plants (Smirnoff, 2008).

Total Non-Structural Carbohydrates of Leaf Tissue

For total non-structural carbohydrates (TNC), no differences were detected for Roundup and Scythe treated trees compared to Control on 7 DAT (F = 3.99, df = (2, 108), $P \geq 0.05$). Among the cultivars, the hybrid had significantly higher TNC compared to OG (F = 2.85, df = (2, 108), $P = 0.041$) (Table 1). The comparison of cultivars within the treatment groups showed BW Control trees had a higher concentration than OG (F = 4.44, df = (3, 36), $P \leq 0.01$). On the other hand, within the herbicide treated trees, AB had a higher TNC concentration than BW and OG (F = 4.06, df = (3,76), $P = 0.0101$) at 7 DAT (Table 3).

AT 28 DAT, TNC concentration in Scythe treated trees was significantly lower than Control trees (F = 6.60, df = (1, 32), $P = 0.015$) (Table 1). Among the cultivars, SV showed significantly lower TNC concentration compared to the hybrid and BW (F = 5.58, df = (3, 32), $P \leq 0.01$). Further, the cultivar effects within the treatment groups demonstrated that within Control trees cultivar BW had significantly higher TNC concentration than OG and SV (F = 8.85, df = (3, 16), $P \leq 0.01$), while no difference was found between the cultivars within herbicide (Scythe) treated trees (F = 2.12, df = (3, 16), $P = 0.138$) (Table 6). The significant interaction between treatment and cultivar showed a significant reduction of TNC in Scythe and Roundup treated BW at 7 DAT (F = 4.26, df = (6,108), $P \leq 0.001$) (Figure 13A) and Scythe treated BW at 28 DAT (F = 6.26, df = (3, 32), $P \leq 0.01$) (Figure 13B) compared to Control trees.

Further, while performing contrast comparison within the treatment, there was no significant difference in the TNC concentration between hybrid (AB) and red maples (BW, OG, and SV) within Control trees at 7 DAT ($F = 0.26$, $df = (1, 38)$, $P = 0.613$) or 28 DAT ($F = 1.07$, $df = 1$, $P = 0.315$). Whereas within herbicide-treated trees, AB had more TNC than all red maple cultivars only at 7 DAT ($F = 6.30$, $df = (1, 78)$, $P = 0.0141$) (Figure 15E).

Non-structural carbohydrates (NSC) are dynamic, and their levels can fluctuate based on the balance between the carbon supply from photosynthesis and its utilization for plant metabolism. Therefore, NSC levels can change depending on various environmental factors and stressors that affect plant growth and metabolism (Richardson et al., 2013). Herbicide treatment led to a significant reduction in non-structural carbohydrates (NSC) in cultivar 'BW' compared to the untreated plant on both 7 and 28 DAT. A reduction in NSC levels after herbicide treatment may also indicate that the trees is using stored energy to deal with the stress caused by the herbicide stress (Czimczik et al., 2006).

Antioxidant Activity of Leaf Tissue

Herbicide treatment did not affect total antioxidant capacity (TAC) in red maple cultivars ($F = 1.60$, $df = (2, 36)$, $P = 0.215$) (Table 1). The analysis of cultivar effects on total antioxidant capacity showed that concentration SV had a significantly higher TAC than hybrid (AB) and OG ($F = 6.29$, $df = (3, 36)$, $P \leq 0.01$). Furthermore, different cultivars showed varying levels of total antioxidant capacity within the Control trees, with SV having more antioxidant activity compared to AB ($F = 3.86$, $df = (3, 12)$, $P = 0.038$). However, exposure to herbicide resulted in a higher antioxidant capacity in SV compared to OG ($F = 4.40$, $df = (3, 28)$, $P = 0.013$) (Table 3). No significant difference was detected between red maples and hybrid (AB) in contrast comparison of both within Control (F = 4.58, df = (1, 14), P = 0.050) and within-herbicide treated trees (F = 0.19, df = (1, 30), P = 0.668).

For CAT, no significant effects were detected for treatment (F = 2.87, df = (2, 84), P = 0.062), or the interaction between cultivar and treatment at 7 DAT (F = 1.23, df = (6, 84), P = 0.299). However, cultivar effects on CAT activity showed that a hybrid (AB) cultivar showed significantly higher CAT activity than red maple cultivar OG (F = 3.41, df = (3, 84), P = 0.0213) (Table 1). Additionally, comparison of cultivars within the treatment groups revealed that a hybrid and cultivar SV showed significantly higher CAT activity than other red maples (BW and OG) within untreated trees (F = 14.48, df = (3, 28), $P \leq 0.001$), whereas, in the herbicide group, all cultivars had similar levels of CAT activity, which were lower than the Control group (F = 2.10, df = (3, 60), P = 0.110) (Table 3). For SOD, there was no significant effect detected for herbicide treatment (F = 1.54, df = (2, 84), P = 0.221) or the interaction of treatment and cultivar (F = 2.34, df = (3, 84), $P \geq 0.05$) at 7 DAT (Table 1). However, the activity of SOD was significantly affected by cultivar (F = 11.02, df = (3, 84), $P \leq 0.001$). The red maple cultivar (OG) showed significantly higher SOD activity than hybrid (AB) and another red maple cultivar (BW). Similarly, there was a significant effect of cultivar on SOD activity within the treatment groups. The cultivar OG had higher SOD activity than cultivar SV and BW within the Control group (F = 3.92, df = (3, 28), P = 0.0186), while the red maple cultivars SV and OG had higher SOD activity than the hybrid (AB) within the herbicide (Roundup and Scythe) group (F = 10.06, df = (3, 60), $P \leq 0.001$) (Table 3).

The present study also investigated the contrast comparison of catalase (CAT) and superoxide dismutase (SOD) activity levels between hybrid and all red maple trees in both

Control and herbicide (Roundup and Scythe) treatment groups. The hybrid (AB) had higher CAT activity compared to red maple trees within the Control treatment (F = 5.80, df = (1, 30), P = 0.022). However, in the herbicide treatment, no difference was detected in CAT activity between the hybrid (AB) and red maples (F = 2.18, df = (1, 62), P = 0.145) (Figure 15F). For SOD activity, red maple trees had higher levels than the hybrid in the herbicide treatment group (F = 21.83, df = (1, 62), $P \leq 0.001$), while there was no difference detected between the tree type in the Control group (F = 0.13, df = (1, 30), P = 0.72) (Figure 15G). Consequently, it appears different tree types have different baseline levels of antioxidant defensive activities, and defensive compound levels may be influenced by stress factors such as herbicide exposure.

Mineral Nutrient from Leaf Tissue

For mineral nutrients, Scythe treatment did not have a detectable effect on most leaf mineral nutrients, except for N (F = 5.99, df = (1, 32), P = 0.0201) and Fe (F = 4.31, df = (1, 32), P = 0.0461), which were significantly lower in the Scythe-treated trees compared to the Control trees (Table 1). Among the different cultivars, significant differences were observed for the nutrients zinc (Zn; F = 5.69, df = (3, 32), $P \leq 0.01$) and potassium (K; F = 4.05, df = (3, 32), P = 0.0151). Cultivar BW had significantly higher K than OG, but not the other cultivars. Cultivar OG had higher Zn than cultivars AB and BW. Further, when cultivars were analyzed separately within the treatment groups, significant differences were observed for the nutrients zinc (Zn; F = 4.85, df = (3, 16), P = 0.0139) and molybdenum (Mo; F = 4.09, df = (3, 16), P = 0.0248), where cultivar OG had significantly higher Mo than only cultivar BW, but not the other cultivars. Cultivar OG also had higher Zn than cultivars AB, SV, and BW within the Control groups. Moreover, within the Scythe treated groups, significant differences were observed only for potassium (K; $F = 6.14$, df = (3, 16), $P \leq 0.01$), where cultivar BW showed significantly higher K than cultivar OG.

In contrast comparison between all red maple vs a hybrid, no significant difference was observed in any nutrient except for copper (Cu; $F = 5.09$, df = (1, 18), $P = 0.0368$). The hybrid AB had a higher concentration of Cu than red maple cultivars in the Control group.

Mineral Nutrient from Bark Tissue

For mineral nutrient from bark tissue, no significant differences were detected between herbicide (Scythe) and Control for elements like nitrogen (N; $F = 0.46$, df = (1, 32), $P = 0.502$), phosphorous (P; $F = 2.59$, df = (1, 32), $P = 0.117$), potassium (K; $F = 0.34$, df = (1, 32), $P = 0.563$), sulfur (S; $F = 3.98$, df = 1, $P = 0.054$), magnesium (Mg; $F = 1.05$, df = (1, 32), $P = 0.312$), iron (Fe; $F = 0.02$, df = (1, 32), $P = 0.888$), cupper (Cu; $F = 0.96$, df = (1, 32), $P = 0.30$), molybdenum (Mo; $F = 2.50$, df = (1, 32), $P = 0.124$), compared to Control (Table 1). In contrast, Scythe treatment increased the concentration of calcium (Ca; $F = 7.45$, df = (1, 32), $P = 0.010$), zinc (Zn; $F = 10.02$, df = (1, 32), $P \leq 0.01$), manganese (Mn; $F = 12.58$, df = (1, 32), $P = 0.01$), boron (B; $F =36.13$, df = (1, 32), $P \leq 0.001$), and carbon (C; $F = 12.35$, df = (1, 32), $P \leq 0.01$) in some maple cultivars (Figure 14). The interaction between cultivar and treatment revealed that Scythe treated ‘OG’ cultivar had higher phosphorous (P) than the Control ($F = 4.44$; df = (3, 32); $P = 0.010$) (Table 1 and Figure 14A). For zinc concentration, a marginal significant difference was observed, with herbicide OG having higher zinc concentration than Control trees ($F = 2.76$; df = (3, 32; $P = 0.058$). However, no significant interaction was observed between the cultivar and treatments for other mineral nutrients.

Contrast comparison between the cultivars (hybrid vs. all red maples) within the treatment revealed that the 'AB' hybrid had significantly higher N (F = 10.21, df = (1, 18), $P \leq 0.01$), P (F = 10.61, df = (1, 18), $P \leq 0.01$), K (F = 17.42, df = 1, $P \leq 0.01$), B (F = 11.23, df = (1, 18), , $P \leq 0.01$), and S (F = 34.07, df = (1, 18), $P \leq 0.01$), and significantly lower Mn (F = 8.29, df = 1, $P \leq 0.01$), C (F = 7.24, df = (1, 18), $P = 0.015$), and Zn (F = 6.50, df = (1, 18), $P = 0.020$) than red maple cultivars within Control conditions. Within herbicide (Scythe) treated trees, the hybrid had significantly higher N (F = 4.60, df = (1, 18), $P = 0.046$), S (F = 5.65, df = (1, 18), $P = 0.028$), and Fe (F = 6.04, df = (1, 18), $P = 0.0243$), than red maples (BW, OG, and SV) (Figure 17).

Twig Water Potential

For twig water potential, no significant effects were detected for treament (F = 0.85, df = (1, 32), $P = 0.362$), cultivar type (F = 1.31, df = (3, 32), $P = 0.287$), or the interaction between cultivar and herbicide (F = 0.48, df = (3, 32), $P = 0.698$) (Table 1). The contrast comparison between all red maples versus hybrid within the treatment groups indicated no significant difference between red maples and hybrid within both Control (F = 1.99, df = (1, 18), $P = 0.175$) and within herbicide (Scythe) treated trees (F = 0.08, df = (1, 18), $P = 0.78$).

Field Study Result

Growth Trait. There was a significant effect of herbicide treatment on growth traits of maple cultivar for height increase (F = 53.21, df = (1, 181), $P \leq 0.001$), and trunk diameter increase (F = 83.56, df = (1, 181), $P \leq 0.001$). The herbicide treated trees had lower height and trunk diameter growth than Control trees (Figure 18). Among the cultivars, hybrid (AB) had higher height increase than red maple BW and SV (F = 11.85, df = (3, 181), $P \leq 0.001$); whereas, for trunk diameter increase, the hybrid had higher diameter increase compared to red maples (SV and OG) (F = 22.42, df = (3, 181), $P \leq 0.001$). The interaction between cultivars and treatment effects on height increase indicated Control hybrid (AB) and other red maple BW and OG had higher height increase compared to herbicide-treated trees (F = 2.90, df = (3, 181), $P = 0.0366$) (Figure 18). Similarly, comparison of cultivars within the treatment groups demonstrated that hybrid had higher height increase compared to red maples BW and SV within Control conditions (F = 9.15, df = (3, 92), $P \leq 0.001$); whereas within herbicide (Scythe) treated trees, hybrid had higher height increase than cultivar SV (F = 3.21, df = (3, 89), $P = 0.0268$). There was a significant effect of cultivar on trunk diameter increase in both Control (F = 9.49, df = (3, 92), $P \leq 0.001$) and herbicide (Scythe) treated trees (F = 15.71, df = (3, 89), $P \leq 0.01$). In Control conditions, AB had greater trunk diameter increase than SV and OG. In herbicide-treated trees, AB had more trunk diameter increase than only OG. In the contrast comparison between the hybrid versus all red maples, the hybrid had greater height increase than red maple cultivars in both Control (F = 18.71, df = (1, 94), $P \leq 0.001$) and herbicide (Scythe) treated trees (F = 7.61, df = (1, 91), $P \leq 0.01$). Additionally, AB had greater trunk diameter increase than red maples within Control (F = 10.40, df = (1,94), $P \leq 0.01$) and within Scythe-treated tree groups (F = 11.37, df = (1, 91), $P \leq 0.01$).

Flatheaded Borer Incidence. In the first-year field study, variations in borer infestations among different cultivars were observed ($\chi^2 = 16.32$, df = 3, $P \leq 0.001$) (Figure 19). The observed frequency of attack for the AB cultivar was 0 which was lower than the expected frequency of 9.5. The hybrid cultivar had the highest negative deviation (-9.5) and relatively higher contribution to the Chi square test compared to other cultivars, suggesting a lower borer attack in hybrid cultivar than expected attack. The observed borer attack frequency for the other red maples such as SV and OG were 14 and 16, which was higher than the expected frequency of 9.5. The positive deviation of 4.5 and 6.5 indicates other red maple have significantly higher attacks than expected and substantial contribution to the overall chi-square statistic. The observed frequency for the red maple cultivar BW was 8 which was close to the expected frequency of 9.5. The small negative deviation (-1.5) for BW indicated less attack than expected and minimum contribution to the Chi square statistic. The Chi-square analysis indicated red maples cultivars OG and SV may have higher susceptibility to borer attack, while the hybrid AB cultivar may have lower susceptibility.

Optimization of Traps for *Chrysobothris* spp. through Evaluation of Shape, Height, and Effective Lure Components

Buprestid Beetle Trapping Results in 2021

Across all trials, a total of 500 buprestids representing seven beetle genera were captured throughout the field season in summer 2021. The most abundant genus was *Agrilu*s followed by *Acmaeodera, Chrysobothris, Phaenops, Ptosima, Anthaxia,* and *Buprestis* ($\chi^2 = 55.69$, df = 6, $P \leq 0.001$) (Figure 20). For the genus *Chrysobothris,* out of total (n=29) specimens captured, 22 were female, while the remaining individual were male. Among the jewel beetles, two genera *Acmaeodera* and *Anthaxia* were classified as flower visitors and the remaining genera were classified as non-flowers visitors. Among the non-flower visitors, more than half of the trapped species belonged to the genus *Agrilus*. Among the identified *Agrilus* species, *Agrilus lecontei* (Saunders) was the most abundant species followed by *Agrilus obsoletoguttatus* (Gory) (Figure

21B). Most of the flower visitors belongs to the genus *Acmaeodera,* including *A. tubulus* (Fabricius) followed by *Acmaeodera pulchella* (Herbst).

Effect of Lure on Buprestidae Capture

The total number of buprestids captured in the lure test was 291 (Table 7). In the Benzaldehyde versus Limonene volatile test, no significant effect of lure treatment was detected on total buprestid capture (χ^2 = 0.76, df = 2, *P* = 0.6825). However, the numbers of trap capture were highest in Limonene (n=53), followed by Benzaldehyde (n=35), and Control (n=34). The study revealed a significant influence of genus on the count data (χ^2 = 17.64, df = 6, *P* = 0.0071) with *Agrilus* and *Acmaeodera* trap captures being higher than *Chrysobothris, Phaenops, Anthaxia, Buprestis,* and *Ptosima. Chrysobothris* was caught in low numbers overall by the Limonene lure (n=5), followed by Benzaldehyde (n=4), and Control (n=3) (χ^2 = 2.00, df = 2, *P* = 0.3679). A similar pattern was observed for the genus *Agrilus* with the most captured in Limonene (n=26), followed by Benzaldehyde (n=12), and Control (n=12) (χ^2 = 2.00, df = 2, *P* = 0.3679), The flower beetle in the genus *Acmaeodera* were trapped equivalently in Control (n=18), Benzaldehyde (n=16), and Limonene (n=15).

In the second lure test, Benzaldehyde versus UHR Ethanol, no lure treatment effect was detected for the total buprestid trap captures and buprestid numbers were similar among the lures including, Ethanol (n=57), Control (n=57), and Benzaldehyde (n=55) (χ^2 = 0.01, df = 2, *P* = 0.996) (Table7). Among genera, *Agrilus* trap captures were higher than the other genera (χ^2 = 14.52, df = 6, *P* = 0.0243). A total of only three *Chrysobothris* specimens were captured (two in Benzaldehyde and one in Ethanol). A similar number of *Agrilus* were trapped in Control (55), Ethanol (55) and Benzaldehyde (52) baited traps ($\chi^2 = 2.00$, df = 2, $P = 0.3679$. Among the *Agrilus* species captured, *A. lecontei* was the most abundant.

Effect of Trap Type and Orientation on Buprestidae Capture

In the trap type and orientation experiment, 113 total buprestids were captured throughout the season (Table 8). Trap captures of *Acmaeodera* were higher than the other genera ($\chi^2 = 14.64$, df = 6, $P = 0.0233$), which had an abundance order of *Agrilus, Chrysobothris, Ptosima, Buprestis, Anthaxia* and *Phaenops*. No differences in beetle capture were detected among the trap treatments ($\chi^2 = 1.61$, df = 2, $P = 0.4467$). The purple sticky panel traps in a horizontal orientation had the highest numerical collection (n=47), followed by the purple traps in a vertical orientation (n=36) and purple pole traps (n=30). Within the horizontal panel trap treatments, *Acmaeodera* (n=42) was the most abundant genus, followed by *Agrilus* (n=4) and *Ptosima* (n=1). In the vertical panel treatment, the most captured genus was *Agrilus* (n=17), followed by *Acmaeodera* (n=14), *Ptosima* (n=3) *Anthaxia* (n=1), and *Chrysobothris* (n=1). For purple pole traps, *Acmaeodera* were the most abundant group (n=11) followed by *Chrysobothris* (n=8), *Agrilus* (n=5), *Ptosima* (n=3), *Buprestis* (n=2), and *Phaenops* (n=1). Although overall captures were low, purple sticky pole traps performed the best for *Chrysobothris* species.

We captured eight *Chrysobothris* species in purple pole traps with *C. femorata* being the most abundant (n=5), followed by *C. pusilla* (n=1), *C. quadriimpressa* (n=1), and *C. sexsignata* (n=1). The genus *Acmaeodera* had higher numerical counts on the horizontal traps (n=42) compared to the vertical (n=14) and pole (n=11) traps, but no significant differences were observed ($\chi^2 = 2.00$, df = 2, $P = 0.3679$). *Agrilus* preferred the vertical traps (n=17) over the horizontal (n=4) and purple pole (n=5) traps.

Effect of UV- and IR-Reflecting Particles on Buprestidae Capture

The total number of buprestid beetles captured in the reflecting particle test was 96 (Table 9). There were no significant effects of reflecting particles treatment on total buprestids capture ($\chi^2 = 0.57$, df = 3, $P = 0.903$). However, among the treatments, the Control (n=32) trap captured the most buprestids, followed by the traps coated with UV (n=27), Combo (n=21), and IR (n=16). *Agrilus* (n=77) was the most abundant genus among trap treatments, followed by the genus *Acmaeodera* (n=14), and *Chrysobothris* (n=5) ($\chi^2 = 24.08$, df = 6, $P \leq 0.001$). The greatest number of *Agrilus* were captured in Control (n=27), followed by UV (n=21), Combo (n=15), and IR (n=14) traps but differences were not significant ($\chi^2 = 3.00$, df = 3, $P = 0.\ 3916$). Overall, few beetles were captured in the genus *Chrysobothris*, including two in Control, one in traps coated with UV, and none in traps coated with IR paint or the Combo IR/UV. The *Acmaeodera* flower beetles were captured in every treatment in low number including Combo (n=6), Control (n=3), UV (n=3), and IR (n=2).

Buprestid Beetle Trapping Results in 2022

The use of purple pole-shaped traps in 2022 improved the capture rates for *Chrysobothris* spp. and reduce bycatch of other buprestid genera. A total of 155 buprestids representing six beetle genera were captured (Table 10). *Chrysobothris* (n=104) were more abundant than other genera (χ^2 =13.59, df = 5, $P = 0.018$) which included *Agrilus* (n=35), *Dicerca* (n=7)*, Acmaeodera* (n=5)*, Spectralia* (n=3)*,* and *Buprestis* (n=1). For the genus *Chrysobothris*, among the captured specimens 75 were female and rest 29 were male. Ten different species were captured under the genus *Chrysobothris* throughout the trapping season. Among them, the most abundant species was *C. quadriimpressa* (n=27), followed by *C. femorata* (n=19), *C. adelpha* (n=17), *C. viridiceps* (n=14), *C. rugosiceps* (n=7), *C. sexsignata* (n=5), *C. shawnee* (n=4), *C. dentipes* (n=3), *C. azurea* (n=3), *C. cribraria* (n=3) (Figure 23A). A total of 10 different *Agrilus* species were captured, including *A. bilineatus* (n=19), *A. lecontei* (n=3), *A. fallax* (n=3), *A. celti* (n=2), *A. planipennis* (n=2), *A. ruficollis* (n=2), *A. cephalicus* (n=1), *A. obsoletoguttatus* (n=1), *A. otiosus* (n=1), and *A. subrobrustus* (n=1) (Figure 23B).

Effect of Volatile Attractants on Buprestidae Capture

The total number of buprestids captured among volatile treatments was 74, representing 5 genera, with *Chrysobothris* (n=56) having higher trap captures than other genera (χ^2 =16.54, df = 5, *P* = 0.005) which included *Agrilus* (n=9), *Acmaeodera* (n=5)*, Dicerca* (n=3)*,* and *Buprestis* (n=1). No total buprestid trap capture differences were detected among lure treatments (χ^2 = 0.71, df = 3, *P* = 0.869). However, the number of trapped buprestids was highest in non-treated control traps (n=23), followed by (Z)-3-hexanol (n=19), combo (Z)-3-hexanol +(Z)-3-hexenyl acetate (n=16), and (Z)-3-hexenyl acetate (n=16). For *Chrysobothris,* specimen captures included more in Control (n=16) followed by (Z)-3-hexanol (n=15), (Z)-3-hexenyl acetate (n=13) and Combo (n=11), but the differences was not statistically significant (χ^2 = 3.00, df = 3, *P* = 0.3916) (Figure 24). Similarly, for the genus *Agrilus*, trap captures were not statistically significant (χ^2 = 3.0, df = 3, *P* = 0.3916) and included Control groups (n=4), Combo (n=3), (Z)-3-hexanol (n=2) and (Z)-3-hexenyl acetate (n=1). At the species level, nine *Chrysobothris* species were captured including *C. quadriimpressa* (n=14) being the most abundant species followed by *C. adelpha* (12), *C. femorata* (9), *C. rugosiceps* (5), *C. viridiceps* (4), *C. dentipes* (4), *C. cribraria* (3), *C. sexsignata* (2)*, C. azurea* (2)*,* and *C. shawnee* (1). The common species of genus *Agrilus* were *Agrilus lecontei* (2), *A. celti* (2), followed by *A. cephalicus* (1), *A. subrobustus* (1), *A. bilineatus* (1), *A.*

fallax (1), *A. planipennis,* and *A. ruficollis* (1). Single specimens of *Acmaeodera tubulus, Dicerca obscura,* and *Buprestis lineata* were captured in the volatile test.

Effect of Trap Type on Buprestidae Capture

Based on the results of our study, the purple pole traps were found to be significantly more effective at capturing *Chrysobothris* beetles than the Lindgren funnel traps ($\chi^2 = 5.23$, df = 1, $P = 0.022$) (Figure 25). Purple Lindgren funnel traps did not capture a single buprestid which further supports the effectiveness of the purple pole traps for this particular beetle genus. In this experiment, a total of 76 buprestid beetles were captured with the majority belonging to the genus *Chrysobothris* (n=47) followed by *Agrilus* (n=25), *Spectralia* (n=3), *and Dicerca* (n=1) However, no statistical differences were detected among the captured genera ($\chi^2 = 2.89$, df = 5, $P = 0.7175$). For *Chrysobothris*, the most abundant species was *C. quadriimpressa* (n=13) followed by *C. femorata* (n=10), *C. viridiceps* (n=10), *C. adelpha* (n=5), *C. shawnee* (n=4), *C. sexsignata* (n=3), *C. rugosiceps* (n=2), and *C. azurea* (n=1). Among the genus *Agrilus,* the most abundant captured species was *A. bilineatus* (n=18) *followed by A. fallax* (n=2), *A. lecontei* (n=1), *A. obsoletoguttatus* (n=1), *A. otiosus* (n=1), *A. planipennis* (n=1), and *A. ruficollis* (n=1). The remaining two species captured in purple pole traps were *Spectralia gracilipes* (n=3) and *Dicerca lusida* (n=1).

Effect of Trap Height on Buprestids Capture

In the trap height test, only four buprestids were captured. In particular, the lower Lindgren funnel trap successfully captured *Dicerca obscura* and one *A. planipennis.* Similarly, the upper purple Lindgren funnel traps also caught two *Dicerca obscura*. No *Chrysobothris* spp.

were captured in any of the traps. The results indicate purple Lindgren funnel traps were not effective for *Chrysobothris* capture.

Chapter 5: Conclusion

The present research aimed to protect woody ornamental trees using sustainable methods. Two objectives related to this research aim were performed, including (1) evaluation of the traits and biochemical responses of red maple cultivars and a hybrid maple to herbicide stress and how these responses might impact their resistance or susceptibility to natural borer infestations and (2) optimization of purple traps for capturing *Chrysobothris* buprestids using volatile attractants and improved trap designs. Herbicide treatments led to decreased growth in maple cultivars, as evidenced by reduced plant height and diameter in herbicide (Scythe)-treated trees compared to Control trees. The current study also demonstrated the degradation of photosynthetic pigments like chlorophyll content, chlorophyll a (chla), chlorophyll b (chlb), and total carotenoids following shortly after herbicide exposure (7 DAT) and at a later date (28 DAT). Cultivars responded differently to the same environmental stress. The red maple cultivar 'SV' showed lower levels of chla in Scythe treated trees compared to the Control at 28 DAT. Interestingly, a reduction in total carotenoids was also observed in the herbicide-treated trees of the cultivar 'SV' on both 7 and 28 DAT. Identifying and quantifying polyphenols (phenolics, tannin and flavonoids) revealed that herbicide-treated trees had more phenolics and tannin concentration whereas flavonoid concentrations were higher in Control trees. The study results demonstrated that exposure to herbicide can have differential effects on different classes of phenolic compounds in different plant cultivars. Phenolic concentrations increased only in the Roundup treated hybrid cultivar 'AB', which indicates these compounds may be upregulated in response to herbicide stress, potentially as a stress defense mechanism. A decrease in total non-structural carbohydrates (TNC) was observed in the herbicide-treated trees compared to Control trees.

Interestingly, the systemic acting Roundup herbicide had a more pronounced effect on biochemical parameters than the contact Scythe herbicide treatment at 7 DAT. The Roundup treated plants were dead within two weeks of treatment, while Scythe treated plants showed significant reductions in growth and alterations in most of the biochemical attributes by 28 DAT. The results support the idea that herbicides with different modes of action have differential effects on plant physiology and biochemistry, thus Roundup having a more immediate and severe effect compared to Scythe.

The study also investigated how herbicide stress influences the growth, physiology, and defense mechanism of hybrid and red maple cultivars, and how these changes might impact their resistance or susceptibility to natural borer infestations. Hybrid maples had a negative deviation from the expected borer attack frequency, indicating it was less susceptible to natural borer attack than the other red maple cultivars under field conditions. In addition, an increase in phenolics and a decrease in flavonoids was detected in Cultivar AB, suggesting that the herbicide may have affected its secondary metabolite production. Furthermore, under Control conditions, cultivar AB showed higher baseline values for several growth and nutrient parameters, including height, trunk diameter growth, as well as nitrogen, phosphorous, potassium, sulfur, boron, copper presence and catalase activity. Consequently, cultivar AB may be better adapted to the specific environmental conditions of this experiment or may have inherent genetic traits from both parent species that contribute to its superior growth and nutrient uptake under normal conditions. Overall, study findings indicate herbicides can have a significant effect on the physiology and growth of tree cultivars, but the responses may vary depending on the parameter studied and the specific cultivars involved. Further research is needed to elucidate the underlying mechanisms in the observed cultivar-specific responses to herbicide treatments. To better understand the hybrid cultivar's defense mechanisms against borer attacks, it may be useful to perform additional biochemical assays focused on bark tissue, such as measuring lignin content and other secondary metabolite profiles. Sap flow assessments in trunk of different cultivars could provide useful information about their ability to withstand or deter larval survival, as sap flow is an important physiological parameter that indicates the movement of water, nutrients, and other substances through the plant's vascular system.

The current study also investigated the optimization of purple traps for *Chrysobothris* collection using different volatile lures, purple trap types and deploying traps at different heights. The results indicated purple pole traps were more effective for capturing *Chrysobothris* spp.; whereas purple Lindgren funnel traps were ineffective (i.e., not a single specimen captured). Other studies have determined that slippery substances like fluon to funnel traps can increase wood-borer captures, so captures of *Chrysobothris* might have been improved in funnel traps with additional treatments like fluon. Among the green leaf volatiles tested, none of the lures were found effective for capturing the *Chrysobothris*. Future studies should consider the use of additional volatiles lure identified from stressed trees or pheromones with some modifications in purple traps to enhance the capture of *Chrysobothris* spp.

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Appendices

Table 1

ANOVA Results Showing Treatment Effect (Control, Roundup, and Scythe) on Responses of Three Red Maple (Acer rubrum) Cultivars ('Brandywine', 'October Glory', 'Sun Valley') and a Hybrid ('Autumn Blaze') on 7 Days After Treatment (DAT) and 28 Days After Treatment (DAT)

Response variable	Treatment			Cultivar			Treatment x Cultivar		
	Df	*F*	*P*	Df	*F*	*P*	Df	*F*	*P*
				Day 7					
Chlorophyll content (SPAD value)	2	33.384	**≤0.001**	3	12.696	**≤0.001**	6	2.045	0.0658
Chlorophyll a (chla)	2	20.998	**≤0.001**	3	8.597	**≤0.001**	6	2.033	0.0673
Chlorophyll b (chlb)	2	10.706	**≤0.001**	3	6.438	**≤0.001**	6	0.965	0.4524
Total carotenoids	2	9.168	**≤0.001**	3	11.196	**≤0.001**	6	3.499	**≤0.01**
Electrolyte leakage	2	0.504	0.606	3	1.463	0.229	6	0.433	0.856
Phenolics	2	17.688	**≤0.001**	3	22.39	**≤0.001**	6	3.719	**≤0.01**
Tannins	2	27.215	**≤0.001**	3	7.160	**≤0.001**	6	1.514	≥0.05
Flavonoids	2	44.872	**≤0.001**	3	2.797	0.043	6	3.841	**≤0.01**
Total non-structural carbohydrates (TNC)	2	3.99	0.0212	3	2.845	**0.041**	6	4.263	**≤0.001**
Total antioxidant	2	1.603	0.2154	3	6.288	**≤0.01**	6	1.350	0.2611

Catalase activity (CAT)	2	2.866	0.0625	3	3.408	**0.0213**	6	1.23	0.2992
Superoxide dismutase activity (SOD)	2	1.535	≥0.06	3	11.019	**≤0.001**	6	2.325	0.0388
					Day 28				
Height	1	4.547	**0.0364**	3	3.575	**0.0180**	3	1.132	0.3419
Diameter	1	24.236	**0.001**	3	2.932	**0.0392**	3	0.305	0.8219
Chlorophyll content (SPAD value)	1	26.447	**≤0.001**	3	7.83	**≤0.001**	3	0.725	0.5402
Chlorophyll a (chla)	1	5.77	**0.0189**	3	3.018	**0.0353**	3	3.515	**0.0194**
Chlorophyll b (chlb)	1	5.792	**0.0187**	3	2.2	0.0954	3	2.686	0.0529
Total carotenoids	1	11.621	**≤0.01**	3	4.38	**≤0.01**	3	4.008	**0.0107**
Electrolyte leakage	1	1.123	0.293	3	0.355	0.786	3	0.227	0.877
Phenolics	1	13.085	**≤0.001**	3	12.892	**≤0.001**	3	0.933	0.4508
Tannins	1	18.259	**≤0.001**	3	12.671	**≤0.001**	3	2.483	0.0746
Flavonoids	1	20.981	**≤0.001**	3	7.052	**≤0.01**	3	0.748	0.527
Total non-structural carbohydrates (TNC)	1	6.597	**0.0150**	3	5.576	**≤0.01**	3	6.258	**≤0.01**
Water potential (bar)	1	0.854	0.362	3	1.314	0.287	3	0.48	0.698
Bark mineral nutrients									
Nitrogen (N%)	1	0.459	0.502	3	8.296	**≤0.001**	3	0.432	0.731
Phosphorous (P%)	1	2.59	0.117	3	17.206	**≤0.001**	3	4.437	**0.0102**
Potassium (K%)	1	0.34	0.563	3	4.723	**≤0.001**	3	0.971	0.4183

Sulphur (S%)	1	3.982	0.0545	3	22.082	**≤0.001**	3	2.021	0.1307
Calcium (Ca%)	1	7.451	**0.010**	3	8.588	**≤0.001**	3	0.125	0.9445
Magnesium (Mg%)	1	1.053	0.312	3	16.539	**≤0.001**	3	0.342	0.795
Zinc (Zn ppm)	1	10.02	**≤0.01**	3	26.84	**≤0.001**	3	2.76	0.058
Iron (Fe ppm)	1	0.02	0.888	3	2.249	0.102	3	1.808	0.166
Manganese (Mn ppm)	1	12.58	**≤0.01**	3	13.82	**≤0.001**	3	2.11	0.118
Cupper (Cu ppm)	1	0.96	0.334	3	5.177	**≤0.001**	3	0.999	0.405
Boron (B ppm)	1	36.125	**≤0.001**	3	17.868	**≤0.001**	3	1.362	0.272
Molybdenum (Mo ppm)	1	2.502	0.124	3	2.067	0.124	3	0.353	0.788
Total Carbon (C%)	1	12.35	**≤0.01**	3	1.258	0.305	3	0.757	0.526
Leaf mineral nutrients									
Nitrogen (N%)	1	5.988	**0.020**	3	1.503	0.232	3	0.247	0.862
Phosphorous (P%)	1	2.075	0.159	3	0.332	0.802	3	0.152	0.927
Potassium (K%)	1	0.302	0.586	3	4.045	**0.015**	3	2.811	0.055
Sulphur (S%)	1	0.002	0.9638	3	2.406	0.085	3	0.04	0.989
Calcium (Ca%)	1	1.056	0.312	3	0.777	0.515	3	0.758	0.526
Magnesium (Mg %)	1	0.002	0.968	3	1.459	0.244	3	0.223	0.880
Zinc (Zn ppm)	1	0.156	0.695	3	5.693	**≤0.01**	3	0.366	0.778
Iron (Fe ppm)	1	4.306	**0.0461**	3	0.603	0.617	3	0.489	0.692
Manganese (Mn ppm)	1	0.253	0.618	3	2.767	0.057	3	1.17	0.336

Cupper (Cu ppm)	1	0.802	0.377	3	1.488	0.236	3	1.22	0.319
Boron (B ppm)	1	0.073	0.788	3	1.441	0.249	3	1.519	0.229
Molybdenum (Mo ppm)	1	0.366	0.549	3	2.533	0.074	3	1.966	0.138
Total Carbon (C %)	1	2.191	0.149	3	1.744	0.178	3	1.036	0.390

Note. Significant P-values are in **bold.**

Table 2

Mean (±SE) Chlorophyll Content, Photosynthetic Pigments (Chlorophyll a, Chlorophyll b and Total Carotenoids), and Electrolyte Leakage of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) within Treatment Groups on 7 Days After Herbicide (Scythe or Roundup) Treatment (DAT)

Treatment	Cultivar	Chlorophyll Content (SPAD)	Chlorophyll a (Chl a) (µg/ml)	Chlorophyll b (Chl b) (µg/ml)	Total Carotenoids (µg/ml)	Electrolyte Leakage (%)
Within						
Control	‘Autumn Blaze’	37.73±0.60 b[w]	9.98±0.51 bc	4.20±0.14 b	1.82±0.15 b	11.86±1.62 a
	‘Brandywine’	37.75±1.03 b	9.21±0.30 c	4.69±0.22 ab	1.55±0.13 b	11.66±0.72 a
	October Glory’	37.61±1.11 b	11.06±0.28 ab	4.74±0.16 ab	1.94±0.09 b	13.17±0.79 a
	‘Sun Valley’	42.74±0.48 a	11.80±0.66 a	5.14±0.30 a	2.60±0.15 a	11.52±0.78 a
	F-value	8.818	6.03	3.269	11.41	0.522
	Df	3	3	3	3	3
	P -value	**≤0.001**	**≤0.01**	**0.0322**	**≤0.001**	0.67
Within						
Herbicide	‘Autumn Blaze’	31.69±0.90 b	8.4±0.71 ab	3.79±0.23 b	1.64±0.17 ab	13.81±3.45 a

‘Brandywine’	32.91±0.72 b	7.22±0.42 b	3.85±0.17 b	1.40±0.11 b	10.01±0.81 a
‘October Glory’	32.53±1.03 b	9.31±0.53 a	3.90±0.14 ab	1.87±0.12 a	11.63±1.67 a
‘Sun Valley’	36.62±0.87 a	9.34±0.39 a	4.55±0.16 a	1.91±0.08 a	10.29±0.81 a
F-value	6.598	4.42	3.956	4.279	1.437
Df	3	3	3	3	3
P -value	**≤0.001**	**≤0.01**	**0.0114**	**≤0.01**	0.909

Note. Significant P-values are in **bold**.

[w] Treatment means that do not share the same letter within in the column are significantly different (alpha=0.05, Tukey's Honestly Significant Difference Test).

Table 3

Mean (±SE) Polyphenols (Phenolic Compound, Tannin, Flavonoid), Total Non-Structural Carbohydrates, Total Antioxidant and Enzymes (Super Oxide Dismutase and Catalase) of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory' and 'Sun Valley') within Treatment Groups on 7 Days After Herbicide (Scythe or Roundup) Treatment (DAT)

Treatment	Cultivar	Phenolic Compound mg/ml (Gallic Acid Equvalent)	Tannin (mm^2)	Flavonoid mg/ml (Quercetin Equvalent)	Total Non-Structural Carbohydrates (TNC%)	Total Antioxidant (nmol/µl)	Super Oxide Dismutase Activity (SOD) (U/ml)	Catalase Activity (CAT) (U/ml)
Within								
Control	'Autumn Blaze'	22.49±2.97 b[w]	57.20±2.99 a	41.86±1.07 a	14.18±1.06 ab	27.86±8.04 b	0.35±0.00 ab	1.09±0.01 a
	'Brandywine'	52.06±4.16 a	53.19±4.50 a	37.98±2.56 a	18.74±2.13 a	42.05±8.58 ab	0.35±0.01 ab	1.05±0.0 b
	'October Glory'	54.29±5.08 a	56.29±7.98 a	38.87±1.30 a	12.46±0.41 b	38.91±5.94 ab	0.37±0.01 a	1.06±0.0 b
	'Sun Valley'	63.31±3.77 a	63.21±5.55 a	39.22±1.65 a	13.90±0.94 ab	60.79±4.51 a	0.35±0.00 b	1.09±0.0 a
	F-value	18.96	0.569	0.921	4.443	3.863	3.923	14.48
	Df	3	3	3	3	3	3	3
	P -value	**≤0.001**	0.642	0.441	**≤0.01**	**0.0381**	**0.0186**	**≤0.001**

Within								
Herbicide	'Autumn Baze'	48.1±5.74 b	71.02±5.0 b	29.74±1.59 b	15.59±0.99 a	52.69±5.12 ab	0.32±0.01 b	1.07±0.01 a
	'Brandywine'	52.89±3.05 b	67.85±7.73 b	35.47±0.97 a	13.07±0.38 b	49.47±4.74 ab	0.35±0.01 ab	1.06±0.0 a
	'October Glory'	61.53±4.44 ab	66.29±8.83 b	31.92±1.26 ab	13.16±0.47 b	35.40±5.58 b	0.38±0.01 a	1.03±0.02 a
	'Sun Valley'	75.06±2.98 a	100.33±9.63 a	35.25±1.16 a	14.74±0.64 ab	63.61±6.22 a	0.37±0.0 a	1.06±0.0 a
	F-value	10.535	6.856	8.454	4.058	4.400	10.057	2.103
	Df	3	3	3	3	3	3	3
	P-value	**≤0.001**	**≤0.001**	**≤0.001**	**0.0101**	**0.0133**	**≤0.001**	0.110

Note. Significant P-values are in **bold**.

[w] Treatment means that do not share the same letter within in the column are significantly different (alpha=0.05, Tukey's Honestly Significant Difference Test).

Table 4

Mean (±SE) Height Increase and Trunk Diameter Increase of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory' and 'Sun Valley') within Treatment Groups on 28 Days After Herbicide (Scythe) Treatment (DAT)

Treatment	Cultivar	Height (cm)[y]	Trunk Diameter (mm)[x]
Within Control	'Autumn Blaze'	44.8±5.66 a[w]	2.87±0.25 a
	'Brandywine'	31.4±3.51 ab	3.17±0.55 a
	'October Glory'	28.2±5.74 ab	2.01±0.53 a
	'Sun Valley'	26.7±3.47 b	2.49±0.28 a
	F-value	3.048	1.402
	Df	3	3
	P-value	**0.041**	0.258
Within Herbicide	'Autumn Blaze'	29.2±3.86 a	1.22±0.26 a
	'Brandywine'	29.2±3.60 a	1.99±0.43 a
	'October Glory'	27.0±5.19 a	1.04±0.15 a
	'Sun valley'	19.3±3.03 a	1.15±0.31 a
	F-value	1.382	2.047
	Df	3	3
	P-value	0.264	0.125

Note. Significant P-values are in **bold**.

[w] Treatment means that do not share the same letter within in the column are significantly different (alpha=0.05, Tukey's Honestly Significant Difference Test).

[y] Height increase was measured by subtracting the height of the plant at the beginning of the experiment from the height of the plant at the end of the experiment.

[x] Trunk diameter increase was measured by subtracting the width of the plant at the beginning of the experiment from the width of the plant at the end of the experiment

Table 5

Mean (±SE) Chlorophyll Content, Photosynthetic Pigments (Chlorophyll a, Chlorophyll b and Total Carotenoids), Electrolyte Leakage and Water Potential of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) within Treatment Groups on 28 Days After Herbicide (Scythe) Treatment (DAT)

Treatment	Cultivar	Chlorophyll Content (SPAD)	Chlorophyll a (chla) (µg/ml)	Chlorophyll b (chlb) (µg/ml)	Total Carotenoids (µg/ml)	Electrolyte Leakage (%)	Water Potential (bar)
Within Control	‘Autumn Blaze’	35.49±0.56 b[w]	11.69±0.64 b	3.07±0.24 b	3.16±0.16 b	9.10±0.44 a	14.0±1.04 a
	‘Brandywine’	35.6±1.02 b	11.09± 0.63 b	3.08±0.28 b	2.96±0.15 b	8.65±0.37 a	17.9±1.24 a
	‘October Glory’	37.96±0.96 ab	12.31±0.48 ab	3.25±0.14 ab	3.43±0.18 ab	8.82±0.83 a	15.7±1.56 a
	‘Sun Valley’	39.59±1.1 a	14.49±0.73 a	4.16±0.34 a	3.88±0.12 a	8.65±0.44 a	14.7±1.04 a
	F-value	4.494	5.607	3.999	6.898	0.151	1.99
	Df	3	3	3	3	3	3
	P -value	**≤0.01**	**≤0.01**	**0.0148**	**≤0.001**	0.928	0.175
Within Herbicide	‘Autumn Blaze’	30.25±0.58 b	10.83±0.34 a	2.92±0.18 a	2.78±0.13 a	9.51±0.45 a	14.40±0.44 a
	‘Brandywine’	33.26±1.26 ab	11.94±0.74 a	3.09±0.25 a	3.21±0.23 a	9.17±0.57 a	15.30±1.54 a

‘October Glory’	33.34±0.71 ab	11.27±0.67 a	2.99±0.24 a	2.78±0.15 a	8.73±0.39 a	14.78±0.87 a
‘Sun Valley’	36.2±1.81 a	11.41±0.52 a	2.95±0.18 a	3.13±0.13 a	9.43±0.64 a	14.40±0.67 a
F-value	4.145	0.6	0.128	1.894	0.447	0.08
Df	3	3	3	3	3	3
P-value	**0.0127**	0.619	0.943	0.148	0.721	0.78

Note. Significant P-values are in **bold**.

[w] Treatment means that do not share the same letter within in the column are significantly different (alpha=0.05, Tukey's Honestly Significant Difference Test).

Table 6

Mean (±SE) Polyphenols (Phenolic Compound, Tannin, flavonoid), Total Non-structural Carbohydrates of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory' and 'Sun Valley') Within Treatment Groups on 28 Days After Herbicide (Scythe) Treatment (DAT)

Treatment	Cultivar	Phenolic Compound mg/ml (Gallic Acid Equvalent)	Tannin (mm^2)	Flavonoid mg/ml (Quercetin Equvalent)	Total Non-Structural Carbohydrates (TNC%)
Within Control	'Autumn Blaze'	108.93±4.65 ab	72.68±10.13 a	33.42±1.69 b	14.01±1.02 ab
	'Brandywine'	85.07±7.03 b[w]	72.78±17.84 a	42.54± 1.08 a	16.1±0.88 a
	'October Glory'	107.96±6.06 ab	77.35±11.86 a	35.53±2.49 b	10.59±1.21 b
	'Sun Valley'	133.22±8.56 a	136.71±27.69 a	43.09±1.5 a	10.37±0.48 b
	F-value	8.285	2.95	8.847	8.85
	Df	3	3	3	3
	P -value	**≤0.001**	≥0.05	**≤0.001**	**≤0.01**
Within Herbicide	'Autumn Blaze'	133.05±9.91 ab	147.13±12.35 b	26.98±2.67 a	11.94±0.57 a
	'Brandywine'	112.54±6.76 b	110.61±24.26 b	34.22±2.93 a	10.50±0.37 a
	'October Glory''	112.14±8.49 b	93.04±14.33 b	31.45±2.02 a	12.39±0.83 a
	'Sun Valley'	154.75±10.02 a	257.09±16.83 a	32.60±2.83 a	9.85±0.43 a
	F-value	5.525	10.58	1.705	2.119

Df	3	3	3	3
P -value	**≤0.01**	**≤0.001**	0.183	0.138

Note. Significant P-values are in **bold**.

[w] Treatment means that do not share the same letter within in the column are significantly different (alpha=0.05), Tukey's Honestly Significant Difference Test).

Table 7

Total Buprestid Beetle Captured in Purple Sticky Panel Traps Treated with Lures Benzaldehyde, Ethanol, and in Summer 2021 in Middle Tennessee

	Experiment 1 (Treatment)			Experiment 2 (Treatment)			
Genus	Control	Benzaldehyde	Ethanol	Control	Benzaldehyde	Limonene	Total
Agrilus	55	52	55	12	12	26	212
Acmaeodera	1	0	1	17	16	15	50
Chrysobothris[a]	0	2[f]	1[f]	3[f]	4[f]	5[2f/3m]	15[12f/3m]
Phaenops	0	0	0	1	3	5	9
Anthaxia	1	1	0	1	0	0	3
Buprestis	0	0	0	0	0	1	1
Ptosima	0	0	0	0	0	1	1
Total	57	55	57	34	35	53	291

Note. f = female, and m = male.

[a] Gender of *Chrysobothris* spp. specimens was determined to provide a measure of trap effectiveness in capturing the reproductive female individuals that contribute to feeding damage and tree losses in commercial nursery and landscape systems.

Table 8

Total Buprestid Beetle Captured in Purple Sticky Pole Traps, and Panel Traps Kept in Vertical and Horizontal Different Direction in Summer 2021 in Middle Tennessee

Genus	Horizontal	Pole	Vertical	Total
Acmaeodera	42	11	14	67
Agrilus	4	5	17	26
Anthaxia	0	0	1	1
Buprestis	0	2	0	2
Chrysobothris [a]	0	8 [5f/3m]	1 [m]	9 [5f/4m]
Phaenops	0	1	0	1
Ptosima	1	3	3	7
Total	47	30	36	113

Note. f = female, and m = male.

[a] Gender of *Chrysobothris* spp. specimens was determined to provide a measure of trap effectiveness in capturing the reproductive female individuals that contribute to feeding damage and tree losses in commercial nursery and landscape systems.

Table 9

Total Buprestid Beetle Captured in Purple Sticky Panel Traps Coated with Reflecting Particles (Ultraviolet, Infrared, and Combination of Both) in Summer 2021 in Middle Tennessee

	Treatment				
Genus	Control	NIR	UV	Combo	Total
Acmaeodera	3	2	3	6	14
Agrilus	27	14	21	15	77
Chrysobothris [a]	2 [f]	0	3 [f]	0	5 [f]
Total	32	16	27	21	96

Note. f = female, and m = male.

[a] Gender of *Chrysobothris* spp. specimens was determined to provide a measure of trap effectiveness in capturing the reproductive female individuals that contribute to feeding damage and tree losses in commercial nursery and landscape systems.

Table 10

Total Buprestid Beetle Captured in Purple Lindgren Funnel Traps Kept at Ground Level and Canopy Level in Summer 2022 in Middle Tennessee

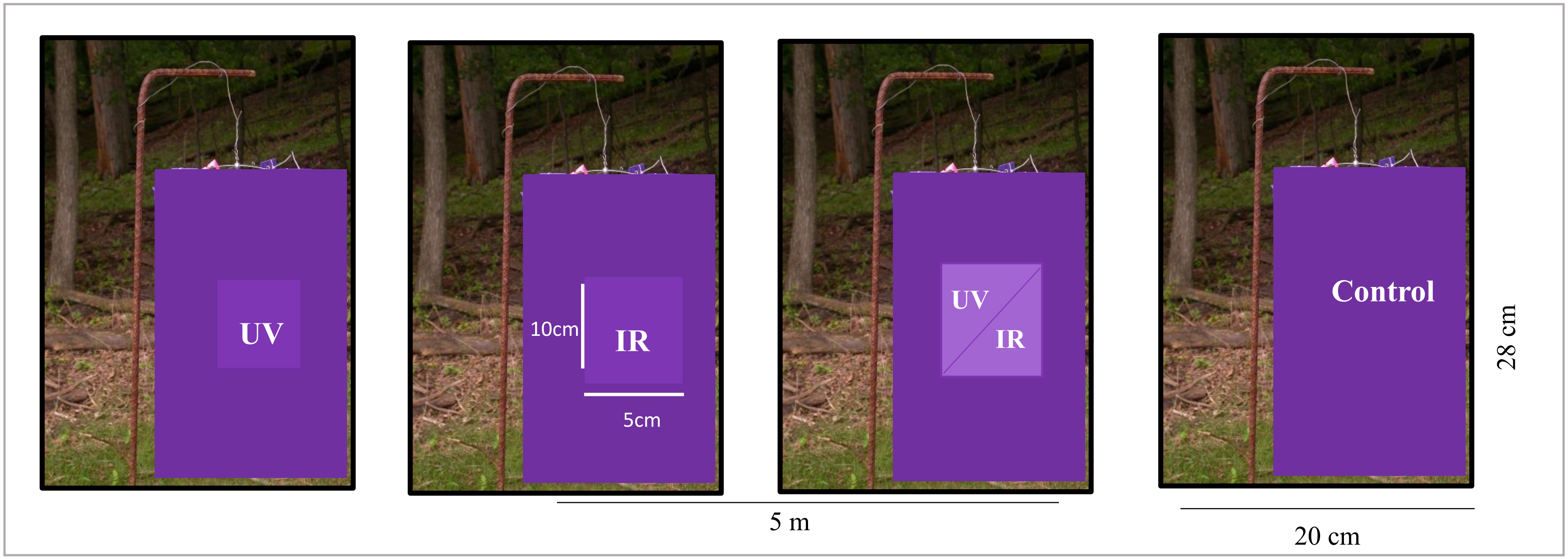
	Volatile Test				Trap Type		Trap Height		Total
Genus	Control	(Z)-3-Hexenyl Acetate	Combo	(Z)-3-Hexanol	Funnel	Pole	Ground Trap	Canopy Trap	
Acmaeodera	2	0	3	0	0	0	0	0	5
Agrilus	4	1	2	2	0	25	1	0	35
Buprestis	0	1	0	0	0	0	0	0	1
Chrysobothris [a]	16[11f/5m]	14[8f/6m]	11[8f/3m]	15[9f/6m]	0	48[39f/9m]	0	0	104 [75f/29m]
Dicerca	1	0	0	2	0	1	1	2	7
Spectralia	0	0	0	0	0	3	0	0	3
Total	23	16	16	19	0	77	2	2	155

Note. f = female, and m = male.

[a] Gender of *Chrysobothris* spp. specimens was determined to provide a measure of trap effectiveness in capturing the reproductive female individuals that contribute to feeding damage and tree losses in commercial nursery and landscape systems.

Figure 1

Purple Trap Painted with Reflecting Paint (UV, IR, or a *Combination of Both) in Summer 2021 in Middle Tennessee*



Note. The experiment consisted of 4 distinct treatments and treatments were spaced at 5 m intervals with at least 10 meters between replicates in a randomized complete block design.

Figure 2

Purple Sticky Panel Trap Deployed Horizontally Facing Skyward (A), Vertically Facing Woodlots (B) and Purple Pole Trap (C) for Capturing Buprestids in Summer 2021 in Middle Tennessee

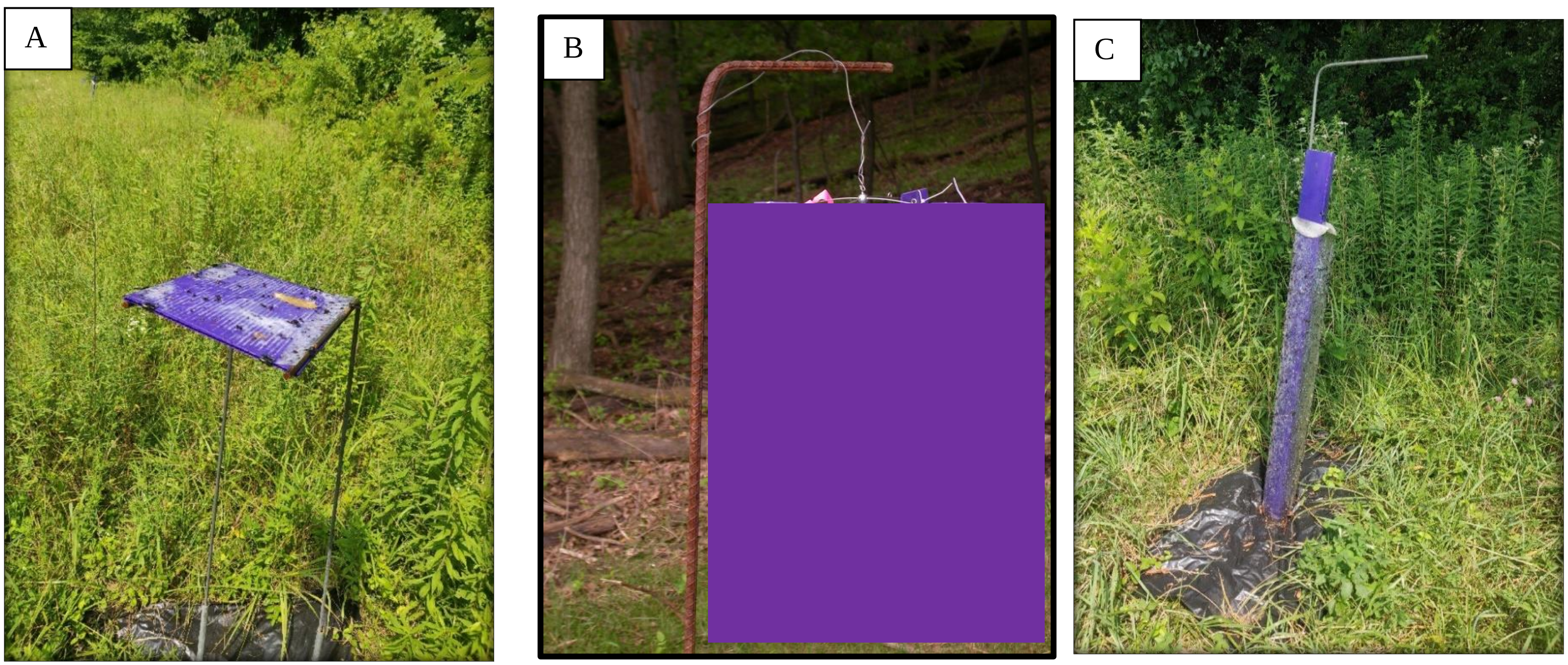
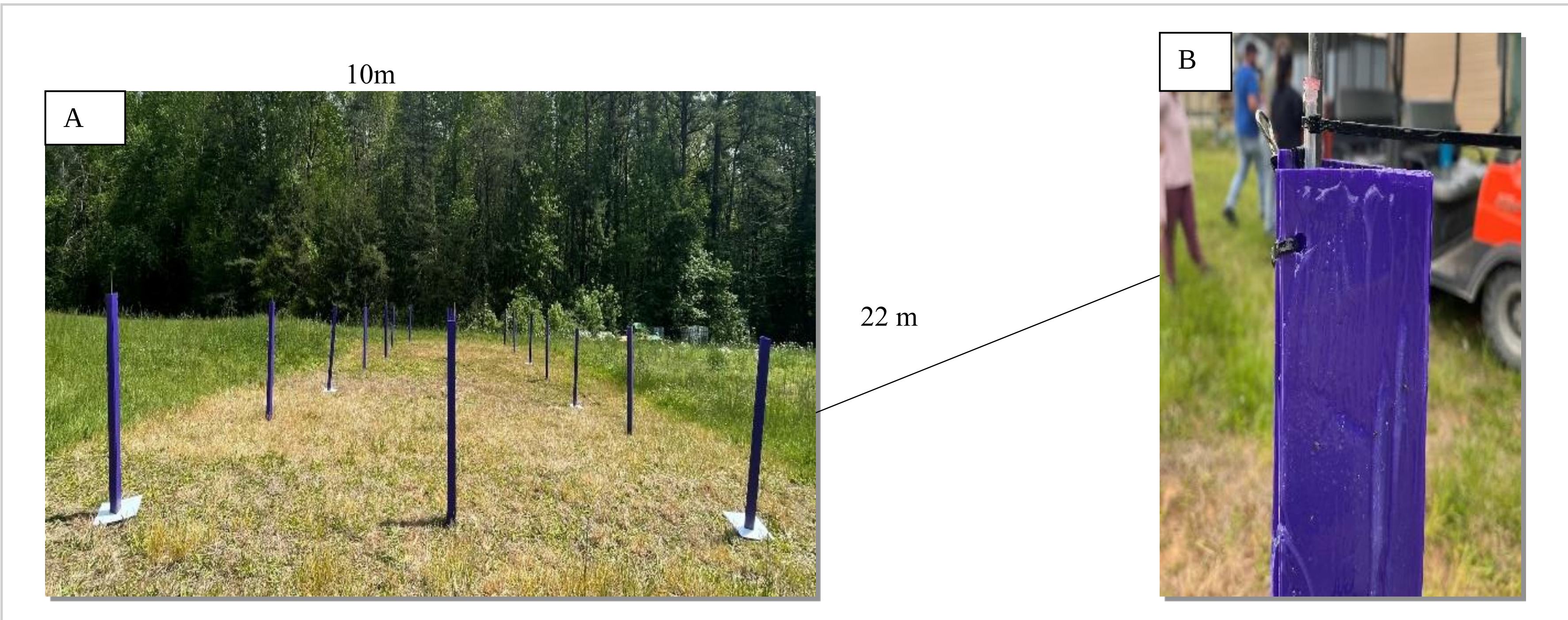


Figure 3

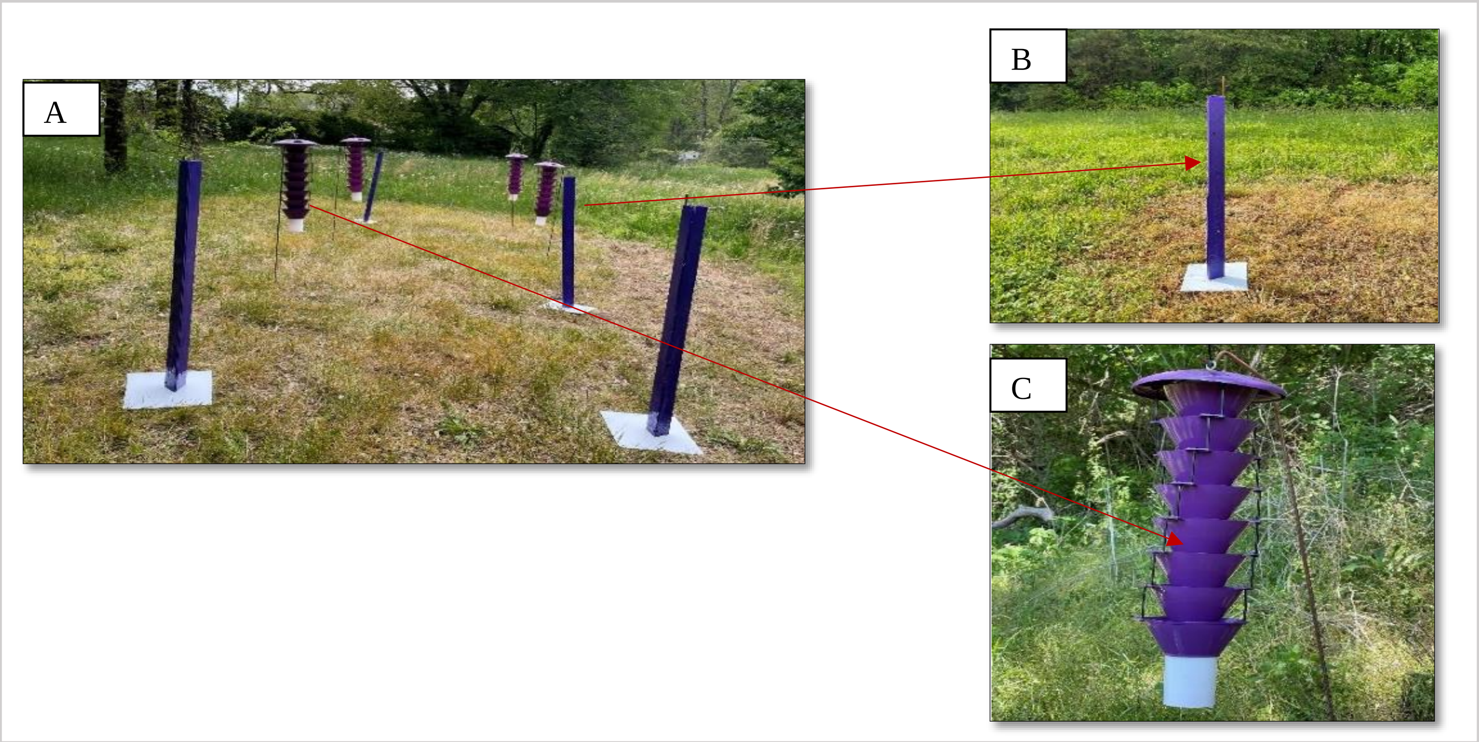
Purple Sticky Pole Traps Baited with Green Volatile Lures that Included (Z)-3-Hexenyl Acetate, (Z)-3-Hexanol, or a Combination of Both (A) and Close-Up View of the Purple Sticky Trap (B) in Summer 2022 in Middle Tennessee



Note. Traps were coated with TAD glue from each side. Baited traps received a volatile lure (100 µl), and unbaited traps were used as controls. Each treatment was replicated two times in a Randomized Complete Block Design.

Figure 4

Purple Sticky Pole Trap (B) and Purple Lindgren Funnel Traps (C) Arranged in 2 × 6 Meters Plot Array (A) in Summer 2022 in Middle Tennessee



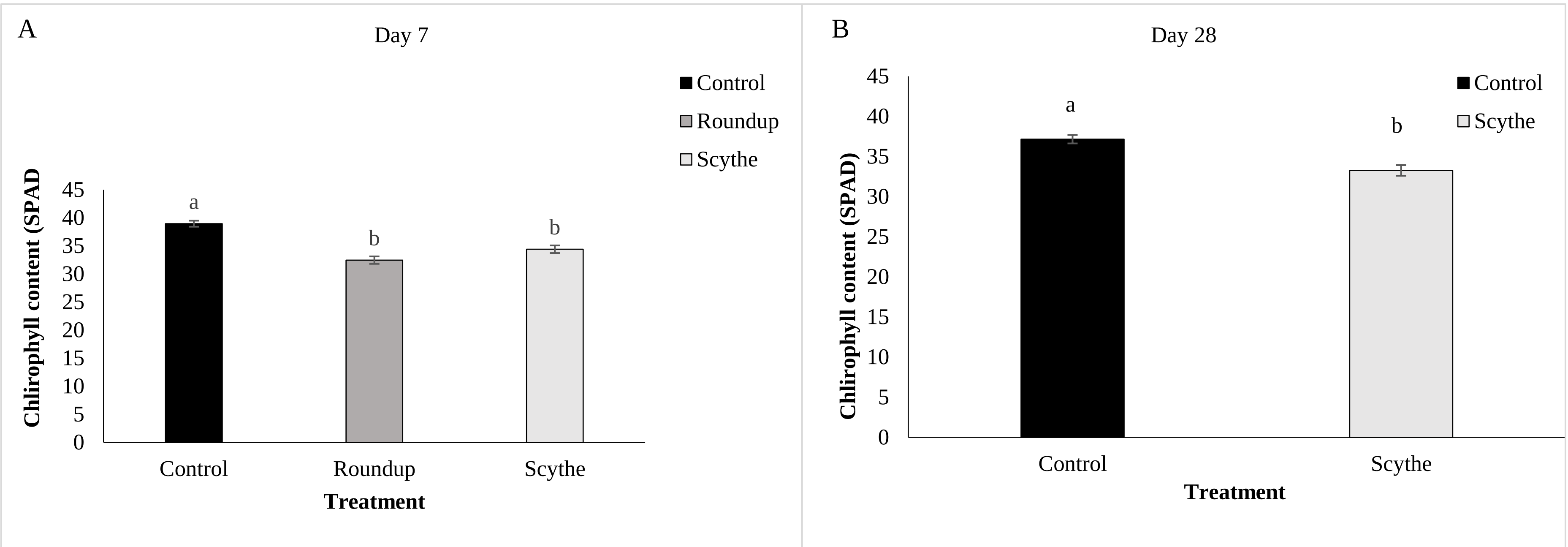
Note. Traps were spaced at 2-m intervals, replicated four times, and arranged in a Randomized Complete Block Design (RCBD).

Figure 5

Purple Lindgren Funnel Traps Suspended Using a Rope in the Tree Canopy (~ 10 Meters) and at Ground Level Using Metal Trap Rods (1.5 Meters) Above Ground Level in Summer 2022 in Middle Tennessee

Figure 6

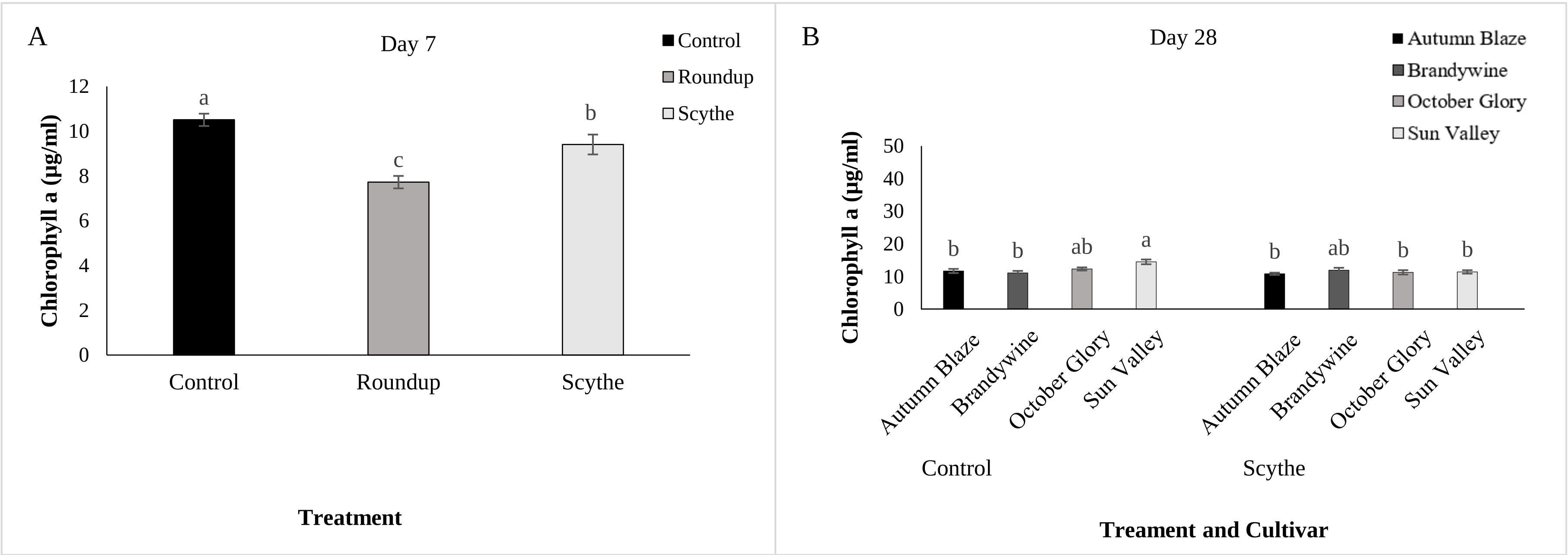
The Chlorophyll Content SPAD (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, And ‘Sun Valley’) Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe Only) After Treatment (DAT (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0.05

Figure 7

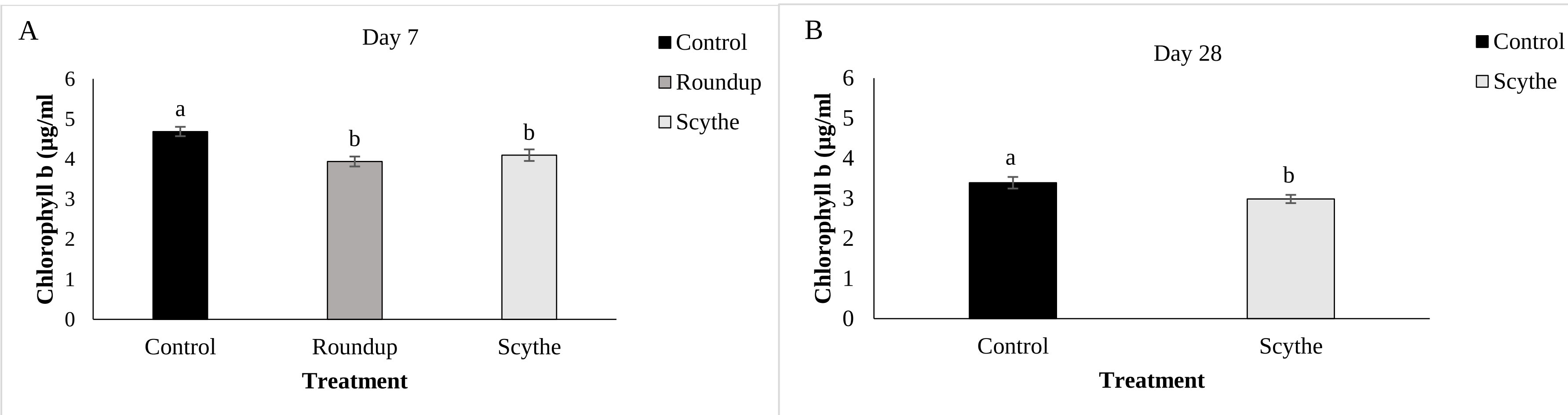
Chlorophyll a (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe only) After Treatment (DAT) (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0.05

Figure 8

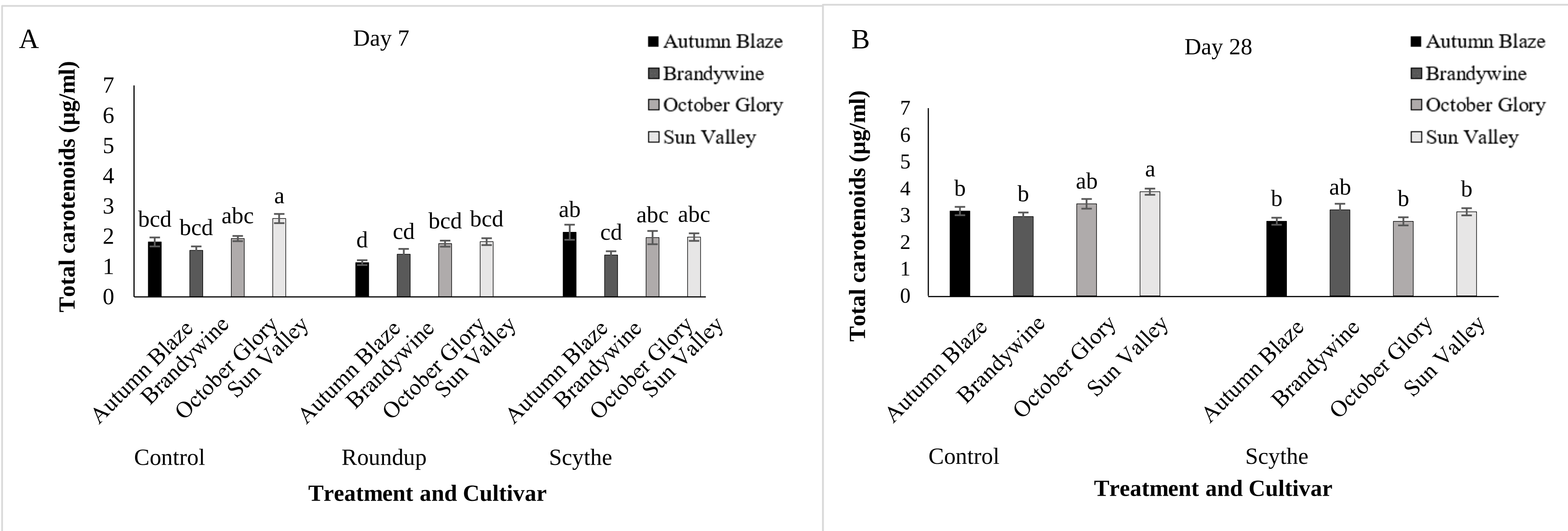
Chlorophyll b (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘’October Glory, and ‘Sun Valley’) Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe Only) After Treatment (DAT) (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0.05

Figure 9

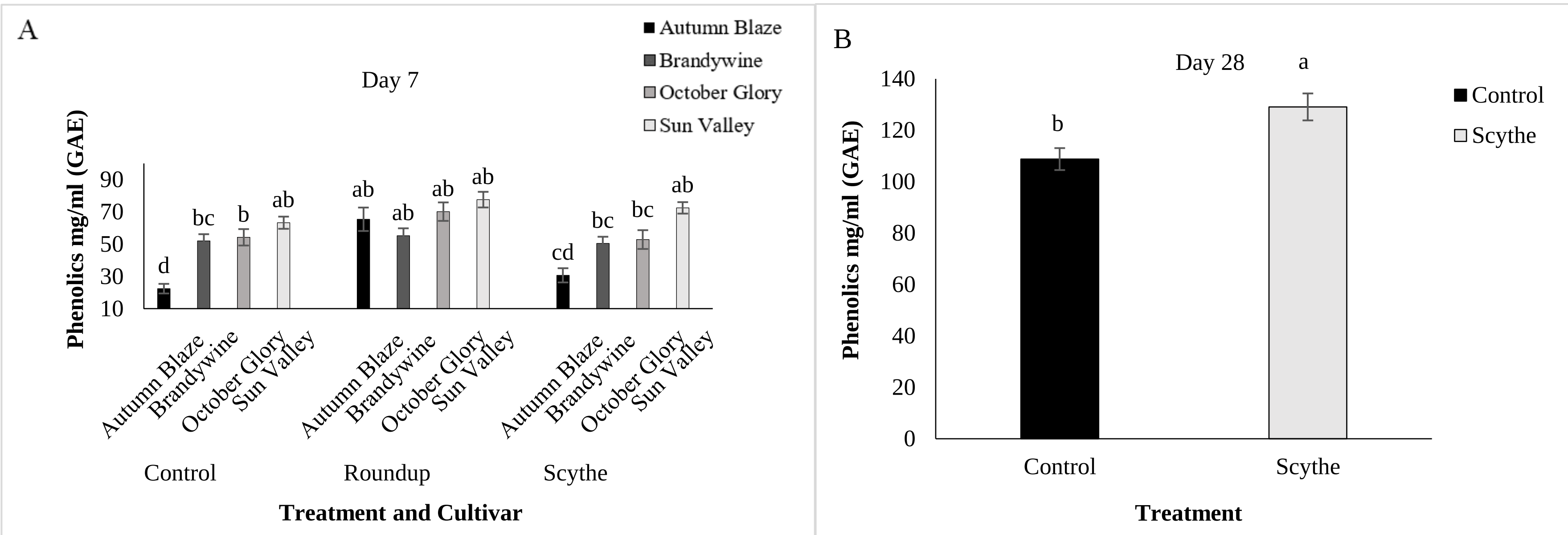
Total Carotenoids (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe only) After Treatment (DAT) (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0.0

Figure 10

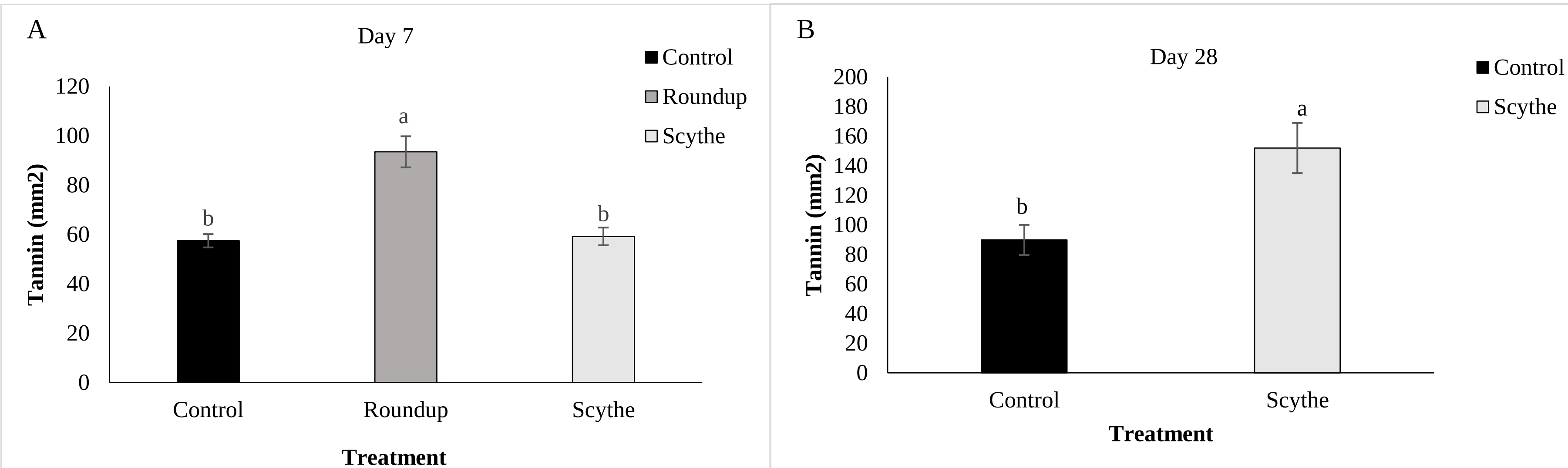
Phenolics Concentration (Mean ± SE) of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe only) After Treatment (DAT) (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey's Honestly Significant Difference Test alpha=0.05.

Figure 11

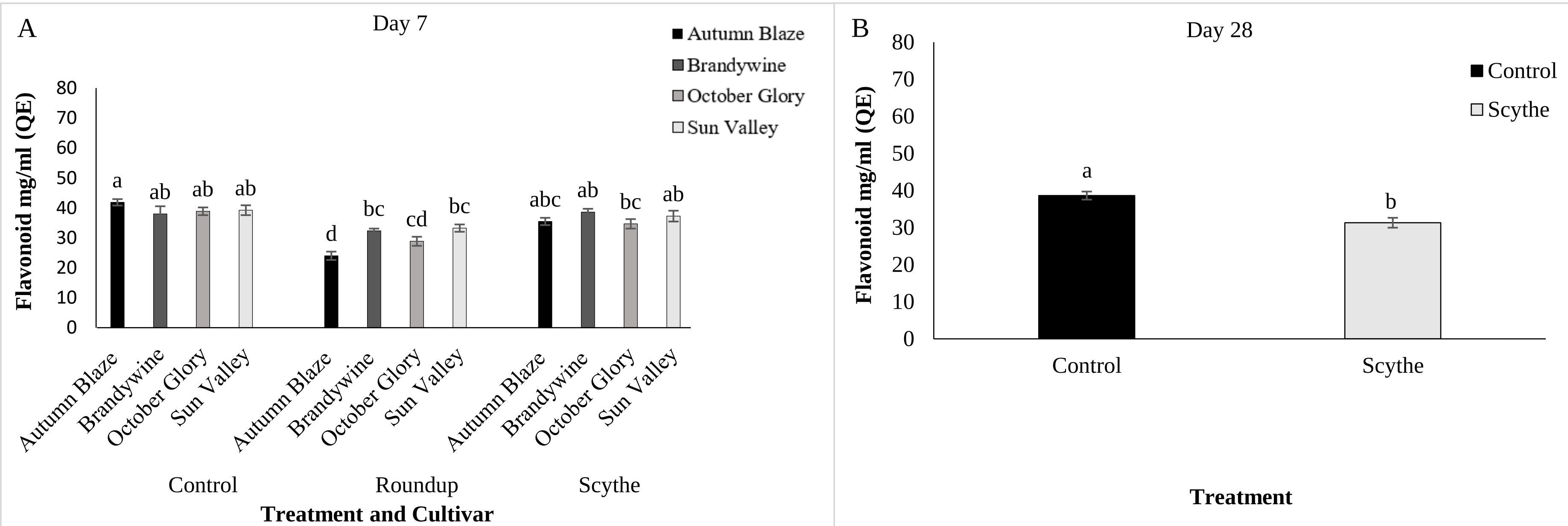
Tannin Concentration (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe Only) After Treatment (DAT) (B)



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0.05

Figure 12

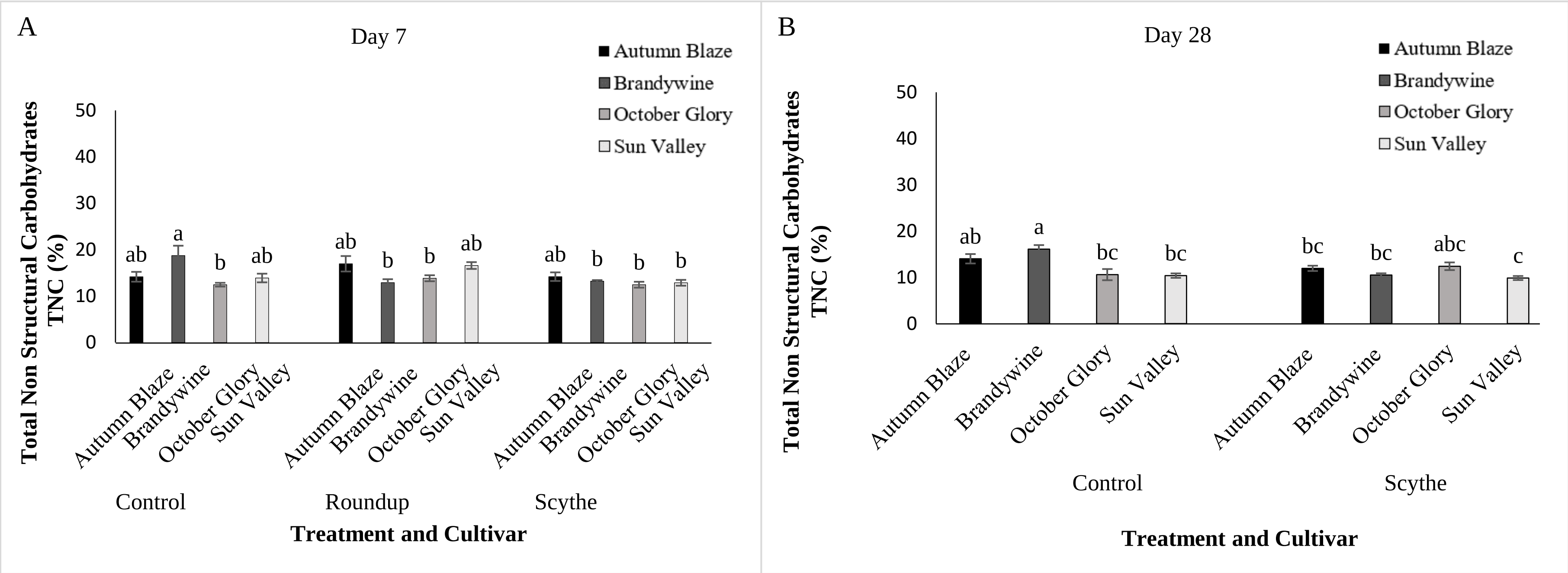
Flavonoid Concentration (Mean ± SE) of *Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 7 Days (Roundup or Scythe) After Treatment (DAT)(A)* and *28 Days (Scythe Only) After Treatment (DAT) (B)*



Note. Bars marked with different lowercase letters are significantly different based on Tukey's Honestly Significant Difference Test at alpha=0.05

Figure 13

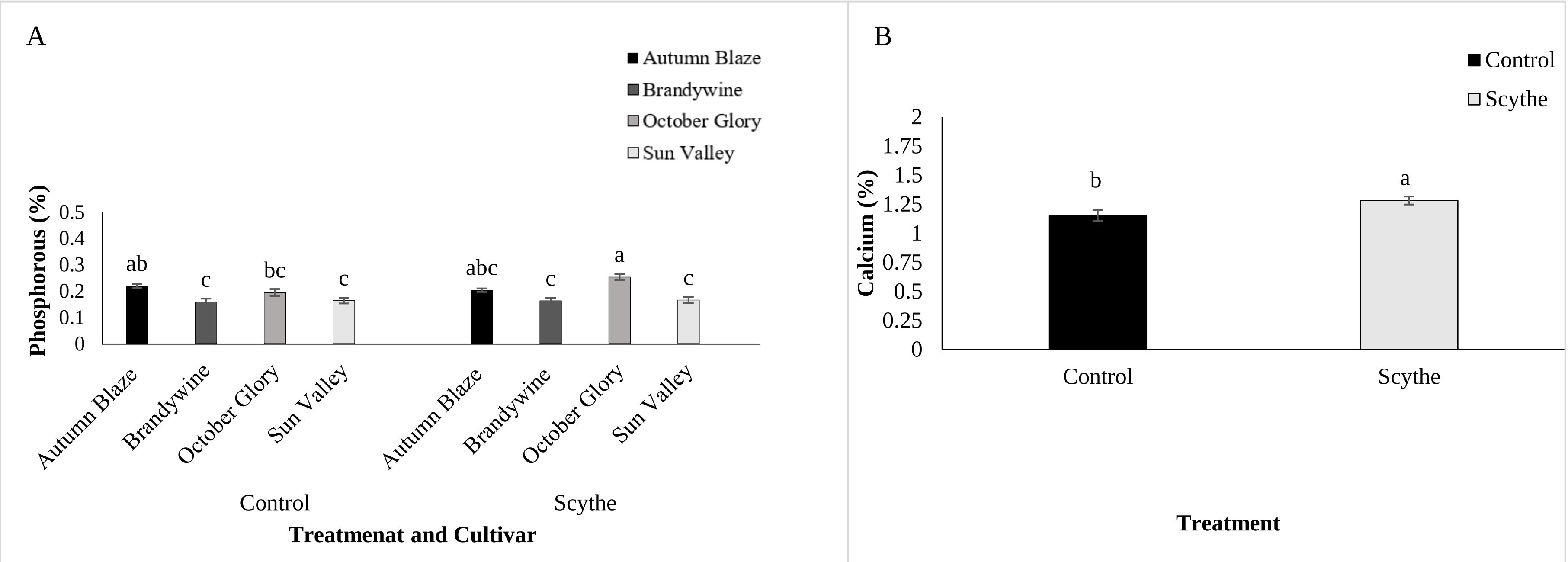
Total Non-Structural Carbohydrates (Mean ± SE) of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 7 Days (Roundup or Scythe) After Treatment (DAT) (A) and 28 Days (Scythe Only) After Treatment (DAT) (B)

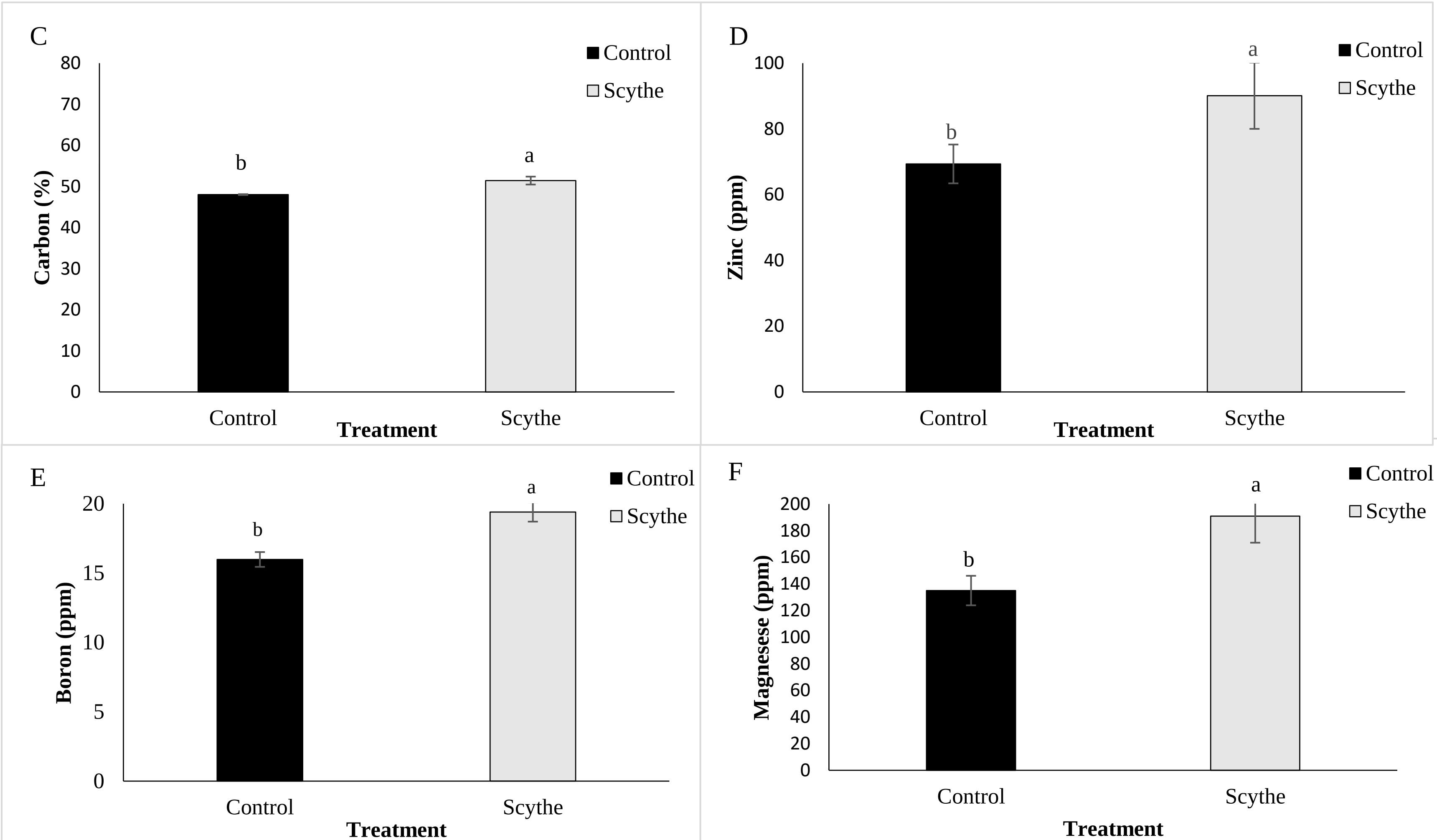


Note. Bars marked with different lowercase letters are significantly different based on Tukey's Honestly Significant Difference Test at alpha=0.05

Figure 14

Concentration (Mean ± SE) of Phosphorous (A), Calcium (B), Carbon (C), Zinc (D), Manganese (E), and Boron (F) in Bark Tissue of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 28 Days After Herbicide (Scythe) Treatment (DAT)

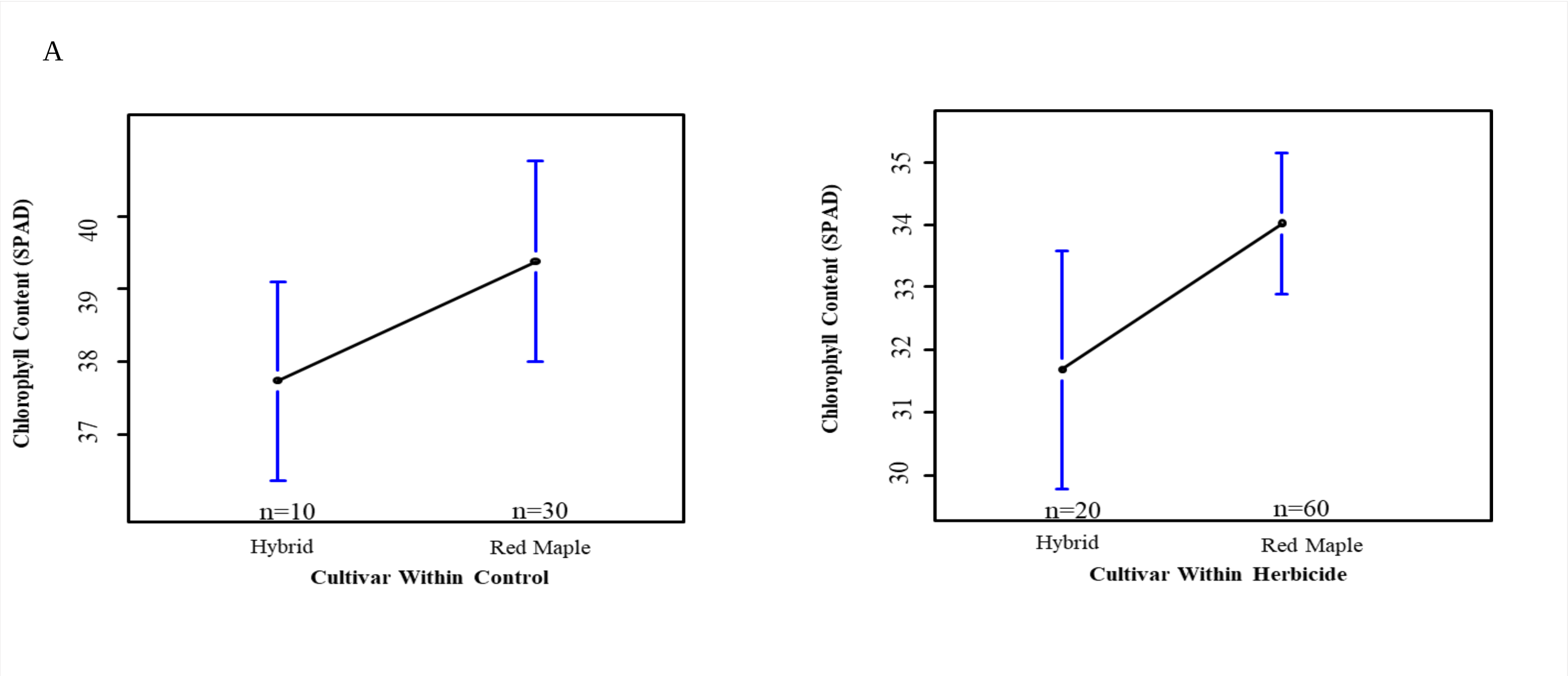




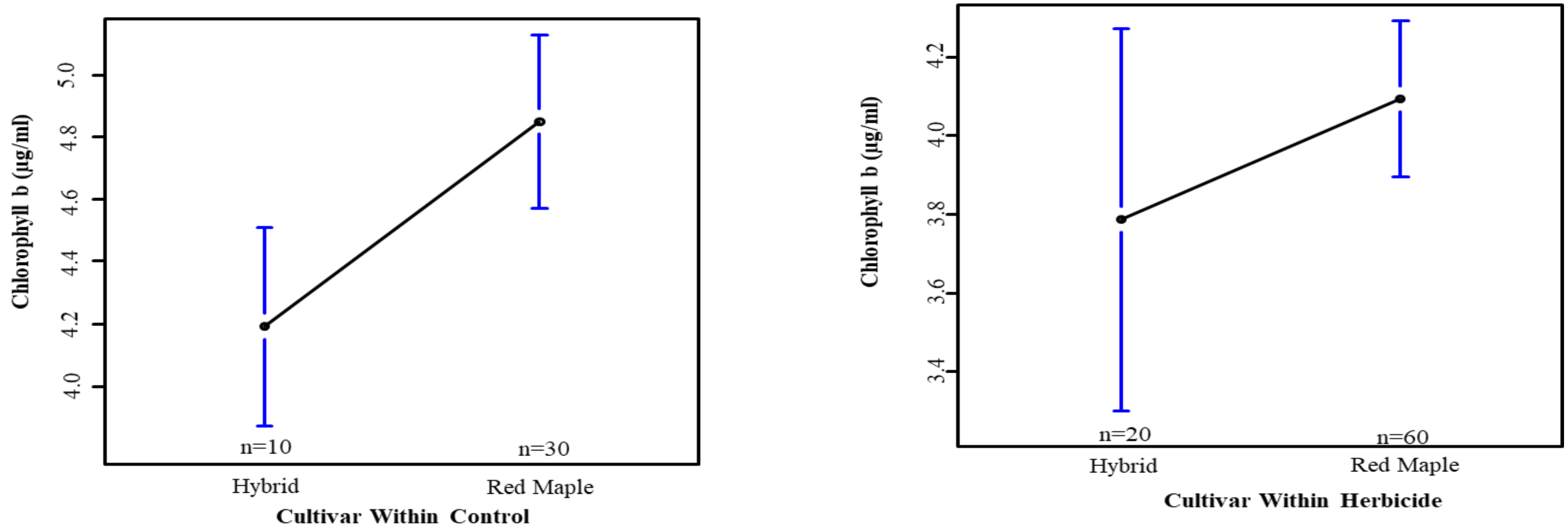
Note. Bars marked with different lowercase letters are significantly different based on Tukey's Honestly Significant Difference Test at alpha=0.05

Figure 15

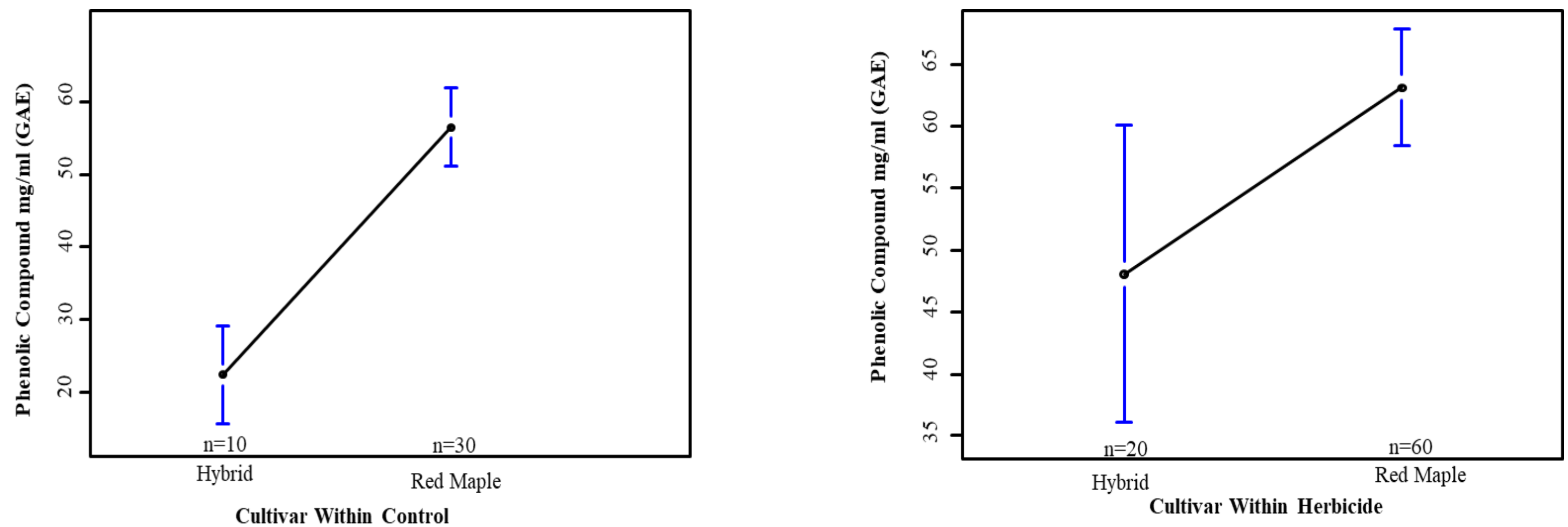
Contrast Analysis of Chlorophyll Content (A), Chlorophyll b (B), Phenolics (C), Flavonoid (D), Total Non-Structural Carbohydrates (E), Catalase (F), and Superoxide Dismutase (G) (Mean ± SE) of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 7 Days After Treatment (DAT) within Untreated Treated Condition (Control) and within Herbicide Treated Conditions (Scythe)

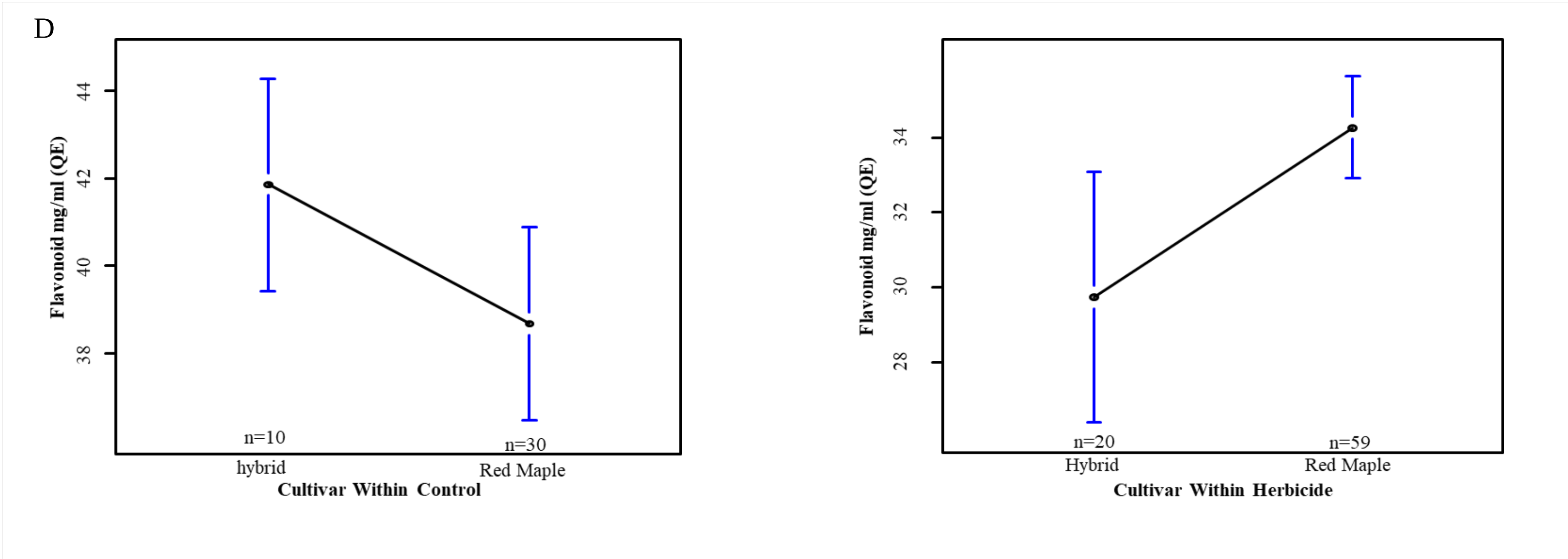


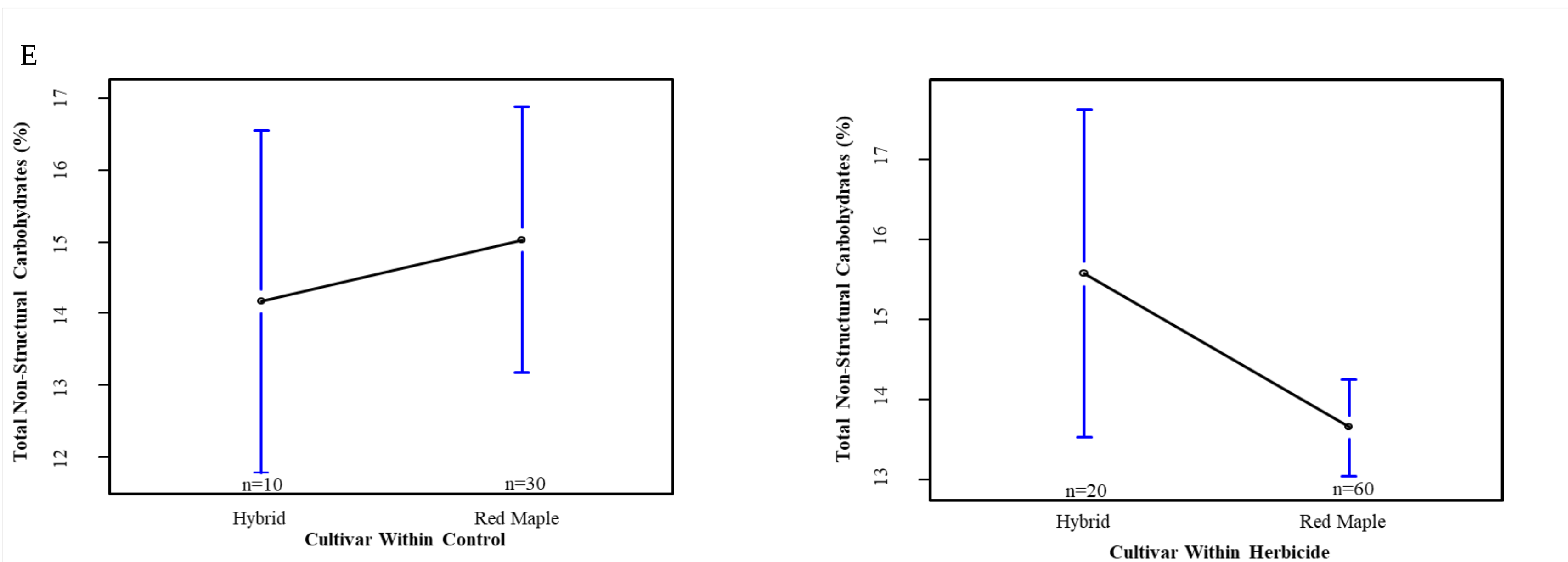
B


Chlorophyll b (μg/ml)
4.0
4.2
4.4
4.6
4.8
5.0
n=10
n=30
Hybrid
Red Maple
Cultivar Within Control
Chlorophyll b (μg/ml)
3.4
3.6
3.8
4.0
4.2
n=20
n=60
Hybrid
Red Maple
Cultivar Within Herbicide

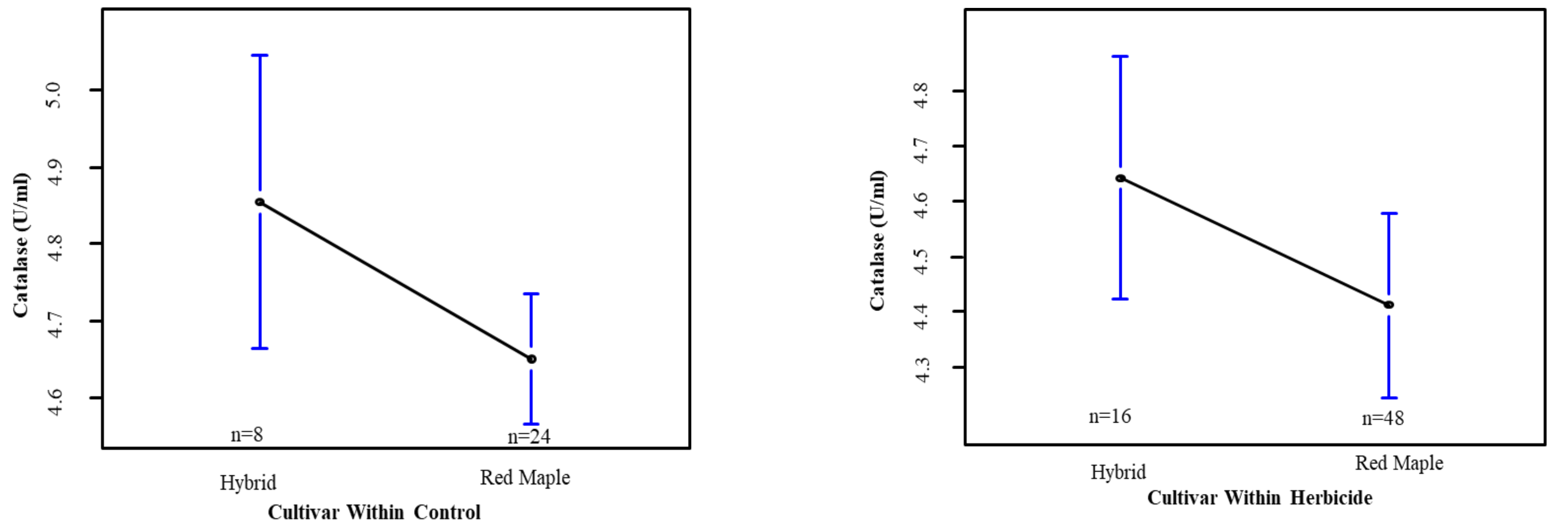
C


Phenolic Compound mg/ml (GAE)
20
30
40
50
60
n=10
n=30
Hybrid
Red Maple
Cultivar Within Control
Phenolic Compound mg/ml (GAE)
35
40
45
50
55
60
65
n=20
n=60
Hybrid
Red Maple
Cultivar Within Herbicide


D
Flavonoid mg/ml (QE)
38
40
42
44
n=10
n=30
hybrid
Red Maple
Cultivar Within Control
Flavonoid mg/ml (QE)
28
30
32
34
n=20
n=59
Hybrid
Red Maple
Cultivar Within Herbicide


E
Total Non-Structural Carbohydrates (%)
12
13
14
15
16
17
n=10
n=30
Hybrid
Red Maple
Cultivar Within Control
Total Non-Structural Carbohydrates (%)
13
14
15
16
17
n=20
n=60
Hybrid
Red Maple
Cultivar Within Herbicide

F


Catalase (U/ml)
4.6
4.7
4.8
4.9
5.0
n=8
n=24
Hybrid
Red Maple
Cultivar Within Control
Catalase (U/ml)
4.3
4.4
4.5
4.6
4.7
4.8
n=16
n=48
Hybrid
Red Maple
Cultivar Within Herbicide

G

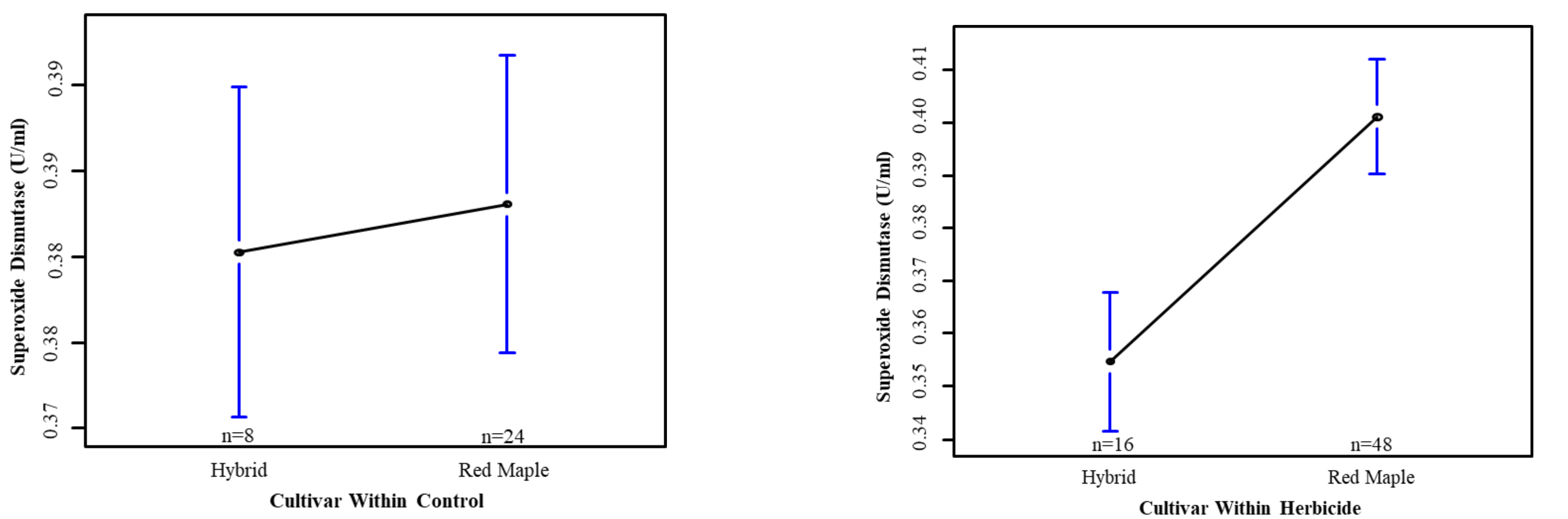
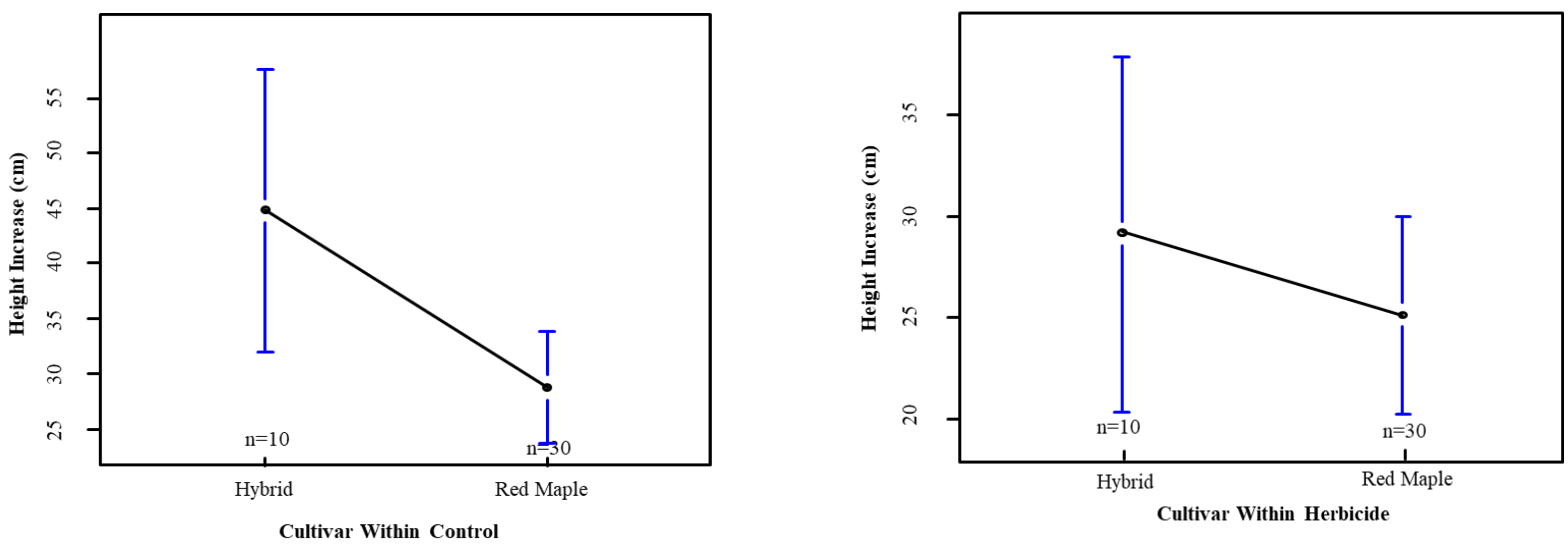

Superoxide Dismutase (U/ml)
0.37
0.38
0.38
0.39
0.39
n=8
n=24
Hybrid
Red Maple
Cultivar Within Control
Superoxide Dismutase (U/ml)
0.34
0.35
0.36
0.37
0.38
0.39
0.40
0.41
n=16
n=48
Hybrid
Red Maple
Cultivar Within Herbicide

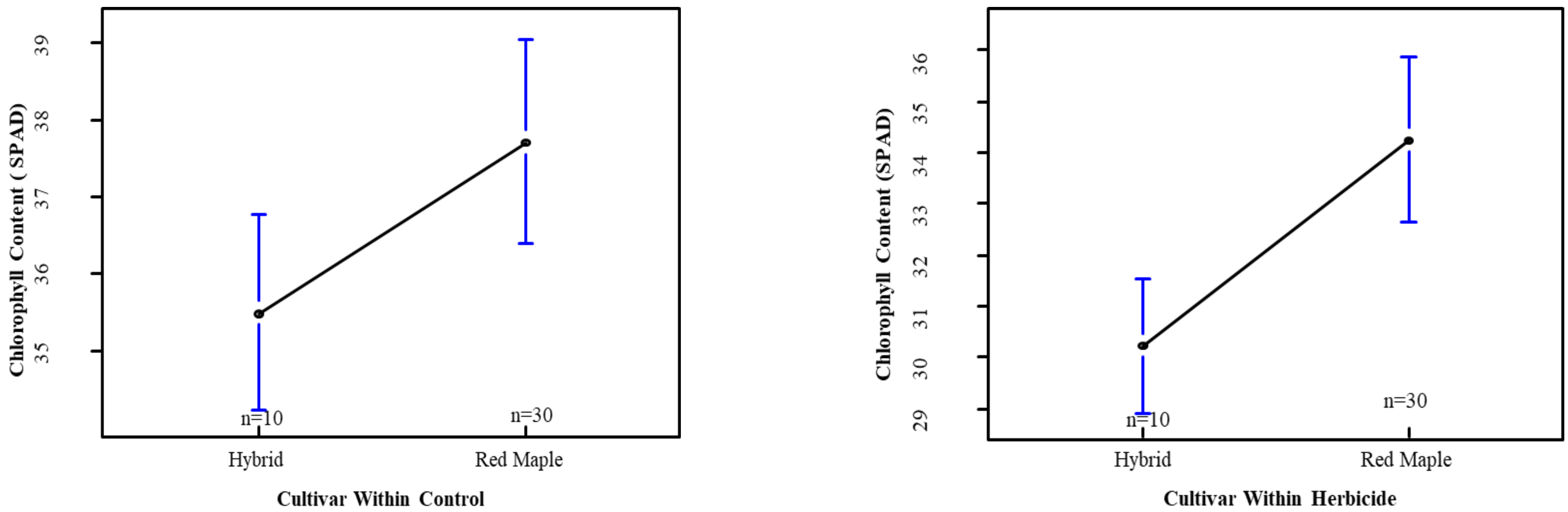
Figure 16

Contrast Analysis of Plant Growth, Chlorophyll Content, and Flavonoid of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') on 28 Days After Treatment (DAT) Measured within Untreated Treated Condition (Control) and within Herbicide Treated Conditions (Scythe)

A



B


Chloropholl Content (SPAD)
39
38
37
36
35
n=10
n=30
Hybrid
Red Maple
Cultivar Within Control
Chlorophyll Content (SPAD)
36
35
34
33
32
31
30
29
n=10
n=30
Hybrid
Red Maple
Cultivar Within Herbicide

C

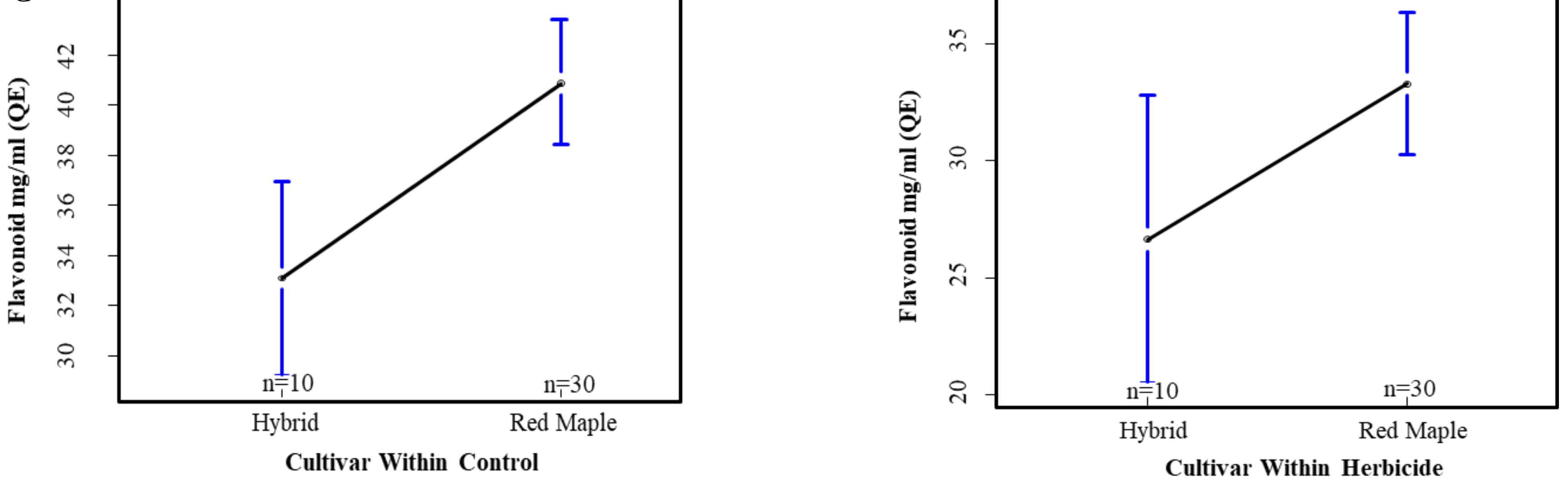
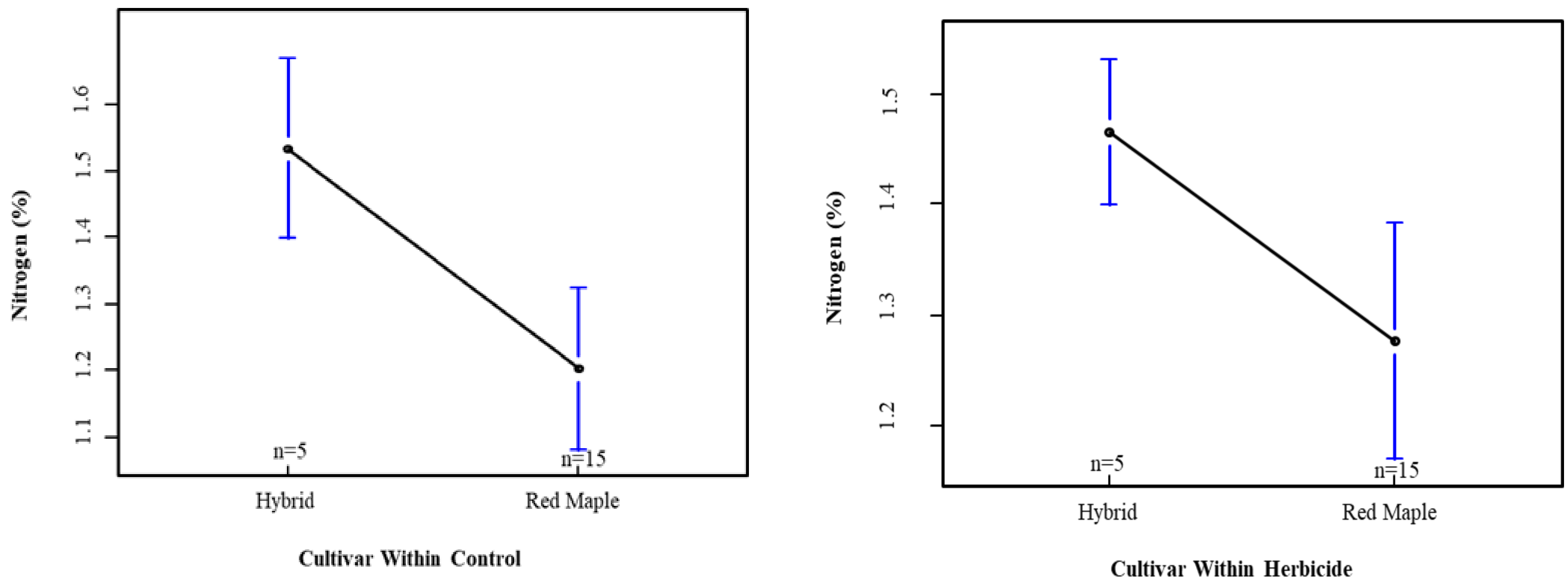
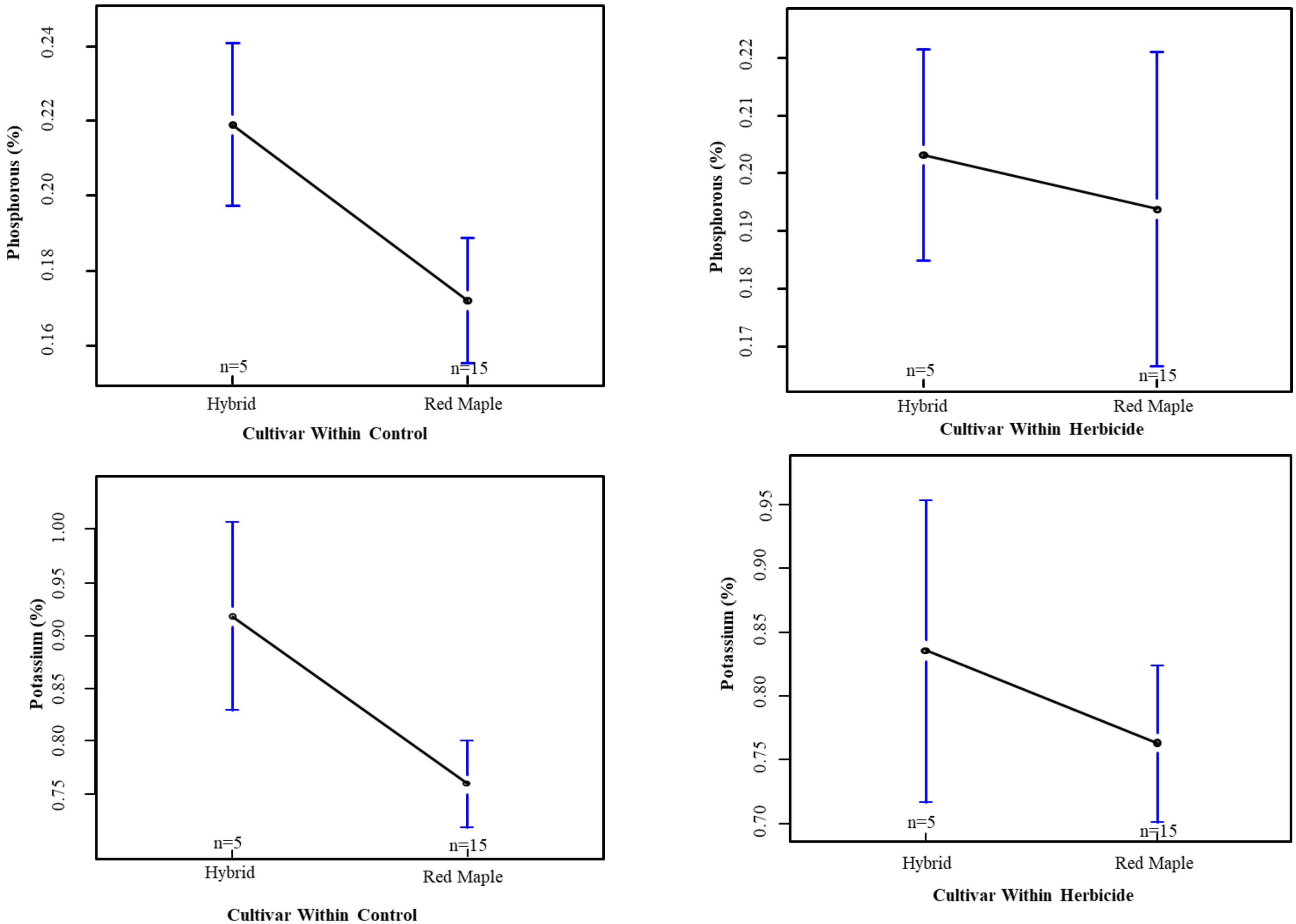
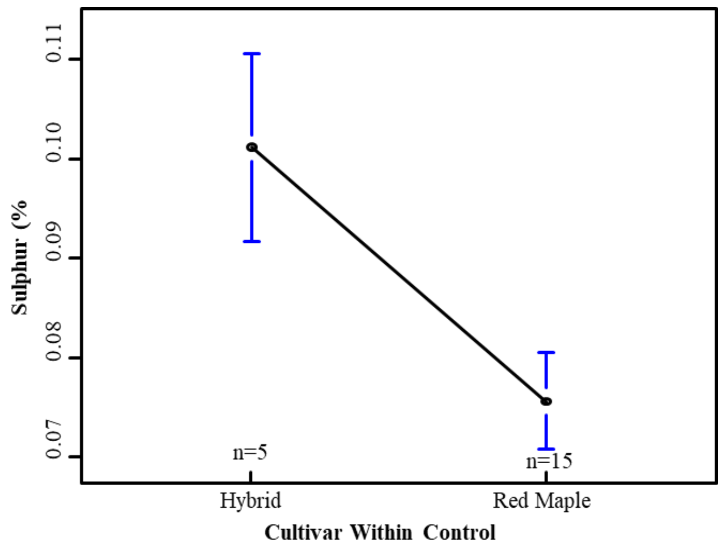

Flavonoid mg/ml (QE)
42
40
38
36
34
32
30
n=10
n=30
Hybrid
Red Maple
Cultivar Within Control
Flavonoid mg/ml (QE)
35
30
25
20
n=10
n=30
Hybrid
Red Maple
Cultivar Within Herbicide

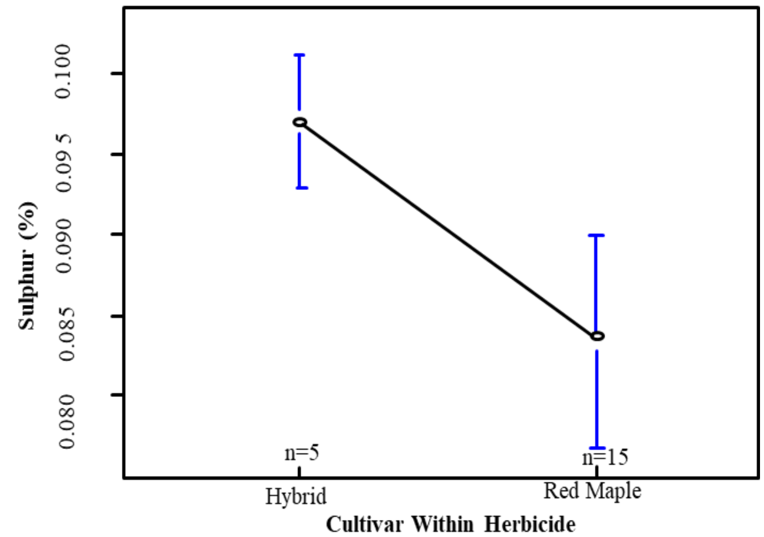
Figure 17

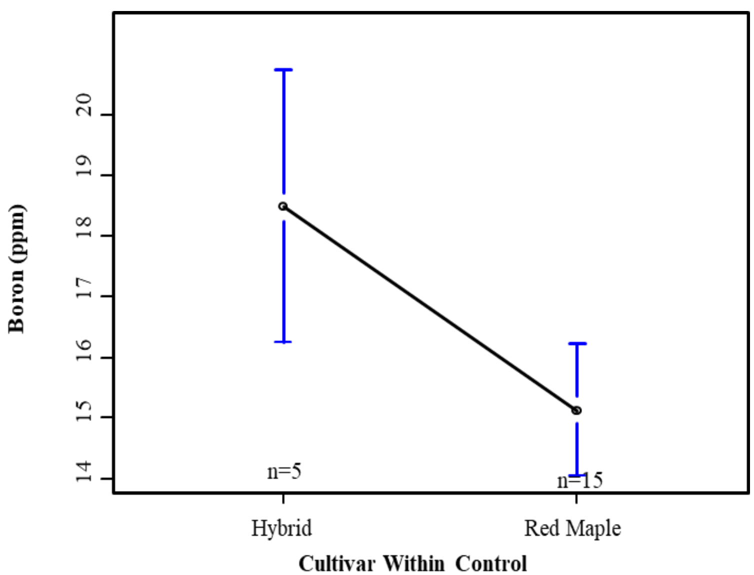
Contrast Analysis of Bark Mineral Nutrient Concentration of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', and 'Sun Valley') Measured on 28 Days After Treatment Measured within Untreated Treated Condition (Control) and within Herbicide Treated Conditions (Scythe)

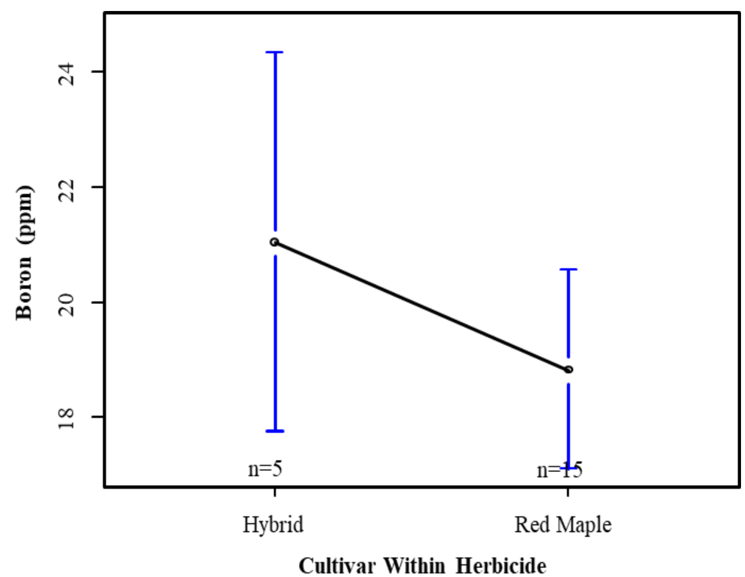


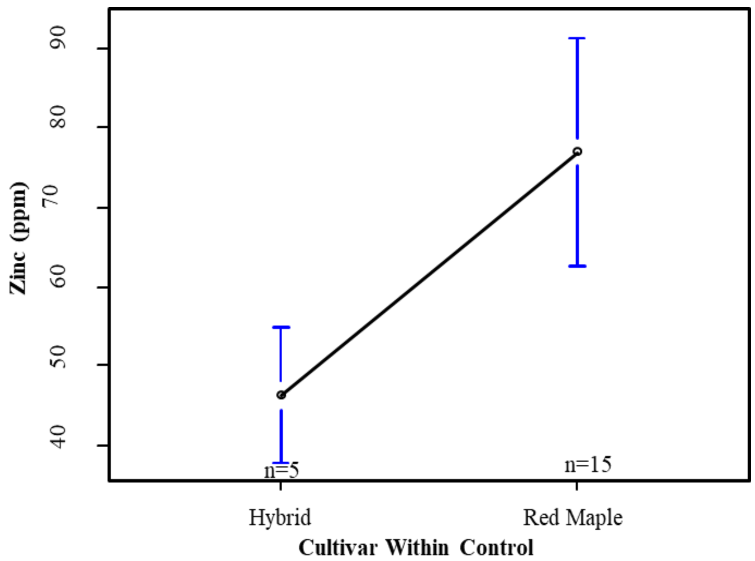

Phosphorous (%)
0.16
0.18
0.20
0.22
0.24
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control
Phosphorous (%)
0.17
0.18
0.19
0.20
0.21
0.22
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide
Potassium (%)
0.75
0.80
0.85
0.90
0.95
1.00
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control
Potassium (%)
0.70
0.75
0.80
0.85
0.90
0.95
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide

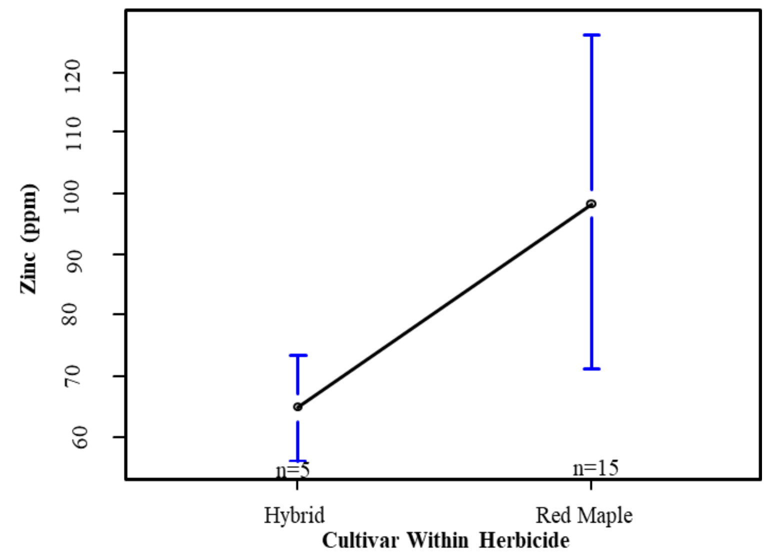

Sulphur (%
0.07
0.08
0.09
0.10
0.11
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control

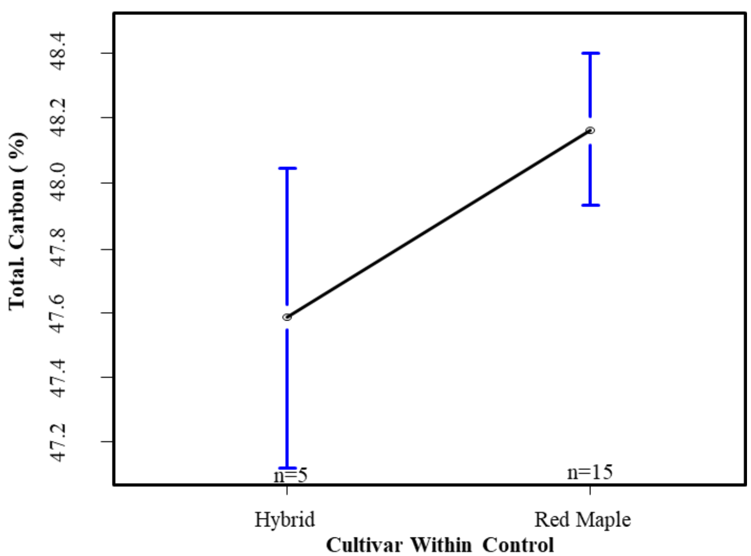

Sulphur (%)
0.080
0.085
0.090
0.09 5
0.100
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide

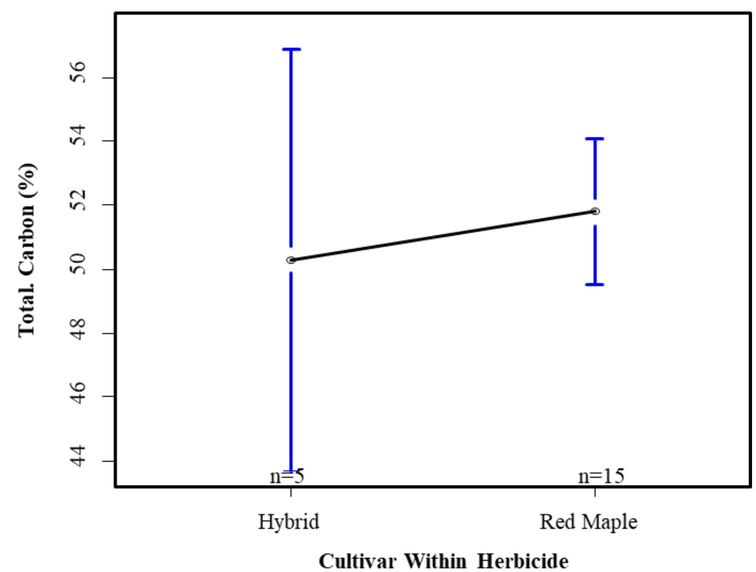

Boron (ppm)
14
15
16
17
18
19
20
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control

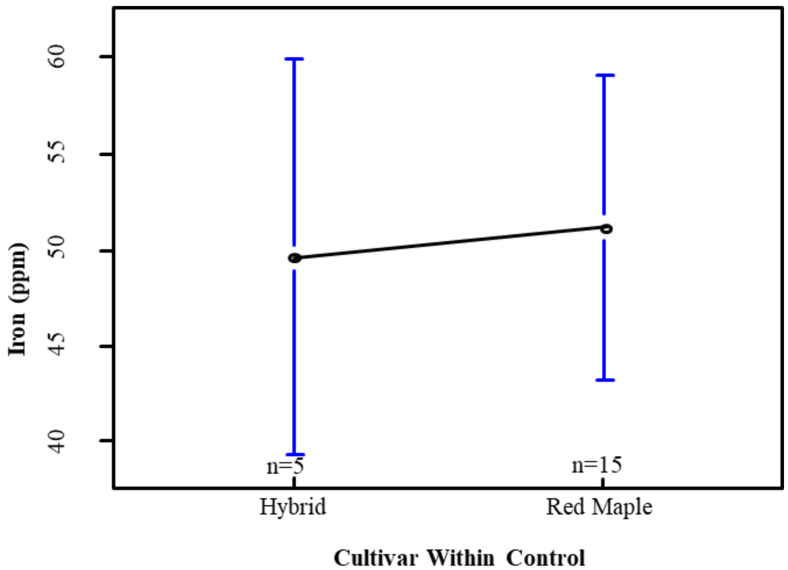

Boron (ppm)
18
20
22
24
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide

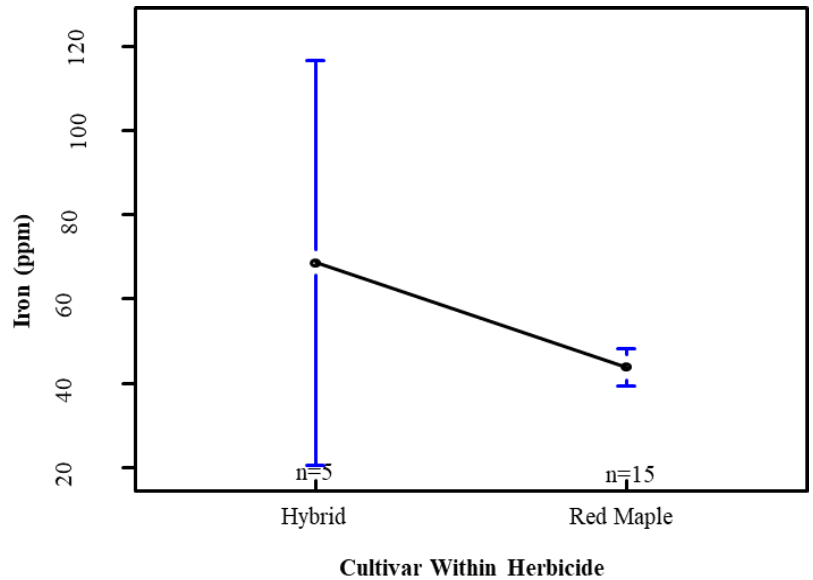

Zinc (ppm)
40
50
60
70
80
90
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control

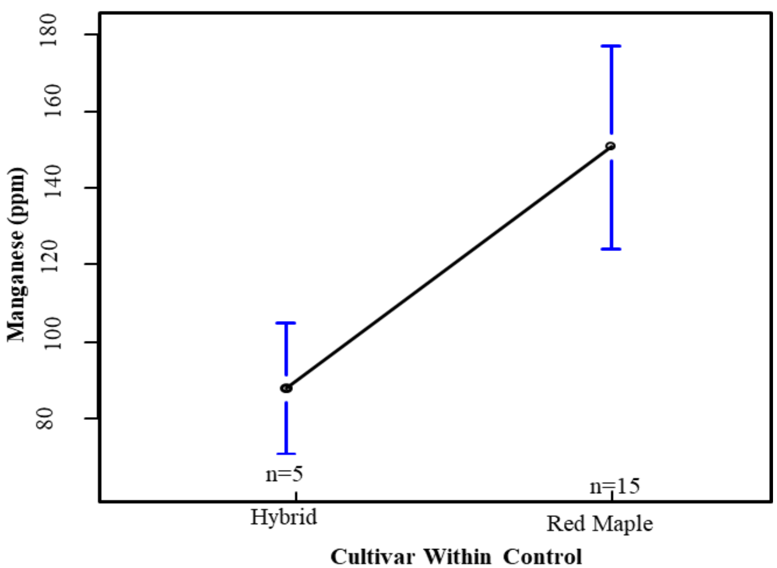

Zinc (ppm)
60
70
80
90
100
110
120
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide


Total Carbon (%)
47.2
47.4
47.6
47.8
48.0
48.2
48.4
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control


Total Carbon (%)
44
46
48
50
52
54
56
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide


Iron (ppm)
40
45
50
55
60
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control


Iron (ppm)
20
40
60
80
100
120
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide


Manganese (ppm)
80
100
120
140
160
180
n=5
n=15
Hybrid
Red Maple
Cultivar Within Control

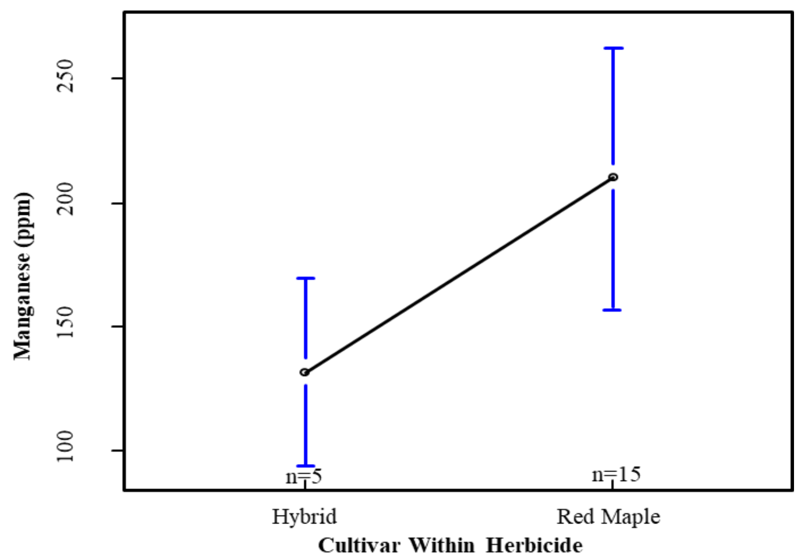
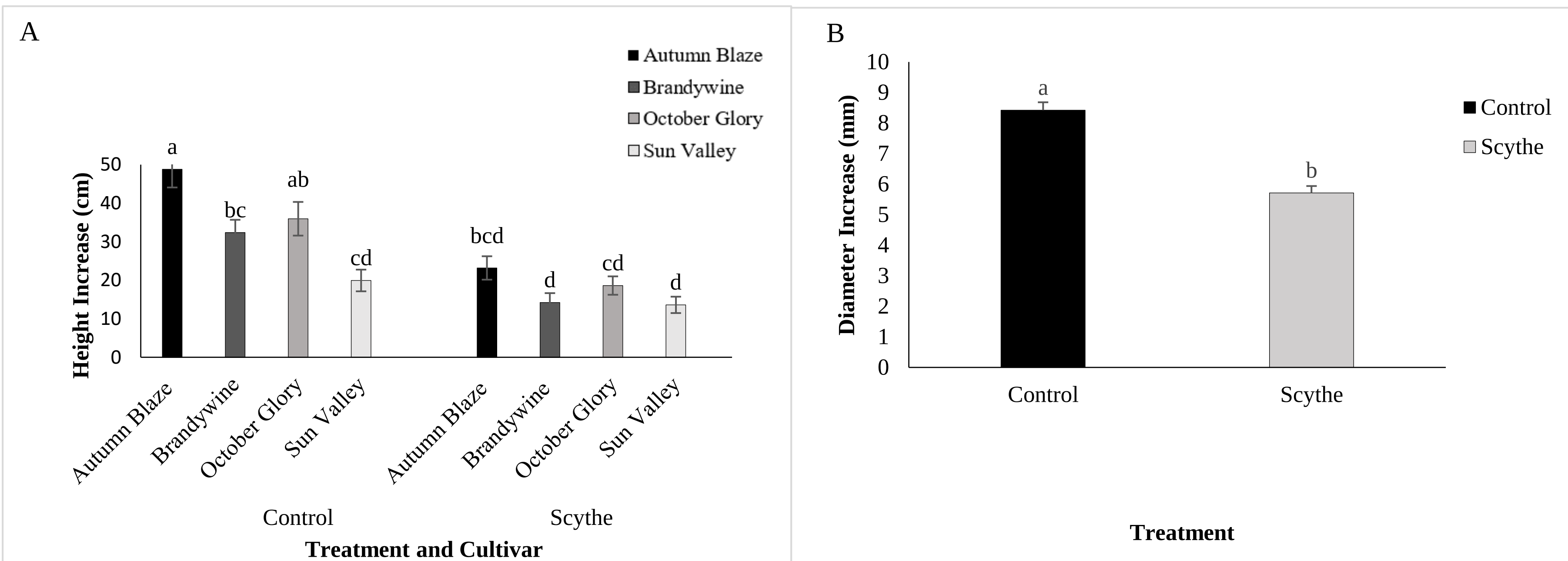

Manganese (ppm)
100
150
200
250
n=5
n=15
Hybrid
Red Maple
Cultivar Within Herbicide

Figure 18

Plant Height (A) and Trunk Diameter (B) Growth (Mean ± SE) of Hybrid (‘Autumn Blaze’) and Red Maple Cultivars (‘Brandywine’, ‘October Glory’, and ‘Sun Valley’) Treated with Scythe Under Field Conditions



Note. Bars marked with different lowercase letters are significantly different based on Tukey’s Honestly Significant Difference Test at alpha=0

Figure 19

Borer Infestation (Deviation) of Hybrid ('Autumn Blaze') and Red Maple Cultivars ('Brandywine', 'October Glory', And 'Sun Valley') Treated with Scythe under Field Conditions

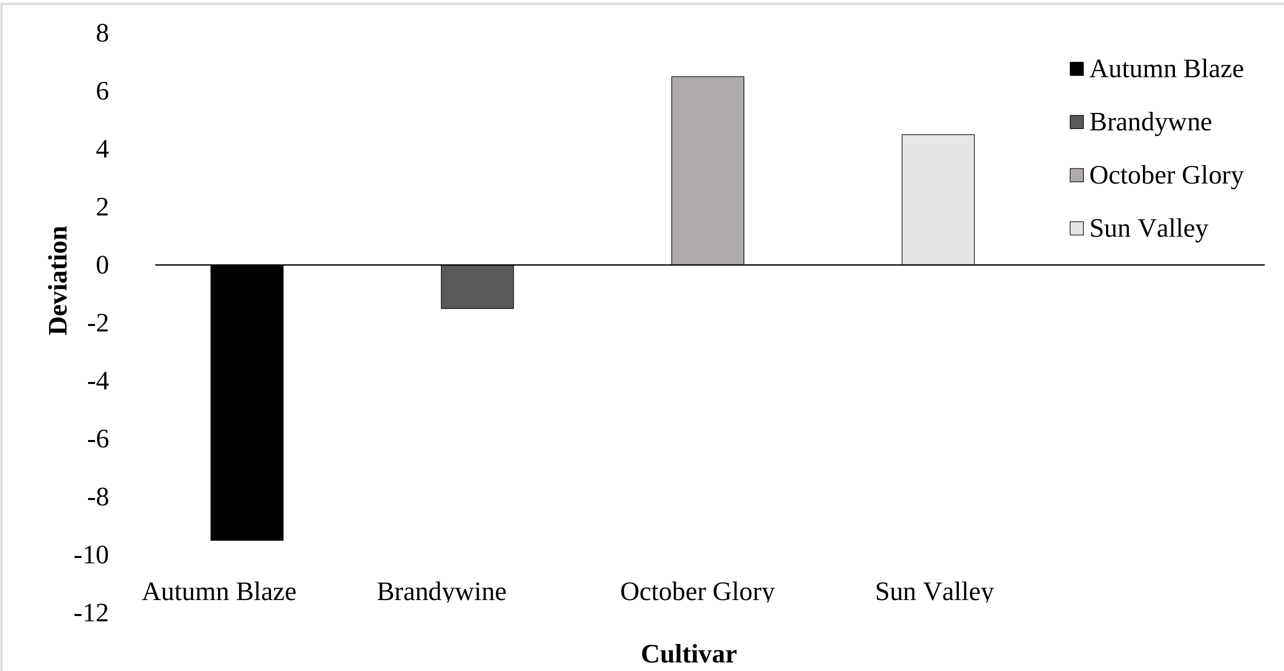


Figure 20

Total Buprestids Captured by Genera in All Traps Throughout the Season in Summer 2021 in Middle Tennessee

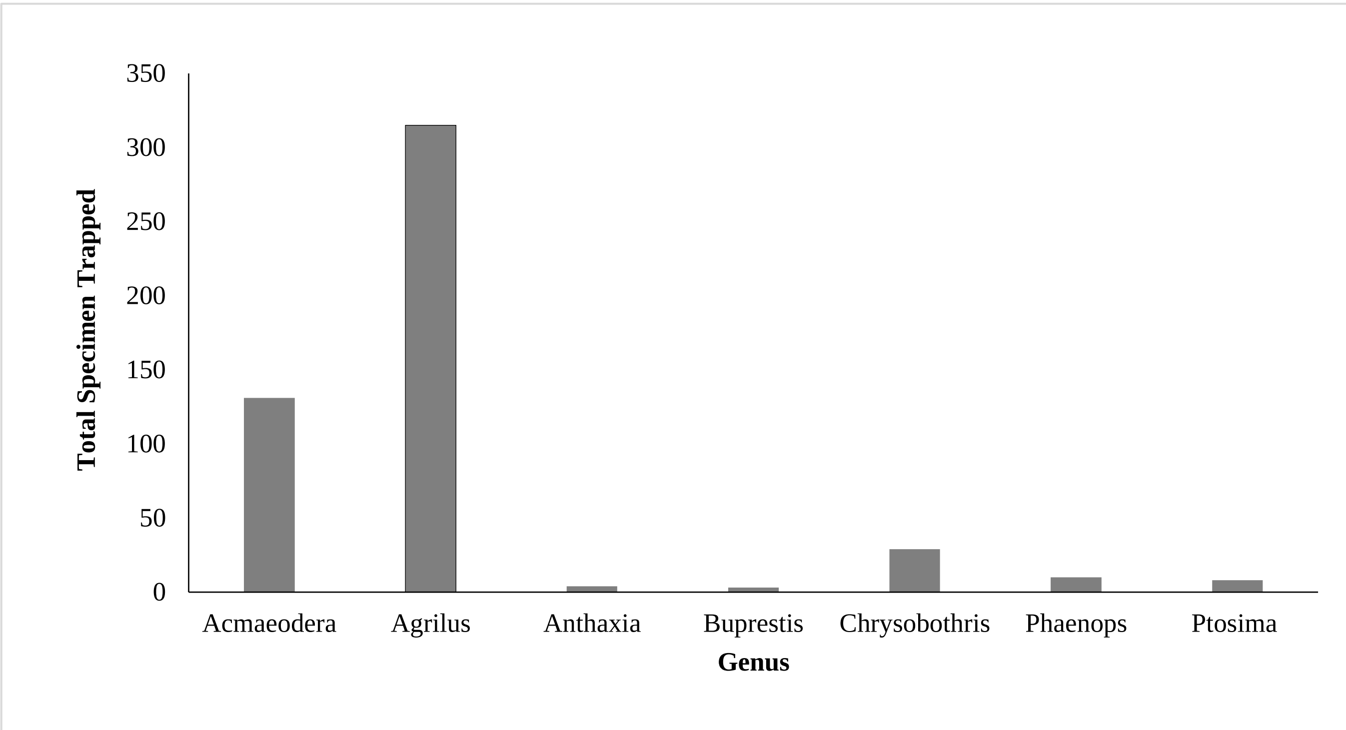


Figure 21

Total number of Chrysobothris (A) and Agrilus (B) Species Captured in All Traps Throughout the Season in Summer 2021 in Middle Tennessee

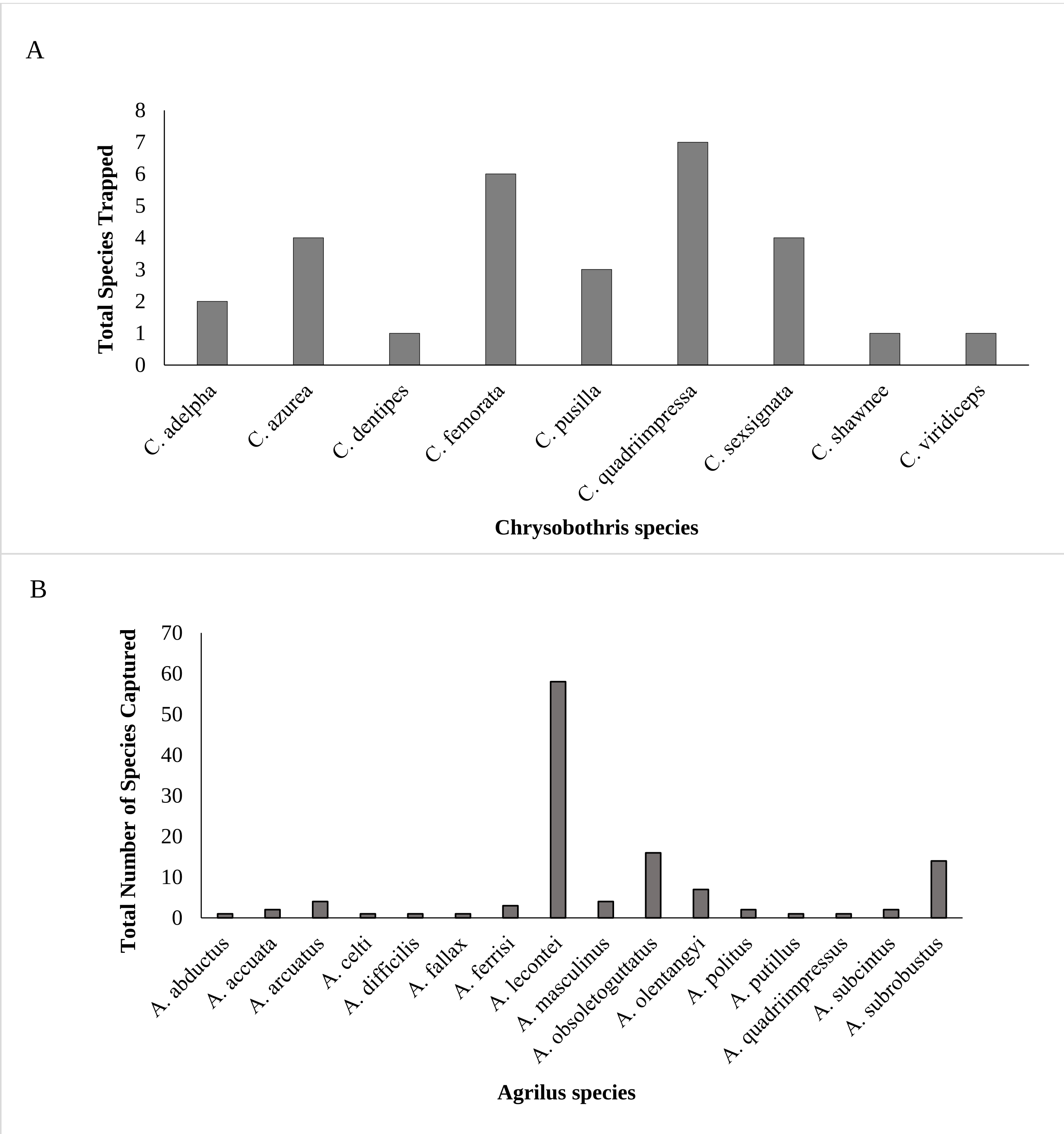


Figure 22

Total Buprestid Captured by Genera in All Traps Throughout the Season in Summer 2022 in Middle Tennessee

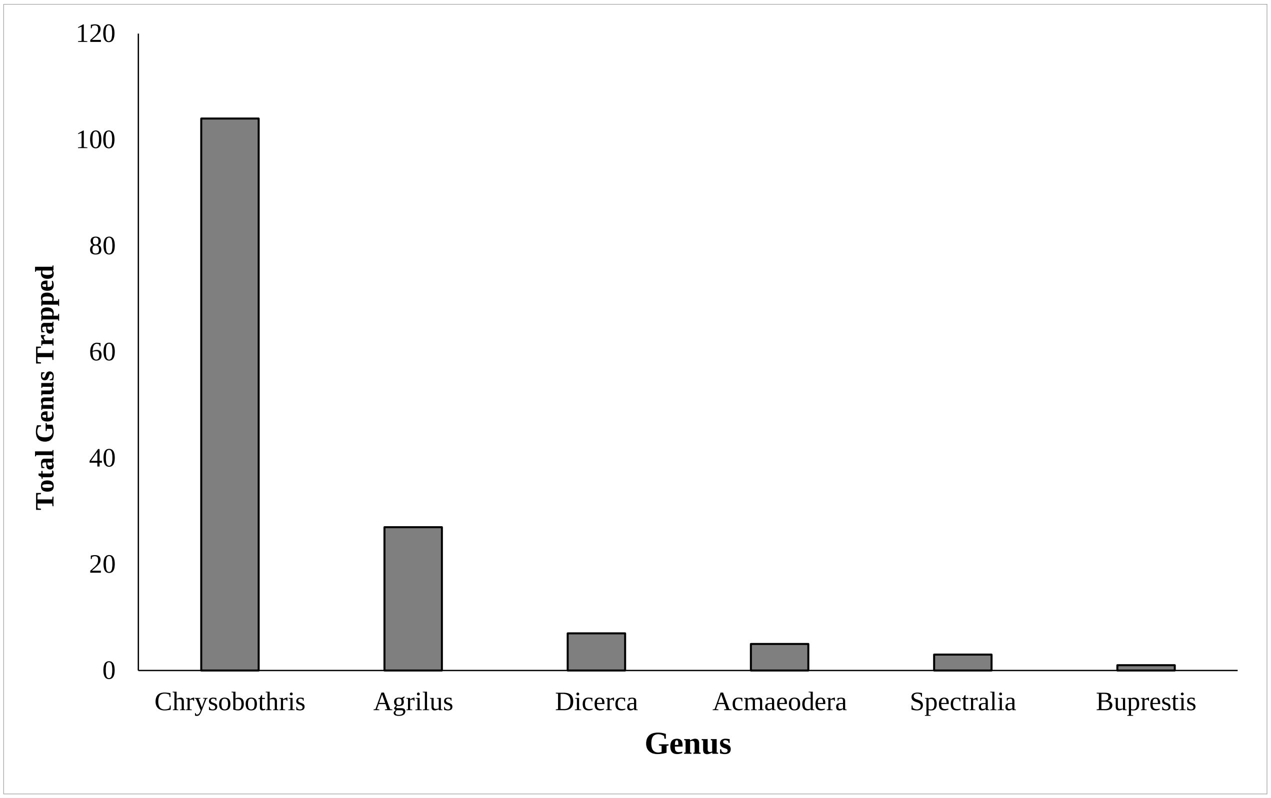


Figure 23

Total Number of Chrysobothris (A) and Agrilus (B) Species Captured in All Traps Throughout the Season in Summer 2022 in Middle Tennessee

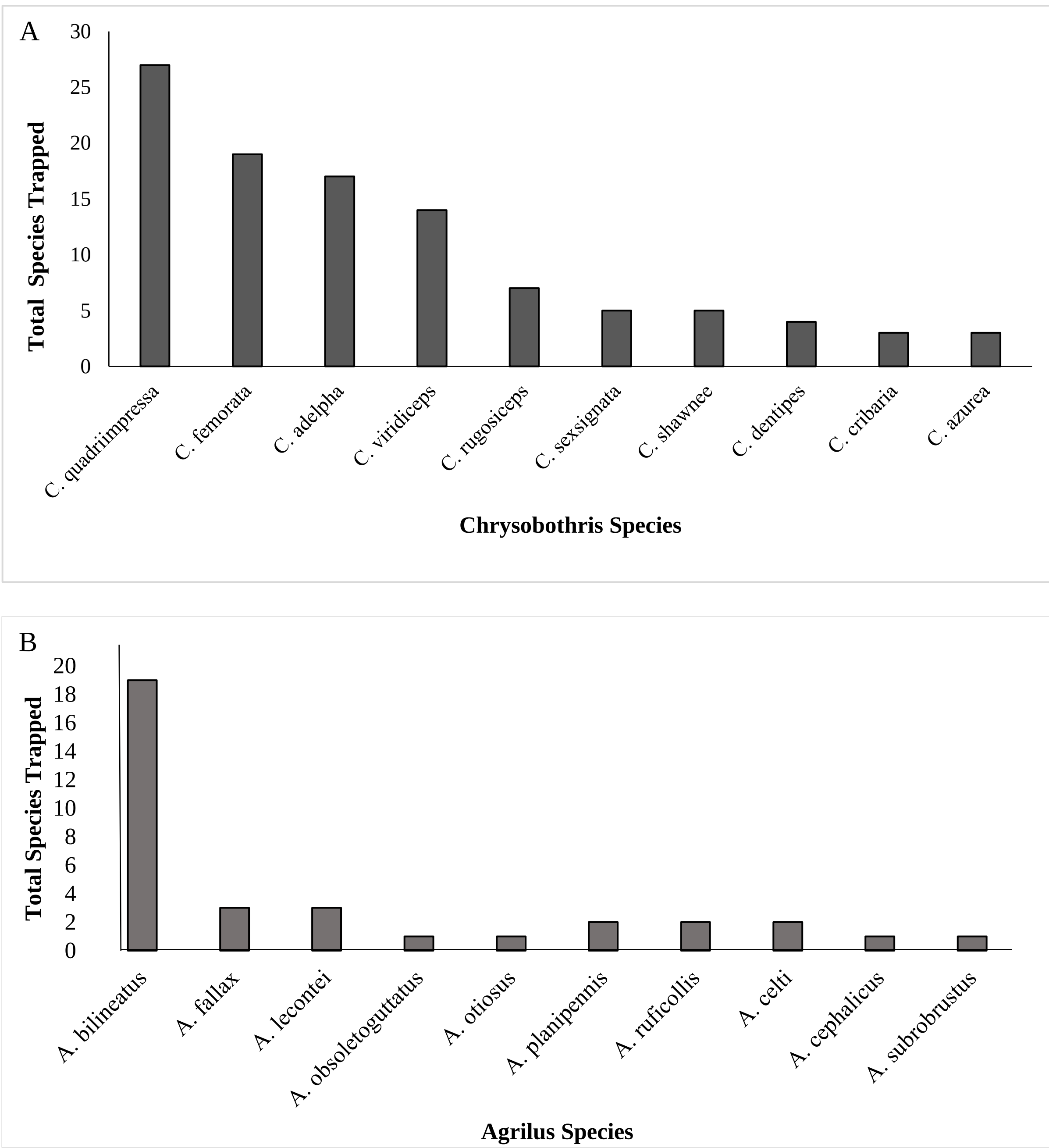


Figure 24

Total Number of Chrysobothris and Agrilus Species Captured in Traps Baited with Green Leaf Volatiles Throughout the Season in Summer 2022 in Middle Tennessee

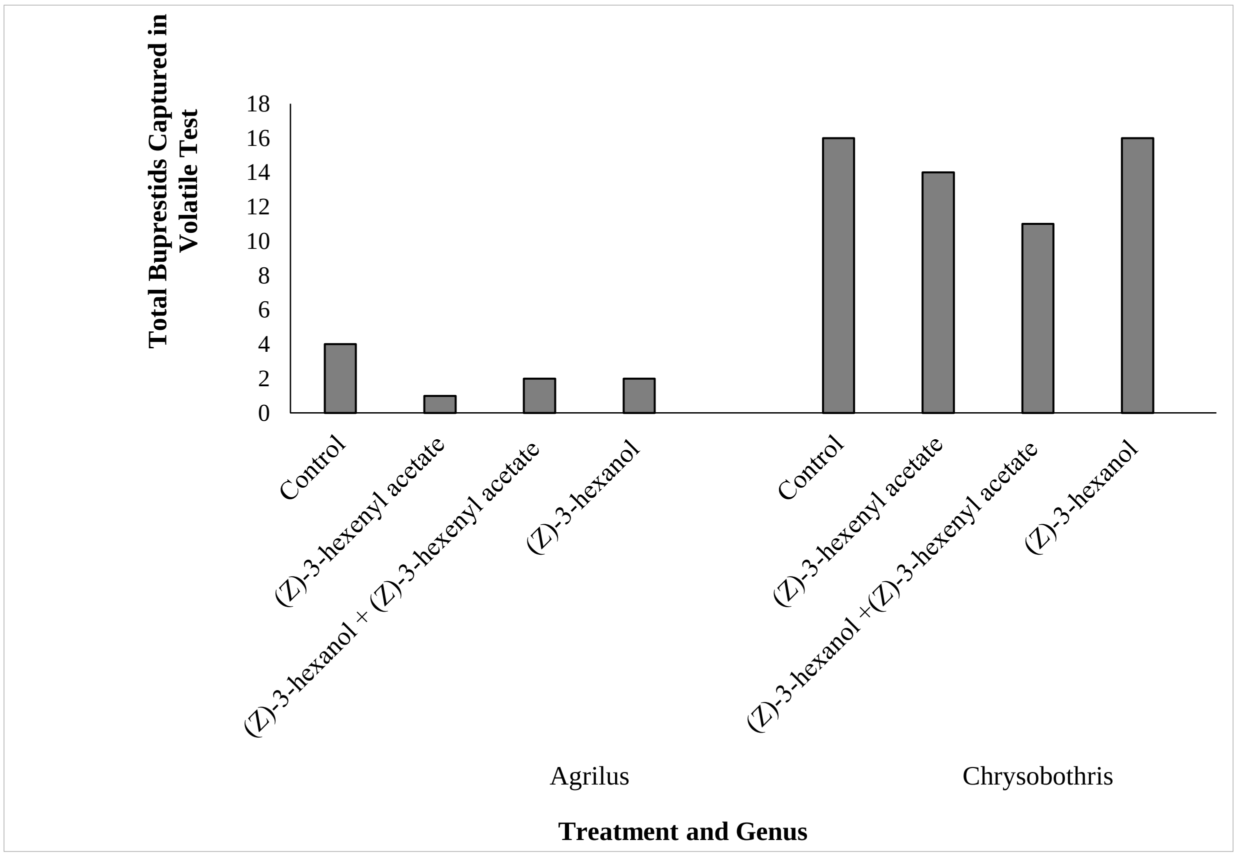
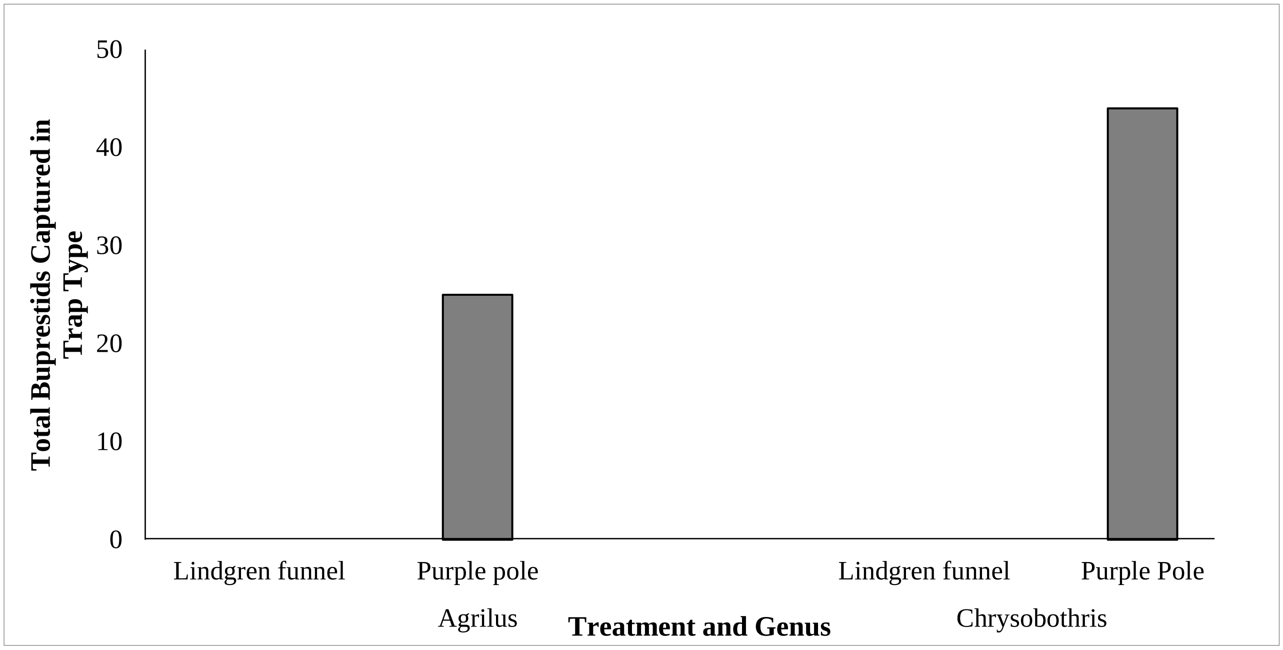


Figure 25

Total Number of Chrysobothris and Agrilus Species Captured in Sticky Purple Pole Traps Throughout the Season in Summer 2022 in Middle Tennessee



CITI Certificate

CITI PROGRAM

Completion Date 19-May-2022
Expiration Date 18-May-2026
Record ID 49026313

This is to certify that:

Asmita Gautam

Has completed the following CITI Program course:

Not valid for renewal of certification through CME.

CITI Conflicts of Interest
(Curriculum Group)
Group 2: Students and Lab Personnel
(Course Learner Group)
1 - Basic Course
(Stage)

Under requirements set by:

Tennessee State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?wb9a3ea66-0bc1-46b0-b926-462218a289b4-49026313

Curriculum Vitae

Asmita Gautam

I am an agriculture scientist with an interest in chemical ecology and entomology. I am a dedicated, hardworking, and proactive researcher with work experience in plant sciences focusing on integrated pest management, plant physiology, and biochemistry, and a specialty in ornamental crop production and management in both controlled and field environments. I am a critical thinker, team player, artistic, and ambitious, a person who works diligently to design experiments to test complex hypotheses and rigorously analyze and interpret data. I can adapt and evolve when working on a broad range of topics with individuals from various backgrounds. I have a strong ability to communicate research in both scientific and extension/outreach settings.

Education

Master's degree. Department of Agricultural and Environmental Sciences, Tennessee State University, Start date: August 2021. Expected Graduation date: December 2023. Current GPA: 4.0

Bachelor of Science in Agriculture. Agriculture and Forestry University Rampur, Chitwan, Nepal. Graduated: 2018. GPA: 3.74

Research and Professional Experience

Graduate Research Assistant (August 2021 to present) Department of Agricultural and Environmental Sciences, Tennessee State University, Nursery Research Center, McMinnville, TN, USA.

Research Assistant (Jan-July 2020) Nepal Agricultural Research Council (NARC), Regional Agricultural Research Station, Plant Pathology Unit, Khajura, Nepal.

Agriculture Assistant Instructor (Feb-Nov 2019) Kerwani Secondary School, Devdaha, Rupandehi, Nepal.

Part-time Agriculture Instructor (Jan- April 2019) Deurali Janata Prabidhik Shikshyalaya, Butwal, Rupandehi, Nepal.

Intern (Jan- July 2018) Completed an internship program as an integral part of my Bachelor of Science in Agriculture at Ginger /Turmeric Zone Office, Nepal.

Researcher (Jan-July 2018) Worked as a principal researcher on "Production and Marketing of Ginger in Salyan, Nepal: An economic analysis. "in Ginger/Turmeric Zone Office, Salyan, Nepal.

Agriculture Trainer (Jan- July 2018) Trained local growers from two VDCs in Salyan in commercial ginger cultivation, making ginger candy, and disease and pest management as a Trainer with the Ginger/Turmeric Zone Office in Salyan, Nepal.

Current Research

Gautam, A., Addesso, K. M., Perkovich, C., Oliver J.B., Witcher. A. (two manuscripts in preparation) Effect of herbicide on morpho-physiological and biochemical attributes in one hybrid and three red maple cultivars

Gautam, A., Addesso, K. M., Oliver J.B., Klingeman, W., O'Neal, P. (in progress) Trap development for *Chrysobothris* flatheaded borers in nursery production

Gautam, A., Addesso, K.M., Oliver J.B., O'Neal, P. Riley C., Chase, K. (in progress) Effectiveness of companion planting to protect newly planted landscape trees from flatheaded borers

Publications

Gautam, A., and Addesso, K.M. (in preparation). Common diseases and insect pest of maple and their integrated management. TSU Extension.

Gautam, A., Addesso, K.M., Poudel, A., and Oliver J.B., 2023. Japanese Beetle (*Popillia japonica*). TSU Extension. https://www.tnstate.edu/extension/documents/Japanese%20Beetle%20Factsheet%2001312023.pdf

Poudel, A., Gautam, A., Addesso, K.M., Oliver J., and Roper, G., 2023. Ambrosia beetles important to Tennessee nurseries. TSU Extension. https://www.tnstate.edu/extension/documents/Ambrosia%20beetle%20Factsheet%2001312023.pdf

Grant Writing

Asmita Gautam and Dr. Karla Addesso. Southern Region SARE 2022 Graduate Student Grant. “Identification of plant traits associated with flatheaded apple tree borer susceptible maples” (Unfunded)

Scientific Presentations

Oral presentation, “Purple trap optimization for *Chrysobothris* spp. in middle Tennessee” Multistate Extension Flatheaded Appletree Borer Training Otis L. Floyd Nursery Research Center McMinnville, TN. (03/30/2023)

Oral presentation, “Herbicide stress, and Flatheaded borer attacks in red maple cultivars, “45th Annual University-wide Research Symposium, Tennessee State University (03/28/2023)

Oral presentation, “Herbicide stress and Flatheaded borer attacks: does differential response to stress by maple cultivars explain susceptibility to flatheaded borers? Southeastern Branch, Entomological Society of America, Little Rock, Arkansas. (03/13/2022)

Oral presentation, “Optimization of Flatheaded borer traps through evaluation of shape, type and effective lure components,” 132nd meeting of the Tennessee Academy of Science, Nashville, Tennessee. (10/07/2022)

Oral presentation, “Effect of herbicide on Morpho-physiological and biochemical attributes in red maple cultivar” Entomological Society of America, Entomological Society of Canada, Entomological Society of British Columbia Joint annual meeting, Vancouver, Canada. (11/14/2022)

Oral presentation “Effect of herbicide on physiological and biochemical attributes in red maple cultivar” Tennessee Entomological Society 49th Annual Conference, Knoxville, Tennessee. (11-18-2022)

Virtual poster presentation, “Companion planting to protect newly planted landscape trees from borers”, Southeastern Branch, Entomological Society of America, Puerto Rico. (03/27/2022)

Oral presentation, “Methods for improving trap capture of buprestid wood-boring beetles,” 2022 Association of 1890 Research Directors Research Symposium, Atlanta, Georgia. (04-03-2022)

Awards and Scholarships

College of Agriculture's 2022- 2023 Outstanding Master of Science (Environmental Sciences) Graduate Student Award from Tennessee State University

Second Place. Oral presentation “Effect of herbicide on physiological and biochemical attributes in red maple cultivar” Tennessee Entomological Society 49th Annual Conference, Knoxville, Tennessee. (11-18-2022)

Second Place. Oral presentation, “Optimization of flatheaded borer traps through evaluation of shape, type and effective lure components” Ecology and Environmental Science Section. 132nd meeting of the Tennessee Academy of Science, Nashville, Tennessee. (10-07-2022)

Agriculture and Forestry University Merit Scholarships for admission of B.Sc. Agriculture, AFU, Rampur, Chitwan Agriculture and Forestry (2013)

University Scholarships during all eight semesters of B.Sc. Ag provided to the top 25% of students to the Faculty of Agriculture (2013-2018)

Workshops and Training

Workshops

Wasp ID Course, Wasp Workshops (2022), Pennsylvania State University, Frost Entomological (3-14 January 2022)

Biweekly R workshop under the guidance of Dr. Cynthia Perkovich (11/17/2022-4/15/2022)

Borer SCRI Symposium “From Culture to Chemistry: Managing Flatheaded Borers in Specialty Crops” Vancouver Convention Centre, Vancouver, Canada (November 13, 2022)

Workshops on Seed Production, Demands and Marketing Organized by DADO, Salyan, Nepal (2018)

Workshop on Research methodology: methods, and techniques organized by the ECO Minions TEM, Rampur, Chitwan, Nepal (2017)

Technical workshop on use of social media by YPARD, Nepal Professional Technical Writing (2016)

Workshop on “Amnesty International’s Campaign and Human Rights” organized by Amnesty International, Nepal (2015)

Trainings

Tree Label Printer Training organized by Carrie Witcher, at Nursery Research Center, McMinnville, Tennessee (February 17, 2022)

Training on Project Concept Note and Proposal Writing organized by Jhee Newa, Nepal (2017)

Training organized by Agriculture Student Liaison Forum -ASLF, Nepal (2016)

Core Competencies

Chemical ecology, plant physiology, and biochemistry in ornamental plants

UV-VIS spectrometry for quantification of chlorophyll, carotenoids, sugar, and starch analysis, antioxidant enzymes (catalase and superoxide dismutase), and plant pigments (phenolics, flavonoids, tannin)

Plant oxidative stress indicator (electrolyte leakage),

Plant vapor pressure quantification using pressure chamber

Identification of buprestid beetle to genus and species level through keywords

Trap designing for insects

Gas chromatography-mass spectrometry for volatile compound identification through SPME and dynamic headspace method

FLIR thermal imaging

Prepare artificial diet for borer

Rearing of insects

Beetle bioassay using Ethovision version 14

Software

R-studio

MS-office (MS-word, MS-Excel, MS-PowerPoint)

SPSS

Volunteering and Service

Volunteered in the Presentation preview room in Entomological Society of America, Entomological Society of Canada, Entomological Society of British Columbia Joint annual meeting, Vancouver, Canada, 2022 (November 13-15th, 2022)

Volunteered at132nd meeting of the Tennessee Academy of Science at Tennessee State University, Nashville, Tennessee (11/17/2022)

Volunteered in an Agriculture extension activity (insect activity for children) in Crossville, Tennessee, 2022 (09/22/2022)

Volunteered in Educational hiking to Stone door for undergraduate students from TSU (07/15/2022)

Society Memberships

Member of the Entomological Society of America (ESA), 2021-present

Member of Tennessee Entomological Society (TES), 2021-present

Member of Minorities in Agriculture, Natural Resources, and Related Sciences (MANRRS) 2022-present

Member of Tennessee Academy of Science (TAS), 2022-present

Member of Nepalese Agricultural Professionals of America (NAPA), 2022-present

Member of TSU Journal Club, NRC, 2021-present

References

Dr. Karla M. Addesso

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Dr. Cynthia Perkovich

Visiting Assistant Professor of Biology

Ashland University

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cperkovi@ashland.edu