RESEARCH/INVESTIGACIÓN

DEVELOPMENT OF WINTER COVER CROPS IN SOIL INFESTED WITH THE NEMATODE *TUBIXABA TUXAUA* IN WESTERN PARANÁ, BRAZIL

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ABSTRACT

Furlanetto, C., J. E. Cares, M. I. F. Cruz and R. Guimarães. 2012. Development of winter cover crops in soil infested with the nematode *Tubixaba tuxaua* in Western Paraná, Brazil. Nematropica 42:314-319.

This work aimed to study the effect of cover crops cultivated in the winter season on the population of *Tubixaba tuxaua* under greenhouse and field conditions. The green manures studied were seeded in pots containing sterile substrate (soil + sand 1:1) with capacity for 5 Kg of substrate. Four plants per pot were inoculated with 40 mixed stages of T. tuxaua when three mature leaves had been developed. The pots were grown under greenhouse condition at 25°C. The experiments, pot and field assays, were run in a completely randomized design with 6 treatments and 5 replications. The treatments (cover crops) were *Raphanus sativus* var. *oleiferus* (oilseed radish), *Lupinus albus* (white lupine), *Lathyrus clymenum* (grass pea), *Vicia villosa* (hairy vetch), *Pisum sativum* var. *arvense* cv. 'Iapar 83' (field pea) and *Avena strigosa* (black oat). Field measurements were plant height, fresh shoot weight, fresh root weight, and initial and final population density of *T. tuxaua*. For the pot assay data collected were shoot height, root length and final population densities of *T. tuxaua*. The results showed that all plants studied, except the oilseed radish, were susceptible to *T. tuxaua*. Oilseed radish significantly decreased the population density of *T. tuxaua* in plots in the field experiment and also in pots. This is the first report of a cover crop (*R. sativus* var. *oleiferus*) with potential to be used in rotation with major crops for controlling *T. tuxaua*.

Key words: Aporcelaimidae, control, crop rotation, ectoparasitic nematode, host suitability.

RESUMO

Furlanetto, C., J. E. Cares, M. I. F. Cruz and R. Guimarães. 2012. Desenvolvimento de culturas de cobertura de inverno em solo infestado com o nematoide *Tubixaba tuxaua* no Oeste do Paraná, Brasil. Nematropica 42:314-319.

Objetivou-se estudar a reação de culturas de cobertura cultivadas em estação de inverno no controle de populações de *Tubixaba tuxaua* em condições de casa de vegetação e campo. Os adubos verdes estudados foram semeados em vasos contendo substrato estéril (solo + areia 1:1) com capacidade para 5 Kg de substrato. Quatro plantas por vaso foram inoculadas com 40 estádios mistos de *T. tuxaua* quando três folhas definitivas haviam sido formadas. Os vasos foram mantidos em casa de vegetação a temperatura de 25°C. Os ensaios em vaso e campo foram desenvolvidos em delineamento inteiramente casualizado com 6 tratamentos (culturas de cobertura) e 5 repetições. Os tratamentos foram *Raphanus sativus* var. *oleiferus* (nabo forrageiro), *Lupinus albus* (tremoço branco), *Lathyrus clymenum* (ervilhaca forrageira), *Vicia villosa* (ervilhaca peluda), *Pisum sativum* var. *arvense* cv. 'Iapar 83' (ervilhaca comum) e *Avena strigosa* (aveia preta). As variáveis analisadas para o ensaio de campo foram altura de plantas, massa fresca da parte aérea, massa fresca do sistema radicular e população inicial e final de *T. tuxaua*. Para o ensaio em vasos as variáveis analisadas foram altura de plantas, comprimento de raízes e população final de *T. tuxaua*. Os resultados mostraram que os adubos verdes estudados, exceto nabo forrageiro, foram suscetíveis a *T. tuxaua*. O tratamento nabo forrageiro foi superior aos demais tratamentos para todas as variáveis estudadas, reduzindo significativamente as populações de *T. tuxaua* em parcelas em campo e também em vasos. Esse é o primeiro relato de uma cultura de cobertura (*R. sativus* var. *oleiferus*) com potencial para ser usada em rotação com culturas econômicas no controle de *T. tuxaua*.

Palabras clave: Aporcelaimidae, controle, rotação de cultura, nematoide ectoparasita, hospedabilidade.

INTRODUCTION

The genus *Tubixaba* Monteiro & Lordello, 1980, belongs to the order Dorylaimida Pearse, 1942, superfamily Dorylaimoidea De Man, 1876, family Aporcelaimidae Heyns, 1965 and subfamily Aporcelaiminae Heyns, 1965, this last composed of 9 genera, including *Tubixaba* (Jairajpuri & Ahmad, 1992).

Four species of *Tubixaba* have been described in the literature: *T. tuxaua* Monteiro & Lordello, 1980, *T. parva* Pretorius, Kruger & Heyns (syn. *T. tswanorum* Nell & Heyns, 1987), *T. minima* Botha & Heyns, 1990 and *T. Saccata* Popovici, 1995. Recently, Chaves *et al.* (2007) reported three species of *Tubixaba* in Argentina.

General aspects concerning the biology of *T. tuxaua* are unknown as well as its trophic role (Procter, 2012). However, the characteristics found in members of Aporcelaimidae suggest that *T. tuxaua* has a long life cycle and reproduces in a low rate. Also, males of *T. tuxaua* are commonly found in field populations (Monteiro & Lordello, 1980; Vovlas *et al*., 1987) lacking information about its reproductive behaviour.

The feeding behaviour in Aporcelaimidae includes predacious and omnivorous nematodes (Yeates *et al.,* 1993). However, there are reports in Brazil that support *T. tuxaua* as a plant parasite which feeds ectoparasitically on the roots of green manures and annual crops (Carneiro & Carneiro, 1983; Furlanetto *et al*., 2008 and 2010). Like *T. tuxaua,* other *Tubixaba* species were described in association with plant root systems. In Africa, *T. parva* was found associated with roots of grasses and Acacia trees, *T. minima* with roots of grasses (Botha & Heyns, 1990) and *T. saccata* was collected in soil from a Montane grassland (Popovici, 1995).

Considering the roles of *T. tuxaua* on crops and its host range known so far, the goal of this work was to study the effect of plant species grown in winter season to control this nematode.

MATERIAL AND METHODS

The development of winter plant species to control the nematode *T. tuxaua* was first studied under greenhouse in pots. Also, a field assay was conducted using the same treatments as the greenhouse study. Both assays were conducted at the Experimental Station of the State University of Western Paraná, located in south of Brazil.

The cover crops tested were *Raphanus sativus* L. var. *oleiferus* Metzg. (Oilseed radish), *Lathyrus clymenum* L. (Grass pea), *Lupinus albus* L. (White lupine), *Vicia villosa* Roth. (Hairy vetch), *Pisum sativum* L. var. *arvense* (L.) Poir. cv. IAPAR 83 (Field pea) and *Avena strigosa* Schreb. (Black oat) as the control. Black oat was selected as positive control due to its susceptibility to the nematode *T. tuxaua* in fields in Western Paraná.

Extraction and identification of Tubixaba tuxaua

Soil samples were collected in areas infested by *T. tuxaua* and located in the municipalities of Marechal Cândido Rondon and Quatro Pontes, Western Paraná. Aliquots with 200 cc of soil were homogenized in 5 L water. The suspensions were poured through layered sieves of 0.84 mm and 0.250 mm. The contents of the 0.250 mm sieve were transferred to a Petri dish (15 X 60 mm) and observed under a stereoscopic microscope. Juveniles and adults of *T. tuxaua* were collected using a handling needle. When necessary, nematodes were examined directly under a light microscope and compared with the description made by Monteiro & Lordello (1980) and Vovlas *et al.* (1987) for species identification.

Greenhouse assay

A mixture of soil and sand 1:1 was autoclaved at 120°C for 60 minutes and fertilized with 10 grams of N(4)-P(14)-K(8) per pot. The experimental design adopted for the pot assay was completely randomized with six treatments (plant species) and five replications. Each plant species was sown at a rate of five seeds per plastic pot filled with 5Kg of soil. After germination, the plants were thinned to four plants per pot. Forty mixed life stages of *T. tuxaua* were inoculated on the root system of plants once they had three mature leaves. Inoculated plants were grown in a glasshouse at 25°C, watered daily and fertilized monthly with 5 g of N(4)-P(14)-K(8). Each treatment was compared with its control (non-inoculated plants) 90 days after ther inoculation. Black oat, white lupine, hairy vetch, grass pea, field pea and oilseed radish were assessed for the variables plant height, root length and final population densities of *T. tuxaua*. A total of twenty plants were measured. The final population of *T. tuxaua* in each pot was estimated by extracting *T. tuxaua* from 5 Kg of soil as described previously.

Field assay

The field assay was performed in a rural property located in the municipality of Quatro Pontes. Plot areas infested with *T. tuxaua* were previously selected based on the presence of 30 day old stunted wheat plants. Control plots were established in areas with symptomless wheat. The experimental design for the field assay was completely randomized with six treatments (plant species) and five replications. Wheat plants (30 days old) were picked up from the soil line, shook to release the soil adhered to the roots and disposed of the experimental area before seeding the winter crops in the plots. The experimental area was 30 m^2 in areas symptomatic of *T. tuxaua* infestation and 30 m^2 in areas without nematode symptoms. Data were collected from 1m^2 within each plot. Soil samples from each replicate in infested and control plots were collected prior to planting test plants to estimate *T. tuxaua* population density. The plants tested were the same used in the pot assay. Black oat was sown at 60 seeds per meter with 25 cm between rows; White lupine was sown at 15 seeds per meter with 50 cm between rows; Hairy vetch was sown at 20 seeds per meter with 40 cm between rows; Field pea was sown at 45 seeds per meter with 50 cm between rows; Grass pea was sown at 18 seeds per meter with 20 cm between rows; and oilseed radish was sown at 25 seeds per meter with 25 cm between rows.

Data collected for each test plant species were plant height, fresh shoot weight, fresh root weight and final population densities of *T. tuxaua* per plot 80 days after seeding. A total of 30 plants per plot were evaluated. For plant height measurement was taken from the top to the base of the plants. Fresh shoot weight was obtained after the plants had been removed from the ground.

Four soil samples were collected from zero to 20 cm deep in each plot, two before the experiment was initiated, and two during the evaluation procedure to estimate the *T. tuxaua* population density. Aliquots with 200 cc of soil were used for extraction and quantification of *T. tuxaua* in each sample, using the methods described in the greenhouse experiments.

Statistical Analysis

Test t (Student) was applied to compare data between infested and control plots and between inoculated and non-inoculated pots. The software ASSISTAT (Silva & Azevedo, 2002) was used for statistical analysis.

RESULTS

Statistical differences were detected between infested and non-infested plots for most of the variables assessed in the field assay, except for the treatment with oilseed radish (Figure 1). Plant shoot height was reduced when grown in infested plots. Black oat height reduction was 28.85% when compared with plants cultivated in control plots. White lupine height reduction was 31.1%, 21.2% for field pea, 51.15% for grass pea and 72.76% for hairy vetch.

There were statistical differences in fresh shoot weight between control and infested plots for the treatments black oat, white lupine, hairy vetch, grass pea and field pea, but no difference was detected for oilseed radish (Figure 2). In infested plots shoot weight reduction for black oat averaged 57.8%, white lupine was reduced 27.58%, field pea 23%, grass pea 74.5% and hairy vetch 80.7%.

Fresh root weight, in control and infested plots, were statistical different with the exception of oilseed radish (Figure 3). Black oat plants had an average fresh root weight reduction of 33.70% in relation to the control plots. White lupine reduction was 35.7%, field pea 73.82%, grass pea 76.17% and 53.80% for hairy vetch.

Populations of *T. tuxaua* were reduced in infested plots in all treatments 80 days after seeding. Despite being reduced, the population density before seeding and 80 days after seeding were not significantly different except for oilseed radish where the initial population was reduced 90.6% (Figure 4).

On tests carried out in pots filled with sterile soil and analyzed 90 days after the inoculation, there was significant reduction of plant height for most treatments in contrast with the controls. Black oat showed reduction of 33.12%, white lupine reduced 31.1%, field pea 21.28%, grass pea 51.68% and hairy vetch 24.82%. However, under the same conditions, plants of oilseed radish inoculated with *T. tuxaua* were 11.29% taller than control plants (Figure 5).

For the variable root length in pots, only oilseed radish (inoculated plants) did not show significant difference when compared to control plants, while for the other crops root length decreased significantly from inoculated to control plants (Figure 6).

In all treatments, the population densities of *T. tuxaua* inoculated in pots decreased after 90 days of inoculation. However, the highest percentage of reduction was obtained in the treatment oilseed radish (98%), followed by hairy vetch (81.5%), white lupine (80%), field pea (72.5%), black oat (67%) and grass pea (63%) (Figure 7).

DISCUSSION

Based on the results of field and pot assays, black oat, white lupine, field pea, grass pea and hairy vetch were susceptible to *T. tuxaua* and should not be recommended for areas infested with this nematode. According to the variables studied in the field experiment, plants in the infested plots were significantly shorter in height, had lower fresh shoot weight and less root development. In the pot assay, the reduction of plant height and root length for most treatments supports the data obtained in the field. Based on the field and pot assays, the growth of oilseed radish plants was stimulated when cultivated in infested plots, showing results compatible with the control plots without nematodes. Data from infested plots and from pots cultivated with oilseed radish had significantly fewer *T. tuxaua* levels at harvest than those of control treatments for field and pot assays, suggesting that, this nematode may be affected when they are in the rhizosphere of these plants. Furlanetto *et al.* (2008) found similar results in field and in pot studies. The authors reported that the dry season in field and the high thermal amplitude in the greenhouse might have contributed to the decrease of *T. tuxaua* populations in field and in pots, respectively. Nevertheless, the authors also mentioned that the long life cycle of *T. tuxaua* and the low rate of reproduction also have contributed to the decrease of nematodes during the experimental

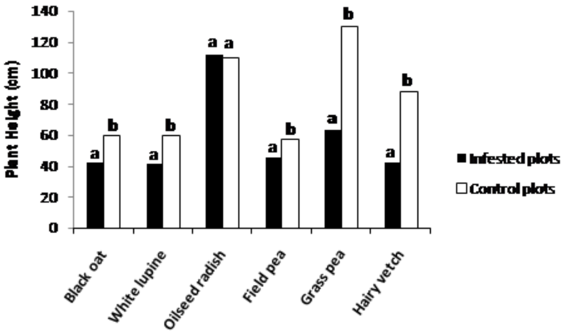

Fig. 1. Height of cover crops grown in *Tubixaba tuxaua* infested and non-infested plots. Black Oat: CV=10.75%, dms=8.00; White lupine: CV=7.19%, dms= 5.30; Oilseed radish: CV=5.84%, dms=9.47; Field pea: CV=12.32%, dms=9.29; Grass pea: CV=12.21%, dms=17.33; Hairy vetch: CV=9.20%, dms=8.74.

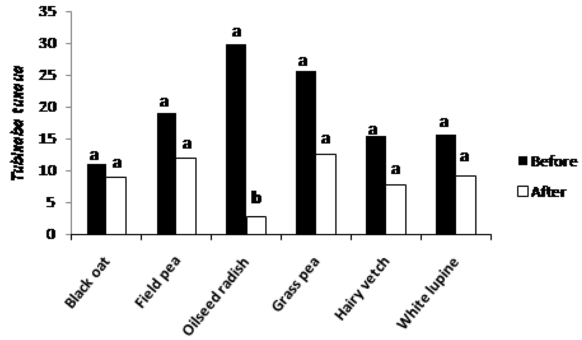

Fig. 4. Evaluation of *Tubixaba tuxaua* population density within plots before sowing cover crops and 80 days after sowing. Black oat: CV=31.85%, dms=4.70; Field pea: CV=41.81%, dms=9,46; Oilseed radish: CV=53.64%, dms=12.77; Grass pea: CV=52.17%, dms= 14.55; Hairy vetch: CV=52.44%, dms=8.88; White lupine: CV=48.89%, dms=8.85.

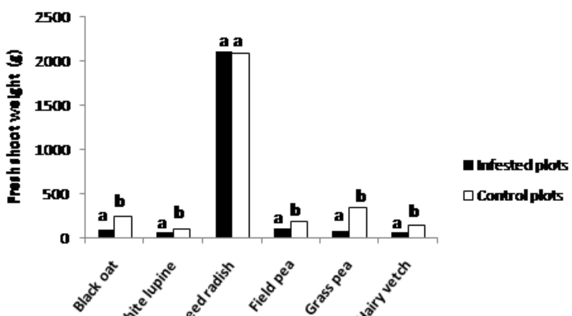

Fig. 2. Fresh shoot weight of cover crops cultivated in *Tubixaba tuxaua* infested and non-infested plots. Black oat: CV=20.10%, dms=28.33; White lupine: CV=7.06%, dms=8.36; Oilseed radish: CV=26.18%, dms=805.97; Field pea: CV=16.44%, dms=37.76; Grass pea: CV=44%, dms=137.87; Hairy vetch: CV=9.37%, dms=14.18.

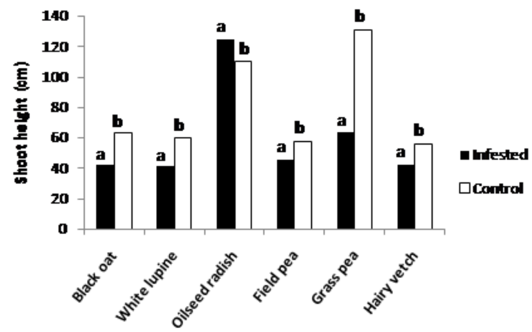

Fig. 5. Shoot height of cover crops cultivated in pots containing sterile soil and inoculated with 40 nematodes (juveniles and adults of *Tubixaba tuxaua*), evaluated 90 days after seeding. Black oat: CV=15.04%, dms=11.62; White lupine: CV=7.19%, dms=5,30; Oilseed radish: CV=6.55%, dms=11.21; Field pea: CV=12.32%, dms=9.20; Grass pea: CV=12.17%, dms=17.25; Hairy vetch: CV=13.70%, dms=9.88.

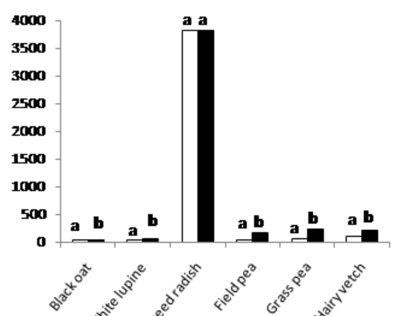

Fig. 3. Fresh root weight (g) of cover crops cultivated in *Tubixaba tuxaua* infested and non-infested plots. Data were multiplied 10X to adequate the graphic. Black oat: CV=20.56%, dms=0.88; White lupine: CV=14.30%, dms=0.74; Oilseed radish: CV=26.17%, dms=145.76; Field pea: CV=16.21%, dms=2.42; Grass pea: CV= 31.89%, dms= 6.77; Hairy vetch: CV=8.56%, dms=1.91.

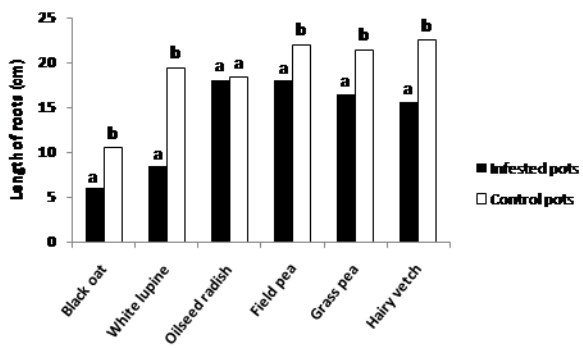

Fig. 6. Root length of cover crops cultivated in pots with sterile soil and inoculated with 40 *Tubixaba tuxaua* (juveniles and adult stages), assessed 90 days after seeding. Black oat: CV=14.26%, dms=1.72; White lupine: CV= 25.23%, dms=5.12; Oilseed radish: CV=21.91%, dms=5.82; Field pea: CV= 17.14%, dms=5.0; Grass pea: CV=14.30%, dms=3.94; Hairy vetch: CV=20.48%, dms=5.71.

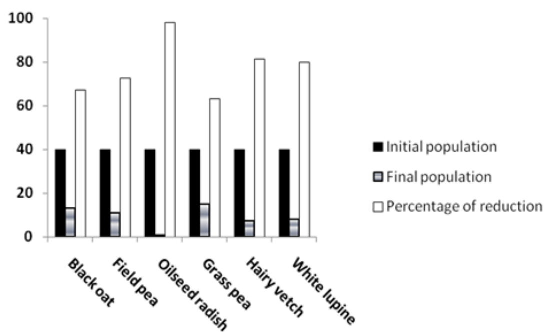

Fig. 7. Percentage of reduction of *Tubixaba tuxaua* population density inoculated in pots and evaluated 90 days after seeding.

period. In this work, the temperature in the greenhouse was controlled (25°C). Therefore, probably the long life cycle of *T. tuxaua* (up to one year or more in certain dorylaimids as *Xiphinema*) seems to be the most probable cause for the decline of the population densities in pots. In dry seasons in the field, *T. tuxaua* probably migrates down in the soil being absent in soils collected at 20 cm deep for analysis. Although, data in the literature suggest that a few nematodes in soil (a minimum of 20 per 200 cc of soil) can cause damage to plants (Furlanetto *et al.*, 2010).

Raphanus sativus var. *oleiferus* (oilseed radish) is a plant that belongs to the Brassicaceae (Cruciferae) family. Plants in this family, used as cover crops, are also known as mustards. *Raphanus sativus* var. *oleiferus* is used in rotation with major crops in winter or autumn seasons. Integration of these plants into cropping systems brings several benefits such as erosion control, soil aeration, weed suppression, nitrate cycling, biomass production and control of insects, nematodes and other plant pathogens (Ngouajio and Mutch, 2004).

The potential of Brassicaceae plants for controlling nematodes relies on the glucosinolate compounds present in their tissues (Rosa *et al.*, 1997). When hydrolyzed, the glucosinolates are converted into isothiocyanates and, once released in soil react with the sulfhydryl groups of proteins present in nematodes and other organisms (Brown & Morra, 1997). The effectiveness of some species of Brassicaceae in the control of plant-parasitic nematodes has been tested previously (Chitwood, 2002). *Brassica nigra*, the black mustard, and *Sinapsis alba*, the white and yellow mustard (Ellenby, 1945 and 1951), were tested successfully against *Globodera rostochiensis*. *Brassica napus* (rapeseed) was used as a green manure in the control of nematodes (Halbrendt, 1996). Also, extracts of leaves and seeds of rapeseed, previously hydrolised, provided a good control of the plant-parasitic nematode *Heterodera schatii* (Lazzeri *et al.*, 1993), but also affected the free-living nematode *Caenorhabditis elegans* (Donkin, 1995).

In Brazil, *R. sativus* var. *oleiferus* was tested before as a green manure in the control of *Heterodera glycines* and *Rotylenchulus reniformis* (Ferraz *et al*., 2010). The effective control of *R. reniformis* in cotton by rotation with *R. sativus* var. *oleiferus* was likewise reported by Asmus *et al*. (2008). Low densities of *R. reniformis* were found in plots and in pots after *R. sativus* had been cultivated. This plant was also recommended to reduce *Meloidogyne incognita* race 4 and race 2 because of low or no reproduction on this host (Asmus *et al*., 2005). However, *R. sativus* var. *oleiferus* was not considered efficient for the control of *Pratylenchus brachyurus* when cultivated in infested areas, despite an RF value close to 1.0 (Inomoto *et al.*, 2006). Carneiro *et al.* (1998) reported oilseed radish as a poor host for *Mesocriconema xenoplax*, *M. incognita* and *M. javanica* parasites of peach plants and thus ideal crop to be planted in winter seasons.

This is the first report of a plant species (*R. sativus* var. *oleiferus*) with potential to be used in rotation with crops for controlling *T. tuxaua*. Further studies will be necessary to confirm whether oilseed radish will increase the yield of crops when integrated into cropping systems affected by *T. tuxaua*.

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