Effect of (*E*)-Chalcone on Potato-Cyst Nematodes (*Globodera pallida* and *G. rostochiensis*)

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Among soil-borne plant diseases, nematodes are responsible for large crop losses. Conventional control methods are currently based on the use of low-specificity neurotoxic compounds, such as carbamates, and phosphorylated and halogenated organic compounds. Some of these substances cause global environmental problems; for example, methyl bromide has a destructive effect on the ozone layer and its production is being restricted. (*E*)-Chalcone (*trans*-1,3-diphenylpropenone) was found highly toxic for phytoparasitic nematodes ($CL_{50} = 33\ \mu M$), acting also as a potent inhibitor of nematode hatch ($HIC_{50} = 7\ \mu M$). (*E*)-Chalcone provides new insight in the search for novel environmentally sound alternatives to conventional nematicidal compounds.

Keywords: *Globodera spp.; trans-chalcone; nematicide; nematostatic; phytotoxicity*

INTRODUCTION

Cyst-forming nematodes (*Globodera* spp. and *Heterodera* spp.) are highly specialized parasites with a narrow host range among the most important economic crops including cereals, potato, soybean, sugar beet, and vegetables (Lamberti and Taylor, 1986). Some of the species can survive for many years in the soil in the absence of a suitable host, with hatching of the infective stages relying on chemical signals released by the host plant (Antoniou, 1989; González and Phillips, 1996). Also, cyst-forming nematodes elicit the transcription of genes encoding several enzymes of the phenylpropanoid pathway, which ends with the production of phytoalexins. Resistance mechanisms in plants have often been related to the activity of this metabolic pathway (Hahlbrock and Scheel, 1989; Bennett and Wallsgrove, 1994; Edens et al., 1995). These features provide alternative approaches to the use of environmentally costly agrochemicals; among these features are the following: (a) phytoalexins or related compounds that could be directly used in plant protection or to regulate gene expression leading to synthesis and accumulation in the plant (Kuc, 1995); (b) molecular signals (Masamune et al., 1982; Fukozawa et al., 1985) to produce nematode hatch in the absence of suitable hosts; and (c) molecules with the opposite effect acting as hatching inhibitors (González et al., 1994).

High activity against the zooparasitic nematodes *Syphacia obvelata* and *Nematospiroides dubius* had been found for (*E*)-chalcone and other related compounds (Laliberté et al., 1967). In a screening for nematicidal activity of metabolites derived from the phenylpropanoid pathway we have found that two naturally occurring lignans (bursehernin and matairesinol) inhibit the hatching of the potato-cyst nematodes *Globodera pallida* and *G. rostochiensis* (González et al., 1994) and that nematicidal activity observed in aromatic compounds related to shikimate pathway was due to the presence of a carbonyl conjugated system in the molecule (González et al., 1995).

This paper describes the nematicidal and hatching inhibition activities of (*E*)-chalcone, including also a study of the phytotoxicity of the compound.

EXPERIMENTAL PROCEDURES

Nematicidal Activity. Batches of up to 1000 cysts of a 50% mixture of the nematodes *G. pallida* and *G. rostochiensis* were induced to hatch using a 10 mM $ZnSO_4$ solution. Every 2 days the cysts were thoroughly washed with distilled water and the hatching solution was replaced with fresh stock. Aliquots (100 μL) containing ~50 newly hatched second-stage juveniles were placed in the test solution containing known concentrations of commercial (*E*)-chalcone (Aldrich catalog no. 13,612-3, 97%) dissolved in aqueous dimethyl sulfoxide (DMSO), the concentration of which never exceeded 1%. At this concentration DMSO alone is without effect on nematodes. The concentration of (*E*)-chalcone was estimated by UV spectroscopy. Appropriate controls were used; the total volume of the test solution was 2 mL, and each treatment was applied to four replicates. Lethal concentration for 50% mortality of second-stage juveniles after 24 h of exposure (LC_{50}) was estimated by extrapolation from the best fitted regression curve between (*E*)-chalcone concentration vs percentage of dead nematodes. The value for LC_{100} was that directly observed in the bioassay.

Hatching Inhibition Analysis. Batches of 10 cysts of a 50% mixture of the nematodes *G. pallida* and *G. rostochiensis* were soaked in distilled water for 2 days. The water was then replaced by 2 mL of the test solutions. Hatched juveniles were counted regularly, the cysts washed thoroughly with distilled water, and the test solutions replaced with fresh stock. Each treatment was applied to four replicates. To test the irreversibility of hatching due to the presence of (*E*)-chalcone in the media, on day 15 the cysts were thoroughly washed and

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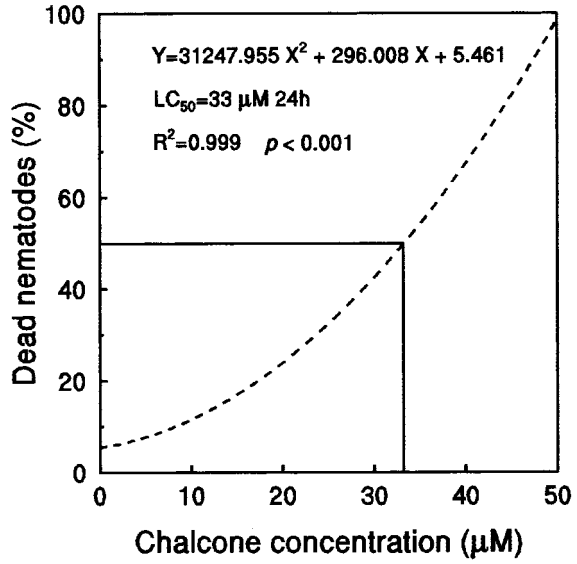

Figure 1. Lethal concentration of (*E*)-chalcone for 50% mortality of second-stage juveniles of the nematode (CL_{50}). Correlation coefficient (R^2) and goodness of the regression (p) are given.

transferred to new vials with 2 mL of 10 mM $ZnSO_4$ as hatching agent. Hatching inhibiting concentration of (*E*)-chalcone for 50% reduction of hatch (HIC_{50}) was estimated by extrapolation from the best fitted regression curve between concentration of compound and percentage of hatched nematodes at day 15 of the experiment.

Phytotoxicity Analysis. Ten tomato seeds were placed in a Petri dish over a filter paper impregnated with different concentrations of (*E*)-chalcone, which was placed in a growth chamber in the dark at 28 °C. Ten watercress seeds (*Lepidium sativum*) were placed in vials with 5 mL of different concentrations of (*E*)-chalcone and placed in a growth chamber at 25 °C. Each treatment was applied to four replicates, and after 7 days, the stem in the tomato and the primary root length in the watercress seedlings were measured. In addition, plants of *Vinca major*, *Adiantum capillus-veneris*, *Begonia rex*, *Hedera helix*, and *Musa acuminata* cv. Cavendish Gran enano were grown under controlled conditions at 25 °C, 80% humidity, and 13 h day length and watered at field capacity with a saturated solution of (*E*)-chalcone. The development of the growing plants was followed and compared with untreated controls.

RESULTS AND CONCLUSIONS

The nematicidal activity of (*E*)-chalcone is very high (LC_{50} = 33 μM, LC_{100} < 50 μM) (Figure 1). Together with the high nematicidal activity, (*E*)-chalcone is also able to inhibit the hatching of second-stage juveniles (infective stage) from nematode cysts. A 7 μM concentration inhibits 50% hatch of *Globodera* spp.; 100% irreversible inhibition occurs for <10 μM of (*E*)-chalcone in the media (Figure 2). No differences were observed in the nematicidal or hatching inhibition activities of (*E*)-chalcone between the two species of potato-cyst nematodes.

No phytotoxicity was observed when plants of *V. major*, *A. capillus-veneris*, *B. rex*, *H. helix*, and *M. acuminata* cv. Cavendish Gran enano were watered at field capacity with a saturated solution of chalcone. When seeds of tomato and watercress were germinated in chalcone-saturated media, only a significant reduction in the elongation of the primary root of watercress seedlings was observed at the highest dose tested (Figure 3).

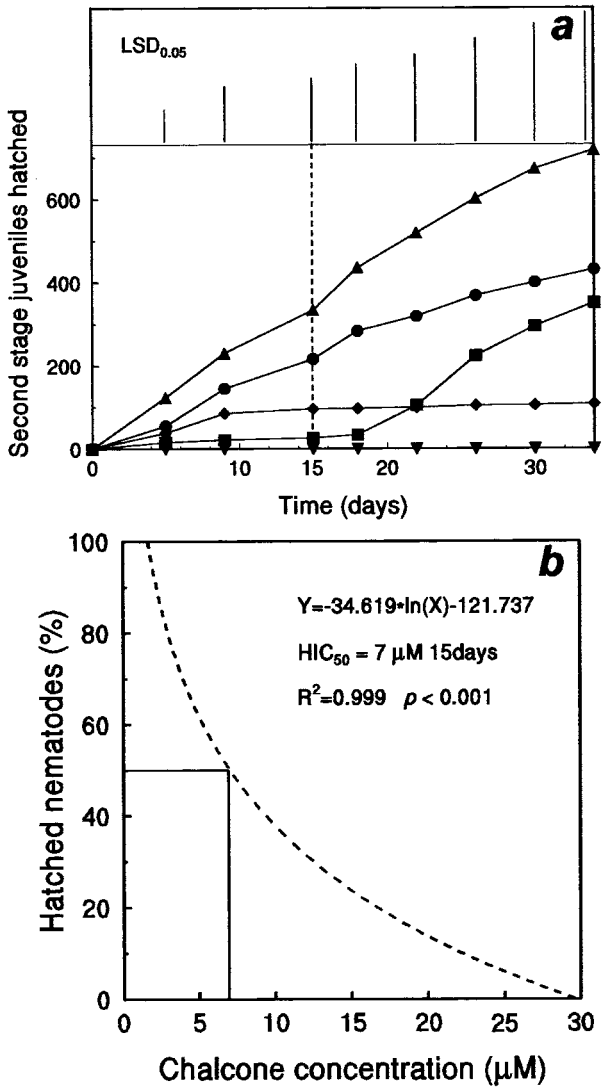

Figure 2. Effect of (*E*)-chalcone on nematode hatch: (a) (▲) 1% DMSO and 10 mM $ZnSO_4$ water solution as hatching agent; (▼) 1% DMSO, hatching agent, and (*E*)-chalcone 30 μM; (◆) 10 μM; (●) 5 μM; (■) control with distilled water and 1% DMSO. Least significant difference for a confidence limit of 0.05 ($LSD_{0.05}$) is given. On day 15 the cysts were transferred to 10 mM $ZnSO_4$ as hatching agent. (b) Hatching inhibiting concentration of (*E*)-chalcone for 50% reduction of hatch (HIC_{50}). Correlation coefficient (R^2) and goodness of the regression (p) are given.

Although various biological activities have been reported for chalcones with substituents in the phenyl groups (Edwards et al., 1990; Edenharder et al., 1993; Christensen et al., 1994; Sato et al., 1994), ours is the first report of a chalcone affecting plant parasitic nematodes. The mode of action of (*E*)-chalcone in the nematodes is unknown but, as it occurs with other chalcones, it may act as an uncoupler of the oxidative phosphorylation processes in the mitochondria (Ravanel et al., 1987).

(*E*)-Chalcone is a substance with high toxicity and hatching inhibition activity for economically important phytoparasitic nematodes. However, it has low toxicity for other organisms; the oral toxicity at 48 h in male Albino-Swiss mice is LD_{50} = 1048 mg kg^{-1} (Alonso and Navarro, 1993). (*E*)-Chalcone and other related compounds are promising alternatives to the hazardous pesticides currently used in agriculture for the control of cyst nematodes.

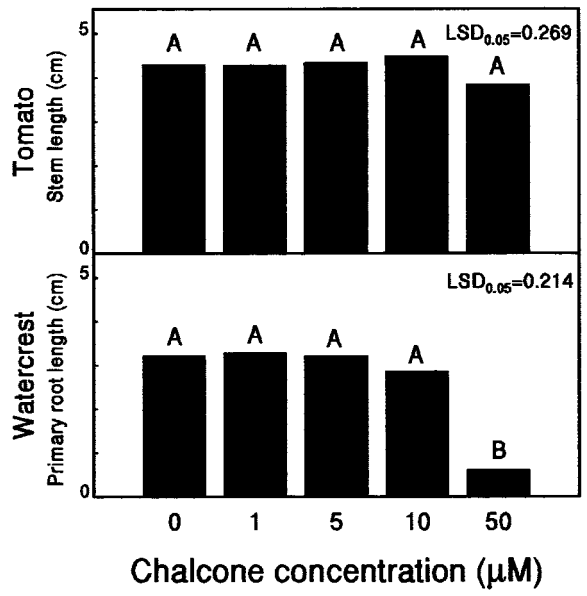

Figure 3. Effect of (*E*)-chalcone in seedlings of tomato and watercress. Least significant difference for a confidence limit of 0.05 ($LSD_{0.05}$) is given, treatments sharing the same letter have nonsignificant differences at 5% confidence level by Duncan's multiple-range test.

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