Supplemental Nickel Corrects Mouse Ear Disorder of American and Interspecific Hybrid Hazelnuts[1]

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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 of (2) Nickel Plus® (169 $mg{\cdot}L^{-1}$ Ni), (3) $NiCl_2$ (169 $mg{\cdot}L^{-1}$ Ni), (4) urea (150 $mg{\cdot}L^{-1}$ N), and (5) combined $NiCl_2$ (169 $mg{\cdot}L^{-1}$ Ni) and urea (150 $mg{\cdot}L^{-1}$ 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). Shoot elongation was the only response that differed between taxa and was influenced by treatments: $NiCl_2$ increased shoot elongation in American hazelnuts, whereas hybrids did not differ across treatments. These data suggest hazelnuts are susceptible to MED, with all nickel treatments mitigating symptoms.

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.

Index words: *Corylus*, nickel deficiency, 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. Sought after for healthful nuts with immense potential as an oilseed crop, this re-envisioned filbert and improved selections of American hazelnut comprise an emerging specialty crop in the Upper Midwest (Braun and 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 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 reinforces 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. The 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 recognized as an essential nutrient for plant growth (The Fertilizer Institute 2019). Nickel deficiency was first documented in soybeans [*Glycine max* (L.) Merr.] 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

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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* (Wangenh.) K.Koch] (Wood et al. 2004a), bitternut hickory [*Carya cordiformis* (Wangenh.) K.Koch] (Miller & Bassuk 2022), river birch (*Betula nigra* L.) (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 the xylem sap of pecan, Bai et al. (2007) demonstrated that nickel deficiency reduces the activity of urease, interrupting regular 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, 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 observed as marginal necrosis of leaves and leaflets in symptomatic plants (Siqueira Freitas et al. 2018). Foliar 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 ppm in foliar tissue (Wood 2015). An early indicator of MED in pecan is chlorosis, similar to iron or sulfur deficiency symptoms, and leaves that later turn dark green (Wood et al. 2004a, 2006). As symptoms become more severe, leaflet tips exhibit rounding or blunting (Fig. 1). Necrotic margins begin to form, leaves will curl, ultimately leading to rosetting and stem dieback (Fig. 1). 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). Soils with a 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, a heavy metal that can disrupt nickel-associated physiological processes because it competes for the same uptake pathway (Wood et al. 2004a). Similarly, in the pecan crop, copper fungicides are frequently utilized to control pecan scab (*Fusicladium effusum* G. Winter), a fungal disease that causes lesions on the leaves and fruit of the pecan plant (Wood et al. 2012, Wood 2015). 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 placed in areas previously inhabited by another tree (Wood et al. 2006, Wood 2015).

Fig. 1. American hazelnut (*Corylus americana*) plants demonstrating symptoms of MED including curling/cupping of leaflets, wrinkled leaflets, and chlorosis of leaflets which later become dark green and then exhibit necrosis of leaf margins.

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 a 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 micronutrients in the experiments of Hand and Reed (2014) shows 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 the nickel nutrition of container-grown plants. 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; Fig. 1). Interactions with researchers (Mark Hamann, Upper Midwest Hazelnut Development Initiative, 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 to test and determine their cause. The objectives of the study were to 1) characterize purported symptoms of nickel deficient American hazelnut and hybrid hazelnut plants and 2) determine if symptoms are corrected by foliar application of supplemental nickel.

Materials and Methods

Two seed lots 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). The Upper Midwest Hazelnut Development Initiative at the University of Minnesota (St. Paul, MN, USA) provided open-pollinated seeds of hybrid hazelnut. Seeds were cold stratified for 90 days in moistened, long-fiber sphagnum moss in a cooler maintained at 4 C (39 F). 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 (4 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 g of slow release fertilizer (Osmocote 14-14-14; ICL Specialty Fertilizers, Dublin, OH), that did not contain urea or nickel, at the time of potting and 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 cooler for 53 days. After meeting chilling requirements, hazelnuts were again moved into a greenhouse (24 July 2023) to break bud. Plants were watered once daily throughout the experiment (45 days; 21 Aug – 05 October 2023). 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 to a maximum of 1438 $\mu mol \cdot m^{-2} \cdot s^{-1}$. 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 (21 August 2023), all plants were treated with either a (1) non-treated control (H_2O spray), or foliar sprays on all leaf surfaces until droplets formed on leaves of (2) Nickel Plus® (169 $mg \cdot L^{-1}$Ni), (3) $NiCl_2$ (169 $mg \cdot L^{-1}$ Ni), (4) urea (150 $mg \cdot L^{-1}$ N), and (5) combined $NiCl_2$ (169 $mg \cdot L^{-1}$ Ni) and urea (150 $mg \cdot L^{-1}$ 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, with the exception of the non-treated control (n=25): seven *C. americana* from Christian Co., nine *Corylus* interspecific hybrids, and eight *C. americana* grown from seed acquired through Sheffield's Seed Company. The additional plant in the non-treated control group was representative of the Sheffield's Seed Company's germplasm. These treatment groups were separated from each other for foliar applications 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, goggles and an N-95 mask that tightly fit their face.

Fig. 2. 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.

One representative stem, which reflected the overall symptom level of each hazelnut plant, was harvested on 5 October 2023 and used to measure the following growth metrics: MED severity rating determined visually (1-5, with "1" representing the most symptomatic plants and "5" representing the least symptomatic plants), 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), and specific leaf area ($cm^2 \cdot g^{-1}$). 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 percentage of new growth displaying MED symptoms: 1 = ∼100%, 2 = ∼75%, 3 = 50%, 4 = ∼25%, 5 = no visible symptoms present on new leaves post-budbreak (Fig. 2). Leaf greenness was also collected in the greenhouse using a SPAD meter (SPAD 502 Plus Chlorophyll Meter, Konica Minolta, Inc., Tokyo, Japan). Leaf greenness was measured on the leaf lamina 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 stems. 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

Table 1. **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 ($\mu S \cdot cm^{-1}$)
Control	6.2	697
Nickel Plus®	6.0	789
$NiCl_2$	6.0	764
Urea	6.3	591
$NiCl_2$ + Urea	6.1	862

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 ($\mu S \cdot cm^{-1}$). To collect leachate, a plastic basin was placed underneath each container and 75 mL (based on a container diameter of 15.24 cm) of deionized 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 on 24 October 2023 using a handheld Apera® meter (Apera® PC60 pH/Cond./TDS/Sal. Tester; Apera® Instruments, Columbus, Ohio). Irrigation water had a pH of 6.6.

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 and then weighed. Leaves were removed from stems, with stems discarded after collecting shoot dry weight (g). If collected leaves from individual plants did not weigh at least 4 g, then they were consolidated with other samples from their treatment group and subsequently placed in paper bags. Samples 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 (Dahlquist & Knoll 1978, Munter and Grande 1981, Munter et al. 1984, Fassel et al. 1974).

Statistical analysis. Data for measured parameters were analyzed using a two-way ANOVA evaluating the effects of treatment and species. The 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 treatment. Data were analyzed using R statistical software (Ver. 4.2.2) (R Core Team 2022). 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 the Upper Midwest Hazelnut Development Initiative). 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*.

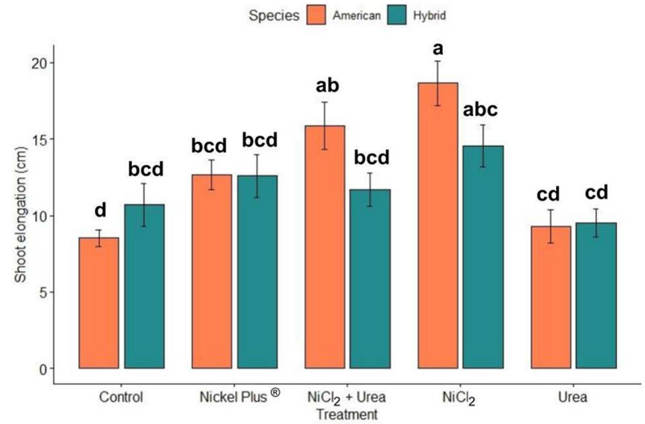

Fig. 3. **Mean shoot elongation (cm) (± standard error) in leaf tissue of one-year old American hazelnut (*Corylus americana*) and interspecific hybrid (*Corylus* spp.) seedlings left untreated (control) or treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).**

Results and Discussion

pH and electrical conductivity (EC). Nutrient deficiencies, like nickel, are often linked to high substrate pH, affecting nutrient availability, especially micronutrients (Torres et al. 2010). Ruter (2005) found that high pH soils may contribute to symptoms of MED. 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 $\mu S \cdot cm^{-1}$. LeBude & Bilderback (2009) state that pour-thru guidelines for most nursery crops suggest leachate EC values should range between 500 to 2,000 $\mu S \cdot cm^{-1}$ 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 of 5.4-6.2. Optimal pH and EC ranges were largely observed in the growth media used for the growth of the plants in these experiments (Table 1).

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) (Fig. 3).

The main effect of treatment was observed for all other parameters. Compared to the non-treated controls, the MED severity rating (1-5) ($P = <2e-16$) increased by 123.9%, 142.4%, 13.6%, and 146.7% for the Nickel Plus®,

Table 2. 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[z].

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	7.04 ± 0.31 b	48.46 ± 2.77 a	42.23 ± 5.97 b	0.41 ± 0.04 c	0.08 ± 0.01 d	99.45 ± 7.11 c
Nickel Plus®	4.12 ± 0.17 a	8.50 ± 0.51 ab	29.97 ± 1.45 b	177.90 ± 17.68 a	1.04 ± 0.11 ab	0.21 ± 0.02 bc	173.77 ± 5.67 b
$NiCl_2$	4.46 ± 0.17 a	9.04 ± 0.48 a	32.86 ± 1.75 b	228.31 ± 21.37 a	1.37 ± 0.15 a	0.33 ± 0.04 a	170.40 ± 7.16 b
Urea	2.09 ± 0.21 b	7.74 ± 0.65 ab	33.18 ± 2.31 b	46.90 ± 6.68 b	0.45 ± 0.06 c	0.10 ± 0.01 cd	108.87 ± 8.95 c
$NiCl_2$ + Urea	4.54 ± 0.12 a	8.62 ± 0.55 ab	31.39 ± 1.63 b	195.03 ± 16.26 a	0.96 ± 0.07 b	0.25 ± 0.04 ab	203.15 ± 7.61 a

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

$NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 2). A similar trend was observed for leaf area (cm^2) ($P = <2e\text{-}16$) 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 2). Continuing this trend, compared to the non-treated controls, specific leaf area ($cm^2 \cdot g^{-1}$) ($P = <2e\text{-}16$) 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 2).

Because shoot length can be stunted due to 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 demonstrates that American hazelnuts treated with $NiCl_2$ or $NiCl_2$ + urea exhibited increased shoot elongation compared to the non-treated controls (Fig. 3) however, American hazelnuts treated with Nickel Plus® were not different from the non-treated controls. Overall, for American hazelnut, treatments containing $NiCl_2$ numerically caused the largest increase in shoot elongation. However, with hybrid hazelnuts, this trend was not observed; rather, shoot elongation for all treatment groups, including the non-treated controls, was not different.

Compared to the non-treated controls, number of leaves ($P = 0.0471$) increased by 20.7%, 28.4%, 9.9%, and 22.4% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 2). A similar trend was observed for leaf dry mass ($P = 7.03e\text{-}12$) compared to the non-treated controls, with increases of 156.2%, 237.4%, 11.1%, and 135.2% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 2). Shoot dry mass ($P = 8.02e\text{-}09$) increased by 166.9%, 332.3%, 24%, and 219% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 2). For leaf greenness ($P = 2.76e\text{-}09$), a decreasing trend was observed. Compared to the non-treated controls, leaf greenness decreased by 38.1%, 32.2%, 31.5%, and 35.3% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Table 2).

In general, the overarching trend observed with these data is that nickel supplementation improved the evaluated growth parameters (Table 2). 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 a lack of current literature regarding use of $NiCl_2$ or urea for the correction or exacerbation of MED within hazelnut. However, results of this study demonstrate that overall, responses between hazelnut seedlings treated with Nickel Plus® and those treated with $NiCl_2$ varied minimally (Table 2). Urea was expected to exacerbate MED symptoms in hazelnuts and was suspected to be detrimental to plants compared to the non-treated control plants due to its the role in the urease pathway, where nickel is necessary for the conversion of urea to ammonia (Bai et al., 2006). Conducting further research on the urease pathway is essential to understand how urea affects symptoms of MED. Urea is utilized worldwide as a significant 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 ($mg \cdot kg^{-1}$) ($P = 2.12e\text{-}12$) increased by 4,752.9%, 2,058.8%, 88.2%, and 2,800% for the Nickel Plus®, $NiCl_2$, urea, and combined $NiCl_2$ + urea treatments, respectively (Fig. 4). The use of Nickel Plus® resulted in the highest leaf nickel concentration compared to all other treatments (Fig. 4). Nickel Plus® may be able to penetrate the leaf cuticle better than other nickel treatments (John Ruter, University of Georgia, Athens, GA, personal communication). Plants treated with Nickel Plus® were statistically different from the other nickel treatments. All nickel treatments were statistically different from the urea and control treatment groups (Fig. 4). These data show that the application of nickel, regardless of the nickel source, improved the nickel concentration in the leaf tissue. The treatment containing $NiCl_2$ and urea has both components necessary to complete the urease pathway. However, based on these results, this combination yielded 40.2% less nickel concentration in foliar tissue compared to treatments involving Nickel Plus® (Fig. 4). Future research should investigate how the urease pathway is affected by the source of nickel applied.

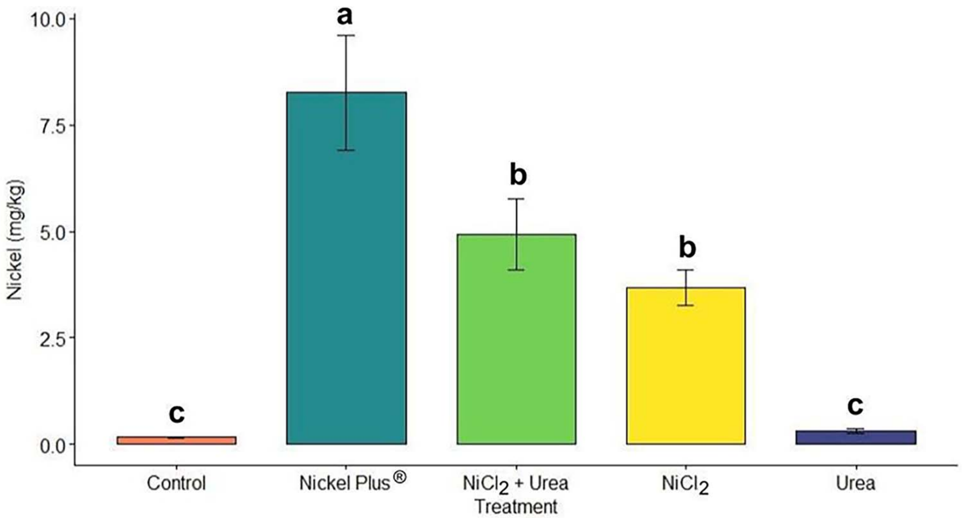

Fig. 4. Mean nickel concentration (± standard error) in leaf tissue of one-year old American hazelnut (*Corylus americana*) and interspecific hybrid (*Corylus* spp.) seedlings left untreated (control) or treated with either Nickel Plus®, $NiCl_2$, urea, or combined $NiCl_2$ and urea. Means across treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

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·kg^{-1} (Liu et al. 2011). Nickel toxicity occurs at concentrations $\geq$ 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 mg·kg^{-1} (Fig. 4). Pecan for example has a relatively high nickel requirement compared to amide-N-dormant species (The Fertilizer Institute, 2019, 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 2019). 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, 2019, Wood et al. 2006, Wood 2015).

Applications 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^{-1} Ni), $NiCl_2$ (169 mg·L^{-1} Ni), and combined $NiCl_2$ (169 mg·L^{-1} Ni) and urea (150 mg·L^{-1} N)] are sufficient for correcting symptoms of MED and increasing nickel content within the leaves. There remain 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 (2022), 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 2022), 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, which should now be recognized as a cause of nickel deficiency when cultivated using soilless substrates. This randomized greenhouse study showed the 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.

Literature Cited

Bai, C., C.C. Reilly, and B.W. Wood. 2006. Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. Plant Physiology 140(2):433–443.

Bai, C., C.C. Reilly, and B.W. Wood. 2007. Nickel deficiency affects nitrogenous forms and urease activity in spring xylem sap of pecan. Journal of the American Society for Horticultural Science 132(3):302–309.

Brown, P.H., R.M. Welch, and E.E. Cary. 1987. Nickel: A micronutrient essential for higher plants. Plant Physiology 85(3):801–803, https://doi. org/10.1104/pp.85.3.801.

Brown, P.H., L. Dunemann, R. Schultz, and H. Marschner. 1989. Influence of redox potential and plant species on the uptake of nickel and cadmium from soils. Z. Pflanzenernähr. Bodenkd. 152:85–91.

Dahlquist, R.L. and J.W. Knoll. 1978. Inductively coupled plasma-atomic emission spectrometry: analysis of biological materials and soils for major trace, and ultra-trace elements. Appl. Spectroscopy 32:1–30.

Eskew, D.L., R.M. Welch, and E.E. Cary. 1983. Nickel: an essential micronutrient for legumes and possibly all higher plants. Science 222(4624):621–623.

Fassel, V.A. and R.N. Kniseley. 1974. Inductively coupled plasma-optical emission spectroscopy. Anal. Chem. 46(13):1110A–1120A.

Hand, C. and B.M. Reed. 2014. Minor nutrients are critical for the improved growth of *Corylus avellana* shoot cultures. Plant Cell, Tissue and Organ Culture 119(2):427–439.

LeBude, A.V. and T.E. Bilderback. 2009. The pour-through extraction procedure: a nutrient management tool for nursery crops. AG-717-W, North Carolina Cooperative Extension, Raleigh, NC. p. 1–8.

Liu, G., E.H. Simonne, and Y. Li. 2020. IFAS Extension. University of Florida. Nickel Nutrition in Plants. https://edis.ifas.ufl.edu/publication/HS1191. Accessed June 22, 2023.

Mattson, N. (n.d.). Testing container media. https://www.css.cornell.edu/courses/260/Me dia%20 testing.pdf. Accessed October 11, 2023.

Miller, B.M. and N.L. Bassuk. 2022. Supplemental nickel corrects mouse ear disorder of bitternut hickory. J. Environ. Hort. 40(1):18–21.

Munter, R.C. and R.A. Grande. 1981. Plant tissue and soil extract analysis by ICP-AES. p. 653-673. *In*: R. M. Barnes (ed.) Developments in atomic plasma spectrochemical analysis. Heydon and Son, Philadelphia, PA.

Munter, R.C., T.L. Halverson, and R.D. Anderson. 1984. Quality assurance for plant tissue analysis by ICP-AES. Comm. Soil Sci. Plant Anal. 15(15):1285–1322.

NIPAN LLC. 2011. Nickel Plus® 5-0-0. http://nickelplus.biz/assets/pdf/Label%20-%20Nickel %20Plus.pdf. Accessed June 3, 2023.

Ruter, J.M. 2005. Effect of nickel applications for the control of mouse ear disorder on river birch. J. Env. Hort. 23(1):17–20.

Siqueira Freitas, D., B. Wurr Rodak, A. Rodrigues dos Reis, F. de Barros Reis, T. Soares de Carvalho, J. Schulze, M.A. Carbone Carneiro, and L.R. Guimarães Guilherme. 2018. Hidden nickel deficiency? Nickel fertilization via soil improves nitrogen metabolism and grain yield in soybean genotypes. Frontiers in Plant Science, 9:614.

The Fertilizer Institute. 2019. Nickel, essential elements. https://www.tfi.org/sit es/default/files/tfi-nickel.pdf. Accessed March 3, 2023.

Torres, A.P., M.V. Mickelbart, and R.G. Lopez. 2010. Measuring pH and EC of large container crops. Greenhouse Product New Magazine. July:52–55.

Wood, B.W. 2015. Nickel. p. 511–535. *In*: Barker, A.V., & Pilbeam, D.J. (Eds.). Handbook of Plant Nutrition (2nd ed.). CRC Press, Boca Raton, FL.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004a. Mouse-ear of pecan: a nickel deficiency. HortScience 39(6):1238–1242.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004b. Mouse-ear of pecan: I. symptomatology and occurrence. HortScience 39(1):87–94.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004c. Mouse-ear of pecan: II. influence of nutrient applications. HortScience 39(1):95–100.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2006. Field deficiency of nickel in trees: symptoms and causes. Acta Horticulturae 721:83–98.

Wood, B.W., C.C. Reilly, C.H. Bock, and M.W. Hotchkiss. 2012. Suppression of pecan scab by nickel. HortScience 47(4):503–508.