Feeding preference of pine sawyer beetle (*Monochamus carolinensis*) on Pinaceae and use of virtual plant walk maps as a tool for teaching plant identification courses

by

Matthew Stephen Wilson

B.S., Auburn University, 2006
M.S., Auburn University, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
College of Agriculture

KANSAS STATE UNIVERSITY
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Abstract

Feeding preference experiments with the pine sawyer beetle (*Monochamus carolinensis* Olivier) were conducted using eleven taxa of Pinaceae. One newly emerged adult beetle (≤ 24 hours) was placed into each feeding arena (*n* = 124) containing three or four shoots of current season's growth from different tree species (one shoot per species) for choice experiments. Beetles were allowed to feed for 48 (2011) or 72 (2012-2014) hours, at which point shoots were removed and data collected on feeding occurrence and percent feeding area. Augmented design analyses of feeding occurrence and percent feeding area of the eleven taxa did not indicate significant evidence for feeding preferences of the pine sawyer beetle on most taxa except for a higher preference for both scots (*Pinus sylvestris* L.) and eastern white (*P. strobus* L.) pines compared to deodar cedar [*Cedrus deodara* (Roxb. ex D. Don) G. Don]. The feeding preference experiments suggest that pine sawyer beetle may feed on a wide-range of Pinaceae taxa.

Virtual plant walk maps were developed using a web-application for two semesters of an ornamental plant identification course (*n* = 87). The maps allowed students to revisit plants and information covered in lecture and laboratory sections at their own convenience, using either a computer or mobile device. Survey results indicated 63% of the students used the virtual plant walk maps as a study resource. Students mainly used the maps to study the most current plant list. Most students who used the virtual maps did so to review the plants online. When asked to rate usefulness of the maps on a scale from slightly useful (1) to very useful (3), 43% of students indicated that the virtual maps study tool was very useful, 25% indicated the maps were useful, and 8% indicated that the maps were slightly useful. A significant dependence between student use frequency and student usefulness ratings of virtual plant walk maps was observed. As students' use of the virtual maps increased, they perceived the maps to be more useful to their studies in preparing for identification quizzes. No differences between plant identification quiz scores were observed between students who used and did not use the virtual plant walk maps or between learning styles. Our survey indicated students used the virtual plant walk maps as a resource and perceived the maps as a useful tool in preparation for identification quizzes.

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Table of Contents

List of Figures

List of Tables

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Preface

Parenthetical documentation, units, and literature citations contained in this dissertation are formatted in accordance with the American Society for Horticultural Science publication submission guidelines.

Chapter 1 - Pine Wilt Introduction and Literature Review

Kansas is an important resource to the United States food supply and economy through the production of commodities such as cattle, wheat, corn, soybeans, and sorghum. The success of these commodities is enhanced by Kansas' forestry products. Along with conservation plantings and evergreen windbreaks, Kansas' forestry products contribute $1.3 billion dollars to the economy and provide $57 million dollars in improved crop yields and annual energy savings in buffering fields and homes from prevailing winds in the winter (KFS, 2012). Kansas' tree resources benefit the environment and its major agriculture products by providing windbreaks for livestock to lower feed intake due to weather-induced stress, providing wind erosion barriers for cropland to reduce loss of soil, and aiding riparian buffers to recover potential groundwater pollutants to improve water quality (KFS, 2012).

Pines (*Pinus* spp. L.) are important to the lumber, conservation, and nursery/landscape industries. Although not native to Kansas (Eckenwalder, 2009), pines grow and acclimate quickly to various growing conditions making them highly valuable tree species for Kansas. Cold tolerance is an issue with pines as nearly 79% of the world's pine species are native to areas receiving average annual minimal winter temperatures warmer than –17.8 to –12.2 °C (0 to 10 °F) (Eckenwalder, 2009) or USDA plant hardiness zone seven (U.S. Dept. Agr., 2012). Scots (*P. sylvestris* L.) and austrian (*P. nigra* Arnold) pines are cold tolerant to USDA plant hardiness zone three (Dirr, 2009), leading to an increase of these exotic pines in Christmas tree, windbreak, and landscape plantings across Kansas. Pine wilt disease was first reported in the U.S. in 1979 in the states of Missouri (Dropkin and Foudin, 1979), Kansas (Kennelly et al., 2009;

KDA, 2014; Robbins, 1979a), and Arkansas (Robbins, 1979b). Since then, scots and austrian pines have substantial mortality due to spread of the disease. Pine wilt is a disease association between a pathogen [pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle] (PWN), an insect vector [sawyer beetle (*Monochamus* spp. Megerle)], and susceptible plant host (taxa within the Pinaceae).

Kansas' evergreen windbreaks, landscape plantings, and Christmas tree operations along the eastern and central regions of the state, are at risk of loss due to pine wilt disease (KDA, 2014). Greenhouse-grown seedlings and mature field-grown scots and austrian pines are susceptible to pine wilt (Dropkin et al., 1981; Linit and Tamura, 1987). While U.S. native conifer species are generally regarded as tolerant to pine wilt, there is contrasting information in the literature as to the extent of resistance/tolerance among native and exotic species (Dropkin et al., 1981; Wingfield et al., 1986). Currently, there are little to no known alternative species of pine suitable for farmers/ranchers and municipalities in Kansas. Field evaluations of various conifer species and ecotypes within currently affected regions of Kansas together with laboratory inoculation and feeding preference experiments are warranted to determine replacements for highly susceptible species. Therefore, the following chapters of the proposed study will describe the pine wilt disease cycle in Kansas, insect vectors, and the results of evaluations and screenings of various conifer species and ecotypes for pine sawyer beetle feeding preferences.

Pine Wilt Pathogen

General Information

There are nearly 100 described species of *Bursaphelenchus* Fuchs worldwide (Burgermeister et al., 2009; Ryss et al., 2005). Approximately 70% of *Bursaphelenchus* species are associated with conifers (Ryss et al., 2005) and 30% associated with angiosperms such as pedunculate oak (*Quercus robur* L.), european hornbeam (*Carpinus betulus* L.), ash (*Fraxinus* spp. L.) (Carletti et al., 2005), figs [*Ficus* spp. L. (Kanzaki, et al., 2000; 2014)], aspen [*Populus* spp. L. (Tomalak and Filipiak, 2010; 2011; Tomalak et al., 2013)], and littleleaf linden [*Tilia cordata* Mill. (Tomalak and Malewski, 2014)]. *Bursaphelenchus cocophilus* (Cobb) Baujard (red ring nematode) and *B. xylophilus* (PWN) are considered economically important plant pests (Jones et al., 2013). Steiner and Buhrer (1934) first collected PWN in the U.S. from samples submitted by lumber mills of longleaf pines (*Pinus palustris* Mill.) in Louisiana and Texas and a shortleaf pine (*P. echinata* Mill.) in Virginia and described the nematode as *Aphelenchoides xylophilus* Steiner and Buhrer. The nematodes were isolated from trees attacked by bark beetles (*Dendroctonus frontalis* Zimmerman and *Ips* spp. De Geer) and associated blue-stain fungi. Nickle (1970) ascribed Steiner and Buhrer's described species to the genus *Bursaphelenchus* based on morphological characteristics (e.g., bursa structure on tail of males). Nickle et al. (1981) then compared the morphologies of *B. xylophilus* from the U.S. and *B. lignicolus* Mamiya and Kiyohara from Japan. *Bursaphelenchus lignicolus* was first observed in Japan in 1905 from investigations into death of pines in Japan and described with species designation by Mamiya and Kiyohara (1972). In a joint species comparative analysis with Mamiya and fellow researchers, Nickle et al. (1981) observed a unified morphology for the two species and recorded successful mating between all U.S. and Japanese populations and progeny successions. The species designation for *B. lignicolus* was synonymized with *B. xylophilus* (Nickle et al., 1981).

Pinewood nematode, *B. xylophilus*, has been documented in 36 states within the Great Plains, Midwest, and Southeast regions of the U.S. and has been reported in Canada, Mexico, China, Taiwan, Japan, Korea (Ferris, 1999) Portugal (Mota et al., 1999) and Spain (Robertson et al., 2011). The presence of PWN and few reported deaths of native conifer species in North America compared to Europe and Asia suggest PWN is indigenous to North America. Additionally, studies of PWN populations have indicated greater genetic diversity between North American populations than European or Asian populations (Metge and Burgermeister, 2006; Zhang et al., 2008) with few introduction events for Europe and Asia (Mallez et al., 2014; Vieira et al., 2007). Pinewood nematode is an aboveground nematode vectored and spread to tree hosts by sawyer beetles such as the pine sawyer, *Monochamus* spp. (Akbulut and Stamps, 2012; Dropkin et al. 1981). The disease cycle is complex and encompasses many phases of the beetle and nematode life cycles.

Feeding Activity

Generally, PWN-infested trees contain adult nematodes feeding on living tree host cells or overwinter-feeding on species of blue-stain fungi within the genus *Ceratocystis* Ellis and Halsted (Himelick, 1982; Wingfield, 1987), previously identified as *Ceratostomella* Sacc. Michelia (Steiner and Buhrer, 1934). Most of the fungal species documented in pine from the aforementioned genera have been reclassified under the genus *Ophiostoma* Syd. and J. Syd. (De Beer et al., 2013; Intl. Mycol. Assn., 2013).

Blue-stain fungi are introduced into trunk tissue on body surfaces, specialized cuticular structures called mycangia, and uncovered cuticular pits located on the head, prosternum, and elytra of ambrosia beetles (e.g., *Gnathotrichus* spp. Eichhoff) and scolytid (Curculionidae: Scolytinae) bark beetles (e.g., *Dendroctonus* Erichson, *Ips*, *Tomicus* Latreille, *Hylurgus* Dejean, and *Orthotomicus* Ferrari) (Davidson, 1979; Ferris, 1999; Harrington, 2005; Lee et al., 2007; Paine et al., 1997).

Fungal feeding (mycophagy) is considered the ancestral feeding strategy of PWN since most species of *Bursaphelenchus* are solely fungivores (Giblin-Davis et al., 2003). Kikuchi et al. (2005) documented that both *B. xylophilus* and *B. mucronatus* Mamiya & Enda genomes contain an endo-β-1,3-glucanase gene closely related to endoglucanase genes of bacteria which can break down polymers that compose cell walls in fungi and structural/storage polysaccharides in some marine algae. Expression of the endo-β-1,3-glucanase gene serves as a mechanism for *Bursaphelenchus* spp. to break down and feed on fungi such as PWN's mycophagous stage feeding on blue-stain fungi in dead and dying trees (Kikuchi et al, 2005). The mycophagous nature of *Bursaphelenchus* and its association with dead or dying trees are not considered traits detrimental for plant health as only two species, *B. xylophilus* and *B. cocophilus*, are considered plant pests. Populations of PWN can be maintained to feed and reproduce in axenic culture and fungal cultures of *Botrytis cinerea* Pers. or blue-stain fungi such as *Ophiostoma* for life cycle completion (Bolla and Jordan, 1982; Kiyohara and Suzuki, 1978). Incorporation of a trait for phytophagous (plant feeding) feeding in combination with mycophagy is believed to contribute to PWN's adaptation for facultative plant parasitism (Giblin-Davis et al., 2003).

Plant parasitic nematodes use a protrusible tube, or stylet, to access the nutrients in the cytoplasm of plant cells. The stylet is considered a morphological adaptation for the transition of bacterivorous feeding ancestors of nematodes to fungal and plant feeding. In addition to other physical adaptations, plant parasitic nematodes may also implement chemical means such as enzymes to depolymerize cell walls for feeding and transport within plants by migratory endoparasite species of nematode (Davis et al., 2000). The ability of plant parasitic nematodes to produce cellulase enzymes for the breakdown of cell wall components of plants (e.g., polysaccharides such as pectin, cellulose, hemicellulose, etc.) is hypothesized to have occurred through horizontal/lateral gene transfer between bacteria or fungi and associated ancestors of plant parasitic nematodes (Davis et al., 2000; Jones et al., 2008; Keen and Roberts, 1998; Mitreva et al., 2009). Comparison of protein structural conformations and phylogenetic analyses of enzymes isolated from plant parasitic nematodes and other organisms, with respect to nematode phylogeny and association with transfer organism candidates, seek to determine the likelihood and source of horizontal transfer of genes between organisms. Without full genomic sequences of nematodes and documentation of all organisms that have genes to produce the same enzymes, it is difficult to confirm horizontal gene transfer even though it is highly suggestive (Mitreva, et al., 2009). Kikuchi et al., (2004) documented a group of glycosyl hydrolase family 45 (GHF-45) cellulase genes for PWN. The cellulases produced from the expression of GHF-45 genes are typically associated with organisms that break down and decompose woody plant material through de-polymerization of cellulose and hemicellulose (Mitreva et al., 2009). Kikuchi et al., (2004) confirmed the expression of the GHF-45 gene and its cellulase activity from the secretions of PWN and recombinant protein expression in *Escherichia coli* (Migula) Castellani and Chalmers. Cellulases of the GHF-45 group have been found in bacteria, fungi, protists, and insects (CAZy, 2015; Mitreva et al., 2009). Phylogenetic analyses of the GHF-45 in PWN indicated a stronger association with fungi than other organisms (Kikuchi et al., 2004). As a migratory endoparasite, the ability to depolymerize components of cell walls to feed on and navigate through phloem, parenchyma, and resin canal tissues throughout trees is an important trait for PWN's facultative phytophagy. A similar cellulase gene (β-1,4-endoglucanase) of the glycoside hydrolase family (GHF-5) is documented for the genomes of four other plant parasitic nematode genera *Globodera* (Skarbilovich) Behrens, *Heterodera* Schmidt (Smant et al., 1998), *Meloidogyne* Göeldi (Rosso et al., 1999), and *Pratylenchus* Filip'ev (Uehara et al., 2001). Cellulases are one of several types of proteins used by plant parasitic nematodes.

Kichuchi et al. (2006) reported the presence and expression of genes for two pectate lysases in the esophageal glands of PWN for the breakdown of pectin-composed matrices anchoring cellulose and hemicellulose fibers within cell walls (Kikuchi, et al., 2006). In 2009 Kikuchi et al., documented the presence of expansin-like genes in the pharyngeal glands of PWN and *B. mucronatus* for the enhancement of cellulase degradation of cell walls. With further exploration of the genes of other species of *Bursaphelenchus* and the complete genomic mapping of PWN in 2011, GHF-45 cellulase genes have been documented for *B. mucronatus* and other species (Palomares-Ruis et al., 2014). With the presence of cellulase and expansin-like protein coding genes in the non-pathogenic *B. mucronatus* and pathogenic PWN, it is believed that these enzymes may be used more for migration between plant cells than detrimental feeding (Shinya et al., 2013b). Recent analyses of the PWN genome and secretome detected genes and proteins, which may serve to elucidate the mechanisms by which PWN evades the defense compounds elicited by susceptible tree hosts. Shinya et al. (2013a) documented several families of peptidase, fungi and plant cell wall degrading enzymes, 12 antioxidant proteins (e.g., peroxiredoxin, glutathione peroxidase, and superoxide dismutase), cysteine peptidase (plant defense regulation) inhibitors, two thaumatin-like proteins (plant anti-fungal defense), and one cystatin-like peptidase (cysteine peptidase control and programmed cell death control) inhibitor. Shinya et al. (2013a) proposed some of the enzymes might reveal the mechanisms PWN uses to mitigate the effects of plant defenses (i.e., antioxidant proteins to combat radical oxygen species), to regulate and inhibit plant defense pathways (i.e., cysteine peptidase inhibition to prevent cell death signaling), and mimic plant enzymes to avoid plant defense initiation within tolerant hosts. However, production and secretion of these enzymes in combination with abiotic (e.g., drought or heat stress) or biotic stresses (e.g., insect or pathogen invasion) may serve to induce hypersensitive protective reactions to induce cell death signaling and defense pathways leading to decline and death in susceptible hosts (Shinya et al., 2013b). Further work is needed to determine the role of recently isolated enzymes in PWN pathogenicity.

Life Cycle and Transmission

Pinewood nematode overwinter on blue-stain fungi in dead trees and logs of tree hosts, alongside beetle larvae and pupae in pupal chambers, where both the nematode and vector may come into contact during life stages conducive for nematode attachment to the beetle vector and dispersal to new host trees (Dwinell, 1997). During overwinter feeding, PWN mate, and lay eggs as part of the propagative phase to maintain populations within tree hosts. In late spring, as blue-stain fungi colonies are reduced by nematode feeding and/or drying of host tissues, PWN enter a dispersal phase of reproduction (Kondo and Ishibashi, 1978). Female PWN lay approximately 80 eggs per nematode within their 15-day life cycle when cultured at 25 °C (77 °F) under laboratory conditions, resulting in several generations per year and exponential population growth (Mamiya and Furukawa, 1977). As during the propagative reproductive stage, dispersal stage PWN undergo three molts as part of four juvenile stages before molting a fourth and final time as adults. However, some molts of dispersal stage PWN feature morphological features to aid survival outside of the tree hosts. The first molt occurs within the egg from the first to second stage juvenile. Second stage juveniles hatch from eggs to feed on resin canal walls and parenchyma cells and begin migrating from tree resin canals toward galleries containing beetle pupae. During this migration, a second and third molt occurs resulting in specialized third stage juveniles and fourth stage juveniles for dispersal (dauer larvae) that surround the sidewalls of the beetle galleries to await beetle molt and emergence. These dauer larvae exhibit thicker cuticles with an increase in the percentage of cuticle thickness allocated to the basal cuticle layer and accumulation of lipid droplets in the intestinal and hypodermal cells in comparison to the propagative stage of PWN (Kondo and Ishibashi, 1978). Thicker cuticle and lipid accumulation allow for increased survival of the dauer larvae in the drier environments outside of tree hosts within the respiratory system and on the body surface of beetle vectors (Kondo and Ishibashi, 1978). Esophageal glands and stylets are reduced in third stage and absent in fourth stage dauer larvae as no nematode feeding occurs for the fourth stage dauer larvae when PWN are attached to vectors for transport to tree hosts (Dropkin et al., 1981;

Kondo and Ishibashi, 1978). As sawyer beetle adults emerge from pupal chambers, nematode dauer larvae attach to the beetles as they exit the tree. Kondo (1986) observed that PWN attached and aggregated at the thoracic (80%) and abdominal (15%) spiracle openings and within the respiratory system (tracheae) of the pine sawyer beetle, *Monochamus carolinensis* Olivier, and to a lesser extent on body surface, appendages and head (5%). Similar observations were documented for the european pine sawyer, *M. galloprovincialis* Olivier (Naves et al., 2006a). Beetles fly to healthy trees with tender shoots for maturation feeding and to stressed/dying or dead trees to mate and lay eggs (oviposition) whereby nematode dauer larvae carried by the beetles may be introduced to the trees through feeding wounds or oviposition pits (Dropkin et al., 1981; Ferris, 1999; Wingfield and Blanchette, 1983). Shortly after transfer to plant hosts, PWN dauer larvae molt a fourth and final time to the adult stage exhibiting stylets and esophageal glands for feeding and migration and reproductive differentiation to enable mating and population of PWN within tree hosts' tissues and vascular systems (Kondo and Ishibashi, 1978).

During maturation feeding, pine sawyer beetles excise needle bundles from the stem and feed on tender tissues and candles of the stem towards the terminal ends of the shoots (personal observation, see Fig. 2.1). Feeding by the pine sawyer beetle adult provides entry points to the stem and vascular system of tree hosts as the beetle feeds and moves towards the ends of shoots whereby nematodes may transfer from the body surface and spiracles to the tree for entry into the plant through the feeding wounds of healthy trees (Ferris, 1999). In a field transmission feeding study, Linit (1990) observed approximately a 50% transmission success rate of PWN for scots pine through feeding wounds of pine sawyer beetle (*M. carolinensis*) with the frequency of successful transmission dependent on beetle age and the number of nematodes extracted per branch correlated with the number of nematodes per beetle. Wingfield and Blanchette (1983) observed similar results for PWN transmission through feeding wounds to eastern white pine (*P. strobus* L.) and documented transmission of PWN to oviposition pits created by females of two species of pine sawyer beetles (*M. carolinensis* and *M. scutellatus* Say). Transmission of PWN through feeding wounds is considered the introductory pathway for tree death due primarily to pine wilt. However, as pine sawyer beetles are attracted to stressed or dying trees for oviposition, PWN transmission through oviposition and subsequent tree death is often considered a secondary pathway of tree death due to pine wilt (Wingfield and Blanchette, 1982). Nematodes introduced within PWN-susceptible and resource-starved/stressed tree hosts overcome plant defenses and induce hypersensitive plant defense pathways (e.g., resin flow, reactive oxygen molecule production, and/or programmed cell death) to survive and reproduce to population levels detrimental to host survival. Declining trees, whether the result of primary or secondary PWN invasion, may elicit stress compounds attractive to secondary insects (e.g., bark beetles) to invade tree hosts and introduce blue-stain fungi. Once inside tree hosts, newly molted PWN adults can undergo propagative phase reproduction and maintain populations until resource availability, environmental conditions, and vector development are conducive for dispersal to new tree hosts.

Pine Wilt Vector Organism

General Information

Most *Bursaphelenchus* are vectored by beetles in the Curculionidae (weevil, bark and ambrosia beetles), Cerambycidae (long-horned beetles), and Buprestidae (metallic borer beetles) families of the order Coleoptera (Ryss et al., 2005) and to a lesser extent by insects within Hymenoptera (Giblin-Davis et al., 2005). Red ring nematode (*B. cocophilus*), an economically important pest of palms, is vectored by American palm and sugarcane weevils (Agrios, 2005). *Bursaphelenchus trypophloei* and *B. masseyi* Tomalak and Filipiak are nematodes associated with dying or dead aspen (*Populus* spp.) and are vectored by bark beetles (Tomalak and Filipiak 2010; Tomalak et al., 2013). Another nematode associated with aspen, *B. populi* Tomalak and Filipiak, is vectored by a long-horned beetle (Tomalak and Filipiak, 2011). A few families of the beetle order, Coleoptera, collected from dead or dying pines have been associated with PWN including Cerambycidae, Curculionidae, and Buprestidae (Linit et al., 1983; Linit, 1988; Akbulut and Stamps, 2012). The carrying capacities for PWN by non-cerambycid beetles are reduced compared to cerambycids (Linit et al., 1983; Wingfield and Blanchette, 1983). Even within the Cerambycidae, other long-horned beetles only carry a fraction of the PWN found on the body surfaces and tracheal system of pine sawyer beetles. Beetles of the genus *Monochamus* are considered the primary vectors of PWN. Linit et al. (1983) observed an average of more than 19,000 and maximum of 67,000 PWN on *Monochamus carolinensis* in Missouri. Wingfield and Blanchette (1983) documented similar numbers of PWN on both *M. carolinensis* and *M. scutellatus* in Minnesota and Wisconsin. Sousa et al. (2001) recorded approximately 2,000 PWN for the european sawyer beetle, *M. galloprovincialis*, but found some beetles carried up to 72,000 PWN. Mamiya and Enda (1972) observed an average of approximately 14,000 PWN with as many as 175,000 PWN on the japanese pine sawyer, *M. alternatus* Hope, in Japan.

Sawyer Beetles (*Monochamus* spp.)

Sawyer beetles (*Monochamus* spp.) are Coleopteran insect members of the Lamiinae subfamily within the Cerambycidae family (long-horned beetles) (ITIS, 2012; Linsley, 1959). With approximately 20 described species throughout the Americas, Asia, and Europe (EOC, 2012), eight species of *Monochamus* are considered native to North America (ITIS, 2012). *Monochamus* are considered wood, phloem, bark, and/or cone, feeding insects of various conifers (Linsley, 1959). Sawyer beetle feeding, oviposition, and development occurs with members of the Pinaceae including: fir (*Abies* Mill.), cedar (*Cedrus* Trew), spruce (*Picea* A. Dietr.), pine (*Pinus*), douglas fir (*Pseudotsuga* Carrière), and hemlock [*Tsuga* (Endl.) Carrière] (Linsley and Chemsak, 1984). Sawyer beetle species are primarily associated with one plant taxon (e.g., *Pinus*), but may include more taxa depending on primary host availability in relation to density and diversity of other taxa (Linsley and Chemsak, 1984). Of the North American species of *Monochamus*, eastern or carolina pine sawyer (*M. carolinensis*), spotted pine sawyer (*M. mutator* LeConte), and northeastern sawyer (*M. notatus* Drury) are associated with pines (Lingafelter, 2007). While pines are the primary hosts for white-spotted sawyer (*M. scutellatus*) and southern pine sawyer (*M. titillator* Fabricius), both species may also use fir and spruce as hosts (Linsley and Chemsak, 1984). *Monochamus scutellatus*, to a lesser extent, may also use larch (*Larix* Mill.) as a host (Akbulut and Stamps, 2012; EPPO, n.d.; Lingafelter, 2007). Balsam fir sawyer (*M. marmorator* Kirby) uses fir and spruce as hosts and obtuse sawyer (*M. obtusus* Casey) primarily uses fir and douglas fir as hosts, but may also use pine as a host. european sawyer (*M. galloprovincialis*) is associated with pine and spruce hosts. *Monochamus alternatus* uses a wide array of taxa for hosts (fir, cedar, larch, spruce, and pine) (Akbulut and Stamps, 2012; EPPO, n.d.). While some species of *Monochamus* are associated with a single taxon, other sawyer beetle species may use several plant taxa as hosts.

Life Cycle- Reproduction and Development

The mating system for *Monochamus* is considered a resource-defense polygyny where, males defend the oviposition sites needed by females to lay eggs as part of the potential mate selection process (Linsley, 1959; Thornhill and Alcock, 1983). While there is evidence pheromones may play a role in mate location, Hughes and Hughes (1982) observed that male body size served as an important and determining factor in mate selection for *M. scutellatus*. Increased body size aids males as they charge and fight with each other using their mandibles in competition for females (Hughes and Hughes, 1982; Linsley, 1959). Once mate selection has occurred, the male and female couple forms a pair-bond where copulation may occur multiple times prior to oviposition. Non-copulatory (male refractory) periods of the pair-bond are often spent with the male attached to the female using the male's prolegs in a partial mating position as a measure to ensure resulting offspring are its progeny (Hughes and Hughes, 1985). These pair-bonds may be considered long or short term pair-bonds depending on the male's success in maintaining the pair-bond without the female escaping from the partial mating position during the male's refractory periods. The male may return to re-form a pair-bond if no competition with other males occurs or if competition does not result in another male being selected as the new mate for the female (Hughes and Hughes, 1985).

Once mating has occurred, females begin to oviposit eggs into pits or "niches" made by the mandibles into the bark of stressed or dead trees. The niches extend through the bark to the phloem and cambium layers of the tree for larval stages to access the wood to feed and create galleries for development. *Monochamus* species vary in the number of eggs laid into each niche. *Monochamus carolinensis* typically lays only one egg per niche, but may occasionally lay more than one egg (Walsh and Linit, 1985). *Monochamus titillator* may lay three to nine eggs into a niche (Dodds and Stephen, 2000; Webb, 1909). Hughes and Hughes (1985) observed variation of fecundity within the species *M. scutellatus* and proposed the variation may be a method employed by females in response to non-receptivity of an undesirable mate when unsuccessful to terminate a pair-bond. Laboratory (Akbulut et al., 2004; Linit, 1985) and controlled field experiments (Alya and Hain, 1985) have observed an average mortality rate for larvae of approximately 85% per generation for pine sawyer (*M. carolinensis*) and southern pine sawyer (*M. titillator*) beetles. Variation in the number of eggs laid into niches between *Monochamus* species may indicate the differing strategies employed to address the high mortality rates of larvae within trees believed to occur as the result of intraspecific competition between larvae (i.e., cannibalism), interspecific competition/predation, and availability of tree host resources. Cannibalism has been documented for larvae of *M. sutor* (Victorsson and Wikars, 1996), *M. carolinensis* (Dodds et al., 2001), and *M. alternatus* (Anbutsu and Togashi, 1997). Additionally, interspecific competition and predation on bark beetles (*Ips* spp.) by *M. carolinensis* and *M. titillator* have been documented (Dodds et al., 2001; Schoeller et al., 2012). There is little information regarding predation on *Monochamus* species by other insects, however insectivorous woodpeckers have been hypothesized (Beal, 1911; Rose, 1957, cited in Victorsson and Wikars, 1996) and documented to predate on sawyer beetles such as *M. scutellatus*

(Murphy and Lehnhausen, 1998; Ross, 1966) and *M. clamator latus* Casey (Wickman, 1965). The single egg per niche strategy implemented by *M. carolinensis* may decrease the likelihood for intraspecific competition and cannibalism by decreasing the number of larvae competing for resources within the tree. Whereas, the multiple egg per niche strategy implemented by *M. titillator* may account and allow for increased intraspecific competition, allowing the increased number of larvae to compete for resources within the tree and allow the population of larvae to reach the maximum threshold supported by the tree. Anbutsu and Togashi (1997) documented cannibalism for larvae of *M. alternatus* when newly hatched larvae were placed under the bark of pine bolts within 2.5 cm compared to 10 cm. The oviposition strategies for *M. carolinensis* and *M. titillator* yielded similar generation survivorship rates in controlled experiments where interspecific competition and predation were prevented (Akbulut et al., 2004; Alya and Hain, 1985; Linit, 1985). Akbulut et al. (2004) found surface area and volume of logs were positively correlated with the number of emerged adult pine sawyer beetles, while the number of eggs per log was not correlated with the number of adult beetles. These findings support the roles of intraspecific competition/cannibalism, resource availability, and other potential density-dependent factors for sawyer beetle survivorship during development within trees (Akbulut et al., 2004; Akbulut and Stamps, 2012; Anbutsu and Togashi, 1997).

Monochamus may vary in the length of development between species and for populations of a single species distributed in different locations. The length of stadia and number of instars during larval development depend on the amount of thermal accumulation over time (e.g., degree day) a population may receive for a specific area (e.g., climate) and whether beetle species undergo an obligatory diapause (Linit, 1985; Pershing and Linit, 1986a; Pershing and Linit, 1988). Linit (1985) observed through continuous laboratory culture of the pine sawyer species *M. carolinensis* that the species did not have an obligatory diapause for development. Pershing and Linit (1986a) determined the degree-days needed for *M. carolinensis* development and calculated that the species may have up to two generations per year in Missouri given the average thermal unit accumulation. Pershing and Linit (1986a) verified the number of generations in Columbia, Missouri as estimated by the development threshold through field observations. Given the distribution of *M. carolinensis* from the central and eastern regions of the United States and Canada (Lingafelter, 2007; Linsley and Chemsak, 1984) and the thermal accumulation units for these areas, *M. carolinensis* requires eight to twelve weeks (Pershing and Linit, 1986a) and up to two years to develop a generation (Akbulut and Stamps, 2012). Similarly, *M. titillator* does not have an obligatory diapause and may have one to three generations per year (Akbulut and Stamps, 2012; Alya and Hain, 1985; Dodds and Stephen, 2000; Webb, 1909). Multivoltine *M. titillator* populations capable of three generations per year are in southern Georgia, Florida, and eastern Texas) of the species' native range in the United States (Linsley and Chemsak, 1984). Species of *Monochamus* requiring one to two years per generation include *M. scutellatus* (Rose, 1957)*, M. alternatus* (Togashi, 1990; Togashi, 1991), *M. sutor* (USDA, 1991), and *M. galloprovincialis* (Francardi and Pennachio, 1996, cited in Naves et al., 2007; Tomminen, 1993). Both larval diapause and location within each species' native range are determining factors for generation time of these four species (Naves et al., 2007; Naves and Sousa, 2008; Rose, 1957; Togashi, 1991). Information regarding thermal unit accumulation and facultative/obligatory diapause of other *Monochamus* species is not well known (Akbulut and Stamps, 2012).

Egg incubation times for sawyer beetles range from five to fourteen days, with ten days serving as an approximate incubation time across species of *Monochamus* and temperature differences experienced in various locations within a distribution or between generations within a season (Akbulut and Stamps, 2012; Pershing and Linit, 1986a; Rose, 1957; Ross, 1966; Webb, 1909). Upon egg hatch, sawyer beetle larvae develop underneath the bark. *Monochamus* larvae generally develop through four instars prior to pupation. Life histories and instars have been reported for *M. scutellatus* (Rose, 1957, Ross, 1966) and *M. alternatus* (Yamane, 1975, cited in Pershing and Linit, 1988) with four instars reported for both species. Larval development for *M. carolinensis* was observed to occur with as few as three and as many as six instars, with four instars being the most common number of larval development stages (Pershing and Linit, 1988). Larvae feed on the phloem and cambium tissues composing the sub-cortex (Pershing and Linit, 1986b). After two weeks of feeding, older instars begin constructing galleries in the xylem tissue (i.e., sapwood) and create pupal chambers at the terminal ends of the larval galleries (Pershing and Linit, 1986b; Shibata, 1984; Webb, 1909). Larvae enlarge the pupal chambers to accommodate the pupae and adult beetles, and occasionally return to feed on sub-cortex tissues prior to pupation (Pershing and Linit, 1986b). Pupation generally occurs four to five weeks after egg hatch and lasts for the remaining development period. Beetle eclosion and sclerotization of the exoskeleton occurs within the pupal chamber (Pershing and Linit, 1986b). Adult beetles emerge from the pupal chamber and tree by chewing through the remaining sapwood, cambium, and bark tissues, and fly to trees for maturation feeding (Pershing and Linit, 1986b). *Monochamus* species unable to complete more than one generation per year, or late season generations overwinter in the egg and larval life stages. Univoltine species such as *M. galloprovincialis* and *M. alternatus* typically overwinter as fourth instars (Naves et al., 2007; Togashi, 1990). Later generations of multivoltine and northern univoltine species such as *M. carolinensis*, *M. titillator*, *M. scutellatus*, and *M. sutor* may overwinter at all instar stages and as eggs too (Alya and Hain, 1985; Pershing and Linit, 1986b; Rose, 1957; Webb, 1909; USDA, 1991).

Monochamus are most active at dusk and dawn in southern regions and during the day and night in northern regions where daylength may be extended during summer months (Linsley, 1959). Adults emerge and fly to trees for maturation feeding during daylight hours and to weakened or stressed trees for mating and oviposition during the evening (Dropkin et al., 1981; Ross, 1966; Wingfield and Blanchette, 1982). Lu et al. (2013) and Wang et al. (1990) observed distinct diurnal cycles of feeding, mating, and oviposition for *Glenea cantor* Fabricius and *Paraglenea fortunei* Saunders, both are cerambycid beetles in the Lamiinae subfamily.

While many cerambycids can mate shortly after emergence, female cerambycids in the Lamiinae subfamily require a period of maturation feeding on host plants for ovary development prior to mating (Hanks, 1999; Li and Liu, 1997; Linsley, 1959; Lu et al., 2013). In laboratory experiments, *M. carolinensis* was observed to undergo maturation feeding for nine days prior to oviposition (Walsh and Linit, 1985; Zhang and Linit, 1998), fourteen days for *M. alternatus* (Zhang and Linit, 1998), and approximately ten days for *M. galloprovincialis* (Akbulut and Stamps, 2008). Ross (1966) confirmed field observations by Rose (1957) and documented a pre-oviposition period between 19 and 38 days for *M. scutellatus* in a controlled field experiment. Zhang and Linit (1999) hypothesized that decreased maturation feeding and increased oviposition periods observed for *M. carolinensis* and *M. alternatus* under laboratory conditions may be due to a continuous supply of plant material available to beetles in laboratory experiments compared to beetles in the field, whereby laboratory beetles can maximize maturation feeding. Maturation feeding periods may be longer for beetles under natural conditions as they spend time and energy reserves searching for suitable feeding hosts (Zhang and Linit, 1999).

Beetle Feeding

Feeding preference of various sawyer beetles to determine potential host tree species and future PWD spread have been done in the U.S. and internationally (Futai et al., 1994; Koutroumpa et al., 2009; Naves et al., 2006; Walsh and Linit, 1984). In Portugal, newly emerged *M. galloprovincialis* Olivier (european pine sawyer) were given 36 h choice feeding preference tests (Naves et al., 2006). Scots pine was most heavily fed upon by males (109 mm^2) and females (91 mm^2) while monterey pine (*P. radiata* D. Don) was fed upon the least by males (10 mm^2) and females (7 mm^2). There was no difference in feeding preference between male and female beetles. In France, european pine sawyer choice feeding tests with scots and maritime (*P. pinaster* Aiton) pine were conducted (Koutroumpa et al., 2009). After 36 h, both species were consumed by the beetle, however, the area of scots pine consumed (0.70 cm^2) was greater than maritime pine (0.15 cm^2). Futai et al. (1994) reported on feeding preference of *M. alternatus* Hope (japanese pine sawyer) in Japan for 72 h following emergence. Beetles reared from logs of Korean (*P. koraiensis* Siebold & Zucc.) or japanese red (*P. densiflora* Siebold and Zucc.) pine fed on a larger area of korean pine (915 mm^2) than japanese red pine (430 mm^2) regardless of the species they were reared from. Similar to the work in Portugal, there was no difference in feeding preference between male and female beetles. In the U.S., Walsh and Linit (1984) investigated feeding preference of the pine sawyer beetle [*Monochamus carolinensis* (PSB)] on scots, jack (*P. banksiana* Lamb.), shortleaf (*P. echinata* Mill.), and eastern white pine. After 24 h for feeding, scots pine was most heavily fed upon (0.49 cm^2) followed by jack (0.26 cm^2), shortleaf (0.14 cm^2) and eastern white (0.05 cm^2) pine. Contrary to previously mentioned research, female beetles consumed more of the pine samples than males.

Cerambycids detect suitable hosts for maturation feeding and mating/oviposition via chemical signals (i.e., volatile organic compounds) (Allison et al., 2004; Hanks, 1999; Kim et al., 1992). Resins of plants serve to defend plants from pests (Langenheim, 2003; Trapp and Croteau, 2001). However, volatile components of these resins may be detected by insects and attract potential pests to host trees. Olfactory reception of host-produced compounds such as monoterpenes and ethanol (Miller, 2006) or pheromones produced by beetles of the same species or other insects, may serve to stimulate or deter/repel activity (Allison et al., 2001; Allison et al., 2012; Fan and Sun, 2006; Fan et al., 2007; Fan et al., 2010; Ibeas et al., 2008; Miller et al., 2013).

The objective of this research was to conduct choice and no-choice feeding preference tests to determine if PSB would avoid feeding upon commonly available Pinaceae taxa. Non-preferred species may be less likely to become infested with PWN and therefore could represent alternative tree species options for green industry professionals. Tree species were selected based on perceived disease susceptibility in Kansas where scots pine is widely recognized as the most susceptible and North American native pines are generally considered tolerant unless otherwise stressed.

Chapter 2 - Feeding Preference of Pine Sawyer Beetle (*Monochamus carolinensis*) on Pinaceae

Abstract

Pine wilt disease is vectored by the pine sawyer beetle (*Monochamus carolinensis* Olivier) from dead or dying trees to healthy trees during feeding. Identifying and planting non-preferred pine tree species for feeding could slow the spread of pine wilt disease and offer green industry professionals alternative species for planting. Therefore, feeding preference trials of pine sawyer beetle were conducted using eleven Pinaceae taxa. One newly emerged adult beetle was placed into each feeding arena (*n* = 124) containing shoots of current season's growth from different tree species (one shoot per species) for choice trials. No-choice trials were conducted with one shoot per taxa. Beetles were allowed to feed for 48 or 72 hours, then shoots were removed and data collected on feeding occurrence and percent feeding. Augmented design analyses of feeding occurrence and percent feeding for the eleven taxa revealed no evidence for a feeding preference of the pine sawyer beetle on most of the taxa with two exceptions. A greater percentage of scots (*Pinus sylvestris* L.) and eastern white (*P. strobus* L.) pines were fed on than deodar cedar [*Cedrus deodara* (Roxb. ex D. Don) G. Don]. The feeding preference trials suggest that pine sawyer beetle is polyphagous and could potentially vector pine wilt disease to many different species of pines and other Pinaceae taxa.

Species Used

Austrian (*Pinus nigra* Arnold), eastern white (*P. strobus*), lacebark (*P. bungeana* Zucc. Ex Endl.), loblolly (*P. taeda* L.), loblolly-pitch hybrid (*P. taeda* x*rigida* Mill.), ponderosa (*P. ponderosa* P. Lawson & C. Lawson), scots (*P. sylvestris*), and southwestern white (*P. strobiformis* Engelm.) pines; and deodar cedar (*Cedrus deodara*), concolor fir [*Abies concolor* (Gordon & Glend.) Lindl. Ex Hildebr.], and white spruce [*Picea glauca* (Moench) Voss]

Additional Index Words: *Bursaphelenchus xylophilus*, Cerambycidae, longhorn beetle, pinewood nematode

Introduction

Pines (*Pinus* spp. L.) are important to the forestry, conservation, and nursery and landscape industries. Although not native to Kansas (Eckenwalder, 2009), pines grow and acclimate quickly to various growing conditions, making them highly valuable tree species for Kansas. Their evergreen foliage is desirable in regions with few native evergreens available for ornamental or windbreak purposes. These qualities have led to an increase in the use of scots (*Pinus sylvestris*) and austrian (*P. nigra* Arnold) pines in Christmas tree and windbreak plantings as well as widespread use in Kansas landscapes. Previously described in Japan, pine wilt disease (PWD) was reported in 1979 for the U.S. states of Missouri (Dropkin and Foudin, 1979), Kansas (Kennelly et al., 2009; Kansas Dept. Agr., 2014; Robbins, 1979a), and Arkansas (Robbins, 1979b). Since then, there has been widespread mortality among scots and austrian pines due to the spread of the disease, a tritrophic relationship between a pathogen [pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (PWN)], an insect vector [sawyer beetles (*Monochamus* spp. Megerle)], and plant host (Pinaceae) (Gleeson et al., 2000). Pinewood nematode and the associated disease complex known as PWD have been documented in 36 states within the U.S as well as Canada, Mexico, China, Taiwan, Japan, Korea, (Ferris, 1999), Portugal (Mota et al., 1999), and Spain (Robertson et al., 2011). Pinewood nematode is an epiterrestrial nematode (Akbulut and Stamps, 2012; Dropkin et al., 1981) that feeds on living tree host cells or species of blue-stain fungi (e.g., *Ophiostoma* Syd. and J. Syd) introduced into trunk tissue by bark beetles (e.g., *Dendroctonus* Erichson, *Ips* De Geer, etc.) and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) (Davidson, 1979; Harrington, 2005; Paine et al., 1997). Pinewood nematode populations increase during a propagative stage of reproduction to colonize tree host tissues such as resin canals for distribution throughout the host, often leading to a decline in tree health as trees experience environmental stress (e.g., heat, water, pest invasion). PWN are introduced to new trees when sawyer beetles fly to healthy trees with tender shoots for maturation feeding (Fig. 2.1), and to stressed, dying, or dead trees to mate and lay eggs (oviposition) (Dropkin et al., 1981; Ferris, 1999; Linit, 1990; Wingfield and Blanchette, 1983).

Feeding preference of various sawyer beetles to determine potential host tree species and future PWD spread have been done in the U.S. and internationally (Futai et al., 1994; Koutroumpa et al., 2009; Naves et al., 2006; Walsh and Linit, 1984). In Portugal, newly emerged *M. galloprovincialis* Olivier (european pine sawyer) were given 36 h choice feeding preference tests (Naves et al., 2006). Scots pine was most heavily fed upon by males (109 mm^2) and females (91 mm^2) while monterey pine (*P. radiata* D. Don) was fed upon the least by males (10 mm^2) and females (7 mm^2). There was no difference in feeding preference between male and female beetles. In France, european pine sawyer choice feeding tests with scots and maritime (*P. pinaster* Aiton) pine were conducted (Koutroumpa et al., 2009). After 36 h, both species were consumed by the beetle, however, the area of scots pine consumed (0.70 cm^2) was greater than maritime pine (0.15 cm^2). Futai et al. (1994) reported on feeding preference of *M. alternatus* Hope (japanese pine sawyer) in Japan for 72 h following emergence. Beetles reared from logs of korean (*P. koraiensis* Siebold & Zucc.) or japanese red (*P. densiflora* Siebold and Zucc.) pine fed on a larger area of korean pine (915 mm^2) than japanese red pine (430 mm^2) regardless of the species they were reared from. Similar to the work in Portugal, there was no difference in feeding preference between male and female beetles. In the U.S., Walsh and Linit (1984) investigated feeding preference of the pine sawyer beetle [*Monochamus carolinensis* (PSB)] on scots, jack (*P. banksiana* Lamb.), shortleaf (*P. echinata* Mill.), and eastern white pine. After a 24 h feeding period, scots pine was most heavily fed upon (0.49 cm^2) followed by jack (0.26 cm^2), shortleaf (0.14 cm^2) and eastern white (0.05 cm^2) pine. Contrary to previously mentioned research, female beetles consumed more of the pine samples than males.

The objective of this research was to conduct choice and no-choice feeding preference tests to determine if PSB would avoid feeding upon any commonly available taxa of Pinaceae. Non-preferred species may be less likely to become infested with PWN and therefore could represent alternative tree species options for green industry professionals. Tree species were selected based on perceived disease susceptibility in Kansas where scots pine is widely recognized as the most susceptible and North American native pines are generally considered tolerant unless otherwise stressed.

Materials and Methods

Beetle Collection

In spring of 2011 to 2014 (April), trunk sections and main lateral branches from pines (approximately 7.6 m tall) previously confirmed by the authors to have died from pine wilt were placed into 162 L transparent, polyethylene containers or a 3.9 x 2.7 m screened enclosure (Ozark Trail, Bentonville, AR) prior to sawyer beetle emergence at the Kansas State University John C. Pair Horticultural Center (Haysville, KS). Due to mold formation on the wood in the polyethylene container, they were not used after 2011 and all beetles were reared and captured from screened enclosures erected inside a polycarbonate greenhouse (Fig. 2.2) to allow for natural photoperiod and temperature control via an evaporative cooling pad. The greenhouse was covered with 50% shade cloth and temperature set at 24 ± 2°C. Trunk sections (approximately 1 m in length) were placed on the enclosure floor in a vertical orientation and branches were stacked in a manner to ensure good airflow. No secondary lateral shoots or needles were placed in the emergence enclosures to prevent beetle feeding upon emergence. Enclosures were inspected daily for beetle emergence. Beetle emergence began in May and continued for approximately six weeks.

Feeding Arena and Taxa Sample Preparation

Feeding arenas (*n* = 124) consisted of 90 L transparent polyethylene containers with transparent lids (unventilated). Individual plant shoots from current season growth, 33 cm in length were collected in the morning and placed into a single 250 ml erlenmeyer flask filled with tap water (Fig. 2.3). The mouths of the flasks were sealed with parafilm to hold the shoot upright and to prevent the beetles from entering the water. Approximately 18 cm of the distal portion of the shoot was maintained above the parafilm for beetle feeding. Each feeding arena contained one flask for each plant specimen evaluated as determined by treatment groupings listed below (Fig. 2.3). Choice feeding trials were conducted by randomly placing one plant sample in each of the four quadrants of the feeding arena. No-choice feeding trials were conducted by placing one plant sample in the middle of the feeding arena. Feeding arenas were arranged in a randomized complete block design, with beetle emergence date as the blocking factor, and placed on tables indoors with fluorescent lighting and temperature set to 24°C. Lights were turned on each morning and off at night providing 10 h of artificial light.

Choice and No-choice Feeding Preference Experiments

Each morning, all beetles were collected from the screened enclosures and one newly emerged beetle (less than 24 h old) was placed into the center of each feeding arena containing plant samples and sealed with the container lid. Beetles remained in the arena to feed for 48 (2011) or 72 (2012-2014) h, which is longer than previous research (Koutroumpa et al., 2009; Naves et al., 2006; Walsh and Linit, 1984). Feeding time was extended to 72 h based on observations that beetles were actively feeding at 48 h but had slowed by 72 h. In previous research Futai et al. (1994) also used a 72 h feeding time. Beetles were removed from the arena and placed into vials and stored at -17.8°C prior to data collection. For each specimen, species was verified according to Lingafelter (2007) and sex determined by visual observation of specific morphological features. Male specimens of *Monochamus spp*. typically have longer antennae and forelegs than females while overall body size of females is greater than males (Benzel, 2015). All captured beetles were determined to be *M. carolinensis* (PSB) and overall male to female sex ratio across all trials and years was 1:1. Specimens used in this research are deposited as voucher number 247 in the Kansas State University Museum of Entomological and Prairie Arthropod Research.

Data Collected

Plant shoots were labeled and placed in plastic zip-top bags after each feeding trial and stored at 4.4°C until data collection. Fascicle bundles of the needles were excised from shoots to prepare the shoots for area analysis. Shoot samples were assessed and scored for feeding occurrence (0 = no feeding; 1 = feeding) and percent feeding. Percent feeding was measured using a leaf area scanning and imaging system (WinFolia, Regent Instruments Inc., Quebec, Canada) connected to a desktop computer. The leaf area scanner captured images and measured the total shoot area for two sides of each shoot (Fig. 2.4). Images of the scans were saved and used to determine the feeding area by drawing exclusion areas around the feeding area on the shoot images and re-analyzing the surface area (Fig. 2.5). Feeding area was calculated based on the difference between the total shoot surface area and shoot surface area fed upon excluded. Percent feeding was calculated as (feeding area ÷ total shoot surface area) × 100.

Year 1

Preliminary choice feeding preference tests were conducted in 2011. PSB were collected from screened enclosures containing scots and austrian pine. Each feeding arena contained one shoot each of loblolly (*P. taeda* L.), ponderosa (*P. ponderosa* P. Lawson & C. Lawson), scots, and southwestern white (*P. strobiformis* Engelm.) pines. This test was repeated nine times.

Authors observed no feeding on southwestern white pine in the choice tests. Therefore, 10 replications of southwestern white pine no-choice feeding were conducted. In both choice and no-choice trials, beetles fed in the arenas for 48 h.

Year 2

In 2012 PSB were collected from screened enclosures containing southwestern white and chinese white pines (*P. armandii* Franch.). Five different choice feeding tests and six different no-choice feeding tests were conducted. The choice trials for 2012 consisted of the following taxa and replications:

Choice 1- loblolly, ponderosa, scots, and southwestern white pines (9 replications)

Choice 2- austrian, loblolly-pitch (*P. taeda* x*rigida* Mill.), and scots pines (17 replications)

Choice 3- deodar cedar [*Cedrus deodara* (Roxb. ex D. Don) G. Don], loblolly-pitch and southwestern white pines (13 replications)

Choice 4- deodar cedar, loblolly, and loblolly-pitch pines (15 replications)

Choice 5- white spruce [*Picea glauca* (Moench) Voss], concolor fir [*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.], and deodar cedar (17 replications)

No-choice feeding trials were conducted for each species in Choice 1 (10 replications) and Choice 2 (15 replications). In both choice and no-choice trials, beetles were allowed to feed for 72 h. Cessation of beetle emergence prevented no-choice trials for species in Choice 3, 4, and 5 trials.

Year 3

In 2013 PSB were collected from the screened enclosures containing austrian and scots pine logs. Two choice feeding tests and four no-choice feeding tests were conducted. The choice trials for 2013 consisted of the following taxa and replications:

Choice 1- loblolly, ponderosa, scots, and southwestern white pines (17 replications)

Choice 2- eastern white, lacebark (*P. bungeana* Zucc. Ex Endl.), and scots pines (16 replications)

No-choice feeding trials were conducted for each species in Choice 1 (5 replications). Cessation of beetle emergence prevented no-choice trials for species in Choice 2. Beetles were allowed to feed for 72 h.

Year 4

In 2014 PSB were collected from screened enclosures containing austrian and scots pine logs. Unfortunately, only 23 beetles emerged from the logs. Due to the shortage of beetles, only one choice and one no-choice feeding trial was conducted. Choice feeding arenas contained one shoot each of loblolly-pitch, ponderosa, scots, and southwestern white pines. This test was repeated 11 times.

Three replications of no-choice feeding trials were conducted for each species of the choice feeding preference trial. In both choice and no-choice feed trials, beetles fed for 72 h.

Statistical Analysis

Choice feeding preference data were analyzed for normality and for year x treatment interaction effects with year designated as a fixed effect to determine if pooling of data across years was appropriate. Data were not normally distributed. Implementation of an arcsin root transformation of the dataset and analysis of year as a fixed factor using the mixed general linear model (MIXED) procedure in SAS (SAS Institute Inc., Cary, NC) indicated no interaction between year and either beetle sex or pine species (treatment) factors. Preliminary analysis indicated a difference in percent feeding between years, so the year factor was subsequently retained in the model as a random effect. Data were analyzed using the PROC GLIMMIX procedure in SAS with treatment differences determined for least square means pairwise comparisons using the Tukey-Kramer adjustment for multiple comparisons with a type I experiment-wise error rate of $\alpha = 0.05$. This analysis was performed for the loblolly, ponderosa, scots, and southwestern white pine choice feeding trials 2011-2014. For the remaining taxa of Pinaceae, an augmented design was used, which included all but one of the four pine species (ponderosa pine) that were evaluated in the first analysis and appeared in combination with the other Pinaceae taxa tested. Ponderosa pine was not included in any other combination of choice tests and so was excluded from the augmented design analysis of the other Pinaceae. Data for the remaining taxa of Pinaceae in the choice feeding preference trials were analyzed using the PROC GLIMMIX procedure in SAS for feeding occurrence and percent feeding of the model based on the distribution of the dataset response variables. The analysis was conducted as an augmented design, with all trials sharing at least one common taxon (check) to allow for pairwise comparison between taxa. The LOGIT link function was used in the GLIMMIX procedure for maximum likelihood estimates of treatment means for comparison between taxa.

Results

Loblolly, Ponderosa, Scots, and Southwestern White Pine Tests 2011-2014

Over the four years *Monochamus carolinensis* (PSB) was the only sawyer beetle recovered from the screened enclosures. The male to female sex ratio in the pine feeding trials was 0.84:1.0 (M:F). Analysis of the fixed effects indicated that neither beetle sex ($P = 0.98$), pine species (treatment) ($P = 0.09$), or the interaction ($P = 0.48$) influenced feeding occurrence. When presented with loblolly, ponderosa, scots, and southwestern white pine, PSB fed 29% of the time on at least one of the taxa (Table 2.1). Pairwise comparison of feeding occurrence showed little evidence of preference by PSB for any of the four pine species (Table 2.3). While not significant, there was some evidence of a slight preference in feeding on loblolly pine compared to scots pine (Tukey-Kramer Adjusted $P = 0.0697$; Table 2.3). Neither beetle sex ($P = 0.65$), pine species (treatment) ($P = 0.76$), nor the interaction ($P = 0.61$) influenced feeding percent on the four pine species tested. PSB fed on 12.4% of the shoot tissue surface area (Table 2.1). Pairwise comparison of percent feeding showed no preference of the PSB for any of the four pine species ($P \geq 0.70$; Table 2.4). In no-choice feeding tests 100% of taxa were fed upon (data not shown).

Augmented Design Analysis for Ten Pinaceae Taxa

The male to female sex ratio for PSB across trial years was 1.17:1.0 (M:F). Analysis of the fixed effects shows that neither beetle sex ($P = 0.99$), Pinaceae taxa (treatment) ($P = 0.08$), or the interaction ($P = 0.92$) influenced feeding occurrence. Averaged across plant species, PSB fed on 38% of the plant shoots presented (Table 2.2). Pairwise comparison of feeding occurrence showed no preference of PSB for any of the ten taxa (data not presented; $P \geq 0.05$). White spruce incurred no feeding in the choice trials, but its presence in a single trial provided insufficient power to detect differences between this species and the other taxa. No-choice tests for white spruce were not conducted due to cessation of beetle emergence after the choice trials. Although not statistically significant, pairwise comparisons of the ten Pinaceae taxa suggested potential PSB preference for both scots and loblolly-pitch hybrid pines compared to deodar cedar based on feeding occurrence (Tukey-Kramer Adjusted $P = 0.0563$ and 0.0570, respectively). The analysis for percent beetle feeding revealed a treatment effect ($P = 0.01$), but neither beetle sex ($P = 0.99$) or the interaction of beetle sex and treatment ($P = 0.92$) influenced percent feeding. Feeding averaged approx. 4% of total shoot area across Pinaceae taxa (Table 2.2). As previously mentioned, white spruce did not incur feeding in the choice trials. Pairwise comparison of the remaining taxa indicated a PSB preference for both eastern white and scots pines over deodar cedar (Tukey-Kramer Adjusted $P = 0.0016$ and 0.0235, respectively).

Discussion

The current study demonstrates that *M. carolinensis* (PSB) is the primary vector of PWD in Kansas. Over a four-year period, no other species of sawyer beetle was recovered from the screened enclosures. This is also the same species use in previous research (Linit, 1990; Walsh and Linit, 1984). Our research suggests that feeding preference of the PSB is not likely the reason for the observed bias in scots pine mortality over other pines. In choice and no-choice feeding preference trials with U.S. native and exotic species of pine, PSB exhibited a wide range of feeding with no particular preference for any species (Tables 2.3 and 2.4). The only exception was a preference for scots and eastern white pines over deodar cedar. This data would suggest that differences in occurrence of PWD among tree species are not related to the behavior of the PSB vector.

Previous work with PSB had shown a strong feeding preference for bark disks of scots pine over jack, shortleaf, and eastern white pines (Walsh and Linit, 1984). Scots pine was consumed twice as much as jack pine and 10x more than eastern white pine. This contrasts with our study in which PSB showed no preference among pine species. Others have investigated feeding preference of european and japanese pine sawyer beetles and similar to our study with PSB, they also reported feeding on all taxa of pine tested (Futai et al., 1994; Koutroumpa et al., 2009; Naves et al., 2006). Additionally, they all report the beetles in their experiments preferred one species to the others. This contrasts with our research where all species of pine were equally fed upon.

Previous research demonstrated that female PSB consume more bark tissue than male beetles (Walsh and Linit, 1984). Our study found both male and female PSB consumed similar amounts of pine shoot tissue. This is similar to reports in which male and female beetles of japanese and european pine sawyer consume equivalent amounts of tissue within the first 72 h after emergence (Futai et al., 1994; Koutroumpa et al., 2009; Naves et al., 2006).

Some of the structural defenses of plants to avoid herbivory include spines, thorns, trichomes, sclerotized leaves, and granular minerals in plant tissues (Hanley et al., 2007). One possible explanation for the lack of observed feeding on white spruce might be due to the presence of sterigmata, a woody stalk attaching each needle to the stem. Spruce needles are rigid and some species end in a sharp apex as a modified spine. White spruce needles do not exhibit a sharp apex as a spine, but are very stiff. The arrangement and rigidity of the needles and presence of sterigmata on spruce may serve as a form of leaf sclerotization to impede beetle attachment and needle removal by PSB.

Our data demonstrate that PSB fed upon several pine species and generally fed upon them equally. This would suggest that PWN would be vectored to many species of pine equally. Therefore, it remains to be determined whether PSB feeding preference plays an important role in PWD etiology.

Figures and Tables

Figure 2.1. Pine sawyer beetle (*Monochamus carolinensis*) feeding on pine shoot.

Figure 2.2. Screened enclosures used for capturing newly emerged pine sawyer beetles (*Monochamus carolinensis*) inside a polycarbonate greenhouse.

Figure 2.3. Feeding arena and arrangement of shoot samples used for choice feeding preference trials.

Figure 2.4. Image of initial scanned ponderosa pine (*Pinus ponderosa*) shoot for feeding area determination.

Figure 2.5. Image of analyzed scanned ponderosa pine (*Pinus ponderosa*) shoot for feeding area determination (black area is feeding area).

Table 2.1. Overall feeding occurrence (%) and feeding area (%) of pine sawyer beetles (*Monochamus carolinensis*) on four species of Pinaceae.

Pinaceae Taxa	***n*[z]**	**Feeding Occurrence (%)[y]**	**Feeding Area (%)[x]**
loblolly pine	56	41.6 ± 6.7	4.3 ± 1.2
ponderosa pine	56	28.8 ± 6.1	3.6 ± 1.1
scots pine	56	19.6 ± 5.3	2.6 ± 1.0
southwestern white pine	56	25.7 ± 6.0	3.7 ± 1.1

[z] Total number of replicate plant samples across all choice trials.

[y] (Number of samples fed on per taxa ÷ *n*)*100.

[x] (Feeding area ÷ shoot area)*100.

Table 2.2. Overall feeding occurrence (%) and feeding area (%) of pine sawyer beetles (*Monochamus carolinensis*) on ten taxa of Pinaceae.

Pinaceae Taxa	n^z	Feeding Occurrence (%)^y	Feeding Area (%)^x
austrian pine	21	48.3 ± 12.2	3.8 ± 1.6
concolor fir	22	47.2 ± 11.4	4.3 ± 1.5
deodar cedar	54	18.6 ± 5.1 *	1.1 ± 0.4 **
eastern white pine	19	53.6 ± 12.0	8.4 ± 2.3
lacebark pine	19	36.6 ± 11.2	5.8 ± 1.9
loblolly pine	32	41.3 ± 9.2	3.1 ± 1.1
loblolly x pitch pine	38	51.8 ± 8.6	4.3 ± 1.1
scots pine	40	51.4 ± 8.4	5.6 ± 1.3
southwestern white pine	15	29.0 ± 13.2	2.1 ± 1.7
white spruce	22	0.0 ± 0.0	0.0 ± 0.0

^{z}Total number of replicate plant samples across all choice trials.

^{y}(Number of samples fed on per taxa ÷ *n*)*100.

^{x}(Feeding area ÷ shoot area)*100.

*, ** indicate that mean was different from scots pine at $P \leq 0.05$ and 0.01, respectively.

Table 2.3. Pairwise comparison of pine sawyer beetle (*Monochamus carolinensis*) feeding occurrence on four species of pine (*Pinus* spp.).[z]

Treatment Comparison	**Estimated Difference[y]**	**Standard Error**	**Adj. *P***
loblolly – ponderosa	0.566	0.404	0.501
loblolly - scots	1.072	0.435	0.069
loblolly – southwestern white	0.725	0.418	0.309
ponderosa – scots	0.506	0.449	0.674
ponderosa – southwestern white	0.159	0.633	0.983
scots – southwestern white	-0.347	0.462	0.876

[z] Error df = 161

[y] Logit scale.

Table 2.4. Pairwise comparison of pine sawyer beetle (*Monochamus carolinensis*) feeding area (%) on four species of pine (*Pinus* spp.).[z]

Treatment Comparison	Estimated Difference[y]	Standard Error	Adj. *P*
loblolly – ponderosa	0.194	0.427	0.968
loblolly - scots	0.529	0.491	0.703
loblolly – southwestern white	0.161	0.423	0.981
ponderosa – scots	0.335	0.507	0.912
ponderosa – southwestern white	-0.033	0.442	0.999
scots – southwestern white	-0.368	0.504	0.885

[z] Error df = 216

[y] Logit scale.

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Chapter 3 - Technological Tools in Plant Identification Courses

Introduction and Literature Review

Resources

Instruction techniques for undergraduate teaching have shifted towards student-centered, facilitated learning as more teaching and learning resources and technologies have become available to instructors and students (Beaudoin, 1990; Davies et al., 1996). Technological resources provide valuable opportunities and resources for traditional in-class and distance horticulture plant identification courses to supplement lecture and lab information. Computer assisted learning and supplemental online resources have been shown to maintain and enhance and increase student learning (Anderson and Walker, 2003; Bing, et al., 2012; Hoch and Dougher, 2011; Peterson and Keeley, 2012; Schittek, et al., 2001). Various computer assisted learning resources include professional society message boards/chat rooms (Paparozzi and Williams, 2000), extensive web-based plant databases from public institutions (e.g., Missouri Botanical Garden, 2014; Oregon State University, 2014; University of Connecticut, 2014; etc.), software databases (Boufford, 1994; Gilman, 1994), dichotomous keys (Shaw, 1993; Wilson and Flory, 2012), interactive quizzes (Campbell et al., 2011) and virtual plant inventories/walks (Polomski and Polomski, 2013; Sabota et al., 1995; Starrett, 2016; Wilson and Danielson, 2005; Wilson and Miller, 2015).

Increasing availability of mobile technologies with Internet-connection capabilities, such as smartphones and tablets, continues to provide students with increased access to information and the ability to study and learn anywhere and anytime.

According to the Pew Research Center, smartphone ownership among U.S. adults rose to 64% in 2015 from 58% in 2014, up from 35% when the research center first began to conduct surveys on smartphone ownership (Pew Research Center, 2014, 2015). It was estimated that 85% of young (18-29 years old) and 79% of middle-aged (30-49 years old) adults in the U.S. own a smartphone (Pew Research Center, 2015); while 42% of all U.S. adults owned a tablet in 2014, compared to 4% in 2010 (Pew Research Center, 2014). These mobile technologies use applications (apps), which can supplement traditional paper-based books, notebooks, and notecards to consolidate data and information from various sources into one resource. Increased availability of data via cellular or wireless local area networks (WLAN/Wi-Fi) on college and university campuses coupled with global positioning capabilities (GPS) of mobile devices allow students to access location-based resources and media for their studies. In 2010, it was estimated that over 80% of U.S. public and private universities, four-year colleges, and over 65% of community colleges have WLAN/Wi-Fi connected classrooms (Green, 2010).

Student Performance and Perceptions of Technology in Horticulture Courses

Correspondence and distance learning horticulture courses have implemented technological and Internet resources for courses such as plant propagation (Gómez, 2004; Wilson and Thetford, 2003), urban horticulture (Martin and Stutz, 1999), plant nutrition (Paparozzi and Williams, 2000), and plant identification (Anderson and Walker, 2003; Campbell et al., 2011; Gilman, 1994, Peterson and Keeley, 2012; Sabota et al., 1995; Seiler et al., 2002; Wilson and Danielson, 2005; Wilson and Flory, 2012; Wilson and Miller, 2015). Plant identification (ID) courses introduce students to numerous plant species and are a significant component of undergraduate horticulture program curricula. In these courses, students are expected to visually recognize many species based on various plant morphological characteristics and depending on the instructor and/or program curricula, are required to provide any combination of the following: plant family, genus, specific epithet, common name and variety/cultivar. In the lecture component, students are introduced to plants to highlight plant ID and cultural characteristics. In the lab portion, students physically observe the different plants through instructor-guided walks around campus grounds, gardens, arboreta, greenhouses, the neighboring community, etc. Peterson and Keeley (2012) found that live-specimen ID quiz scores of students in a web-based approach to teaching turfgrass ID were not different than those of students enrolled in a traditional classroom, however web-based students performed worse on knowledge-based ID questions than traditionally taught students. Anderson and Walker (2003), McCaslin and Na (1994), and Seiler et al. (2002) found computer-based instruction as effective in teaching plant ID. Kahtz (2000) found the ID quiz scores of students experiencing computer-assisted instruction were similar to quiz scores of traditionally taught students regardless of cognitive learning style, but students preferred classroom based instruction in contrast with the computer-assisted program. Taraban et al. (2004) and Teolis et al. (2007) found that traditionally taught students had better plant ID quiz scores than web-based taught students.

Gómez (2004) found no differences in the time required to perform a plant propagation assignment or students’ perceptions of the assignment’s level of difficulty between students receiving instruction using a video or from an instructor, but video-instructed students scored better on the assignment quiz than instructor-guided students.

Vavala et al. (2010) observed the perceptions on learning and grade point averages of students enrolled in web-based in introductory plant science, soil science, and entomology courses were not significantly different from students in these classroom-based introductory courses, although students' perceptions of community and peer to peer connectedness were lower for online students compared to campus-based students. Kahtz (2000) reported students of a plant ID course preferred computer-assisted instruction as a supplemental resource instead of a replacement for traditional classroom-based instruction.

Learning Styles

There are many theories to categorize student learning by various learning styles (Barkley, 1995; Lehman, 2011). Some of the most prominent learning style instruments are: Kolb's experiential learning style inventory, Gregorc's style delineator, Myers-Briggs' indicator, Witkin's group embedded figures test, and Visual-Auditory, and Kinesthetic Assessments.

Kolb's Experiential Learning Style Inventory

Kolb categorizes student-learning preferences in how they experience learning along two dimensions or spectrums: concrete to abstract and experimentation to observation. Students' responses to Kolb's questionnaire place students along each of the two dimensions (axes) to cluster responses into one to four categories (i.e., convergent, divergent, assimilative, accommodating) (Baker et al., 2012; Barkley, 1995; Kolb, 1984). Students classified along the concrete portion of the concrete-abstract spectrum of experiential learners are categorized into either divergent or accommodating learning styles, as these students prefer to fit theories or relationships to already-known facts and information contributed by others. Students along the abstract portion of the spectrum (i.e., convergent and assimilative learning styles) prefer solve problems by creating theoretical models through reasoning and testing theories through making decisions (Baker et al., 2012; Barkley, 1995; Kolb, 1984). Students classified along the experimentation portion of the experimentation-observation spectrum are active learners and are categorized into either accommodating or convergent learning styles as these students prefer to actively problem-solve by actively carrying out hypothesis testing and performing technical tasks. Students along the observational portion of the spectrum (i.e., divergent and assimilative learning styles) prefer conceptualizing ideas and implications through brainstorming and imagination (Baker et al., 2012; Barkley, 1995; Kolb, 1984). Several studies have used Kolb's experiential learning style inventory in seeking to characterize the learning styles of students based on demographics in agricultural courses (Baker et al., 2012; McKim et al., 2013; Ricketts et al., 2005).

Gregorc's Style Delineator

Like Kolb, Gregorc's learning styles consist of two dimensions (spectrums) and shares the concrete to abstract spectrum of Kolb's experiential learning theory (Gregorc, 1979). Gregorc's style delineator differs for the second spectrum, where students are ranked on a continuum of sequential to random in their approach to problem solving and following processes (Gregorc, 1982). Student learning styles are categorized by where their scores appear on each of the two spectrums falling into one of four categories: concrete sequential, concrete random, abstract sequential, or abstract random (Gregorc, 1979 and 1982). Numerous studies have used Gregorc's style delineator to characterize the learning styles of students based for student demographic data in agricultural courses (Cartmell et al., 2007; Kappes and Schmidt, 2002; Moss et al., 2002), with some studies evaluating student performance in relation to learning styles (Lehman, 2007 and 2011).

Myers-Briggs Learning Style Theory

The Myers-Briggs indicator is a theory of learning styles based on personality traits first described by Jung (1971) and modified by Myers-Briggs (1980). The Myers-Briggs indicator examines where students place on four dimensions/spectrums (e.g., sociability, information, decisions, and, structure) to categorize students into one of 16 personality types. Students classified as extroverts on the sociability spectrum are oriented to learning by movement or activities such as group discussions or collaborative projects. Introverts prefer teacher-centered instruction and individual assignments (Barkley, 1995; Myers-Briggs, 1980). Students classified as sensing on the information spectrum are focused on concrete facts and putting existing knowledge to use, whereas intuition classified students focus on concepts and theories and prefer open-ended assignments and problem-solving (Barkley, 1995; Myers-Briggs, 1980). Students oriented to thinking on the decision spectrum prefer to develop an in-depth understanding of how things work and rule-based reasoning, whereas feeling-based students prefer to know how potential solutions will affect people and focuses on convictions and values (Barkley, 1995; Myers-Briggs, 1980). Students oriented to judgement-based learning on the structure spectrum prefer structure, goals, deadlines, and achievement of assignments, whereas perception oriented learners prefer less structured learning environments and open-ended/adjustable goals (Barkley, 1995; Myers-Briggs, 1980). Numerous studies have been conducted at agricultural colleges and courses to characterize student personality types to obtain demographic data (Barkley, 1995; Johnson et al., 1993; Skaggs, 1992; Sorensen, 1998; Young, 1997; Zimmerman et al., 1994), with one study evaluating crossword and fill in the blank exam formats in an animal science course (Hallman et al., 1992).

Witkin's Group Embedded Figures Test

Witkin et al. (1971) approached learning styles through their simplified theory of field dependence/independence learning styles. Field dependent learners rely on external guidance through structured learning environments, whereas field independent students are more self-reliant and prefer to analyze and restructure concepts as a part of their learning experience (Witkin et al., 1971). Several studies have been conducted using Witkin's group embedded figures test to characterize student-learning styles in agricultural courses (Garton and Ball, 2005; James et al., 2004; Kahtz, 2000; Miller, 1997; Whittington and Raven, 1995; Witkin et al., 1971).

Visual, Auditory, and Kinesthetic (VAK) Assessments

Sensory modalities theory of learning styles bases categories of learning styles on three of the five senses, (vision, sound, and touch). Unlike the other learning styles previously mentioned, sensory assessments are based on students' perceptions of the environment around them and what mode (modality) students best process received information using from the environment around them (Barbe and Milone, 1981). Mills (1970) created a learning methods test for teachers to determine which modality strengths students process information efficiently. Barbe et al. (1979) added a kinesthetic modality to the visual and auditory modalities previously evaluated by learning style researchers. Barbe et al. (1979) and Barkley (1995) described that visual learners prefer to learn by reading information or using images such as pictures, graphs/tables, or timelines. Auditory learners prefer to learn through listening to lectures or engaging in class discussion. Kinesthetic or tactile learners prefer to engage in hands-on activities that replicate or accomplish the skill to be learned, whereby information is learned physically through troubleshooting/trial-and-error (Barbe et al., 1979; Barkley, 1995). Dunn et al. (1975) created a learning style inventory for students to self-test and self-report modality preferences. Barbe et al. (1979) conducted a study involving over 1,000 people including elementary school children in southern California, their teachers, music studies high school students, elementary students from a midwestern city, and teachers across the country to characterize learning modality strengths of these populations. Barbe et al. (1979) observed that visual and mixed (co-dominant) modalities each accounted for 30% of the population measured with around 25% of the population having auditory learning as their strongest modality and the remaining 15% having kinesthetic learning as their strongest modality. Most VA and VAK studies have been conducted with primary school children and rarely with infants or adults (Barbe and Milone, 1981; Barkley, 1995). Review of agricultural education articles revealed one study evaluating VAK for students enrolled in two agricultural courses. Contreras et al. (2013) conducted a VAK learning style assessment for two plant ID courses using the instrument from Fleming and Mills (1992) as modified by Chislett and Chapman (2005). The VAK assessment consisted of 30 situational questions with three possible responses. Responses were categorized into each of the learning style preferences. Student learning preferences were calculated as the dominant percentage of responses for a learning style type. For example, a student responding to the VAK with 40% visual, 30% auditory, and 30% kinesthetic would be classified as a predominantly visual learner. No significant correlations between student learning style, frequency of study resource use, or course performance were observed (Contreras et al., 2013).

While supplemental resources have been shown to increase learning, the effectiveness of these resources/tools to increase learning beyond traditional teaching approaches have mostly proven ineffective, but were comparable for agriculture courses (Anderson and Walker, 2003; Contreras et al., 2013; Hallman et al., 1992; Kahtz, 2001; Kappes and Schmidt, 2002; McCaslin and Na, 1994; Peterson and Keeley, 2012; Seiler et al., 2002; Taraban et al., 2004; Teolis et al., 2007). Technological resources have the potential to provide valuable opportunities and resources for plant ID courses to supplement lecture and lab information comparably to traditional in-class instruction. The objectives of the following activities were to introduce the Google Maps web-application tool, describe the creation of the maps as a supplemental resource for students, and to characterize student use and perceptions of the virtual plant walk maps as a plant ID course study resource.

Chapter 4 - Using Google Maps Web-application to Create Virtual Plant Maps for Use as an Online Study Tool in Plant Identification Courses

Abstract

Virtual plant maps were developed using a web-application for plant identification courses with the goal of providing an additional study resource to students. Each map plots the plants covered for the given weekly plant list, providing photographs of specimens, correct nomenclature, and additional identification and cultural information. The virtual plant maps provide students an opportunity to review and revisit plants covered in lecture and laboratory sections on their own and at their convenience. An additional advantage of the virtual plant maps is that they can be easily created using a free web-application via any Internet browser, without the need for rigorous understanding of software and webpage design.

Introduction

Plant identification courses introduce students to numerous plant species and are a significant component of undergraduate horticulture program curricula. In these courses, students are expected to visually recognize many species based on various plant morphological characteristics and depending on the instructor and/or program curricula, are required to provide any combination of the following: plant family, genus, specific epithet, common name and variety/cultivar. Similar to other horticulture plant identification course formats, the Landscape Plants I and II courses (HORT 374 and HORT 375, respectively) at Kansas State University (Manhattan, KS) consist of two 50-

min lectures and one 2 h lab each week. In the lecture component, students are introduced to plants using PowerPoint (Microsoft Corp., Redmond, WA) to highlight plant identification and cultural characteristics. In the lab portion, students physically observe the different plants through instructor-guided walks around campus grounds, gardens, arboreta, greenhouses, the neighboring community, etc.

Technological resources provide valuable opportunities and resources for traditional in-class and distance horticulture plant identification courses to supplement lecture and lab information. Computer assisted learning and supplemental online resources have been shown to enhance and increase student learning (Bing, et al., 2012; Schittek, et al., 2001), though Contreras et al. (2013) found little correlation between study methods use frequency and course grade performance. Various computer assisted learning resources include extensive web-based plant databases from public institutions (e.g., Missouri Botanical Garden, 2014; Oregon State University, 2014; University of Connecticut, 2014; etc.), software databases (Boufford, 1994; Gilman, 1994), dichotomous keys [e.g., FloraGator (Wilson and Flory, 2012)], interactive quizzes (Campbell et al., 2011) and virtual plant walks (Sabota et al., 1995; Wilson and Danielson, 2005).

Moreover, increasingly available mobile technologies, such as smartphones and tablets, may increase student access to information and the ability to study and learn anywhere and anytime their schedules allow. As of Jan. 2014, it is estimated that 83% of young (18-29 years old) and 74% of middle-aged (30-49 years old) adults in the U.S. own a smartphone; while 42% of all U.S. adults owned a tablet in 2014, compared to 4% in 2010 (Pew Research Center, 2014). These mobile technologies use applications (apps), which can supplement traditional paper-based books, notebooks, and notecards to consolidate data and information from various sources into one resource. Increased availability of data via cellular or wireless local area networks (WLAN/Wi-Fi) on college and university campuses coupled with global positioning capabilities (GPS) of mobile devices allow students to access location-based resources and media for their studies. In 2010, it was estimated that over 80% of U.S. public and private universities, four-year colleges, and over 65% of community colleges have WLAN/Wi-Fi connected classrooms (Green, 2010).

Students in plant identification labs often rely on locations of plant materials to relocate reference specimens covered in the course for study purposes. In the Landscape Plants I and II courses at Kansas State University, the authors developed virtual plant walk maps using the Google Maps web-application (Google Inc., Mountain View, CA) as an additional study tool for students. The virtual plant walk maps provided students with plant specimen locations, plant description data, and visual media. The maps were accessible to all students with data-connected mobile devices or computer access. The objective of this paper is to introduce the Google Maps web-application tool and to describe the creation of the maps as a supplemental resource for students.

Creating a Virtual Plant Map with the Google Maps Web-application

Plant walk maps were created using the classic version of Google Maps web-application via an Internet browser. To create custom maps, users are required to have a Google account which is free and available to anyone able to access the web-application via an Internet browser. Using the satellite view (Figs. 4.1A, 1B, and 2A) of campus, points on maps were marked or “pinned” according to the plant specimen locations observed in lab. For each plant specimen pinned, various data were included. Each mark or “pin” provided a “Title”, which consisted of the plant scientific and common name (Fig. 4.1A). Text in the title field was limited to plain text formatting. A “Description” field for each pin allowed for rich text formatting and the proper citation for binomial nomenclature (italic or underline) (Fig. 4.1A). In the description field, additional relevant information and media were included for each plant including; taxonomic family name, key identification characteristics, important cultivars, unique features, and plant specimen image(s) and video media (Fig. 4.1B).

Original photographs captured of exact plant specimens observed in lab were taken by map collaborators and uploaded to Google+ (Google, Inc., Mountain View, CA), a social network website with a media storage feature, to obtain the uniform resource locator (URL) necessary to include the images within the description field. All media forms included within the description data field were required to be from websites or social network websites with media storage features, [e.g., Facebook (Facebook, Inc., Menlo Park, CA), Flickr (Yahoo!, Inc., Sunnyvale, CA), Vimeo (Vimeo, LLC., New York City, NY), Google+ and YouTube (Google, Inc., Mountain View, CA)] that provide a URL associated for each photograph. When images and content not original to the collaborators were used, web addresses and copyright information were cited in the description text. Web videos (e.g., Vimeo, YouTube, etc.) were imbedded into the description field using hypertext markup language (HTML) code provided by video host websites’ “share/embed” feature and were cited as appropriate.

Individual plant pins were modified by color to represent various plant types included on the plant list (e.g., green = evergreen species, blue = deciduous species, yellow = herbaceous perennial species, and pink = annual species) (Fig. 4.2A). Plant lists were mapped weekly, according to the walk and plants observed in lab. Instructors provided students web-links for individual plant walk maps, which were posted on the course website (K-State Online; Axio Learning, Manhattan, KS). Virtual maps were protected which allowed students to view the map but restricted their ability to modify the content. Students were provided with a printed plant list in addition to the web-link.

Benefits/Challenges of Google Maps Virtual Plant Maps

There are numerous ways virtual plant walk maps may benefit instructors and students. Instructors may develop “master maps” containing all plants with associated locations to serve as inventories of the plant specimens on or around campus (Polomski and Polomski, 2013). These inventory maps benefit instructors by providing a visual and spatial way to organize plant walks efficiently and effectively. New plant walks can be easily created using the “Save to map” or “Keyhole Markup Language (KML)” import features in which plants may be pinned to new maps without recreating individual plant pins or a loss of plant information/locations to the master map (Fig. 4.1B). Students have an opportunity to benefit by using the maps as a study tool. Students can relocate and review, at their convenience, in person or online, the exact plants observed in lab along with the following information: family names, identification feature descriptions, and media such as photographs. Additionally, students can review maps numerous times throughout the semester.

The Google Maps web-application is free and accessible through any Internet browser. Maps were accessible on desktop and laptop computers (Fig. 4.2A), tablets, and smartphone Internet browsers (Fig. 4.2B). There are advanced versions of the Google Maps web-application available for purchase; however, for the context of the courses discussed herein, the free version was used by the authors and was more than suitable. The web-application is compatible across all operating system platforms, easily accessible, and does not require special or unique software with multiple purchase licenses.

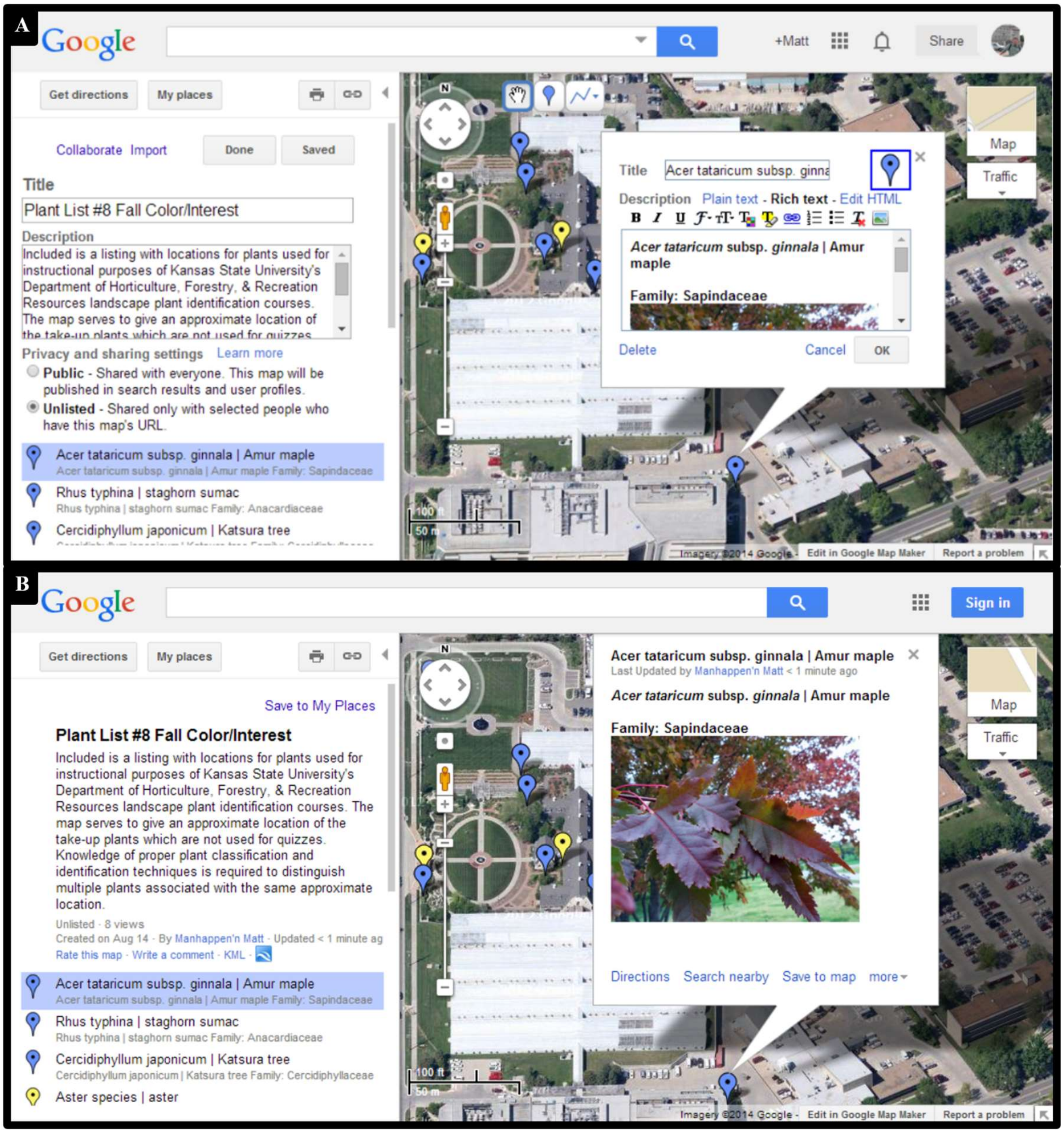
Another major advantage of virtual plant walk maps created using the Google Maps web-application is the ease in which they can be created and viewed. Previously, virtual plant walks required expensive software programs and complex technology skills for image processing and coding of webpages (Sabota et al., 1995; Wilson and Danielson, 2005), whereas the Google Maps web-application is free and user friendly.

Along with benefits, there are potential challenges in creating and implementing virtual plant walks. While the time to create a map is relatively quick, a challenge for instructors may be the initial time required to pin plants to maps, write identification descriptions, and capture original photographs and video media for plant pins. However, collection of most of the items would be required of the instructor for plant identification classes and labs regardless of whether the maps are used. As with all technologies, companies release system upgrades, incorporate new features, improve aesthetics, etc., which can be a challenge as well. The maps were developed using the classic version of the Google Maps web-application. A pre-release version, Google Maps beta, is currently available to the public alongside the classic version. Most, if not all features along with all classic maps are to be included and upgraded within the “My Maps” feature of the final release of the Google Maps web-application.

Conclusions

In conclusion, the Google Maps web-application allows users to create customized virtual plant walk maps as an additional study resource for students enrolled in plant identification courses. The maps can be quickly created using information already prepared for presentation in the classroom. The Google Maps web-application is a free tool teachers of plant identification can employ to increase students' exposure to the plant materials. As an online resource, the virtual maps provide students an opportunity to revisit plants covered in lecture and laboratory sessions at their convenience.

Figures



Title: Plant List #8 Fall Color/Interest

Description: Included is a listing with locations for plants used for instructional purposes of Kansas State University's Department of Horticulture, Forestry, & Recreation Resources landscape plant identification courses. The map serves to give an approximate location of the take-up plants which are not used for quizzes

Privacy and sharing settings Learn more

○ **Public** - Shared with everyone. This map will be published in search results and user profiles.

● **Unlisted** - Shared only with selected people who have this map's URL.

Acer tataricum subsp. ginnala | Amur maple
Acer tataricum subsp. ginnala | Amur maple Family: Sapindaceae

Rhus typhina | staghorn sumac
Rhus typhina | staghorn sumac Family: Anacardiaceae

Cercidiphyllum japonicum | Katsura tree

Title: Acer tataricum subsp. ginna

Description Plain text - Rich text - Edit HTML

***Acer tataricum* subsp. *ginnala* | Amur maple**

Family: Sapindaceae

Plant List #8 Fall Color/Interest

Included is a listing with locations for plants used for instructional purposes of Kansas State University's Department of Horticulture, Forestry, & Recreation Resources landscape plant identification courses. The map serves to give an approximate location of the take-up plants which are not used for quizzes. Knowledge of proper plant classification and identification techniques is required to distinguish multiple plants associated with the same approximate location.

Unlisted · 8 views
Created on Aug 14 · By Manhappen'n Matt · Updated < 1 minute ag
Rate this map · Write a comment · KML ·

Acer tataricum subsp. ginnala | Amur maple
Acer tataricum subsp. ginnala | Amur maple Family: Sapindaceae

Rhus typhina | staghorn sumac
Rhus typhina | staghorn sumac Family: Anacardiaceae

Cercidiphyllum japonicum | Katsura tree
Cercidiphyllum japonicum | Katsura tree Family: Cercidiphyllaceae

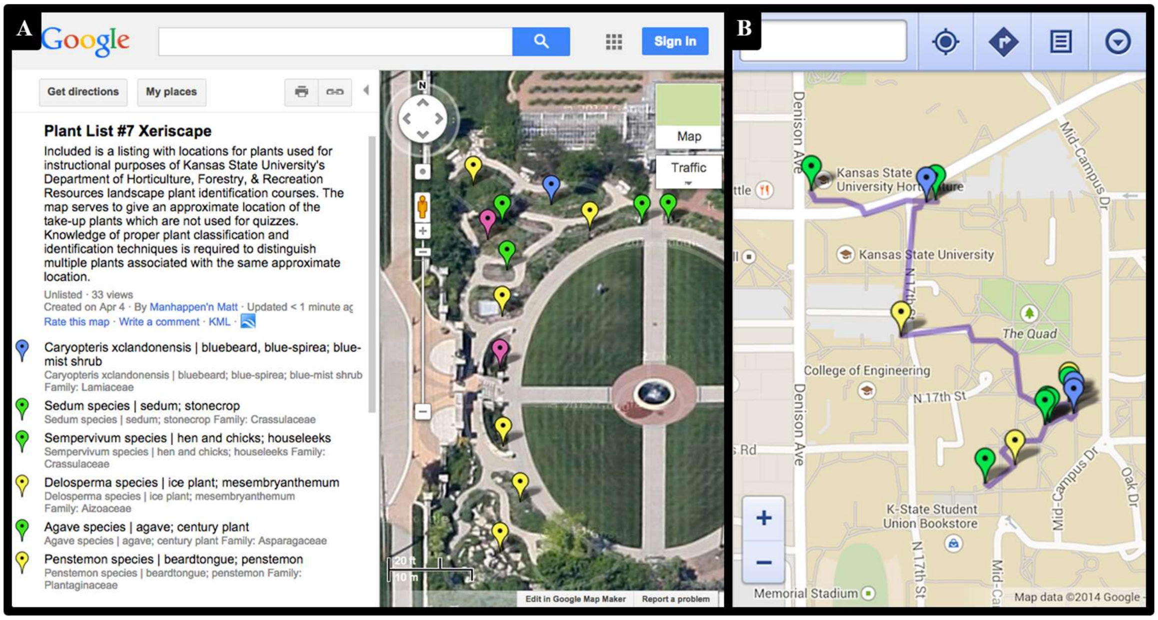
Aster species | aster

Acer tataricum subsp. ginnala | Amur maple
Last Updated by Manhappen'n Matt < 1 minute ago

***Acer tataricum* subsp. *ginnala* | Amur maple**

Family: Sapindaceae

Figure 4.1. Individual plant specimen description field with scientific, common and family names, and image media as viewed in editor (A) and view modes (B) of Google Maps web-application (Google, Inc., Mountain View, CA) using a computer Internet browser. Imagery Copyright 2014 Google. Google and the Google logo are registered trademarks of Google Inc.



Plant List #7 Xeriscape

Included is a listing with locations for plants used for instructional purposes of Kansas State University's Department of Horticulture, Forestry, & Recreation Resources landscape plant identification courses. The map serves to give an approximate location of the take-up plants which are not used for quizzes. Knowledge of proper plant classification and identification techniques is required to distinguish multiple plants associated with the same approximate location.

Unlisted · 33 views
Created on Apr 4 · By Manhappen'n Matt · Updated < 1 minute ag
Rate this map · Write a comment · KML ·

- Caryopteris xclandonensis | bluebeard, blue-spirea; blue-mist shrub
  Caryopteris xclandonensis | bluebeard; blue-spirea; blue-mist shrub Family: Lamiaceae
- Sedum species | sedum; stonecrop
  Sedum species | sedum; stonecrop Family: Crassulaceae
- Sempervivum species | hen and chicks; houseleeks
  Sempervivum species | hen and chicks; houseleeks Family: Crassulaceae
- Delosperma species | ice plant; mesembryanthemum
  Delosperma species | ice plant; mesembryanthemum Family: Aizoaceae
- Agave species | agave; century plant
  Agave species | agave; century plant Family: Asparagaceae
- Penstemon species | beardtongue; penstemon
  Penstemon species | beardtongue; penstemon Family: Plantaginaceae

Figure 4.2. Plant walk maps as viewed with Google Maps web-application (Google, Inc., Mountain View, CA) desktop (A) and mobile (B) Internet browsers. Plant list is shown to the left in A, with the different colored pins in both maps. Panel A also shows a satellite image view, while panel B shows a simple map view with plant pins and an outlined walking route. Imagery Copyright 2014 Google. Google and the Google logo are registered trademarks of Google Inc.

Chapter 5 - Student Use and Perceptions of Virtual Plant Walk Maps as a Study Tool in Plant Identification Courses

Abstract

Virtual plant walk maps were developed for an ornamental plant identification course, with the goal of providing an additional study resource to potentially enhance student learning. The maps provided students an opportunity to revisit plants covered in lecture and laboratory sections at their own convenience, using either a computer or mobile device. Each map plotted the locations of the plants from the corresponding list and provided: photographs of specimens, plant family, common and scientific names, and plant type information. An end of the course survey collected information about student use and perceptions of the virtual plant walk maps for two fall semesters (*n* = 87). Survey results indicated 63% of the students used the virtual plant walk maps as a study resource. Students who used the maps reported accessing the maps an average of 3.2 times between receiving the maps and taking the plant identification quiz in lab. Students mainly used the maps to study the most current plant list and accessed previous plant list maps to a lesser extent. Approximately 67% of students who used the virtual maps, used the maps to visually review the plants online only, while 31% of students, used the maps for both visual review and to physically re-trace the plant walk to view the live specimens. Of the students who did not use the maps, most found other study resources/methods more useful or they forgot about the maps as a resource. When asked to rate usefulness of the maps on a scale from slightly useful (1) to very useful (3), 43% of students indicated that the virtual maps study tool was very useful, 25% indicated the maps were useful, and 8%

indicated that the maps were slightly useful. A significant dependence between student use frequency and student usefulness ratings of virtual plant walk maps was observed. As students' use of the virtual maps increased, they perceived the maps to be more useful to their studies in preparing for identification quizzes. No differences between plant identification quiz scores were observed between students who used and did not use the virtual plant walk maps or between learning styles. Our survey indicated students used the virtual plant walk maps as a resource and perceived the maps as a useful tool in preparation for identification quizzes.

Introduction

Plant identification (ID) courses are a significant component of undergraduate horticulture program curricula. Students are introduced to numerous plant species in these courses, often through instructor-guided walks around campus during the laboratory (lab) sessions. Students are quizzed on plants from previously covered plant lists, with the expectation that students can visually identify the plants based on various morphological characteristics using live specimens and/or photographs (photos). In our course, weekly identification quizzes account for approximately 60% of the course grade. Exams and projects aimed to test cultural and landscape use knowledge of the plant material accounted for the remaining portion.

Technology Use in Education

Instruction techniques for undergraduate teaching have shifted towards student-centered, facilitated learning as more teaching and learning resources and technologies have become available to instructors and students (Beaudoin, 1990; Davies et al., 1996).

Technological resources provide valuable opportunities and resources for traditional in-class and distance horticulture plant identification courses to supplement lecture and lab information. Computer assisted learning and supplemental online resources have been shown to maintain and enhance and increase student learning (Anderson and Walker, 2003; Bing, et al., 2012; Hoch and Dougher, 2011; Peterson and Keeley, 2012; Schittek, et al., 2001). Various computer assisted learning resources include professional society message boards/chat rooms (Paparozzi and Williams, 2000), extensive web-based plant databases from public institutions (e.g., Missouri Botanical Garden, 2014; Oregon State University, 2014; University of Connecticut, 2014; etc.), software databases (Boufford, 1994; Gilman, 1994), dichotomous keys (Shaw, 1993; Wilson and Flory, 2012), interactive quizzes (Campbell et al., 2011) and virtual plant inventories/walks (Polomski and Polomski, 2013; Sabota et al., 1995; Starrett, 2016; Wilson and Danielson, 2005; Wilson and Miller, 2015).

Increasing availability of mobile technologies with Internet-connection capabilities, such as smartphones and tablets provide students with increased access to information and the ability to study and learn anywhere and anytime. According to the Pew Research Center, smartphone ownership among U.S. adults rose to 64% in 2015 from 58% in 2014, up from 35% when the research center first began to conduct surveys on smartphone ownership (Pew Research Center, 2014, 2015). Increased availability of data via cellular or wireless Internet on college and university campuses coupled with global positioning capabilities (GPS) of mobile devices allow students to more readily access location-based resources and media for their studies. In 2010, it was estimated that over 80% of U.S. public and private universities, four-year colleges, and over 65% of community colleges have wireless local area networks (WLAN/Wi-Fi) connected classrooms (Green, 2010). These technological resources have the potential to provide valuable opportunities and resources for plant ID courses to supplement lecture and lab information. Students in plant ID courses can rely on several techniques to learn the plants, including revisiting exact plant specimens in a given area (e.g., campus, adjacent neighborhood, arboreta, etc.) that were covered in the course. Web-based mapping technologies allow both instructors and students to create virtual plant walk maps containing the locations, identification and descriptive information, as well as photos and videos for each plant specimen plotted on the maps (Wilson and Miller, 2015).

Learning Styles

Sensory modalities theory of learning styles bases categories of learning styles on three of the five senses, (vision, sound, and touch). Unlike the other learning styles theories, sensory assessments are based on students' perceptions of the environment around them and what mode (modality) students best process received information using from the environment around them (Barbe and Milone, 1981). Mills (1970) created a learning methods test for teachers to determine which modality strengths students process information efficiently. Barbe et al. (1979) added a kinesthetic modality to the visual and auditory modalities previously evaluated by learning style researchers. Barbe et al. (1979) and Barkley (1995) described that visual learners prefer to learn by reading information or using images such as pictures, graphs/tables, or timelines. Auditory learners prefer to learn through listening to lectures or engaging in class discussion. Kinesthetic or tactile learners prefer to engage in hands-on activities that replicate or accomplish the skill to be learned, whereby information is learned physically through troubleshooting/trial-and-error (Barbe et al., 1979; Barkley, 1995). Dunn et al. (1975) created a learning style inventory for students to self-test and self-report modality preferences. Barbe et al. (1979) observed that visual and mixed (co-dominant) modalities each accounted for 30% of the population measured with around 25% of the population having auditory learning as their strongest modality and the remaining 15% having kinesthetic learning as their strongest modality. Most VA and VAK studies have been conducted with primary school children and rarely with infants or adults (Barbe and Milone, 1981; Barkley, 1995). Review of agricultural education articles revealed one study evaluating VAK for students enrolled in two agricultural courses (Contreras et al., 2013).

Virtual plant walks using the Google Maps web-application (Google, Mountain View, CA) were created and implemented in two semesters of the Landscape Plants I course at Kansas State University (Manhattan, KS). Students were provided with links to the plant maps with the plotted locations of the plants observed in the weekly lab period. The maps allowed students to either physically re-walk the lab route on campus and view live specimens alongside the map photos on their smartphone or tablet and/or virtually re-walk the plant walk observing photos of the plants on the lab route using mobile devices or computer. The objective of this study was to characterize student use and perceptions of the virtual plant walk maps as a plant ID course study resource.

Materials and Methods

Virtual plant walk maps were created weekly for two fall semesters (2013 and 2014) of the HORT 374 Landscape Plants I course, by the teaching assistant and faculty, using the classic version of the map web-application (Wilson and Miller, 2015). The plant species observed in the weekly lab portion of the courses were marked or “pinned” on the maps. Each plant pin included information such as nomenclature (i.e., family name, genus, specific epithet, and common name), plant type (e.g., annual, perennial, deciduous, or evergreen), and plant specimen images. Plant images were added to provide a systematic approach to plant identification, including photos of overall plant form/texture, leaf type, arrangement, venation, and margin, reproductive structures (e.g., flower and fruit), and other unique characteristics (e.g., buds, bark, etc.). No written identification information was included in the description field for the maps as the images were meant to replicate the identification process discussed and observed during lab walks. Following observation of the weekly plant list in lab, instructors emailed the students with a web-link for the corresponding plant walk map via the course management website (K-State Online; Axio Learning, Manhattan, KS). Students were not given map modification privileges, only the ability to view the maps. Individual map links remained active for the entire semester to allow students to use the maps to study plants from previous plant lists, because once a plant was introduced in the course, students could be quizzed on that species any given week, thereafter.

A voluntary survey instrument was administered at the completion of each course, both semesters (*n* = 87), to assess if students used the maps as a study resource and whether they perceived the maps as a valuable resource. Along with student demographics, the survey included questions about the number of hours students spent studying for weekly quizzes (Fig. C.1), usage [frequency, (Fig. C.2 and 3)], and perceived usefulness (Fig. C.4) of the virtual plant walk maps. Four questions were included to characterize how students did or did not use the virtual plant walk maps (Fig.

C.2): 1) How many times did you access the virtual plant maps between receiving the maps and taking the quiz?; 2) Which maps did you primarily access (i.e., "previous plant lists", "current plant list", or "both" previous and current plant lists)?; 3) For what purpose did you use the maps (i.e., visual review online, physical re-walking lab route, or both online and physical review)?; and 4) If you did not use the maps, why not? Students were also asked to rate how often they used the virtual plant walk maps resource on a scale from: did not use (0), rarely (1), occasionally (2), to frequently (3). Students rated the usefulness of the virtual plant walk maps resource as: did not use (0), slightly useful (1), useful (2), or very useful (3).

In addition to the study resource survey, students completed a visual, auditory, and kinesthetic learning style assessment (VAK) at the end of both semesters using the instrument from Fleming and Mills (1992) as modified by Chislett and Chapman (2005) and used by Contreras et al. (2013). The VAK assessment consisted of 30 situational questions with three possible responses. Responses were categorized into each of the learning style preferences. Student learning style preferences were calculated as the dominant percentage of responses for a learning style type. For example, a student responding to the VAK with 40% visual, 30% auditory, and 30% kinesthetic would be classified as a predominantly visual learner. Most students exhibited a preference for a learning style to some extent in the VAK assessment (Fig. 5.1). The learning style with the largest percentage of points was used to classify each student's dominant learning style preference. Students exhibiting equal percentages in two or more learning style categories ($n = 9$) were combined and categorized into a co-dominant group, as no primary learning style preference could be attributed to the student. Three responses were omitted from analyses associated with the VAK assessment, as no learning style could be attributed to two students who did not complete the VAK assessment and one student gave highly suspect answers in answering 100% of the VAK questions for one learning style. One response was omitted from comparisons of quiz scores between virtual plant walk map use and learning styles, as the individual's quiz grade was greater than four standard residuals away from the mean quiz score of the study. The study resource survey and VAK assessment data were entered and coded for each student participant by a third party to anonymize the data and ensure objectivity in analysis of the data. The study resource survey, VAK assessment, and data collection procedures were evaluated and exempted by Kansas State University's Committee on Research Involving Human Subjects/ Institutional Review Board (IRB proposal no. 6911) under the criteria set forth in the Federal Policy for the Protection of Human Subjects.

Descriptive statistics, including frequencies were counted and percentages calculated for course demographics, student resource use, and resource perceptions. We separated map use characteristics, use frequency ratings, and usefulness ratings by the dominant learning style preferences reported by students in the VAK learning assessment survey. Demographic information and map survey data, were combined, containing all 87 student responses and where separated by learning style preference, 84 student responses. Data were analyzed using SAS (version 9.4; SAS Institute, Cary, NC) statistical analysis software program with a level of significance set at $\alpha = 0.05$. A test of independence [chi-square analysis using a Fisher's exact test (i.e., Freeman-Halton test) for contingency tables larger than two by two] was performed between student use frequency and student usefulness ratings of virtual plant walk maps. A generalized linear mixed model with a βdistribution and LOGIT link function was used for maximum likelihood estimates of means for comparison of quiz scores between virtual plant walk map usage and dominant learning style types and their two-way interaction. Semester was included the model as a random effect with map usage and learning styles as fixed effects. Differences between fixed effects were determined for least square means pairwise comparisons using the using the Tukey-Kramer adjustment for multiple comparisons with a level of significance set at α = 0.05

Results and Discussion

Eighty-seven students participated in the voluntary survey resulting in a participation rate of 92.5% (total students enrolled = 94). The total number of survey participants for both semesters, consisted of 40 female and 47 male students comprising 35 sophomores, 32 juniors, 18 seniors, and 2 graduate students. Most the students were horticulture majors (*n* = 57), followed by landscape architecture (*n* = 20), and other majors (*n* = 10; e.g., agriculture education, agronomy, parks management, etc.). Approximately 76% of the class had not previously taken a plant ID course (Table 5.1). Percentages from the VAK learning style assessment scores (*n* = 84) identified 28 (33%) students as mostly visual learners, 8 (10%) students as auditory learners, 39 (46%) students as kinesthetic learners, and 9 (11%) students as co-dominant or balanced learners (Fig. 5.1). With exception of the presence of co-dominant learners, the percentages of learning styles were similar to those of Barbe et al. (1979) and Contreras et al. (2013).

Students reported studying for an average of 4.1 h for the weekly identification quizzes (Table 5.2). When surveyed whether the students used the virtual plant walk maps, 63% (*n* = 55) of the respondents indicated they used the maps while 37% (*n* = 32) did not (Table 5.2). The percent of total students who reported to use the maps in the map use characterization question (63%) of the survey (Table 5.2) differed from those in the map use frequency rating question, in which 77% of students reported to use the maps resource (Fig. 5.2). It is believed that not all students continuously used the maps resource throughout the semester but may have used them at least once, hence contributing to the discrepancy. This may be explained that although 77% (*n* = 67) of the students tried the maps as a study tool at least once (Fig. 5.2), 55 students (63%) continued to use the maps throughout the semester (Table 5.2). Students used the virtual plant walk maps an average of 3.2 times per week before taking the quiz (Table 5.2). Considering those who reported using the maps, 68% of visual learners, 50% auditory, 59% as kinesthetic and 67% characterized as co-dominant learners used the virtual plant walk maps resource.

When asked how they used the maps in preparation for weekly identification quizzes, 47% of students (across all learning styles) indicated they used the maps to primarily study the current plant list, and 47% of students to study both current and previous lists prior to the quiz (Table 5.2). Very few students used the maps to primarily study only previous plant lists. Student responses to the question, “for what purpose did you use the maps (i.e., visual review online, physical re-walking lab route, or both online and physical review)” indicated most of the students (67%) viewed the maps online for visual review of the plants using the images included for each plant pinned to the map and 31% of students used the maps for visual review combined with physically re-walking (Table 5.2). Seventy-nine percent of visual and 74% of kinesthetic learners mainly used the maps for visual review of the plant list specimens online. Approximately 21% of visual and 22% of kinesthetic learners used the maps to both review the plant list online and follow the map to physically re-walk the lab route (Table 5.2). Auditory learners mostly used the virtual plant walk maps to both visually review the plant lists online and physically re-walk the lab route (75%) with 25% using the maps only to review the plant material online (Table 5.2). Considering the maps had no audio information associated with a pinned plant, the use of two implementation strategies may have proven useful for auditory learners. In future maps, it may be beneficial to include audio information to enhance the study resource for auditory learners. Students who did not use the maps indicated that they found other study resources/methods more useful, while the second most cited reason for not using the digital maps was that they forgot about them as a resource, despite receiving a weekly email and verbal reminders (Table 5.2).

Students who reported using the virtual plant walk maps resource were asked to rate how often they used the resource; did not use (0), rarely (1), occasionally (2), or frequently (3), results indicated students used the maps occasionally to frequently. Approximately 28% of students used the maps frequently, 31% occasionally, and 18% rarely. Co-dominant learners (66.7%) and visual learners (60.7%) reported using the maps occasionally to frequently, whereas, 56.4% of kinesthetic and 50% of auditory learners reported using the maps resource occasionally to frequently (Fig. 5.2).

Student perceptions of the usefulness of the virtual plant walk maps resource, rated on a scale from did not use (0), slightly useful (1), useful (2), or very useful (3), indicated that most students who used the study resources perceived virtual plant walk maps as very useful. Nearly 43% of students rated the virtual plant walk maps as very useful with 50% of visual learners rating the maps as very useful compared to 37.5% of auditory learners and 38.5% of kinesthetic learners (Fig. 5.2). Twenty-one percent of students classified as visual learners, 12.5% of auditory, and 30.8% of kinesthetic learners rated the maps as useful. Eight percent of students rated the maps as slightly useful. No auditory learners rated the maps as slightly useful (Fig. 5.2). A test of independence [chi-square analysis using a Fisher's exact test (i.e., Freeman-Halton test) for contingency tables larger than two by two] indicated a significant dependence between student use frequency and student usefulness ratings of virtual plant walk maps ($P < 0.0001$; df = 4). The significant dependency suggests as students' use of the virtual maps increased, they perceived the maps to be more useful to their studies in preparing for identification quizzes (Table 5.3).

The mean quiz scores of students who did not use and those who used the virtual plant walk maps resource were similar and similar across learning styles (Table 5.4). No differences were found between the quiz scores of students who did not use and those who used the virtual plant walk maps resource (Table 5.5). Additionally, no differences were found between dominant learning styles (Table 5.5) or the two-way interaction between map use and learning style ($P = 0.4795$, data not shown). It must be noted this analysis is of the data from an observational study rather than an experiment and is a measure of the possible differences between quiz scores associated with map use or learning style rather than differences attributed to experimental units, where subjects are assigned to treatment groups (e.g., no map use or map use) and tested using standardized exams (e.g., pre-tests and post-tests). Further work using pre-tests and post-tests for control and map use groups should be performed to assess the effect of the virtual plant walk map resource on student performance in plant identification quizzes.

Students were open to using the virtual plant walk maps as a study tool, as nearly 77% of the students tried the maps (Fig. 5.2) and 63% of the class continued to use the maps for study purposes as based on the map use characterization portion of the survey (Table 5.2). The virtual plant walk maps were used by students mainly as a visual study resource and to less extent, a resource to retrace the plant walk to view live specimens. Nearly all students who used the maps studied the most current plant list during the semester and around half of them used the maps to study previous plant lists in addition the most current list. Virtual plant walk maps were viewed approximately three times before the quiz each week. Students who used the maps perceived the study tool to be very useful in their preparation for identification quizzes as were Internet, notes, and flashcard study resources (data not shown). Students who did not use the maps perceived other resources (e.g., Internet, notes, flashcards, etc.) as more useful to helping them study for plant identification quizzes. No differences between plant identification quiz scores were associated with virtual plant walk map use or learning style. The virtual plant walk maps can be modified to include audio, videos, and text in the description field of the pins and be implemented as interactive activities such as scavenger hunts to locate, identify, and pin plants on their own or collaborative map for matching descriptions provided by unnamed pins on a map. With inclusion of such enhancements, instructors may be able to encourage more students to use virtual plant walk maps as a study resource and provide multiple methods for students of all learning styles to learn plant identification suited to their learning style preference. While supplemental resources have been shown to increase learning, the effectiveness of these resources/tools to increase learning beyond traditional teaching approaches have mostly proven ineffective, but were comparable for agriculture courses (Anderson and Walker, 2003; Contreras et al., 2013; Hallman et al., 1992; Kahtz, 2001; Kappes and Schmidt, 2002; McCaslin and Na, 1994; Peterson and Keeley, 2012; Seiler et al., 2002; Taraban et al., 2004; Teolis et al., 2007). Technological resources have the potential to provide valuable opportunities and resources for plant ID courses to supplement lecture and lab information comparably to traditional in-class instruction. Our survey results indicated students used the virtual plant walk maps as a resource and perceived the maps as a very useful tool in preparation for identification quizzes. Virtual plant walk maps as a study tool in plant identification courses may be a resource to use in addition to traditional study resources.

Figures and Tables

Table 5.1. Student demographics for two fall semesters (2013 and 2014) of an ornamental plant identification course at Kansas State University.

Student demographics	**Fall 2013** *n*=49	**Fall 2014** *n*=38	**Total** *n*=87
Academic standing		**Students (%)**	
Sophomore	53	24	40
Junior	25	52	37
Senior	18	24	21
Graduate Student	4	0	2
Major		**Students (%)**	
Horticulture	43	95	66
Landscape Architecture	41	0	23
Other[z]	16	5	11
Previous course in plant identification		**Students (%)**	
No	80	71	76
Yes	20	29	24

[z] Other majors included: Agronomy, Agricultural Education, Park Management, Life Sciences, etc.

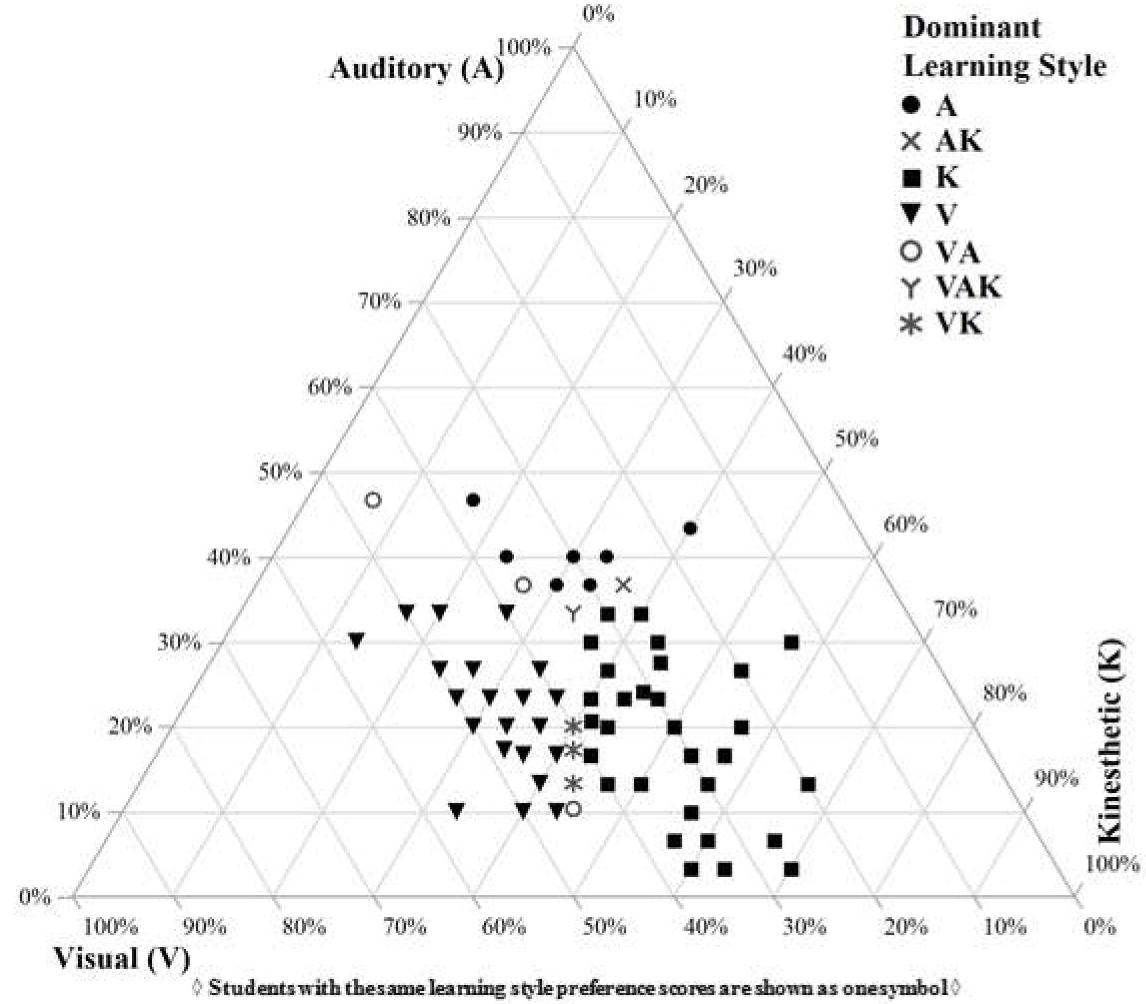


Figure 5.1. Ternary plot of learning style preference percentages for students of two semesters of an ornamental plant identification course (*n* = 84).

Table 5.2. Characterization of student study habits and use of virtual plant walk maps to study for plant identification quizzes based on student responses to survey questions.

		Learning Style Preferences[y]			
Student Study and Map Adoption Characteristics	**All[z]**	**Visual**	**Auditory**	**Kinesthetic**	**Co-Dominant**
	***n*=87**	***n*=28**	***n*=8**	***n*=39**	***n*=9**
Average hours spent studying before quiz	4.1	4.3	4.1	3.9	4.9
Percentage of students that used the maps	63	68	50	59	67
Student Map Use Characteristics	***n*=55[x]**	***n*=19**	***n*=4**	***n*=23**	***n*=6**
Average map accessions before quiz	3.2	3.6	2.3	2.8	4.0
When students used the maps[w]					
Primarily to review previous plant lists	6	5	0	9	0
Primarily for the current plant list	47	53	75	43	33
Both current and previous plant lists	47	42	25	48	67
How students used the maps					
Visually review (online)	67	79	25	74	50
Physically re-walk lab route	2	0	0	4	0
Both visually and physically	31	21	75	22	50
Reasons for not using the maps[v]	***n*=32**	***n*=9**	***n*=4**	***n*=16**	***n*=3**
Other study methods more useful	63	78	75	56	33
Forgot about the maps as a resource	34	22	25	38	67
Could not find or access the maps	3	0	0	6	0

[z] Students from two fall semesters of a plant identification course (*n*=87).

[y] Students characterized by dominant or co-dominant learning style from the visual, auditory, kinesthetic assessment (VAK; *n*=84).

[x] Number of students who reported using the maps resource.

[w] All values reported below are percentages of student responses to questions (rows and sub-rows) by column categories.

[v] Number and percentages of student responses to questions (row and sub-rows), by column categories, for student respondents who reported not using the maps resource.

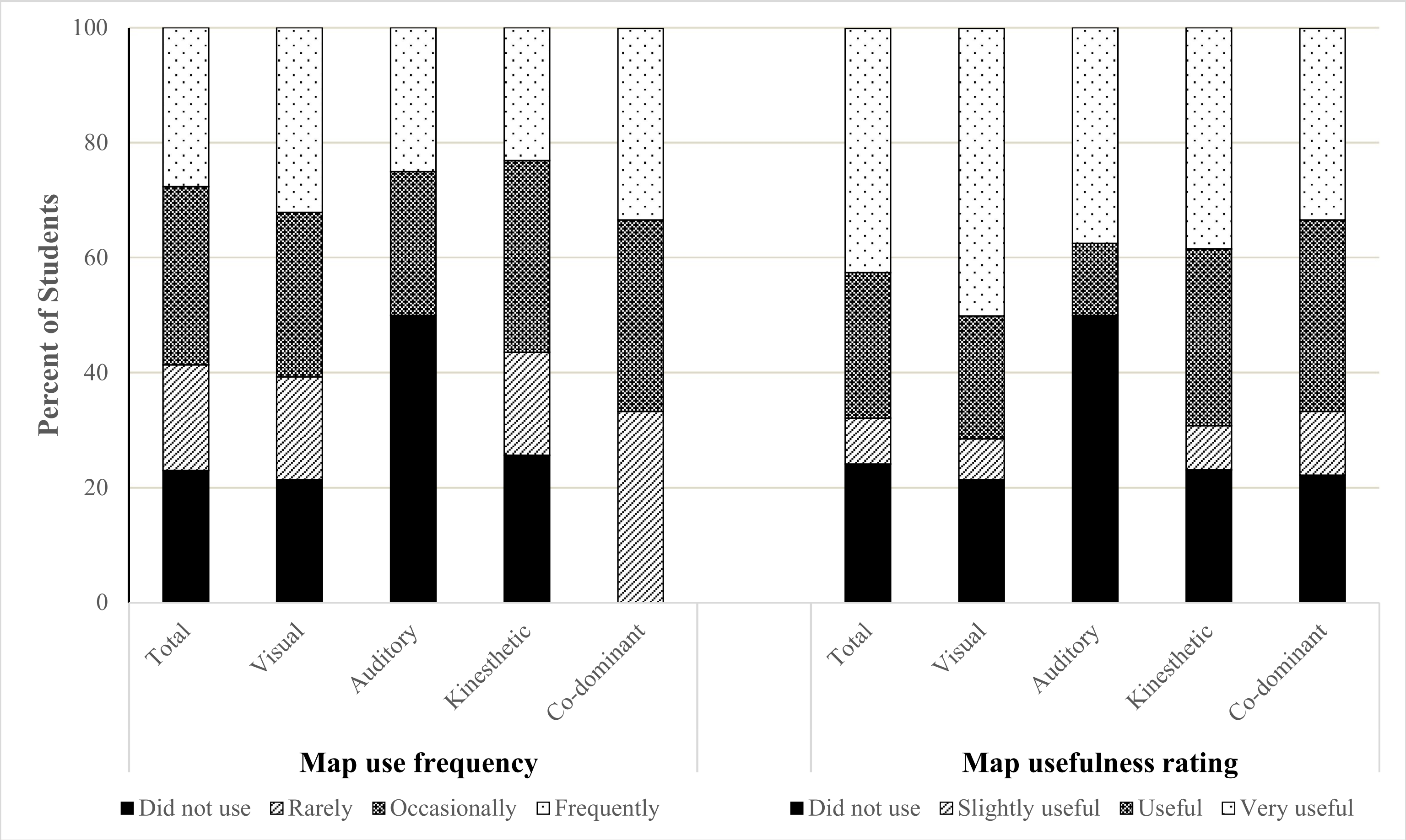


Figure 5.2. Percentages of self-reported student use and usefulness of virtual plant walk maps for two semesters of an ornamental plant identification course combined (n = 87; “Total”) and separated by dominant learning style preference (n = 84).

Table 5.3. Contingency table of frequencies for student ratings use frequency and usefulness of virtual plant walk maps for two semesters of a plant identification course.[z]

Map Use Frequency	Map Usefulness			Total
	Slightly Useful	Useful	Very Useful	
Rarely	6	5	2	13
Sometimes	0	10	16	26
Frequently	0	5	19	24
Total	6	20	37	63

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated significant dependence (*P* < 0.0001; df = 4) for student use frequency and usefulness ratings of virtual plant walk maps.

Table 5.4. Mean quiz scores for virtual plant walk map usage and dominant learning styles for two semesters of a plant identification course.[z]

Map Usage	**Mean Quiz Score (% ± SE)**
Did not use plant walk maps	82.4 ± 2.8
Used plant walk maps	82.9 ± 2.4
Dominant Learning Style	
Visual	85.2 ± 2.5
Auditory	78.4 ± 4.7
Kinesthetic	80.3 ± 2.3
Co-dominant	85.9 ± 3.9

[z] Error df = 74

Table 5.5. Pairwise comparisons of student quiz scores for virtual plant walk map usage and dominant learning style for two semesters of a plant identification course.[z]

Map Use Comparison	**Estimated Difference**[y]	**Standard Error**	**Adj. *P***
Students who did not use maps – Students who used maps	-0.038	0.234	0.873
Dominant Learning Style Comparison			
Visual – Auditory	0.460	0.326	0.499
Visual – Kinesthetic	0.344	0.228	0.436
Visual – Co-dominant	-0.061	0.362	0.998
Auditory – Kinesthetic	-0.116	0.299	0.980
Auditory – Co-dominant	-0.520	0.410	0.585
Kinesthetic – Co-dominant	-0.405	0.336	0.626

[z] Error df = 74

[y] Logit scale

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Appendix A - Pinewood Nematode Pathogenicity for Six Pine Species

A critical aspect of the pine wilt disease life cycle is the susceptibility of the tree species to the pinewood nematode [*Bursaphelenchus xylophilus* (PWN)]. Therefore, the following study was conducted to determine the relative susceptibility of six pine species (1 to 2 years old) to PWN. Species included *Pinus nigra* (austrian pine), *P. sylvestris* (scots pine), *P. flexilis* (limber pine), *P. ponderosa* (ponderosa pine), *P. strobiformis* (southwestern white pine), and *P. taeda* (loblolly pine). All species were planted into 6.0 L trade #2 containers (Nursery Supplies, Chambersburg, PA) containing 6:1 (by vol.) pine bark: sand substrate amended with 6.59 $kg \cdot m^{-3}$ of 18-16-12 (18N–2.6P–9.9K) Osmocote Classic fertilizer (Scotts, Marysville, OH), 0.89 $kg \cdot m^{-3}$ of Micromax (Scotts, Marysville, OH) on 5 and 6 May 2011 except scots pine, which was planted on 12 May. Plants were then placed outside on a gravel container pad at the John C. Pair Horticultural Research Center, Haysville, KS (37°31’N, 97°18’W) and hand-watered after planting. Plants were maintained with overhead irrigation using approximately 25.4 $mm \cdot day^{-1}$ of water via (2) 12.7 mm cycles for two weeks. Plants were moved into a polycarbonate greenhouse to avoid exposure to PWN from endemic emerging vector [pine sawyer beetle (*Monochamus carolinensis*)]. The greenhouse was covered with 50% shade cloth and temperature set at 24±2°C. The plants were micro-irrigated using spray stakes 0.19 $L \cdot min^{-1}$ (Netafim Fresno, CA) equivalent to 12.7 mm overhead irrigation as needed (approximately 2 $min \cdot 2$ $times \cdot day^{-1}$). Plants were arranged in a randomized complete block design with stem caliper as the blocking factor.

Scots pine trees affected with pine wilt disease were identified prior to the experiment. These trees were used for PWN inoculum collection following established methods (James et al., 2006). Primary lateral branches approximately 7-14 cm in diameter and exhibiting the early symptoms of pine wilt were cut into 5-10 cm^2 segments, approximately 2 cm thick, and placed in 500 ml beakers containing tap water and air-agitated for 24 h at 20°C in Manhattan, KS. After 24 h, the PWN inoculum suspension was filtered with a 38 µm screen to collect PWN on the screen. The PWN were then rinsed from the screen with water into a beaker. Final beaker volume contained approximately 40 ml of water for PWN concentration determination. PWN concentration was determined using 1 ml of nematode rinse water and placing on a separated plate, counting slide. All PWN in the aliquot were counted at 40x magnification and adjusted to a concentration of 1100 $PWN \cdot ml^{-1}$. Final suspensions containing PWN were placed in centrifuge tubes and stored 12 h for inoculation at John C. Pair Horticulture Center in Haysville, KS on 12 Aug. 2011. Each species/inoculum treatment combination contained 10 replications that were blocked randomly throughout greenhouse (6 species·3 treatments·10 replications, $n = 180$). The treatments consisted of:

1. Control (distilled water only)
2. Inoculum suspension solution, filtrate (no nematodes)
3. 1100 $PWN \cdot ml^{-1}$ suspension

To conduct the inoculation, the main central leader of all the plants was pruned at approximately 5 cm below the apical bud. A 4.8 mm diameter wood drill bit was used to create a reservoir capable of containing 0.5 ml of treatment solution by boring down through the center of the stem from the cut surface (approximately 2.8 cm deep). Treatments of water, filtrate, or PWN suspension from the stored centrifuge vials were pipetted into the reservoirs. The pruning wound and treatment reservoir were sealed using parafilm following treatment. Pine wilt disease progression symptoms were recorded for each species four weeks after treatment on 7 Sept. 2011 using a scale of the following criteria:

0 = no symptoms

1 = greying/drooping of needles

2 = death

Samples were collected from saplings showing symptoms of pine wilt disease and processed to determine the presence or absence of PWN using the procedures for collecting PWN inoculum.

Results

A test of independence [chi-square analysis using a Fisher's exact test (i.e., Freeman-Halton test) for contingency tables larger than two by two] did not indicate a significant dependence between inoculation treatment and disease progression symptoms for any of the pine species evaluated ($P > 0.05$; df = 4). Austrian pine exhibited one death of a control (i.e., water treatment) sapling and greying of needles for two saplings of both the filtrate and PWN inoculation treatments. The remaining saplings exhibited no symptoms of pine wilt disease four weeks post-treatment (Table C.1). Similarly, loblolly pine exhibited one death of a control treatment sapling with the remaining saplings exhibiting no symptoms or death due to pine wilt disease (Table C.2). Three saplings of scots pine inoculated with PWN exhibited pine wilt symptoms with one dying within four weeks of treatment (Table C.3). One southwestern white pine inoculated with PWN exhibited greying/browning of needles, but remained alive four weeks after treatment (Table C.4). Limber pine saplings did not exhibit any pine wilt disease symptoms or death for any of the inoculation treatments (Table C.5). All ponderosa pine saplings exhibited greying and browning of needles for all treatments (Table C.6). Deaths incurred for control treatments of the pine species were likely the result of pruning injury. Removal of the main leader for the saplings resulting in increased resin flow from the pruning site and likely resulted in detrimental water loss and death of the saplings. Similarly, greying and browning of needles for control treatment saplings for ponderosa pine suggests pruning injury likely to be the greatest influencing factor in symptoms observed. Additionally, the continuous flow of resin exuding from the pruning site may have prevented PWN inoculation and infection of the pine species as inoculum treatment solutions and PWN were exuded with resin flow from the pruning site. No PWN were recovered from any of the shoots of the brown/grey or dead saplings. Disease symptoms and deaths for this PWN pathogenicity trial were likely the result of pruning injury incurred resulting from the inoculation procedure of this trial which, was adapted from mature tree inoculation protocol (James et al., 2006). Further PWN pathogenicity trials of saplings should follow inoculation procedures suited for sapling/seedling specimens.

Table A.1. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for austrian pine (*Pinus nigra*).[z]

Treatment	Disease Symptoms			Total
	No Symptoms	Grey Needles	Dead	
Water	6	0	1	7
Filtrate	5	2	0	7
Nematode	5	2	0	7
Total	16	4	1	21

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated $P > 0.05$ (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Table A.2. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for loblolly pine (*Pinus taeda*).[z]

Treatment	Disease Symptoms			Total
	No Symptoms	Grey Needles	Dead	
Water	6	0	1	7
Filtrate	7	0	0	7
Nematode	7	0	0	7
Total	20	0	1	21

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated *P* > 0.05 (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Table A.3. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for scots pine (*Pinus sylvestris*).[z]

Treatment	**Disease Symptoms**			**Total**
	No Symptoms	**Grey Needles**	**Dead**	
Water	7	0	0	7
Filtrate	7	0	0	7
Nematode	4	2	1	7
Total	18	2	1	21

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated $P > 0.05$ (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Table. A.4. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for southwestern white pine (*Pinus strobiformis*).[z]

Treatment	Disease Symptoms			Total
	No Symptoms	Grey Needles	Dead	
Water	7	0	0	7
Filtrate	7	0	0	7
Nematode	6	1	0	7
Total	20	1	0	21

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated $P > 0.05$ (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Table. A.5. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for limber pine (*Pinus flexilis*).^{z}

Treatment	Disease Symptoms			Total
	No Symptoms	Grey Needles	Dead	
Water	7	0	0	7
Filtrate	7	0	0	7
Nematode	7	0	0	7
Total	21	0	0	21

^{z}Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated $P > 0.05$ (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Table A.6. Contingency table of frequencies for nematode inoculation treatments and pine wilt disease symptoms for ponderosa pine (*Pinus ponderosa*).[z]

Treatment	Disease Symptoms			Total
	No Symptoms	Grey Needles	Dead	
Water	0	7	0	7
Filtrate	0	7	0	7
Nematode	0	7	0	7
Total	0	21	0	21

[z] Fisher's exact test (Freeman-Halton test) for tables larger than 2x2 indicated $P > 0.05$ (df=4) for nematode inoculation treatment and pine wilt disease progression symptoms.

Appendix B - Survey of Insects Associated with Pines in Kansas

A statewide survey of potential *Monochamus* spp. (sawyer beetles) and other potential vectors of pinewood nematode [*Bursaphelenchus xylophilus* (PWN)] was conducted in 2013. This effort was an attempt to determine which species of pine sawyer beetles may be responsible for vectoring pine wilt disease within the state of Kansas. Six sites representing all the geographic regions of Kansas were selected. Survey collaborators were requested to maintain and collect trap contents for the duration of the survey. Sites included in the survey were: northwest-Colby, southwest-Garden City, north central-Hays, northeast-Manhattan and Olathe, and southeast-Parsons. Two Lindgren funnel wet-traps (8-funnels) were used to conduct the survey at each site (Contech Enterprises, Victoria, BC, Canada). Traps were baited with two chemical lures, alpha-pinene and ethanol ultra-high-release lures (Contech Enterprises, Victoria, BC, Canada) as prescribed by the Cooperative Agricultural Pest Survey (CAPS) reference for pine commodity-based surveys (CAPS, 2013). The base of the funnels contained a collection cup (wet-trap) filled with 50:50 (by vol.) marine/recreation vehicles grade antifreeze (polypropylene glycol and ethanol) and water. Funnel traps and collection cups were labeled for each site. Collaborators were provided with insect storage vials and adhesive labels providing site name, trap number, and collection date for storage vial identification. A materials and instruction guide detailing the setup, collection, and storage of insects with photographs and written guidelines were provided to collaborators. The principal investigator travelled to each site to deliver survey materials and setup up traps prior to survey initiation (May 22-23). Supply issues from manufacturer and distributor backorder of traps and lures prevented an earlier initiation of the survey. Traps were attached from the upper, lateral branches of pines at sites with the bottom of the funnel trap reaching the midpoint of the tree trunks using polypropylene baling twine to create a pulley system for collaborators to easily collect from traps from the ground. Pine species varied as permitted by site availability. Collaborators baited and filled collection cups on their best day for consistent weekly collection (i.e., every Monday morning, every Tuesday afternoon, etc.). Insects were collected weekly from collection cups, stored with the antifreeze solution in vials, and frozen until survey termination and retrieval of traps from all sites. The survey was conducted for eight weeks from June through July of 2013. The information below lists and describes the survey locations with the first tree species listed used as the trap tree for the corresponding trap number:

Northeast- Northeast Research and Extension Center - Olathe, KS

Traps 1 & 2: Double row of scots pine (*Pinus sylvestris*) windbreak

Northeast- Kansas Forest Service - Manhattan, KS

Traps 1 & 2: Ponderosa pine (*Pinus ponderosa*) grove

North central- Agricultural Research Center - Hays, KS

Trap 1: Austrian (*Pinus nigra*) and ponderosa pine mix grove

Trap 2: Ponderosa and austrian pine mix grove

Northwest- Northwest Research-Extension Center - Colby, KS

Trap 1: Austrian pine single row windbreak planting between one row of ponderosa pines north and one row south

Trap 2: Ponderosa pine single row windbreak planting between one row of eastern redcedar (*Juniperus virginiana*) north and one row of austrian pine south

Southwest- Research and Extension- Finney County Office and Fairgrounds, Garden City, KS

Trap 1: Scots planting in municipally managed landscape

Trap 2: Single row windbreak of scots pine bordering municipal fairgrounds

Southeast-Parsons Arboretum - Parsons, KS

Traps 1 & 2: Austrian pine planting remnant of mixed composition arboretum

Results

Of the insects associated with pine, the only insects collected capable of penetrating bark and potentially infecting the trees with PWN were beetles (Table B.1). The eastern pine sawyer beetle, *Monochamus carolinensis*, was the only species of sawyer beetle (Lingafelter, 2007) collected and only known PWN vector for one site in northeast Kansas (Table B.1). The remaining buprestid, cerambycid, curculionid, and scolytid, beetles have been associated with PWN but not confirmed to successfully vector PWN [Linit et al., 1983; Linit, 1988; Akbulut and Stamps, 2012; (Table B.1)]. The nematode carrying capacity for these beetles are low compared to those of sawyer beetles (Linit et al., 1983; Mamiya and Enda, 1972; Sousa et al., 2001; Wingfield and Blanchette, 1983). Their association with PWN are likely more a function of their prevalence in stressed or dying trees as plant defenses weaken and allow for secondary infestations rather than serving as a vector of PWN and causing pine wilt disease-induced decline/death. In addition to insects capable of penetrating the bark, there were other insects and non-insect arthropods collected in the traps (Table B.2 and 3). Other pests of pines were collected in the traps including pine sawflies and some moths (Table B.3), but are not likely vectors of PWN as the larvae of these pests are foliage feeders and cause damage to the needles rather than the shoots. Additionally, some predators of pests were collected as some spiders; checkered, clown, and ladybird beetles; assassin bugs; various wasps; and lacewings were collected in the traps (Table B.2 and 3). The diversity of the arthropods collected suggests an assorted array of predator and pests were living on or near the pines containing the traps. *Monochamus carolinensis* was collected at only one site for the survey. The lack of diversity and absence of sawyer beetles collected for the other sites are likely a function of the delayed start of the survey due to a shortage of supplies available prior to the survey and the absence of the sawyer beetle from western locations in the state, where pine wilt disease has not spread and been recorded. The results from this survey and from collections from south-central Kansas for beetle feeding preference trials (Chapter 2) suggest that *M. carolinensis* is likely the only *Monochamus* vector in central to eastern Kansas.

Table B.1. Insects associated with pines (*Pinus* spp.) at six collection sites in Kansas.

Coleopteran Family Subfamily	**Genus**	**Common Name**	**Number of Insects by Trap Site Locations**						**Total**
			Olathe	**Manhattan**	**Hays**	**Colby**	**Garden City**	**Parsons**	
Buprestidae	*N/A*	metallic borers	0	0	0	16	1	2	19
Cerambycidae									
Aseminae	*Arhopalus*	longhorned beetles	0	2	0	0	0	0	2
Cerambycinae	*Clytus*	longhorned beetles	0	0	0	1	0	0	1
Lamiinae	*Monochamus*	pine sawyer beetles	5	0	0	0	0	0	5
	Urographis	longhorned beetles	0	0	0	0	0	1	1
Curculionidae									
Scolytinae	*Hylastes*	bark beetles	0	1	0	6	0	0	7
	Ips	ips bark beetles	81	317	231	26	1	2	658
	Scolytus	bark beetles	0	0	0	0	1	0	1
Molytinae	*Hylobius*	pales weevils	10	0	0	0	0	0	10
N/A	*N/A*	weevils	2	0	5	0	1	0	8

Table B.2. Insects captured in wet-traps in pines (*Pinus* spp.) at six collection sites in Kansas.

Order Family Subfamily	Genus	Common Name	Number of Arthropods/Insects by Trap Site Locations						Total
			Olathe	Manhattan	Hays	Colby	Garden City	Parsons	
Araneae	*N/A*	spiders	1	1	0	2	2	5	11
Blattaria	*N/A*	roaches	6	0	9	3	2	17	37
Coleoptera									
Carabidae	*N/A*	ground beetles	4	1	4	0	7	0	16
Cerambycidae	*N/A*								
Cerambycinae	*Elaphidion*	longhorned beetles	0	0	0	0	0	0	1
	Parelaphidion	longhorned beetles	1	0	0	0	0	0	1
Lamiinae	*Astylopsis*	longhorned beetles	0	1	0	0	0	0	1
	Dorcasta	longhorned beetles	0	0	0	1	0	0	1
Lepturinae	*Typoceus*	longhorned beetles	0	0	1	0	0	0	1
Chrysomelidae	*N/A*	leaf beetles	0	0	0	2	0	2	4
Cleridae	*N/A*	checkered beetles	0	2	1	14	1	2	20
Coccinellidae	*N/A*	ladybird beetles	1	0	4	12	5	1	23
Dermestidae	*N/A*	skin/carpet beetles	0	0	2	0	0	0	2
Elateridae	*N/A*	click beetles	4	2	13	5	7	10	41
Histeridae	*N/A*	clown beetles	1	2	0	11	81	0	95
Lampyridae	*N/A*	lightning bugs	0	0	16	0	0	8	24
Scarabaeidae	*N/A*	June beetles	1	0	2	3	2	0	8
Diptera	*N/A*	flies	2	3	6	10	69	1	91

Table B.3. Insects captured in wet-traps in pines (*Pinus* spp.) at six collection sites in Kansas.

Order Family Subfamily	Genus	Common Name	Number of Arthropods/Insects by Trap Site Locations						Total
			Olathe	Manhattan	Hays	Colby	Garden City	Parsons	
Hemiptera									
Alydae	*N/A*	broadhead bugs	0	2	0	0	0	0	2
Cicadidae	*N/A*	cicadas	0	5	0	0	0	0	5
Coreidae	*N/A*	leaf footed bugs	1	0	0	0	0	0	1
Dictyopharidae	*N/A*	plant hoppers	0	1	0	0	0	0	1
Lygaeidae	*N/A*	seed bugs	0	1	3	1	6	0	11
Miridae	*N/A*	plant bugs	0	0	0	1	0	0	1
Nabidae	*N/A*	damsel bugs	0	0	0	0	1	0	1
Pentatomidae	*N/A*	plant bugs	0	0	0	1	0	1	2
Reduviidae	*N/A*	assassin bugs	14	1	0	0	0	2	17
Tyngidae	*N/A*	lacebugs	0	2	0	0	0	0	2
Hymenoptera									
Apidae	*N/A*	bees	0	1	0	0	0	0	1
Diprionidae	*N/A*	confer sawflies	4	0	0	3	0	0	7
Evaniidae	*N/A*	ensign wasps	0	0	0	0	3	1	4
Formicidae	*N/A*	ants	0	1	0	0	1	2	4
Pompillidae	*N/A*	spider wasps	0	0	1	0	0	2	3
Sphecidae	*N/A*	solitary wasps	0	0	2	2	0	0	4
Vespidae	*N/A*	social wasps	1	0	1	0	0	1	3
Isopoda	*N/A*	wood lice	0	0	0	0	0	1	1
Lepidoptera	*N/A*	moths	4	1	13	96	8	5	127
Neuroptera	*N/A*	lacewings	0	0	1	2	4	0	7

Appendix C - Survey of Study Resource Use and Perceptions for Landscape Plant Identification Courses

1. What is your academic standing? (*circle one*)

Freshman Sophomore Junior Senior Graduate

2. What is your major? (*circle one*)

Horticulture Landscape Architecture Other___________

If in Horticulture, what is your emphasis? (*circle one*)

Landscape Design
Landscape Management
Professional Horticulture
Greenhouse/Nursery Management
Fruit & Vegetable Production
Sports Turf Operations
Golf Course Management

3. Have you taken a plant identification course before? (*circle one*) Yes No

4. On average, how many hours did you STUDY each week for the plant quizzes? (*circle one*)

0 1 2 3 4 5 6 7 8 9 10 10+

Figure C.1. Student demographic and study characterization section for survey of study resource use and perceptions for landscape plant identification courses.

5. A. Did you use the Google Maps for studying? (*circle one*) Yes No

B. If YES, how many times do you think you accessed them between receiving the map and taking the quiz?

1 2 3 4 5 6 7 8 9 10 10+

C. If YES, when did you use the Google Maps?

a. Primarily for the ***current*** weekly plant list
b. Primarily to review ***old*** plant lists
c. Mixed purposes; review ***current*** weekly plant list and ***old*** plant lists

D. If YES, how did you use the Google Maps? (*circle one*)

a. Mostly for ***visual*** identification reminders (did *NOT* physically re-walk lab walk)
b. Mostly for ***physically re-walking*** the lab walk
c. Both; ***visual*** identification and ***physically re-walking*** the lab walk

E. If NO, why did you not use Google Maps? *(circle all that apply)*

a. Not worth the time
b. Did not know where to find them
c. Forgot about them as a resource
d. Other methods more useful to me
e. Other (*please specify*)____

Figure C.2. Student map use characterization section for survey of study resource use and perceptions for landscape plant identification courses.

6. How OFTEN did you access or refer to the following resources for your studying purposes? Please rate each resource; if you did not use it, circle 'DID NOT USE'.

RESOURCE	**DID NOT USE**	**RARELY**	**OCCASIONALLY**	**FREQUENTLY**
Textbooks	**0**	**1**	**2**	**3**
Apps	**0**	**1**	**2**	**3**
Websites/Internet	**0**	**1**	**2**	**3**
Personal Notes/ Observations	**0**	**1**	**2**	**3**
Google Plant Walk Maps	**0**	**1**	**2**	**3**
Review Sessions	**0**	**1**	**2**	**3**

Figure C.3. Student resource use frequency ratings section for survey of study resource use and perceptions for landscape plant identification courses.

7. Rate the EFFECTIVENESS or USEFULNESS of the following resources in aiding you to learn or remember the plant identifications. Please rate each resource.

RESOURCE	**DID NOT USE**	**SOMEWHAT USEFUL**	**USEFUL**	**VERY USEFUL**
Textbooks	**0**	**1**	**2**	**3**
Apps	**0**	**1**	**2**	**3**
Websites/Internet	**0**	**1**	**2**	**3**
Personal Notes/ Observations	**0**	**1**	**2**	**3**
Google Plant Walk Maps	**0**	**1**	**2**	**3**
Flashcard Apps (e.g., Online; Study Blue, Quizlet, etc.)	**0**	**1**	**2**	**3**
Flashcard Traditional (e.g., Index cards/paper, etc.)	**0**	**1**	**2**	**3**

Figure C.4. Student resource usefulness ratings section for survey of study resource use and perceptions for landscape plant identification courses.