

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 that 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, whereas urea supplementation will exacerbate symptoms. Our objectives were to characterize symptoms of MED in American persimmon (*D. virginiana* L.), Japanese persimmon (*Diospyros kaki* Thunb.), and dateplum persimmon (*Diospyros lotus* L.), as well as to determine whether 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, symptomatic seedlings of each species were evaluated by comparing a nontreated control (H₂O spray), to foliar spray treatments of Nickel Plus® (169 mg/L Ni), NiCl₂ (169 mg/L Ni), urea (150 mg/L N), and combined NiCl₂ (169 mg/L Ni) and urea (150 mg/L N). Following treatment, the plants were evaluated using a MED severity rating scale including leaf characterization metrics (chlorophyll content index, count, surface area, dry mass, and specific area), as well as metrics characterizing stem traits (elongation and dry mass). The results suggest that the addition of supplemental nickel is effective at correcting MED for *Diospyros* species displaying symptoms; however, application should reflect species differences and grower conditions to optimize growth on a case-by-case basis. 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.

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 commonly referred to as the Japanese persimmon or Chinese persimmon (Chamberlain 2020; Ojha et al. 2023). *D. kaki* is a deciduous tree originating from Asia, offering desirable fruits with a red or orange

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 foliage 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 (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 *Diospyros lotus* (L.) in China due to the compatibility of the taxon and its tolerances to abiotic stresses, including drought (Liu et al. 2023); however, in the southeastern United States, *Diospyros virginiana* is more commonly used as a rootstock. *Diospyros lotus* is commonly referred to as dateplum 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 northeast 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 postharvest is less desirable than in *D. kaki* or *D. virginiana* (Ojha et al. 2023).

While the cultivation of Japanese persimmon in the United States is limited to US Department of Agriculture (USDA) zones 7 to 10 (Missouri Botanical Garden [date unknown]a), the American persimmon (*D. virginiana* L.) is considered hardy in USDA zones 4 to 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 2009; 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 drought-prone, highly disturbed sites including fence rows, along highways, and even top-soil stripped mining sites (Dirr 2009; Elias 1987; Peattie 1966). The species offers a pyramidal to oval crown, yellow to purple fall color, and attractive blocky bark (Dirr 2009). American persimmon can also exhibit glossy or 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 that can withstand difficult conditions and offers specialty fruits and ornamental value, yet the species is not readily available in the nursery trade (Dirr 2000). One potential explanation for limited availability is that American persimmon develops a substantial taproot, which makes it an effective choice for erosion control but can lead to challenges for successful transplanting (Nesom 2006). In many instances, trees that 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 (Burhenn Z, personal communication). 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 identifying a solution could support broader adoption of this emerging specialty crop. Nickel deficiency has not been reported previously in the literature, and more research is needed to document its occurrence in the landscape.

Mouse ear disorder, also known as little leaf disorder, represents the suite of symptoms associated with nickel deficiency and 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, potentially ammonia, 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 budbreak has been shown to correct symptoms of MED in pecan (Wood et al. 2006).

Nickel is the most recent element 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 that 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, research has evaluated select species of *Betula* (Miller 2021; Ruter 2005), *Carya* (Miller and Bassuk 2022b), and *Corylus* (Headley et al. 2025). As consumer preferences continue to shift toward more diverse nursery crops to increase landscape diversity and resiliency (Hilbert et al. 2023), it is becoming increasingly important to be able to recognize the symptoms and understand the conditions that make specific taxa, such as American persimmon, more susceptible to MED than others (Wood 2015), which may ultimately influence their availability in commerce (Miller and Bassuk 2022b).

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 using artificial potting substrates (Ruter 2005). Similar to the signs of iron and sulfur 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 rounder with blunt edges and curl, thus resembling a mouse's ear (Fig. 1). The leaf margins darken due to necrosis, which progresses to rosetting (Fig. 2) and stem dieback (Fig. 3). Nickel supplementation



Fig. 1. American persimmon (*D. virginiana*) exhibiting symptoms of mouse ear disorder (MED) such as leaflet tips becoming more round in shape with blunt edges and curling, thus resembling a mouse's ear.

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 (Fertilizer Institute 2019a). The use of copper fungicides in pecan orchards, 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 the deficiency in pecan plants and can cause problems in these orchards when trees are replaced with transplants (Wood et al. 2004b, 2006). Excess copper reduces the ability of the transplants to take up nickel. The transplants are unlikely to survive for more than a few years, and the problem is exacerbated by replanting into areas that were previously inhabited by large pecan trees that may have mined much of the plant-available nickel (Wood et al. 2006; Wood 2015).

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

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Fig. 2. *D. virginiana* plant exhibiting symptoms of mouse ear disorder (MED) such as stunted leaves and leaf curling.

Materials and Methods

Nickel and urea supplementation experiment. Seeds of *D. virginiana*, *D. kaki*, and *D. lotus* (Table 1) were cold, moist stratified for 90 d 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 medium (Premier Tech, Rivière du Loup, Canada) and subsequently moved to a glass greenhouse located in Saint Paul, MN, USA (lat. 44°59'15"N, long. 93°11'0"W), where germination began on 21 Jun 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 27 Oct 2022 to 6 Jun 2023. After dormancy, the plants were potted singly (6 Jun 2023 to 20 Jun 2023) into black plastic #1 nursery containers (1 gallon pots; 17 cm × 15.5 cm) filled with Pro Mix BRK substrate. The containers were top dressed with 10 g of slow release fertilizer that did not contain urea or any nickel component (Osmocote 14N-14P-14K; ICL Specialty Fertilizers, Dublin, OH, USA) at the time of potting-up, as well as each subsequent spring. The plants were moved into a glass greenhouse in Saint Paul, with an average temperature of 22.6°C (minimum of 10.1°C and maximum of 41.7°C) as monitored by an ARGUS Titan 900 9.0.15.0



Fig. 3. *D. virginiana* plant exhibiting symptoms of mouse ear disorder (MED) such as interveinal chlorosis, darkening of leaf margins due to necrosis, progressing to shortened internodes, rosetting, and stem dieback.

Table 1. Germplasm sources of *Diospyros kaki*, *Diospyros lotus*, and *Diospyros virginiana* acquired as open-pollinated seed and cultivated for this experiment.

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

NA = not applicable; USDA-GRIN = US Department of Agriculture Germplasm Resources Information Network.

system (Argus Controls, Langley, Canada), with plants watered once daily. Persimmon plants were once again overwintered beginning on 31 Oct 2023 in the aforementioned cooler for 92 d. After chilling requirements were met, the persimmons were again moved into a greenhouse (31 Jan 2024) to begin deacclimating. The plants were watered once daily throughout the experiment. Every 15 min, an Apogee SQ-500 full-spectrum quantum sensor (Apogee Instruments, Inc., Logan, UT, USA) recorded the average photosynthetically active radiation of 302.6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, ranging from a minimum of 0.001 to a maximum of 2002.7 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The average temperature was 22.4°C, with minimum and maximum values of 18.4 and 30.9°C, respectively. Relative humidity averaged 41.2%, ranging from 11.1% to 82.0%, with a dew point of 8.5°C, varying between 0.03 and 19.7°C, as monitored by the HOBOconnect MX2302A (version: 1.6.1; Onset Computer Corporation, Bourne, MA, USA).

Uniform budbreak of *D. kaki* and *D. lotus* occurred on 12 Feb 2024, and budbreak of *D. virginiana* occurred on 19 Feb 2024. Two weeks post-budbreak, *D. kaki*, *D. lotus*, and *D. virginiana* plants were treated with either a (1) nontreated control (H_2O spray) or foliar sprays on all leaf surfaces until beading of (2) Nickel Plus® (169 mg/L Ni), (3) NiCl_2 (169 mg/L Ni), (4) urea (150 mg/L N), and (5) combined NiCl_2 (169 mg/L Ni) and urea (150 mg/L N). Nickel Plus® (5N-0P-0K) (NIPAN LLC, Valdosta, GA, USA) is derived from urea and nickel lignosulfonate (5% N, 3% S, 5.4% Ni) (NIPAN LLC 2011). The nickel content in each treatment containing nickel matched the composition of Nickel Plus®. Similarly, the urea treatment maintained the same urea concentration as found in Nickel Plus®.

Each treatment group for *D. kaki* and *D. lotus* comprised 20 or 30 seedlings, respectively. *D. virginiana* treatment groups each comprised 25 seedlings, with the exception of the nontreated control ($n = 30$). Seedlings were randomly assigned a treatment with near-equal distribution across cultivars, provenances, or genotypes within each taxon. At the time of foliar treatment, the groups were physically separated in separate greenhouse bays to avoid drift. After leaves dried following the foliar treatments (sprayed with fine mist until beading), the 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 37 d post-treatment beginning on 3 Apr 2024 (*D. lotus* and *D. kaki*) and 10 Apr 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 percentage of new growth displaying MED symptoms: 1 = ~100%, 2 = ~75%, 3 = 50%, 4 = ~25%, and 5 = no visible symptoms present on new leaves post-budbreak (Figs. 4–6). 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) 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 bud scar adjacent to the main stem. Collected stems were moved to the laboratory 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), and specific leaf area (cm^2/g). The leaves were individually



Fig. 4. *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 ~100% of the plant affected, bottom right (2) represents roughly 75% of the plant affected, upper left (3) corresponds to 50% affected, upper middle (4) indicates 25% affected, and upper right (5) signifies little to no visible symptoms present.



Fig. 5. *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 ~100% of the plant affected, bottom right (2) represents roughly 75% of the plant affected, upper left (3) corresponds to 50% affected, upper middle (4) indicates 25% affected, and upper right (5) signifies little to no visible symptoms present.

separated from the stems and analyzed using a LI-COR 3100 leaf area meter (LI-COR Biosciences Inc., Lincoln, NE, USA) 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 h in the dryer, shoot and leaf dry weights were collected on 11 Apr 2024, 13 Apr 2024, and 14 Apr 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 ($\mu\text{S}/\text{cm}$) of the substrate using the pour-through technique (Wright 1986). 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



Fig. 6. *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 ~100% of the plant affected, bottom right (2) represents roughly 75% of the plant affected, upper left (3) corresponds to 50% affected, upper middle (4) indicates 25% affected, and upper right (5) signifies little to no visible symptoms present.

handheld meter (Apera PC60 pH/Conductivity/TDS/salinity tester; Apera Instruments, Columbus, OH, USA).

Leaf tissue analysis. One stem with leaves deemed representative of the overall symptom level of each plant in the experiment was removed and placed in a labeled paper bag between 3 Apr 2024, and 11 Apr 2024. The paper bags were subsequently placed in a forced air dryer maintained at 50°C for 72 h and then weighed. The leaves were separated from the stems and discarded after measuring the shoot dry weight (g). Leaves from individuals were combined within their treatment group if the dried leaves from a single individual weighed less than 4 g. A total of 80 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 18 Apr 2024, where an elemental analysis by inductively coupled plasma (ICP) optical emission spectrometry was performed. The elemental analysis for nickel, zinc, copper, and iron in leaf tissue was conducted using the dry ashing method at 485°C ashing temperature (Dahlquist and Knoll 1978; Fassel and Kniseley 1974; Munter and Grande 1981; Munter et al. 1984).

Statistical analysis. The data were analyzed using a two-way analysis of variance (ANOVA) (treatment \times species) in R statistical software (version 4.2.2). The data analyzed met the assumptions of the ANOVA model. A square-root transformation was used to meet model assumptions for analysis of leaf tissue concentration of nickel, nontransformed data are presented. When an interaction effect was not detected (shoot elongation, shoot dry mass, CCI, leaf count, or 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

Leaf tissue analysis. For leaf tissue concentration of nickel (mg/kg), there was an interaction between treatment \times species ($P < 0.001$). The use of Nickel Plus[®] resulted in the highest leaf nickel concentration compared with all other treatments (Fig. 7). The application of Nickel Plus[®] increased leaf tissue nickel concentration for *D. kaki* and *D. lotus* compared with *D. virginiana* (Fig. 7). The application of Nickel Plus[®] increased leaf tissue nickel concentration for *D. kaki* and *D. lotus* compared with the other two nickel treatments and the nontreated control and urea treatments (Fig. 7). For *D. virginiana*, the application of supplemental nickel increased nickel concentration compared with the urea treatment. The application of Nickel Plus[®] and NiCl_2 increased nickel concentration compared with the control; however, there was no difference in nickel concentration between the NiCl_2 + urea treatment and urea treatment compared with the nontreated control (Fig. 7).

The leaf tissue concentrations of zinc (mg/kg) were significantly affected by the main effects of treatment ($P = 0.016$) and species ($P < 0.001$), while the treatment \times species interaction was not significant. Compared with the nontreated controls, leaf tissue concentrations of zinc decreased by 13.3%, 12.2%, 17.7%, and 7.9% for the Nickel Plus[®], NiCl_2 , urea, and combined NiCl_2 + urea treatments, respectively (Table 2), with the urea treatment group and the nontreated control group being different from each other

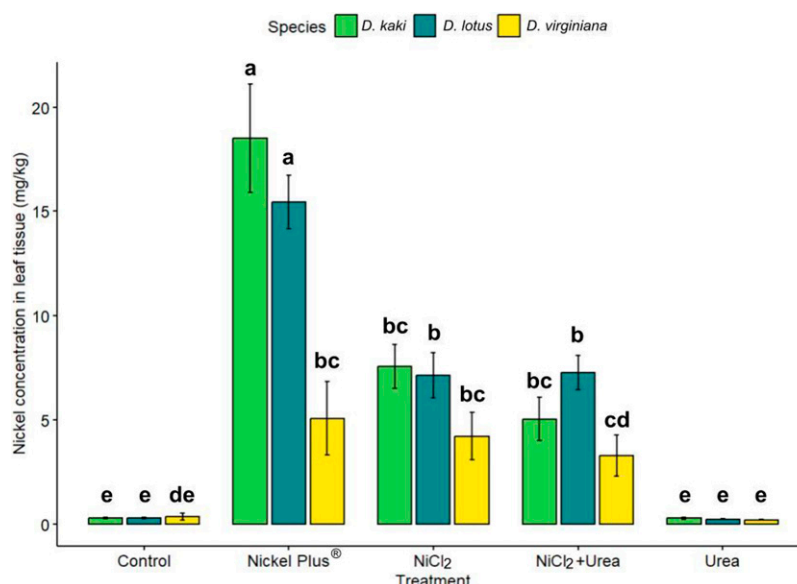


Fig. 7. Mean nickel concentration (\pm standard error) in leaf tissue of three species of 1-year-old *Diospyros* seedlings left untreated (control) or treated with either Nickel Plus[®], NiCl_2 , urea, or combined NiCl_2 and urea. The data were square root transformed for analysis; nontransformed values are presented. Means marked with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Table 2. Leaf tissue zinc concentrations (mg/kg) of 1-year-old Japanese persimmon (*Diospyros kaki*), dateplum persimmon (*Diospyros lotus*), and American persimmon (*Diospyros virginiana*) seedlings.

Treatment	Zinc (g/kg)
Control	22.3 ± 1.79 a
Nickel Plus®	19.3 ± 1.49 ab
NiCl ₂	19.5 ± 1.11 ab
Urea	18.3 ± 1.25 b
NiCl ₂ + urea	20.5 ± 1.36 ab

Means with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$). The data were pooled across species to reflect the main effect of treatment.

(Table 2). Averaged over treatment, all three species were different from each other, with the highest zinc concentrations in *D. virginiana* and the lowest in *D. lotus* (Table 3). The results of this study show that the presence of urea and the absence of nickel applied to the plants affected the leaf concentration of zinc; however, each species uniquely accumulated zinc at different concentrations (Tables 2 and 3).

Leaf tissue concentration of copper (mg/kg) was only affected by species ($P < 0.001$); no interaction exists for treatment × species. For leaf tissue concentration of copper, there was a species difference between *D. lotus* and *D. kaki* and a species difference between *D. lotus* and *D. virginiana*. *D. kaki* and *D. virginiana* were not different from each other, with the highest copper concentration in *D. lotus*.

The leaf tissue concentration of iron (mg/kg) was only affected by species ($P < 0.001$); no interaction exists for treatment × species. For leaf tissue concentration of iron, there was a species difference between *D. kaki* and *D. virginiana* and between *D. lotus* and *D. virginiana*. *D. kaki* and *D. lotus* were not different from each other, with the lowest iron concentration in *D. virginiana*.

Nickel and urea supplementation. An interaction between treatment × species was observed for MED severity rating ($P = 0.006$), leaf surface area (cm²) ($P = 0.001$), and leaf dry mass (g) ($P = 0.002$) (Table 4). For *D. virginiana*, the three nickel treatments improved the parameter studied compared with the nontreated control and urea treatments for MED severity rating, leaf surface area (cm²), and leaf dry mass (g), although the three nickel treatments were not different. *D. lotus* exhibited higher ratings overall, showing the least amount of MED symptoms when left untreated compared with *D. kaki*

Table 4. 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 Japanese persimmon (*Diospyros kaki*), dateplum persimmon (*D. lotus*), and American persimmon (*D. virginiana*) after foliar treatment with H₂O (control), NiCl₂, urea, NiCl₂ + urea, and Nickel Plus®.

Response	Main effects	DF	F ratio	P value ¹
Rating	Treatment	4	26.139	<2e ⁻¹⁶
	Species	2	62.636	<2e ⁻¹⁶
	Treatment × species	8	2.719	0.00639
Shoot elongation	Treatment	4	2.748	0.0281
	Species	2	55.407	<2e ⁻¹⁶
Leaf count	Treatment	4	29.03	1.87e ⁻¹²
	Species	2	29.03	1.87e ⁻¹²
Leaf surface area	Treatment	4	7.534	7.68e ⁻⁶
	Species	2	18.817	1.66e ⁻⁸
Chlorophyll content index (CCI)	Treatment × species	8	3.351	0.00102
	Species	2	44.57	<2e ⁻¹⁶
Leaf dry mass	Treatment	4	4.975	0.000647
	Species	2	12.270	6.96e ⁻⁶
Shoot dry mass	Treatment × species	8	3.219	0.001502
	Treatment	4	2.732	0.0289
Specific leaf area	Species	2	16.078	2e ⁻⁷
	Treatment	4	8.573	1.25e ⁻⁶
	Species	2	7.727	0.000515

¹ Significance at $P \leq 0.05$.

DF = degrees of freedom; MED = mouse ear disorder.

and *D. virginiana*, which were not different from each other (Fig. 8). There was a trend that supplemental nickel improved the MED severity rating compared with the nontreated control and urea treatments for all three species (Fig. 8). All treatments, including the nontreated control, did not influence mean leaf surface area (cm²) for *D. kaki* or *D. lotus* (Fig. 9). There was a trend that nickel improved leaf surface area (cm²) compared with the nontreated control and urea treatments for *D. virginiana* (Fig. 9). All treatments, including the nontreated control, did not influence mean leaf dry mass (g) for *D. kaki* or *D. lotus* (Fig. 10). There was a trend that nickel improved leaf dry mass (g) compared with the nontreated control and urea treatments for *D. virginiana* (Fig. 10).

Of the remaining responses for which an interaction was not observed, the main effect of species affected CCI ($P < 0.001$), leaf count ($P < 0.001$), specific leaf area (cm²/g) ($P = 0.001$), shoot elongation ($P < 0.001$), and shoot dry mass ($P < 0.001$) (Table 5). The data for these responses were pooled across treatments to demonstrate species differences (Table 5). The main effect of treatment affected specific leaf area (cm²/g) ($P < 0.001$), shoot elongation ($P = 0.028$), and shoot dry mass ($P = 0.029$); these data were pooled across species to demonstrate treatment differences (Table 6).

For shoot elongation, NiCl₂ + urea treatment was the only treatment different from

the nontreated controls (Table 6). Compared with the nontreated controls, shoot elongation increased by 13.87%, 18.98%, 15.33%, and 21.17% for the Nickel Plus®, NiCl₂, urea, and combined NiCl₂ + urea treatments, respectively. Species also affected shoot elongation (cm) (Table 4). Nickel application is expected to increase shoot elongation in symptomatic plants. Reduced shoot elongation in a nontreated control plant shows severe MED symptoms (Fig. 3).

Compared with the nontreated controls, shoot dry mass increased by 17.62%, 37.82%, 20.73%, and 34.72% for the Nickel Plus®, NiCl₂, urea, and combined NiCl₂ + urea treatments, respectively. NiCl₂ treatment increased shoot dry mass compared with the nontreated control (Table 6). Other nickel treatments and the urea treatment were not different from each other or the nontreated control. *D. lotus* and *D. virginiana* exhibited improved shoot dry mass compared with *D. kaki*.

Compared with the nontreated controls, specific leaf area (cm²/g) increased by 23.42%, 7.37%, 2.37%, and 10.75% for the Nickel Plus®, NiCl₂, urea, and combined NiCl₂ + urea treatments, respectively. Nickel Plus® was different from all treatments for this parameter including the nontreated control (Table 6). Species also significantly affected specific leaf area (Table 4). *D. lotus* and *D. virginiana* had improved specific leaf area (cm²/g) compared with *D. kaki*.

pH and EC. The overall growing substrate pH averaged overall plants in this study was 6.37, with minimum and maximum values of 5.61 and 7.59. The EC averaged overall plants in his study was 931.141 μS/cm, with minimum and maximum values of 531 and 2350 μS/cm.

Discussion

Nickel and urea supplementation. Different forms of nickel have been used for crops studied in regard to MED. Bitternut hickory [*Carya cordiformis* (Wangenh.) K. Koch] was

Table 3. Leaf tissue zinc, copper, and iron concentrations (mg/kg) of 1-year-old Japanese persimmon (*Diospyros kaki*), dateplum persimmon (*Diospyros lotus*), and American persimmon (*Diospyros virginiana*) seedlings.

Treatment	Zinc (g/kg)	Copper (g/kg)	Iron (g/kg)
<i>D. kaki</i>	18.5 ± 0.56 b	2.0 ± 0.09 b	57.0 ± 2.64 a
<i>D. lotus</i>	15.6 ± 0.42 c	2.3 ± 0.08 a	54.5 ± 1.65 a
<i>D. virginiana</i>	25.4 ± 0.85 a	1.7 ± 0.08 b	45.9 ± 1.42 b

Means with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$). The data were pooled across treatments to reflect the main effect of species.

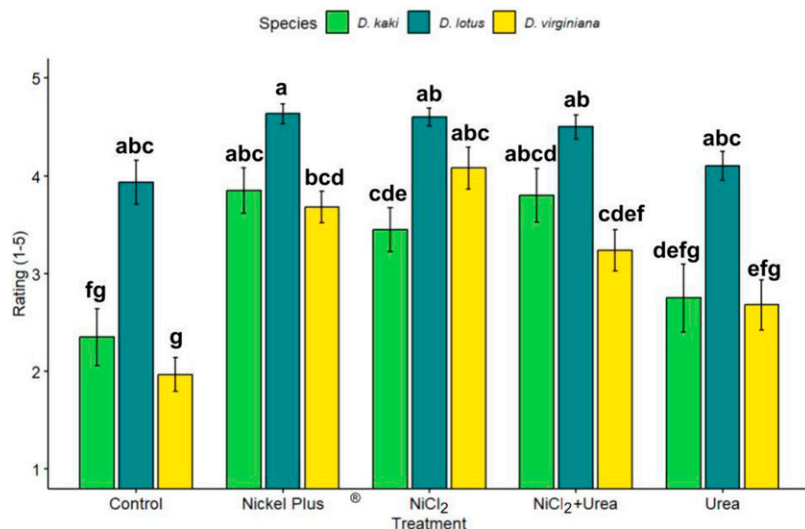


Fig. 8. Mean overall MED severity rating scale (1 to 5, where 1 signifies ~100% of the plant affected, 2 represents roughly 75% of the plant affected, 3 corresponds to 50% affected, 4 indicates 25% affected, and 5 signifies little to no visible symptoms present) (\pm standard error) of leaf tissue of three species of 1-year-old *Diospyros* seedlings left untreated (control) or treated with either Nickel Plus®, NiCl₂, urea, or combined NiCl₂ and urea. Means across species and treatments marked with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

supplemented with nickel in the form of nickel ligandsulfonate (Nickel Plus®) (Miller and Bassuk 2022b), river birch was supplemented with nickel in the form of nickel sulfate (Ruter 2005), and hazelnut was supplemented with NiCl₂ and nickel ligandsulfonate (Nickel Plus®) (Headley et al. 2025). Nickel product formulation may affect how much leaf area a plant gains in regard to the amount of leaf biomass. In production, foliar sprays are used preferentially compared with soil drench methods. It is suggested that Nickel Plus® may be able to penetrate the leaf cuticle better than other nickel treatments (Ruter J, personal communication). Miller and Bassuk (2022b) used a

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 with bitternut hickories treated with a foliar spray of Nickel Plus® (2.5 mL/L). Persimmons treated with Nickel Plus® showed a 55.72% increase in leaf area (compared with nontreated controls). The 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 ligandsulfonate fertilizer, is one of the only nickel fertilizers on the market for woody plants.

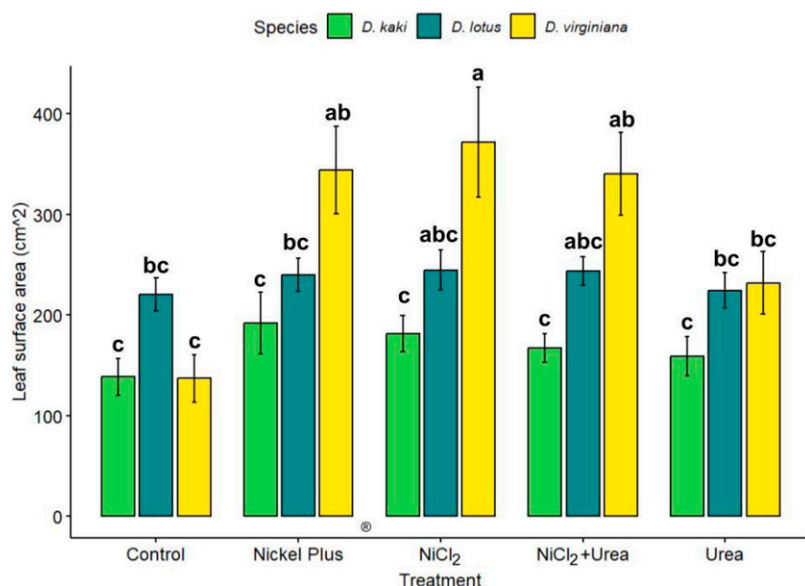


Fig. 9. Mean leaf surface area (cm²) (\pm standard error) of leaf tissue of three species of 1-year-old *Diospyros* seedlings left untreated (control) or treated with either Nickel Plus®, NiCl₂, urea, or combined NiCl₂ and urea. Means across species and treatments marked with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

However, it is not the only foliar-applied supplemental nickel that has been studied. Nickel ligandsulfonate, 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 in correcting MED (Wood et al. 2004a, 2004b). Nickel ligandsulfonate fertilizers, such as Nickel Plus®, have been deemed safer for applicators than other nickel fertilizers such as nickel nitrates and nickel sulfates (Wood 2015). In this study, we also used NiCl₂, 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 was used in this study for several reasons. Urea is a component of Nickel Plus® and supplies urea to persimmon, which is a ureide-transporting plant (Wood et al. 2006). Urea is also used worldwide as a major source of nitrogen for crops (Wood 2015). It was hypothesized that urea applied without nickel would be more detrimental than the nontreated 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 exhibited MED symptoms; however, the manifestation of MED symptoms varied among the species (Figs. 8–10). In a randomized greenhouse study, *D. lotus* showed fewer MED symptoms than *D. virginiana* and *D. kaki* (Figs. 8–10). Foliar applications of supplemental nickel in this study were effective at correcting symptoms of MED in *D. virginiana*, *D. kaki*, and *D. lotus*. *D. kaki* and *D. lotus* are grown more extensively in horticulture than *D. virginiana* and appear less susceptible to MED. *D. kaki* is also frequently grafted onto *D. lotus* (Liu et al. 2023). Based on these observations, future research should investigate whether grafting *D. kaki* on *D. lotus* affects how or whether MED symptoms occur in these species.

In river birch (*Betula nigra*), Ruter (2005) reported a 53% to 60% increase in shoot elongation for plants treated with nickel sulfate compared with the nontreated controls. In bitternut hickory (*C. cordiformis*), Miller and Bassuk (2022b) observed an 83.5% increase in shoot elongation for plants treated with a foliar application of Nickel Plus® compared with the nontreated controls. Headley et al. (2025) observed a 35.8% increase in shoot elongation for American and interspecific hybrid hazelnuts. The percentage of increase for shoot elongation between nontreated controls and differing nickel treatments in this *Diospyros* study is less pronounced compared with river birch and bitternut hickory, suggesting that shoot elongation in persimmon is less affected by supplemental nickel treatment compared with other ureide transporting species.

Leaf tissue analysis. The typical nickel concentration in most plant leaf material ranges from 0.1 and 5 ppm (on a dry weight basis) (Fertilizer Institute 2019a). In this study, leaf tissue nickel concentrations among

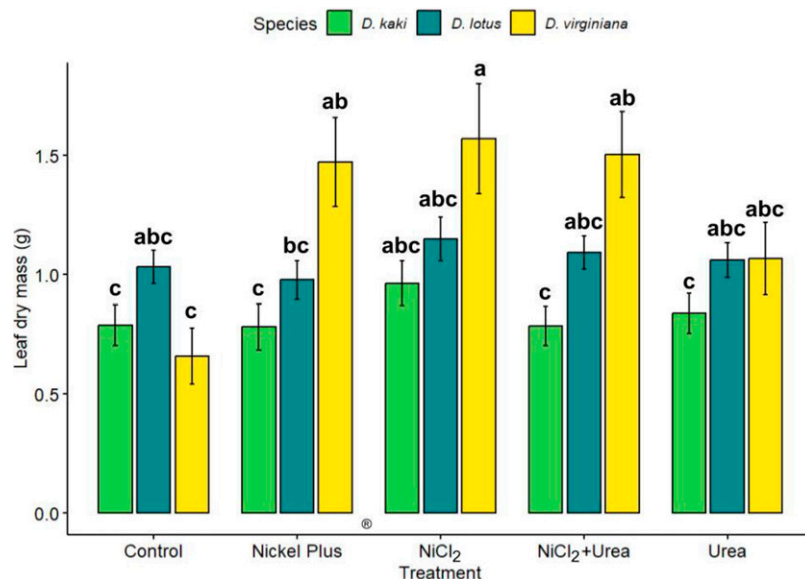


Fig. 10. Mean leaf dry mass (g) (\pm standard error) of leaf tissue of three species of 1-year-old *Diospyros* seedlings left untreated (control) or treated with either Nickel Plus[®], NiCl₂, urea, or combined NiCl₂ and urea. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Table 5. Plant growth metrics (mean \pm standard error) of 1-year-old Japanese persimmon (*Diospyros kaki*), dateplum persimmon (*Diospyros kaki*), and American persimmon (*Diospyros virginiana*) seedlings cultivated in a soilless substrate 37 d after foliar treatment with H₂O (control), NiCl₂, urea, NiCl₂ + urea, and Nickel Plus[®].

Species	Shoot elongation (cm)	Leaf count	Chlorophyll content index (CCI)	Shoot dry mass	Specific leaf area (cm ² /g)
<i>D. kaki</i>	10.8 \pm 0.49 c	7.9 \pm 0.27 c	24.6 \pm 0.96 a	0.16 \pm 0.01 b	204.9 \pm 6.18 b
<i>D. lotus</i>	18.9 \pm 0.52 a	9.8 \pm 0.24 b	16.5 \pm 0.35 b	0.27 \pm 0.01 a	227.0 \pm 4.56 a
<i>D. virginiana</i>	15.4 \pm 0.55 b	11.7 \pm 0.45 a	16.2 \pm 0.75 b	0.25 \pm 0.02 a	232.5 \pm 5.18 a

Means with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$). The data were pooled across treatments to reflect the main effect of species.

treatments ranged from 0.2 mg/kg within the urea-only treatment group to 11.9 mg/kg for Nickel Plus[®]. In moderately nickel-tolerant species, nickel becomes toxic at concentrations exceeding 50 mg/kg (Fertilizer Institute 2019a). In a study on bitternut hickory (*C. cordiformis*), Miller and Bassuk (2022) found that foliar nickel applications resulted in higher leaf tissue nickel concentrations than soil drenches. This raises questions concerning whether the measured nickel from the leaf samples may have been a result of nickel residue on the leaf surface from the foliar spray as opposed to nickel

found within leaf tissue after being fully absorbed. Further research is needed to determine whether the high nickel concentration shown by the application of Nickel Plus[®] reflects true biological uptake or is influenced by nonabsorbed foliar residues, which could affect the accuracy of ICP analyses and our understanding of plant response to foliar supplementation.

The typical concentration of zinc in most plant leaf material ranges from 20 to 100 ppm (Fertilizer Institute 2019c). In this study, leaf tissue zinc concentrations among treatments ranged from 18.3 mg/kg within the urea

treatment group to 22.3 mg/kg in the control treatment group, which represents the lower end of the typical range. Among species, the leaf tissue zinc concentrations ranged between 15.6 mg/kg for *D. lotus* and 25.4 mg/kg for *D. virginiana*, representing the lower end of the typical range. Future research should look into the relationship between nickel and zinc concentration in leaf tissue when zinc is also supplemented to the plants to better understand the relationship between the two elements and their combined impact on MED symptoms.

Overall, nickel concentration in persimmon leaf tissue was increased by the application of nickel treatment, regardless of the nickel source (Fig. 7). The treatment combination of NiCl₂ and urea has both of the necessary components for the completion of the urease pathway; however, it resulted in a 57% decrease in nickel concentration in persimmon foliar tissue compared with the Nickel Plus[®] treatment (Fig. 7).

The results of this study show that the addition of nickel in any form did not affect the leaf tissue concentration of copper; however, each species accumulated copper at different concentrations. The typical concentration of copper in most plant leaf material ranges from 5 to 20 ppm (Fertilizer Institute 2024). In this experiment, leaf tissue copper concentrations between species ranged from 1.7 for *D. virginiana* to 2.3 for *D. lotus*, below the typical range.

Similar to studies with copper, the results of this study also indicated that adding nickel in any form did not affect the leaf tissue concentration of iron; however, each species accumulated iron at different concentrations. The typical iron concentration in plant leaf material varies between species; however, it is generally between 50 to 250 ppm (on a dry weight basis) (Fertilizer Institute 2019b). Toxicity levels of iron may be observed at concentrations exceeding 500 ppm (Fertilizer Institute 2019b). In this experiment, leaf tissue iron concentrations between species ranged from 45.9 mg/kg for *D. virginiana* and 57 mg/kg for *D. kaki*, which represents the lower end of the typical range.

pH and EC. 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 to 6.2 in soilless substrates; however, each nutrient has its own optimal pH range (Torres et al. 2010). Nickel is most 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 to 6.2 range; however, it is below the 6.5 threshold for nickel. For the majority of nursery crops, the EC values of the leachate should range from 500 to 2000 μ S/cm during periods of active growth (LeBude and Bilderback 2009). The overall EC value for this study is within this suggested range.

MED in specialty crops. There is very little literature regarding MED in woody specialty crops, including some taxa with significant

Table 6. Plant growth metrics (mean \pm standard error) of 1-year-old Japanese persimmon (*Diospyros kaki*), dateplum persimmon (*Diospyros lotus*), and American persimmon (*Diospyros virginiana*) seedlings cultivated in a soilless substrate 37 d after foliar treatment with H₂O (control), NiCl₂, urea, NiCl₂ + urea, and Nickel Plus[®].

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

Means with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$). The data were pooled across species to reflect the main effect of treatment.

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