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EFFECTIVENESS OF DIFFERENT DOSES OF *HETERORHABDITIS BACTERIOPHORA* AGAINST *POPILLIA JAPONICA* 3rd INSTARS: LABORATORY EVALUATION AND FIELD APPLICATION

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Paoli F., Marianelli L., Binazzi F., Mazza G., Benvenuti C., Sabbatini Peverieri G., Bosio G., Venanzio D., Giacometto E., Klein M., Roversi P.F. – Effectiveness of different doses of *Heterorhabditis bacteriophora* against *Popillia japonica* 3rd instar larvae: laboratory evaluation and field application.

The recent introduction into Italy of the Japanese beetle (*Popillia japonica* Newman) has triggered new research on biological control agents aimed at limiting the spread of this alien pest. From the scientific literature it is known that the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar is very effective against *P. japonica*. Here we present a laboratory test comparing the lethal activity at three different concentrations of the commercial product Nematop® (CBC, Europe), containing *H. bacteriophora*, against *P. japonica* 3rd instars. The results showed that the lower dose (25 IJs/cm²) maintained an effectiveness of about 90%, thus not differing on the whole from the values of the other concentrations. Afterwards, this dose was tested in the field both in the spring and summer with grub killing values of 25 and 45%, respectively.

KEY WORDS: pest management, insect pest, alien species, Japanese beetle, turf, Scarab, entomopathogenic nematode.

INTRODUCTION

In 2014 the Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) was spotted in the Natural Park of the Ticino Valley (Northern Italy), along the Piedmont and Lombardy regions (PAVESI, 2014). Since its discovery, the Italian outbreak has been a biological invasion (REGIONE PIEMONTE BU6S1, 2016). The number of captures obtained by standard Japanese beetle lure traps reported a high density of individuals thriving in this territory (REGIONE PIEMONTE BU6S1, 2016), and therefore needed to be limited. The methods for controlling the Japanese beetles have often included the use of chemicals (COWLESS & VILLANI, 1996; GREWAL *et al.*, 2001, 2005; POTTER & HELD, 2002). However, the growing concern about the environmental pollution caused by pesticides raised the issue of the safety of these products, promoting research on biological alternatives (AKTAR *et al.*, 2009). To limit the biological invasion in the Italian territory, several experiments have been carried out to evaluate both the best biocontrol agents and the conditions and doses of their use (e.g. PAOLI *et al.*, 2017). According to scientific literature, the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar has proven to be very effective against *P. japonica* larvae (VILLANI & WRIGHT 1988; KLEIN, 1990; GEORGIS & GAUGLER, 1991; ALM *et al.*, 1992; POWER *et al.*, 2009; MORRIS & GREWAL, 2011). Therefore in the current paper, we report the results of a laboratory trial carried out in Italy to ascertain, between three different concentrations, the best

dose of *H. bacteriophora* to be tested in the field. Accordingly, this dose then was tested in the field in two different seasons.

MATERIALS AND METHODS

LARVAL COLLECTION AND STORAGE

Popillia japonica third instars were collected for laboratory experiments during field surveys in February 2016 in the sandy soil of a perennial meadow located in Oleggio (Novara, Piedmont) (45°36' N, 08°38' E, altitude ca. 230 m a.s.l.). The study site was delimited by water channels regularly opened every week during spring-summer time to irrigate the hayfield. The grubs were maintained in groups at 4°C in native sandy soil.

NEMATODES

The commercial product Nematop® (CBC, Europe) made from *H. bacteriophora* was used for the tests. Nematop® was first dissolved in water, then IJs were counted and used for laboratory and field tests after an evaluation of viability.

LABORATORY TEST

The laboratory test involving EPNs started at the end of the winter. The test was carried out about 10 days after larval collection. Three different doses consisting of about 2300 nematodes/insect (100 IJs/cm² - double dose), 1150 nematodes/insect (50 IJs/cm² - standard dose, as reported by

manufacturer's indication), 570 nematodes/insect (25 IJs/cm² - half dose) were used. Each of the three doses, plus the control, were tested as follows: thirty plastic containers without lids (4.5 cm diam., 7 cm deep), filled with 100 cc of native sandy soil, each containing one 3rd instar larva, were used as experimental units. Soil from the collection site was sterilised by autoclaving at 121°C for 20 min before use. Larvae were acclimated at 20°C for a week before nematode inoculation. The nematodes were applied onto the soil surface of each container in 1 ml of sterile water. Then, 5 ml of water were added to each unit to avoid soil desiccation, thus allowing nematode survival (final soil moisture: 21% w/w). In the controls, only sterile water was added. The containers were incubated at 20 ± 1°C and 90 ± 5% RH in the dark. The grubs were left without food for the whole 3-weeks experiment. Two ml of sterile water was added every 2 days to guarantee continuous soil moisture. Insect mortality was checked at the end of the experiment.

LARGE-PLOT FIELD TEST

In field experiments, two five-week bioassays were performed in the same area where the Japanese beetle larvae had been previously collected. The product concentration selected for field evaluation was the lowest (=25 IJs/cm² equivalent to 2.5×10⁹ IJs/ha) among those tested in laboratory. The bioassays were carried out at two different seasons: the first one was performed in spring (April 13 - May 18, 2016) while the second one in summer (August 11 - September 8, 2016). The initial infestation was evaluated counting the number of live larvae from five soil samples (size: 20×25×15 cm) randomly extracted with a shovel. In the spring trial, two treated plots and two control plots of 100 m² (20×5 m) separated each other by 1 m wide untreated buffers were defined. In the summer trials, the arrangement of the experiment was the same as in spring, except for the number of plots, which were three treated and three controls. The application was carried out by a sprayer bar 5 m width mounted on a farm tractor. Flood irrigation the day before treatments, plus a regular flood irrigation (once a week in summer) guaranteed continuous moderate to high moisture of the soil. The nematode suspension was sprayed at 2-bar

pressure. Powder formulated nematodes were stirred into a bucket containing 10 litres of water (15-20°C) before the suspension was transferred into the spray tank into another 20 litres of water. The tank contents were continuously agitated to prevent settling of the IJs. Each plot was uniformly sprayed. Control plots were treated only with water following the same procedure. The evaluation of nematodes efficacy was carried out after 3 weeks by counting the number of live larvae of *P. japonica* from 15 soil samples (20×25×15 cm) randomly extracted from each plot.

DATA ANALYSIS

At the end of the laboratory experiment, the association between overall insect survival and dose of entomopathogenic nematodes was assessed by Fisher's exact test (statistic: F). This test was preferred over the chi-square test of independence due to the small total sample size (McDONALD, 2014). Post hoc tests were then performed through pairwise comparisons by 2X2 Fisher's exact tests (MACDONALD & GARDNER, 2000; McDONALD, 2014). A Bonferroni correction was applied with statistical significance accepted at $P < 0.0083$. In the field trial, data were first tested for normality and equality of variances with the Shapiro-Wilk and Levene tests, respectively. Student's *t* (statistic: *t*) tests were then carried out to assess the statistical significance between the treated plots and controls.

RESULTS

LABORATORY BIOASSAY

The laboratory bioassay, which targeted 3rd instars, resulted in significant differences among the tested doses and the control ($P < 0.0001$), with the following levels of efficacy: *H. bacteriophora* double and standard dose killed 93% of *P. japonica* larvae, while the half dose killed 90% of larvae (Fig. I). The mortality in the control was 7%. Pairwise comparisons between groups showed statistically significant differences between each treatment and the control ($P < 0.0001$), but no differences between the different intervention theses ($P = 1$).

Fig. I – Mortality percentages of *Popillia japonica* 3rd instars treated in the laboratory at different doses of *Heterorhabditis bacteriophora* (2HB= Double dose; HB= Standard dose; 1/2HB = Half dose).

LARGE-PLOT FIELD BIOASSAYS

In April, the grub density was 12.3 ± 2.8 (mean $\pm$ SE larvae per sample). The field bioassay performed in the spring targeted 3rd instars and showed a significant difference between the treatments and the control ($t=-3.279$; $df=58$; $P=0.002$) with an efficacy of 25%. The mean temperature of this period was 14.6°C and the precipitation 131.8 mm. In August the grub density was 6.67 ± 0.6 (mean $\pm$ SE larvae per sample). The summer field bioassay, which targeted mainly the 2nd instars, had a significant difference between the treatments and the control ($t=-2.046$; $df=88$; $P=0.044$) with an efficacy of 45%. The mean temperature during the summer treatment was 23.2°C. The total precipitation was 28 mm.

DISCUSSION

Mortality percentages analysed by the Fisher's exact test, showed no statistically significant difference between the efficacies of the various doses used in the laboratory experiments. All the tested doses attained about 90% effectiveness. This lead us to consider the *H. bacteriophora* half dose (25 IJs/cm², equivalent to 2.5 billion nematode/ha) retaining a good effectiveness rate, and so needed to be evaluated in the field. This information, namely the absence of differences between the tested doses, was important also from the economical point of view: in fact, with equal effectiveness, the lower the dose the lower the economical expense to be supported.

Moreover, it is likely that in territories with dense infestations, such as the case of the Natural Park of the Ticino Valley, EPNs can largely disseminate by recycling into the numerous hosts (KLEIN and GEORGIS, 1992; KLEIN, 1993). In fact, within few weeks, every grub encountered by the released nematodes is a source of new cruiser EPNs seeking new hosts for new infestations. Thus, in highly infested territories lower doses are presumably still effective.

Therefore, the field trials were carried at the dose of 2.5 billion IJ nematodes/ha. The trial carried out in spring targeted against the post-wintering 3rd instars, which are known to have low susceptibility to *H. bacteriophora* (POWER *et al.*, 2008, PAOLI *et al.*, 2017). This trial reported a low mortality (about 25%) thus proving, as expected, this period to be unsuitable for Japanese beetle control purposes by EPNs.

The trial made in the summer, on the contrary, targeted mainly 2nd instars, known to be more susceptible to EPNs (KOPPENHÖFER & FUZY, 2004; POWER *et al.*, 2009). This trial had an effectiveness higher than the spring trial, attaining a value of 45%. This could be considered a promising initial result, consistent with previous works (GEORGIS & GAUGLER, 1991; GREWAL *et al.*, 2004), needed to be integrated with some other measures of pest control, such as for example, the mass trapping by the lure traps.

It is worth mentioning the natural resilience of the soil in the infested area. In this territory, a new species of mermithid nematode belonging to the genus *Hexamermis* has been observed parasitizing the Japanese beetle grubs (MAZZA *et al.*, in press).

In conclusion, a cost-benefit balance indicates that a dose of 2.5 billion *H. bacteriophora* IJ nematodes/ha applied on sandy soils gives noteworthy results when used against the more susceptible stages of *P. japonica* larvae occurring in summer.

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