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First report about the trapping activity of *Stropharia rugosoannulata* acanthocytes for Northern Root Knot Nematode

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Summary

This study summarises the results of in vitro screening of the nematophagous activity of *Stropharia rugosoannulata* and *Arthrobotrys oligospora*. The tests were conducted with *Meloidogyne hapla* plant parasitic nematode juveniles placed into Petri dishes containing cultures of the tested fungal species. Immobilisation of the nematodes was observed after 4 and 24 hours. Both species of fungi showed nematophagous activity, however it was much stronger and faster in the case of *S. rugosoannulata.*

Keywords: nematophagous fungus; *Stropharia; Arthrobotrys*; *Meloidogyne hapla*; biocontrol; plant parasitic nematodes

Introduction

Soil organisms represent a complex system, with many interspecific relationships. Soil nematodes (Nematoda) constitute an important component of the soil fauna. This group of invertebrates contains representatives of most trophic groups, including species utilising decomposing organic material, bacteria, other soil invertebrates, including other nematodes, and plant material. Nematodes alone serve as a source of energy for number of soil organisms, such as mites (Acarina) (Walter & Kaplan, 1991), springtails (Collembola) (Lee & Widden, 1996) and even the roots of carnivorous higher plants (genus *Philcoxia*) (Pereira *et al.,* 2012). However, the most important group of soil organisms feeding on nematodes are soil fungi (Janson & Lopez-Llorca, 2004). This group of organisms has developed a broad range of different trapping strategies and specialised organs e.g., constrictive and nonconstrictive rings (Gray, 1987), adhesive knobs and branches (Jafee, 2004) and hyphae nets (Gray, 1987). Numerous attempts have been conducted to utilise nematophagous fungi for the control of plant parasitic nematodes e. g. Bourne and Kerry (1998); Van Damme *et al.* (2005); Singh *et al.* (2007). Indeed, a commercial biological treatment based on nematophagous fungi recently became available (Atkins *et al.,* 2005).

Genus *Stropharia* belongs to nematophagous fungi occurring in woods, grasslands and composts (Luo *et al.,* 2006), and some of its members are characterised by the formation of large spiked cells called acanthocytes (Farr, 1980). The function of acanthocytes was not previously known, but it was recently established that they act as nematotrapping structures (Luo *et al.,* 2006). Luo et al. (2006) demonstrated the nematophagous activity of *S. rugosoannulata* on free-living nematode *Panagrellus redivivus* and the serious phytoparasitic nematode *Bursaphelenchus xylophilus*. Because agricultural practices lack efficient management strategies for plant parasitic nematodes, we tested the nematophagous activity of *S. rugosoannulata* on Northern Root Knot Nematode (*Meloidogyne hapla*), a significant pest of root vegetables worldwide. In our previous study we tested pathogenic effect of six species of fungus to three quarantine plant parasitic nematode species, *Ditylenchus dipsaci*, *Globodera rostochiensis* and *Meloidogyne hapla* (Zouhar *et al.,* 2010). Because of species *A. oligospora* was recorded as the most pathogenic fungus to all three tested species of nematodes, in current experiment *A. oligospora* treatment was used for the comparison of the nematophagous effect of *S. rugosoannulata* as well. Moreover nematode trapping activity of *A. oligospora* was confirmed many times in past, e.g. with *M. enterolobii* (Dupponois *et al*., 1998), animal nematode parasite *Heligmosomoides polygyrus* (Morgan *et al*., 1997) or *Ditylenchus myceliophagus* (Sukhjeet & Kaul, 2007). The trials were conducted under in vitro conditions, and the evaluation of the potential of *S. rugosoannulata* as biocontrol agent was performed.

Material and methods

Stropharia rugosoannulata and Arthrobotrys oligospora strains used in the experiment and their cultivation

The *S. rugosoannulata* (Fig. 1) strain was isolated from infected straw obtained from a commercially available source. The obtained isolate was cultivated on malt extract agar (MEA - Himedia) media plates at 23 ºC for 5 days. The subsequent cultivation was repeated six times to ensure that a pure fungal culture was used in the following trials. The final culture plates were stored at 4 ºC for 10 days before testing.

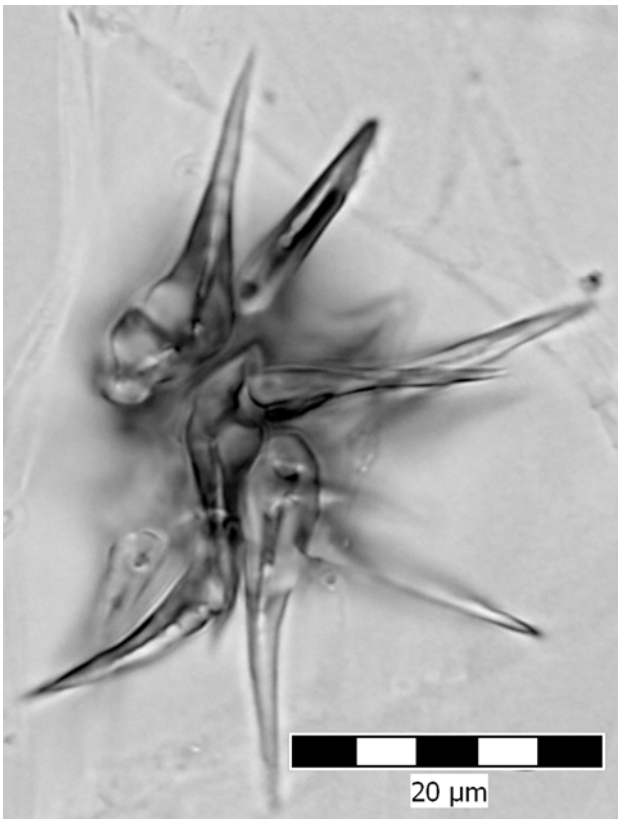

Fig. 1. Acanthocyte of *S. rugosoannulata*

Strains of *A. oligospora* were isolated from soil samples collected from an open field (locality Hradišťko, GPS 50°9'46.001"N 14°53'47.022"E). The soil samples were dried at 23 °C for five days, and the dry soil particles were inoculated on 1.5 % water agar plates. A 1 ml suspension containing approximately 1000 *Caenorhabditis elegans* nematodes was used to stimulate the sporulation of *A. oligospora*; the suspension was pipetted onto the surface of each plate. After three days incubation at 24 ºC, a stereomicroscope and sterile needle were used for the isolation of *A. oligospora* spores, which were transferred on MEA plates. The spores were incubated at 23 ºC for seven days. The final culture plates were stored at 4 ºC for 10 days before testing.

Nematodes used for experiment

Soil samples from *M. hapla* infested growths of carrot were collected from the Litol region (50°11'6.573"N, 14°50'38.933"E) and used for the reproduction of *M. hapla* nematodes. Tomato plants (cv. Tornádo, SEMO) were used as the hosts. After three months of cultivation, the roots containing egg sacs of *M. hapla* were stained with 0.01 % eosin at 4 ºC for 3 hours. The stained egg sacs were handpicked using a nematological needle under 20 x magnification and stored in physiological solution at 4 °C. The acquired egg sacs were transferred on a 25 µm sieve placed in a 50 ml Petri dish containing distilled water and allowed to hatch for five days. Second development-stage juveniles were collected and concentrated by centrifugation at 3000 x *g* for 15 minutes. The collected nematodes were directly used for the experiments.

Bioassay on cultures

The *Stropharia rugosoannulata* and *Arthrobotrys oligospora* strains subjected to the bioassay were grown under the conditions described above on 60 mm polycarbonate Petri plates until acanthocyte formation, in the case of *S. rugosoannulata*, and after the *A. oligospora* mycelia reached a diameter of 20 mm. Second-stage juveniles of *M. hapla* were rinsed twice in distilled water prior to the trials, and five *M. hapla* juveniles were individually transferred to prepared Petri dishes using the nematology needle. A control blank variant was arranged in a similar fashion: the same number of nematodes was transferred to Petri plates containing pure cultivation media. The dishes were incubated at 23 °C. The experiment was performed using twenty replicates.

The evaluation of results

The mobile and immobile *M. hapla* nematodes were counted after 4 and 24 hours using a stereomicroscope. The control variants were observed for the same periods as the experimental nematodes Data were expressed as a percentage and transformed using the arcsine function. A statistical analysis was performed using ANOVA, followed by Tukey's test (Statistica 9.0).

Results and discussion

Large restriction and revision of the using of pesticides in agricultural production is opportunity for researchers to undertake and find new environmental, human and animal health safety methods of plant pest's reduction. So, the research on low environmental impact alternatives to chemicals has received a strong impulse with a wide range of options. Among these alternatives, the organic amendments (Renčo *et al*., 2011; Sasanelli, 2009; D'Addabbo *et al*., 2011), natural plant-derived compounds (Maistrello *et al.* 2010; Renčo *et al.,* 2012) as well as the use of nematode-trapping fungus as nematode biological control (Zouhar, *et al.,* 2010) has been found as the most suitable in organic agriculture.

According to our knowledge no tests with *Stropharia* sp. and gall nematodes were conducted so far. As showed our results, both of the tested nematophagous fungi exhibited a

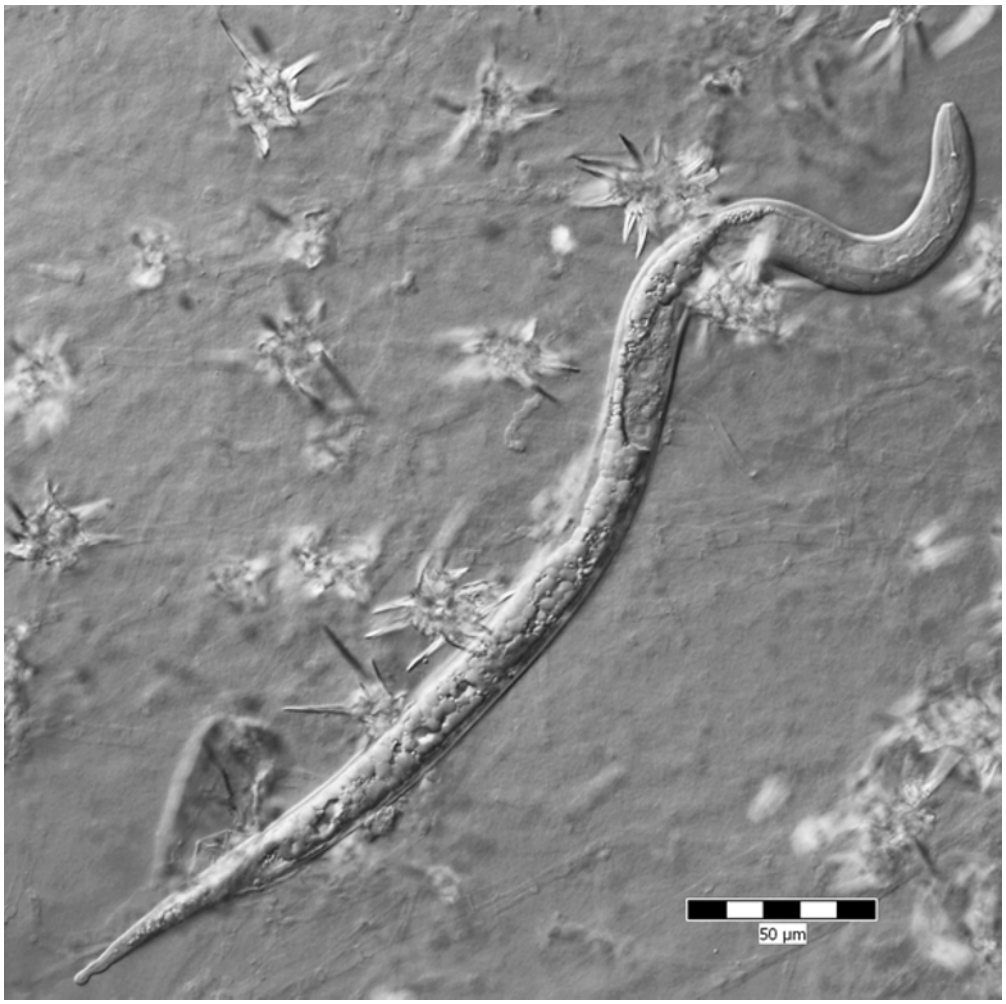

Fig. 2. Second stage juvenile *M. hapla* immobilized by acanthocytes *S. rugosoannulata*

statistically significant carnivorous activity (Fig 2). However, *S. rugosoannulata* was much more effective than *A. oligospora*; in the case of the treatment with *A. oligospora* for 4 h, the efficacy of the fungus was rather low but still statistically significant. The nematodes treated with *S. rugosoannulata* showed almost 100 % immobilisation after 24 h (Table 1). These results confirm the findings of Luo *et al.* (2006) who found over 90 % of *Bursaphelenchus xylophilus* nematodes immobilised after 15 minutes in the vicinity of *S. rugosoannulata* mycelium.

The species *A. oligospora* have been found as the first nematode-trapping fungus by Duddington (1954) inside the cysts of *Heterodera rostochiensis* and now is recognized as the most commonly isolated and by far the most abundant nematode-trapping fungus in the environment. Since then it has been found as pathogenic to several species of plant parasitic nematodes, e.g. *D. dipsaci, G. rostochiensis* and *M. hapla* (Zouhar *et al.,* 2010) or to *Pratylenchus peneterans* (Timper & Brodie, 1993). In addition to these a lot of other fungal species have been found as parasite of root-knot nematodes *M. hapla* and *M. incognita* as well, e.g. *Fusarium, Alternatia, Verticillium psalliotae, V. chlamydosporium, Monacrosporium* sp*., Arthrobotrys* sp*., Hirsutella rhossiliensis, Paecilomyces lilacinus; Pochonia suchlasporia var. catenata, Aphanocladium album* (De Leij *et al.,* 1993; Bonants *et al.*, 1995; Viaene & Abawi, 1998; 2000; Xu *et al.,* 2008; Sasanelli *et al.* 2008); species *Arthrobotrys oligospora, Dactylella oviparasitica, Dactylellina candida, Dactylellina lysipag, Dactylellina phymatopaga* and *Pochonia chlamydosporia* var. *chlamydosporia* as pathogenic to *Ditylenchus dipsaci*, *Globodera rostochiensis* and *Meloidogyne hapla* nematodes (Zouhar *et al.,* 2010). The species *Botryotrichum piluliferum, Scolecobasidium constrictum, Gliocladium roseum* and *Phoma finetii* were isolated from cysts and eggs of *G. rostochiensis* (Trifonova & Karadjova, 2003) or *V. chlamydosporium*, *Fuzarium oxysporum* and *Cylindrocarpon destructans* from infected females of *Heterodera schachtii* and *H. avenae* (Tribe, 1979; Crump, 1987) and many others.

Our results suggest that *S. rugosoannulata* may be used for the biological control of plant parasitic nematodes. However, its utilisation should be assessed more precisely. The main problem when assessing the development of novel fungi for biological nematicide substances is generally the low saprophytic competiveness in soil because mycelial

Table 1. Immobilization of *Meloidogyne hapla* juveniles by *Stropharia rugosoannulata* and *Arthrobotrys oligospora*

Fungi species	Incubation time	Average no. of immobile nematodes ± SD	Average no. of mobile nematodes ± SD	Average % immobilized nematodes ± SD	P value of ANOVA for immobile nematodes
Stropharia rugosoanulata	4 h	4.1 ± 1.4	0.9 ± 1.4	81.7 ± 27.6	0.000121
Arthrobotrys oligopora	4 h	0.9 ± 0.8	4.1 ± 0.8	17.8 ± 15.6	0.021786
Control	4 h	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	-
Stropharia rugosoanulata	24 h	4.9 ± 0.3	0.1 ± 0.3	98.3 ± 5.8	0.000121
Arthrobotrys oligopora	24 h	3.0 ± 1.0	2.0 ± 1.0	60.0 ± 20.0	0.000121
Control	24 h	0.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	-

growth and acanthocyte formation are energy-requiring processes that precede predation (Gray, 1987). The ability to colonise the rhizosphere of crop plants quickly is an important factor when considering the possibility of utilising certain fungal species for crop protection treatments (Persson & Jansson, 1999). Thus, an effective and inexpensive method of nematophagous fungi cultivation is of crucial importance so that we can expect a rapid colonisation when delivering a large amount of fungal biomass to the field. Considering this factor, there could be an advantage in using *S. rugosoannulata* because this species is cultivated as an edible mushroom and cultures are commercially available for this purpose (Poppe, 2000).

Therefore, the nematophagous activity of *S. rugosoannulata* on the plant parasitic nematode *M. hapla* was confirmed for the first time. Additional tests are necessary to evaluate the potential use of *S. rugosoannulata* for the biological control of *Meloidogyne hapla* and the other root-knot nematode species. Such test should focus especially the practical aims as optimizing of high volume fungi cultivation and development of carrier suitable for field application. Evaluation of the effect on other plant parasitic nematodes would be desirable as well.

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