Supplemental Nickel Corrects Mouse Ear Disorder of Bitternut Hickory[1]

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Abstract

While sought after for use in managed landscapes, bitternut hickory [*Carya cordiformis* (Wang.) K. Koch] remains underutilized in horticulture due its reputation for difficulty with production and transplanting. After learning of issues experienced by growers and observing deformed leaf development of container-grown stock, we examined effects of supplemental nickel applications on seedlings of bitternut hickory. We hypothesized that, like the closely related species pecan [*Carya illinoinensis* (Wang.) K. Koch], bitternut hickory would be similarly limited in production by a susceptibility to mouse ear disorder as a function of nickel deficiency. Seedlings cultivated with a soilless substrate in 3.8L (#1) nursery pots were treated with either a substrate drench or foliar spray of Nickel Plus® two weeks after budbreak. After 30 days, plants treated with a drench or foliar spray exhibited increased leaf area and decreased number of leaves per unit shoot extension and leaf greenness compared to nontreated controls. Foliar treatments resulted in an 83.5% increase in shoot extension over nontreated controls. Whereas seedlings provided supplemental nickel displayed healthy foliage after treatment, nontreated seedlings exhibited traditional symptoms of mouse ear disorder. These data indicate bitternut hickory is susceptible to mouse ear disorder, an issue remedied by supplementing nickel.

Index words: foliar spray, substrate drench, soilless substrate, nursery production, ornamental plants.

Chemicals used in this study: nickel lignosulfonate (Nickel Plus®).

Species used in this study: bitternut hickory, *Carya cordiformis* (Wang.) K. Koch.

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Significance to the Horticulture Industry

Between the 1970s and early 2000s, mouse-ear disorder limited the production of river birch (*Betula nigra* L.) (Ruter 2005). The discovery that nickel deficiency causes the disorder identified the core issue and prompted the development of a commercial product to correct the problem. Nickel has since been recognized as an essential element for plant growth and the understanding of nickel requirements of different taxa continues to grow. As species diversity of managed landscapes becomes a principal issue in the green industry, growers are looking for new crops to produce. Bitternut hickory is a species gaining the attention of growers, horticulturists, and urban foresters for its horticultural merit. The introduction of this taxon as a nursery crop requires new techniques to enhance production and availability to consumers. Because nickel is a heavy metal, careful recommendations of its application in the nursery are substantiated to maintain safe, sustainable, and efficient production. Growers interested in adopting bitternut hickory into production should anticipate the occurrence of mouse ear disorder when cultivated with soilless substrates. Nickel can be adequately supplemented with Nickel Plus® applied as either a foliar spray or substrate drench shortly after bud break and in accordance with label-recommended rates for pecan.

Introduction

Superior ornamental traits and broad adaptive capabilities are among the reasons why bitternut hickory is sought after for use in managed landscapes. Endemic to much of the eastern United States and useful in USDA hardiness zones 4-9 (Dirr 2009), bitternut hickory could be used to diversify landscapes and should be considered as one option for replacing ash (*Fraxinus* spp. L.) species decimated by the emerald ash borer (*Agrilus planipennis* Fairmaire) (Poland and McCullough 2006). While desirable, bitternut hickory and many of its congeners have remained underutilized due to limitations with production in the nursery and transplanting to the landscape (Miller 2017). As species diversity and abiotic stress resilience become forefront issues for urban forests and green spaces, horticulturists have begun to assess the potential of underutilized taxa (Hirons et al. 2021) and the needs for their successful production in the nursery (Miller and Graves 2019). Yet much is left unknown and needs to be studied before hickories will become commonplace in the green industry.

Nursery production of pecan, a closely related taxon, has already been streamlined for use in nut orchards. Among the issues that afflict pecan is mouse ear disorder, a function of nickel deficiency (Wood et al. 2004a). Symptoms of mouse ear disorder begin with curling of the lamina on developing foliage and the presence of a necrotic margin. As symptoms become more severe, leaves fail to expand, branches begin to rosette, and stems die back. Previously documented and thoroughly studied on pecan (Wood et al. 2004a, Wood et al. 2004b, Wood et al.

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2004c, Wood et al. 2006), mouse ear disorder is infrequently encountered with most nursery crops except river birch (Ruter 2005). Aside from these studies, mouse ear disorder has not been reported in other major nursery crop species. The condition occurs rarely on field sites, except for locales with sandy soils, sites with a high pH (Ruter 2005), and in the case of pecan, in orchards at the time of replanting, especially those with soils comprising a high zinc:copper ratio (Wood et al. 2004a). Most commonly, mouse ear disorder is observed on plants grown in soilless substrates (Ruter 2005).

Of the woody perennials documented as highly susceptible to mouse ear disorder, one common theme among them is their characterization as ureide-transporters (Bai et al. 2006). Mouse ear disorder has since been associated with disrupted metabolism of ureides, amino acids, and organic acids (Bai et al. 2006). Further, nickel deficiency reduces urease activity and through disruption of ureide metabolism, interrupts normal nitrogen cycling (Bai et al. 2007). Given the common theme of vulnerability of ureide-transporters to mouse ear disorder, Wood et al. (2006) predicted other species with similar physiological and metabolic pathways are likely candidate taxa to monitor for susceptibility to mouse ear disorder.

In 2019, the authors observed symptoms consistent with mouse ear disorder on seedlings of bitternut hickory grown in a peat-based substrate (unpublished data). Communication with industry partners that produce bitternut hickory in containers corroborated the occurrence of these symptoms (Ben French, Johnson's Nursery, Menomonee Falls, WI, personal communication). An experiment was designed with the intention of determining the cause of the symptoms. The objectives of the study were to 1) provide evidence that bitternut hickory is susceptible to mouse ear disorder, and 2) characterize growth responses of symptomatic plants after treatment with the commercial product Nickel Plus® (Nipan LLC., Valdosta, GA) [5% N (derived from urea), 3% S, and 5.4% Ni (derived from nickel lignosulfonate)] as either a substrate drench or foliar spray to assess if supplemental nickel ameliorated symptoms.

Materials and Methods

Seeds of bitternut hickory were wild collected in the fall of 2018 from multiple individual trees in Ithaca, NY. Exocarps were first excised by peeling away the mature husk. Seeds were surface sterilized by entirely coating with 95% ethanol using a spray bottle, then rinsing with tap water. Seeds were cold-moist stratified at 4 C (39.2 F) for 90 days then shallowly sown [≈3 cm (1.2 in) deep] in trays filled with a peat-based substrate in January of 2019. Trays were maintained on a greenhouse bench until germination. Seedlings were potted singly into 0.5 L (4 in) containers filled with Lamberts LM-6 (≈3:1 peat:perlite) soilless substrate (Lambert Peat Moss, Inc., Riviere-Ouelle, Quebec, Canada) and grown in a greenhouse in Ithaca, NY. After overwintering in a cooler maintained at 4 C (39.2 F), plants were up-potted in April of 2020 into 3.8 L (#1) nursery pots filled with the same soilless substrate and returned to the greenhouse for the 2020 growing season. In the greenhouse (2019 and 2020) plants were irrigated as needed with municipal tap water and fertilized twice monthly with Peters Professional® Acid Special 21N–7P–7K (JR Peters, Allentown, PA) water-soluble fertilizer [21% N (derived from ammonia and urea), 7% P_2O_5, 7% K_2O, 0.6% Mg, 13% S, 0.0262% B, 0.0262% Cu, 0.15% Fe, 0.05% Mn, 0.01% Mo, and 0.05% Zn] applied via fertigation at a rate of 150 $mg \cdot L^{-1}$. Each time plants were up-potted, all containers were top dressed with a low rate (3 g for 0.5 L, 18 g for 3.8 L) of Osmocote® Plus 15-9-12 (ICL Specialty Fertilizers, Dublin, OH) slow release fertilizer [15% N (derived from ammonia and nitrate), 9% P_2O_5, 12% K_2O, 1.3% Mg, 5.9% S, 0.02% B, 0.05% Cu, 0.46% Fe, 0.06% Mn, 0.02% Mo, and 0.05% Zn]. Shortly after budbreak in 2020, authors began observing consistent symptoms of mouse ear disorder, including leaf curling and development of a necrotic leaf margin.

Uniform plants between 4-6 mm caliper [diameter measured 2.5 cm (1 in) above cotyledon scar] were randomly selected and assigned to treatments prior to overwintering in a cooler maintained at 4 C (39.2 F). On April 24, 2021, plants were moved to an unheated polyhouse located at the Bluegrass Lane Turf and Landscape Research Center, in Ithaca, NY (latitude 42.48 N, longitude 76.4° W, elevation 335 m). Plants were randomly placed and attached to drip irrigation, where they were watered twice daily [≈1 L per day (1.1 qt)]. Bud break occurred on all plants on May 3, 2021 (defined as exhibiting at least one expanding leaf). On May 17, two weeks after bud break, twelve single-plant replicates each were treated with either 59.14 ml (1.9 fl oz) of a substrate drench [37.85 ml Nickel Plus® per 3.79 L H_2O (1.28 fl oz per gal)]) or a fine-mist foliar spray using a handheld sprayer on all leaf surfaces until beading [9.46 ml Nickel Plus® per 3.79 L H_2O (0.32 fl oz per gal)]. Included also were twelve nontreated controls. Rates and application times were based on label recommendations for treating mouse-ear disorder of pecan.

Plants were evaluated 30 days after treatment on June 16, 2021. Leaf greenness (relative amount of chlorophyll determined by measuring leaf absorbance in the red and near-infrared wavelength regions) was measured using a SPAD 502 handheld spectrophotometer (Konica-Minolta, Osaka, Japan) determined for each plant by averaging measurements of three fully expanded leaves selected at random. The dominant shoot of each plant was excised at the site where growth initiated in 2021 and used to record and calculate remaining growth metrics. Shoot extension was determined by the length of the excised shoot from the base to the distal end of the terminal bud. Leaf area was calculated as the total surface area of leaves removed using a LI-COR 3100 leaf area meter (LI-COR Biosciences Inc., Lincoln, NE). The number of leaves per shoot was divided by the mean shoot extension to calculate the number of leaves per unit shoot extension. Excised shoots and their leaves were then dried in an oven at 65 C (149 F) for three days. Subsequently, leaf and shoot dry weights were measured independently. Specific leaf area was measured by dividing leaf area by leaf dry weights. An MX2303 data logger (Onset HOBO, Bourne, MA) recorded air temperature and relative humidity every fifteen minutes for the

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Table 1. **Growth analysis of three-year old bitternut hickory (*Carya cordiformis)* seedlings grown in a soilless substrate 30 days after treatment with Nickel Plus®, a formulation containing 5% N (derived from urea), 3% S, and 5.4% Ni (derived from nickel lignosulfonate)[z].**

Treatment	Leaf greenness (SPAD units)	Shoot extension (cm)	Number of leaves per unit shoot extension (#/cm)	Leaf area (cm^2)	Specific leaf area ($cm^2 \cdot g^{-1}$)
Control	29.8±1.5 a	7.9±1.2 b	1.162±0.15 a	316.5±64 b	144.1±17.1 b
Drench	23.5±1.5 b	13.0±1.8 ab	0.693±0.11 b	769.6±99 a	213.5±9.8 a
Foliar	23.8±1.1 b	14.5±1.5 a	0.584±0.05 b	718.2±126 a	191.1±7.1 a

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

duration of the experiment. Mean (Min., Max) temperature and relative humidity (%) within the polyhouse were 20.4 (5.7, 41.5 C) [68.7 (42.3, 106.7 F)] and 74.2% (29.1%, 99.3%), respectively. Mean ±SE photosynthetic photon flux of ≈272 ±6.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height was measured with a quantum sensor (SQ-520 Apogee Instruments, Logan, UT). Substrate pH was determined using pour-thru methodology on three randomly selected single-plant replicates from each treatment at the time of data collection, ranging from 6.9-7.6. Using a 0.05M DTPA and water saturated media extract, it was determined that the soilless substrate consisted of ≈0.006 $mg \cdot kg^{-1}$ Ni. Oven-dried foliar samples of four replicates from each treatment were selected at random and evaluated for foliar nickel content using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Statistical analysis. All growth response data were analyzed using a one-way ANOVA. To meet the assumptions of the model some data were square root (shoot extension, leaf area) or log (number of leaves per unit shoot extension) transformed. Post-hoc mean comparisons were made using Tukey's Honestly Significant Difference Test. Statistical analysis was conducted using JMP Pro 15 software (JMP Version 15, SAS Institute, Inc., Cary, NC).

Results and Discussion

Compared to nontreated controls, leaf greenness ($P = 0.0046$) and number of leaves per unit shoot extension ($P = 0.0015$) decreased respectively by 21.1% and 40.4% for plants treated with a nickel substrate drench or 20.1% and 49.7% for those treated with a foliar spray (Table 1). As opposed to nontreated controls, leaf area ($P = 0.0010$) increased by 143.2% and 126.9% in response to treatment with a drench or foliar spray, respectively (Table 1). Specific leaf area ($P = 0.0010$) of plants treated with a substrate drench improved by 47.9% whereas the foliar treatment resulted in a 32.6% increase (Table 1). Shoot extension ($P = 0.0093$) was not different between nontreated controls and the substrate drench treatment; however, those treated with the foliar spray exhibited an 83.5% increase over nontreated plants (Table 1). ICP-AES test results indicated all four nontreated controls had undetectable amounts of nickel whereas mean nickel content of plants treated with either a drench or foliar spray comprised ≈2.5 $mg \cdot kg^{-1}$ or 83.6 $mg \cdot kg^{-1}$, respectively.

As an essential element for plant growth (Mengel et al. 2001), the occurrence of nickel deficiency can theoretically manifest itself in any plant species. However, because nickel is a co-factor for the urease enzyme and is essential for its activity (Ruter 2005), the occurrence of nickel deficiency is more likely to occur among species with ureide-transporting nitrogen metabolism (Wood et al. 2006). The classic symptoms of mouse ear disorder, wherein the lamina becomes curled and exhibits a necrotic margin, eventually leading to rosetting of the stems (Fig. 1), is thought to be the result of toxic levels of urea

Fig. 1. **Top to bottom: a nontreated seedling of bitternut hickory exhibiting mouse ear disorder and an asymptomatic seedling of bitternut hickory 30 days after treatment with a foliar spray of Nickel Plus®, a formulation containing 5% N (derived from urea), 3% S, and 5.4% Ni (derived from nickel lignosulfonate).**

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accumulating in the tissue (Eskew et al. 1983, Krogmeier et al. 1989). When nickel deficient, urease activity decreases, resulting in the toxic build-up of urea in the foliar tissue and subsequent mouse ear symptoms.

The correction of mouse ear disorder by supplementing nickel has previously been demonstrated with field-grown pecan (Wood et al. 2004a), container-grown river birch (Ruter 2005), and now with bitternut hickory (Fig. 1). Of these examples, pecan has been successfully treated with a foliar spray application, whereas mouse ear disorder for the remaining taxa has been corrected with either a foliar spray or substrate drench. Like the findings of Ruter's (2005) study of correcting mouse ear on river birch, we found supplementing nickel with a foliar spray or substrate drench increased leaf area and specific leaf area while also decreasing the number of leaves per unit shoot elongation compared with nontreated controls (Table 1).

Important for better understanding the effects of the treatments in this study are the nutrient components of the fertilizers, substrates, and supplement tested. Both fertilizer sources (Peters Professional® and Osmocote® Plus) contained comparable micronutrients but lacked Ni. Except Ni, all components of Nickel Plus® [S and N (derived from urea)] were present in the fertilizers used to grow the plants leading up to the study. S and N (derived from urea) are purportedly used in the formulation of Nickel Plus® because they serve as good carriers and are effective at permeating the epidermal layer of leaves when used as a foliar spray (John Ruter, University of Georgia, Athens, GA, personal communication). While the pH of the soilless substrate was moderately high (6.9-7.6) and could have played a role in the development of mouse ear disorder of the plants used in this study, the Ni content of the soilless substrate used was negligible ($\approx$0.006 $mg \cdot kg^{-1}$ Ni).

Of the nursery issues shared among the species that belong to the genus *Carya*, susceptibility to mouse ear disorder appears to be consistent for both pecan and bitternut hickory, thus providing some empirical evidence for the prediction set forth by Wood et al. (2006). Both species belong to the section Apocarya (Wood and Grauke 2011) and are closely related. Susceptibility of bitternut hickory to mouse ear disorder may partially explain the reputation held by members of the genus for their difficulty in production in the nursery. Currently no other studies have assessed the susceptibility of any other species of *Carya* to mouse ear disorder, however, it may be required for integrating the genus into the green industry. Furthermore, this issue should be assessed in other candidate genera with ureide-transporting nitrogen metabolism to determine if this issue is more commonplace than previously thought. Once production is streamlined, bitternut hickory will be a useful addition to the plant palette as an ornamental tree for a variety of managed landscapes.

Literature Cited

Bai, C., C.C. Reilly, and B.W. Wood. 2007. Nickel deficiency affects nitrogenous forms and urease activity in spring xylem sap of pecan. J. Amer. Soc. Hort. Sci. 132:302–309.

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 Physiol. 140:433–443.

Dirr, M. 2009. Manual of woody landscape plants: Their identification, ornamental characteristics, culture, propagation and uses. 6th ed. Stipes Publishing, Champaign, IL. p. 211.

Eskew, D.L., R.M. Welch, and E.E. Cary. 1983. Nickel: an essential micronutrient of legumes and possibly all higher plants. Sci. 222:621–624.

Hirons, A.D., J.H.R. Watkins, T.J. Baxter, J.W. Miesbauer, A. Male-Muñoz, K.W. Martin, N.L. Bassuk, and H. Sjöman. 2021. Using botanic gardens and arboreta to help identify urban trees for the future. Plants, People, Planet 3:182–193.

Krogmeier, M.J., G.W. McCarty, and J.M. Bremner. 1989. Phytotoxicity of foliar-applied urea. Proc. Natl. Acad. Sci. 86:8189–8191.

Mengel, K., E.A. Kirkby, H. Kosegarten, and T. Appel. 2001. Principles of Plant Nutrition. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 1.

Miller, B.M. 2017. The horticultural potential of six species of North American hickories, MS Thesis. Iowa State University, Ames, IA. p. 2.

Miller, B.M. and W.R. Graves. 2019. Root pruning and auxin alter root morphology of hickories. HortScience 54:1517–1520.

Poland, T.M. and D.G. McCullough. 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. J. Forestry 104:118–124.

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

Wood, B.W. and L.J. Grauke. 2011. The rare-earth metallome of pecan and other Carya. J. Amer. Soc. Hort. Sci. 136:389–398.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2006. Field deficiency of nickel in trees: Symptoms and causes. Acta Hort. 721:83–97.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004a. Mouse-ear of pecan: I. Symptomology and occurrence. HortScience 38:87–94.

Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004b. Mouse-ear of pecan: II. Influence of nutrient applications. HortScience 38:95–100.

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

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