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Multifactorial Nutrient Interactions Contribute to Mouse-Ear Symptoms in Greenhouse-Grown 'Giles' Pecan Seedlings

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Abstract. Mouse ear (ME) is a growth disorder that poses a significant challenge in pecan seedlings. This study investigates nutrient uptake as a potential cause of ME disorder in 6-month-old 'Giles' pecan seedlings grown in soilless media. Based on visual observations, seedlings were categorized into normal leaves (control) and ME groups. Nutrient analysis of leaf samples showed that nickel concentrations were critically low, and copper levels were excessively high in both groups. ME-affected leaves had higher levels of phosphorus, zinc, sulfur, and magnesium, along with lower nitrogen compared with normal leaves. The nutrient analysis of soilless media indicated that media from ME seedlings had significantly higher levels of most nutrients including sodium, potassium, calcium, chloride, nitrate, and sulfate, compared with media from normal seedlings. The electrical conductivity (EC) of ME group media was also significantly higher. These findings support a multifactorial model of ME, in which limited nickel availability interacts with imbalance of other nutrients in the leaves. In this study, elevated EC, along with increased levels of calcium, chloride, sulfate, and magnesium in the media, may exacerbate the deficiency and contribute to the ME symptoms observed in pecan seedlings. Overall, our results suggest that ME symptoms arise from a complex interplay of deficiencies, toxicities, and disrupted nutrient uptake dynamics. These findings highlight the need for comprehensive nutrient management, including micronutrient balancing and substrate optimization, to prevent ME during the early developmental stages of pecan seedlings grown in greenhouse

Mouse ear (ME) is a distinct growth disorder in pecan [Carva illinoinensis (Wangenh.) K. Koch] seedlings and mature trees, characterized by small, wrinkled, often dark green, commonly cupped, and thickened leaves with necrotic margins, stunted growth, delayed budbreak, and impaired root development (Ruter 2005; Wood et al. 2004a). Mouseear symptoms are commonly observed in newly transplanted pecan seedlings during their second or third year and can lead to severe developmental issues, even causing seedling mortality. This disorder poses a significant economic challenge for pecan growers, as it can reduce orchard productivity and increase management costs (Wood et al. 2004c). Mouse-ear disorder has been reported in other trees, such as river birch (Betula nigra L.) and bitternut hickory [Carya cordiformis

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(Wang.) K. Koch] (Miller and Bassuk 2022; Ruter 2005).

Although early research investigated multiple potential causes of ME, including cold injury, rootstock incompatibility, and general micronutrient deficiencies, later studies increasingly pointed toward a nutritional basis, with nickel (Ni) deficiency emerging as one of the key factors (Wood et al. 2004a, 2004b, 2004c). Wood et al. (2004a) found higher concentrations of calcium (Ca), magnesium (Mg), phosphorus (P), and zinc (Zn) and lower concentrations of copper (Cu) and nickel (Ni) in pecan orchard soils associated with ME symptoms, suggesting that both nutrient imbalances and toxicities may be involved. Building on this, Wood et al. (2004b) evaluated the effects of various foliar nutrient applications and found that treatments with Cu, sulfur (S), and P moderately corrected deficiency after 3 years. In contrast, foliar applications of Ca and Zn induced or worsened symptoms in newly planted pecan trees. This study again indicated the ME linked to deficiency of Cu and Ni at budbreak time or soil Zn accumulation. In the following study, Wood et al. (2004c) reported that Cu foliar application in a greenhouse increased ME severity the following spring, effectively ruling out Cu deficiency as a primary cause of the ME disorder, whereas Ni foliar applications (0.8 g·L⁻¹ Ni) significantly reduced

ME symptoms in pecan trees (5 to 10 years old). Similarly, results have been observed in bitternut hickory and river birch, where Ni application alleviated ME symptoms and improved growth (Miller and Bassuk 2022; Ruter 2005). Ni deficiency was identified as one of the primary causes of ME (Wood et al. 2004c).

Ni is a critical micronutrient for plants, required in very small amounts (1 to 100 ng·g⁻¹ dry weight or 5 to 15 mg·L⁻¹) (Bai et al. 2006). The first reported case of Ni deficiency in field crops was in pecan orchards by Wood et al. (2004b), where researchers observed growth abnormalities in 5- to 10-yearold pecan trees attributed to Ni scarcity (Bai et al. 2007). While low Ni is a known trigger for ME, Ni uptake and functionality are likely influenced by high concentrations of competing or antagonistic nutrients in soil such as Ca, Mg, Zn, Cu, and NO₃, further indicating a multifactorial model (involves the combined influence of interacting factors such as nutrient deficiencies, toxicities, and imbalance conditions rather than a single cause) of ME (Wood et al. 2004c; Yusuf et al. 2011). Furthermore, excessive Cu accumulation in leaves can be toxic and competitively inhibits Ni uptake through shared transport pathways in roots (Wood et al. 2004c).

While prior studies have confirmed Ni's importance in preventing ME, this experiment is the first to investigate early onset of ME symptoms in very young, 6-month-old 'Giles' pecan seedlings grown in a greenhouse setting with soilless media. The present study aimed to evaluate whether ME symptoms observed in about 6-month-old potted 'Giles' pecan seedlings were solely associated with Ni deficiency or whether they arose from broader nutrient imbalances and potential toxicities. By analyzing nutrient concentrations in both leaf tissue and growth media, we aimed to clarify the nutritional context surrounding early ME symptom development in pecans.

Materials and Methods

Plant materials and sampling. 'Giles' pecan seedlings about 6 months old were used in this study. The 'Giles' nuts, harvested as a mixed lot from multiple trees at Cimarron Valley research station (Perkins, OK), were initially germinated for about 6 weeks in soilless media [BM6; Berger, Saint-Modeste, Canada; composition: coarse sphagnum peatmoss (70% to 80%), perlite] within potato grow bags (5 gallons; Gardzen, City of Industry, CA, USA) in the Oklahoma State University Research Greenhouse facility near Stillwater, OK, USA (lat. 36°08′0″.9″N, long. 97°05′1″.9″W). In Apr 2024, all seedlings (about 60 to 70 d old) were transplanted into individual plastic pots $(27.94 \times 10.16 \times 10.16 \text{ cm})$, one seedling per pot, filled with new soilless media (BM6) of similar composition. All seedlings were grown under uniform greenhouse conditions, with the same pot size, media type, temperature, irrigation, and other environmental factors. They were

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subsequently transferred to an open-air area on the same day, where similar conditions were maintained. A slow-release fertilizer, Osmocote[®] (18N–6P–12K; Everris NA Inc., Dublin, OH, USA), was purchased from American Plant Products & Services Inc. (Oklahoma City, OK, USA) and applied to all seedling pots (\sim 10 g/pot) 2 months after transplanting at end of June. Some seedlings began exhibiting symptoms of stunted young compound leaves growth (Fig. 1). Based on the visible ME symptoms in compound leaves, the seedlings were categorized into two groups: normal (control) and ME (Fig. 1). By the end of Aug 2024, three composite leaves samples and three corresponding composite media samples were collected for each group (plants exhibiting normal compound leaves growth and plants exhibiting ME symptoms). Each leaf sample consisted of pooled central leaflets (younger and expanded leaves) collected from 10 different seedlings, to ensure sufficient biomass for nutrient analysis. While we technically collected leaflets, we refer to the pooled leaflet samples as leaf sample throughout this article for consistency and simplicity. Three such composite leaf samples were collected per group (normal and ME), representing a total of 30 seedlings/group. Media samples were also taken from the same pots from which leaves samples were collected to ensure consistency. All leaf samples were thoroughly washed (first rapidly with tap water followed by quick wash in 0.1 N Cl and then washed three times with distilled water), oven-dried (48 h at approximate 75 °C), and, along with the media samples, sent to the Soil, Water and Forage Analytical Laboratory (SWFAL), Oklahoma State University (Stillwater, OK, USA) for nutrient analysis.

Plant tissue and media sample testing. Total nitrogen (N) of leaf tissues was measured by combustion using a carbon/nitrogen analyzer (836 series; LECO Europe, Geleen, The Netherlands). All other leaf nutrients (phosphorus, calcium, potassium, magnesium, sulfur, boron,

iron, zinc, nickel, copper, and manganese) were analyzed using inductively coupled plasma (ICP) spectroscopy (Thermo Fisher Scientific Waltham, MA, USA). For the soilless growth media, the saturated media extract (SME) method was employed (SWFAL laboratory, OSU). This involved saturating the media sample with distilled water, allowing it to equilibrate overnight, and then running the solution through a filter. The extracted solution was analyzed for pH, electrical conductivity (EC), sodium, potassium, calcium, magnesium, sulfur, nickel, copper, and boron analyzed using ICP spectroscopy. Nitrate and chloride were measured with a flow injection analyzer using the cadmium reduction method for nitrate and the ferricyanide method for chloride.

Statistical analysis. All statistical analyses were performed in R (version 4.4.3; R Foundation for Statistical Computing, Vienna, Austria) using RStudio (version 2024.12.1 + 563; Posit Software, PBC, Boston, MA, USA). Means and standard errors (SE) were calculated for each group (normal and ME) for both leaf and soilless media nutrient concentrations. One-way analysis of variance was used to compare nutrient concentrations between the normal and ME groups. Post hoc comparisons were conducted using Tukey's honestly significant difference test (at P < 0.05), implemented via the *multcomp* and *multcompView* packages in R. Compact letter displays (CLDs) were generated to visualize significant differences between treatments.

Results and Discussion

Leaf nutrients. The nutrient analysis of leaves showed that macronutrients such as N, P, Ca, and S levels were within their sufficiency ranges (Wells and Harrison 2024) in both normal and ME leaves, with a few notable exceptions (Table 1). N was significantly lower in ME leaves (2.64%) compared with normal leaves (2.92%). However, P and S

were significantly higher in ME leaves (0.23% and 64.93 mg·L⁻¹, respectively), compared with normal leaves (0.18% and 49.87 mg·L⁻¹, respectively). Potassium (K) was slightly below sufficiency (recommended 1.3% to 2.5%) at 0.96% in normal and 1.03% in ME leaves. While Mg was high in both groups, leaves with ME exhibited higher Mg 0.85% vs. recommend (0.35% to 0.6%).

For micronutrients, boron (B) and iron (Fe) were within normal ranges for both ME leaves. Zn was significantly higher in ME leaves (0.27%) compared with normal leaves (0.24%), even though it was closer to the lower recommended levels. Notably, manganese (Mn) levels were slightly below the recommended range in both groups of leaves. However, Cu was exceptionally high, with concentrations of 155.03 mg·L⁻¹ in normal and 141.20 mg·L⁻¹ in ME leaves (far exceeding the sufficiency range of 6 to 30 mg· L^{-1}). While Cu was not higher in ME seedlings, its excessive levels in both groups are concerning, as such concentrations can interfere with Ni-dependent physiological functions by reducing physiologically available Ni or disrupting its utilization in enzymatic processes (Wood et al. 2004c). Copper can also compete with Ni for uptake through shared ion channels in feeder roots, thereby limiting Ni absorption and availability for plant functions (Durham 2007; Yusuf et al. 2011). This suggests that Cu toxicity itself may not be the distinguishing factor between normal and ME seedlings; however, the overall excessive Cu background likely contributed to Ni limitation in both groups.

Ni levels were extremely low across both groups of leaves with 0.13 and 0.27 mg·L⁻¹ in normal and ME leaves, respectively. Although ME leaves had slightly higher Ni concentrations, the difference was not statistically significant. Also, this Ni level was far below the sufficiency range of 3 to 15 mg·L⁻¹ (Wells 2024) in both normal and ME leaves. This Ni deficiency aligns with the typical ME





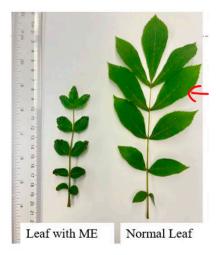


Fig. 1. (A) Normal compound leaves growth in 'Giles' pecan seedlings in pot. Red lines indicate the central leaflets collected for nutrient analysis.

(B) Mouse-ear symptoms displayed by young compound leaves of 'Giles' seedlings. (C) Comparison between normally growing compound leaf (on right) and stunted grown compound leaf affected by mouse-ear disorder (Amandeep Kaur).

Table 1. Leaf nutrients analysis of 'Giles' seedlings with normal leaves and mouse-ear (ME) symptoms grown in soilless media compared with sufficiency range.

$\begin{array}{ c c c c c c c c }\hline Nitrogen (\%) & 2.92 \pm 0.02 \ a & 2.64 \pm 0.05 \ b & 2.5-3.3 & Normal & 0.007 \\ Phosphorus (\%) & 0.18 \pm 0.00 \ b & 0.23 \pm 0.01 \ a & 0.12-0.3 & Normal & 0.01 \\ Calcium (\%) & 1.27 \pm 0.10 \ a & 1.40 \pm 0.07 \ a & 1.3-1.5 & Normal & 0.351 \\ Potassium (\%) & 0.90 \pm 0.01 \ a & 1.03 \pm 0.05 \ a & 1.25-2.5 & Low & 0.054 \\ Magnesium (\%) & 0.74 \pm 0.04 \ a & 0.85 \pm 0.01 \ a & 0.35-0.6 & High & 0.05 \\ Sulfur (\%) & 0.24 \pm 0.00 \ b & 0.27 \pm 0.00 \ a & 0.25-0.5 & Low & 0.007 \\ Boron (mg·L^{-1}) & 37.87 \pm 2.90 \ a & 36.73 \pm 1.81 \ a & 50-100 & Normal & 0.757 \\ Iron (mg·L^{-1}) & 60.70 \pm 2.08 \ a & 71.13 \pm 5.39 \ a & 50-300 & Normal & 0.145 \\ Zinc (mg·L^{-1}) & 49.87 \pm 0.79 \ b & 64.93 \pm 3.34 \ a & 50-100 & Normal & 0.011 \\ Nickel (mg·L^{-1}) & 0.13 \pm 0.07 \ a & 0.27 \pm 0.15 \ a & 3-15 & Very Low & 0.451 \\ Copper (mg·L^{-1}) & 155.03 \pm 8.10 \ a & 141.20 \pm 5.79 \ a & 6-30 & Very High & 0.237 \\ Manganese (mg·L^{-1}) & 80.33 \pm 8.50 \ a & 78.33 \pm 2.49 \ a & 100-800 & Low & 0.832 \\ \end{array}$	Nutrients	Normal leaves ⁱ	ME leaves	Sufficiency range of leaf nutrients ⁱⁱ	Nutrient status in ME 'Giles' leaves (relative to sufficiency range)	P value ⁱⁱⁱ
Calcium (%) 1.27 ± 0.10 a 1.40 ± 0.07 a $1.3-1.5$ Normal 0.351 Potassium (%) 0.90 ± 0.01 a 1.03 ± 0.05 a $1.25-2.5$ Low 0.054 Magnesium (%) 0.74 ± 0.04 a 0.85 ± 0.01 a $0.35-0.6$ High 0.05 Sulfur (%) 0.24 ± 0.00 b 0.27 ± 0.00 a $0.25-0.5$ Low 0.007 Boron (mg·L ⁻¹) 37.87 ± 2.90 a 36.73 ± 1.81 a $50-100$ Normal 0.75 Iron (mg·L ⁻¹) 60.70 ± 2.08 a 71.13 ± 5.39 a $50-300$ Normal 0.145 Zinc (mg·L ⁻¹) 49.87 ± 0.79 b 64.93 ± 3.34 a $50-100$ Normal 0.011 Nickel (mg·L ⁻¹) 0.13 ± 0.07 a 0.27 ± 0.15 a $3-15$ Very Low 0.451 Copper (mg·L ⁻¹) 155.03 ± 8.10 a 141.20 ± 5.79 a $6-30$ Very High 0.237	Nitrogen (%)	2.92 ± 0.02 a	$2.64 \pm 0.05 \text{ b}$	2.5-3.3	Normal	0.007
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Phosphorus (%)	$0.18 \pm 0.00 \text{ b}$	$0.23 \pm 0.01 \text{ a}$	0.12-0.3	Normal	0.01
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Calcium (%)	$1.27 \pm 0.10 \text{ a}$	$1.40 \pm 0.07 \text{ a}$	1.3-1.5	Normal	0.351
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Potassium (%)	$0.90 \pm 0.01 \text{ a}$	$1.03 \pm 0.05 \text{ a}$	1.25-2.5	Low	0.054
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Magnesium (%)	$0.74 \pm 0.04 a$	$0.85 \pm 0.01 \text{ a}$	0.35-0.6	High	0.05
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Sulfur (%)	$0.24 \pm 0.00 \text{ b}$	$0.27 \pm 0.00 \text{ a}$	0.25-0.5	Low	0.007
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Boron $(mg \cdot L^{-1})$	$37.87 \pm 2.90 \text{ a}$	$36.73 \pm 1.81 \text{ a}$	50-100	Normal	0.757
Nickel (mg·L $^{-1}$) 0.13 ± 0.07 a 0.27 ± 0.15 a 3–15 Very Low 0.451 Copper (mg·L $^{-1}$) 155.03 ± 8.10 a 141.20 ± 5.79 a 6–30 Very High 0.237	Iron $(mg \cdot L^{-1})$	$60.70 \pm 2.08 \text{ a}$	$71.13 \pm 5.39 \text{ a}$	50-300	Normal	0.145
Copper (mg·L ⁻¹) 155.03 ± 8.10 a 141.20 ± 5.79 a $6-30$ Very High 0.237	Zinc $(mg \cdot L^{-1})$	$49.87 \pm 0.79 \text{ b}$	$64.93 \pm 3.34 \text{ a}$	50-100	Normal	0.011
	Nickel (mg·L ⁻¹)	$0.13 \pm 0.07 \text{ a}$	$0.27 \pm 0.15 \text{ a}$	3–15	Very Low	0.451
	Copper $(mg \cdot L^{-1})$	$155.03 \pm 8.10 a$	$141.20 \pm 5.79 \text{ a}$	6–30	Very High	0.237
	Manganese (mg·L ⁻¹)					

The data represent means \pm standard error (N=3) for normal and ME leaf groups. Each sample consisted of a pooled set of central leaflets collected from younger, fully expanded compound leaves of 10 different seedlings. Thus, three pooled leaf samples were prepared per group (leaflets from a total of 30 seedlings/group). Different lowercase letters indicate significant differences among means within each nutrient between normal and ME leaves, as determined by Tukey's honestly significant difference test (P < 0.05).

symptoms, as Ni is crucial for ureide metabolism and overall seedling growth. The stunted growth and leaf deformities observed in our ME-affected seedlings (Fig. 1B and 1C) are consistent with previous reports of Ni deficiency symptoms (Wells 2004; Wood et al. 2004a). However, the fact that normal leaves also exhibited similarly low Ni concentrations suggests that Ni deficiency alone may not be sufficient to induce ME symptoms in young pecan seedlings. Instead, it is likely that ME results from the combined effects of Ni deficiency and other nutrient imbalances such as lower N and higher Mg, P, S, and Zn levels, which may interfere with Ni uptake, transport, or function.

Soilless media nutrients. Nutrient content was analyzed from the transplant soilless media (BM6); fresh media were used after germination to grow the seedlings in individual pots. This analysis does not reflect the initial

germination media but specifically pertains to the new BM6 media used during the transplant phase. Soilless media analyses further support a multifactorial cause of ME by revealing elevated nutrient concentrations in ME-affected seedlings pots, many of which exceeded sufficiency thresholds (Table 2). Media from ME seedlings had significantly higher EC (3706.67 µS·cm⁻¹) than normal seedlings (2580.00 µS·cm⁻¹). Although EC remained within the upper sufficiency limit (750 to 3500 μS·cm⁻¹), localized salt accumulation may impair root function and nutrient selectivity, contributing to the development of ME symptoms. It is also possible that the higher residual nutrient levels observed in ME seedling pots are a consequence of reduced nutrient uptake rather than a cause of ME symptoms. In other words, poor root development or impaired physiological function in symptomatic seedlings may have limited nutrient absorption, leaving more nutrients behind in the media. This interpretation is supported by the observed mismatch between media and leaf nutrient levels, which is discussed in more detail below.

Among the macronutrients, Ca and SO₄ were significantly higher in media from seedling with ME symptoms (309.40 and 428.80 mg·L⁻¹, respectively) compared with media from normal seedlings (213.50 and 277.63 mg·L⁻¹, respectively). Both Ca and SO₄ exceeded optimal sufficiency ranges for Ca (80 to 200 $\text{mg} \cdot \text{L}^{-1}$) and SO₄ (20 to 200 mg L^{-1}) (Hu et al. 2021). Similarly, Cl andnitrate-nitrogen (NO₃ N) were also significantly higher in media from seedling with ME symptoms (44.43 and 344.90 mg·L $^{-1}$, respectively) compared with media from normal seedlings (33.47 and 249.93 $\text{mg}\cdot\text{L}^{-1}$). This trend was particularly notable when considered alongside the leaf nutrients data,

Table 2. Nutrient profile of soilless media from 'Giles' pecan seedlings with normal and mouse-ear (ME) symptoms, with sufficiency ranges as reference.

Soilless media nutrients	Normal ⁱ	ME	Sufficiency range of media nutrients ⁱⁱ	Nutrient status in ME 'Giles' media (relative to sufficiency range)	P value ⁱⁱⁱ
pH	4.60 ± 0.20 a	4.63 ± 0.12 a	5.5-6.3	Low	0.893
EC (μS·cm ⁻¹)	$2580.0 \pm 187.7 \text{ b}$	$3706.6 \pm 252.2 \text{ a}$	750–3500	Normal	0.023
Na $(\text{mg} \cdot \text{L}^{-1})$	$48.30 \pm 2.06 \text{ b}$	$56.27 \pm 1.30 \text{ a}$	<160	Normal	0.03
$K (mg \cdot L^{-1})$	$140.00 \pm 9.87 \text{ b}$	$215.33 \pm 16.56 \text{ a}$	60–249	Normal	0.017
$Ca (mg \cdot L^{-1})$	$213.50 \pm 8.63 \text{ b}$	$309.40 \pm 21.37 \text{ a}$	80–200	High	0.014
$Mg (mg \cdot L^{-1})$	$114.20 \pm 6.32 \text{ a}$	$149.83 \pm 12.14 a$	30–100	High	0.059
$Cl (mg \cdot L^{-1})$	$33.47 \pm 0.75 \text{ b}$	$44.43 \pm 3.15 \text{ a}$	_	_	0.027
$NO_3^- N (mg \cdot L^{-1})$	$249.93 \pm 17.45 \text{ b}$	$344.90 \pm 26.99 a$	40–199	High	0.041
$SO_4 (mg \cdot L^{-1})$	$277.63 \pm 15.12 \text{ b}$	$428.80 \pm 17.11 \text{ a}$	20-200	High	0.002
$B (mg \cdot L^{-1})$	$0.18 \pm 0.02 \text{ a}$	0.28 ± 0.06 a	0.05-1.0	Normal	0.162
$NH_4 (mg \cdot L^{-1})$	$0.60 \pm 0.25 \text{ a}$	$0.37 \pm 0.19 \text{ a}$	0–20	Normal	0.497
Cu (mg·L ⁻¹)	$0.18 \pm 0.02 \text{ a}$	$0.20 \pm 0.04 \text{ a}$	0.5-1.5	Low	0.68
Ni (mg·L ⁻¹)	$< 0.08 \pm 0.00 \text{ a}$	$< 0.08 \pm 0.00 \text{ a}$	$3-1000^{iv}$	Low	0.374

The data represents standard error (N=3) for normal and ME groups. Each sample consisted of soilless media collected from 10 different seedling pots from which leaves were sampled, resulting in three pooled media samples/group (media from a total of 30 pots/group). Different lowercase letters indicate significant differences between normal and ME groups for each nutrient, as determined by Tukey's honestly significant difference test (P < 0.05).

ii Sufficiency range is from range (Wells 2024; Wells and Harrison 2024).

iii P values obtained from a one-way analysis of variance using treatment (normal vs. ME group) as the main factor. Bold values are statistically significant ($P \le 0.05$).

ii Sufficiency range is from Hu et al. (2021).

iii P values obtained from a one-way analysis of variance using treatment (normal vs. ME group) as the main factor.

iv Nickel range is for soils not media (Iyaka 2011).

EC = electrical conductivity.

where N and S concentrations were lower in ME-affected seedlings despite higher availability of these nutrients in the media. This discrepancy suggests a disruption in nutrient uptake efficiency or translocation mechanisms in symptomatic seedlings, potentially due to root stress or antagonistic interactions among nutrients.

Mg concentrations, while not significantly different between treatments, were notably high in ME media at 149.83 mg·L⁻¹, exceeding the recommended range of 30 to 100 mg·L⁻¹. This elevated Mg level, in conjunction with high Ca concentrations, may contribute to nutrient imbalances. Wood et al. (2004a) observed slightly different patterns in second-generation orchard soils, where high soil levels of Ca and Mg were associated with ME symptoms in pecan trees. In another study, Ca application induced ME symptoms in pecans (Wood et al. 2004b). In contrast, the association in our study was less pronounced, possibly due to the younger age of seedlings compared with the bigger trees studied by Wood et al. (2004a, 2004b).

Interestingly, Cu content did not differ significantly between normal and ME but remained below the expected range in the media. However, Cu was found at excessively high levels in the leaves of both normal and ME seedlings, indicating that Cu was either preferentially taken up or inadequately regulated within the plant. Ni content was also very low in the media ($<0.08 \text{ mg}\cdot\text{L}^{-1}$) in both groups, and no supplemental Ni was applied during the experiment. Consistently, Ni content in the leaves of both ME and normal seedlings were also extremely low, well below the established sufficiency range. This suggests that limited Ni availability was a primary constraint on uptake but likely not the sole driver of ME symptoms. Although the pH in both treatments was below the optimal range, this was not necessarily critical, as a study by Keever et al. (1991) reported the best growth of pecan seedlings (compared with other treatments) at a pH of 4.3, showing that low pH can still support healthy growth.

In summary, media from ME seedlings displayed significantly elevated levels of Ca, SO₄, Cl, and NO₃ N. These elevated concentrations may have contributed to nutrient uptake disruption or competitive inhibition, particularly in relation to Ni. Mg levels, although not significantly different between groups, were higher in ME media pots and could have further compounded ionic imbalance. In contrast, Cu and Ni remained low in the media across all treatments, indicating that their extreme leaf concentrations likely resulted from root-level uptake dynamics rather than media abundance. These findings support the idea that nutrient imbalances, rather than excesses alone, played a role in limiting Ni uptake and inducing ME symptoms. These observations align with findings in Alyssum bertolonii desv., where Ca and Mg ions in the growth medium were shown to suppress Ni ion uptake due to competition for a shared uptake system in the roots (Gabbrielli and Pandolfini 1984).

Additionally, excessive fertilizer applications can induce Ni deficiency in pecan trees (Wood et al. 2006). This may be relevant to our study since a slow-release NPK fertilizer (Osmocote 18N–6P–12K) was applied in June (at the recommended rate per manufacturer instructions on the fertilizer bag) to all seedling pots. Even though the application rate was not excessive by standard guidelines, the combination of young seedlings, confined root area, and soilless media may have created localized nutrients concentrations that potentially contributed to the excessive buildup of certain nutrients in the media and to the observed Ni deficiency in both normal and ME seedlings.

Conclusions

The study suggests that ME symptoms in 'Giles' pecan seedlings are not solely caused by single nutrient deficiency but rather arise from a complex interplay of nutrient imbalances, uptake limitations, and potential toxicities. While Ni levels were critically low in both normal and ME-affected seedlings, and Cu levels were excessively high in both groups of leaves, only a subset of seedlings developed ME symptoms. This indicates that although Ni deficiency and Cu accumulation may contribute to ME, they are not the sole causes. The ME symptoms appear to be correlated with elevated concentrations of competing nutrients such as Ca, Cl, K, SO₄, and nitrate NO₃ in the media of ME seedlings symptoms compared with normal seedlings. Nutrient uptake dynamics could have been disrupted by excessive accumulation of these nutrients, potentially contributing to Ni deficiency and broader nutrient imbalances in the leaves. Given that Ni was not included in the fertilizer and was present at very low concentrations in the media, its overall availability was already limited, and competition from other cations may have further restricted its uptake.

Furthermore, elevated Zn, P, and Mg in ME-affected leaves, along with significantly higher media EC in ME pots, reflect additional factors that may impair nutrient selectivity and transport. The observed mismatch between nutrient supply in the media and actual uptake into leaf tissue (e.g., high media N vs. low leaf N) points to impaired root nutrient uptake or transport.

Overall, these findings support a multifactorial model of nutrient dynamics in young pecan seedlings exhibiting ME in which a combination of Ni deficiency, nutrient antagonism, possibly salt stress, and unbalanced nutrient uptake were correlated with ME symptom development. Future efforts to manage ME in nursery settings should emphasize not only Ni supplementation but also careful monitoring of substrate EC and cationic nutrient ratios to ensure balanced nutrient availability and uptake. This study also highlighted the importance of early detection and nutritional management to prevent ME symptom development during the initial stages of pecan establishment. Although this study did not evaluate recovery, previous research suggests that early correction of Ni deficiency and nutrient imbalances may alleviate ME symptoms (Wood et al. 2006). Future research should test whether targeted nutrient adjustments, particularly through Ni supplementation and EC regulation, can reverse ME symptoms once they appear in nursery-grown pecan seedlings.

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