

Phytoprotection



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J.W. Potter and A.W. McKeown.

Volume 83, Number 2, 2002

URI: <https://id.erudit.org/iderudit/706234ar>

DOI: <https://doi.org/10.7202/706234ar>

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Publisher(s)

Société de protection des plantes du Québec (SPPQ)

ISSN

0031-9511 (print)

1710-1603 (digital)

[Explore this journal](#)

Cite this document

Potter, J. & McKeown, A. (2002). Inhibition of *Pratylenchus penetrans* by intercropping of *Rudbeckia hirta* and *Lycopersicon esculentum* in pot cultivation. *Phytoprotection*, 83(2), 115–120. <https://doi.org/10.7202/706234ar>

Article abstract

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La société de protection des plantes du Québec, 2002

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Inhibition of *Pratylenchus penetrans* by intercropping of *Rudbeckia hirta* and *Lycopersicon esculentum* in pot cultivation

John W. Potter¹ and Alan W. McKeown²

Received 2001-07-26; accepted 2002-04-18

PHYTOPROTECTION 83 : 115-120

In greenhouse pot culture over a 6-week period, population density of *Pratylenchus penetrans* declined pronouncedly both under *Rudbeckia hirta* alone and under a *R. hirta* - tomato combination, whereas tomato alone supported the nematode population. Population reduction is attributed in part to the known nematicidal effect of thiarubrine C in *R. hirta*. Overall, the presence of *R. hirta* in the tomato pot only slightly suppressed growth of either plant during the 6 weeks.

[Inhibition du *Pratylenchus penetrans* par culture intercalaire de *Rudbeckia hirta* et *Lycopersicon esculentum* en culture en pot]

Dans la culture en pot en serre chaude sur une période de 6 semaines, la densité de population du *Pratylenchus penetrans* a diminué sévèrement avec *Rudbeckia hirta* seul et avec un mélange de *R. hirta* et de tomate, tandis que la tomate seule a supporté la population de nématodes. La réduction de population est attribuée en partie à l'effet nématicide connu du thiarubrine C de *R. hirta*. De façon générale, la présence du *R. hirta* dans le pot de tomate a simplement supprimé légèrement la croissance de l'une ou l'autre des plantes durant les 6 semaines.

The root-lesion nematode *Pratylenchus penetrans* Cobb is an important pest in production of tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.) and other vegetables in Ontario (Potter and Olthof 1977, 1993), requiring remedial control by chemical or other means (McKeown and Potter 2001). Suppression of *P. penetrans* by the native sand-prairie plant species *Rudbeckia hirta* L. (brown-eyed susan) and *Rudbeckia serotina* Nutt. (black-

eyed susan) was reported previously (McKeown and Potter 1994; McKeown et al. 1994). In addition, *Ambrosia artemisiifolia* L. (common ragweed) was considered a poor host for *P. penetrans* and a non-host for the northern root-knot nematode *Meloidogyne hapla* Chitwood (McKeown and Potter 1994).

In several studies on the Asteraceae family, various authors commented on the presence of polyacetylene-deriva-

1. Agriculture and Agri-Food Canada, Research Station, Box 6000, Vineland Station, Ontario, Canada L0R 2E0; e-mail: potterj@em.agr.ca

2. University of Guelph, Department of Plant Agriculture, Box 587, Simcoe, Ontario, Canada N3Y 4N5.

tive compounds, including nematicidal thiophenes and thiarubrine, in *Rudbeckia* spp. and *Ambrosia* spp. (Freeman *et al.* 1993; Gomez-Barrios *et al.* 1992; Guillet *et al.* 1997; Lu *et al.* 1993; Sanchez de Viala *et al.* 1998), and changes to sex ratios of nematodes (El-Zawahry *et al.* 1998). Since the active nematicidal chemical in *Tagetes marigolds* (*Tagetes erecta* L., *T. patula* L., *T. tenuifolia* Cav.) was identified as a thiophene, α -terthienyl (Gommers and Voor in't Holt 1976), it seemed reasonable that the active antihelmintic compound in *Rudbeckia* spp. might also be either a thiophene or a closely related thiarubrine. Interestingly, Gommers and Voor in't Holt (1976) mentioned nematicidal red-coloured dithio compounds in *Rudbeckia* or *Ambrosia* spp., which they suggested were probably acetylenic but did not identify as α -terthienyl. Recently, Sanchez de Viala *et al.* (1998) have shown that the compound thiarubrine C in *R. hirta* was toxic to *P. penetrans* and *M. incognita* Kofoed & White, and pointed out that such compounds are prevalent in roots of plants in the family Asteraceae. Soil fertility may also impact nematodes, as Walker (1971) found that concentrations of nitrite over $70 \mu\text{L L}^{-1}$ can be toxic to nematodes. This could have implications for various nematological studies performed in greenhouse pot culture with inorganic fertilization.

Notwithstanding the modes of lethal action demonstrated above, they must be translated into practical solutions in the field environment. The potential of Asteraceae as rotation crops with assorted solanaceous agricultural crops has been studied recently (El-Zawahry *et al.* 1998; Kimpinski *et al.* 2000; Reynolds *et al.* 2000; Riga and Potter 1998). In most cases, the concept has taken the traditional agricultural approach of planting a rotational cover crop for most or part of a growing season, i.e. growing the nematode-suppressive plant prior to the desired agricultural crop. A preferable alternative approach might be to intercrop the suppressive plant at the same time and in adjacent space with the preferred agricultural crop, thus eliminating the problem of devoting agricultural land to plants with no di-

rect economic return. Since other suppressive plant species require soil incorporation for activity (McKeown and Potter 2001), a crop that was nematicidal *in situ* would be most useful for a no-till high residue soil conservation production system.

The objective of this research was to determine the effect on changes in population densities of the root-lesion nematode *P. penetrans*, of intercropping brown-eyed susan (*R. hirta*) in the same pot with the lesion nematode-susceptible tomato cv. Bonny Best. Pots with soil alone were used as a reference to account for any physical effects on populations.

Seeds of *R. hirta* and *L. esculentum* cv. Bonny Best were planted on 14 March 1995 in 200-cell Blackmore plug trays (Blackmore Co. Inc., Beltsville, MD) in a commercial soilless mix (Promix; Plant Products, Brampton, Canada) and the seedlings were grown to transplanting stage (fourth expanded leaf). A sufficient volume of Scotland sandy loam to fill 48 15-cm diam plastic pots was obtained from a field infested with *P. penetrans* at the Simcoe, Ontario, campus of the University of Guelph, Department of Plant Agriculture. The presence of root-lesion nematodes in this soil, at a level of approximately 1100 nematodes kg^{-1} , was verified by using the Baermann pan extraction method (Townshend 1963). This soil was mixed in a concrete mixer before filling 48 pots, and plants were transplanted into pots containing 500 g of unsterilized soil on 4 April 1995. Water-soluble greenhouse fertilizer, consisting of 200 mL of 20-20-20 with trace elements (Plant Products, Brampton, Canada) at $100 \mu\text{L L}^{-1}$, was applied on 4, 13, 20 April and 2 May, to half the pots; the other pots were not fertilized. Plants were grown in a greenhouse with day/night set temperatures of 25/15°C. Plants were harvested on 17 May 1995, when fresh and dry weights were measured.

The experiment was designed to compare the two levels of fertilization at a single nematode population density, under each of four plant treatments: 1) tomato alone; 2) *Rudbeckia* alone; 3)

Table 1. Nematode population of tomato and *Rudbeckia hirta* grown with or without fertilization^a

Treatments	Nematode number		Total number
	per root system	in soil, per pot	
Fertilizer -	57.8	26.7	84.4
Fertilizer +	79.5	10.8	90.3
<i>R. hirta</i>	2.4	0.4	2.8
Tomato	134.9	37.1	172.0
Anova (<i>P</i> values)			
Fertilizer	0.3842	0.1943	0.8834
Plant	< 0.0001	0.0040	< 0.0001
Fertilizer * plant	0.4586	0.1724	0.9578

^a Means of six replications.

tomato + *Rudbeckia*; 4) no host plant. For treatments 1 and 2, a single seedling of the appropriate plant was transplanted into each pot. For treatment 3, one seedling of each plant species was transplanted into the same pot. Treatment 4 contained no plant, to test the survival of the nematode in the absence of a plant. Treatments were arranged in a randomized complete block factorial design with six replications.

After harvesting the top growth from each pot, roots were removed from the soil, and nematode numbers in a 50-g soil subsample were determined by Baermann pan extraction (Townshend 1963). Nematodes were extracted from the weighed fresh root system by mist extraction (Goodey 1963) for 2 wk, following which the roots were dried and weighed. Total number of nematodes per pot was derived from summation of the calculated number in the soil + the total number extracted from each root. Fresh and dry top weights of both the tomato and *Rudbeckia* were determined. Data were analyzed as a fertility x plant species factorial using the General Linear Model procedure in SAS (SAS Institute Inc. 1985); pots with soil alone (treatment 4) were not included in the factorial analysis but were used to monitor any physical effects on nematode population.

As expected, the nematode population in pots containing no host plant declined substantially over the course of the experiment from an initial num-

ber of 550 nematodes 0.5 kg⁻¹ soil; a residual population of 45 and 37 nematodes 0.5 kg⁻¹ soil persisted in the "unfertilized soil" and "fertilized soil" pots, respectively. However, in pots with plants, fertilization had no detectable effect on nematodes (Table 1). *Rudbeckia hirta* reduced the number of nematodes per root system, per pot, and total nematodes, compared to tomatoes (Table 1). No or very few nematodes were recovered from the soil from any treatment in which *Rudbeckia* was planted alone or co-planted with tomato. Thus, in almost all pots where *Rudbeckia* was planted, *P. penetrans* was nearly eliminated from the soil within 42 d of transplanting, irrespective of the presence or absence of a concomitant tomato plant.

Tomato had higher fresh and dry weights of shoots than *R. hirta* (Table 2). There was more recoverable fresh and dry root weight of *R. hirta* than tomato. However, there was a differential effect of fertilization and plant species for shoots. Tomato shoots had higher fresh and dry weights when fertilized, but *R. hirta* shoots did not appear to respond to fertilization (Table 3). There was no observable phytotoxic effect of *R. hirta* on the growth of coexistent tomatoes.

Our observation of nematode suppression by native sand-prairie species of Asteraceae, along with published reports by other nematologists, stimulated our curiosity about practical agri-

Table 2. Effect of co-planted *Rudbeckia* and tomato on fresh and dry weights (g) of shoots and roots in pots^a

Treatment	Shoots		Roots	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Fertilizer -	21.3	2.6	12.0	0.8
Fertilizer +	29.3	14.6	13.8	0.9
<i>R. hirta</i>	20.7	2.6	19.8	1.3
Tomato	29.9	12.8	6.0	0.3
Anova (<i>P</i> values)				
Fertilizer	0.0149	0.0521	0.5006	0.6053
Plant	0.0058	0.0021	< 0.0001	< 0.0001
Fertilizer * plant	0.0376	0.0128	0.7215	0.8932

^a Means of six replications.

cultural uses of this effect and led to the present study. The demonstration by Sanchez de Viala *et al.* (1998), that thiarubrine C was active as a nematocidal agent against plant-parasitic nematodes when the pure compound was added to soil containing nematodes and a susceptible host (tomato), was an important advance in understanding the modes of action of species of Asteraceae. When Gommers and Voor in't Holt (1976) commented on the presence of strongly nematocidal unstable red-coloured dithio compounds in *Ambrosia artemisiifolia* and *Ambrosia trifida* L., they were very close to implicating nematocidal thiarubrine in ragweeds, perhaps the same thiarubrine C which Sanchez de Viala *et al.* (1998) identified as the active nematocidal material in *Rudbeckia hirta*. The importance to agriculture of nematode-suppressive weeds should be fairly obvious.

However, investigation of a mechanism of action must be transferred into practical terms, to become a benefit to "real-world" agriculture. Our principal concern was whether protectant activity against nematodes could be demonstrated by co-planting *Rudbeckia* along with a nematode-susceptible host. Because our previous field studies had already demonstrated that nematode suppression occurred in the presence of *R. hirta* for an entire growing season (McKeown *et al.* 1994), we did not emphasize studying its mode of action. In investigating protectant activity, we imposed the additional constraint that the coexistence must not be detrimental to either plant partner; this concern has been allayed by the finding that root growth and top growth of both partners was not seriously diminished by the co-planting approach.

Table 3. Differential response of shoot growth of *Rudbeckia hirta* and tomato in response to fertilizer^a

Fertilizer	<i>R. hirta</i>	Tomato
Fresh weight		
Fertilizer -	20.1	22.5
Fertilizer +	21.3	37.3
Dry weight		
Fertilizer -	2.7	3.0
Fertilizer +	2.5	4.9

^a Means of six replications. LSD (*P* = 0.05) for fresh weight = 9.0; dry weight = 1.2.

The nematode data obtained in the present study require interpretation to evaluate the reasons for the rapid changes from the initial population of 550 nematodes per pot to the much lower populations presented in Table 1. Quite possibly, the nematodes remaining in the unplanted soil represent those which survived the initial mechanical soil mixing as well as 6 wk without a food source. Walker's (1971) observation on nitrite toxicity effects might explain somewhat lower numbers observed in the fertilized, unplanted controls, and could be investigated. The reason for the near-complete eradication of nematodes in the *Rudbeckia*-planted pots is partly explained by the research of Sanchez de Viala *et al.* (1998). However, given that thiophenes and thiarubines are not very water-soluble (B. McGarvey, personal communication), the question of how nematodes could be killed at some distance from a *Rudbeckia* root, or in a tomato root, remains unexplained. In the tomato-alone pots, it appears that nematodes probably entered the roots in considerable numbers, and may have reproduced to some extent, as the 6-wk experimental period is about the length of one life cycle. If so, does this imply that they were killed *in situ* in the tomato roots in the "tomato + *R. hirta*" pots? If a water-soluble antihelminthic compound occurs in *R. hirta*, biocontrol could be achieved without incorporating the plant tissue into the soil, as is done now with most cover crops.

The reduction of nematode numbers in a confined pot is evidence that a similar mechanism may function in the field. Extensive field sampling of *R. hirta*, a remnant sand-prairie species (McKeown *et al.* 1994), over several yr (McKeown *et al.* unpublished) has demonstrated that this plant is highly suppressive; in fact, no nematodes were collected under it in 6 yr of sampling effort. As well, Kimpinski *et al.* (2000) have shown suppression of field populations of root-lesion nematodes by *R. hirta*, as a precursor to potato cultivation. This suggests that intercropping with *R. hirta* or similar species may protect susceptible hosts. We contend that, in their natural sand-prairie habi-

tat, *Rudbeckia* spp. and possibly other Asteraceae may function as protectants for highly nematode-susceptible species such as wild Leguminosae (legumes) and Labiatae (mints) (McKeown *et al.* 1994). Even common ragweed has its ecological niche and might prove useful as a model for studying suppressive mechanisms or as a source of nematode-suppressive genes, although its use for controlling nematodes in agriculture and/or home gardens is limited unless it could be rendered non-allergenic. The opportunity and challenge is to utilize our ecological knowledge to develop practical nematode suppressive cropping systems.

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