Addressing Production Bottlenecks of Emerging Nursery Crop Species in the Upper Midwest

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BY

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Dedication

Dedicated to my parents, whose unwavering support for my dreams has shaped me into the person I am today. I love you both immensely!

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CHAPTER 1: Literature Review

Species Diversity

Underutilized nursery crops may serve not only as opportunities for economic growth, but also as a means for expanding species diversity within the green industry (Hilbert et al., 2023). The issue of limited species diversity in managed landscapes has garnered attention from horticulturists and landscape managers alike (Hilbert et al., 2023). As consumers increasingly prefer native and resilient plants, the nursery industry is hindered by its focus on easily grown traditional crops (Hilbert et al., 2023). This limits the utilization of adaptable and superior ornamental species. To meet changing demands and offer more diverse options, underutilized species with horticultural value should be more broadly considered in production.

Ornamental Landscapes - Managed Landscapes

Species diversification in managed landscapes can increase overall landscape resilience, buffering managed landscapes against abiotic and biotic stressors (Miller, 2024). One way to increase species diversity is through the introduction of underutilized taxa (Hilbert et al., 2023). Unlike conventional nursery crops, which are widespread and readily available in commerce, underutilized species are infrequently available in the trade because they are often understudied and lack protocols for propagation and production. Selection of plants for use in managed landscapes should not be based solely on their native range but should take into consideration ornamental features throughout the year and species tolerance to stressors present at the selected planting site (Miller & Bassuk, 2022a). The introduction of new species as nursery crops will likely require new techniques to enhance their production and availability to growers and consumers (Hilbert et al., 2023). For example, species such as kingnut hickory (*Carya laciniosa* (F.Michx.) G.Don), American persimmon (*Diospyros virginiana* L.), and Chinese wingnut (*Pterocarya stenoptera* C.DC.) are suspected to be great candidates for landscape application due to their ornamental appeal and resilience to a variety of landscape conditions, but they have been neglected in horticulture due to production limitations (Dirr and Warren, 2019).

Managed Landscapes - Urban Forests

Urban forests are threatened by low tree diversity due to over-reliance on just a few species (Hilbert et al., 2023). This is exacerbated by the limited options in nursery for practitioners to choose and the legacy of monocultures in cities and neighborhoods (Hilbert et al., 2023). The changing climate presents a multitude of challenges for landscapes. In the Upper Midwest, low winter temperatures are common, however, less typical climate conditions have been observed recently, including flooding in the spring and severe summer droughts (Miller, 2024). Not only is it important to look for species resilient to abiotic stresses, but also species less vulnerable to biotic stresses, such as invasive insects and diseases, that are ever changing and unpredictable (Miller, 2024).

One introduction of a biotic stressor by human activity is the emerald ash borer (EAB) (*Agrilus planipennis*), which is a genus level threat (Hilbert et al., 2023). The instance of over reliance on ash (*Fraxinus* spp.) trees for urban planting underscores the problems associated with insufficient diversity of urban tree species (Hilbert et al., 2023). Additionally, it can pose challenges for the nursery trade, given the significant decrease in demand due to the emergence of EAB (Hilbert et al., 2023). A more diverse selection of landscape plants can decrease the complete loss of trees in a landscape caused by monocropping and pest pressures. The introduction of fruit and nut producing woody landscape plants can attract birds that can keep insect pressures in check in a natural manner (Josiah & Lackey, 2014).

Managed landscapes, especially those in urban environments, pose a multitude of challenges which can limit plant establishment and growth (Allen et al., 2017). Among those challenges include high soil pH, extreme temperatures, limited soil volumes, limited water, and nutrient availability, as well as soil compaction as a result of the built environment they inhabit (Allen et al., 2017). As such, with urbanization can come the loss of species diversity. While the built environment presents many challenges for plant growth, trees contribute ecosystem services which benefit those that dwell in urban settings (Sjöman et al., 2015). For example, trees serve as a major component of green infrastructure in towns and cities (Sjöman et al., 2015). Site selection is key to plant performance and choosing a species not suited to the environmental conditions reduces benefits like ecosystem services (Sjöman et al., 2015). Drought is a limiting factor for tolerance for a tree in an urban planting (Sjöman et al., 2015). *Acer* genotypes, found in varying climates and landscapes in Europe, Asia, and North America, were studied for their drought tolerance which correlates to their potential to survive in differing urban planting sites (Sjöman et al., 2015). A variety of hickories (*Carya* spp.) have also been studied for their use in managed landscapes because of purported drought tolerance within the several true hickory species (Miller & Bassuk, 2022a). To fully address reforestation needs in the

United States, tree nurseries in the U.S. must increase the production of seedlings by an extra 1.7 billion annually, which is a 2.4-fold increase in production compared to the current nursery output (University of New Mexico, 2021). To address this greater need in the production pipeline, investments need to be made not only in expanding production capacity, but also in the creation of protocols, especially for underutilized taxa or taxa that require production bottlenecks to be addressed before they are widely distributed in the nursery industry.

Ornamental Landscapes - Green Infrastructure

There is potential for diversification of plants that are currently marked for green infrastructure, such as for hedges, ground cover, windbreaks, and shelterbelts. The genus *Forestiera* encompasses species that could be a native replacement as a deciduous shrub for invasive privet species in the *Ligustrum* genus. Both swamp privet (*Forestiera acuminata*) and desert olive (*Forestiera pubescens*), formerly known as *F. neomexicana*, are underutilized species in the *Forestiera* genus and the Oleaceae family. These species could be used in riparian plantings, with swamp privet being tolerant to wet soils, and desert olive having purported tolerances to both flooding and drought (Geneve et al., 2019, Jacobs, 2009). Adaptable plants will be necessary in the landscape as the climate continues to change. Both swamp privet and desert olive are diecious, which allows for the selection of male plants that do not set seed, thus keeping the plant from spreading outside of its planting environment by seed.

Edible Landscapes

Woody edible landscapes can be utilized in environments that are being more highly urbanized as a way to connect the people that live there to where their food comes from, the wildlife that inhabits them, and also can serve as a food source (Josiah & Lackey, 2014). A list of northern adapted woody plants that would be great candidates for an edible landscape was produced by University of Nebraska - Lincoln School of Natural Resources and Cooperative Extension (Josiah & Lackey, 2014). The taxa listed includes fruit and nut crops, some more novel than others, that can be enjoyed by the community as well as are beneficial to wildlife. Nut crops include hybrid hazelnuts, northern pecan, shagbark hickory, and shellbark hickory to name a few (Josiah & Lackey, 2014). Fruit crops include some more novel to the Upper Midwest, such as persimmon and paw paw, and others like black cherry, black or red raspberry, and elderberry (Josiah & Lackey, 2014).

Food System Landscapes

According to Brainard et al. (2021), agronomic, row-crop agriculture spans 3.28 billion acres of land worldwide. Due to the extensive amount of land utilized for conventional agriculture, it becomes more important for the adoption of perennial crops that can produce edible products as well as provide ecosystem functions for a more sustainable and biodiverse future (Kreitzman et al., 2022). Perennial agriculture has the potential to implement principles from ecology, such as heterogeneity and functional diversity, that are essential for sustaining biodiversity, ecosystem services, and resilience (Kreitzman et al., 2022). The hazelnut industry could help diversify cropping systems in the Upper Midwest. While perennial cropping systems do not deliver their fruits as quickly as agronomic crops such as corn and soybean, their latent potential can provide many ecological benefits, especially in the face of a shifting climate (Brainard et al., 2021). These benefits include carbon sequestration, an expansive underground root system for nutrient uptake, and permanence in the landscape providing soil stability and habitat (Brainard et al., 2021).

Emerging Nursery Crops - *Forestiera*

Botany and Taxonomy

Desert olive, otherwise known as Stretchberry, New Mexico privet, New Mexico forestiera, and New Mexico olive, was formerly known as *Forestiera neomexicana* Gray, and is still known as such at various public gardens and institutions (Nesom, 2009). The currently accepted nomenclature for desert olive is *Forestiera pubescens* Nutt. var. *pubescens* (Scianna & Hybner, 2009). Desert olive is in the Oleaceae family, consisting of taxa such as olives and privets.

Background and potential applications

Desert olive is endemic to the southwestern United States, including California, Nevada, Arizona, Utah, Colorado, New Mexico, Texas, and Oklahoma (Scianna & Hybner, 2009). Desert olive is adapted to annual precipitation zones of approximately 23 to 61 centimeters with a soil pH ranging from 7.0 to 8.5 (Scianna & Hybner, 2009). Desert olive grows from lower elevations of 3,000 feet along stream courses and moist valleys to evaluations of 7,000 feet along hillsides and mesas (Jacobs, 2009). It is described as needing a minimum of 160 frost-free days, which is applicable to the number of frost days in USDA zone 4b at the Minnesota Landscape Arboretum where two male desert olive plants have been growing for close to 20 years with no signs of winter damage (Jacobs, 2009).

Plant Morphology

Desert olive is a deciduous, multi-stemmed, perennial shrub with a medium to tall stature. Desert olive commonly forms a dense, seemingly impenetrable hedge with its interlocking branches (Scianna & Hybner, 2009). It is capable of sprouting from the base, and suckering from the base is common (USDA-SCS, 1976). Desert olive plants also tend to form a tap root. It provides good cover for several species of birds and small animals (USDA-SCS, 1976).

Leaves are simple, oblong, light green to gray, and opposite or clustered on the stem (Jacobs, 2009). New bark is smooth, light gray to brown, while old bark is smooth and light gray in color (Jacobs, 2009). Desert olive is a dioecious plant, having separate male and female plants and flowers on the separate plants (Scianna & Hybner, 2009). Flowering occurs January through March, and sometimes into April, with fruits maturing in May through June (Nesom, 2009). The male flowers are yellow and form in inconspicuous, crowded clusters in the axils of the previous year's leaves (Jacobs, 2009). Female flowers are green (Breen, [date unknown]). Fruits are abundant and form as round to oblong drupes (5-8 mm) which mature in color from dark bluish-black to black (Breen, [date unknown]). Plants have moderate retention of drupes, however most fruit is lost to the primary consumer, birds, before falling to the ground (Scianna & Hybner, 2009).

'Jemez' Cultivar

'Jemez' is the current preferred and recommended seed source for growing desert olive where applicable in restoration projects (USDA-SCS, 1976). 'Jemez' originates from seed collected near Jemez Springs, New Mexico, from a single seed source in 1939 (USDA-SCS, 1976). ‘Jemez’ was more formally released in 1978 by the Los Lunas, New Mexico Plant Materials Center for use as a plant for conservation applications in its native region of the southwestern United States (Scianna & Hybner, 2009). ‘Jemez’ is said to obtain heights of 3.66 meters or more under favorable conditions (USDA-SCS 1976). Quality formal hedges can be formed with ‘Jemez’ (USDA-SCS, 1976). Along with conservation plantings, it can also be utilized in beautification plantings (USDA-SCS, 1976).

Propagating ‘Jemez’ through Los Lunas, New Mexico Plant Materials Center is by seed (USDA-SCS, 1976). Seeds should be planted sometime during the fall or prior to February in soil that is kept moist for natural stratification to occur (USDA-SCS, 1976). A good stand of these seedlings should be seen by May (USDA-SCS, 1976). Stratifying seeds in moist sand in a cooler at 3°C for 30 days at minimum (USDA-SCS, 1976). Regarding spring planting, seeds can be planted from the cooler into moist soil in May (USDA-SCS, 1976). Seedlings can be used as bare root plantings or potted plants. Smaller roots along with the tap root are important to preserve for the best survival rate (USDA-SCS, 1976).

Field Testing in Bridger, Montana

Seedlings of ‘Jemez’ were planted in Bridger, Montana (USDA zone 5a), along with ‘Big Horn’ skunkbush sumac in 1976 (Scianna & Hybner, 2009). Desert olive is comparable to skunkbush sumac *Rhus trilobata* in function and use (Scianna & Hybner, 2009). The planting purpose was to build desert olive into a living snow fence and field windbreak as well as document desert olive in a conservation planting (Scianna & Hybner, 2009). Over the 33-year period this trial was observed, there were no recorded signs of cold temperature injury, winter desiccation damage, or drought (Scianna & Hybner, 2009). No serious insect pest or disease problems were recorded (Scianna & Hybner, 2009). Fields adjacent were agronomic and cultivated with supplemental moisture and fertility (Scianna & Hybner, 2009).

Conservation Uses

Desert olive has multiple conservation applications including windbreaks, living snow fences, shelterbelts, as well as application in wildlife plants for nesting, cover, and food (Scianna & Hybner, 2009). Desert olive could also be used in Xeriscapes® (as a multi-stem shrub or a small, single stemmed tree with pruning) and for reclamation of mined landscapes (Scianna & Hybner 2009). Desert olive can also seed into a landscape, producing roughly 35,800 seeds per pound (Scianna & Hybner, 2009). No evaluations have determined if this species exhibits invasive tendencies in the Upper Midwest.

Management

Although desert olive is considered drought tolerant and flood tolerant, it performs best when managed with supplemental watering and fertility (Jacobs, 2009). During establishment, as with other woody plants, it is important to control weeds with tillage, weed cloth, or careful application of herbicides (Jacobs, 2009).

Swamp Privet

There is little known about the propagation of desert olive (*F. pubescens*), and the only known publication on propagation in this genus is by Geneve et al. (2019) at the University of Kentucky. This paper by Geneve et al., (2019) focuses on swamp privet [*Forestiera accuminata* (Michx.) Poir.], which is a North American native plant in the Oleaceae family for which the nursery crop potential is currently underutilized. Swamp privet, like desert olive, is utilized for riparian plantings in southwestern North America (Geneve et al., 2019). Swamp privet could be utilized to replace invasive privets (*Ligustrum* spp.) that are used as ornamental shrubs. Privets in the *Ligustrum* genus are an ecological threat to grasslands, forests, riparian sites, barren fields, roadsides, and sites that are disturbed (Panke & Renz, 2012). These privets readily escape cultivation, which is why a native alternative needs to be developed.

For propagation of swamp privet, semi-hardwood cuttings were taken from mature (flowering-age) male and female stock plants that were field-grown as well as from juvenile (non-flowering age) container grown seedlings (Geneve et al., 2019). Mature cuttings were treated with 0, 2.1, 4.1 or 20.5 mM (0, 500, 1000 or 5000 mg 1-1) potassium salt of indole 3-butyric acid (K-IBA); whereas juvenile cuttings were treated with 0 or 20.5 mM (0 or 5000 mg1-1) K-IBA (Geneve et al., 2019). Treatments were applied utilizing an aqueous three-second quick dip method of K-IBA and were then placed in six-pack containers with a substrate of Pro-Mix and perlite substrate (Geneve et al., 2019). These cuttings in containers were placed under intermittent mist with bottom heat (Geneve et al., 2019). Geneve et al. observed 90% rooting with or without auxin application using semi-hardwood cuttings from seedling juvenile stock plant sources. Treatment of K-IBA utilizing the quick-dip method improved rooting of these cuttings to nearly 100% with a three-fold increase in the number of roots.

Nursery production of specialty container crops

Diversification of the landscape is of current importance, not just due to changes in consumer preferences, but also due to the need of replacing invasive species that can displace native plants, causing both economic and environmental damage (Noyszewski & Smith, 2020). This is where specialty container crops make their way into the horticultural market. In the United States, over 754,000 metric tons of plastic are used per year for specialty crop containers (Schrader, 2013), showing the increase in use of containers to grow specialty crops. This metric is based on plant/container units of specialty crops sold in 2009 (Schrader, 2013).

To produce specialty container crops, asexual propagation techniques can be utilized. The current rooting threshold for a plant to be considered a viable commercial nursery crop is 80% rooting success (Griffith Gardner et al., 2019). Many factors influence asexual propagation success, including the substrate used (Schwab et al., 2021). Additionally, exogenous auxin in the form of indole-3-butyric acid (IBA) has been used to promote rooting in cuttings as early as the 1930s (Pincelli-Souza et al., 2024). The success and efficacy for adventitious rooting is species specific (Stokes et al., 2023). Improving the techniques that produce adventitious rooting can promote diversity in the taxa that are cultivated and distributed by the green industry, thus having both environmental and economic benefit (Pincelli-Souza et al., 2024).

Emerging Nursery Crops - *Diospyros*

Background and potential applications

The genus *Diospyros* belongs to the Ebenaceae family. The most widely studied species of the *Diospyros* genus include *D. kaki*, *D. lotus*, and *D. virginiana* (Ojha et al., 2023). These taxa are grown in their respective tropical or temperate regions for fruit production. According to the Food and Agriculture Organization (FAO), more than 4.2 million tons of persimmon fruits were produced in 2019 (Ojha et al., 2023). However, these species are not only important for fruit production, but also boast many ornamental features.

Diospyros virginiana L., referred to as both American or common persimmon, has a native range from Connecticut to southern Florida and west into Kansas and Oklahoma, as well as southern Ohio, southern Indiana, and central to southeast Iowa. It is a slow growing medium-sized tree (Chamberlain, 2020; Halls, 1990). American persimmon is hardy in USDA zone 4-9 (Dirr, 1975). American persimmon is found to grow under a wide range of conditions, most abundant in the rich bottomlands of the Mississippi River and associated tributaries, as well as in abandoned fields and farrowed croplands with poor soil (Chamberlain, 2020). American persimmon is a multi-faceted plant. The fruit produced by the American persimmon plant is the primary consumer product. The fruits produced by an American persimmon tree are generally 2.5 to 5 cm (1 to 2 in) in diameter with a round to oblong shape (Missouri Botanical Garden [date unknown] b). The wood is desirable for products such as golf club heads (Chamberlain, 2020). It can also be found as a specimen tree from personal yards to botanical gardens such as the Morton Arboretum in Lisle, IL. As a specimen tree, American persimmon has ornamental value in its glossy and leatherlike leaves (Chamberlain, 2020). Its exfoliating bark is also an attractive ornamental feature that can be appreciated year-round.

The current market for persimmon is dominated by Japanese persimmon (*D. kaki*) (Chamberlain, 2020). Japanese persimmon, also known as oriental persimmon and Chinese persimmon, is native to China, and is commonly cultivated in Japan, South Korea, Spain, and Nepal (Ojha et al., 2023). Japanese persimmon produces fruits larger than those of American persimmon and sweeter (less astringent) (Chamberlain, 2020). The fruits of Japanese persimmon have an average size of 120 to 180 grams and range in both shape and color (Matheus et al., 2020). Japanese persimmon trees are smaller than those of American persimmon and are hardy in USDA zones 7-10, but perform best in USDA zones 8-10 (Chamberlain, 2020; Missouri Botanical Garden [date unknown] a). The major producer for Japanese persimmon in the United States is the state of California, which produced about $8 million in 2014 (Chamberlain, 2020). Not only does the U.S. produce persimmon, but also exports this fruit. In 2016 the US exported roughly $6 million worth of cultivated persimmon (Chamberlain, 2020). Even with Japanese persimmon being of the most economic value between these three species, the American persimmon has the potential to be a specialty crop for local consumption (Chamberlain, 2020).

The native range of *D. lotus*, commonly known as dateplum persimmon, Caucasian persimmon, or lilac persimmon, extends from the subtropical areas of southwest Asia and southeast Europe, and is also found growing and in cultivation throughout north-east and southern Anatolia (Ojha et al., 2023). The fruits produced by the dateplum persimmon trees are 1.5 to 2 cm in diameter with a globular shape and yellow color (Ojha et al., 2023). These fruits are also highly astringent, containing high amounts of soluble tannins in the immature (unripe) stage, which makes consumption directly after harvesting less desirable than in *D. kaki* or *D. virginiana* (Ojha et al., 2023). Due to this astringency, the fruits are dried prior to consumption (Ojha et al., 2023). Dried persimmon can be eaten as is or used as an ingredient in consumer products such as baked goods (Ojha et al., 2023).

While there is limited published studies that have investigated mouse ear disorder (MED) in persimmon, knowledge that the genus *Diospyros* involves ureide transporters leads to the belief that *Diospyros* will show symptoms of MED when deficient in nickel resources (Wood et al., 2006). Images from persimmon growers in the United States via Facebook correspondence show classic MED symptoms such as: rosetting of branches, curling/cupping of leaf lamina, necrotic leaf margins, stem dieback, etc. These observations prompted the investigation into whether MED occurs as a function of nickel deficiency in American, Japanese, and dateplum persimmon varieties.

Emerging Nursery Crops - *Corylus*

Background and potential applications

American hazelnuts and hybrid hazelnuts have potential as versatile landscape shrubs due in part to their vibrant red autumn foliage (Dirr 1975.). Moreover, they've garnered recent interest for their suitability in northern hazelnut production systems (Brainard et al. 2021). *Corylus* is another genus predicted by Wood et al. (2006) as a likely candidate to be affected by mouse ear disorder. As a globally traded commodity, hazelnuts boast a market value approaching $5 billion annually (University of Wisconsin-Madison Extension [date unknown]). According to the USDA Foreign Agricultural Service in 2004, the United States only accounts for approximately 2% of hazelnut production worldwide (Fischbach et al., 2011). The Pacific Northwest currently monopolizes commercial hazelnut production in the United States, producing cultivars of European hazelnuts (*Corylus avellana*) (Fischbach et al., 2011). These cultivars are unable to be produced in the Upper Midwest due to poor cold tolerance and their susceptibility to native fungal disease in the Upper Midwest, Eastern Filbert Blight, which could lead to complete loss of the crop (Fischbach et al., 2011). Since the 1990s growers in the Upper Midwest have tested seedlings of hybrids between the European (*Corylus avellana*) and American hazelnut (*Corylus americana*). Due to hazelnuts being endemic to Wisconsin and the Upper Midwest, in 2007, the University of Minnesota and University of Wisconsin formed the Upper Midwest Hazelnut Development Initiative (UMHDI) which helps guide farmers through the implementation of hazelnuts suitable to the landscape onto their farms (University of Wisconsin-Madison Extension [date unknown]). The hybrid varieties currently being trialed in the Upper Midwest are selected for their bushy-stature, cold-hardiness, and disease tolerance of the American hazelnut and the larger yield, nut size, and thin shell of the European hazelnut (Fischbach et al., 2011).

There is little published literature that investigates the nickel requirements for hazelnuts. Hand and Reed (2014) studied nickel in a micropropagation study of *Corylus avellana* (European hazelnut). In general, many European hazelnut cultivars are difficult to grow *in vitro* on standard growth medium (Hand & Reed, 2014). According to Hand and Reed

(2014), nickel is not used in many growth media common for hazelnut production including: Driver and Kuniyuki (DKW) medium, woody plant medium (WPM), or Murashige and Skoog (MS), but the introduction of nickel may improve the culturing of this plant and many others. Nickel is the most recent element to be listed as an essential nutrient for plant growth, which may contribute to the lack of nickel present in commonly used growth media (Liu et al., 2020). Nickel was given to hazelnut plants from 0.0 to 6.0 µM in the nutrient solution in DKW medium (Hand and Reed 2014). A diverse response to differing levels of all minor nutrients in the experiments of Hand and Reed (2014) show that nickel requirements *in vitro* must continue to be explored. *In vitro* micropropagation is not the only facet of propagation that must be studied regarding nickel nutrition to overcome potential nickel deficiency in the production pipeline of both American and European hazelnuts, as well as hybridizations of the two.

Production Bottlenecks for Emerging Crops - Asexual propagation

Developing protocols for novel nursery crops

Commercial cultivation of ornamental perennial crops depends on three methods of plant propagation: seeds, stem (vegetative) cuttings, or tissue culture (Lewis et al., 2020). Stem cuttings preserve the genotype of the plant they were taken from, making them clonal propagules (Lewis et al., 2020). The physiological differences among species or cuttings collected at various developmental stages may determine the extent to which a crop is sensitive to substrate composition (Peterson et al., 2020). Woody plants exhibit significant diversity in their reactions to asexual propagation methods (Stokes et al., 2023). While certain species readily develop roots from stem cuttings, others pose greater challenges, potentially constraining their commercial cultivation despite their appealing landscape attributes (Stokes et al., 2023). Adventitious rooting through stem cuttings treated with auxin has been studied in many taxa that exhibit horticultural merit and could be used to diversify the landscape, such as sweetgale (*Myrica gale*), rhodora (*Rhododendron canadense),* and catberry (*Ilex mucronata*) (Peterson et al., 2020). These studies also include taxa where selection to the customer is limited such as Amur maple (*Acer ginnala*) where the seedless selections rooting efficacy was evaluated (Noyszewski and Smith, 2020), or where the taxon is difficult to root such as the northern spicebush (*Lindera benzoin*) (Stokes et al., 2023). Difficulty to root can be a consequence of any of the following or a combination of application/choice of plant growth regulator (Peterson et al., 2020), timing of cutting (Kidwell-Slak et al., 2014), and substrate choice (Schwab et al., 2021). Improving the understanding of asexual propagation techniques will allow for the green industry to pursue greater clonal selections based on desirable ornamental traits, thereby expanding the market for underutilized woody plants (Stokes et al., 2023).

Asexual propagation of *Shepherdia canadensis* using hardwood cuttings utilizing auxin was studied by Krishnan and Hughes (1991). *Shepherdia canadensis* is of the Oleaster family and is a hardy shrub with the capability to endure harsh winter temperatures and drought conditions (Krishnan & Hughes, 1991). Rooting potential of *S. canadensis* has largely been understudied. Hardwood cuttings of 7 to 13 cm were made and one-third of the basal portion of the cuttings were dipped into IBA concentrations of 0 ppm (control), 1000 ppm, 3000 ppm, and 8000 ppm before being placed in a 1:1 perlite:vermiculite medium (Krishnan & Hughes, 1991). The 3000 ppm exogenous auxin resulted in the highest rooting percentage (46.5%), whereas the 8000 ppm treatment inhibited rooting (Krishnan & Hughes, 1991).

Box huckleberry [*Gaylussacia brachycera* (Michx.) Gray] is an evergreen groundcover plant native to eight states of the Mid-Atlantic region of the United States with the potential to be a niche landscape plant (Kidwell-Slak et al., 2014). With little known about its asexual propagation behavior, softwood stem cuttings approximately 7 cm in length were dipped in 8000 ppm IBA-talc and placed in flats containing 50% milled sphagnum peat and 50% coarse perlite (Kidwell-Slak et al., 2014). The authors concluded that the rooting proportion was highest from stem cuttings taken in May, June, and July, with greater than 90% rooting percentage (Kidwell-Slak et al., 2014). In contrast, the lowest proportion of stem cuttings rooted from cuttings taken September through December. Semi-hardwood and softwood cuttings are optimal cutting times for asexual propagation of box huckleberry (Kidwell-Slak et al., 2014).

The formation of adventitious rooting on stem cuttings of mountain fly honeysuckle (*Lonicera villosa*) was studied by Hayes and Peterson (2019). Mountain fly honeysuckle could serve as a native alternative in North America for Eurasian honeysuckle species that are in high consumer demand but tend to escape cultivation and are banned in some parts of the United States (Hayes & Peterson, 2019). Softwood terminal cuttings were taken from 17 healthy plants of mountain fly honeysuckle in this experiment. Basal ends were 5 second quick-dipped into water or exogenous auxin solutions of 400, 8000, 12000 $mg \cdot L^{-1}$ K-IBA dissolved in water (Hayes & Peterson, 2019). Cuttings were inserted into horticultural perlite at 50%, 75%, or 100%, with the remaining percentage occupied by professional-grade peatmoss (Hayes & Peterson, 2019). It was concluded that the root system quality increased as concentration of K-IBA increased, as well as using coarse perlite without peatmoss also increased root system quality (Hayes & Peterson, 2019).

Many species that may bolster landscape diversity have little to no reports on their ability to be propagated from vegetative cuttings, such as Lewis et al. (2020) which published the first report of such with butterfly milkweed (*Asclepias tuberosa*). Vegetative cuttings were taken at two maturities and treated with either K-IBA liquid dip at 0 ppm, a 50-ppm basal soak for 24 h, and 5-second quick dip (1000 ppm, 3000 ppm,5000 ppm, and 8000 ppm) or NAA as a 5-second quick dip (500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm) (Lewis et al., 2020). The exogenous auxin treatments had no effect on the cutting survival, root number, or root length (Lewis et al., 2020). Lewis et al. (2020) found that *Asclepias tuberosa* can be propagated at a commercially acceptable level without exogenous auxin application.

Amur maple (*Acer ginnala*) offers high horticultural value, such as its attractive red foliage, however, it is classified as a noxious weed, which limits propagation efforts and sale of the taxa (Noyszewski & Smith, 2020). To control the spread of this taxon, propagation of seedless plants can allow this plant to re-enter the nursery industry (Noyszewski & Smith, 2020). Efficacy of adventitious rooting was studied with seedless selections through treatment with IBA (500, 1500, 5000, and 10000 ppm) as well as NAA (100, 500, 1000, and 5000 ppm) (Noyszewski & Smith, 2020). Softwood cuttings were the easiest to root

(Noyszewski & Smith, 2020). Cuttings treated with 5000 or 1000 ppm IBA as well as 5000 ppm NAA produced the highest rooting percentage, mean number of roots, and mean root length of the longest root (Noyszewski & Smith, 2020).

Low-bush blueberries (*Vaccinium* spp.) were evaluated for rooting efficacy in soilless substrates (Schwab et al., 2021). Blueberries traditionally have cultural requirements of low soil pH and salt loads, for which soilless substrates may allow for better management options for producers in areas that do not meet these requirements (Schwab et al., 2021). Schwab et al. (2021) studied apical and basal semi-hardwood stem cuttings of *Vaccinium corymbosum* × *angustifolium* 'Northland' rooted in rockwool cubes, shredded rockwool, or 3:1 perlite:peat moss (Schwab et al., 2021). There is little research reported on asexual propagation of woody species utilizing stem cuttings in rockwool substrates (Schwab et al., 2021). 'Northland' cuttings were treated with 0.1% IBA, 1:1 95% ethyl alcohol, or untreated (water) (Schwab et al., 2021). In a second experiment, basal cuttings of 'Northland' and *V. angustifolium* 'Brunswick' were rooted in the same soilless substrates as 'Northland', with the addition of coco coir, and treated with 1000 ppm K-IBA (Schwab et al., 2021). 'Northland' was found to root well in peat:perlite and coco coir substrates, and 'Brunswick' rooted less proficiently with these substrates (Schwab et al., 2021). 'Northland' rooted at around 50% in rockwool substrates, with 'Brunswick' rooting very poorly in these substrates at <2% (Schwab et al., 2021). For plant growth hormone application, 'Northland' rooting efficacy was not improved by a 0.1% auxin application (Schwab et al., 2021). Stem position influenced rooting in 'Northland', with apical cuttings having a higher rooting success compared to basal stem cuttings (Schwab et al., 2021).

Being able to successfully root blueberry cuttings in rockwool or coco coir would contribute to establishing successful container production and hydroponic systems for blueberries (Schwab et al., 2021).

Comptonia peregrina is a native plant that exhibits horticultural merit and can survive in challenging landscapes, however; it cannot be produced using softwood stem cuttings (Griffith Gardner et al., 2019). As an alternative, the authors attempted to propagate *C. peregrina* by young shoots with IBA treatment of 3000 and 8000 ppm and grown in an intermittent mist nursery setting. Rooting percentage and shoot count was improved by application of IBA (Griffith Gardner et al., 2019). The highest number of roots was observed in cuttings treated with 8000 ppm IBA (Griffith Gardner et al., 2019).

Sweetgale (*Myrica gale*), rhodora (*Rhododendron canadense*), catberry (*Ilex mucronata*) are shrubs native to eastern North America that hold promise for wider utilization in horticultural settings, even though the commercial potential and landscape performance of rhodora and catberry is not well understood (Peterson et al., 2020). There is limited information regarding asexual propagation of these three taxa, therefore, a two-year experiment was conducted to gather data regarding the timing of cutting collection, wounding, substrate composition, and concentration of applied K-IBA on each taxon (Peterson et al., 2020). Cuttings were collected at softwood or semi-hardwood stages, unwounded or wounded with a razor blade, treated with K-IBA at concentrations ranging from non-treated (0 mg/L) to 15000 mg/L, and then placed in substrates of 100%, 75%, or 50% perlite with the remaining percent (v/v) occupied by peat moss (Peterson et al., 2020).

Peterson et al. (2020) found that no exogenous auxin is necessary to propagate sweetgale; however, rhodora and catberry benefit from 5000 to 10000 mg/L K-IBA (Peterson et al., 2020). Semi-hardwood and softwood cuttings are effective for rooting all three taxa (Peterson et al., 2020). Substrate ratios of perlite: peat are important to rooting these three taxa, with sweetgale having the ability to root in perlite alone and rhodora and catberry requiring the addition of peat moss for acceptable rooting (Peterson et al., 2020).

Production Bottlenecks for Emerging Crops - Plant Nutrition (Mouse Ear Disorder)

History of Nickel Nutrition in Plants

Mouse ear disorder (MED) is an issue commonly encountered and documented in container production of river birch (*Betula nigra*) (Ruter, 2005) and pecan (*Carya illinoinensis*) (Wood et al., 2004a, 2004b, 2004c), however, this function of nickel deficiency may be a more widespread problem than previously thought. Nickel nutrition management has also been studied with a variety of important food crop species, including pomegranate (Dichala et al., 2018), wheat (Gajewska and Sklodowska, 2008), tomato (Kumar et al., 2015), soybean (Eskew et al., 1983) and peach (Nyczepir and Wood, 2012). Prior to the 1980s, nickel was a known component of the urease enzyme but was not regarded as essential for plant health (Welch, 1981). Agricultural practitioners have previously ignored nickel nutrition because of its classification as a micronutrient needed in low concentrations for plant nutrition and its relative abundance in the soil (Wood, 2015). Eskew et al. (1983) identified nickel as an essential micronutrient for higher plants through studies with soybean, a ureide transporting crop plant. Eskew et al. (1983) observed that soybeans grown in nickel deprived solutions accumulated toxic levels of urea in the leaves resulting in necrotic lesions on leaflet tips. Eskew et al. (1983) presented evidence of nickel as an essential element for higher plants by meeting these criteria: An element may be considered essential (i) if a plant grown in a medium adequately purged of that element fails to grow normally and to complete its life cycle or (ii) if the element is a constituent of a molecule that is known to be an essential metabolite. In 1987 the necessity of nickel for the completion of the barley plant life cycle was observed by Brown et al., which certified that nickel functioned as an essential nutrient in higher plants. The essentiality of nickel as a plant nutrient was further identified by Wood et al. (2004b) who observed that pecan (*Carya illinoinensis*) transplants died in nickel deficient orchards. Nickel has since been recognized as an essential element for plant growth and the understanding of nickel requirements of different taxa continues to expand. Even after forty years since the Eskew et al. (1983) publication, little is known regarding nickel deficiency in plants.

Mouse ear disorder (MED), also known as little leaf, leaf curl, and squirrel ear is the term used in regard to nickel deficiency seen in a handful of woody perennials (Ruter, 2005; Wood et al., 2006). Between the 1970s and early 2000s, a poorly understood phenomenon, referred to as mouse ear disorder, limited container production of river birch (Ruter, 2005) and was experienced with nursery stock and replanted orchards of pecans (Wood et al., 2004a). Symptoms of mouse ear disorder include overall plant stunting, shortened internodes, leaf cupping accompanied by a necrotic margin, as well as reduced leaf size, dark green color, and rosetting of the stem (Ruter, 2005).

Nickel deficiency leading to mouse ear disorder symptoms has been an issue for river birch grown in containers, for several decades (Ruter, 2005). Ruter (2005) published that mouse ear disorder may be accelerated by container grown plants and provided evidence for mouse ear in *Betula nigra* (river birch). Symptoms of MED in river birch were not always uniform, with random plants in a block being affected, while others only expressed symptoms on one branch (Ruter, 2005). Symptomatic plants seemed to be correlated to plants that had been container grown for an extended period and had become rootbound (Ruter 2005). MED is rarely seen on river birch plants growing in the field, with river birch plants transplanted into the field outgrowing their symptoms within a single growing season (Ruter, 2005). River birch (*Betula nigra*) is an important ornamental crop, readily grown as a nursery crop for its use in a diversity of landscapes (Jeffers et al., 2002). Mouse ear disorder symptoms in river birch include stunted plants with shortened internodes and the appearance of a witch's broom (Ruter, 2005). The leaves appear darker green in color, as well as small, cupped, and wrinkled with necrotic margins (Ruter, 2005). The necrotic leaf margins as a result of mouse ear disorder are thought to be a result of urea toxicity. Eskew et al. (1983) found that soybean plants deficient in nickel accumulated urea at toxic concentrations in their leaflet tips, causing necrotic margins to develop. Nickel is essential for nitrogen metabolism and is the essential cofactor for urease activity (Ruter, 2005; Wood et al., 2006). Bai et al. (2007) demonstrated in xylem sap of pecan that a shortage of nickel leads to the interruption of normal nitrogen cycling. If urease activity declines, it is thought that urea and/or organic acids necessary for the urease pathway build to a toxic level in leaf tissue, ultimately leading to cell death (Bai et al., 2006; Wood, 2015).

Prior to Ruter (2005), publications regarding mouse ear disorder in pecan [*Carya illinoinensis* (Wangenh.) K. Koch] were published by Wood et al. (2004). Symptoms of mouse ear disorder in pecan include but are not limited to early-season leaf chlorosis, dwarfing of foliage, blunting of leaf/leaflet tips, necrosis of leaf or leaflet tips, curled leaf/leaf margins, dwarfed internodes, and diminished root systems (Wood et al., 2006). Mouse ear disorder of pecan is most frequently observed as a replant problem, but also occasionally occurs at sites where pecan has not previously been grown (Wood et al., 2006). Ruter (2005) also found that river birch plants with MED that were transplanted into a field seemed to outgrow the problem. This suggests that there is an element in native soil that is not being supplied to the container grown plants in their fertilization or substrate (Ruter, 2005).

Nickel nutrition in nursery crops beyond river birch and pecan has largely been ignored (Wood, 2015). However, characteristics of plants afflicted by nickel deficiency appear to vary by species (Wood, 2015). An array of symptoms has been recorded with other taxa, including leaf chlorosis, arrested development of the lamina, pronounced expansion of petiole wings, and rosetting (Wood et al., 2006). Foliage of MED affected plants is thicker, less pliable, and brittle (Wood, 2015). Other taxa claimed to have exhibited mouse ear disorder include species belonging to the genera *Prunus*, *Pyracantha*, and *Citrus* (Wood et al., 2006). According to Wood (2015), the majority of cases of nickel deficiency are in the form of 'hidden hunger', with no visual symptoms, which can make diagnosis even more difficult. Visible symptoms present in woody perennials when the foliar concentration of nickel falls below $\simeq$ 1.0 $\mu g \times g^{-1}$ (Wood, 2015). For non-hyperaccumulating plants, nickel concentration in the foliage at a range of 0.05 to 10 $\mu g \times g^{-1}$ dry weight (DW) and is species dependent as well as dependent on the concentration of the above competing cations (Wood, 2015).

The discovery that nickel deficiency causes MED prompted the development of a commercial product to correct the problem. This commercial fertilizer, known as Nickel Plus®, was developed by NIPAN, LLC, and is a urea and nickel lignosulfonate (NIPAN LLC 2011). Organic nickel ligands, such as lignosulfonates and heptogluconates, are proven to be highly efficient as nickel foliar fertilizers (Wood, 2015). It's noteworthy that nickel sulfonates, found in products like Nickel Plus®, are favored over nickel nitrate and nickel sulfate fertilizers due to safety considerations for workers (Wood, 2015). Application of Nickel Plus® via a substrate drench or foliar application were effective in treating MED of bitternut hickory (*Carya cordiformis*). Nickel application via a nickel spray and nickel drench were seen as effective in correcting MED symptoms in the symptomatic river birch plants (Ruter, 2005). Application of nickel to soilless substrates to be taken up by the root system and transported to the foliage of river birch is supported by this study (Ruter, 2005). Correction of MED in pecan is accomplished by timely foliar applications of nickel salts or ligands (Wood et al., 2006). Applications of nickel soon after bud break are found to be effective in ameliorating nickel deficiency symptoms (Wood et al., 2006).

Role of Plant Metabolism in MED Occurrence

Mouse ear disorder frequently occurs in ureide-transporting woody-perennial species (Bai et al., 2006) and has been linked to disrupted metabolism of ureides, amino acids, and organic acids (Bai et al., 2006). Using xylem sap of pecan, Bai et al. (2007) demonstrated that nickel deficiency reduces the activity of urease, interrupting normal nitrogen cycling via disruption of ureide metabolism. Therefore, the urease enzyme requires nickel as a cofactor (Bai et al., 2006). According to Gerendás et al. (1998), other metal ions appear unable to substitute for nickel as the cofactor for the urease enzyme. Wood (2015) found that if urea cannot convert to ammonia in the urease pathway, excess urea and/or organic acids may build to toxic levels in leaf tissue, leading to cell death in leaves and leaflets (Figure 1). Given this understanding of the mechanism controlling MED, Wood et al. (2006) predicted likely candidate genera to monitor for susceptibility to mouse ear disorder. Those genera include: *Acer*, *Alnus*, *Annona*, *Betula*, *Carpinus*, *Carya*, *Cercis*, *Chamaecyparis*, *Cornus*, *Corylus*, *Diospyros*, *Juglans*, *Nothofagus*, *Ostrya*, *Platanus*, *Populus*, *Pterocarya*, *Salix*, and *Vitis*. Ureide-transporting species not only are probable candidates for MED, but also might have a greater requirement for nickel compared to amide-transporting species. (Wood et al., 2006). Both the morphological and growth characteristics of nickel-deficient woody perennials, such as birch, pecan, river birch, and more, indicate that nickel impacts plant metabolism by hindering the production of essential intermediates required for various metabolic processes in these plants (Wood, 2015) (Figure 1).

Instances of Symptom Presence and Factors Influencing MED

Mouse ear disorder has been observed on plants cultivated on sandy soils (personal communication, Brandon Miller, University of Minnesota, 1 July 2022), soils with high pH (Ruter, 2005), and, in the case of pecan, it frequently occurs when replanting orchards which have been heavily fertilized or treated with copper-heavy fungicides (Wood et al., 2003a; 2003b; 2004). Nickel availability is also dependent on conditions, such as soil water, and high soil concentrations of other divalent heavy metal cations such as Zn^{2+}, Cu^{2+}, Fe^{2+}, and Co^{2+} can inhibit root uptake (The Fertilizer Institute [date unknown]; Wood, 2015). In regard to pH, the application of lime in container-grown pecan trees, which increases the pH of the media, was shown to increase the presence of MED (Goff & Keever, 1991). In acidic soils (pH < 6.5), nickel bioavailability tends to be high, whereas it is less available in relatively alkaline soils (pH > 6.7) (Brown et al., 1989).

The disorder is most frequently observed in container-grown crops with artificial potting substrates and rarely occurs in trees grown in native soil, with the exception of pecan (Ruter, 2005). River birch crops cultivated with peat-based (McNamara et al., 2003) or pine-bark based substrates (Ruter, 2005) have produced trees afflicted by MED with more frequent occurrences on pot-bound plants (Ruter, 2005). This suggests symptoms occur when plants have depleted the potting medium of available nickel. McNamara et al. (2003) suggested that container-grown river birch plants capable of rooting into the native soil do not display symptoms of MED. The severity of MED in river birch container production has previously resulted in growers removing it from production (Ruter, 2005).

Miller and Bassuk (2022b) demonstrated an underutilized landscape species, bitternut hickory (*Carya cordiformis*), which responded to supplemental nickel when cultivated in containers. Further, Miller (2021) characterized and tested a treatment protocol for two underutilized species of birch [yellow birch (*Betula alleghaniensis*) and sweet birch (*B. lenta*) which appear sensitive to nickel deficiency when container grown. Interestingly, this trend of susceptibility of birch species appears to only occur with taxa endemic to North America while Asian and European species, such as silver birch (*B. pendula*), have long been produced in containers without issue. While solutions for correcting MED of river birch and other select woody-perennial species have been previously studied, a thorough examination of production considerations for many other desirable nursery crops are lacking.

Birch

Common symptoms of MED in river birch include small, cupped leaves that are darker green in color with necrotic margins. In the Ruter (2005) study, symptomatic river birch plants in their second growing season in containers were treated with a control, 789 ppm nickel spray, 394 ppm nickel spray, 000.5 lbs Ni/yd^3 as a drench, 26 g/pot triple superphosphate (0-46-0), or 130 g/pot Milorganite. The nickel applied is in the form of nickel sulfate, with applications of triple superphosphate and Milorganite only containing trace amounts of nickel (Ruter, 2005). Results by Ruter (2005) show that all plants treated with nickel sulfate resumed normal growth rate at a 100%. Plants treated with trace amounts of nickel provided by triple superphosphate and Milorganite continued to suffer from MED (Ruter, 2005).

MED has also been previously linked to other heavy metal insufficiencies such as zinc (Wood et al., 2004). Göransson (1999) studied zinc nutrition in *Betula pendula* (silver birch). Zinc deficiency in *B. pendula* has produced mouse ear disorder-like symptoms, such as rosetting, but the rosetting from zinc deficiency looks different than mouse ear disorder (Göransson, 1999). The plants in the Göransson (1999) study were given free access to all other nutrients excluding zinc, and then dosed zinc in limited supply. It was concluded that low zinc concentrations do limit the growth rate of *B. pendula*. However, this study failed to apply nickel in the same concentrations of "all other nutrients," which neglects to determine if nickel deficiency affects *B. pendula* (Göransson, 1999).

Seedlings of *Betula costata* (Korean birch) showed symptoms of MED within their first growing season in containers in Saint Paul, MN. Ornamental features of *B. costata* include its light gray-brown to creamy white bark, as well as young trees display bark peeling in moderately thick flakes, which becomes plated and rough on mature trees (McAllister & Ashburner, 2013). *Betula costata* 'CinnDak' (Cinnamon Curls® Dwarf Korean Birch) developed at North Dakota State University is the newest cultivar of *B. costata* and features robust summer foliage, golden fall color, ornamental exfoliating bark resembling cinnamon-colored undersides (West et al. 2016). 'CinnDak' is marketed for USDA zones 3a-7 (West et al., 2016). With its compact dwarf habit, 'CinnDak' is suitable as a landscape plant (West et al. 2016). This cultivar is unique because compared to most plants of this species, this selection has proven to perform well during periodic drought events (West et al., 2016). Protocols for nickel deficiency in this taxon and its cultivars are important for increased use in the greenhouse industry as well as to bring diversity to the birch industry.

Species such as paper birch, silver birch, and gray birch are common representatives of the genus in the green industry, but their production could be enhanced and expanded while the adoption of their underutilized relatives would expand plant palettes for landscape practitioners (Wyman, 1962). To promote and develop these underutilized species for horticultural use, a thorough understanding of their cultural needs should be comprehensively understood (Hilbert et al., 2023).

CHAPTER 2: Asexual Propagation of *Forestiera pubescens* (Nutt.) Using Stem Cuttings

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Abstract

Desert olive (*Forestiera pubescens*), a large shrub or small tree native to the southwestern United States, exhibits ornamental value and potential for broader application in managed landscapes. Purported tolerances to both drought and flooding suggest *F. pubescens* is well-suited for use in urban settings and in green infrastructure. Further, this taxon has demonstrated cold hardiness in USDA zone 4b and may be useful as an option for the diversification of landscapes in the Upper Midwest. However, asexual propagation protocols for producing this taxon are nonexistent. If this species is to be adopted by the nursery trade and produced on a large scale, propagation protocols need to be developed. We examined the rooting of desert olive when propagated using softwood, semi-hardwood, or dormant hardwood stem cuttings. The objective of this study was to generate foundational knowledge on asexual propagation of *F. pubescens* and explore the factors that influence rooting for different types of stem cuttings. Results confirm that *F. pubescens* can be successfully propagated using stem cuttings. For example, semi-hardwood cuttings collected from the Minnesota Landscape Arboretum were left without treatment (control; ETOH only) or were treated using a three second quick dip of Indole-3-butyric acid (1000, 2000, or 3000 ppm). Rooting percentage, root length, and number of roots were evaluated. After 35 days under intermittent mist, 21%, 62%, 74%, and 87% rooting occurred with the non-treated controls, 1000, 2000, and 3000 ppm IBA, respectively. Compared to non-treated controls, a ~764% increase of the mean number of roots and a ~122% increase of mean root length (combined across IBA treatments) were observed. Dormant hardwood cuttings were not a viable technique for propagating this taxon. Semi-hardwood cuttings stuck in 100% perlite substrate resulted in 97% rooting, whereas the use of 50/50 peat/perlite substrate yielded 40% rooting. Cultivar was determined to play a critical role in the rooting response of this species. Auxin application was not necessary for rooting to take place; however, the number of roots and root length were improved by treatment with IBA.

Additional index words: Desert olive, IBA (Indole-3-butyric acid), adventitious roots, underutilized native plants

Introduction

Underutilized nursery crops could serve as a means towards expanding species diversity within the green industry. *Forestiera pubescens* (Nutt.), more commonly known as desert olive, stretchberry, or New Mexico privet, (Breen, [date unknown]; Jacobs, 2009; Scianna & Hybner, 2009), is an ornamental shrub with a multitude of conservation applications and may be utilized as an alternative landscape option that replaces invasive privets (Ligustrum spp.). Conservation applications include windbreaks, shelterbelts, and living snow fences (Scianna & Hybner 2009). *Forestiera pubescens* has purportedly demonstrated drought tolerance (Jacobs, 2009) and flood tolerance (Tasker & Schulz, 2017), making it applicable to landscapes in the Upper Midwest. *Forestiera pubescens* grows along stream courses, moist valleys, hillsides, and mesas at elevations of 3,000 to 7,000 feet (Jacobs 2009). While considered drought tolerant, supplemental water and fertilizer improve performance in the landscape (Jacobs, 2009). *Forestiera pubescens* grows in USDA zones 4-9 with a mature spread of 0.9-3 m tall (Jacobs, 2009; Breen, [date unknown]). Production of a beautiful yellow fall leaf color also adds to its horticultural merit. There are no observed pest or disease problems with this taxon (Jacobs, 2009).

The commercialization of emerging nursery crops and their introduction into cultivation requires an assessment of production limitations and solutions for growers. There is little known about the propagation of *F. pubescens*, and the only known publication on asexual propagation within this genus was determined by Geneve et al., (2019). They evaluated asexual propagation of swamp privet (*Forestiera acuminata*), a North American native plant, and close relative of *F. pubescens*, with nursery crop potential (Geneve et al., 2019). The protocol developed by Geneve et al. (2019) for asexually propagating swamp privet serves as a foundation for determining best practices with other species of *Forestiera*.

While desert olive has largely been neglected in horticulture, the taxon was evaluated for landscape performance in Bridger, Montana (USDA zone 4) (Scianna & Hybner, 2009). Seedlings of the cultivar ‘Jemez’ were planted in a living snow fence and field windbreak in Bridger, Montana. In this field trial, desert olive plants were determined to perform well for landscape and conservation applications over a 33-year period, with no serious insect or disease problems (Scianna & Hybner 2009). 'Jemez' is the current recommended and preferred seed source for growing desert olive (Scianna & Hybner 2009) in Montana, especially for conservation and restoration purposes.

Due to its cold hardiness, adaptability, and resistance to abiotic and biotic stresses, desert olive merits horticultural attention and could support the development of resilient landscapes in the Upper Midwest. While little is known about how to asexually propagate *F. pubescens*, we hypothesize that it can be propagated by cuttings at different growth stages (semi-hardwood, dormant hardwood, softwood) utilizing standard levels of exogenous auxin typically employed in the green industry for other staple nursery crop species. The objective of this study is to develop an asexual propagation protocol for *F. pubescens* and explore the factors that optimize rooting for different types of stem cuttings.

Materials and Methods

Experiment 1

Semi-hardwood cuttings (derived from partially mature woody tissue) were collected on 22 July 2022, from accession 20050530 at the Minnesota Landscape Arboretum in Chanhassen, MN (lat. 44.8°N, long. 93.6°W). Five-node terminal cuttings (~9 cm in length) were collected using a bypass pruner. Cuttings were immediately placed in a plastic bag with a moist paper towel to maintain hydration, transported to the UMN Twin Cities

St. Paul campus, treated, and inserted into substrate. Propagules collected were 5-node terminal cuttings, ~9 cm in length. Cuttings were treated with 95% ETOH (control only); 1,000 ppm IBA; 2,000 ppm IBA; 3,000 ppm IBA (each dissolved in 95% ETOH) using a three-second quick dip of the basal ⅓ of the cutting. The cuttings were inserted in individual cells of 72 cell trays (T.O. Plastics; Otsego, Minnesota; 27.94 cm × 53.8988 cm × 5.72 cm) using a completely randomized design with four experimental units (comprising six single cuttings each) per treatment (N=336, n=84). Cell trays were filled with 100% perlite (Midwest Perlite, Appleton, WI). Trays were placed in a mist bay greenhouse where intermittent mist sprayed for eight second intervals, every 4.5 minutes, in Saint Paul, MN using a completely randomized design. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 128.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 889.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Greenhouse temperature and relative humidity were monitored by a HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). The average temperature was 25.1°C, with minimum and maximum values of 20.1°C and 35.3°C, respectively. Relative humidity averaged 72.3%, ranging from 30.7% to 97.6%. Rooting data were collected 35 days post-treatment with exogenous auxin. Individual cuttings were carefully removed from the cell, rinsed, and examined. The total number of roots as well as length of each root were recorded. A root was counted if it measured ≥ 0.25 cm in length.

Experiment 2

Dormant hardwood cuttings were collected on 23 February 2023 from the USDA North Central Regional Plant Introduction Station in Ames, IA (lat. 42°N, long. 93.6°W). The cuttings were inserted into a 50% perlite (Midwest Perlite, Appleton, WI) and 50% peat (Berger® BP Series Professional Sphagnum Peat Moss; Saint-Modeste, Québec) mix on 02 March 2023. Cuttings were ~23 cm in length and ~3 mm in diameter. Dormant hardwood cuttings were collected from two accessions: Accession 27629 and Accession 303300. For each accession, 50 cuttings were included in each experiment. Treatments included a non-treated control (95% ETOH only); 1,000 ppm IBA; 3,000 ppm IBA; 8,000 ppm IBA (each dissolved in 95% ETOH) using a three-second quick dip of the basal ⅓ of the cutting. Stem cuttings were placed into 50 cell count trays (T.O. Plastics; Otsego, Minnesota; 27.94 cm × 53.8988 cm × 6.03 cm). Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for eight second intervals, every 4.5 minutes, in Saint Paul, MN using a completely randomized design. The average temperature was 22.8°C, with minimum and maximum values of 10.3°C and 31.7°C, respectively. Relative humidity averaged 56.33%, ranging from 11% to 92.6%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation, Bourne, MA). Rooting data were collected at 50 days and again at 85 days post-treatment with exogenous auxin. A root was counted if it measured ≥ 0.25 cm in length.

Experiment 3

To test the effects of bottom heat, additional dormant hardwood cuttings grown at the Horticultural Research Center in Chaska, MN were collected 23 February 2023 from the

USDA North Central Regional Plant Introduction Station (Ames, IA). The cuttings were approximately 27.13 cm in length and 6.47 mm in diameter. There were 64 cuttings from two accessions: Accession 27629 and Accession 303300. These cuttings were placed in 36 cell count trays (T.O. Plastics; Otsego, Minnesota; 27.7876 cm × 54.4576 cm × 7.62 cm) with a bark-based potting substrate on 04 March 2023. There were 16 cuttings from each accession per treatment group. Cuttings were treated with or without exogenous auxin (3000 ppm IBA) as well as with or without basal heat. Propagules rooted in a glass greenhouse in Chaska, MN using a completely randomized design. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 162.38 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 1348.93 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 23.1°C, with minimum and maximum values of 10.3°C and 33.3°C, respectively. Relative humidity averaged 56.1%, ranging from 11% to 92.6%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). Rooting data were collected 90 days post-treatment with exogenous auxin. A root was counted if it measured ≥ 0.25 cm in length.

Experiment 4

The semi-hardwood cuttings from the first experiment underwent a dormancy period from 27 October 2022 to 02 February 2023. Once they produced semi-hardwood, two node (~6 cm long and ~3 mm in diameter) cuttings were taken to perform a second semi-hardwood experiment with exogenous auxin with substrate being the treatment. The cuttings were approximately 6.18 cm in length and 3.13 mm in diameter. The cuttings were inserted in 50 cell count trays (T.O. Plastics; Otsego, Minnesota; 27.94 cm × 53.8988 cm × 6.03 cm) on 02 June 2023. Cuttings were treated with 3,000 ppm IBA (dissolved in 95% ETOH) using a three-second quick dip of the basal ⅓ of the cutting. The substrate treatments were 100% perlite (Midwest Perlite, Appleton, WI), 100% peat (Berger® BP Series Professional Sphagnum Peat Moss; Berger Peat Moss Ltd; 121, 1er rang, Saint-Modeste, Québec), and 50/50 peat to perlite (N=160 cuttings: n=60 cuttings in 100% perlite, n=60 cuttings in 50/50 peat/perlite, n=40 cuttings in 100% peat). Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for eight second intervals, every 4.5 minutes, in Saint Paul, MN using a completely randomized design. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 192.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.02 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.9°C, with minimum and maximum values of 21.2°C and 36.3°C, respectively. Relative humidity averaged 55.2%, ranging from 20.8% to 90.4%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). Rooting data were collected 40 days post-treatment with exogenous auxin. A root was counted if it measured ≥ 0.25 cm in length.

Experiment 5

Softwood cuttings were taken from living collections at the Minnesota Landscape Arboretum in Chaska, MN on 14 June 2023. The propagules were 3 node cuttings (~11 cm long, ~2mm in diameter). The cuttings were inserted in 50 cell count trays (T.O. Plastics; Otsego, Minnesota; 27.94 cm × 53.8988 cm × 6.03 cm) filled with 100% perlite (Midwest Perlite, Appleton, WI) on 14 June 2023. Cuttings were treated with 95% ETOH (control only); 1,000 ppm IBA; 3,000 ppm IBA; 5,000 ppm IBA (each dissolved in 95% ETOH) using a three-second quick dip of the basal ⅓ of the cutting. Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for eight second intervals, every 4.5 minutes, in Saint Paul, MN using a completely randomized design.

Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 181.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.016 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.8°C, with minimum and maximum values of 21.2°C and 36.3°C, respectively. Relative humidity averaged 57.8%, ranging from 27.3% to 90.4%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). Rooting data were collected 35 days after treatment. A root was counted if it measured ≥ 0.25 cm in length.

Experiment 6

Container grown plants of *F. pubescens* (‘Berry Girl’ and ‘Silver Satin’) were obtained from High Country Gardens (Hotchkiss, CO). Softwood cuttings were collected from ‘Berry Girl’ and ‘Silver Satin’ and inserted into 100% perlite (Midwest Perlite, Appleton, WI) in 50 cell count trays (T.O. Plastics; Otsego, Minnesota; 27.94 cm × 53.8988 cm × 6.03 cm) on 14 June 2023. Cuttings of ‘Berry Girl’ and ‘Silver Satin’ were 2 node cuttings ~9 cm long and ~1 mm in diameter. Cuttings were treated with 95% ETOH (control only); 1,000 ppm IBA; 3,000 ppm IBA; 5,000 ppm IBA (each dissolved in 95% ETOH) using a three-second quick dip of the basal ⅓ of the cutting. There were three replicates of each treatment for both ‘Berry Girl’ and ‘Silver Satin’. Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for eight second intervals, every 4.5 minutes, in Saint Paul, MN using a completely randomized design. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 181.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.016 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.8°C, with minimum and maximum values of 21.2°C and 36.3°C, respectively. Relative humidity averaged 57.8%, ranging from 27.3% to 90.4%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). Data were collected 35 days after treatment. A root was counted if it measured ≥ 0.25 cm in length.

Statistics

Analysis of Variance models were applied to each dataset to evaluate mean root length, employing a one-way ANOVA or two-way ANOVA model dependent on the number of variables assessed. Where appropriate, data were transformed to meet model assumptions. To evaluate the number of roots per stem cutting, Poisson regression models were utilized. Data were analyzed utilizing R Statistical Software (Ver. 4.2.2). The R package *ggplot2* was used for graphing the mean root lengths and number of roots. The R package *emmeans* was used for group comparisons. Mean separation among treatments was obtained by polynomial contrasts using Tukey's test and a 5% rejection level. The R package *pscl* was used for creating zero-inflated Poisson regression models which were then used to compare the number of roots between treatment groups. The R package *MASS* was used for fitting and estimating linear models. The R package *ggpubr* was used for the creation of bar graphs.

Results and Discussion

Experiment 1

Semi-hardwood stem cuttings rooted at 21%, 62%, 74%, and 87% for non-treated controls (95% ETOH only), 1000 ppm, 2000 ppm, and 3000 ppm IBA, respectively (Figure 2). Mean root length data were log transformed to fit model assumptions with significance at P = < 2.2e-16 (Table 1). All treatment groups had improved mean root length when compared to the control group (Table 1). The 3000 ppm group had improved mean root length when compared to the 1000 ppm group (Table 1). Compared to the non-treated controls, root length was improved by 342.6%, 485.2%, 641.9% for the 1000 ppm, 2000 ppm, and 3000 ppm IBA treatments, respectively.

Number of roots per stem cutting data were analyzed using a zero-inflated Poisson regression model (P = 0.0222) (Table 1). The exogenous auxin treatment groups are not different from each other but are all different from the non-treated controls (Table 1). Compared to the non-treated controls, the number of roots per stem cutting was improved by 667.8%, 778.9%, 1237.6% for the 1000 ppm, 2000 ppm, and 3000 ppm IBA treatments, respectively.

Every treatment involving the application of IBA increased the number of roots and root length compared to non-treated controls. Similar rooting was observed across treatments with IBA. It is recommended that growers apply 3000 ppm IBA to maximize overall rooting percentage, root length, and number of roots.

Experiment 2

Results for mean root length were inconclusive from this study, as rooting observed was very low. Collected data could not fit model assumptions. At 30 days, no rooting was observed in any treatments. At 50 days, Accession 27629 rooted at 4%, 20%, 0%, and 24% for control (95% ETOH only), 1000 ppm, 3000 ppm, and 8000 ppm, respectively. At 50 days, Accession 303300 rooted at 0%, 0%, 4%, and 4% for control, 1000 ppm, 3000 ppm, and 8000 ppm, respectively. At 85 days, Accession 27629 rooted at 12%, 12%, 4%, and 36% for control (95% ETOH only), 1000 ppm, 3000 ppm, and 8000 ppm, respectively. At 85 days, Accession 303300 rooted at 8%, 16%, 24%, and 16% for control, 1000 ppm, 3000 ppm, and 8000 ppm, respectively.

Experiment 3

Dormant hardwood cuttings in both Accession 27629 and Accession 303300 did not root successfully. Out of the 128 cuttings between the two accessions, only five cuttings rooted. Four cuttings from Accession 27629 with hormone and heat added rooted, and one cutting from Accession 303300 with hormone and heat added rooted.

Experiment 4

Semi-hardwood stem cuttings rooted at 97%, 40%, and 0% for substrate treatments of 100% perlite, 50/50 peat/perlite, and 100% peat, respectively. Mean root length data were square root transformed to fit model assumptions with significance at $P = < 2.2e\text{-}16$ (Table 2). All treatment groups are different from each other, where the 100% perlite treatment group had the most improvement for root length compared to the other treatment groups (Table 2). Number of roots per stem cutting data were analyzed using a Poisson regression model without inflation ($P = 0.80452$) (Table 2).

Experiment 5

Softwood stem cuttings rooted at 34%, 63%, 87%, and 81% for control (95% ETOH only), 1000 ppm, and 3000 ppm, 5000 ppm, respectively. Mean root length data were log transformed to fit model assumptions with significance at $P = < 2.2e\text{-}16$ (Table 3). The 5000 ppm IBA treatment group improved root length compared to the non-treated control and 1000 ppm IBA treatment groups (Table 3). Compared to the non-treated controls, root length was improved by 83.8%, 236.9%, 214.2% for the 1000 ppm, 3000 ppm, and 5000 ppm IBA treatments, respectively. Number of roots per stem cutting data were analyzed using a Poisson regression model without inflation (P = 0.982324) (Table 3). There is a trend that increasing the exogenous auxin improved the number of roots per stem cutting.

Experiment 6

The cultivar 'Berry Girl' rooted at 53%, 53%, 87% and 47% for control (95% ETOH only), 1000 ppm, 3000 ppm, and 5000 ppm IBA, respectively. The cultivar 'Silver Satin' rooted at 13%, 47%, 80% and 73% for control, 1000 ppm, 3000 ppm, and 5000 ppm IBA, respectively. This study was implemented to determine if there is a difference between cultivar in number of roots and root length produced utilizing exogenous auxin. A two-way ANOVA analysis was utilized to look at cultivar differences as well as the differences in treatment. For mean root length, Treatment × Cultivar was significant at P = 0.011183 (Figure 3). For mean root length data, cultivar 'Berry Girl' and 'Silver Satin' are different from each other at a significance of P = 0.000929 (Figure 3). Cultivar 'Berry Girl' applied with 1000 ppm exogenous auxin and cultivar 'Silver Satin' applied with 5000 ppm exogenous auxin are statistically different from cultivar 'Silver Satin' non-treated control and 1000 ppm exogenous auxin application (Figure 3). All other treatments are not different from each other.

Number of roots per stem cutting data were analyzed using a two-way ANOVA. Treatment × Cultivar was significant at P = 0.011183 (Figure 4). For the number of roots data, cultivar 'Berry Girl' and 'Silver Satin' are different from each other at a significance of P = 0.000202 (Figure 4). For the number of roots data, treatments are different from each other at a significance of P = 2.73e-07 (Figure 4). Cultivar 'Berry Girl' non-treated control, cultivar 'Silver Satin' non-treated control and 1000 ppm exogenous auxin application are different from cultivar 'Berry Girl' applied with 3000 ppm exogenous auxin (Figure 3). All other treatments are not different from each other.

The current rooting threshold for a plant to be considered a viable commercial nursery crop is 80% rooting success (Griffith Gardner et al., 2019). This threshold was met for semi-hardwood cuttings in 100% perlite substrate with application of 3000 ppm exogenous auxin as well as for softwood cuttings including the cultivars of 'Berry Girl' and 'Silver Satin' under the same conditions.

Woody plants show considerable variation in their responses to asexual propagation techniques (Stokes et al., 2023). *Shepherdia canadensis,* an extremely hardy shrub plant, was asexually propagated by stem cuttings. Hardwood cuttings of *S. canadensis* rooted best with the application of 3000 ppm IBA, and rooting was inhibited by the application of 8000 ppm IBA (Krishnan & Hughes, 1991). Asexual propagation of *Comptonia peregrina* with the application of 3000 ppm and 8000 ppm IBA improved rooting percentage compared to non-treated stem cuttings of this taxon (Griffith Gardner et al., 2019). For desert olive, 3000 ppm IBA was also the optimal level of exogenous auxin out of the treatments utilized. When looking at using higher levels of exogenous auxin, 8000 ppm was utilized for dormant hardwood cuttings which did not have any significant rooting across treatments. Further studies are necessary to determine an exogenous auxin level for semi-hardwood and softwood cuttings of desert olive that inhibits root growth or reduces root length or number of roots per stem cutting.

Contrary to the results of this study, semi-hardwood cuttings of swamp privet (*Forestiera acuminata*) rooted above the commercial nursery crop rooting threshold at 90% for both non-treated cuttings and cuttings treated with potassium salt of indole butyric acid (K-IBA) (Geneve et al., 2019). Peterson et al. (2020) also found that application of K-IBA is not always necessary for adventitious rooting, with sweetgale (*Myrica gale*) root rating, root dry weight, and root length all being reduced by the application of K-IBA (Peterson et al., 2020).

Asexual propagation of low-bush blueberries by stem cuttings showed that substrate can have a significant effect on rooting (Schwab et al., 2021). These stem cuttings rooted best in a more traditional soilless substrate of peat:perlite compared to other less traditional substrates in the study. With desert olive, substrate also influenced rooting. Even with the utilization of traditional soilless substrates, peat was seen to not be a viable option for rooting of stem cuttings even in combination with perlite. Peat was also deemed not viable as a substate for the propagation of mountain fly honeysuckle, an underutilized plant with horticultural potential. Mountain fly honeysuckle produced the greatest rooting in 100% perlite compared to the addition of peatmoss to the substrate (Hayes and Peterson 2019). The root size was diminished as the concentration of peatmoss increased in an overhead mist irrigation system (Hayes and Peterson 2019).

In line with desert olive, collection of stem cuttings of box huckleberry (*Gaylussacia brachycera*) collected in late spring to early summer will produce the highest rooting percentage (Kidwell-Slak et al., 2014). This timing of stem cutting collection corresponds with softwood and/or semi-hardwood production.

The results of both semi-hardwood experiments show that semi-hardwood cuttings treated with 3000 ppm exogenous auxin and grown in 100% perlite growing medium maximizes overall rooting percentage, root length, and number of roots. It is not recommended that growers attempt to root dormant stem cuttings of *F. pubescens*. The softwood auxin experiment shows that softwood cuttings root best with either 3000 ppm or 5000 ppm exogenous auxin for maximization of root length and the number of roots per stem. The softwood cultivar study showed the cultivar ‘Silver Satin’ has significantly less roots and shorter root lengths compared to cultivar ‘Berry Girl’. Optimization of a rooting protocol for desert olive can allow for the introduction of this environmentally adaptable species into the nursery industry to replace invasive privets in the *Ligustrum* genus.

CHAPTER 3: Supplemental Nickel Corrects Mouse Ear Disorder of American and Interspecific Hybrid Hazelnuts

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Abstract

Cold hardy hazelnut [*Corylus* (L.) spp.], an emerging crop in the Upper Midwest, has been reported to exhibit symptoms aligned with Mouse Ear Disorder (MED) when cultivated using soilless substrates in containers. To test these claims, we questioned if supplemental nickel could correct symptomatic growth. Our objectives were to 1) characterize purported symptoms of non-treated American hazelnut [*C. americana* (Walter)] and hybrid hazelnut plants [*C. americana* × *C. avellana* (L.)] and 2) determine if symptoms are corrected by supplementing nickel. Seedlings of both taxa were treated with either (1) a non-treated control (H_2O spray) or foliar sprays on all leaf surfaces until beading of (2) Nickel Plus® (169 mg/L), (3) $NiCl_2$ (169 mg/L), (4) urea (150 mg/L N), and (5) combined $NiCl_2$ (169 mg/L) and urea (150 mg/L N). Forty-five days post-treatment, plants were evaluated using a rating scale, leaf characterization metrics (greenness, count, surface area, dry mass, and specific area), and metrics characterizing stem traits (elongation and dry mass). Stem elongation was the only response that differed between taxa and was affected by the

treatments, whereas all other metrics were influenced solely by treatment, regardless of taxon. These data suggest hazelnuts are susceptible to MED, which can be mitigated by supplementing nickel.

Index Words: *Corylus*, nickel deficiency, urea

Species used in this study: American hazelnut [*Corylus americana* (Walter)]; Interspecific hybrid hazelnut [*C. americana* (Walter) × *C. avellana* (L.)]

Chemicals used in this study: $NiCl_2$; Nickel Plus®; urea

Significance to the Horticulture Industry

Hybrid hazelnuts [*C. americana* (Walter) × *C. avellana* (L.)] combine the cold hardiness and disease resistance traits of the locally native American hazelnut with the kernel size and thin shells of the globally popular European hazelnut. This re-envisioned filbert, along with improved selections of American hazelnut, comprise an emerging specialty crop in the Upper Midwest, sought after for healthful nuts with immense potential as an oilseed crop (Braun & Wyse 2019). However, hybrid and American hazelnuts remain underutilized due to plant-production pipeline bottlenecks like purported susceptibility to Mouse Ear Disorder (MED). Reports from nursery growers of deformed leaf development of container-grown stock strongly align with symptoms of MED reported for other members of the Betulaceae family (Ruter 2005) and may suggest hazelnuts, like some other ureide transporting woody plants, may require supplemental nickel when cultivated with soilless substrates. However, hazelnuts have not previously been evaluated for susceptibility to this unusual deficiency and protocols for best practices are non-existent. The data presented reinforce the suspicion that hazelnuts are susceptible to MED when cultivated in nursery containers with soilless substrates and that symptoms of MED on hazelnuts can be corrected by supplementing plants with either Nickel Plus®, $NiCl_2$, or combined $NiCl_2$ and urea treatments, but not urea alone. Results of this study serve as a resource for improving the production of hybrid and American hazelnuts in the nursery and may support further development of this emerging crop.

Introduction

Nickel is the most recent element to be recognized as an essential nutrient for plant growth (The Fertilizer Institute [date unknown]). Nickel deficiency was first documented in soybeans after a report of plant responses to a controlled depletion of available nickel by Eskew et al. in 1983. Brown et al. (1987) later confirmed that nickel functioned as an essential nutrient in higher plants. Forty years later, nickel has yet to be comprehensively evaluated, especially in woody plant species produced in nurseries. Nickel deficiency in nursery crops is a topic of discussion as growers become more familiar with the plant genera that exhibit a higher risk of displaying deficiency symptoms under otherwise standard conditions, producers are better able to recognize symptoms, and research further explores the environmental conditions that lead to occurrences of deficiency (Wood 2015). A more comprehensive understanding of nickel nutrition in plants could enable specialty crop growers to overcome this production pipeline problem that seemingly predominates in ureide transporting species including pecan (*Carya illinoinensis*) (Wood et al. 2004a), bitternut hickory (*Carya cordiformis*) (Miller & Bassuk 2022b), river birch (*Betula nigra*) (Ruter 2005), and potentially in several other perennial angiosperm genera (Wood 2015).

Nickel deficiency in woody plants is commonly referred to as “Mouse Ear Disorder” (MED), which is known to occur in ureide-transporting woody-perennial species and has been linked to disrupted metabolism of ureides, amino acids, and organic acids (Bai et al. 2006). Using xylem sap of pecan, Bai et al. (2007) demonstrated that nickel deficiency reduces the activity of urease, interrupting normal nitrogen cycling via disruption of ureide metabolism. The urease enzyme requires nickel as a cofactor, allowing urea to be converted into ammonia, a usable form of nitrogen in plants, and releasing carbon dioxide. When nickel is deficient, urease activity declines. Subsequently, urea that is not broken-down, or certain organic acids, or both, are thought to build to toxic levels in the leaf tissue causing cell death in leaves or leaflets (Wood 2015). Increasing urease activity through nickel supplementation is expected to reduce the leaf urea accumulation that is observed as marginal necrosis of leaves and leaflets in symptomatic plants (Siqueira Freitas et al. 2018). Applications of nickel soon after bud break are known to be effective at ameliorating nickel deficiency symptoms (Wood et al. 2006).

The nickel threshold for visual symptoms of nickel deficiency in woody perennials is $\simeq$ 1.0 $\mu g \times g^{-1}$ in foliar tissue (Wood 2015). An early indicator of MED in pecan is chlorosis, appearing similar to iron or sulfur deficiency symptoms that later turns dark green (Wood et al. 2004a, 2006). As symptoms become more severe, leaflet tips begin to exhibit rounding or blunting (Figure 7). Necrotic margins begin to form, leaves will curl, ultimately leading to rosetting and stem dieback (Figure 7). The disorder is frequently observed in container-grown crops of susceptible species grown with artificial potting substrates (Ruter 2005). Nickel availability is also dependent on soil (or substrate) pH, type, water availability, temperature, and competition between other divalent cations of heavy metals such as zinc, cobalt, copper, and iron (Wood 2015). Relatively alkaline soils with a soil pH greater than 6.5 decrease the availability of nickel (Brown et al. 1989). The competition between these cations suggests that there may be a shared uptake system into the plant amongst these heavy metals.

Many fungicides used in orchards contain zinc, another heavy metal like copper, that can disrupt nickel-associated physiological processes because it competes for the same uptake pathway (Wood et al. 2004a). In the pecan crop, copper fungicides are frequently utilized to control pecan scab (*Fusicladium effusum*), a fungal disease that causes lesions on leaves and fruit of the pecan plant (Wood et al. 2012). Due to these management practices, copper accumulates in the soil, and as a result, nickel deficiency is frequently observed during the replanting process of orchards or groves (Wood et al. 2004b, 2006). Trees displaying severe nickel deficiency symptoms are unlikely to survive a few years after transplanting into the orchard, particularly if they are placed in areas that were previously inhabited by another tree (Wood et al. 2006; Wood 2015).

Previous research on nickel nutrition in hazelnut is limited to an evaluation of *in vitro* nutrient solutions in conjunction with other essential plant nutrients (Hand and Reed 2014). Many European hazelnut cultivars are difficult to culture *in vitro* on standard growth medium (Hand and Reed 2014). Nickel is not incorporated with *in vitro* growth media commonly used for hazelnut production, including: Driver and Kuniyuki (DKW) medium, woody plant medium (WPM), or Murashige and Skoog (MS), but the introduction of nickel may improve *in vitro* culture of this crop and many others (Hand and Reed 2014). A diverse response to differing levels of all minor nutrients in the experiments of Hand and Reed (2014) show that nickel requirements *in vitro* should be explored further. In addition to recognizing the importance of nickel nutrition *in vitro* for successful propagation, a lack of knowledge exists for nickel nutrition of container grown plants and controlled experiments could further support the development of hazelnut crops in horticulture.

In 2021, the authors observed symptoms akin to MED with container grown plants of American hazelnut and interspecific hybrid hazelnuts grown in nursery containers filled with a peat and bark substrate mix (unpublished data). Interactions with researchers (Mark Hamann, University of Minnesota Hazelnut Breeding Program, Saint Paul, MN, personal communication) and industry collaborators (Brent McKown, Knight Hollow Nursery Inc., Middleton, WI, personal communication) within the Upper Midwest Hazelnut Development Initiative corroborated the routine occurrence of these symptoms in container-grown plants. A study was designed to replicate the conditions under which symptoms occur in order to test and determine their cause. The objectives of the study were to 1) characterize purported symptoms of nickel deficient American hazelnut [*C. americana* (Walter)] and hybrid hazelnut plants [*C. americana* × *C. avellana* (L.)] and 2) determine if symptoms are corrected by foliar application of supplemental nickel.

Materials and Methods

Two lots of seeds of American hazelnut were sourced from wild populations in Ontario, Canada (Sheffield's Seed Company, Locke, NY, USA) or Christian County, Illinois (Starhill Forest Arboretum, Petersburg, IL, USA). Open-pollinated seeds of hybrid hazelnut were provided by the hazelnut breeding program at the University of Minnesota (St. Paul, MN, USA). Seeds were cold stratified for 90 days in moistened, long-fiber sphagnum moss in a cooler maintained at 4 C. After stratification, seeds were sown just below the substrate surface in flats (25.72 cm × 25.72 cm × 6.03 cm) containing Pro-Mix BRK soilless media (Premier Tech, Quebec, Canada) and subsequently moved to a glass greenhouse (located at 44° 59' 15'' N, 93° 11' 0'' W) where germination began on 21 June 2022. Following germination and initial growth, plants entered a quiescent state and were moved to a cooler (40 C) to acquire chilling hours for their first dormancy period from 12 October 2022 to 17 January 2023. After dormancy, plants were potted singly (07 February 2023) into black plastic 6" Azalea pots (15.24 cm × 11.43 cm) filled with Pro Mix BRK substrate. Containers were top dressed with 10 grams of slow release fertilizer that did not contain urea or any nickel component applied with a foliar spray in this study (Osmocote 14-14-14; ICL Specialty Fertilizers, Dublin, OH) at the time of potting-on as well as each subsequent spring. Plants were moved into a glass greenhouse maintained at 23 C where the plants were watered once daily. Hazelnuts were once again overwintered in the aforementioned cooler for 53 days. After chilling requirements were met, hazelnuts were again moved into a greenhouse (24 July 2023) to break bud. Plants were watered once daily throughout the experiment. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average

Photosynthetically Active Radiation (PAR) of 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 1438.156 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 24 C, with minimum and maximum values of 12 C and 43 C, respectively. Relative humidity averaged 61%, ranging from 23% to 92%, with a dew point of 15.45 C, varying between 2.13 C and 27.40 C, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation, Bourne, MA).

Two weeks after bud break (21August 2023), all plants were treated with either a (1) non-treated control (H_2O spray), or foliar sprays on all leaf surfaces until beading of (2) Nickel Plus® (169 mg/L), (3) $NiCl_2$ (169 mg/L), (4) urea (150 mg/L N), and (5) combined $NiCl_2$ (169 mg/L) and urea (150 mg/L N). Nickel Plus® (5-0-0) is derived from urea and nickel lignosulfonate (5% N, 3% S, 5.4% Ni) (NIPAN LLC, Valdosta, GA).

For each treatment, the plants were grouped and assigned a flag color representing each treatment. Each treatment group comprised 24 plants: 7 from *C. americana* Christian Co., 9 from *Corylus* hybrid, and 8 from *C. americana* Sheffield's Seed Co. These treatment groups were separated from each other for foliar application to avoid drift and were then spread out in a completely randomized design across two greenhouse benches after treatment. To protect from inhalation and exposure to nickel, the operator wore a disposable coverall suit with a hood and boot covers, as well as goggles and an N-95 mask that tightly fit their face.

One representative stem, which reflected the overall symptom level of each hazelnut plant, was harvested on 05 October 2023 and used to measure the following growth metrics: rating (1-5), shoot elongation (cm) measured from where buds broke after dormancy to their most apical position of the newly extended shoot, number of leaves per unit shoot extension (calculated as the total number of leaves present on the harvested stem), leaf surface area (cm^2), leaf greenness using a SPAD meter, leaf dry mass (g), shoot dry mass (g), specific leaf area (cm^2/g). First, an overall observational symptom rating of each plant was collected in the greenhouse, using a rating scale of 1 to 5 which reflected the approximate percent of new growth displaying MED symptoms: 1 = ~100%, 2 = ~75%, 3 = 50%, 4 = ~25%, 5 = no visible symptoms present on new leaves post-budbreak (Figure 8). Chlorophyll content was also collected in the greenhouse using a SPAD meter (SPAD 502 Plus Chlorophyll Meter, Konica Minolta, Inc., Tokyo, Japan). Leaf Greenness was measured as an average of three measurements for three separate leaves each (n=3 per plant). After rating and leaf greenness were recorded, one representative stem with its leaves was collected from each plant in the experiment for a total of 121 cuttings. Leaves were individually separated from the stems and analyzed using a LI-COR 3100 Leaf Area Meter (LI-COR Biosciences Inc., Lincoln, NE) to obtain leaf surface area in cm^2. Leaves and stems were placed in paper bags and set in a dryer maintained at 50 C. After 72 hours in the dryer, shoot and leaf dry weights were collected on 10 October 2023. These data were used to calculate specific leaf area, determined as the leaf area (cm^2) divided by the leaf dry weight (g).

pH and electrical conductivity (EC)

For 15 representative samples from each treatment group, leachate was collected using the pour-thru technique (Mattson [date unknown]) to measure substrate pH and EC (µS). To collect leachate, a plastic basin was placed underneath each container and 75 mL (based on a container diameter of 15.24 cm) of DI water was subsequently poured evenly across the top of the substrate within the pot. From each basin, 50 mL of leachate was collected and measured using a handheld Apera® meter (Apera® PC60 pH/ Cond./TDS/Sal. Tester; Apera® Instruments, Columbus, Ohio).

Leaf tissue analysis

One stem with leaves deemed representative of the overall symptom-level of the plant was removed and placed in a labeled paper bag between 31 October 2023, and 03 November 2023, from each plant in the experiment. Paper bags were subsequently placed in a forced air dryer maintained at 50 C for 72 hours, then weighed. Leaves were removed from stems, with stems discarded after collecting shoot dry weight (g). Leaves from individuals were consolidated within their treatment group if the leaves collected did not weigh at least 4 grams. A total of 96 paper bags containing dried leaf tissue were submitted for leaf tissue analysis at the Research Analytical Laboratory on the University of Minnesota campus in Saint Paul, MN on 17 September 2023, where an elemental analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was performed. The elemental analysis for nickel in leaf tissue was performed utilizing the dry ashing method at 485 C ashing temperature.

Statistical analysis

Data for measured parameters were analyzed using a two-way ANOVA evaluating the effects of treatment and species. Data analyzed met the assumptions of the ANOVA model. When the interaction was not significant, the interaction term was removed from the model and the analysis was rerun to evaluate the main effects of species and/or treatment. Data were analyzed using R statistical software (Ver. 4.2.2). Species were defined as "American hazelnuts" [*C. americana* (Christian Co. and germplasm sourced from Sheffield's Seed Co.)] as well as "hybrid hazelnuts" (interspecific *Corylus* hybrids sourced from University of Minnesota Hazelnut Breeding Program). Post-hoc mean comparisons were made using Tukey's Honestly Significant Difference Test. Packages in R included *dplyr*, *ggplot2*, *ggpubr*, *tidyr*, *agricolae*, *plotrix*, and *multcompView*.

Results and Discussion

pH and electrical conductivity (EC)

Nutrient deficiencies, like nickel, are often linked to high substrate pH, which affects the availability of nutrients, especially micronutrients (Torres et al. 2010). Ruter (2005) found that high pH soils may contribute to MED disorder symptoms. Electrical conductivity (EC) is a measure of dissolved soluble salts in solution, as nutrients in solution exist as salts which can conduct electricity (Torres et al. 2010). It is important to monitor pH and EC of substrates to correct nutrient availability issues before they become a detriment to a crop (Torres et al. 2010). For each treatment group in this study, the average pH values ranged from 6.0-6.3 and the average EC values were between 697-862 µS/cm. LeBude & Bilderback (2009) state that pour-thru guidelines for most nursery crops suggest leachate EC values should range between 500 to 2000 µS/cm during periods of active growth.

Torres et al. (2010) states that each plant species has an optimal pH range with nutrients in soilless substrates usually available at a pH range of 5.4-6.2. Guidelines for optimal pH and EC ranges were observed with the plants used in these experiments (Table 4).

Nickel and urea supplementation

There was a significant interaction between treatment and species for shoot elongation (cm) (P = 0.0468). Not all responses that involved nickel treatment were statistically different from the non-treated control, however, there is a general trend that nickel supplementation improved shoot length (cm) (Figure 5).

The main effect of treatment was observed for all other parameters. Compared to the non-treated controls, rating increased (P = <2e-16) by 123.9%, 142.4%, 13.6%, and 146.7% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 5). A similar trend was observed for leaf area (cm^2) (P = <2e-16) compared to the non-treated controls, with increases of 321.8%, 440.3%, 18.2%, and 362.1% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 5). Continuing this trend, compared to the non-treated controls, specific leaf area (cm^2/g) (P = <2e-16) increased by 74.9%, 70.9%, 9.5%, and 104% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 5).

Because shoot length can be stunted as a result of nickel deficiency, it is generally expected that supplemental nickel application will yield an increase in shoot elongation. The interaction between treatment and species for shoot elongation showed that American hazelnuts treated with $NiCl_2$ or $NiCl_2$ + urea were significantly different from the non-treated controls (Figure 5) however, American hazelnuts treated with Nickel Plus® were not different from the non-treated controls. Although treatments containing $NiCl_2$ best improved shoot elongation in American hazelnut, there is a trend that nickel supplementation, regardless of the form of nickel, increased shoot elongation in American hazelnuts (Figure 5). This trend was not observed with hybrid hazelnuts; instead, shoot elongation for all treatment groups, including the non-treated controls, was not different (Figure 5).

The overarching trend observed with these data is that nickel supplementation improved the evaluated growth parameters (Table 5). Because treatment and taxon differences were observed, growers should utilize supplemental nickel on a case-by-case basis considering the cost, availability, and the unique production requirements of their operation. For example, $NiCl_2$ may be harder for a practitioner to acquire compared to Nickel Plus®, which is sold commercially as a fertilizer. Nickel Plus® is derived from urea and nickel lignosulfonate. According to Wood (2015), organic nickel ligands, including nickel lignosulfonates, are particularly effective nickel foliar fertilizers, especially in field conditions due to worker safety concerns compared to other nickel fertilizers derived from nickel-nitrates and nickel-sulfates. There is no current literature regarding use of $NiCl_2$ and/or urea for the correction or exacerbation of MED within hazelnut. Urea was expected to exacerbate MED symptoms in hazelnuts, and also suspected to be more detrimental to plants compared to the non-treated control plants due to it's the role in the urease pathway, where nickel is necessary for the conversion of urea to ammonia (Bai et al., 2006). Urea is utilized throughout the world as a major nitrogen fertilizer for crops, which furthers the need to understand urea metabolism and a plant's ability to rapidly metabolize urea to increase crop productivity, especially in ureide-N transporting crops (Wood 2015).

Leaf tissue analysis

Compared to the non-treated controls, leaf tissue concentration of nickel (mg/kg) (P = 2.12e-12) increased by 4752.9%, 2058.8%, 88.2%, and 2800% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 6). The use of Nickel Plus® resulted in the greatest leaf nickel concentration compared to all other treatments (Figure 6). Nickel Plus® may be able to better penetrate the leaf cuticle compared to the other nickel treatments (John Ruter, University of Georgia, Athens, GA, personal communication). Nickel Plus® was statistically different from the other nickel treatments. All nickel treatments were statistically different from the urea and control treatment groups (Table 6). These data show that the application of nickel, regardless of the nickel source, improved the nickel concentration in the leaf tissue. Treatment involving $NiCl_2$ and urea has both components necessary to complete the urease pathway, but based on these results, this combination yielded 40.2% less nickel concentration in foliar tissue compared to treatments involving Nickel Plus® (Table 6). Future research should investigate how the urease pathway is affected by the nickel source applied.

All three nickel treatments in this study were sufficient for correcting MED symptoms and improving nickel nutrition in American and interspecific hybrid hazelnuts. The typical range for nickel concentration in healthy plant leaves is 0.05 to 5 $mg \times kg^{-1}$ (Liu et al. 2011).

Nickel toxicity levels occur at concentrations ≥ 10 ppm in sensitive plant species or cultivars (Liu et al. 2011). As an essential micronutrient, nickel is needed in low quantities in plants; however, there may be an optimal nickel threshold in leaf tissue specific to plant species. Based on these results, MED symptoms are overcome in American and hybrid hazelnuts when leaf tissue concentration ranges from ~3.7-8.3 g/kg (Table 6). Pecan for example has a relatively high nickel requirement compared to amide-N-dormant species (The Fertilizer Institute [date unknown], Wood et al., 2004a, 2004b, 2004c, 2006). The adequate range for nickel content in pecan tissue is between 2.5 to 30 ppm (The Fertilizer Institute [date unknown]). The nickel requirement for ureide transporting species is also dependent on endogenous concentrations of competing cations, such as the divalent cations of heavy metals: zinc (Zn^{2+}), copper (Cu^{2+}), and iron (Fe^{2+}) (The Fertilizer Institute [date unknown], Wood et al. 2006, Wood 2015).

Application utilizing foliar sprays are important to investigate, as foliar sprays are more readily used in production compared to a soil drench method. In this study, all three nickel treatments [Nickel Plus® (169 mg/L), $NiCl_2$ (169 mg/L), and combined $NiCl_2$ (169 mg/L) and urea (150 mg/L N)] are sufficient for correcting symptoms of MED and increasing nickel content within the leaves. There are still many other species worth studying regarding nickel deficiency, including ureide transporting genera: *Acer* (L.), *Alnus* (Mill.), *Annona* (L.), *Betula* (L.), *Carpinus* (L.), *Carya* (Nutt.), *Cercis* (L.), *Chamaecyparis* (Spach), *Cornus* (L.), *Corylus* (L.), *Diospyros* (L.), *Juglans* (L.), *Nothofagus* (Blume), *Ostrya* (Scop.), *Platanus* (L.), *Populus* (L.), *Pterocarya* (Kunth), *Salix* (L.) and *Vitis* (L.) (Wood et al. 2006) and several other woody angiosperm genera: *Coffea* (L.), *Prunus* (L.), *Pyracantha* (M.Roem.) and *Rosa* (L.) (Wood 2015). Compared to Miller and Bassuk (2022b), who reported a 126.9% increase in leaf area (cm^2) with bitternut hickories treated with a foliar spray of Nickel Plus® [9.46 ml Nickel Plus® per 3.79 L H_2O (0.32 fl oz per gal)], this study revealed a 321.8% increase in leaf area (cm^2). Previous research has explored the application of Nickel Plus® in addressing MED (Miller and Bassuk, 2022b), along with the utilization of nickel salts and ligands for corrective measures (Wood et al., 2004a, 2004b). However, this study marks the inaugural investigation into the use of $NiCl_2$ for alleviating symptoms associated with MED.

In addition to previously evaluated taxa, American and interspecific hybrid hazelnuts should be considered particularly susceptible to MED that should now be recognized as a cause of nickel deficiency when cultivated using soilless substrates. Results from this randomized greenhouse study showed significant effects of nickel treatment on various metrics, including stem elongation and leaf characteristics. Overall, this study highlights the significance of nickel nutrition for hazelnut cultivation in containers with soilless media, offering valuable guidance for growers aiming to optimize plant health and yield in this emerging crop.

CHAPTER 4: The Effects of Supplemental Nickel on Mouse Ear Disorder of Three *Diospyros* Species

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Abstract

American persimmon (*Diospyros virginiana* L.) is a multipurpose tree endemic to the eastern United States with potential for broader use in managed landscapes or for producing desirable fruits. Whereas most members of this genus originate from tropical and subtropical regions, the American persimmon could expand landscape diversity and fruit production applications in northern climates. Due to purported challenges with transplant success, American persimmon is likely best suited to production in containers. Recent observations of container-nursery crop production indicate American persimmon is susceptible to mouse ear disorder (MED), a function of nickel deficiency. We hypothesized that American and Asian-origin persimmon species are susceptible to MED and that supplementing nickel with a foliar spray will ameliorate the disorder. Our objectives were to characterize symptoms of non-treated MED with American persimmon (*D. virginiana*), Japanese persimmon (*D. kaki* Thunb.), and dateplum persimmon (*D. lotus* L.) as well as to determine if MED could be corrected by foliar application of nickel or other compounds presumed to interact with the urease metabolic pathway, such as urea. In a randomized greenhouse study, seedlings of each species were evaluated by comparing a non-treated control (H_2O spray), to foliar spray treatments of Nickel Plus® (169 mg/L), $NiCl_2$ (169 mg/L), urea (150 mg/L N), and combined $NiCl_2$ (169 mg/L) and urea (150 mg/L N). Following treatment, plants were evaluated using a MED severity rating scale, leaf characterization metrics [chlorophyll content index (CCI), count, surface area, dry mass, and specific area], as well as metrics characterizing stem traits (elongation and dry mass). Compared to the non-treated control, leaf surface area increased by ~56%, ~61%, ~25%, and ~52% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively, across species. These results suggest nickel supplements are effective at correcting MED for *Diospyros* species displaying symptoms. This study offers valuable insights for improving the cultivation of persimmons in container nursery production settings, contributing to the development and advancement of American persimmon as an emerging specialty crop.

Introduction

The genus *Diospyros* (L.) comprises 778 species with an overall cosmopolitan distribution (Kew Science [date unknown]). The most widely cultivated species, *Diospyros kaki* (Thunb.), is primarily valued for fruit production and is commonly referred to as the Japanese persimmon, oriental persimmon, or the Chinese persimmon (Chamberlain 2020;

Ojha et al. 2023). *Diospyros kaki* is a deciduous tree originating from Asia, offering desirable fruits with a red exterior and yellow to orange flesh (Yakushiji and Nakatsuka 2007). Aside from its fruits, Japanese persimmon is also valued as an ornamental tree due to its brilliant red fall color display (Yakushiji and Nakatsuka 2007). This species commands the persimmon fruit market with more than 4.2 million tons of persimmon fruits produced worldwide in 2019 according to the Food and Agriculture Organization (FAO) (Ojha et al. 2023). In 2014, Japanese persimmon produced in the United States (primarily cultivated in California) was valued at $8 million (Chamberlain 2020). Japanese persimmon is frequently grafted onto *D. lotus* (L.) due to the compatibility of the taxon and its tolerances to abiotic stresses, including drought (Liu et al. 2023). *Diospyros lotus* is commonly referred to as dateplum persimmon, Caucasian persimmon, or lilac persimmon. The native range extends from the subtropical areas of southwest Asia and southeast Europe and is also found growing and in cultivation throughout north-east and southern Anatolia (Ojha et al., 2023). Dateplum persimmon fruits are highly astringent, containing high amounts of soluble tannins in the immature (unripe) stage (Ojha et al., 2023). For this reason, consumption directly post-harvest is less desirable than in *D. kaki* or *D. virginiana* (Ojha et al., 2023).

Whereas the cultivation of Japanese persimmon in the United States is limited to USDA zones 7-10 (Missouri Botanical Garden [date unknown]a), the American persimmon (*D. virginiana* L.) is considered hardy in USDA zones 4-9 (Missouri Botanical Garden [date unknown]b), suggesting the taxon is a candidate for broader application, including in the Upper Midwest. Commonly referred to as American or common persimmon, *D. virginiana* is endemic to the eastern United States, occurring from Florida, north to Connecticut, central Ohio, Indiana, Illinois, and Missouri, and west to eastern parts of Kansas, Oklahoma, and Texas (Dirr 1975; Elias 1987; Peattie 1966), excluding higher elevations in the Appalachian Mountains (Peattie, 1966). American persimmon is known for its resiliency and adaptability, frequently found growing in adverse sites ranging from standing water of bottomland habitats to highly drought prone, highly disturbed sites including fence rows, along highways, and even top-soil stripped mining sites (Dirr 1975; Elias 1987; Peattie 1966). The species offers a pyramidal to oval crown, yellow to purple fall color, and attractive blocky bark (Dirr 1975). American persimmon can also exhibit glossy and leatherlike leaves (Chamberlain 2020). The primary consumable from the American persimmon plant is the fruit, however, the wood has been used historically for golf club heads (Chamberlain 2020).

American persimmon is a resilient plant, able to withstand difficult conditions and offering specialty fruits, as well as ornamental value, yet the species is not readily available in the nursery trade (Dirr 2000). One potential explanation for limited availability is because American persimmon develops a substantial taproot, making it an effective choice for erosion control, but lending to challenges for successful transplanting (Nesom 2006). In many instances, trees which develop strong taproots are initially grown in containers to reduce transplant shock (Miller and Bassuk 2023). Container production could better support the development of this emerging specialty crop; however, the authors have observed unusual symptoms of a nutritional deficiency on American persimmon cultivated in nursery containers with soilless substrates. These symptoms are characteristic signs of nickel deficiency, also called Mouse Ear Disorder (MED), and they include chlorosis, stem dieback, rosetting, leaf curling, wrinkling of leaves, and necrotic leaf margins (Wood et al. 2004b). These observations have been corroborated by specialty nursery growers that produce American persimmon in containers (Personal communication, Zach Burhenn, Iowa Native Tree and Shrub Nursery, May 2022). Further, images of persimmon plants displaying these symptoms have emerged on social media platforms, such as Facebook, by home growers. Combined, these observations and images indicate that MED may be a more widespread issue than previously thought and determining a solution could support broader adoption of this emerging specialty crop.

Mouse ear disorder, also known as little leaf disorder, describes nickel deficiency, which has been best documented in ureide-transporting woody plants (Bai et al. 2006; Wood et al. 2006). The occurrence of MED has been associated with the disruption of ureides, amino acids, and organic acids (Bai et al. 2006). Using xylem sap, which exhibited a reduction of urease activity and disruption of ureide metabolism, Bai et al. (2007) demonstrated that a shortage of nickel leads to the interruption of normal nitrogen cycling. Nickel is a necessary cofactor for urease activity, such that if nickel is deficient, urease activity declines (Bai et al., 2006). If urea is not converted to ammonia in this process, it is thought that the urea and/or specific organic acids will build to a toxic level in the leaf tissue and ultimately cause cell death in leaves or leaflets (Wood 2015). Applying supplemental nickel shortly after bud break has shown to be effective at correcting symptoms of MED (Wood et al. 2006).

Nickel was most recently acknowledged as an essential nutrient for plant growth, making it the latest element to be recognized as essential for plants (Liu et al. 2020). In 1983, Eskew et al. documented nickel deficiency using a controlled nickel availability experiment with soybeans. Brown et al. (1987) later made the case that nickel should be considered an essential element for higher plants. While evidence and arguments stating the importance of nickel nutrition in higher plants have been presented, the significance of nickel in agriculture, especially with woody plants in horticultural applications, has largely been undervalued and understudied. Although nickel is now accepted as essential for the growth of all higher plants, some species may be particularly susceptible to deficiency due to their metabolic pathways. Wood et al. (2006) proposed many important woody plant genera may be particularly susceptible to MED because they are ureide transporters, including: *Acer* (L.), *Alnus* (Mill.), *Annona* (L.), *Betula* (L.), *Carpinus* (L.), *Carya* (Nutt.), *Cercis* (L.), *Chamaecyparis* (Spach), *Cornus* (L.), *Corylus* (L.), *Diospyros* (L.), *Juglans* (L.), *Nothofagus* (Blume), *Ostrya* (Scop.), *Platanus* (L.), *Populus* (L.), *Pterocarya* (Kunth), *Salix* (L.) and *Vitis* (L.). In addition, *Coffea* (L.), *Prunus* (L.), *Pyracantha* (M.Roem.) and *Rosa* (L.) have also been suspected of exhibiting symptoms of MED in horticultural settings (Wood 2015). Many of these genera have yet to be formally studied for their susceptibility to MED, however, recent research has initiated this process with *Betula* (Miller 2021), *Carya* (Miller & Bassuk 2022b), and *Corylus* (unpublished data). It is becoming increasingly important to be able to recognize the symptoms and understand the conditions that make specific taxa more susceptible to MED than others (Wood 2015).

The availability of nickel for uptake by plants is thought to be affected by soil or substrate pH, type, water availability, and temperature (Wood 2015). High pH is seen as a contributor to the occurrence of MED, with soil pH greater than 6.5 causing a decrease in plant available nickel (Brown et al. 1989). MED is most commonly observed in container-grown crops utilizing artificial potting substrates (Ruter 2005). Similar to the signs of iron (Fe) and sulfur (S) deficiency, an early symptom of MED observed in some crops like pecan is chlorosis, however, over time the leaves become dark green, presumably as the leaf surface area reduces (Wood et al. 2004a, 2006). As MED increases in severity, leaflet tips will become more round in shape with blunt edges and curl, thus resembling a "mouse's ear" (Figure 14). The leaf margins darken due to necrosis, which progresses to rosetting (Figure 15) and stem dieback (Figure 16). Nickel supplementation increases urease activity and thus is expected to reduce the accumulation of urea/and or organic acids to toxicity levels in the leaves, which leads to cell death or is observed as marginal necrosis of leaflets (Siqueira Freitas et al. 2018; Wood, 2015).

Nickel competes with the same uptake pathway in plants as other heavy metals such as zinc, copper, and iron (The Fertilizer Institute [date unknown]). The use of copper fungicides in pecan orchards, which are used to control pecan scab, compete with nickel for uptake and have the potential to disrupt physiological processes associated with nickel (Wood et al. 2004a). Due to this heavy metal fungicide use, uptake competition between the copper and nickel cations can exacerbate deficiency of these essential nutrients to the pecan plants in these orchards causing a transplant issue (Wood et al. 2004b, 2006). Transplants are unlikely to survive for more than a few years, which is exacerbated by replanting into areas that were previously inhabited by a previous pecan tree (Wood et al. 2006; Wood 2015).

Because American persimmon has potential for broader application in horticulture and is likely suited to production in containers, we examined the conditions under which MED occurs with container grown plants of three species of horticultural importance and investigated the cause of these symptoms. The objectives of the study were to 1) characterize the purported symptoms on non-treated American (*D. virginiana*), Japanese (*D. kaki*), and dateplum (*D. lotus*) persimmon, 2) determine if foliar application of supplemental nickel can correct these symptoms, and 3) create a comprehensive protocol for foliar application of supplemental nickel that could be utilized in the green industry.

Materials and Methods

Nickel and Urea Supplementation Experiment

Seeds of *Diospyros virginiana*, *D. kaki*, and *D. lotus* (Table 10) were cold moist stratified for 90 days in moistened, long-fiber sphagnum moss in a cooler maintained at 40°C. After stratification, seeds were sown just below the substrate surface in flats (25.72 cm × 25.72 cm × 6.03 cm) containing Pro-Mix BRX soilless media (Premier Tech, Quebec, Canada) and subsequently moved to a glass greenhouse (located at 44° 59' 15" N, 93° 11' 0" W) where germination began on 21 June 2022. Following germination and initial growth, plants entered a quiescent state and were moved to a cooler (40°C) to acquire chilling hours for their first dormancy period from 27 October 2022 to 06 June 2023. After dormancy, plants were potted singly (06 June 2023 – 20 June 2023) into black plastic #1 nursery containers (1 gallon) pots (17 cm × 15.5 cm) filled with Pro Mix BRX substrate. Containers were top dressed with 10 grams of slow release fertilizer that did not contain urea or any nickel component applied with a foliar spray in this study (Osmocote 14-14-14; ICL Specialty Fertilizers, Dublin, OH) at the time of potting-on as well as each subsequent spring. Plants were moved into a glass greenhouse in Saint Paul, MN with an average temperature of 22.6°C (Min: 10.1°C, Max: 41.7°C) as monitored by ARGUS Titan 900 9.0.15.0 (Argus Controls®), with plants watered once daily. Persimmon plants were once again overwintered beginning on 31 October 2023, in the aforementioned cooler for 92 days. After chilling requirements were met, persimmons were again moved into a greenhouse (31 January 2024) to begin deacclimating. Plants were watered once daily throughout the experiment. Every 15 minutes, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT) recorded the average Photosynthetically Active Radiation (PAR) of 302.611 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 2002.742 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 22.39°C, with minimum and maximum values of 18.37°C and 30.9°C, respectively. Relative humidity averaged 41.19%, ranging from 11.1% to 82%, with a dew point of 8.5°C, varying between 0.03°C and 19.65°C, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation, Bourne, MA).

Uniform budbreak of *D. kaki* and *D. lotus* occurred on 26 February 2024, and of *D. virginiana* on 04 March 2024. Two weeks post-budbreak, *D. kaki*, *D. lotus*, and *D. virginiana* plants were treated with either a (1) non-treated control (H_2O spray), or foliar sprays on all leaf surfaces until beading of (2) Nickel Plus® (169 mg/L), (3) $NiCl_2$ (169 mg/L), (4) urea (150 mg/L N), and (5) combined $NiCl_2$ (169 mg/L) and urea (150 mg/L N). Nickel Plus® (5-0-0) is derived from urea and nickel lignosulfonate (5% N, 3% S, 5.4% Ni) (NIPAN LLC, Valdosta, GA).

Each treatment group for *D. kaki* and *D. lotus* comprised 20 or 30 seedlings, respectively. *Diospyros virginiana* treatment groups each comprised 25 seedlings, with the exception of the non-treated control (n=30). Seedlings were assigned a treatment at random with near-equal distribution across cultivars, provenances, or genotypes within each taxon. At the time of foliar treatment, groups were physically separated from each other in separate greenhouse bays to avoid drift. After leaves dried following the foliar treatments, plants were returned to one greenhouse and arranged in a completely randomized design spread across four greenhouse benches with equal spacing.

Final data were collected 35 days post-treatment beginning on 03 April 2024 (*D. lotus* and *D. kaki*) and 10 April 2024 (*D. virginiana*). First, an overall observational symptom rating of each plant was collected in the greenhouse, using a rating scale of 1 to 5 which reflected the approximate percent of new growth displaying MED symptoms: 1 = ~100%, 2 = ~75%, 3 = 50%, 4 = ~25%, 5 = no visible symptoms present on new leaves post-budbreak) (Figure 11, Figure 12, Figure 13). Chlorophyll content index (CCI) was collected in the greenhouse using a chlorophyll concentration meter with standard calibration (Apogee MC-100 Chlorophyll Concentration Meter; Apogee Instruments®, Inc., Logan, UT) by measuring three separate leaves per plant. Subsequently, one representative stem (growth from the current season) indicative of the overall symptom level for each plant was harvested by removal using a bypass hand pruner at the site of the budscar, adjacent to the main stem. Collected stems were moved to the lab and evaluated to measure: shoot elongation (cm) measured from where buds broke after dormancy to their most apical position of the newly extended shoot, number of leaves per unit shoot extension (calculated as the total number of leaves present on the harvested stem), leaf surface area (cm^2), CCI, leaf dry mass (g), shoot dry mass (g), specific leaf area (cm^2/g). Leaves were individually separated from the stems and analyzed using a LI-COR 3100 Leaf Area Meter (LI-COR Biosciences Inc., Lincoln, NE) to obtain leaf surface area in cm^2. Leaves and stems were placed in paper bags and set in a dryer maintained at 50°C. After 72 hours in the dryer, shoot and leaf dry weights were collected on 11 April 2024, 13 April 2024, and 14 April 2024. These data were used to calculate specific leaf area, determined as the leaf area (cm^2) divided by the leaf dry weight (g).

pH and electrical conductivity (EC)

Leachate was collected from nine representative samples from each species group to measure substrate pH and EC (μS) of the substrate using the pour-thru technique (Mattson [date unknown]). To collect leachate, a plastic basin was placed underneath each container and 150 mL (based on a container diameter of 17 cm) of DI water was subsequently poured evenly across the top of the substrate within the pot. From each basin, 50 mL of leachate was collected and measured using a handheld Apera® meter (Apera® PC60 pH/ Cond./TDS/Sal. Tester; Apera® Instruments, Columbus, Ohio).

Statistical analysis

Data were analyzed using a two-way ANOVA (Treatment × Species) in R statistical software (Ver. 4.2.2). Data analyzed met the assumptions of the ANOVA model. When an interaction effect was not detected (shoot elongation, shoot dry mass, CCI, leaf count, specific leaf area), the interaction term was removed from the model and the analysis was rerun to evaluate the main effects of Treatment and Species, separately. Post-hoc mean comparisons were made using Tukey's Honestly Significant Difference Test. Packages in R included *dplyr*, *ggplot2*, *ggpubr*, *agricolae*, and *plotrix*.

Results and Discussion

pH and electrical conductivity (EC)

The overall growing substrate pH averaged over all plants in this study was 6.37. High pH has been associated with the presence of MED symptoms (Ruter 2005). This is because the pH of a substrate can affect nutrient uptake, especially of micronutrients (Torres et al. 2010). Most nutrients for plant growth are readily available at a pH range of 5.4-6.2 in soilless substrates, however, each nutrient has its own optimal pH range (Torres et al. 2010). Nickel is available to the plant at a soil pH that is less than 6.5 (Brown et al. 1989). The overall pH averaged in this study is just outside of the 5.4-6.2 range, however, it is below the 6.5 threshold for nickel. The electrical conductivity (EC) averaged over all plants in his study was 931.141 µS. For the majority of nursery crops, the EC values of the leachate should range from 500 to 2000 µS/cm during periods of active growth (LeBude & Bilderback, 2009). The overall EC value for this study is within this suggested range.

Nickel and Urea Supplementation

An interaction was observed for MED severity rating (P = 0.00639), leaf surface area (cm^2) (P = 0.00102), and leaf dry mass (g) (P = 0.001502) (Table 7). For *D. virginiana*, the three nickel treatments improved the parameter studied compared to the non-treated control and urea treatments for MED severity rating, leaf surface area (cm^2), and leaf dry mass (g), though the three nickel treatments were not different from each other. *D. lotus* had higher ratings overall, showing the least amount of MED symptoms, when left untreated compared to *D. kaki* and *D. virginiana*, which were not different from each other (Figure 17). There was a trend that supplemental nickel improved MED severity rating compared to the non-treated control and urea treatments for all three species (Figure 17). All treatments, including the non-treated control, did not influence mean leaf surface area (cm^2) for *D. kaki* or *D. lotus* (Figure 18). There was a trend that nickel improved leaf surface area (cm^2) compared to the non-treated control and urea treatments for *D. virginiana* (Figure 18). All treatments, including the non-treated control, did not influence mean leaf dry mass (g) for *D. kaki* or *D. lotus* (Figure 19). There was a trend that nickel improved leaf dry mass (g) compared to the non-treated control and urea treatments for *D. virginiana* (Figure 19).

Of the remaining responses for which an interaction was not observed, the main effect of species affected CCI (P = <2e-16), leaf count (P = 1.87e-12), specific leaf area (cm^2/g) (P = 0.000515), shoot elongation (P = <2e-16), and shoot dry mass (P = 2e-07) (Table 9). Data for these responses were pooled across treatments to demonstrate species differences (Table 9). The main effect of treatment affected specific leaf area (cm^2/g) (P = 1.25e-06), shoot elongation (P = 0.0281), and shoot dry mass (P = 0.0289); these data were pooled across species to demonstrate treatment differences (Table 8).

For shoot elongation, $NiCl_2$ + urea treatment was the only treatment group that was different from the non-treated controls (Table 8). Compared to the non-treated controls, shoot elongation increased by 13.87%, 18.98%, 15.33%, and 21.17% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively. In river birch (*Betula nigra),* Ruter (2005) reported a 53 to 60% increase in shoot elongation for plants treated with nickel sulfate compared to the non-treated controls. In bitternut hickory (*Carya cordiformis*), Miller and Bassuk (2022b) observed an 83.5% increase in shoot elongation for plants treated with a foliar application of Nickel Plus® compared to the non-treated controls. Headley and Miller (In Prep) observed a 35.8% increase in shoot elongation for American and interspecific hybrid hazelnuts. The percent increase for shoot elongation between non-treated controls and differing nickel treatments in this *Diospyros* study is less pronounced compared to river birch and bitternut hickory, suggesting that shoot elongation in persimmon is less affected by supplemental nickel treatment compared to other ureide transporting species. Species also impacted shoot elongation (cm) (Table 7). Nickel application is expected to increase shoot elongation in symptomatic plants. Reduced shoot elongation can be seen in a non-treated control plant showing severe MED symptoms (Figure 16).

Compared to the non-treated controls, shoot dry mass increased by 17.62%, 37.82%, 20.73%, and 34.72% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively. $NiCl_2$ treatment increased shoot dry mass compared to the non-treated control (Table 8). Other nickel treatments and the urea treatment were not different from each other or the non-treated control. *D. lotus* and *D. virginiana* exhibited improved shoot dry mass compared to *D. kaki*.

Compared to the non-treated controls, specific leaf area (cm^2/g) increased by 23.42%, 7.37%, 2.37%, and 10.75% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively. Nickel Plus® was different from all treatments for this parameter including the non-treated control (Table 8). Nickel product formulation may have an effect on how much leaf area a plant gains in regard to the amount of leaf biomass. Species also had a significant effect on specific leaf area (Table 7). *D. lotus* and *D. virginiana* had improved specific leaf area (cm^2/g) compared to *D. kaki*.

Different forms of nickel have been utilized for crops studied in regard to MED. Bitternut hickory (*Carya cordiformis*) was supplemented with nickel in the form of nickel lignosulfonate (Nickel Plus®) (Miller & Bassuk 2022b), river birch was supplemented with nickel in the form of nickel sulfate (Ruter 2005), and hazelnut was supplemented with $NiCl_2$ and nickel lignosulfonate (Nickel Plus®) (unpublished data). In production, foliar sprays are used preferentially compared to soil drench methods. Miller and Bassuk (2022b) used foliar application of supplemental nickel for the correction of MED in bitternut hickory. Miller and Bassuk (2022b) reported a 126.9% increase in leaf area (cm^2) with bitternut hickories treated with a foliar spray of Nickel Plus® (0.32 fl oz per gal). Persimmons treated with Nickel Plus® showed a 55.72% increase in leaf area (cm^2)

compared to non-treated controls. Leaf area of river birch (*Betula nigra*) increased 80 to 83% for plants with nickel sulfate applied using a foliar spray or via a soil drench (Ruter 2005).

The use of Nickel Plus®, a urea and nickel sulfonate, is one of the only nickel fertilizers on the market for woody plants. Nickel Plus® is not the only foliar applied supplemental nickel that has been studied. Nickel lignosulfonate, an organic nickel ligand, was effective as a nickel foliar fertilizer (Wood, 2015). Nickel salts and nickel ligands have also been studied for their effectiveness to correct MED (Wood et al. 2004a, 2004b). Nickel lignosulfonate fertilizers, such as Nickel Plus®, have been deemed safer for applicators compared to other nickel fertilizers that are derived from nickel-nitrates and nickel-sulfates (Wood 2015).

In this study we also utilized $NiCl_2$, a nickel salt, for supplemental nickel, which has not been studied for correction of MED in persimmon. MED symptoms of pecan have been ameliorated by foliar applications of nickel salts (Wood et al. 2006). Urea is utilized in this study for several reasons. Urea is a component of Nickel Plus® and it supplies urea to persimmon which is a ureide-transporting plant (Wood et al. 2006). Urea is also utilized worldwide as a major source of nitrogen for crops (Wood 2015). It was suspected that urea applied without nickel would be more detrimental than the untreated control; however, this was not observed. Understanding urea metabolism in ureide-transporting crops will help producers optimize plant growth with these crops (Wood 2015).

All three species in this study, *Diospyros virginiana*, *D. kaki*, and *D. lotus* exhibited MED symptoms; however, the manifestation of MED symptoms varied among the species (Figure 17, 18, 19). Dateplum persimmon (*D. lotus*) showed less MED symptoms compared to *D. virginiana* and *D. kaki* in this randomized greenhouse study (Figure 17, 18, 19). Foliar applications of supplemental nickel in this study were effective at correcting symptoms of MED in American persimmon (*Diospyros virginiana*), Japanese persimmon (*D. kaki*), and dateplum persimmon (*D. lotus*). Plants readily absorb nickel from soil, and this absorption is directly linked to the concentration of nickel in the soil (Ruter 2005). *D. kaki* and *D. lotus* are grown more extensively in horticulture compared to *D. virginiana*, and also appear to be less susceptible to MED. Japanese persimmon is also frequently grafted onto dateplum persimmon (Liu et al. 2023). Based on these observations, future research may investigate if grafting Japanese persimmon on dateplum persimmon impacts how or if MED symptoms occur in the production of persimmon in horticulture.

There is very little literature regarding mouse ear disorder in woody specialty crops, including some taxa with significant economic value. One study of a fruit-bearing woody plant that is improved by the supplementation of nickel is pomegranate (*Punica granatum*) (Dichala et al. 2018). Other than traditional foliar symptoms of MED, they found that foliar application of nickel nitrate has the potential to reduce pomegranate fruit cracking leading to crop loss (Dichala et al. 2018). Another woody perennial crop susceptible to MED is citrus (Wood 2006). Foliar blunting in citrus was associated with an accidental double foliar application of urea during shoot expansion in orchard soils containing high Ca and Mg (Wood 2006). To introduce a specialty crop species into the green industry, new techniques should be utilized to enhance production and availability of the taxon (Hilbert et al. 2023). The introduction of a new species bolsters species diversity in managed landscapes, which can increase resilience of the landscape and lessen the effects of abiotic and biotic stressors (Miller 2024).

Persimmons are not only grown for fruit but are also multifaceted with exceptional features for use in managed landscapes. In the landscape, persimmon trees can be utilized as specimen trees (Chamberlain, 2020). This is seen in both Tokyo, Japan with *D. kaki* (Figure 10) and at the Morton Arboretum (Lisle, IL) with *D. virginiana* (Figure 9). Woody landscape plants, such as persimmon, can be a novelty when introduced as a component of edible landscapes (Josiah & Lackey, 2014). As a woody edible landscape plant, persimmon can be utilized as a local fruit source, but also as an educational tool to connect community members with where their food comes from (Josiah & Lackey, 2014).

While the majority of members within the *Diospyros* genus are tropical or sub-tropical, such as *D. kaki* and *D. lotus* in this study, the American persimmon (*D. virginiana*) offers growers in northern climates an avenue to establishing a fruit industry in the Upper Midwest. The ornamental appeal and resilience in the landscape makes American persimmon a great candidate for landscape application, however, production limitations, such as MED, have slowed its introduction to the horticultural market. This research supports the establishment of this fruit market with American persimmon by developing a protocol to overcome production limitations regarding MED and nickel nutrition.

Increased presence of American persimmon, not only in edible landscapes, but also in urban landscapes, can increase species diversity in both sectors.

This study reinforces those of pecan (*Carya* spp.) (Wood et al. 2004a, 2004b, 2004c), river birch (*Betula* spp.) (Ruter 2005), and bitternut hickory (*Carya* spp.) (Miller & Bassuk 2022b) and the report of Wood et al. (2006) which states that ureide transporting genera are candidates for mouse ear disorder. As demonstrated in previous studies, supplemental nickel was found to have a significant effect on both leaf and growth characteristics of persimmon in this randomized greenhouse study. Treatment and species also interact to affect leaf characteristics and how we rate MED symptoms of each individual plant in the study. Overall, supplementing nickel for each of these species could improve leaf and growth metrics, but application should reflect species differences and grower conditions to optimize growth on a case-by-case basis.

FIGURES

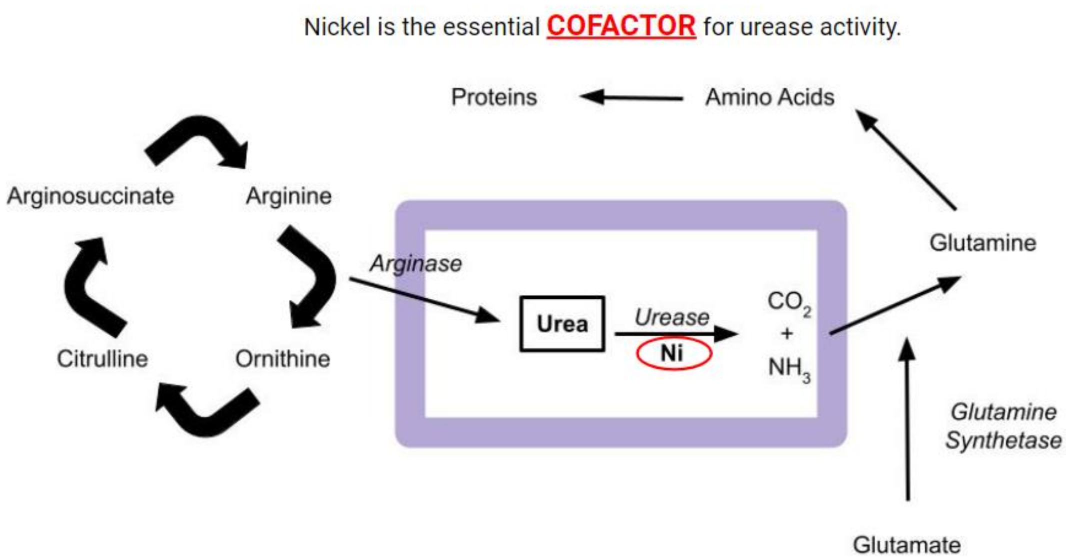

Figure modified from http://www.nickelplus.biz

Figure 1: Nickel is the cofactor for the urease pathway in ureide-transporting plants. Nickel deficiency reduces the activity of the urease enzyme, interrupting normal nitrogen cycling via disruption of ureide metabolism.

Figure 2: Semi-hardwood stem cuttings treated with (left to right): Control (95% ETOH only), 1000 ppm IBA, 2000 ppm IBA, and 3000 ppm IBA.

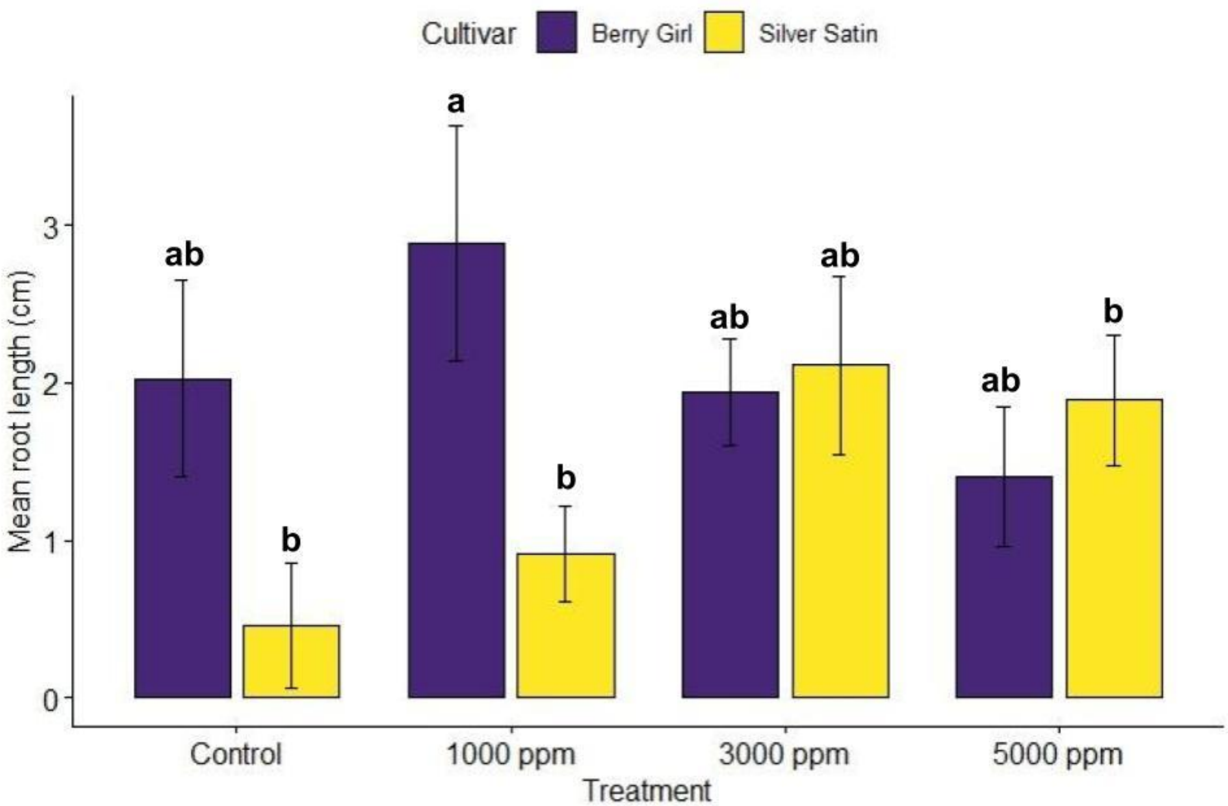

Figure 3: Mean root length (cm) (± standard error) of softwood stem cuttings of Forestiera pubescens 'Berry Girl' and F. pubescens 'Silver Satin' 35 days after treatment with non-treated control (95% ETOH only), 1000 ppm IBA, 3000 ppm IBA, and 5000 ppm IBA (95% ETOH solution) via a 3-sec quick dip. Roots were counted if there were ≥ 0.25 cm. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

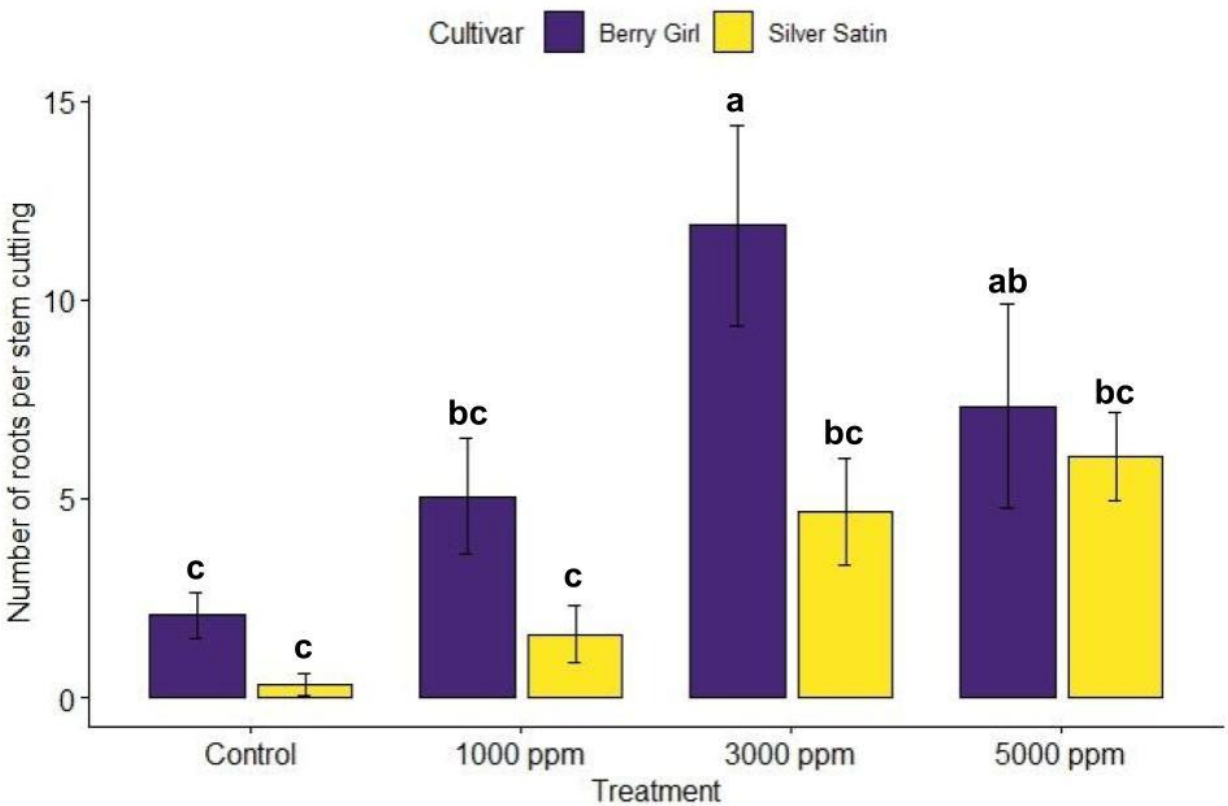

Figure 4: Mean number of roots per stem cutting (± standard error) of softwood stem cuttings of Forestiera pubescens 'Berry Girl' and F. pubescens 'Silver Satin' 35 days after treatment with non-treated control (95% ETOH only), 1000 ppm IBA, 3000 ppm IBA, and 5000 ppm IBA (95% ETOH solution) via a 3-sec quick dip. Roots were counted if there were ≥ 0.25 cm. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

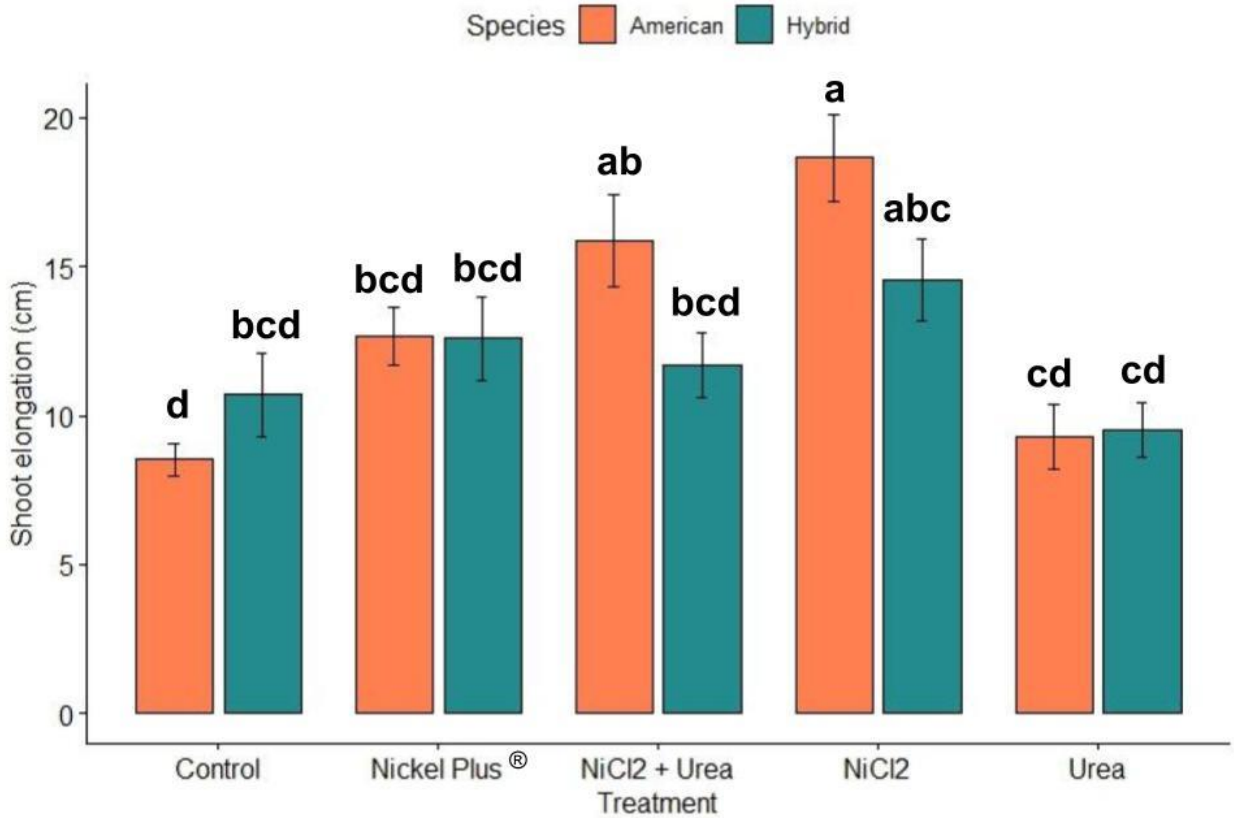

Figure 5: Mean shoot elongation (cm) (± standard error) in leaf tissue of one-year old American hazelnut (Corylus americana) and interspecific hybrid (Corylus spp.) seedlings left untreated (control) or treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

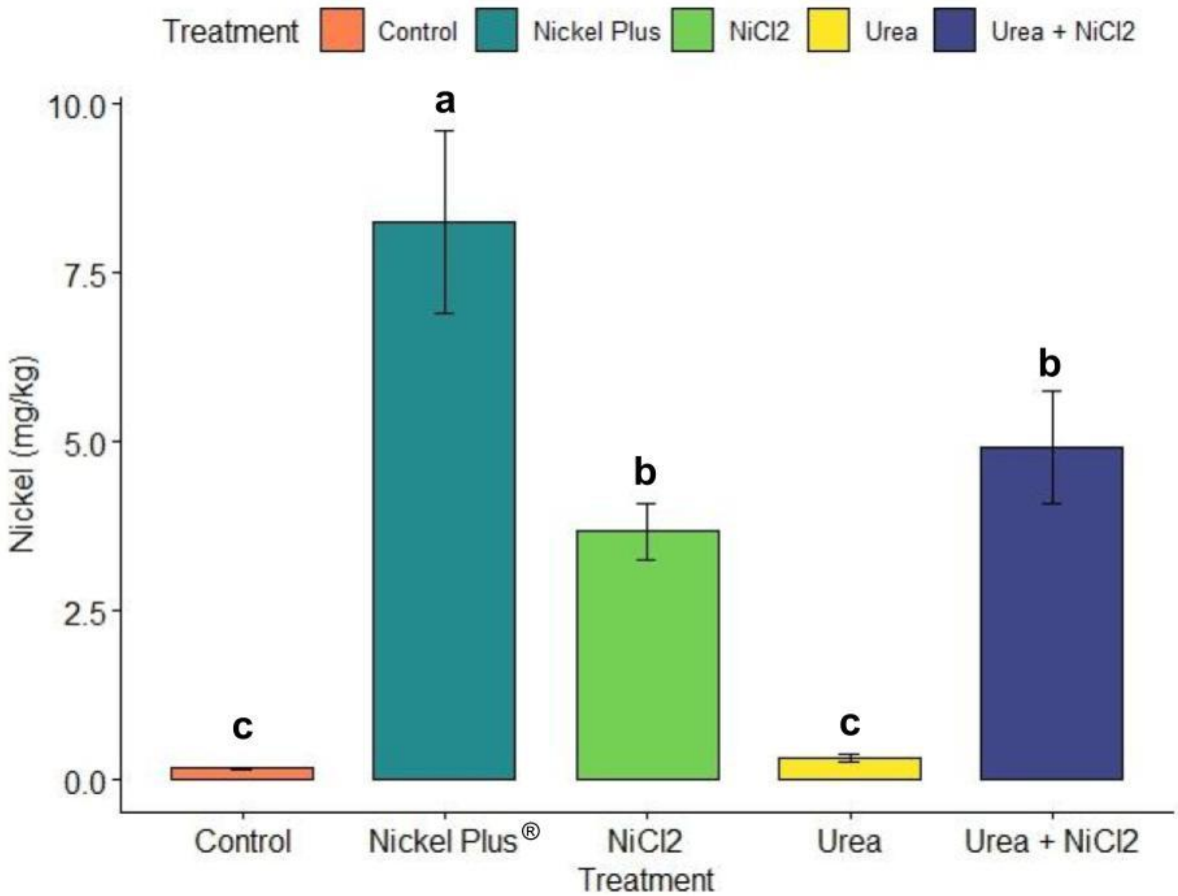

Figure 6: Mean nickel content (± standard error) in leaf tissue of one-year old American hazelnut (Corylus americana) and interspecific hybrid (Corylus spp.) seedlings left untreated (control) or treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Figure 7: American hazelnut (Corylus americana) plants demonstrating symptoms of MED including curling/cupping of leaflets, wrinkled leaflets, chlorosis of leaflets which later become dark green in color, and then exhibit necrosis of leaf margins.

Figure 8: Rating scale depicting the severity of plant symptoms. The scale ranges from 1 to 5, with the following interpretations: bottom left (1) signifies approximately 100% of the plant affected, bottom middle (2) represents roughly 75% of the plant affected, bottom right (3) corresponds to 50% affected, top left (4) indicates 25% affected, and top right (5) signifies no visible symptoms present.

Figure 9: Mature specimen tree (D. virginiana) in an ornamental landscape at the Morton Arboretum (Lisle, IL).

Figure 10: D. kaki (Japanese persimmon) planted as a specimen tree on a street corner in Tokyo, Japan (right). Fruit production as well as a potential nutritional deficiency (leaf cupping, marginal necrosis) can be observed (left).

Figure 11: D. lotus (dateplum persimmon) rating scale depicting the severity of plant symptoms. The scale ranges from 1 to 5, with the following interpretations: bottom left (1) signifies approximately 100% of the plant affected, bottom middle (2) represents roughly 75% of the plant affected, bottom right (3) corresponds to 50% affected, top left (4) indicates 25% affected, and top right (5) signifies little to no visible symptoms present.

Figure 12: D. kaki (Japanese persimmon) rating scale depicting the severity of plant symptoms. The scale ranges from 1 to 5, with the following interpretations: bottom left (1) signifies approximately 100% of the plant affected, bottom middle (2) represents roughly 75% of the plant affected, bottom right (3) corresponds to 50% affected, upper left (4) indicates 25% affected, and upper right (5) signifies little to no visible symptoms present.

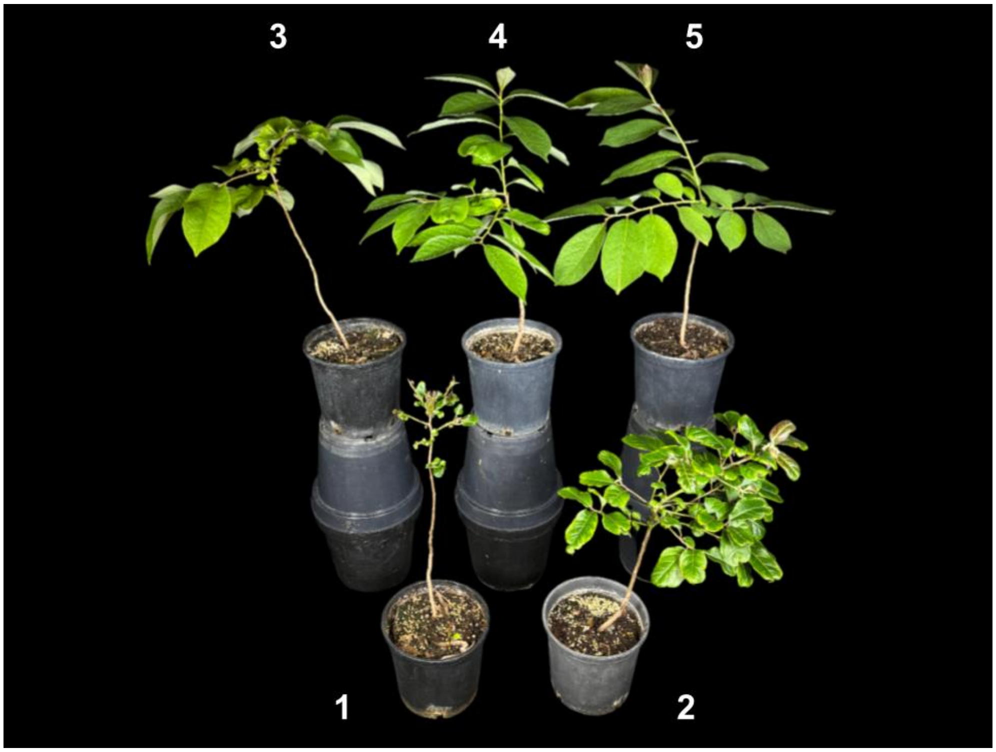

Figure 13: D. virginiana (American persimmon) rating scale depicting the severity of plant symptoms. The scale ranges from 1 to 5, with the following interpretations: bottom left (1) signifies approximately 100% of the plant affected, bottom middle (2) represents roughly 75% of the plant affected, bottom right (3) corresponds to 50% affected, upper left (4) indicates 25% affected, and upper right (5) signifies little to no visible symptoms present.

Figure 14: American persimmon (D. virginiana) exhibiting symptoms of MED such as leaflet tips becoming more round in shape with blunt edges and curling, thus resembling a "mouse's ear".

Figure 15: D. virginiana plant exhibiting symptoms of MED such as stunted leaves and leaf curling.

Figure 16: D. virginiana plant exhibiting symptoms of MED such as interveinal chlorosis, darkening of leaf margins due to necrosis, progressing to shortened internodes, rosetting, and stem dieback.

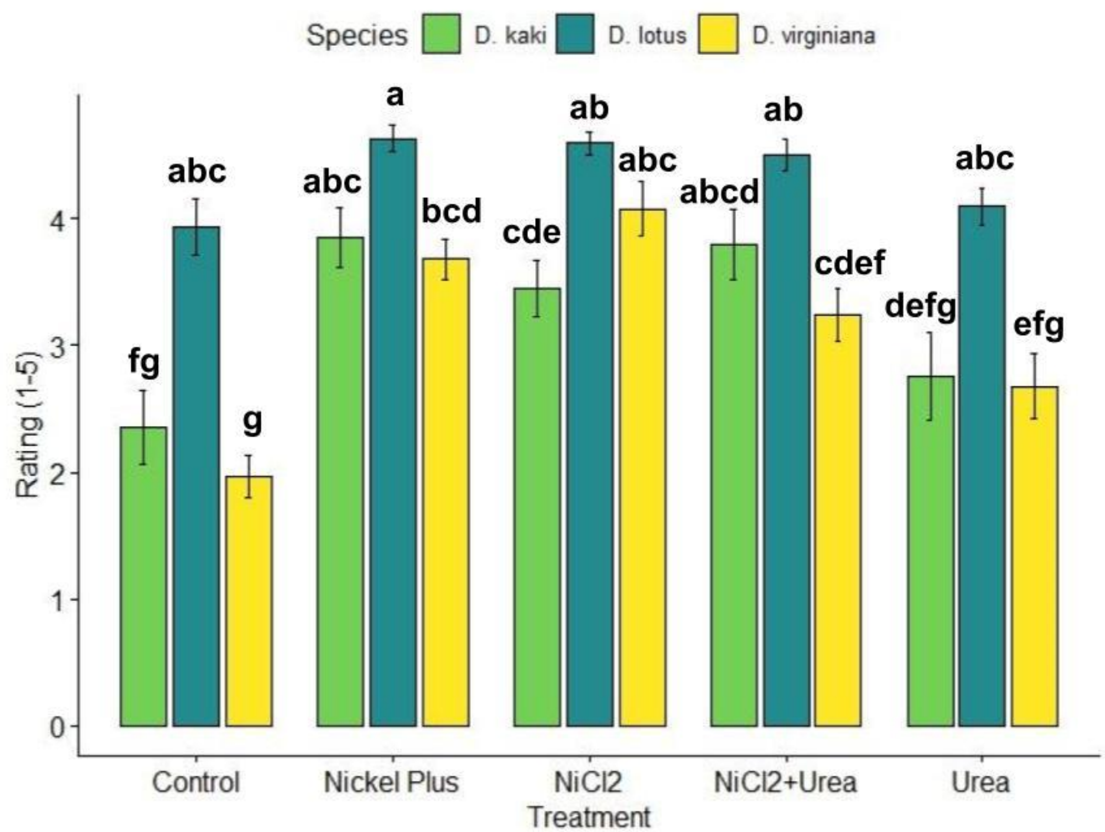

Figure 17: Mean overall MED severity rating scale (1 to 5; (1) signifies approximately 100% of the plant affected, (2) represents roughly 75% of the plant affected, (3) corresponds to 50% affected, (4) indicates 25% affected, (5) signifies little to no visible symptoms present) (± standard error) of leaf tissue of three species of one-year old Diospyros seedlings left untreated (control) or treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

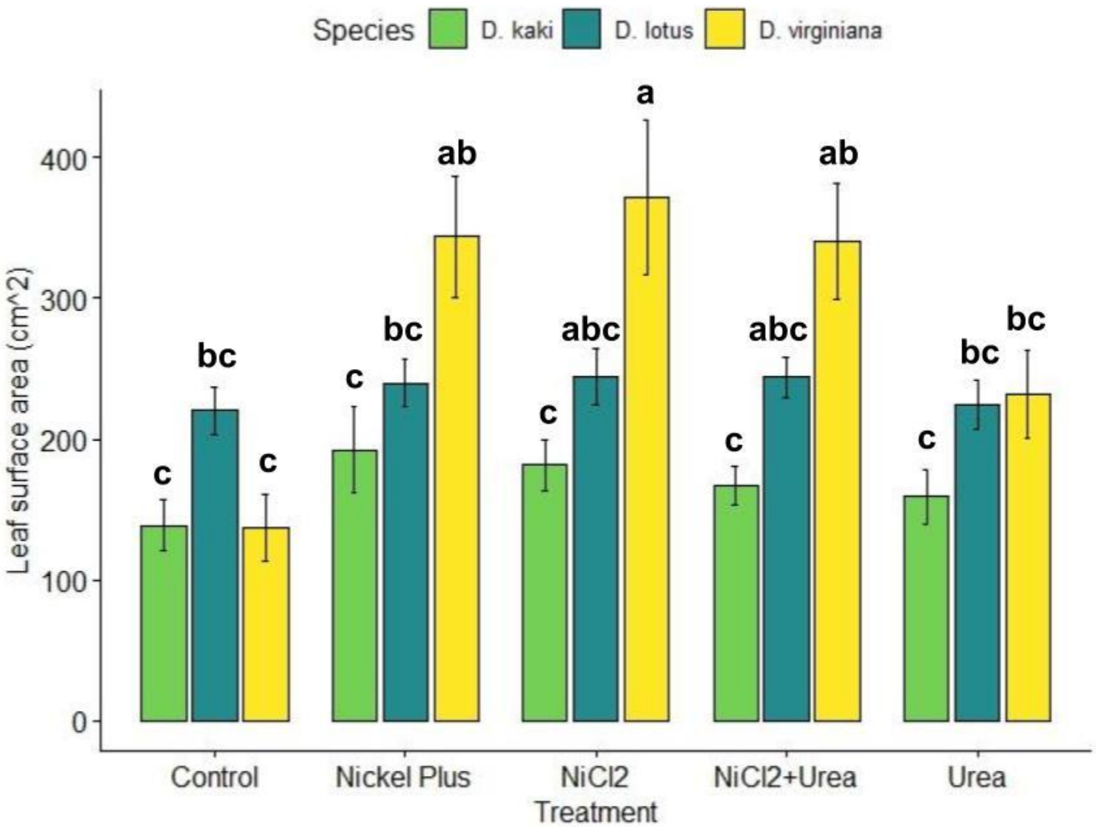

Figure 18: Mean leaf surface area (cm^2) (± standard error) of leaf tissue of three species of one-year old Diospyros seedlings treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

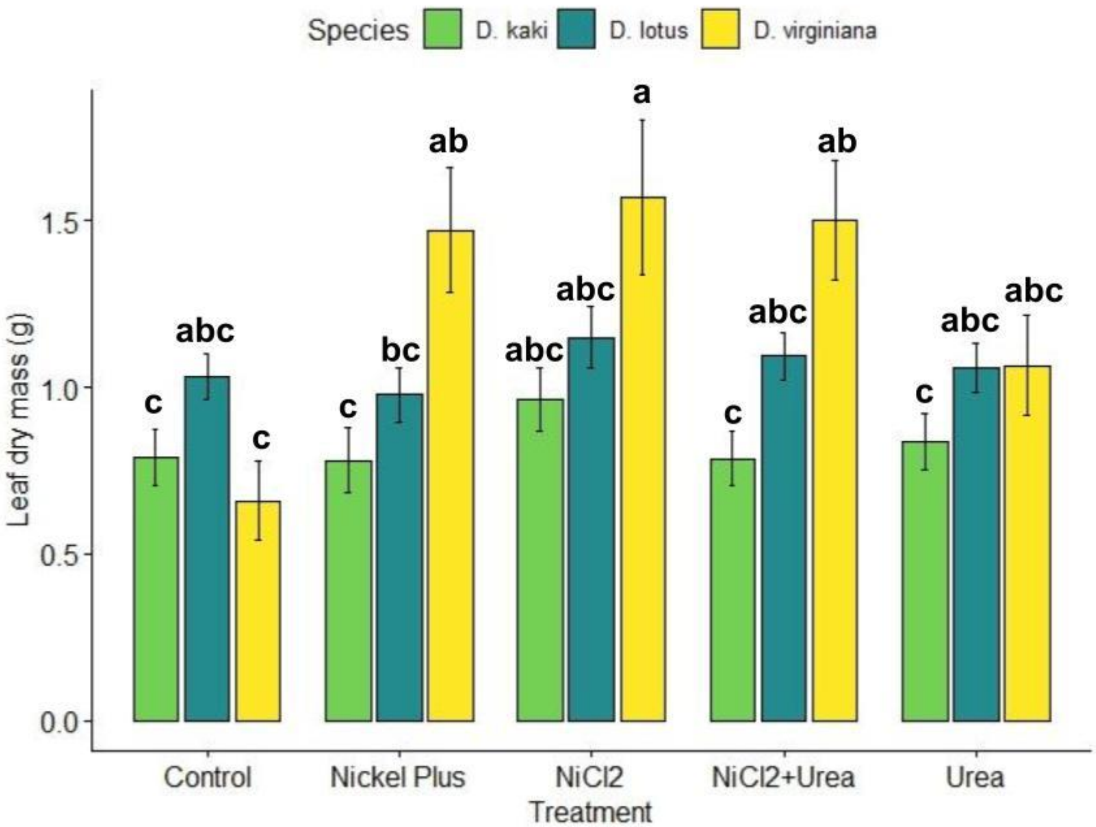

Figure 19: Mean leaf dry mass (g) (± standard error) of leaf tissue of three species of one-year old Diospyros seedlings treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

TABLES

Table 1. Rooting responses (number of roots per stem cutting and root length) of semi-hardwood stem cuttings of Forestiera pubescens 35 days after treatment with non-treated control (95% ETOH only), 1000 ppm IBA, 2000 ppm IBA, and 3000 ppm IBA (95% ETOH solution) via a 3-sec quick dip. Roots were counted if they were ≥ 0.25 cm.

Treatment	Number of roots per stem	Mean root length (cm)
Control	0.70±0.21 b[z]	0.16±0.04 c
1000 ppm	5.39±0.66 a	0.69±0.07 b
2000 ppm	6.17±0.65 a	0.91±0.08 ab
3000 ppm	6.60±0.51 a	1.15±0.09 a

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 2. Rooting responses (number of roots per stem cutting and root length) of semi-hardwood stem cuttings of Forestiera pubescens 40 days after substrate treatments of 100% peat, 100% perlite, 50/50 peat/perlite. Roots were counted if there were ≥ 0.25 cm.

Treatment	Number of roots per stem	Mean root length (cm)
100% peat	0.00±0.00	0.00±0.00 c[z]
100% perlite	8.38±0.75	4.83±0.34 a
50/50 peat/perlite	2.73±0.61	0.25±0.05 b

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 3. Rooting responses (number of roots per stem cutting and root length) of softwood stem cuttings of Forestiera pubescens 35 days after treatment with non-treated control (95% ETOH only), 1000 ppm IBA, 3000 ppm IBA, and 5000 ppm IBA (95% ETOH solution) via a 3-sec quick dip. Roots were counted if there were ≥ 0.25 cm.

Treatment	Number of roots per stem	Mean root length (cm)
Control	1.04±0.22	0.75±0.17 a[z]
1000 ppm	2.97±0.47	1.38±0.17 a
3000 ppm	6.81±0.93	2.53±0.24 ab
5000 ppm	9.61±0.83	2.36±0.18 b

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 4: Substrate analysis of pH and electrical conductivity (EC) of one-year old American hazelnut (Corylus americana) and interspecific hybrid (Corylus spp.) seedlings utilizing the pour-thru procedure.

Treatment	pH	EC (µS/cm)
Control	6.2	697
Nickel Plus®	6.0	789
$NiCl_2$	6.0	764
Urea	6.3	591
$NiCl_2$ + Urea	6.1	862

Table 5: Plant growth responses of one-year old American hazelnut (Corylus americana) and interspecific hybrid (Corylus spp.) seedlings cultivated in a soilless substrate 45 days after foliar treatment with H_2O (Control), Nickel Plus®, $NiCl_2$, urea, or $NiCl_2$ + urea.

Treatment	Rating (1-5)	Number of Leaves	Leaf Greenness	Leaf Area (cm^2)	Leaf Dry Mass (g)	Shoot Dry Mass (g)	Specific Leaf Area (cm^2/g)
Control	1.84±0.17 b[z]	7.04±0.31 b	48.5±2.77 a	42.2±5.97 b	0.41±0.04 c	0.08±0.01 d	99.5±7.11 c
Nickel Plus®	4.12±0.17 a	8.50±0.51 ab	30.0±1.45 b	178.0±17.7 a	1.04±0.11 ab	0.21±0.02 bc	174.0±5.67 b
$NiCl_2$	4.46±0.17 a	9.04±0.48 a	32.9±1.75 b	228.0±21.4 a	1.37±0.15 a	0.33±0.04 a	170.0±7.16 b
Urea	2.09±0.21 b	7.74±0.65 ab	33.2±2.31 b	49.9±6.68 b	0.45±0.06 c	0.10±0.01 cd	109.0±8.95 c
$NiCl_2$ + Urea	4.54±0.12 a	8.62±0.55 ab	31.4±1.63 b	195.0±16.3 a	0.96±0.07 b	0.25±0.04 ab	203.0±7.61 a

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 6: Leaf tissue nickel concentration (mg/kg) of one-year old American hazelnut (Corylus americana) and interspecific hybrid (Corylus spp.) seedlings.

Treatment	Nickel (g/kg)
Control	0.17 ± 0.02 c[z]
Nickel Plus®	8.25 ± 1.36 a
$NiCl_2$	3.67 ± 0.41 b
Urea	0.32 ± 0.05 c
$NiCl_2$ + Urea	4.93 ± 0.83 b

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 7: Significance of species and treatment main effects on the MED severity rating scale, leaf characterization metrics and stem trait metrics (elongation and dry mass) on seedlings of American persimmon (D. virginiana), Japanese persimmon (Diospyros kaki), and dateplum persimmon (D. lotus) after foliar treatment with H_2O (control), $NiCl_2$, urea, $NiCl_2$ + urea, and Nickel Plus®.

Response	Effects [z]	DF	F ratio	*P*-value [y]
Rating	Treatment	4	26.139	< 2e-16
	Species	2	62.636	< 2e-16
	Treatment x Species	8	2.719	0.00639
Shoot elongation	Treatment	4	2.748	0.0281
	Species	2	55.407	<2e-16
Leaf count	Species	2	29.03	1.87e-12
Leaf surface area	Treatment	4	7.534	7.68e-06
	Species	2	18.817	1.66e-08
	Treatment x Species	8	3.351	0.00102
Chlorophyll content index (CCI)	Species	2	44.57	< 2e-16
Leaf dry mass	Treatment	4	4.975	0.000647
	Species	2	12.270	6.96e-06
	Treatment x Species	8	3.219	0.001502
Shoot dry mass	Treatment	4	2.732	0.0289
	Species	2	16.078	2e-07
Specific leaf area	Treatment	4	8.573	1.25e-06
	Species	2	7.727	0.000515

[z] Main effects.

[y] Significance at $P \leq 0.05$.

Table 8: Plant growth metrics (mean ± SE) of one-year-old American persimmon (Diospyros virginiana), Japanese persimmon (D. kaki), and dateplum persimmon (D. lotus) seedlings cultivated in a soilless substrate 37 days after foliar treatment with H_2O (control), $NiCl_2$, urea, $NiCl_2$ + urea, and Nickel Plus®. Data were pooled across species to reflect the main effect of treatment.

Treatment	Shoot elongation (cm)	Shoot dry mass	Specific leaf area (cm^2/g)
Control	13.7±0.756 b	0.193±0.016 b	205.35±6.60 b
Nickel Plus®	15.6±0.786 ab	0.227±0.019 ab	253.44±8.37 a
$NiCl_2$	16.3±0.736 ab	0.266±0.021 a	220.49±6.30 b
Urea	15.8±0.799 ab	0.233±0.018 ab	210.22±6.25 b
$NiCl_2$ + Urea	16.6±0.790 a	0.260±0.020 ab	227.43±4.80 b

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 9: Plant growth metrics (mean ± SE) of one-year-old American persimmon (Diospyros virginiana), Japanese persimmon (D. kaki), and dateplum persimmon (D. lotus) seedlings cultivated in a soilless substrate 37 days after foliar treatment with H_2O (control), $NiCl_2$, urea, $NiCl_2$ + urea, and Nickel Plus®. Data were pooled across treatments to reflect the main effect of species.

Species	Shoot elongation (cm)	Leaf count	Chlorophyll content index (CCI)	Shoot dry mass	Specific leaf area (cm^2/g)
D. kaki	10.8±0.488 c	7.9±0.268 c	24.6±0.958 a	0.161±0.012 b	204.93±6.18 b
D. lotus	18.9±0.521 a	9.8±0.242 b	16.5±0.354 b	0.270±0.012 a	226.98±4.56 a
D. virginiana	15.4±0.552 b	11.7±0.445 a	16.2±0.754 b	0.251±0.017 a	232.53±5.18 a

[z] Means with the same letter (within a column) are not significantly different according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

Table 10: Germplasm sources of Diospyros kaki, D. lotus, and D. virginiana acquired as open-pollinated seed and cultivated for this experiment.

Species	Cultivar/Provenance/ Reference Name	Source	Accession Number
Diospyros kaki	'Fennio'	USDA-GRIN	DDIO 216
Diospyros kaki	'Mishirasu'	USDA-GRIN	DDIO 241
Diospyros lotus	'Budogaki'	USDA-GRIN	DDIO 161
Diospyros lotus	880392	USDA-GRIN	DDIO 53
Diospyros lotus	880446	USDA-GRIN	DDIO 56
Diospyros virginiana	Columbia, MO	Stephens Lake Park Arboretum	-
Diospyros virginiana	Baring, MO	George O. White State Forest Nursery	-

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