Research

Nematotoxicity of *Paeonia* spp. Extracts and *Camellia oleifera* Tea Seed Cake and Extracts to *Heterodera glycines* and *Meloidogyne incognita*Yanhua Wen,¹ Susan L. F. Meyer,^{2†} Margaret H. MacDonald,² Liuchun Zheng,¹ Chunyue Jing,¹ and David J. Chitwood²¹ Guangdong Province Key Laboratory of Microbial Signals and Disease Control, College of Agriculture, South China Agricultural University, Guangzhou 510642, China² United States Department of Agriculture, Agricultural Research Service, Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD 20705, U.S.A.

Abstract

Tea-oil camellia (*Camellia oleifera*) is grown for tea seed oil production, with tea seed cake produced as a byproduct. Rather than disposing of the cake, agricultural uses increase the value of oil production. Constituents of *C. oleifera* are also utilized for traditional Chinese medicine, as are compounds produced by tree peony roots. Consequently, the unused *C. oleifera* cake, and stems from two tree peony species, *Paeonia rockii* and *Paeonia suffruticosa*, were studied for compounds antagonistic to soybean cyst nematode (*Heterodera glycines*) and root-knot nematode (*Meloidogyne incognita*). Extracts from *C. oleifera* cake and *P. rockii* stems suppressed hatch and were nematotoxic to second-stage juveniles (J2) of both nematode species. *P. rockii* extracts were more effective than *P. suffruticosa* extracts for decreasing *M. incognita* hatch and J2

viability. In greenhouse trials with soybean (*Glycine max* 'Essex'), powdered *C. oleifera* cake applied as a soil amendment suppressed *H. glycines* cysts/g root by up to 66% compared with nonamended controls. These results indicate that the extracts and cake contain compounds active against *H. glycines* and *M. incognita*, with activity varying between the two *Paeonia* species. *C. oleifera* tea seed cake, and constituents of the cake or of *P. rockii*, are candidates for further studies on management of these nematodes.

Keywords: *Camellia*, *Heterodera*, management, *Meloidogyne*, natural product, nematode, *Paeonia*, plant extract, root-knot nematode, soil amendment, soybean cyst nematode

Deregistration of synthetic nematicides has led to development of novel nematocidal compounds, often with a focus on plant-derived compounds. Plants with traditional uses in herbal medicine have been a rich source of materials for research in this area. Two plants that originated in Asia, *Camellia oleifera* Abel and *Paeonia rockii* (S.G. Haw & Lauener) T. Hong & J.J. Li, have demonstrated nematocidal activity, and were therefore selected for further studies. In addition, a cultivar of the domesticated *Paeonia suffruticosa* Andrews was studied for comparison with the naturally occurring *P. rockii*.

C. oleifera is grown for production of tea seed oil, and tea seed cake (also called tea seed meal) is a byproduct of oil production. Approximately 7 tons of this cake are produced when a ton of seed oil is extracted, and in China alone, several million tons of the cake are discarded annually (Gong et al. 2018; Hu et al. 2012; Liu et al. 2016). Utilization of the tea seed cake, rather than disposal, increases the value of tea seed oil production.

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The cake produces compounds, including saponins, that are active against many organisms. For example, a saponin-rich fraction from *C. oleifera* cake inhibited growth of the bacteria *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* and the fungi *Aspergillus oryzae*, *Mucor racemosus*, *Penicillium glaucum*, *Rhizopus stolonifer*, and *Saccharomyces cerevisiae* (Hu et al. 2012). The cake is active against mollusks, and the meal has been applied to fields to control snails (Kijprayoon et al. 2014). Ethanol and methanol extracts, volatile compounds, and saponins from *C. oleifera* cake were nematocidal to *Meloidogyne javanica* second-stage juveniles (J2) and suppressed egg hatch, and cake reduced galling and/or numbers of this root-knot nematode in pot trials with banana, Malabar spinach, and water spinach (Li et al. 2013a; Yang et al. 2015). Tea seed cake and/or saponin extracts were also active against *Meloidogyne incognita* (Kofoed & White) Chitwood and *Bursaphelenchus xylophilus* in laboratory assays, suppressed *M. incognita* on tomato in pot studies, and reduced *M. incognita* populations in a field test with *Trichosanthes kirilowii* (Wang et al. 2013). In greenhouse experiments, tea seed cake application to soil also increased abundance of soil microbes and free-living nematodes, and decreased numbers of *Meloidogyne* spp. and *Helicotylenchus* spp. (Yang et al. 2018).

The genus *Paeonia* has long been cultivated in China for ornamental value and use in traditional Chinese medicine (Bao et al. 2018). Species of peony have not been widely investigated for production of nematocidal constituents, but several studies have indicated that such compounds are present in tree peonies. *Paeonia suffruticosa* and *Paeonia moutan* were reported as susceptible hosts for *M. incognita* (Park et al. 2007); however, root bark reduced gall formation by *M. incognita* on tomato and increased shoot weights and plant heights (Kim et al. 2003). Essential oil from *P. suffruticosa* was also nematocidal to *B. xylophilus* (Choi et al. 2007). Because *Paeonia* roots are utilized in traditional Chinese herbal medicine, the unused stems were studied as a potential source of beneficial plant compounds (Wen et al. 2001). The authors reported that extracts from stems of *P. rockii* (originally listed in the paper as *P. suffruticosa*), an ancestral species found in the

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mountains of northwestern China (Bao et al. 2018), were active against *B. xylophilus*, *Hirschmanniella oryzae*, and *Meloidogyne arenaria*. These results warrant further investigations of *Paeonia* spp. stem extracts for activity against other nematodes.

The current study was conducted to investigate activity of extracts from *C. oleifera* cakes and *P. rockii* stems against Maryland isolates of *M. incognita* and *Heterodera glycines* Ichinohe. To our knowledge, extracts from these plant sources have not been tested against *H. glycines*. Additionally, stem extracts from two tree peony sources, *P. suffruticosa* and *P. rockii*, were compared for nematotoxicity to *M. incognita*. Because *C. oleifera* cake is used as a soil amendment, it was also applied in the greenhouse to determine effects on *H. glycines* on soybean (*Glycine max* (L.) Merr.).

Materials and Methods

Nematode cultures. *Meloidogyne incognita* (root-knot nematode) race 1 was obtained as previously described (Meyer et al. 2016). Roots were harvested from 2- to 3-month old, greenhouse-grown, susceptible pepper (*Capsicum annuum*) cv. PA-136 plants that had been inoculated with *M. incognita* (originally isolated in Maryland), and egg masses were picked from roots. The plants were maintained in a greenhouse at 24 to 29°C, with natural and supplemental lighting combined for a 16-h day length. The same greenhouse was used for all nematode cultures and greenhouse plants described in this paper.

Heterodera glycines (soybean cyst nematode) race 3, originally isolated in Maryland, was maintained on greenhouse-grown soybean (*Glycine max* cv. Essex). Mature females and cysts were dislodged by gently rubbing roots in water. Egg suspensions were poured through nested sieves (#20/#60; pore sizes 850/250 µm diameter), collected in a 1-liter graduated cylinder, mixed with 454 g/liter sucrose solution, and females and cysts that floated to the top after 15 min were collected and washed to remove the sucrose. A rubber stopper was used to crush females and cysts on a sieve (#60; 250-µm-diameter pore size) that was partially submerged in a small tray of distilled water, egg suspensions from the tray were washed

through nested sieves (#230/#500; pore sizes 63/25 µm diameter), and eggs were collected in distilled water.

For bioassays with nematodes, *H. glycines* eggs or *M. incognita* egg masses were placed in 0.6% sodium hypochlorite for 5–6 min and rinsed with sterile distilled water (SDW). To obtain J2, eggs were placed in a sterile hatching chamber for 3 days.

Preparation of tree peony (*P. suffruticosa* cv. Shimanishiki) extracts in the United States. Plants were obtained from Sun Nursery (Woodbine, MD), and were maintained in the greenhouse for 7 months. Stems and petioles from 20 plants were cut into 5-cm-long pieces, freeze dried, coarsely ground (Thomas-Wiley, Laboratory Mill Model 4, Swedesboro, NJ), and then finely ground in a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) equipped with a 1-mm-diameter pore sieve. To prepare extract, 40 g powdered material was placed into 320 ml of 95% ethanol (12.5% wt/vol) and maintained at room temperature for 7 days. The extract was filtered through Whatman No. 1 filter paper (Whatman, Clifton, NJ), collected, and the residue was re-extracted in 160 ml of 95% ethanol for 7 days with gentle stirring on a stir plate. The second extract was also filtered through Whatman No. 1 filter paper, combined with the first extract, and then dried in a rotary evaporator (Heidolph 2 Rotavac, Schwabach, Germany) to nearly dry. The remaining ethanol was removed with a CentriVap Benchtop Concentrator (Labconco, Kansas City, MO). The weight of the dried ethanolic extract was 6.57 g.

Preparation of tree peony (*P. rockii*) extracts in China. Stems of naturally growing tree peony plants were collected from mountains in Hubei Province, China, air-dried, and ground in an electric mill (Xulang model WN-200, Guangzhou, China) to a powder fine enough to pass through a #10-mesh sieve (2-mm-diameter pore size). To prepare extracts, 1,100 g of powder was soaked in 8.8 liters of 95% ethanol (12.5% wt/vol) for 7 days at room temperature, and the ethanol extract was then filtered through Whatman filter paper (Whatman International Ltd., Hangzhou, China). The residue in the flask was re-extracted with 4.4 liters of 95% ethanol for 5 days. This second extract was then filtered as described above and combined with the first extract. The pooled extracts were concentrated in a

Table 1. *Heterodera glycines* second-stage juvenile (J2) activity and viability in extracts from camellia (*Camellia oleifera*) tea seed cake and tree peony (*Paeonia rockii*) stems. Previously hatched J2 were immersed in the extracts.

Treatment mg/ml ^x	<i>Camellia oleifera</i>				<i>Paeonia rockii</i>			
	Day 1 ^y , % active	Day 2, % active	Day 3, % active	Days 4-6 rinsed % viable	Day 1, % active	Day 2, % active	Day 3, % active	Days 4-6 rinsed % viable
Water	80.1 a	76.6 a	73.2 a	71.4 a	80.1 a	76.6 a	73.2 a	71.4 a
1.0	77.7 a (97.0%) ^z	61.3 b** (80.0%)	61.9 b*** (84.6%)	52.0 b*** (72.8%)	79.9 a (99.8%)	62.2 b*** (81.2%)	48.4 b*** (66.1%)	55.7 b** (78.0%)
2.5	20.0 b**** (25.0%)	12.1 c*** (15.8%)	13.7 c*** (18.7%)	39.4 c**** (55.2%)	19.0 b**** (23.7%)	17.9 c*** (23.4%)	2.7 c**** (3.7%)	24.3 c**** (34.0%)
5.0	0.8 c**** (1.0%)	0.0 d**** (0%)	0.0 d**** (0%)	0.5 d**** (0.7%)	0.0 c**** (0%)	0.0 d**** (0%)	0.0 d**** (0%)	0.2 d**** (0.3%)

^x Weight extract per volume water.

^y For Day 1 and Days 4-6 rinsed, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For Day 2 and Day 3, means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). Significance levels of treatment means versus control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05, 0.005, 0.001$, and 0.0001 , respectively). Values are means of five replicate wells in each of two trials, for a total of $N = 10$. Nematodes were considered viable if they were active following the water rinse, and nonviable if they were not active.

^z Numbers in parentheses are percentages of the corresponding water control.

Table 2. *Heterodera glycines* egg hatch and second-stage juvenile activity in extracts from camellia (*Camellia oleifera*) tea seed cake and tree peony (*Paeonia rockii*) stems. Eggs were immersed in the extracts.

Treatment mg/ml ^x	<i>Camellia oleifera</i>					
	Day 3 ^y Hatch	Day 3, % active	Day 5 Hatch	Day 5, % active	Day 7 Hatch	Day 7, % active
Water	31.2 a	87.3 a	34.3 a	70.3 a	36.4 a	62.9 a
1.0	24.3 ab (77.9%) ^z	51.2 b**** (58.6%)	25.7 ab (74.9%)	43.1 b**** (61.3%)	35.9 a (98.6%)	45.7 b* (72.7%)
2.5	19.5 ab (62.5%)	51.9 b**** (59.5%)	20.7 b* (60.3%)	28.6 c**** (40.7%)	27.8 a (76.4%)	29.4 c**** (46.7%)
5.0	14.7 b** (47.1%)	42.1 b**** (48.2%)	16.6 b** (48.4%)	19.2 c**** (27.3%)	17.2 b**** (47.3%)	6.5 d**** (10.3%)

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^x Weight extract per volume water.

^y For all days, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). Significance levels of treatment means versus control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05, 0.005, 0.001$, and 0.0001 , respectively). Values are means of five replicate wells in each of two trials, for a total of $N = 10$.

^z Numbers in parentheses are percentages of the corresponding water control.

rotary evaporator (EYELA N-1100 Rotary Evaporator, Japan). The weight of the dried ethanolic extract was 63.14 g.

Preparation of *C. oleifera* tea cake extracts in China. The tea cakes were purchased from Guangdong Baohua Biotechnology Co. Ltd. (Guangdong Province, China), air-dried, ground in an electric mill, and prepared as described for tree peony in China. A total of 1,000 g powder was used to make extracts; weight of the dried ethanolic extract was 63.09 g.

Laboratory bioassays. Extracts for assays were dissolved in 40°C SDW at 0.02 wt/vol (e.g., 0.15 g extract in 7.5 ml water). Extracts were then filtered through 1.0, 0.45, and 0.2 µm filter syringes (Nalgene, Rochester, NY) as needed, depending on the extract density at the time of preparation, and diluted in SDW to prepare treatment concentrations.

Laboratory assays with previously hatched J2 were conducted in 48-well or 96-well polystyrene plates, modified from procedures in Meyer et al. (2006). Wells each received an equal amount of extract and J2 suspension in SDW (250 µl of each in 48-well plates, 100 µl of each in 96-well plates), resulting in ca. 0.2 J2 per µl in each well. J2 activity was counted in extracts for at least 2 days, wells were rinsed with SDW on Day 2 or Day 3, and J2 activity counted again after 1 to 3 days of incubation in SDW to determine whether treatments were nematotoxic or nematostatic. J2 were counted as active if they showed any movement within 5 s, and inactive if there was no movement during that time. J2 that were inactive after the water rinse were considered dead.

For laboratory assays with immersed *M. incognita* eggs, aqueous suspensions of eggs at various developmental stages were adjusted to include 50 eggs that each contained either a first-stage juvenile (J1) or J2 per 100 µl. Each well of a 96-well polystyrene plate received 100 µl of egg suspension and 100 µl of extracts. Assays with immersed *H. glycines* eggs were similar, except that each well contained ca. 100 total eggs. The total hatched J2, and numbers of inactive/active J2 that had hatched, were counted at Day 2 or Day 3, Day 5, and Day 7.

Treatments were 1.0, 2.5, and 5.0 mg extract/ml for *M. incognita* and *H. glycines* J2 assays with camellia cake and *P. rockii* extracts, and 0.25, 0.5, 1.0, and 5.0 mg/ml for assays comparing *P. rockii* and *P. suffruticosa* extracts. All assays included a SDW control. Water + 50 µg/ml kanamycin monosulfate (Phytotechnology Laboratories, Shawnee Mission, KS) was also added as a control to assays with immersed *M. incognita* eggs, due to bacterial contamination in initial assays. For all assays, each treatment was replicated in 5 wells in each of two trials, for a total of $n = 10$ per treatment. The microwell plates were covered by plastic adhesive sealing film (Excel Scientific, Inc., Victorville, CA) and the lids sealed with Parafilm (Bemis, Neenah, WI). The plates were incubated at 27°C.

Greenhouse trials with camellia tea cake soil amendment, *H. glycines*, and soybean. Camellia cake powder, ground as described for extract preparations, was mixed with steamed and air-dried loamy sand enriched soil (16 parts sand:9 parts compost, vol/vol; 85.1% sand, 7.2% silt, 7.6% clay, pH 6.9; 0.6% organic matter). Three soil amendment rates were hand-mixed into the soil: 1.5 g/300 ml soil (0.5% wt/vol), 2.25 g/300 ml soil (0.75% wt/vol) and 3.0 g/300 ml soil (1.0% wt/vol), placed into 10-cm-diameter pots, and the soil was watered. Soybean (cv. Essex) seedlings

(9–11 days old) were then removed from Promix PGX (Premier Tech Horticulture, Quakertown, PA), the roots were dipped in water to remove the potting mix, and one seedling was transplanted into each pot. Each *H. glycines*–inoculated plant received 2,000 total *H. glycines* eggs in 2 ml water (pipetted into 3 holes around the base of each plant). The treatments were: 1) 1.5 g camellia powder + *H. glycines* eggs; 2) 2.25 g powder + eggs; 3) 3.0 g powder + eggs; 4) Water + eggs; and 5) Water, No *H. glycines*. Pots were arranged in a randomized complete block design. Plants were harvested 33 days after transplant. Shoot fresh weights were recorded, and shoots were then placed in a 60°C oven for 7 days and shoot dry weights recorded. Cysts were collected from root balls as described above for *H. glycines* inoculum, except that the cysts were not crushed. Instead, the cyst suspension was poured onto on a 90-mm-diameter ruled filter paper in a Buchner funnel under a vacuum. The filter paper was then placed in the lid of a 100-mm-diameter petri dish and cysts were counted under a dissecting microscope. Roots were damp-dried with paper towels and weighed. Two trials were conducted, with 10 pots per treatment in each trial, for a total $n = 20$ per treatment.

Statistical analyses. Data were analyzed with the statistical package JMP 11.2.0 (SAS Institute, Cary, NC). Differences among treatments were determined by ANOVA, and means were compared using Tukey Kramer's adjustment for multiple comparisons ($P \leq 0.05$). For nonparametric data, a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons was used to determine differences ($P \leq 0.05$) among means. The analysis used for each treatment is indicated in the footnote of each table.

Results

Activity of camellia tea seed cake extract and *P. rockii* stem extract against *H. glycines* in vitro. Both types of extracts were nematotoxic to previously hatched *H. glycines* J2 (Table 1). By Day 1, the two highest concentrations of camellia extract, 2.5 mg/ml and 5.0 mg/ml, reduced J2 activity by 75 and 99%, respectively. By Day 2, all three tested concentrations suppressed J2 activity. The 5.0 mg/ml was the most effective, resulting in 100% loss of *H. glycines* J2 activity. Camellia extract at 5.0 mg/ml was the most nematotoxic, resulting in 99% reduction in J2 viability compared with the water control after a SDW rinse.

The *P. rockii* extracts were the most effective at the 2.5 and 5.0 mg/ml concentrations, with 0% J2 active on Days 1–3 in 5.0 mg/ml, and a 99.7% reduction in viable J2 after the water rinse (Table 1). Immersion of J2 in 2.5 mg/ml *P. rockii* extract resulted in up to a 96.3% loss of J2 activity by Day 3, but the reduction in J2 viability after the water rinse was 66%.

Extracts from both plants also inhibited *H. glycines* hatch and viability of J2 that hatched from immersed eggs (Table 2). In camellia extracts, hatch was decreased by the highest concentration (5.0 mg/ml) by Day 3, and was suppressed by 53% by Day 7. The lowest concentration did not inhibit hatch, while the 2.5 mg/ml showed a significant suppression on Day 5. However, all three concentrations inhibited J2 activity on all days (Table 2). The two highest concentrations were the most effective, with the 5.0 mg/ml suppressing activity by 90% on Day 7. Although effects on previously hatched J2 versus immersed J2 were not tested in the same assays, the results

Table 2. (Continued from previous page)

<i>Paeonia rockii</i>					
Day 3 Hatch	Day 3, % active	Day 5 Hatch	Day 5, % active	Day 7 Hatch	Day 7, % active
31.2 a	87.3 a	34.3 a	70.3 a	36.4 ab	62.9 a
27.7 a (88.8%)	85.6 a (98.1%)	35.8 a (104.4%)	60.6 ab (86.2%)	39.5 a (108.5%)	48.2 b* (76.6%)
25.8 a (82.7%)	79.5 a (91.1%)	28.0 a (81.6%)	51.4 bc* (73.1%)	29.3 b (80.5%)	40.8 b**** (64.9%)
15.0 b** (48.1%)	67.7 b**** (77.5%)	15.9 b*** (46.4%)	39.1 c**** (55.6%)	16.1 c**** (44.2%)	26.5 c**** (42.1%)

suggested that the camellia extracts were more effective against previously hatched J2 that were directly immersed in the treatments (Tables 1 and 2).

With *P. rockii*, the 5.0 mg/ml extract was the most effective against *H. glycines* in immersed egg assays (Table 2). This concentration inhibited hatch by 52% (Day 3) to 56% (Day 7). The lower concentrations were not effective in decreasing hatch. By Day 3, activity of hatched J2 was decreased by 23% in the highest concentration of *P. rockii* extract. By Day 7, all three concentrations inhibited J2 activity, with 5.0 mg/ml resulting in the least J2 activity (58% decrease compared with the water control). As with the camellia extract, the results suggested that the *P. rockii* extract decreased viability of previously hatched *H. glycines* J2 more than viability of J2 that hatched from immersed eggs (Tables 1 and 2).

Activity of camellia tea seed cake extract and *P. rockii* stem extract against *M. incognita* in vitro. Extracts from *C. oleifera* cake and *P. rockii* stems were nematotoxic to previously hatched J2 immersed directly into the treatments (Table 3). On Day 1, all concentrations of camellia extracts resulted in small but significant decreases in J2 activity. By Day 2, J2 activity in camellia extracts was less than half that in the water control. The 2.5 mg/ml treatment resulted in the fewest active J2 on Day 3. All three camellia extract treatments resulted in high mortality (Day 6 after rinse), with the two highest rates resulting in 94 to 96% greater J2 mortality than in the water control.

P. rockii extracts also reduced activity of immersed J2 by Day 1 (Table 3). The highest rate (5.0 mg/ml) was the most effective in

inhibiting J2 activity on Days 1, 2, and 3, resulting in 62 to 70% decreases compared with the water control (Table 3). All three rates of tree peony extract resulted in similar numbers of nonviable J2, decreasing viability by 57 to 69%.

Camellia cake and *P. rockii* extracts also inhibited *M. incognita* hatch and activity of J2 that hatched from immersed eggs (Table 4). Because the antibiotic kanamycin monosulfate was used in all extract treatments to stop growth of bacteria, effects on hatch and J2 activity were compared with the kanamycin control. By Day 5, the two highest concentrations of camellia extract inhibited hatch. All three concentrations were effective by Day 7, with hatch inhibited by 41 to 69% in the extract treatments. Activity of J2 that hatched from the eggs was decreased by all camellia extract treatments on all days, with 5.0 mg/ml resulting in no active J2 on Days 5 and 7, and 2.5 mg/ml extract causing a 99% reduction in J2 activity by Day 7.

With *P. rockii* extracts, the highest concentration had the greatest effect, with little or no effect at the lower concentrations (Table 4). On Day 3, 5.0 mg/ml suppressed hatch and J2 activity by 87 and 92%, respectively. By Day 7, hatch in the 5.0 mg/ml extract was decreased by 94%, and J2 activity by 80%.

Assays comparing activity of stem extracts from two tree peony sources against *M. incognita* in vitro. Extracts from two tree peony taxa were compared for activity against previously hatched *M. incognita* J2 (Table 5). As with the *P. rockii* extract assays tested at the same time as camellia (Table 3), the *P. suffruticosa* and *P. rockii* extracts were consistently effective on all days for reducing

Table 3. *Meloidogyne incognita* second-stage juvenile (J2) activity and viability in extracts from camellia (*Camellia oleifera*) tea seed cake and tree peony (*Paeonia rockii*) stems. Previously hatched J2 were immersed in the extracts.

Treatment mg/ml ^x	<i>Camellia oleifera</i>				<i>Paeonia rockii</i>			
	Day 1 ^y % active	Day 2 % active	Day 3 % active	Day 6 rinsed % viable	Day 1 % active	Day 2 % active	Day 3 ^y % active	Day 6 rinsed % viable
Water	89.4 a	86.6 a	86.8 a	71.7 a	89.4 a	86.6 a	86.8 a	71.7 a
1.0	75.8 b** (84.8%) ^z	37.6 b**** (43.4%)	46.1 bc**** (53.1%)	11.3 b**** (15.8%)	60.4 b**** (67.6%)	58.2 b**** (67.2%)	47.2 b**** (54.4%)	30.6 b**** (42.7%)
2.5	76.5 b* (85.6%)	42.4 b**** (49.0%)	28.2 c**** (32.5%)	4.2 c**** (5.9%)	50.0 b**** (55.9%)	55.4 b**** (64.0%)	40.9 b**** (47.1%)	26.8 b**** (37.4%)
5.0	72.2 b*** (80.8%)	39.9 b**** (46.1%)	50.0 b*** (57.6%)	2.5 c**** (3.5%)	27.3 c**** (30.5%)	32.8 c**** (37.9%)	29.2 c*** (33.6%)	22.6 b**** (31.5%)

^x Weight extract per volume water.

^y For all days except *P. rockii* Day 3, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For *P. rockii* Day 3, means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). Significance levels of treatment means versus control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of five replicate wells in each of two trials, for a total of $N = 10$. Nematodes were considered viable if they were active following the water rinse, and nonviable if they were not active.

^z Numbers in parentheses are percentages of the corresponding water control.

Table 4. *Meloidogyne incognita* egg hatch and second-stage juvenile activity in extracts from camellia (*Camellia oleifera*) tea seed cake and tree peony (*Paeonia rockii*) stems. Eggs were immersed in the extracts, which contained kanamycin (K) to prevent bacterial contamination.

Treatment mg/ml ^x	<i>Camellia oleifera</i>					
	Day 3 ^y Hatch	Day 3, % active	Day 5 Hatch	Day 5, % active	Day 7 Hatch	Day 7, % active
Water	25.8 a	95.7 a	45.6 a	95.1 a	63.3 a	88.2 b**
Water + K	16.4 b	92.1 a	35.6 ab	96.0 a	52.2 a	94.1 a
1.0	13.7 b (83.5%) ^z	51.9 b**** (56.4%)	23.4 bc (66.0%)	24.8 b**** (25.8%)	30.6 b** (58.6%)	19.9 c*** (21.1%)
2.5	13.9 b (84.8%)	17.9 c**** (19.4%)	20.1 c* (56.5%)	4.3 c**** (4.5%)	21.0 b**** (40.2%)	1.1 d**** (1.2%)
5.0	14.4 b (87.8%)	5.2 d**** (5.6%)	15.8 c*** (44.4%)	0.0 c**** (0%)	16.0 b**** (30.7%)	0.0 d**** (0%)

(Continued on next page)

^x Weight extract per volume water.

^y For all columns except *C. oleifera* Day 7, percent active, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For *C. oleifera* Day 7, percent active, means within a column followed by the same letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). Significance levels of treatment means versus water + K control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of five replicate wells in each of two trials, for a total of $N = 10$.

^z Numbers in parentheses are percentages of the corresponding water + kanamycin control.

J2 activity at 1.0 mg/ml and 5.0 mg/ml. After the water rinse, all treatments reduced J2 viability. However, at 5.0 mg/ml, the *P. rockii* extract was more effective than the *P. suffruticosa* extract against J2. In that concentration, reductions in J2 activity on Day 1 and Day 2 were 41 and 39%, respectively, for *P. suffruticosa*, and loss in viability was 26% after the water rinse. In *P. rockii* extracts, reductions in activity/viability for Days 1, 2, and 3 were 96, 94, and 80%, respectively.

In assays with the *P. suffruticosa* and *P. rockii* extracts and immersed *M. incognita* eggs, hatch was decreased by *P. rockii* extracts, with a 95% hatch reduction in 5.0 mg/ml by Day 7 (Table 6). This was similar to results from the assays conducted with *P. rockii* and camellia (Table 4). In both assays, *P. rockii* extracts suppressed hatch at the highest rates; rates below 2.5 mg/ml were not effective (Tables 4 and 6). *P. suffruticosa* extracts did not significantly reduce hatch (Table 6). *P. rockii* 5.0 mg/ml extracts were highly effective from Day 1; hatch was low on Day 2 and remained low throughout the assay.

In the same assays, *P. rockii* extracts affected activity of *M. incognita* J2 that hatched from the eggs (Table 7). The greatest decreases on all days occurred in 5.0 mg/ml, and J2 activity was decreased by 100% on Day 7. In general, there was no effect on J2 activity with *P. suffruticosa* extracts (Table 7). This was similar to results with previously hatched J2, in which *P. rockii* extract had a greater effect than *P. suffruticosa* extract on J2 activity and viability (Table 5).

Greenhouse trials with camellia tea seed cake powder and *H. glycines*. Soybean shoot fresh and dry weights tended to be lower with the two highest camellia powder amendment rates (0.75% wt powder/vol soil and 1.0% wt/vol) when compared with controls with and without *H. glycines* (Table 8). There was an exception in Trial 1: shoot fresh weights with these two treatments were similar to weights from control plants without *H. glycines*. Results with the lowest amendment rate, 0.5% wt/vol, were also variable. This treatment suppressed shoot fresh weights compared with the *H. glycines* control in Trial 1, did not suppress shoot fresh weight in Trial 2, and resulted in shoot dry weights lower than those in the control without *H. glycines* in both trials. Unlike results with shoots, root fresh weights in Trial 1 were lower in all nematode-inoculated plants than in the plants without nematodes. However, in Trial 2, most root fresh weights were not significantly different among treatments. Only 0.75% wt/vol camellia powder amendment resulted in lower root fresh weights than in the control without *H. glycines*.

Numbers of cysts/root system were decreased by all camellia cake powder treatments (Table 8). Numbers tended to be the lowest in the highest amendment rate, which decreased cysts per root system by 60% in Trial 1 and 67% in Trial 2. However, all three rates decreased cyst/root system numbers compared with the control without camellia cake powder. Cysts/g root were also decreased by amendment with camellia powder, with decreases of 52% in Trial 1 and 66% in Trial 2 at the highest amendment rate, compared with the control

without camellia cake powder. The lowest amendment rate, 0.5% wt/vol, decreased cysts/g root by 33 to 34%.

Discussion

Extracts prepared from *C. oleifera* tea seed cake and *P. rockii* stems inhibited *H. glycines* and *M. incognita* egg hatch and were toxic to J2. At the highest rates, both types of extracts decreased *H. glycines* hatch by more than half, and resulted in death of nearly all previously hatched *H. glycines* J2. With *M. incognita*, the high-rate *C. oleifera* cake extracts rendered most previously hatched J2 nonviable and reduced hatch by more than 2/3. *P. rockii* stem extracts had greater activity against *M. incognita* than *P. suffruticosa* cv. Shimanishiki stem extracts, with the highest *P. rockii* concentration resulting in more than three times as many nonviable J2 and reducing hatch to less than a tenth of hatch in *P. suffruticosa* extracts. This is the first report on effects of camellia cake extracts and *Paenonia* spp. stem extracts on *H. glycines*, and the first comparing activity of two tree peony species against any nematode.

Nematotoxicity of camellia tea seed cake extracts has been found with other phytoparasitic nematodes. At concentrations of 50 mg/ml and 150 mg/ml, ethanol extracts from tea seed cake killed 40 to 70% of *M. javanica* J2, respectively, and inhibited hatch by 66 to 85% (Li et al. 2013a). *C. oleifera* cake extract at 10 mg/ml resulted in death of up to 90% of *B. xylophilus* and an average of 55% of *M. incognita* J2 (Wang et al. 2013). Water extracts from *C. oleifera* cake killed 99% of *M. javanica* J2 at doses of 5 to 100 g/liter (5 mg/ml to 100 mg/ml) (Yang et al. 2015), similar to our results with *H. glycines* and *M. incognita* J2 that were immersed directly in ethanolic extracts.

Camellia has many economically important uses, so studies have reported on the phytochemistry of plants in this genus (Cui et al. 2018). Camellia is known for production of triterpenoid saponins, referred to as theasaponins, that are considered environmentally friendly. Hundreds of these saponins have been identified from bark, flower buds, fruit peels, leaves, roots, seeds, and stems of *Camellia* spp. (Cui et al. 2018). Saponins comprise 15 to 20% of *C. oleifera* cake (Liu et al. 2016), and 11 theasaponins were identified from the cakes (Cui et al. 2018). Much of the camellia tea seed cake activity is attributed to saponins, which are also derived from other plants and have been shown to be nematocidal to plant-parasitic nematodes, including *M. incognita*, *Rotylenchulus reniformis*, and *Xiphinema index* (Ntalli and Caboni 2012). For example, saponins isolated from *Pulsatilla koreana*, the Korean pasque flower, were active against *M. incognita* in laboratory assays, with LC₅₀ values of 69.7 to 77.6 µg/ml (Li et al. 2013b). Saponins in alfalfa (*Medicago sativa*) acted by disrupting membranes (Ntalli and Caboni 2012), and saponin extracts from alfalfa were active against *M. incognita*, *Globodera rostochiensis*, and *Xiphinema index* in vitro (D'Addabbo et al. 2011). Soil amended with dry alfalfa tops or roots reduced *M. incognita* populations on tomato and *G. rostochiensis* on potato (D'Addabbo et al. 2011). The authors postulated that saponins and other active metabolites, including canavanine and phenolics, may have been involved

Table 4. (Continued from previous page)

<i>Paenonia rockii</i>					
Day 3 Hatch	Day 3, % active	Day 5 Hatch	Day 5, % active	Day 7 Hatch	Day 7, % active
25.8 a	95.7 a	45.6 a	95.1 a	63.3 a	88.2 ab
16.4 b	92.1 a	35.6 ab	96.0 a	52.2 a	94.1 a
22.1 ab (134.8%)	88.6 a (96.3%)	37.0 ab (103.9%)	85.2 ab (88.8%)	53.1 a (101.7%)	78.6 b* (83.5%)
15.5 b (94.5%)	87.8 a (95.3%)	26.7 b (75.0%)	77.1 b**** (80.3%)	32.4 b* (62.1%)	81.2 ab (86.3%)
2.1 c**** (12.8%)	7.5 b**** (8.1%)	3.4 c**** (9.6%)	20.4 c**** (21.3%)	3.0 c**** (5.7%)	18.7 c**** (19.9%)

in the suppression, along with ammonia release as the amendments decomposed, and increases in numbers of microbes active against plant-parasitic nematodes. Saponins were also considered to have contributed to *M. incognita* suppression when soil was amended with 20 or 40 ton/ha of alfalfa meal in a pellet form, which also increased tomato yield (Leonetti et al. 2011).

Studies with *C. oleifera* cake as a soil amendment, and saponins from the cake, have demonstrated nematocidal activity. In our greenhouse trials, powdered *C. oleifera* cake suppressed *H. glycines* cyst numbers by up to 66%. The amendment rates, when applied to individual plants in a field at 5.0, 7.5, and 10.0 g/liter, would correspond to ca. 150 kg/ha, 225 kg/ha, and 300 kg/ha. *Camellia sasauqua* cake powder amended into soil at 9–11 g/liter reduced egg numbers by 70 to 80% on water spinach and Malabar spinach (Li et al. 2013a). A saponin extract from *C. oleifera* cake, applied at 0.75 and 1.5 g/kg to soil in a pot experiment, reduced *M. incognita* galling on tomato (Wang et al. 2013). The same authors found that *C. oleifera* cake applied at 2.5 t/ha reduced reproduction of *M. incognita* on Chinese cucumber. Saponin from *C. oleifera* cake was nematocidal to *M. javanica*, with 100% mortality in 70 g/liter, while volatile compounds from cake extract killed nearly 100% of J2 at 50 g/liter (Yang et al. 2015). In pot experiments, soil application rates of 1, 2, and 5 g cake/kg soil generally resulted in decreased nematode densities on banana plants (Yang et al. 2015). No phytotoxicity was observed,

likely because the banana seedlings were transplanted 7 days after soil amendment with *C. oleifera* cake. Eight volatile compounds identified from the seed cake were nematocidal to *M. javanica*, including 1-butanol, 2-methyl-butanoic acid, 1-octen-3-ol, butylester butanoic acid, 2-methylbutanebutyl, 3,3-dimethyloctane, 4-methylphenol, and 2-bromo dodecane. The authors concluded that these also contributed to nematode suppression, and that nutrients in the cake played a role in improving plant vigor (Yang et al. 2015). Additional studies with *C. oleifera* cake also indicated that banana seedling transplant should take place 7 days after soil amendment to avoid phytotoxicity; transplanting after that time improved plant vigor with a cake amendment of 5 g/kg soil. The seed cake increased numbers of free-living nematodes and soil microbes, and decreased numbers of plant-parasitic nematodes (Yang et al. 2018). Some phytotoxicity to soybean was observed in our greenhouse experiments. However, even the lowest tested seed cake application rate resulted in suppression of *H. glycines*. Future studies would indicate whether low rates in the field, applied a week or more before seeding, would result in nematotoxicity without phytotoxicity.

Our study demonstrated that tree peony stem extracts were nematocidal to *H. glycines* and *M. incognita*. In the only prior investigation with tree peony stem components and phytoparasitic nematodes, *P. rockii* (reported as *P. suffruticosa*) stem extracts were tested against *B. xylophilus*, *M. arenaria*, and *Hirschmanniella oryzae*

Table 5. *Meloidogyne incognita* second-stage juvenile (J2) activity and viability in extracts from stems of two tree peony species: *Paeonia suffruticosa* cv. Shimanishiki (nursery-grown in the United States) and *Paeonia rockii* (naturally growing in China). Previously hatched J2 were immersed in the extracts.

Treatment mg/ml ^x	<i>Paeonia suffruticosa</i>			<i>Paeonia rockii</i>		
	Day 1 ^y , % active	Day 2, % active	Day 3, rinsed % viable	Day 1, % active	Day 2, % active	Day 3, rinsed % viable
Water ^y	96.8 aA	94.4 aA	81.5 aB	96.8 aA	94.4 aA	81.5 aB
0.25	93.1 abA (96.2%) ^z	74.9 bB**** (79.3%)	55.6 bC**** (68.2%)	84.2 bcAB**** (87.0%)	76.0 bB**** (80.5%)	57.0 bC**** (69.9%)
0.5	78.1 cA**** (80.7%)	65.3 bcAB**** (69.2%)	45.4 bC**** (55.7%)	80.7 bcA*** (83.4%)	64.7 bcAB**** (68.5%)	51.6 bBC**** (63.3%)
1.0	71.0 cdA**** (73.3%)	61.1 cAB**** (64.7%)	49.1 bB**** (60.2%)	63.6 deAB**** (65.7%)	62.4 cAB**** (66.1%)	59.7 bAB**** (73.3%)
5.0	56.7 eA**** (58.6%)	57.3 cA**** (60.7%)	60.5 bA*** (74.2%)	3.9 fC**** (4.0%)	5.7 dBC**** (6.0%)	16.0 cB**** (19.6%)

^x Weight extract per volume water.

^y For all days except Day 1, means within the two columns for that day (one column for each tree peony species) followed by the same lowercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For Day 1, means within the two columns for that day (one column for each tree peony species) followed by the same lowercase letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). For all treatments, means within a row followed by the same uppercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). Within columns, significance levels of treatment means versus water control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of five replicate wells in each of two trials, for a total of N = 10. Nematodes were considered viable if they were active following the water rinse, and nonviable if they were not active.

^z Numbers in parentheses are percentages of the corresponding water control.

Table 6. *Meloidogyne incognita* egg hatch in extracts from stems of two tree peony species: *Paeonia suffruticosa* cv. Shimanishiki (nursery-grown in the United States) and *Paeonia rockii* (naturally growing in China). Eggs were immersed in the extracts, which contained kanamycin (K) to prevent bacterial contamination.

Treatment mg/ml ^x	<i>Paeonia suffruticosa</i>			<i>Paeonia rockii</i>		
	Day 2 ^y Hatch	Day 5 Hatch	Day 7 Hatch	Day 2 Hatch	Day 5 Hatch	Day 7 Hatch
Water ^y	5.1 abC	20.4 abB	34.9 aA	5.1 abC	20.4 abB	34.9 aA
Water + K	3.2 bcC	17.0 abB	24.6 abcA	3.2 bcC	17.0 abB	24.6 abcA
0.25	5.9 abC	18.5 abB	28.9 abcA	4.1 bC	19.1 abB	27.8 abcA
	(184.4%) ^z	(108.8%)	(117.5%)	(128.1%)	(112.4%)	(113.0%)
0.50	6.0 abC	20.0 abB	30.9 abA	5.2 abC	20.0 abB	30.6 abA
	(187.5%)	(117.6%)	(125.6%)	(162.5%)	(117.6%)	(124.4%)
1.0	7.1 aDE***	23.5 aB	33.1 aA	3.8 bE	13.6 bCD	20.9 bcBC
	(221.9%)	(138.2%)	(134.6%)	(118.8%)	(80.0%)	(85.0%)
5.0	4.8 abC	13.9 bB	19.7 cA	0.8 cD	1.1 cD****	1.3 dD****
	(150.0%)	(81.8%)	(80.1%)	(25.0%)	(6.5%)	(5.3%)

^x Weight extract per volume water.

^y For all days, means within the two columns for that day (one column for each tree peony species) followed by the same lowercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For all treatments, means within a row followed by the same uppercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). Within columns, significance levels of treatment means versus water + K control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of five replicate wells in each of two trials, for a total of N = 10. Nematodes were considered viable if they were active following the water rinse, and nonviable if they were not active.

^z Numbers in parentheses are percentages of the corresponding water + kanamycin control.

(Wen et al. 2001). With a dose of 1 mg/ml, proliferation of *B. xylophilus* mixed stages was reduced by 84%, and activity of *M. arenaria* and *Hirschmanniella oryzae* J2 by 100 and 86%, respectively. In other research with tree peonies, essential oil from *P. suffruticosa* (reported by the syn. *P. moutan*) roots killed 100% of *B. xylophilus* males, females, and adults at 2 mg/ml (Choi et al. 2007). Tannins (1, 2, 3, 4, 6-penta¹²)-*O*-galloyl- β -D-glucose and a condensed tannin (proanthocyanidin) polymer isolated from fresh *P. suffruticosa* leaves and tested against *Caenorhabditis elegans* had LC₅₀ values of 15.4 and 5.3 μ g/ml, respectively (Mohamed et al. 2000). *P. suffruticosa* root bark ground into a powder and amended into soil at 0.2% (weight dried plant material/volume soil) reduced *M. incognita* root galling on tomato by 95.3%. The authors of the latter study suggested that the rates might be high for field use but applicable to more commercially valuable crops (Kim et al. 2003). These studies indicate the possibility that tree peony constituents might act as natural nematocides.

While there is reason for further work on these natural products, it is notable that extracts from the two tree peony species had different effects on *M. incognita*. *P. rockii* stem extract exhibited greater nematotoxicity, killing up to 80% of previously hatched J2, as opposed to 26% in the *P. suffruticosa* stem extract. Hatch in *P. rockii* extract at 5.0 mg/ml was 7% of the hatch in *P. suffruticosa* extract. J2 that hatched from immersed eggs were not affected by *P. suffruticosa* stem extract, while none were active in *P. rockii* stem extract. These

results were likely due to such factors as differences in plant genotypes, growth conditions of the plants, or even some slight variations in extract preparation. While chemical constituents of flowers, leaves, and roots of tree peony have been studied (Bao et al. 2018; Li et al. 2018), chemistry of the stems has not been investigated. However, a study on roots of 372 tree peony cultivars identified 42 metabolites, and demonstrated that there were seven groups based on variations from low to high amounts of metabolites (Li et al. 2018). The tree peony taxa that we used for our assays were not tested by Li et al. (2018), but their results clearly showed that metabolites vary with tree peony cultivar.

The current research indicates that *C. oleifera* tea seed cake has potential for use as a soil amendment for managing nematodes on crop plants. Tests can be conducted to determine if phytotoxicity occurs on crops in the field, and if so, various times between soil amendment and planting or transplanting can be tested for eliminating any phytotoxic effects. Field studies will indicate whether suppression of nematode populations occurs at an amendment rate that will be useful for a field crop like soybean, or better applied to a specialty crop. Extracts from *Paenonia* spp. stems also demonstrate activity against these nematodes, with different nematotoxicity between the wild type plant and the cultivar. While stems are not being investigated for use as soil amendments, further work on identification of active compounds from *P. rockii* may yield metabolites that can be applied as biobased nematocides.

Table 7. *Meloidogyne incognita* second-stage juvenile activity after hatch from eggs immersed in extracts from stems of two tree peony species: *Paenonia suffruticosa* cv. Shimanishiki (nursery-grown in the United States) and *Paenonia rockii* (naturally growing in China)

Treatment mg/ml ^x	<i>Paenonia suffruticosa</i>			<i>Paenonia rockii</i>		
	Day 2 ^y , % active	Day 5, % active	Day 7, % active	Day 2, % active	Day 5, % active	Day 7, % active
Water ^y	73.1 abA	94.5 aA	94.8 aA	73.1 abA	94.5 aA	94.8 aA
Water + K	50.8 abB	92.1 aA	94.0 abA	50.8 abB	92.1 aA	94.0 abA
0.25	74.8 bA (147.2%) ^z	89.6 aA (97.4%)	84.0 dA* (89.4%)	74.7 abA (147.0%)	91.3 aA (99.2%)	92.2 abcA (98.1%)
0.50	79.5 abAB (156.5%)	90.4 aA (98.3%)	84.9 dAB* (90.3%)	70.3 abB (138.4%)	89.5 aA (97.3%)	85.9 cdAB* (91.4%)
1.0	87.8 aA (172.8%)	90.4 aA (98.3%)	84.5 bcdA (89.9%)	74.8 abAB (147.2%)	87.3 aA (94.9%)	68.9 fB*** (73.3%)
5.0	80.5 abA (158.5%)	77.9 aA (84.7%)	79.9 abcdeA*** (85.0%)	10.0 cB* (19.7%)	15.0 bB**** (16.3%)	0.0 gB**** (0%)

^x Weight extract per volume water.

^y For Day 5, means within the two columns for that day (one column for each tree peony species) followed by the same lowercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For Days 2 and 7, means within the two columns for that day (one column for each tree peony species) followed by the same lowercase letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). For treatments 0.25 mg/ml and 0.5 mg/ml, means within a row followed by the same uppercase letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). For treatments water, water + K, 1.0 mg/ml and 5.0 mg/ml, means within a row followed by the same uppercase letter are not significantly different according to a Kruskal-Wallis test with Wilcoxon each pair nonparametric multiple comparisons ($P \leq 0.05$). Within columns, significance levels of treatment means versus water + K control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of five replicate wells in each of two trials, for a total of $N = 10$. Nematodes were considered viable if they were active following the water rinse, and nonviable if they were not active.

^z Numbers in parentheses are percentages of the corresponding water + K control.

Table 8. Effect of soil amendment with powdered camellia (*Camellia oleifera*) tea seed cake on soybean (*Glycine max* cv. Essex) plant vigor, with and without *Heterodera glycines*, and on cyst numbers on soybean roots. Amendments were applied on the day of seedling transplant. Soybean plants were harvested 33 days after transplant.

Treatment ^y	Shoot fresh weight (g) ^z		Shoot dry weight (g)		Root fresh weight (g)		Cysts/root system		Cysts/g root fresh weight	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0 g, No <i>H. glycines</i>	10.8 ab	11.3 a	2.8 a	3.2 a	11.1 a	5.4 a	NA	NA	NA	NA
0 g + <i>H. glycines</i>	11.8 a	11.3 a	2.8 ab	3.0 ab	8.4 b***	4.8 ab	107.3 a	977.0 a	12.9 a	209.5 a
1.5 g (0.5% w/v) + <i>H. glycines</i>	9.9 b	9.6 ab	2.4 bc*	2.5 bc**	7.9 bc****	4.6 ab	62.6 b****	613.0 b*	8.5 b*	141.3 ab
2.25 g (0.75% w/v) + <i>H. glycines</i>	9.5 b	8.5 b**	2.2 c***	2.3 c****	7.0 c****	4.1 b*	50.9 b****	374.0 bc****	7.5 b**	93.1 b***
3.0 g (1.0% w/v) + <i>H. glycines</i>	10.0 b	9.1 b*	2.2 c***	2.2 c****	7.2 bc****	4.4 ab	43.5 b****	320.0 c****	6.2 b***	71.7 b****

^y Grams powder per 300 cm³ soil (followed by % weight/volume).

^z For each plant part and trial, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). Means are not comparable among columns. Within columns, significance levels of treatment means versus No *H. glycines* control means are indicated by *, **, ***, and **** (denoting $P \leq 0.05$, 0.005, 0.001, and 0.0001, respectively). Values are means of 10 replicate pots per trial for each treatment.

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