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Macrolophus pygmaeus induces systemic resistance in tomato against *Meloidogyne*

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The ability of *Macrolophus pygmaeus* to induce systemic resistance in susceptible and *Mi*1.2 resistant tomato against *Meloidogyne* spp. was evaluated in 200cm^3 pot experiments. The susceptible cv. Roma and the resistant cv. Caramba were exposed to 15 *M. pygmaeus* nymphs per plant in mesh bags for 48 h and then were inoculated with 200 stage juveniles (J2) of *M. incognita* or 600 J2 of a mixed community of *M. arenaria*, *M. hapla*, and *M. javanica*. Tomato plants were maintained in a growth chamber during 40 days. Then the number of egg masses and eggs per plant were determined. In addition, the preference of the insect was evaluated confronting nematode-infected vs. non-infected plants in a Y-tube olfactometer and in insect cages, where 10 females were released into each cage containing resistant or susceptible tomato plants. After 1, 2, 4, 24, 48 and 72 h, the number of *M. pygmaeus* was counted as well as the offspring after 14 days. The infectivity and reproduction of *M. incognita* were reduced by 37% and 53%, respectively, in susceptible tomato plants inoculated with *M. pygmaeus*. Inoculation with the nematode community resulted in a 52% reduction in infectivity and a 37% reduction in reproduction. However, no effect was observed in the *Mi*1.2 resistant tomato plants, regardless of the nematode inoculum. The preference and the offspring of *M. pygmaeus* was not negatively affected by the nematode infection or the tomato cultivar. In conclusion, pre-induction of tomato plants with *M. pygmaeus* reduces RKN infectivity and reproduction in susceptible but not in *Mi*1.2 resistant tomato.

Keywords RKN, Biological control, Induced resistance, Tomato

Meloidogyne spp. is the most challenging plant-parasitic nematode (PPN) genus affecting global plant production[1]. More than 100 species have been described, however, the root-knot nematodes (RKN) tropical species *M. arenaria*, *M. incognita*, and *M. javanica*are the most widespread and limiting for vegetable production in tropical and subtropical climates[2]. These species are obligate parasites, which reproduce parthenogenetically. The second stage juvenile (J2) penetrates the root near the elongation zone, moves intercellularly and induce a hypertrophied and multinucleate giant cells (GC) in the vascular cylinder becoming a feeding site, supplying nutrients to the nematode. After that, the J2 undergoes three moults until it reaches the adult stage. The adult female feeds from these giant multinucleated cells, that form a gall in the root, and produces a gelatinous matrix containing hundreds of eggs that protrudes from the gall, known as the egg mass[3]. Because of that, plant roots become galled, interfering with the correct uptake of water and nutrients, which causes yellowing, wilting, and dwarfism in aboveground parts of the plant, leading to plant death in severe attacks. In tomato, one of the main cash crops in the Mediterranean area, RKN can reduce crop yield until 62% and 72%, depending on the cropping season and crop duration[4,5]. Among sustainable nematode control methods, plant resistance stands out as one of the most effective, economically profitable and environmentally safe[4,6,7]. In tomato, the resistance against *Meloidogyne* is mediated by the *Mi*1.2 resistance gene and conferred resistance against *M. arenaria*, *M. incognita* and *M. javanica*[8], *M. luci* and *M. ethiopica*[9] but not to *M. hapla*[8] or *M. enterolobii*[10]. This gene induces a hypersensitive reaction (HR)-mediated cell death around the feeding site, involving an oxidative burst by reactive oxygen species (ROS) that prevents nematode infection and development[11]. Nevertheless, its effectiveness is reduced or lost after repeated cultivation due to the emergence of virulent populations[4,12,13]. An

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alternative strategy to reduce nematode building up populations is to induce resistance in susceptible germplasm by biotic or abiotic factors and thus, reduce the selection pressure on resistant germplasms.

Among biotic elicitors, bacteria and fungi have been previously described to induce plant resistance against RKN[14–19]. Recently, the ability of other organisms, such as insects, to be used in biological pest control has been investigated. For instance, zoophytophagous mirid bugs such as *Macrolophus pygmaeus* and *Nesiodiocoris tenuis* (Heteroptera: Miridae), known predators of different aboveground pests, such as whiteflies, caterpillars or thrips have been observed to trigger plant resistance against several key pests: the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), the South American tomato pinworm *Tuta absoluta* Meyrick (Lepidoptera: Gelichiidae), the Western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), and the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). This resistance is induced when these mirid bugs engage in phytophagous behavior and puncture the plants[20–25]. However, the effect of puncturing of these insects on plants infected with RKN in susceptible and resistant germplasm remains unknown, and also the possibility that the induced resistance could be additive to the genetic resistance mediated by the *Mi*1.2 resistance gene, as reported for the resistance conferred by *Trichoderma asperellum*T-34[19].

Moreover, PPN infection can modify the host preference and development of certain phytophagous insects and mites, affecting the performance of their development stages and offspring. For example, *Pratylenchus penetrans* enhanced the production of leaf phenolics and glucosinolates in *Brassica nigra*, where the caterpillar *Pieris rapae* (Lepidoptera: Pieridae) larvae grew slower and produced fewer pupae compared to non-infested plants[26]. Likewise, root infection by the cyst nematode *Heterodera schachtii* reduced the population density of the aphid *Brevicoryne brassicae* (Hemiptera: Aphididae) and induced changes in the composition of glucosinolates in *Brassica oleracea*[27]. Moreover, infection of *Arabidopsis thaliana* by *Heterodera schachtii* increased the attractiveness of the spider mite *T. urticae*, while simultaneously improved its fitness and performance[28], whereas *P. penetrans* reduced its fertility on *Phaseolus vulgaris*[29]. The effect of RKN infection can also affect the aboveground phytophages. For example, the leaf miner *T. absoluta* preferred uninfected RKN tomato plants for its oviposition, and that root infection negatively affected its pupation process[30]. Interestingly, positive effects of RKN infection on leaf-chewing herbivores have also been observed. For example, *Spodoptera exigua* (Lepidoptera: Noctuidae) benefited from *M. incognita* infection, increasing its weight compared to non-infected plants, depending on the development stage of the nematode[31]. Furthermore, *M. incognita* infection inhibited the production of root nicotine as a chemical defense in response to foliar herbivory by *Manduca sexta* (Lepidoptera: Sphingidae) and *Trichoplusia ni* (Lepidoptera: Noctuidae), resulting in low-leaf nicotine levels in tobacco[32].

This study aims to investigate the potential of *M. pygmaeus* in enhancing plant resistance against root-knot nematodes (RKN) in susceptible and resistant tomato cultivars. *Macrolophus pygmaeus* was selected for its known safety profile for plants, contrasting with *Nesidiocoris. tenuis*, which has been associated with adverse effects such as necrotic rings on stems and flowers, as well as fruit punctures[33,34]. Additionally, this research explores the insect's preference between RKN-infected and non-infected plants using a vertical Y-tube olfactometer and insect cages, providing insights into the ecological interactions between predator behaviour and plant defence mechanisms.

Materials and methods

Plant material, insects and nematodes

The experiments were conducted at the Institute of Agrifood Research and Technology (IRTA) (Cabrils, Spain) during 2023. The susceptible tomato cv. Roma (Fitó Seeds) and the *Mi*1.2 resistant cv. Caramba (Seminis Seeds) were used in the experiments. The resistance of the tomato cv. Caramba has been previously described in pot and field experiments[35]. Tomato plants were germinated in a seedling tray containing a mixture consisting of pit: perlite (95:5; v: v) and placed inside insect cages to avoid insect interferences and maintained in a glasshouse. Subsequently, the plants were transplanted into 200 cm^3 pots filled with sterile river sand when they had developed four true leaves. They were allowed to establish roots for one week under controlled conditions in a growth chamber previous to perform the experiments. (25 ± 2ºC; RH% 70 ± 10%; 16:8 h L: D photoperiod).

M. pygmaeus N4-5 nymphs of 4–7 days and mated females used in the experiments were supplied by the SELMAR (Federació d'Agrupacions de Defensa Vegetal) mass rearing located at Santa Susanna (Barcelona, Spain).

The RKN used in the experiments consisted in a *M. incognita* (Agropolis) population isolated from an experimental greenhouse[7] and a mixed community containing *M. arenaria*, *M. hapla*, and *M. javanica* (Community) isolated from grafted tomato in a commercial field production. Both the *M. incognita* population and the mixed community were reproduced and maintained in the susceptible tomato cv. Durinta (Seminis Seeds) and J2 were obtained from nematode eggs produced in tomato roots and extracted by maceration in a 5% commercial bleach solution (40 g L^{-1}NaOCl)[36]. After that, the egg suspension was placed in Baermann trays, which consist of a tray with a filter and paper where the egg suspension is placed on top of the paper and water at the bottom of the tray, so that the juveniles emerge and migrate to the water to be collected[37]. J2 collected during the first 24 h were discarded to avoid the potential effect of bleach on the juveniles present at the time of extraction, and the subsequent ones were collected daily and stored at 9 ºC until use. The RKN species were identified using specific SCAR PCR for *M. arenaria*, *M. incognita* and *M. javanica*[38] and specific PCR tests for *M. hapla*, *M. chitwoodi* and *M. fallax*[39].

Inducing resistance of tomato to RKN in pot experiments

After a week of transplanting, plants were covered with a 100 microns mesh bag of $20 \times 5.1 \times 27.9$ cm, and 15 N4-5 nymphs of 4–7 days old of *M. pygmaeus* per plant were released inside the bag and fixed to the pot using an elastic rubber. Forty-eight hours later, bags and the insects were removed, and plants were inoculated with 200 stage juveniles (J2) of *M. incogita* (Agropolis) or 600 J2 of a mixed community of *M. arenaria*, *M. hapla*, and *M.*

javanica (Community) to increase the possibilities that all RKN species could infect the plant. The J2 suspension was applied in two opposite holes, 2 cm deep and 2 cm apart from the plant, and covered with the soil. Plants not exposed to *M. pygmaeus* were included for comparison, and each combination of tomato cultivar-RKN-mirid exposure was repeated 12 times, and the experiment was conducted twice. Plants were watered as needed during the experiments and fertilized once a week with a Hoagland solution.

Forty days after nematode inoculation (DANI), the aboveground part of the plants were removed, and the roots were carefully washed. The RKN infectivity was assessed by counting the egg masses after being stained with a 0.01% erioglaucine solution for 30 min[40]. RKN eggs were extracted by immersing roots in a 10% commercial bleach solution (40 g L^{-1}NaOCl)[36] and then, full and empty eggs were counted. The nematode fertility was expressed as the mean number of eggs per egg mass. The reproduction index (RI) was calculated as the percentage of the number of eggs produced in the resistant germplasm in relation to the number of eggs produced in the susceptible germplasm in the plants not exposed to *M. pygmaeus* (RI% = (eggs resistant / eggs susceptible) x 100). The level of resistance was categorized as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%), moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%), or susceptible (RI ≥ 50%)[41].

Preference experiments

Vertical Y-tube olfactometer

The preference of *M. pygmaeus* females for odours from RKN infected or non-infected plants were investigated in a Y-tube olfactometer (Nathura, ECIS, Bessanvido, Italy). The susceptible tomato cv. Roma or the *Mi*1.2 resistant cv. Caramba plants were evaluated after 14 DANI with 600 J2 using 7-day-old females of *M. pygmaeus*. The olfactometer consisted of a Y-shaped glass tube with an internal diameter of 3.5 cm and 17 cm long arms. Both arms were connected to an air pump in the upper part. The Y-tube was positioned vertically, producing controlled air that flowed from the arms to the bottom. The air was controlled using an anemometer (TESTO, Barcelona, Spain), at the ends of both pump tubes and maintained at 2.7 ± 0.1 m s^{-1}. The airflow at the exit of the olfactometer was maintained at 0.20 ± 0.02 m s^{-1}. At the beginning of the trial, a *M. pygmaeus* female was allowed to walk onto a mesh lid placed at the base of the olfactometer. After the female emerged from the lid, the lid was removed and the time was counted. Each individual was observed until it crossed a line drawn on the lower third of the olfactometer arm or until 5 min had elapsed, after which the insect was discarded. Sixty insects were tested per each treatment, and each individual was used only once. To minimize potential experimental biases from environmental variables or location-specific effects, the olfactometer was cleaned with 96% alcohol after every five insects tested. Additionally, the positions of the olfactometer arms were alternated between the two plants, and the orientation of the jars was rotated after every ten insects[42,43]. The trials were conducted at the same location, under uniform light conditions, and at the same time of the day (between 9:00 h and 16:00 h) to avoid circadian variations in the insect behaviour[44]. The first choice of the insect was noted as well as its final choice after 5 min. If during these 5 min it reached the end of the arm of its choice, this was regarded as a final choice, as proposed by Du et al.[45], to prevent the inclusion of random choices resulting from the exploration of the arms by the insects. At the end of the experiment, the root infection by RKN was confirmed by staining the nematodes inside the roots in acid fuchsine[46].

Insect cages experiments

Three experiments were carried out to assess the preference and the offspring of *M. pygmaeus* for non-infected or infected plants with 3 J2 cm^{-3} of soil by *M. incognita* or the RKN community after 14 DANI. The susceptible tomato cv. Roma and the *Mi*1.2 resistant cv. Caramba were germinated, transplanted, cultivated, and inoculated as previously described. After 14 DANI, one inoculated and one non-inoculated plant of the same cultivar were transferred to a 250 microns mesh cage of 30 × 30 × 30 cm and placed in a growth chamber (25°C ± 2; 70% RH;16: 8 h L: D photoperiod). Then, 10 mated females of *M. pygmaeus* of, 7- days-old, were released into the cage. After, 1, 2, 4, 24, 48, and 72 h, the number of *M. pygmaeus* females on and outside the plant was counted. After that, the aboveground part of the plant was removed, cut into pieces, and transferred to 480 mL insect pots of 12 cm diameter with a 100 micron mesh at the top to allow air circulation and placed in the growth chamber. After 14 days, the number of nymphs produced in each plant was evaluated under a stereomicroscope. Each treatment was repeated 10 times. At the end of the experiment, RKN infection was confirmed by staining the roots in acid fuchsine[46].

Statistical analyses

Data of nematode infectivity (egg masses), reproduction (eggs per plant), and fertility (eggs per egg mass) belonging to the inducing of tomato resistance experiments, and data of the number of insects per plant and period along with the insect offspring in the cage experiments were assessed for normality and homogeneity of variances. Data were compared using the Student-t test if no differences ($P > 0.05$) between variances were observed or using the Welch test otherwise. Data were compared between experiments and pooled if there were not significant differences ($P > 0.05$). Significant differences in the proportion of *M. pygmaeus* choosing a particular host plant in the olfactometer were tested using a two-sided binomial test. Females that did not make choice were discarded in the statistical analysis. Statistical analyses were performed using JMP 16.2.0 (SAS Institute inc.).

Results

Inducing resistance of tomato to RKN in pot experiments

Both *M. incognita* (Agropolis) and the community of RKN used in the experiments overcome the resistance (RI > 50%) to the *Mi*1.2 resistance gene of the tomato cv. Caramba performing as susceptible in both experiments

(Agropolis: 89% and 116%; Community: 52% and 63%) (Fig. 1). The number of eggs masses per plant (infectivity) and the number of eggs per plant (reproduction) of *M. incognita* in the first experiment in the susceptible tomato cv. Roma exposed to *M. pygmaeus* was reduced ($P<0.05$) by 40 and 62% respectively. In addition, the number of eggs per egg mass (fertility) was reduced by 39% in plants infected with *M. incognita* and induced by *M. pygmaeus* (Fig. 1A). The infectivity of *M. incognita* was reduced by 50% in the resistant tomato cv. Caramba induced by *M. pygmaeus* ($P<0.05$), but not the nematode reproduction ($P>0.05$), increasing the nematode fertility by 49%. The nematode reproduction and fertility were 42% and 57% lower ($P<0.05$) respectively, in the susceptible compared to the resistant germplasm in the plants exposed to *M. pygmaeus*, but not in plants not exposed to the insect (Fig. 1A). In the second experiment, in the susceptible germplasm, the nematode infectivity was reduced by 34%, and the reproduction by 43% in the induced plants, respectively ($P<0.05$). However, in the case of resistant plants, no differences were found ($P>0.05$). The nematode reproduction in plants exposed to *M. pygmaeus* were 38% lower in the susceptible compared to the resistant germplasm, but not in the plants not exposed. Concerning nematode fertility in both exposed and non-exposed plants, a 44% and 33% reduction was observed, respectively, in the susceptible germplasm in comparison to the resistant one. (Fig. 1A).

In the susceptible plants of the first experiment infected with the nematode community strain, the nematode infectivity in plants exposed to *M. pygmaeus* were 65% lower ($P<0.05$) in relation to non-exposed plants, but the reproduction was not affected ($P>0.05$), increasing its fertility by 132% in the exposed plants ($P<0.05$). Regarding the resistant cv. Caramba, both nematode reproduction and fertility increased by 196% and 220% ($P<0.05$) in plants exposed to *M. pygmaeus* compared to non-exposed plants. The nematode infectivity and reproduction were 49 and 49% lower ($P<0.05$) respectively, in the susceptible plants induced by the insect compared to the resistant, but not in plants not exposed ($P>0.05$). In the second experiment, both nematode infectivity and reproduction were reduced by 39% and 53% ($P<0.05$) respectively in the susceptible plants exposed to *M. pygmaeus*, but not in the resistant germplasm ($P>0.05$). Finally, the nematode infectivity was 43% higher ($P<0.05$) in the susceptible compared to the resistant plants non-exposed to *M. pygmaeus*, but not in the exposed ones ($P>0.05$) (Fig. 1B).

Vertical Y-tube olfactometer

The olfactometer setup showed that 97, 87, 85 and 75% of the *M. pygmaeus* females used responded to the odors from tomato plants. However, their preference was not affected by the nematode infection irrespective of the tomato cultivar neither the RKN inoculum used in the experiment ($P>0.05$) (Fig. 2).

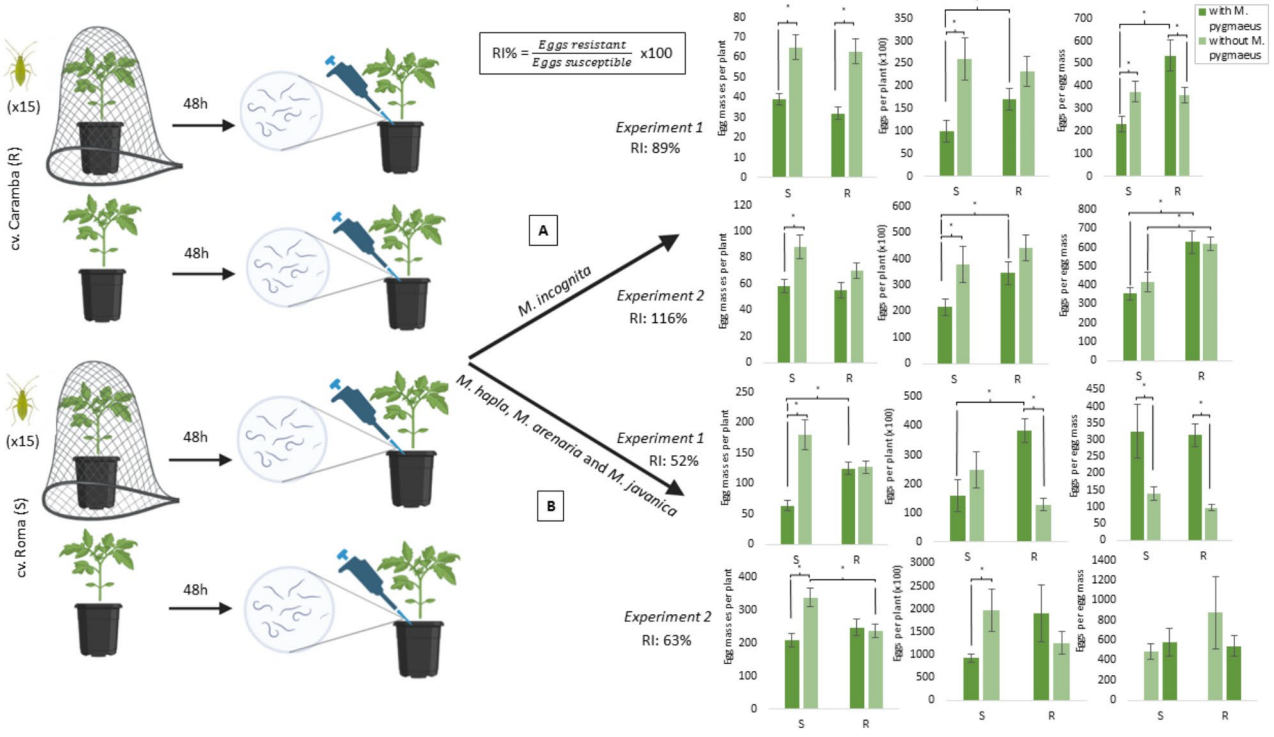

Fig. 1. Infectivity (egg masses per plant), reproduction (eggs per plant), and fertility (eggs per egg mass) (mean ± SE) were assessed on both susceptible tomato cv. Roma (S) and the *Mi*1.2 resistant cv. Caramba (R) in 200 cm^3 pot experiments. Plants were either induced (with) or not (without) with 15 nymphs of *Macrolophus pygmaeus* over a 48-hour period and subsequently inoculated with 1 J2 cm^{-3} of soil of *Meloidogyne incognita* (Agropolis strain) (**A**) or 3 J2 cm^{-3} of soil containing a mixed community of *M. hapla*, *M. arenaria*, and *M. javanica* (Community strain) (**B**). Data followed by * are different according to the Student-t test or the Welch test ($P<0.05$).

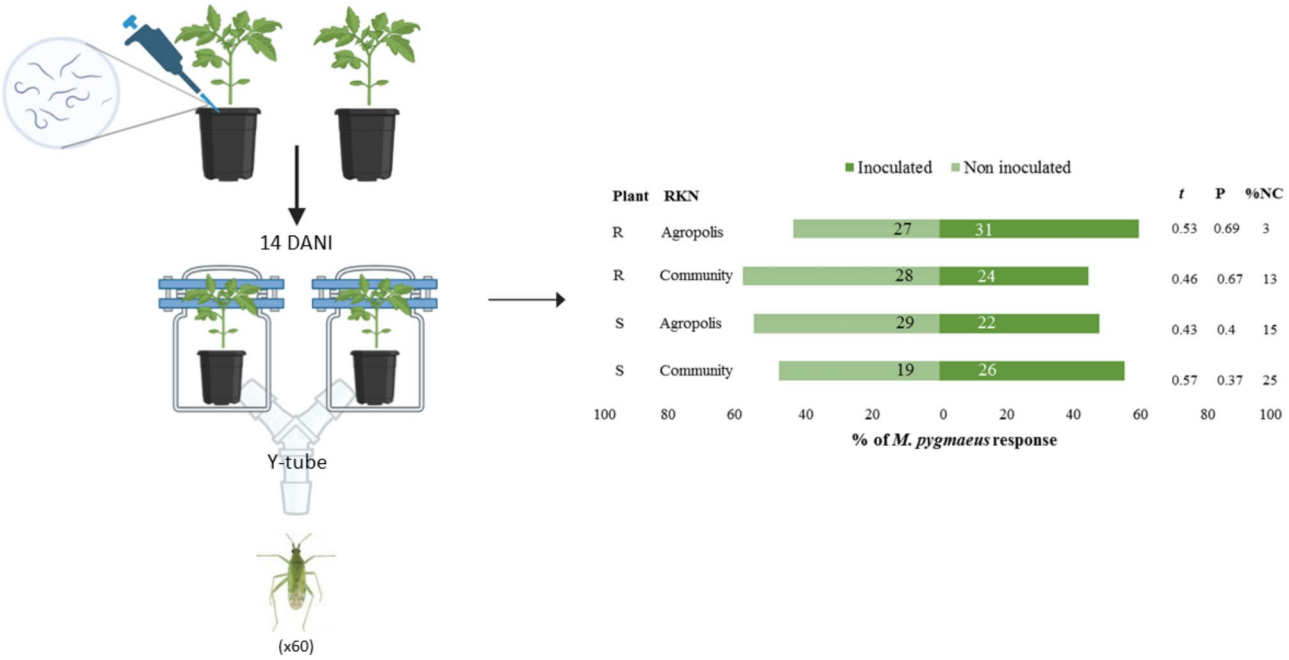

Fig. 2. Preference of *M. pygmaeus* females to nematode infected or non-infected plants in a vertical Y-tube olfactometer, 14 days after nematode inoculation. The susceptible tomato cv. Roma (S) or the *Mi*1.2 resistant cv. Caramba (R) were inoculated with 3 J2 cm^{-3} of soil of M. incognita (Agropolis strain) or a mixed community of *M. hapla* , *M. arenaria* and *M. javanica* (Community strain). NC indicates the number of tested individuals that did not respond. Significant differences using a two side binomial test are marked with (*) ($P < 0.05$).

Insect cages experiments

In the first two experiments inoculated with *M. incognita* there were no differences between experiments ($P > 0.05$), therefore data were pooled. The number of *M. pygmaeus* in RKN infected resistant tomato compared to non-infected were 2-fold and 1.7-fold higher after 1 h and 48 h of releasing the insects into the cages, respectively ($P < 0.05$), but no differences were recorded in the susceptible cultivar ($P > 0.05$) (Fig. 3A). In addition, no differences in the offspring were recorded irrespective of the nematode inoculation or the tomato cultivar ($P > 0.05$) (Fig. 4A). In the third, experiment inoculated with the nematode community, no differences in the number of *M. pygmaeus* choosing infected versus non-infected plants were recorded irrespective of the time and the tomato cultivar ($P > 0.05$) (Fig. 3B). Moreover, no significant differences in the offspring were recorded irrespective of the nematode inoculation or the tomato cultivar ($P > 0.05$) (Fig. 4B).

Discussion

The present study reveals *M. pygmaeus* ability to induce plant resistance against RKN, effectively reducing nematode infectivity and reproduction in susceptible tomato plants in both *M. incognita* and the mixed community, probably by activating the jasmonic acid (JA) pathway[20,21], inducing resistance irrespective of the *Meloidogyne* specie. However, in resistant tomato plants, only the initial pot experiment showed a reduced infectivity with *M. incognita*, while reproduction remained unaffected. Moreover, neither infectivity nor reproduction was impacted in the subsequent pot experiment. These findings underscore the influence of plant genetics on the phenotypic response to RKN when exposed to *M. pygmaeus*. The effect of plant feeding by *M. pygmaeus* has been previously reported to upregulate the genes related to the JA pathway, increasing the concentration of 12-oxo-phytodienoic acid and jasmonic acid–isoleucine in the punctured leaves, affecting the performance of *T. urticae* and *F. occidentalis* in other leaves[24]. Besides, the JA-related genes were also upregulated in tomato leaves induced by the mirid bug *N. tenuis*[20]. For that, the JA pathway seems responsible for mediating the resistance to RKN. In addition, Wang et al.[47] demonstrated through a series of grafting experiments using mutants lacking the *GLUTAMATE RECEPTOR-LIKE* 3.5 or the *RESPIRATORY BURST OXIDASE HOMOLOG 1*, key for ROS and JA accumulation in the upper stems and leaves, that basal resistance of roots against RKN relies significantly on JA synthesis in shoots but not in roots. The JA is then transported from the shoots to the roots to help trigger defense responses. The exogenous application of JA and its derivatives, such as methyl jasmonate, have also been demonstrated to reduce nematode infection, probably by increasing toxic compounds to nematodes produced by roots such as hytoectosteroids, flavonoids and proteinase inhibitors[48–51].

Salicylic acid (SA) seems to be an important signaling compound associated with the hypersensitive reaction to prevent nematode establishment in the *Mi*-mediated resistance[52]. Furthermore, SA and JA could interact antagonistically depending on the combination of their respective concentrations and could be exploited by pathogens to enhance plant susceptibility[53]. For instance, the bacterium *Pseudomonas syringae* uses coronatine,

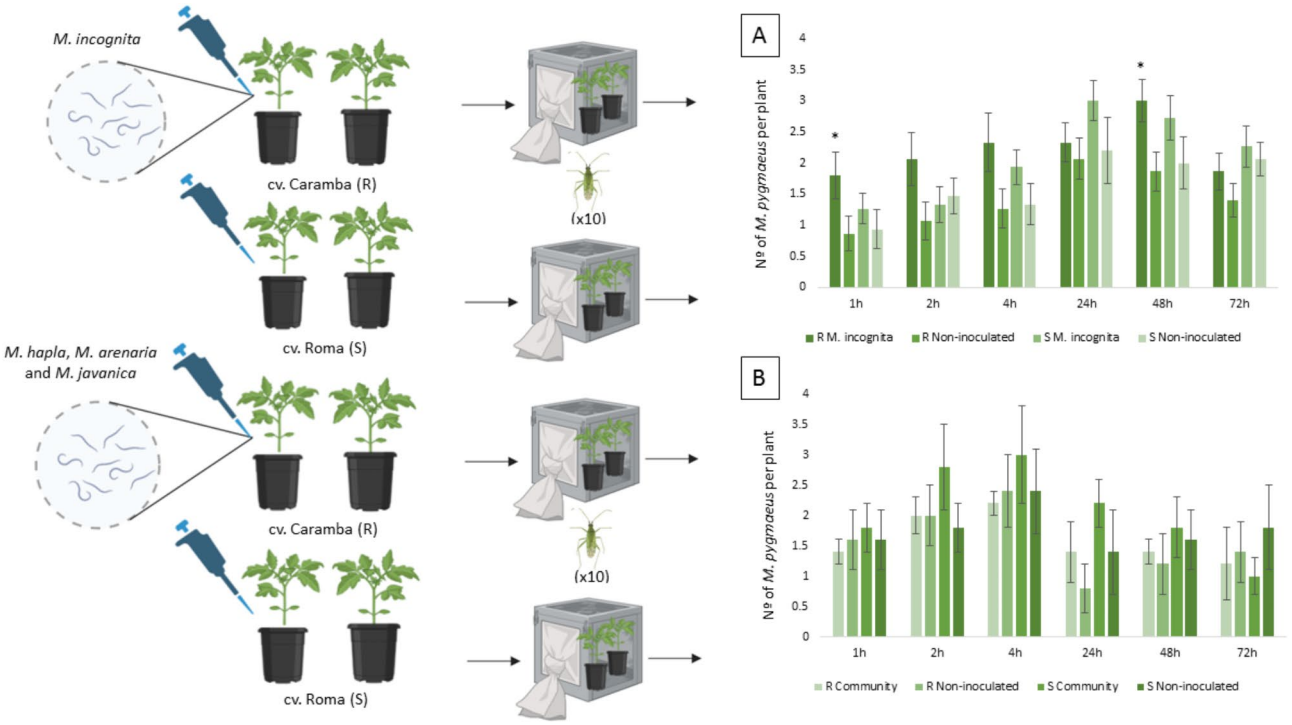

Fig. 3. *Macrolophus pygmaeus* (mean ± SE) per plant counted in insect cages 1, 2, 4, 24, 48 and 72 h after releasing the insects in the susceptible cv. Roma (S) or the *Mi*1.2 resistant tomato cv. Caramba (R), inoculated or not with 3 J2 cm^{-3} of soil of *M. incognita* (Agropolis strain) (**A**) or a mixed community of *M. hapla*, *M. arenaria* and *M. javanica* (Community strain) (**B**) 14 days after nematode inoculation (DANI). Data followed by * in the same plant germplasm are different according to the Student-t test or the Welch test ($P < 0.05$).

a substance similar to jasmonate-isoleucine (JA-I1e) to repress the SA-mediated defense pathway[54]. Thus, a negative crosstalk between the SA and JA signaling pathways may occur leading to a deficiency to induce resistance in the *Mi*1.2 plants. Interestingly, Copper et al.[50] found a reduction in nematode infection when JA application was performed on susceptible tomato plants at 25ºC and 32ºC one day prior to inoculation and 7 days after, using avirulent nematode populations compared to non-treated plants. When *Mi*-resistant plants were used, no effect of the JA application at 25ºC or 32ºC was found, although the resistance at 32ºC was partially lost due to high temperature. When *Mi*1.2 virulent nematode populations were used, the JA application did not reduce the nematode infection in susceptible and resistant plants. Therefore, the interaction between the JA application, the (a)virulent status of the nematode, and the plant genetic background affects the plant response against the nematode. Thus, further studies related to the gene expression in susceptible and resistant plants, including grafting, must be explored to understand the potential interaction of susceptible scions induced by *M. pygmaeus* grafted onto resistant rootstocks and its interactions with *Mi*1.2-virulent and avirulent RKN. Furthermore, the mechanisms related to defense induction by *M. pygmaeus* seem to differ from those mediated by the resistance conferred by the *Mi*1.2 gene and therefore alternating these strategies would be advisable to reduce nematode populations and avoid the selection of virulence to R genes.

In our work, no significant differences were found between *M. pygmaeus* choice of nematode-infected or non-infected plants and the offspring produced, regardless of the plant background or the nematode used. The preference of *M. pygmaeus* for tomato plants is significantly influenced by the Herbivore Induced Plant Volatiles (HIPVs) emitted when the plants are infested by various pests, such as spider mites, aphids, whiteflies, and caterpillars[55,56]. Additionally, RKN infection is known to alter the volatile organic compounds (VOC) profile, potentially affecting the preference and development of both pests and their predators. Arce et al.[30] reported a strong suppression of 8 out of 33 compounds emitted by RKN infected plants, including α-terpinene, β-phellandrene β-caryophyllene, α-pinene, and α-humulene, affecting the oviposition and development of *T. absoluta* and thus, the biological control of *T. absoluta* with *M. pygmaeus* would be enhaced, since *M. pygmaeus* would not be affected by the nematode infection, as our results show. Moreover, the root infection of wheat plants by *M. incognita* reduced the feeding of *Sitobion avenae* (Hemiptera: Aphididae) 7 days after inoculation and interestingly, its aphid predator *Harmonia axyridis* (Coleoptera: Coccinellidae) preferred plants co-damaged by *M. incognita* and *S. avenae* from those only infested by the aphid[57]. In addition, the accumulation of other compounds in RKN-infected roots, such as α-tomatine, a steroidal glycoalkaloid related to herbivory defense, is associated with the nematode development stage, increasing its concentration at the nematode reproduction stage[31,58]. In our study, as no prey were introduced in the plant, the response of the predator was not affected by the nematode infection. Those results are encouraging since the presence of the predator will not be affected by the nematode, ensuring the integrated pest management in these conditions.

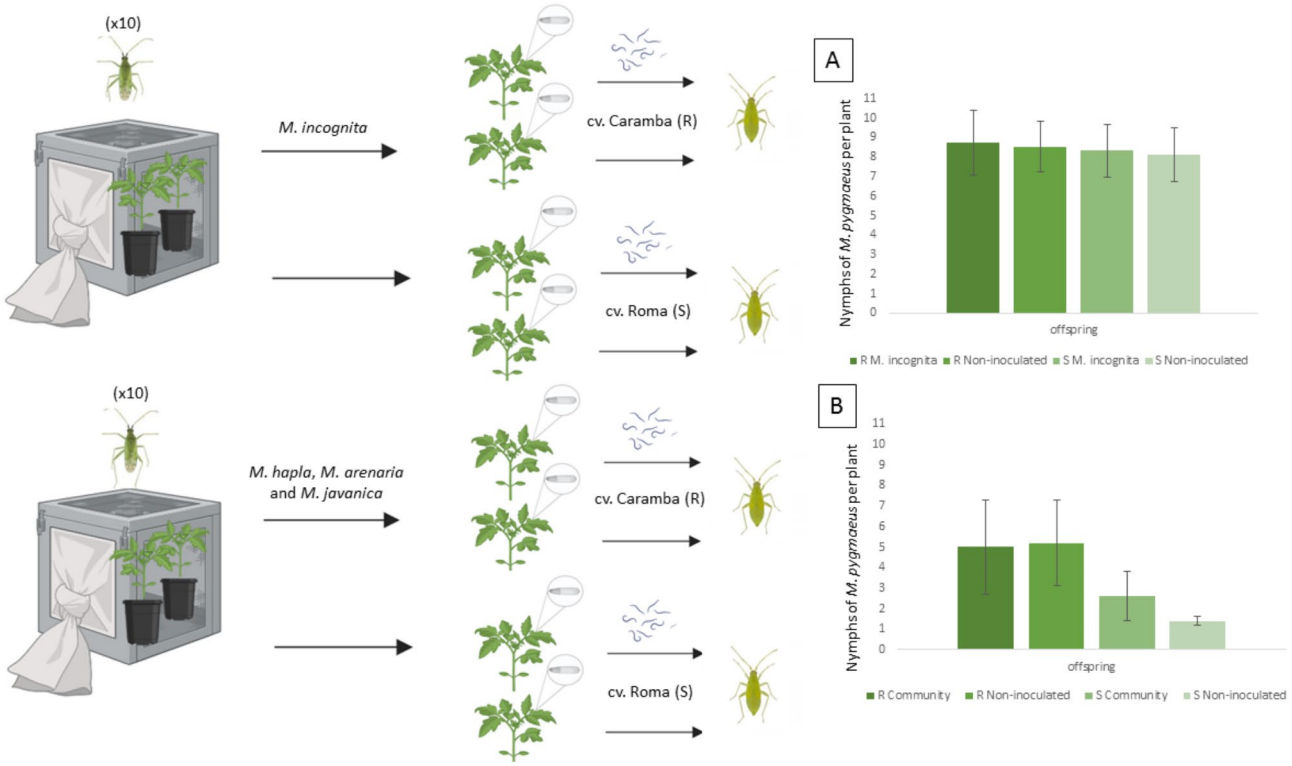

Fig. 4. *Macrolophus pygmaeus* (mean ± SE) offspring nymphs per plant produced in separated boxes after 14 days of the last count in each plant of the susceptible cv. Roma (S) or the *Mi*1.2 resistant tomato cv. Caramba (R), inoculated or not with 3 J2 cm-^{3} of soil of *M. incognita* (Agropolis strain) (**A**) or a mixed community of *M. hapla*, *M. arenaria* and *M. javanica* (Community strain) (**B**). Data followed by * in the same plant germplasm are different according to the Student-t test or the Welch test ($P < 0.05$).

In conclusion, the induction of susceptible tomato plants with *M. pygmaeus* prior to planting significantly reduces RKN infectivity and reproduction. Given that the preference of *M. pygmaeus* is influenced by plant-emitted HIPVs, but not for those altered by RKN infection, the biological control of pests will not be affected and is proposed as a tool to include into integrated pest management.

Data availability

The data supporting this study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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