Association of *Meloidogyne partityla* with Nickel Deficiency and Mouse-ear of Pecan

A.P. Nyczepir,^{1,4} B.W. Wood,² and C.C. Reilly³

U.S. Department of Agriculture, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008

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Abstract. Pecan [*Carya illinoensis* (Wangenh.) K. Koch] trees exhibit nickel (Ni) deficiency in certain orchard situations. The symptoms are manifest as either mouse-ear or replant disorder and in certain situations are associated with nematode parasitism. A field microplot study of pecan seedlings treated with either *Meloidogyne partityla* or *Criconeimoides xenoplax* or both found that parasitism by *M. partityla* can result in enhancement in the severity of mouse-ear symptoms and a reduction in foliar Ni concentration. The Ni threshold for triggering morphological symptoms in young developing foliage was between 0.265 and 0.862 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight, while the threshold for rosetting was between 0.007 and 0.064 $\mu\text{g}\cdot\text{g}^{-1}$ dw. Results indicate that parasitism by *M. partityla* is a contributing factor to the induction of Ni deficiency in pecan and raises the possibility that nematode parasitism and Ni nutrition can be contributing factors to many plant maladies.

Pecan is a major worldwide tree-nut crop that is North America's most valuable native tree-nut, with worldwide cultivation and substantial production in the United States and Mexico (Wood, 1994; Wood et al., 1990). It is also becoming an increasingly common crop in several other Western Hemisphere countries (e.g., Argentina, Brazil, Chile, Peru, and Uruguay) and is a significant horticultural crop in Australia and South Africa. This tree crop is attacked by a wide variety of disease and insect pests that greatly reduce tree productivity if improperly managed. Pecan is also prone to exhibit certain trace element deficiencies (e.g., Zn) when grown in nonhydrophilic *oligotrophic* soils; consequently, such soils are alien to the hydrophilic *eutrophic* soils that typify its ecological niche in the river bottoms of the central and southern United States.

The mouse-ear (ME) or little leaf malady of pecan is a nutrient disorder that has manifest itself as far back as the early 1900's (Demaree, 1926) and has increasingly manifest itself more recently as an orchard "replant" disorder (Wood et al., 2004a). Both the ME and replant disorders have recently been found to be due to a nickel (Ni) deficiency, with timely foliar application of Ni correcting both disorders (Wood et al., 2004a; 2004b). Examination of Ni deficient trees sometimes, but not always, exhibited evidence of nematode damage, especially that of root-knot nematode.

The nematode species, *Meloidogyne incognita* (Kofoid and White) Chitwood and

M. arenaria (Neal) Chitwood are known to attack pecan in Georgia. However, in 2002, the pecan root-knot nematode, *M. partityla* Kleynhans, was found on pecan in the southeastern U.S. and was associated with stressed trees exhibiting dead branches in the upper canopy and/or typical ME associated foliar symptoms (Nyczepir et al., 2002). This was the first report of *M. partityla* on pecan in Georgia and the third report of this nematode outside of South Africa (Kleynhans, 1986); which was where the original description occurred. Starr et al. (1996) first reported *M. partityla* from pecan in the U.S. in 1996. Additionally, the ring nematode, *Criconeimoides xenoplax* Raski [= *Mesocriconeima xenoplax* (Raski) Loof & de Grisse], was detected in the same soil as *M. partityla* in certain of these ME orchards in Georgia. In 2003–04, a survey was conducted in the major pecan growing regions of Georgia to determine distribution of *M. partityla* and other *Meloidogyne* spp. (Nyczepir et al., 2004). *Meloidogyne partityla* and two unknown *Meloidogyne* spp. were the only root-knot nematode species found parasitizing pecan. *Meloidogyne partityla* was found in a greater number of samples and appears to be the dominant root-knot nematode species in pecan.

The occasional association of nematode damaged pecan root systems with symptoms of ME and pecan replant disorder indicates that nematode parasitism is potentially a factor that triggers ME and Ni deficiency on certain sites. The present study demonstrates that root parasitism by *M. partityla* influences the severity of ME symptoms in pecan trees.

Materials and Methods

Inoculum source and production. The *C. xenoplax*, which originated from a pecan orchard with ME symptoms in Leary, Ga., was

cultured on 'Desirable' pecan seedlings; and *M. partityla* eggs, originating from a commercial pecan orchard with ME symptoms in Cobb, Georgia, were extracted directly from the roots of an individual tree. Identification of the root-knot nematode as *M. partityla* was confirmed via the esterase phenotype technique (Esbenshade and Triantaphyllou, 1985). Root-knot nematode eggs were extracted from pecan roots using the method described by Hussey and Barker (1973), whereas *C. xenoplax* was extracted from the culture medium using centrifugation (Jenkins, 1964).

Field microplot study. Approximately 4-week-old open-pollination 'Desirable' pecan seedlings were planted singly in bucket microplots (Barker, 1985) (25-cm-diameter $\times$ 31-cm-deep) containing 15,000 cm^3 of steam pasteurized soil (96% sand, 0% silt, 4% clay, pH 7.0, 1.27% organic matter) in May 2002. The soil was obtained from under pecan trees exhibiting typical ME symptoms in a commercial orchard located in Sumter County, Ga. Nickel (Ni) concentration in the soils within the immediate area of this orchard ranged between 15.41 to 20.02 ppm and is usually sufficient for plant needs. Microplots were established in a shaded area (30% shade) in the field in Byron, Georgia. In July 2002, after seedling survival was evident, the following nematode treatments were added per microplot: 1) 4,000 *M. partityla* eggs (Mp); 2) 4,000 *C. xenoplax* adults and juveniles (Cx); 3) 4,000 *C. xenoplax* adults and juveniles + 4,000 *M. partityla* eggs (Cx + Mp); and 4) an untreated control. The soil in each microplot was infested with the respective nematode inoculum in 40 ml total solution added to two furrows (10 cm long $\times$ 3 cm wide $\times$ 7 cm deep) around each seedling. The soil in the untreated control microplots was inoculated with an egg/nematode-free soil extract suspension from the same *M. partityla* pecan orchard and *C. xenoplax* culture. Note that an unpasteurized field soil treatment also was included which contained 15 *C. xenoplax* per 100 cm^3 soil and no detectable *M. partityla*. The experiment was arranged as a randomized complete block design with nine treatment replicates. Plants were watered as needed and received no fertilizer for the duration of the experiment, as the orchard soil possessed both macro and micronutrients at levels to satisfy the needs of pecan seedlings. Annual applications of fertilizer were not made because most fertilizers contain nickel as a trace contaminate and would thus confound the experiment. Also, limited space did not allow for additional treatments. Treatment trees were evaluated for ME severity in April 2003, May 2004, and May 2005. ME severity was based on the following scale derived for identification of Ni deficiency symptoms by Wood et al. (2004a, 2004b): 1 = no Ni associated morphological distortions of leaflets or leaves (i.e., normal); 2 = 1% to 25% of leaflets on the seedling exhibiting Ni deficient morphological distortions (i.e., slightly blunted); 3 = 26% to 50% of leaflets exhibiting some degree of Ni associated morphological distortions; 4 = >50% of leaflets exhibiting morphological distortions; 5 = #4, plus leaflet cupping; 6 =

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¹Research nematologist.

²Research horticulturist.

³Research plant pathologist.

⁴To whom reprint requests should be addressed; e-mail anycep@ars.usda.gov.

#5, plus necrosis of leaflet tips; 7 = #6, plus necrosis of leaflet margins, crinkled leaflets, and dwarfed leaflets; 8 = #7, plus dwarfed shoots; 9 = #8, plus rosetting; and 10 = #9, plus tree death. Data were subjected to analysis of variance with the general linear model (GLM) procedure of SAS (SAS Institute, Cary, N.C.). Treatment means were separated with Fisher's protected least significant difference (LSD) test following a significant *F*-test. Nickel content of foliage from among the test trees exhibiting different degrees of ME symptoms (i.e., specifically ME rating 1, 2, 7, 9; see above), regardless of nematode treatment, was determined by atomic absorption spectroscopy using standard techniques (Wood et al., 2004a) in May 2005, and was done to link ME severity class to tissue Ni concentration. Data were subjected to analysis of variance and treatment means separated with Student's *t* test. In Aug 2005, nematode soil populations were determined by collecting and thoroughly mixing four soil cores (2.5 × 30 cm deep) from each microplot. Nematodes were extracted from a 100-cm³ subsample by elutriation (Byrd et al., 1976) and centrifugation (Jenkins, 1964) and then counted.

Results and Discussion

Nematode effect on severity of foliar ME symptoms was evident among treatments during the first two growing seasons (Table 1). In April 2003 (9 months following inoculation) ME severity was greater ($P \leq 0.05$) for trees growing in *M. partityla* infested soil than for trees in the untreated control, unpasteurized soil, or *C. xenoplax* plots. In May 2004 (21 months following nematode inoculation) ME symptom severity was also greater for trees growing in *M. partityla* infested soil than that of the untreated control. No differences in ME severity were detected among the unpasteurized soil, *C. xenoplax* and untreated control plots during either of these two growing seasons. By May 2005 (33 months following inoculation), no differences ($P = 0.17$) in ME symptom severity were detected among the five treatments. However, the trend among the means was consistent in that the *M. partityla* trees exhibited the more severe ME symptoms as compared to the untreated control or the other treatments. The presence of *M. partityla* or *C. xenoplax* in the inoculated treatments was verified following ME severity rating and Ni

leaflet analysis. Average nematode soil population densities were as follows: 1) *M. partityla* (23 Mp J2/100 cm³ soil), 2) *C. xenoplax* (1,052 Cx/100 cm³ soil), 3) Mp + Cx (17 Mp J2 & 38 Cx/100 cm³ soil, respectively), and 4) unpasteurized field soil (5 Mp J2 and 183 Cx/100 cm³ soil, respectively). The presence of *M. partityla* appeared to suppress the population density of *C. xenoplax* 33 months after inoculation as compared to *C. xenoplax* alone. The results indicate biological antagonism between the nematode species and that host specificity is shifted in favor of *M. partityla*, since more severe ME symptoms occurred in the presence of this nematode alone. Further investigations are warranted to clarify these observations.

The severity of ME symptoms in the current study was inversely proportional ($P \leq 0.05$) to Ni concentration in the pecan leaflets (Table 2), thus supporting earlier findings that ME severity in pecan is due to a physiological nickel deficiency (Wood et al., 2004a). In that same study, Wood et al. (2004a) also demonstrated that severe ME symptoms can be reversed in subsequent shoot, leaf and leaflet growth following Ni application immediately after budbreak in the spring. Seedlings exhibited morphologically normal canopy (leaflet and shoot) growth when foliar Ni content was $\geq 0.862 \mu\text{g}\cdot\text{g}^{-1}$ dry weight under the conditions of our trial (Table 2). The foliar Ni content of leaflets exhibiting slight morphological foliar distortions (i.e., leaflet blunting) contained Ni at $0.265 \mu\text{g}\cdot\text{g}^{-1}$ dry weight; thus it appears that the threshold leading to morphological distortion of young developing foliage is a Ni concentration between 0.265 and $0.862 \mu\text{g}\cdot\text{g}^{-1}$ dry weight. The Ni threshold for marginal necrosis and wrinkled lamina (crinkling) is between 0.064 and $0.265 \mu\text{g}\cdot\text{g}^{-1}$ dry weight, whereas that for rosetting appears to be between 0.007 and $0.064 \mu\text{g}\cdot\text{g}^{-1}$ dry weight. These threshold concentrations indicate that morphological evidence of Ni deficiency is not manifested until Ni concentrations are below ca. $0.862 \mu\text{g}\cdot\text{g}^{-1}$ dry weight during the time of leaf expansion. One explanation for nickel deficiency, or ME, in certain pecan orchards is that root parasitism by *M. partityla* is potentially affecting the absorption and/or translocation of the Ni or other metals that influence the physiological availability of Ni. Identification of the mechanism of action therefore requires additional research.

The present study indicates that severity of ME symptoms, and thus Ni deficiency, in pecan trees can potentially be triggered or enhanced by parasitism of roots by *M. partityla*. The parasitic habit of *M. partityla* likely affects the timely absorption and subsequent translocation of the Ni to aerial organs, thus disrupting plant metabolism and visually detectable growth processes when the Ni concentration of young expanding foliage is between $0.265 \mu\text{g}\cdot\text{g}^{-1}$ and $0.862 \mu\text{g}\cdot\text{g}^{-1}$ dry weight. Results also indicate that parasitism by *C. xenoplax* appears to have little or no influence on ME severity or Ni nutrition of pecan. The interactions of nematode parasitism and plant Ni nutrition merits further study in regards to several complex

Table 1. Mouse-ear (ME) severity in open pollinated 'Desirable' pecan seedlings grown in field microplots and evaluated nine, 21, and 33 months after inoculation with *Meloidogyne partityla* or *Crictonemoides xenoplax* alone and in combination (n = 9).^a

Treatment	Mouse-ear severity rating ^b		
	Apr. 2003	May 2004	May 2005
<i>M. partityla</i> (Mp)	8.3 a ^x	8.2 a	9.2 a
Mp + Cx	7.6 ab	5.2 c	7.4 a
Unpasteurized soil	5.9 bc	7.3 ab	6.9 a
<i>C. xenoplax</i> (Cx)	5.9 bc	6.7 abc	6.8 a
Untreated control	4.6 c	6.6 bc	6.4 a

^aInitial population density of *Meloidogyne partityla* = 267 eggs/100 cm³ soil, *Crictonemoides xenoplax* = 267 juveniles and adults/100 cm³ soil, and Mp + Cx = 267 Mp + 267 Cx/100 cm³ soil.

^bME severity was based on the following foliar symptom class scale: 1 = no Ni associated morphological distortions of leaflets or leaves (i.e., normal); 2 = 1% to 25% of leaflets on the seedling exhibiting Ni deficient morphological distortions (i.e., slightly blunted); 3 = 26% to 50% of leaflets exhibiting some degree of Ni associated morphological distort; 4 = >50% of leaflets exhibiting morphological distortion; 5 = #4, plus leaflet cupping; 6 = #5, plus necrosis of leaflet tips; 7 = #6, plus necrosis of leaflet margins, crinkled leaflets, and dwarfed leaflets; 8 = #7, plus dwarfed shoots; 9 = #8, plus rosetting; and 10 = #9, plus tree death.

^cMeans in columns followed by the same letters are nonsignificant ($P \leq 0.05$), Fisher's protected LSD.

Table 2. Nickel concentrations in young leaflets of open pollinated 'Desirable' pecan seedlings exhibiting different degrees of mouse-ear (ME) symptoms due to nematode treatment and grown in field microplots 33 months after inoculation with *Meloidogyne partityla* or *Crictonemoides xenoplax* alone and in combination^a.

Foliar symptom class ^b	Ni concn ($\mu\text{g}\cdot\text{g}^{-1}$ dry wt)
Normal (Class = 1)	0.862 a ^x
Slightly blunted leaflets (Class = 2)	0.265 b
Dwarfed and blunted leaflets with tip necrosis, curved margins, and crinkling (Class = 7)	0.064 c
Class 7, plus dwarfed shoots and rosetting (Class = 9)	0.007 d

^aLeaflet sample taken near the end of canopy expansion in late May 2005, from both the untreated control plots and nematode-inoculated plots with an initial population density of *Meloidogyne partityla* = 267 eggs/100 cm³ soil, *Crictonemoides xenoplax* = 267 juveniles and adults/100 cm³ soil, and Mp + Cx = 267 Mp + 267 Cx/100 cm³ soil. Leaflet samples were composited within a respective foliar symptom class regardless of treatment.

^bME severity was based on the following foliar symptom class scale: 1 = no Ni associated morphological distortions of leaflets or leaves (i.e., normal); 2 = 1% to 25% of leaflets on the seedling exhibiting Ni deficient morphological distortions (i.e., slightly blunted); 3 = 26% to 50% of leaflets exhibiting some degree of Ni associated morphological distort; 4 = >50% of leaflets exhibiting morphological distortion; 5 = #4, plus leaflet cupping; 6 = #5, plus necrosis of leaflet tips; 7 = #6, plus necrosis of leaflet margins, crinkled leaflets, and dwarfed leaflets; 8 = #7, plus dwarfed shoots; 9 = #8, plus rosetting; and 10 = #9, plus tree death.

^cMeans in columns followed by the same letters are nonsignificant ($P \leq 0.05$), Student's *t* test.

replant diseases or disorders exhibited by a wide variety of woody perennial crops.

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