

From the H2020 IPM-Popillia Project: an ongoing field experiment with entomopathogenic fungi and nematodes

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Abstract: *Popillia japonica* (Pj) is a polyphagous alien pest established in Northern Italy since 2014. Its pre-imaginal life is spent entirely in the soil. A large three-year field trial to assess the potential control of Pj larvae using entomopathogenic fungi and nematodes showed the latter to be highly effective and able to survive in the soil. Fungi had a lesser and slower effect.

Key words: biocontrol, pest management, Japanese beetle, white grubs

Introduction

Popillia japonica (Pj) is a polyphagous alien pest established in the Ticino Valley Natural Park (Northern Italy) since 2014 (Pavesi, 2014). Its pre-imaginal life is spent entirely in the soil, while adults are good fliers and feed and mate on many trees, shrubs, and crops (Potter and Held, 2002). Both larval and adult stages of this scarabaeid are naturally susceptible to entomopathogenic fungi (EPF) like *Beauveria* and *Metarhizium* (Ramoutar et al., 2010; Graf et al., 2023), while entomopathogenic nematodes (EPNs) have shown great potential for the biological control of Pj larvae (Marianelli et al., 2017).

Materials and methods

An extensive survey was carried out in 2017 to assess the natural occurrence of EPF and EPNs in the newly invaded territories (Barzanti et al., 2020). As a result, a *Metarhizium robertsii* and a *Heterorhabditis bacteriophora* isolate were selected as promising biological control agents (BCAs) to control Pj larvae in the soil (Torrini et al., 2020; Barzanti et al., 2023). As soon as the Horizon2020 project “IPM-POPILLIA” was funded, a field trial was set up in Piedmont (Northern Italy) to test the real efficacy of these BCAs in an agricultural environment, over time. Three meadows managed under three different irrigation regimes (irrigated, partially irrigated, and non-irrigated) were selected as experimental sites.

The *Metarhizium robertsii* isolate Mro17/T02 was mass-produced as fungus-colonized barley kernels (FCBK) and sodium alginate pellets (SAPs). A commercial *Metarhizium* reference strain (MA43, syn. BIPESCO5 / F52) was also mass-produced as FCBK. In addition to fungi, the naturally occurring *H. bacteriophora* isolate POP16 and a commercial isolate were also tested.

Ten 5 × 10 m plots were assigned to each treatment in each field, together with a negative control (no treatment). Fungi granules (FCBK and SAPs) and EPNs suspensions were applied to the watered soil in early September 2021. Entomopathogenic fungi were applied during the day using a mechanical seed drill, while EPNs were applied at sunset with a backpack sprayer to avoid problems related to heat stress.

One week before treating the fields, soil samples were taken from each plot to assess the natural occurrence of *Metarhizium* and *Heterorhabditis* and the initial number of Pj larvae in the soil. After counting, the Pj larvae were returned to the soil so as not to alter the experiment results. The same sampling scheme was used for subsequent periodic sampling.

Soil samplings in the treated plots have been carried out two months after the treatments (November 2021), after the first winter season (April 2022), and after one and two years (November 2022 and November 2023). A last set of soil samples was collected in October 2024, three years after treating the plots. At the time of submission of this article, data of the final evaluation of these samples were not yet available.

Results and discussion

All treatments significantly reduced the number of Pj larvae in the soil compared to pre-treatment levels and to the control. EPNs reached a maximum mean efficacy (Henderson-Tilton) of 92 % while EPF reached 48 %. As of November 2023, the best-performing BCA (*H. bacteriophora* POP16) still showed a mean efficacy as high as 47 %, while the *Metarhizium* SAPs treatment had a slightly lower one (46 %).

When examining the three experimental fields closely, it's evident that the hot and dry summer of 2022 significantly impacted the Pj larval population. This effect was especially notable in Field 2 (not irrigated), where the grubs almost disappeared, even in the control plots. As a result, it was impossible to assess any efficacy for this field after April 2022. In any case, the strong impact of nematodes immediately after the treatment and after the first winter season is clearly visible in all three fields, most of all that of the indigenous strain POP16. On the other hand, EPF had a lower and slower effect on Pj larvae numbers.

As of November 2023, in Fields 1 and 3 (partially irrigated and irrigated, respectively), the FCBK formulations seemed to lose their effect, while EPNs and SAPs treatments still showed a significant reduction of Pj larvae if compared to the control (Figure 1).

Regarding the survival of the entomopathogens in the soil, after a peak in November 2021, the nematode count showed a drastic reduction in November 2022. Still, a new significant increase was recorded in November 2023. This was especially true for the indigenous POP16 strain. A similar trend, but with a less strong reduction in November 2022, was observed for the CFUs count of FCBK treatments and again the local strain showed a better performance. Despite the significant larval population reduction, a different and unclear situation was observed in the SAPs-treated plots whose CFU count never significantly differed from the control plots.

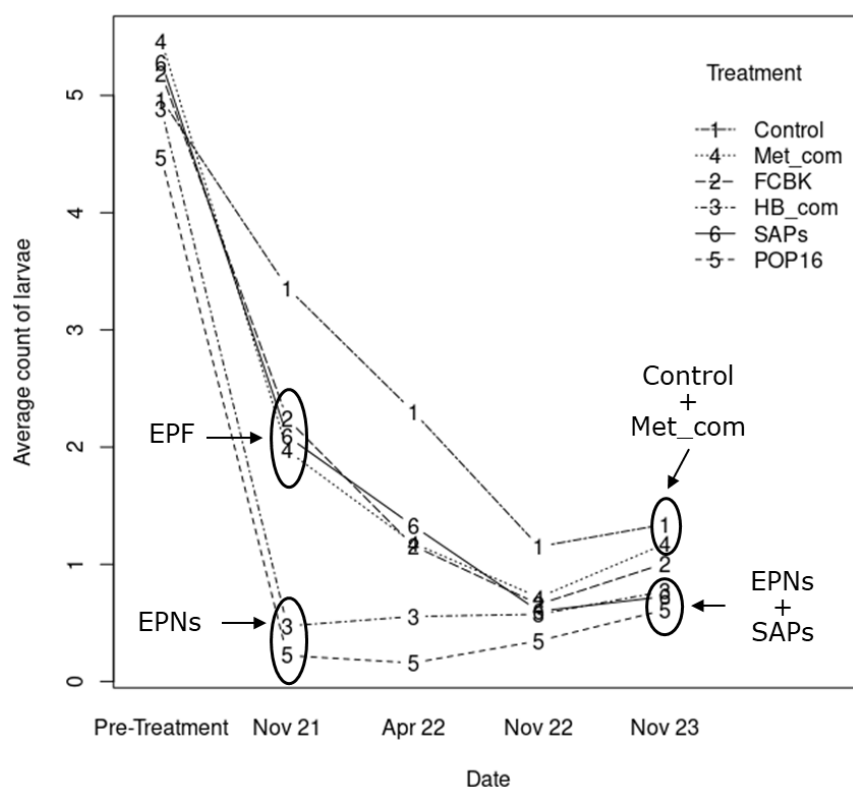


Figure 1. Average impact of EPNs and EPF treatments on Pj larval populations of the three experimental fields. Met_com and HB_com are fungal and nematode reference treatments, respectively.

In conclusion, we can affirm that *Heterorhabditis bacteriophora* nematodes are highly effective and fast at reducing Pj larval populations in the soil, especially the indigenous strain POP16. Two years after a single treatment, their average efficacy remains almost 50 %. Our experiment also demonstrated that EPNs can survive in the soil, even under environmental constraints. Again, this is particularly true for the indigenous strain.

On the other hand, due to their different “behavior”, fungi have a lower and slower, but still significant effect on larval populations. FCBK formulations only maintain a low mean efficacy level two years after a single treatment, unlike SAPs, whose mean efficacy appears to improve over time, and two years after their distribution in the soil it is only slightly lower than that of POP16. Fungal survival dynamics in SAPs-treated plots remain however totally unclear. As mentioned above, the last survey was conducted in October 2024, three years after the treatment. Given these promising results, it will be very interesting to evaluate the outcomes of this last survey. Hopefully, we will also get a clearer picture of the survival of nematodes and fungi in the soil.

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References

- Barzanti, G. P., Enkerli, J., Benvenuti, C., Marianelli, L., Paoli, F., Sabbatini Peverieri, G., Mazza, G., Bosio, G., Venanzio, D., Giacometto, E., Roversi, P. F. 2020. Preliminary analysis of genetic variability of *Metarhizium* isolates from Parco del Ticino (Northern Italy). IOBC-WPRS Bull. 150: 6-8.
- Barzanti, G. P., Enkerli, J., Benvenuti, C., Strangi, A., Mazza, G., Torrini, G., Simoncini, S., Paoli, F., Marianelli, L. 2023. Genetic variability of *Metarhizium* isolates from the Ticino Valley Natural Park (Northern Italy) as a possible microbiological resource for the management of *Popillia japonica*. J. Inv. Path. 197: 107891.
- Graf, T., Scheibler, F., Niklaus, P. A., Grabenweger, G. 2023. From lab to field: biological control of the Japanese beetle with entomopathogenic fungi. Front. Insect Sci. 3: 1138427. doi: 10.3389/finsc.2023.1138427
- Marianelli, L., Paoli, F., Torrini, G., Mazza, G., Benvenuti, C., Binazzi, F., Sabbatini Peverieri, G., Bosio, G., Venanzio, D., Giacometto, E., Priori, S., Köppenhöfer, A. M., Roversi, P. F. 2017. Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (Coleoptera, Scarabaeidae) in Piedmont Region (Italy). J. Appl. Entomol. 142: 311-318.
- Pavesi, M. 2014. *Popillia japonica* specie aliena invasiva segnalata in Lombardia (*Popillia japonica* – Alien invasive species found in Lombardy). L'Informatore Agrario 32: 53-55.
- Potter, D. A., Held, D. W. 2002. Biology and management of the Japanese Beetle. Annu. Rev. Entomol. 47: 175-205.
- Ramoutar, D., Legrand, A. I., Aim, S. R. 2010. Field performance of *Metarhizium anisopliae* against *Popillia japonica* (Coleoptera: Scarabaeidae) and *Listronotus maculicollis* (Coleoptera: Curculionidae) larvae in turfgrass. J. Entomol. Sci. 45(1): 20-26.
- Torrini, G., Paoli, F., Mazza, G., Simoncini, S., Benvenuti, C., Strangi, A., Tarasco, E., Barzanti, G. P., Bosio, G., Cutino, I., Roversi, P. F., Marianelli, L. 2020. Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (Coleoptera: Scarabaeidae) in Northern Italy. Insects 11(11): 804; doi:10.3390/insects11110804.