

The effect of linalool on second-stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*

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Abstract: Linalool is either a toxic compound to a few species of plant parasitic nematodes or attractive to entomopathogenic nematodes. This compound is produced and emitted by several host plants of *Globodera rostochiensis* and *G. pallida*, the potato cyst nematodes (PCN). With the aim to reveal the effect of linalool on PCN, laboratory assays were carried out. Survival of PCN second-stage juveniles (J2s) in water + linalool control did not differ; thus, proving linalool to be nontoxic to PCN. Behavioral assays carried out in Petri dishes revealed attractiveness in the form of positive response of J2s of both PCN species towards linalool. Based on these behavioral assays, sensitivity to linalool of *G. rostochiensis* J2s was higher compared with that of *G. pallida* J2s. Linalool is the first compound of plant origin to elicit positive response in both PCN species.

Key words: Chemoattraction, behavior, *Globodera rostochiensis*, *Globodera pallida*, linalool, potato cyst nematodes, second-stage juvenile, *Solanaceae*.

Eggs of potato cyst nematodes (PCN), *Globodera rostochiensis* (Wollenweber) Behrens and *G. pallida* (Stone) Behrens, can remain unhatched for many years in the soil (Turner, 1996). In the presence of a suitable *Solanaceae* host plant after overwintering, PCN second-stage juveniles (J2s) emerge from the cysts and search for host plant roots using chemoreception (e.g. Steiner, 1925; Devine and Jones, 2003). Weischer (1959) and Clarke and Hennessy (1984) have demonstrated that potato root diffusates stimulated locomotory activity, movement and migration, of *G. rostochiensis* J2s. Behavioral assays carried out by Devine and Jones (2003) revealed the presence of chemoattractants, chemostats and chemorepellents for *G. rostochiensis* and *G. pallida* juveniles in several potato root diffusate fractions. Identification of bioactive compounds that elicit behavioral responses to PCN J2s could provide us with better understanding of their behavior and might lead to novel control strategies. *Solanaceae* release a great variety of chemicals (Eich, 2008) into the soil, many of which are common amongst the major host plants of PCN. One of these chemicals is linalool (3,7-dimethyl octa-1,6-dien-3-ol) and it is known to be toxic to some plant parasitic nematodes such as *Meloidogyne incognita* (Ibrahim et al., 2006), *M. arenaria* (Walker and Melin, 1996), *M. javanica*, *Anguina tritici*, *Heterodera cajani*, *Tylenchulus semipenetrans* (Malik et al., 1987; Sangwan et al., 1990) and *Bursaphelenchus xylophilus* (Kong et al., 2007). On the other hand, linalool is known as attractant to the following entomopathogenic nematode species: *Diplogasteroides magnus* (Hong et al., 2008), *Heterorhabditis megidis* (Köllner et al., 2008), and *Pristionchus maupasi* (Hong and Sommer, 2006). Thus, the role of this

compound in host plant-PCN interaction remains unknown. Our aim was to reveal the effect of linalool on J2s of both PCN species.

MATERIALS AND METHODS

Potato cyst nematodes: *Globodera rostochiensis* Ro1 (Ecosse population, Germany) and *G. pallida* Pa2 (Kalle population, Germany) were supplied by Julius Kühn Institute (Quedlinburg, Germany). Cysts were placed on a filter paper moistened with distilled water (dW) and kept at 21°C for seven days and then the cysts were gently crushed to expose hatched J2/unhatched eggs that were washed by dW to a Petri dish with its bottom covered by a layer of 1.5 % agar (Carl Roth, Karlsruhe, Germany). In a few hours the water was absorbed and actively moving J2s were used for testing.

Potato root diffusate: Potato root diffusate (PRD) was prepared according to Pridannikov et al. (2007), except filtering of PRD. Three Désirée variety potato tubers were planted in 400 ml pots filled with autoclaved sand and were maintained under 60% relative humidity, 14:10 hr light:dark period and 22°C in a greenhouse. After three weeks the potato tubers were pulled out and the intact roots were rinsed with dW to remove sand particles. Intact roots were then immersed into 50 ml dW for 24 hr at 22°C and the obtained PRD was kept at 4°C.

Linalool: Linalool (Sigma-Aldrich, Seelze, Germany) was racemic with purity 97%. Due to its limited solubility in water, an aqueous linalool emulsion of 0.56×10^{-1} M concentration was made. A dilution series to 0.56×10^{-6} M was prepared; the pH differed from 6.57 to 6.82, depending on the concentration. To calculate the content of linalool in PRD, headspace solid-phase microextraction (polydimethylsiloxane fiber - Supelco, Bellefonte, USA) (Pawliszyn, 1997) and gas chromatography with flame ionization detector (Clarus 500, Perkin Elmer, Shelton, USA), Stabilwax column (Restek, Bellefonte, USA), and external standard method (Rouessac and Rouessac, 2007) were used.

Toxicity test: Fifty *G. rostochiensis* and *G. pallida* J2s were placed in separate watch glasses containing 2 µl of dW.

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Two milliliters of 0.56×10^{-3} M linalool solution or dW (control) was added. The watch glasses were placed inside Petri dishes and kept at room temperature in the dark. To minimize evaporation, both linalool solution and control were replaced every three days. Mortality of J2s was recorded daily for 12 days. The nematodes were defined as dead if their body was straight or did not move after mechanical prodding while observed under a binocular microscope (Kong et al., 2006). Mortality percentages were counted from four replicates.

Behavioral tests: Behavioral tests for both *G. rostochiensis* and *G. pallida* were carried out following Rühm et al. (2003). Petri dishes, 32-mm-diam., with their bottom coated by 1–2-mm thick film of 1.5 % agar (Carl Roth, Karlsruhe, Germany) were used. A filter paper disk (Schleicher & Schuell MicroScience, Niedersachsen, Germany) 5-mm-diam. was placed in the center of each dish. One microliter either of linalool, dW, or 7 μ l of PRD were spread on the filter paper. In order to increase the number of attracted J2s, another 7 μ l of PRD were spread on a filter after 1 hr. After 2 hr, the paper was removed and four J2s were placed at equal distances from each other on a circular line (marked on the outer surface), which divided dish area into two equal parts; and the nematode position was recorded after 1 hr. For nematodes located in the inner area of the behavioral arena, attractiveness to the stimulus was considered and counted as a positive response, and for those found in the outer area as a negative response. Potato root diffusate and dW were used as controls. Four nematodes in 5 replicates were tested, the test was repeated 6 times (120 nematodes in total) to evaluate the effect of each linalool concentration, PRD and dW. Data was analyzed by χ^2 -test using software Statistica 6.0 (StatSoft, Tulsa, USA). Behavioral response of PCN J2s to dW was considered and counted as 'no attraction'.

RESULTS AND DISCUSSION

Toxicity: In water, *G. rostochiensis* and *G. pallida* J2s survived for 12 days, i.e. during the period of observation

with survival up to 99%. In water + linalool (linalool solution equal to 0.56×10^{-3} M) during the same period, survival of *G. rostochiensis* J2s was $95 \pm 2.1\%$, and that of *G. pallida* J2s was $97 \pm 1.7\%$. No statistically significant difference in J2 survival in water + linalool compared with the water control was recorded. The high concentration of linalool solution had no toxic effect to J2s, so lower concentrations were not tested. Therefore, we hypothesize that in case host plants would emit this compound in high concentrations, the compound would not be hazardous. Based on this hypothesis, we assume linalool is unlikely to be a *Solanaceae* plant defensive (toxic) compound against PCN.

Behavior: In our study, linalool was not repellent to J2s; in fact, this compound was attractive to both PCN species (Fig. 1). Our findings contradict earlier reports on the toxic properties of linalool to some plant parasitic nematodes (Malik et al., 1987; Sangwan et al., 1990; Walker and Melin, 1996; Ibrahim et al., 2006; Kong et al., 2007). However, some interspecific behavioral differences were noted in response to linalool. The lowest concentration of linalool attractive to *G. rostochiensis* J2s was 0.56×10^{-4} M; J2s exhibited similar response to PRD ($P > 0.05$). However, a higher concentration of linalool, 0.56×10^{-3} M, was needed to evoke a positive response to *G. pallida* J2s. Thus, sensitivity to linalool by *G. rostochiensis* J2s was higher compared with that of *G. pallida* J2s. The range of linalool concentrations eliciting a positive response was differed as well. For example, *G. rostochiensis* J2s responded to linalool concentrations ranging from 10^{-2} to 10^{-4} M, while *G. pallida* J2s responded from undiluted linalool to 0.56×10^{-3} M concentration. In addition, high concentrations of linalool (undiluted and emulsion 0.56×10^{-1} M) did not elicit a positive response to *G. rostochiensis* J2s, as no statistically significant difference was found compared with the water control ($P > 0.05$). The reduced response at very high concentrations of an allelochemical due to possible saturation of chemoreceptors has been reported previously (Diez and Dusenbery, 1989; Stamps and Linit, 1998). In *G. pallida* the same effect, loss of attractiveness at higher concentrations, was absent, due to less sensitivity to the compound compared with sensitivity of *G. rostochiensis*. The

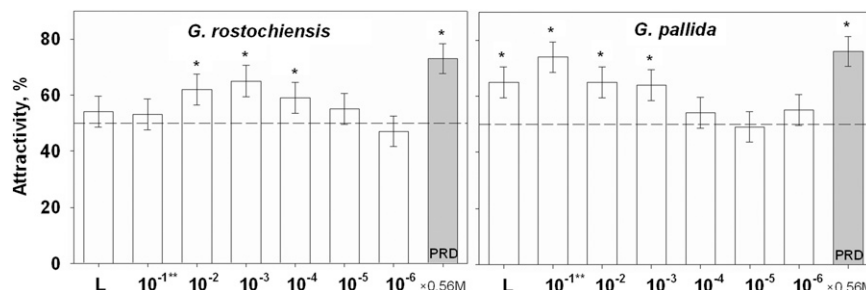


FIG. 1. Chemoattraction in the form of positive response of *Globodera rostochiensis* and *Globodera pallida* J2s to different linalool (L, undiluted) concentrations (**emulsion and solutions) (M) and potato root diffusate (PRD). Open column indicates response to linalool; grey column to PRD. Response to water was counted as zero and was detracted from that to linalool and PRD solutions (in water). Columns marked with asterisk and containing no mark differs significantly (χ^2 -test $P < 0.05$).

behavioral threshold to linalool was 0.56×10^{-3} M in *G. pallida* and 0.56×10^{-4} M in *G. rostochiensis*.

Devine and Jones (2003) demonstrated that potato root diffusate-hatched juveniles of both PCN species were attracted to different fractions of the diffusate, whereas water-hatched J2s were attracted to the same potato root diffusate fractions. Therefore, water-hatched juveniles were more homogenous in their response to potato root diffusate chemicals compared with potato root diffusate-hatched juveniles. Water-hatched juveniles used in our test differed in their sensitivity, i.e. threshold,) to a single compound, thus, interspecific differences even within water-hatched *G. rostochiensis* J2s and *G. pallida* J2s were revealed. If behavioral response to linalool differs in potato root diffusate-hatched and water-hatched juveniles remains to be investigated.

The linalool concentration in PRD used in the assay was 9.7×10^{-6} M, i.e. much lower than that attractive in the assay (i.e. 10^{-3} – 10^{-4} M). However, we assume under natural conditions roots could emit higher linalool concentrations compared with those obtained in our PRD, because the PRD-making procedure included removal of sand by washing, thus leading to some losses of chemicals on a root surface including linalool.

Several compounds have been reported to stimulate egg hatching in PCN (e.g. Devine et al., 1996; Byrne et al., 2001; Maher, 2001), as well as few compounds attractive to adult males (Riga et al., 1997). However, no plant origin compounds that elicit a chemoresponse to PCN juveniles have been identified, yet. Our tests revealed that linalool released by *Solanaceae* roots does not function as a defensive compound to PCN J2s but it may function as a kairomone enabling the nematodes to detect plants and may serve as a nonspecific attractant. Several fractions of potato root diffusate contain compounds that elicit chemoattraction to PCN J2s (Devine and Jones, 2003). Thus, many other compounds in addition to linalool could be involved in nematode attraction to *Solanaceae* roots, and increasing response to host specificity. Linalool is the first compound present in host plants that elicits chemoattraction to juveniles of both PCN species.

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