



## *Duddingtonia flagrans* preying a plant parasitic nematode<sup>1</sup>

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The root-knot nematodes (*Meloidogyne* spp. Goeldi) are among the plant parasitic nematodes that cause the greatest losses in agriculture. In order to reduce the damage caused by these pathogens, biological control has become popular because it can be used with other control methods, does not leave residues in food and, in most cases, does not cause an impact on the environment.

*Duddingtonia flagrans* has been studied in the control of parasitic nematodes of animals. This biological control agent is a soilborne fungus that promotes the growth of plants, produces chlamydo spores and its nematophagous activity is guaranteed by adhesive hyphae and three-dimensional adhesive networks that capture the nematodes (Larsen, 2000; Monteiro et al., 2018). *Duddingtonia flagrans* also produces important proteases during the interaction with the nematode cuticle, as observed in parasitic trichostrongylid larvae of domestic animals (Cruz et al., 2015). The ability of this antagonist to prey on plant parasitic nematode was observed by Pandit et al. (2017), *in vitro*, with *Meloidogyne* spp., but information on the reduction of these nematodes populations in soil by the fungus has not been provided. The purpose of this work was to study the ability of *D. flagrans* to prey on *Meloidogyne javanica* in microcosm soil and to capture images of the live action of the fungus on the nematode.

The nematode inoculum was composed of eggs of *M. javanica*, collected from tomato roots maintained in a greenhouse. The mass production of chlamydo spores of *D. flagrans* was performed in crushed corn. *D. flagrans* (AC001) fungal isolate belongs to the collection of the Parasitology Laboratory of the Department of Veterinary at the Universidade Federal de Viçosa.

Predation in microcosm soil: in this experiment, the predatory capacity of fungus *D. flagrans*, tested at different concentrations, on *M. javanica* was performed *in vitro*. 200 mL polypropylene pots were filled with 50 g of sterile soil (microcosms) infested with 1,500 eggs of nematode. Each pot received 0, 2,500, 5,000, 10,000, 15,000, 20,000 or 25,000 chlamydo spores of *D. flagrans* per gram of soil and the pots were then incubated for 15 days at 26°C. After this time juveniles were collected from the

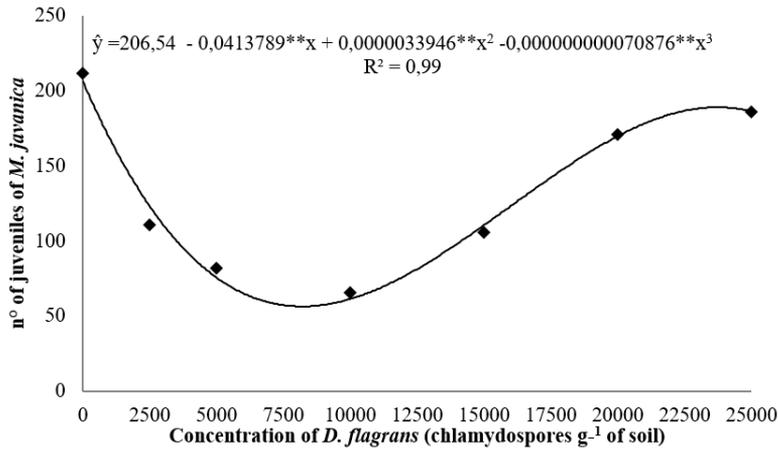
soil using the Baermann funnel technique. The volume was transferred to a Peters counting chamber and the number of juveniles were quantified by counting under a light microscope. The treatments were replicated four times and arranged in a completely randomized design.

The capturing images of the interaction between fungus and nematode were taken in petri dishes with 2% of Water-Agar (WA) containing streptomycin. Each petri dish received, on the same day, *D. flagrans* and an average of 37 juveniles of *M. javanica*, previously disinfested with ampicillin and chloramphenicol suspension, at concentrations of 200 and 300 ppm, respectively. The control of the treatment was performed in petri dishes containing only the fungus. The petri dishes were stored at 28°C for 48 hours. The culture medium with *D. flagrans* and nematodes was sectioned and observed using a stereoscopic microscope with image capture. The data from “Predation in microcosm soil” was evaluated by means of regression, at 1% of probability.

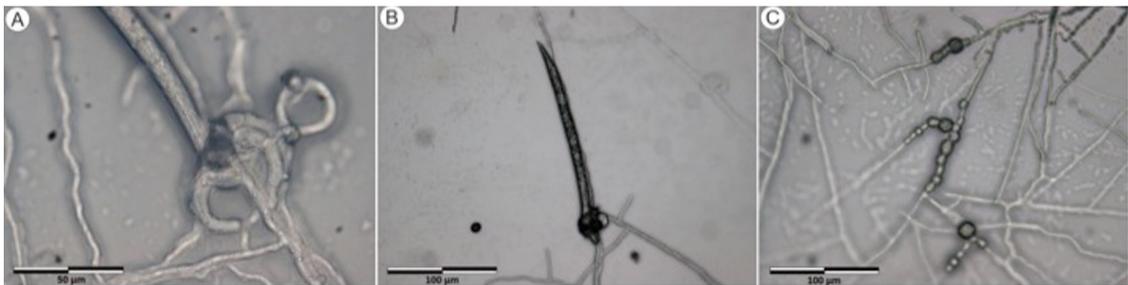
The increase in doses of *D. flagrans* reduced the number of second stage juveniles up to the predicted concentration of 8,201 chlamydo spores g<sup>-1</sup> of soil (estimated value using the regression model), meaning a 72.7% reduction of *M. javanica* (Figure 1). The reduction of the number of juveniles with the application of *D. flagrans* can be explained by the ability of this fungus to form traps in response to the presence and migration of nematodes or substances secreted by them.

After 48 hours of *D. flagrans* fungus being in contact with *M. javanica* juveniles, in WA, the fungus produced adhesive nets that captured root-knot nematode juveniles (Figure 2). In addition, it was possible to observe a large production of chlamydo spores, when compared with the absence of the nematode (data was not quantified).

When placed in the presence of *Meloidogyne* spp. for 24h, the fungus *D. flagrans* had increased the protease activity (Pandit et al., 2014). These enzymes may have an effect on nematodes and are related to the predation of *M. javanica*, and consequently, the reduction of this nematode in the microcosm soil, as seen in this work. However, it should be highlighted that more studies are



**Figure 1.** Number of juveniles of *Meloidogyne javanica* 15 days after application of different concentrations of chlamydospores of *Duddingtonia flagrans* in soil infested with 1,500 eggs. \*\*significant at 1% by the F test.



**Figure 2.** Images from the interaction between *Duddingtonia flagrans* and *Meloidogyne javanica*; (A) Adhesive nets formed by *D. flagrans* to capture root-knot nematode; (B) *M. javanica* second-stage juvenile captured by *D. flagrans*; (C) chlamydospores of the fungus *D. flagrans*.

needed to better understand the interactions between *D. flagrans* and plant parasitic nematodes.

The fungus *D. flagrans* produces nets that capture *M. javanica* and, consequently, predate the nematode reducing its viability in soil.

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