University of São Paulo
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Meloidogyne incognita and melon plants: host status, induced resistance and biological control

Victor Hugo Moura de Souza

Thesis presented to obtain the degree of Doctor in Science. Area: Plant Pathology

Piracicaba,
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To my beloved wife and family

For the unconditional support and for the kind words during the hardest moments

To my dear friends,

Who have always motivated me and inspired me with their dedication and their lives

To my great grandmother Severina Anunciada de Jesus (*in memoriam*)

The loveliest person I have ever met that now lives in our hearts

To my grandfather José Zacarias Sobrinho (*in memoriam*)

The ironsmith who has fought fiercely to raise and to take care of his family

For you all, I dedicate this work

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“Smooth sea never made a skilled sailor”

Franklin D. Roosevelt

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RESUMO

Meloidogyne incognita e meloeiro: reação de hospedeiro, indução de resistência e controle biológico

Atualmente, o melão (*Cucumis melo* L.) consiste na fruta mais exportada do Brasil, consistindo em importante agronegócio para os produtores da região Nordeste do país, tendo como maiores produtores os estados do Ceará e Rio Grande do Norte. Dentre os fatores limitantes para essa cultura, o nematoide das galhas (*Meloidogyne* spp.) se destaca entre as principais doenças do meloeiro. Plantas sob alta infestação apresentam deficiência nutricional, baixo desenvolvimento da porção aérea e deformação do sistema radicular devido as galhas, que consiste no sintoma mais característico dessa doença. Com a falta de ferramentas de manejo, medidas alternativas são necessárias para evitar maiores perdas. Nesse contexto, o presente trabalho teve por objetivo avaliar a influência de agentes de controle biológico e indutores de resistência no controle de *M. incognita* em plantas de melão, bem como a influência desses agentes no desenvolvimeto do meloeiro em casa de vegetação, além de seus efeitos em diferentes estádios de *M. incognita*. Dessa forma, experimentos de casa-de-vegetação foram conduzidos, sendo um para verificar a reação de hospedeiro de híbridos de melão à *M. incognita*, três para verificar a influência de indutores de resistência e agentes de controle biológico no desenvolvimento do meloeiro e no controle de *M. incognita* (Exerimento 1, 2 e 3). Adicionalmente, um experimento foi conduzido para verificar a dose-resposta de *Pochonia chlamydosporia* (Rizotec®) para controle de *M. incognita* e dois para verificar o uso potencial de filtrados obtidos a partir de organismos de controle biológico no desenvolvimento do meloeiro e no controle de *M. incognita*. Nesses experimentos, o fator de reprodução (valor R), população final (Pf) e nematoides por grama de raiz (Nem/g) foram obtidos ao final dos ensaios. Adicionalmente, foram avaliados a massa fresca e seca da parte aérea, altura das plantas, massa das raízes, massa dos frutos por planta, diâmetro do caule (base, meio e ápice) e conteúdo de clorofila. Ademais, realizou-se um experimento para se verificar o efeito de filtrados nas sementes de melão e três para verificar o efeito dos filtrados e da taxtomina parcialmente purificada (TPP) na eclosão dos ovos de *M. incognita*. Adicionalmente, três experimentos foram realizados para verificar o efeito de indutores de resistência na penetração e desenvolvimento de juvenis J2 de *M. incognita* nas raízes de meloeiro. Como resultados, todos os genótipos testados foram suscetíveis a *M. incognita*. A Pf variou de 2.381 à 7.806 nematoides, o valor R variou de 5,7 à 19,5 e o Nem/g foi de 271 à 1.791. No experimento 1, meloeiros tratados com indutores de resistência apresentaram menor altura, embora, nenhuma diferença estatística foi encontrada nas massas frescas e secas das plantas. Adicionalmente, plantas tratadas com acibenzolar-S-metil (ASM) (inoculadas e não inoculadas), *P. lilacinus* (-N) e *P. chlamydosporia* (+N) apresentaram frutos mais pesados. Com relação ao experimento 2, todos os tratamentos inoculados apresentaram maior peso das raizes, quando comparada aos tratamentos não inoculados, causado pela deformação da porção radicular. Adicionalmente, plantas tratadas com agentes de controle biológico apresentaram raízes com menos sintomas, quando comparados ao controle. Ademais, plantas tratadas apresentaram maior quantidade de clorofila em folhas jovens, quando comparadas aos controles. Todos os tratamentos, com exceção a *P. chlamydosporia* no experimento 1, reduziram a população final de *M. incognita*. Quando testado separadamente, todas as doses de *P. chlamydosporia* reduziram a Pf de *M. incognita*, embora as mais eficientes tenham sido a dose 4 (1g/planta) e dose 5 (2g/planta) Com relação aos filtrados, resultados promissores foram obtidos. Os filtrados obtidos a partir de *B. amyloliquefaciens* e *P. lilacinus* reduziram as populações finais de *M. incognita*, embora apenas *B. amyloliquefaciens* tenha diferido estatisticamente. Adicionalmente, aumento na massa das raízes e na massa dos frutos foram observados para plantas tratadas com filtrados de *B. amyloliquefaciens* e *P. lilacinus*, respectivamente. Ademais, todos os filtrados aceleraram a germinação das sementes quando comparado ao meio batata-dextrose, que suprimiu a germinação. Sementes tratadas com o filtrado obtido a partir de *P. chlamydosporia* apresentaram radículas pilosas, o que não foi observado nos outros tratamentos. Com relação aos experimentos de penetração, juvenis J2 de *M. incognita* foram observados no interior das raízes em todos os ensaios. Nenhum efeito do ASM foi observado em ambos os experimentos. Com relação a TPP, os dados apontam que o tratamento acelerou o ciclo do nematoide no interior das raízes. Conclui-se, que os dados obtidos apontam para o potencial dos indutores de resistência e agentes de controle biológico e seus filtrados no controle de *M. incognita* em meloeiro.

Palavras-chave: Nematoide das galhas; *Cucumis melo*; Controle alternativo; Organismos de controle biológico; Indutores de resistência; Filtrados de meio

ABSTRACT

Meloidogyne incognita and melon plants: host status, induced resistance and biological control

The melon crop (*Cucumis melo* L.) is the most exported fruit of Brazil and consist in an important agribusiness to the producers on Northeastern Region of Brazil, having as the main producers the Rio Grande do Norte and Ceará states. Among the limiting factors of this crop, root-knot nematode (*Meloidogyne* spp.) stand out as major treat, that causes on severed attacked plants malnourishment, poor development of the above-ground portion and shorter root system due to the galls, which is the most characteristic symptom of this disease. With the lack of management tools to the root-knot nematode, alternative measures are needed to avoid losses. In this context, the main objective of the present work was to evaluate the influence of resistance inducers and biological control agents on the control of *M. incognita* on melon plants, as the influence of these agents on melon plant development and their effects on different *M. incognita* stages. For these purposes, greenhouse experiments were performed. The first one aimed to verify the host status of melon hybrids to *M. incognita* and additional three were carried out to verify the influence of resistance inducers and biological control organisms on melon development and on the control of *M. incognita* on this crop (trials #1, #2 and #3). Also, an additional greenhouse trials were carried out to verify different doses of *P. chlamydosporia* (Rizotec®) on the control of *M. incognita* in melon plants and to verify the potential of culture filtrates (Cf) obtained from biological control organisms on the control of *M. incognita* and on the melon plant development. In these greenhouse experiments, the reproductive variables final population (Pf), reproduction factor (R value) and nematodes per gram of root (Nem/g) were obtained at the end of the experiments. Additionally, it was evaluated the fresh and dry weight of the aerial portion, the plant height, root weight, fruit weight, stem diameter (measured on stem basis, middle and apex) and chlorophyll content. Additionally, *in vitro* assays were performed to verify the effect of Cfs on melon seeds and to verify the effect of the Cfs and the partially purified thaxtomin A (PPT) on *M. incognita* egg hatching. Moreover, three additional assays were carried out to verify the effect of resistance inducers acibenzolar-S-methyl (ASM) and PPT in the penetration and post-penetration of *M. incognita* second stages juveniles in melon roots. As results, all tested genotypes were susceptible to *M. incognita*. The Pf values ranged from 2,381.06 to 7,806 nematodes, the R value ranged from 5.95 to 19.5. Also, Nem/g values ranged from 271 to 1,791. In trial #1, melon plants treated with resistance inducers presented lower height; despite no statistically differences were found for fresh and dry weight. Also, plants treated with ASM (inoculated and non-inoculated), *Paecilomyces lilacinus* (non-inoculated) and *Pochonia chlamydosporia* (inoculated) produced heavy fruits. On trial #2, all inoculated plants differed statistically on root weight and presented heavier roots than non-inoculated plants, which was due the large amount of galls caused by *M. incognita*. In addition, treated plants with the biological control agents presented fewer symptoms than control plants. Treated melon plants exhibited higher chlorophyll content on young leaves, when compared with both controls. Moreover, on both treatments the *M. incognita* population was reduced, except by *P. chlamydosporia* on the experiment 1. When tested separately, all *P. chlamydosporia* doses reduced the Pf, however the most efficient were the dose 4 (1g/plant) and dose 5 (2g/plant). Regarding Cf, promising results were obtained. Both Cf from *P. lilacinus* and *Bacillus amyloliquefaciens* reduced the *M. incognita* population, in greenhouse experiment. Additionally, *P. lilacinus* Cf increased the fruit weight, and *B. amyloliquefaciens* Cf increased the root weight, despite being inoculated or not. Furthermore, the tested Cfs presented suppressive effect on *M. incognita* egg hatching, but further evidence is necessary due to lack of statistical differences with the potato-dextrose broth medium (PD). Additionally, the filtrates improved the germination of melon seeds, despite the suppressed effect of PD broth medium on them. Also, *P. chlamydosporia* treatment induced hairy roots, which were not observed on the other treatments. Regarding the penetration assays, juveniles were not observed at 3 days after inoculation (DAI). No effect of ASM in penetration and post-penetration of *M. incognita* J2 was

observed on both experiments. Concerning PPT, penetration was not observed at 3 DAI, but it was observed on the other assessed periods. Furthermore, our data points out that the TPP may speed the nematode cycle on melon roots. In conclusion, the obtained data point out the potential of the resistance inducers, biological control organisms and their culture filtrates on the control of *M. incognita* on melon plants.

Keywords: Root-knot nematode; *Cucumis melo*; Alternative control; Biological control organisms; Resistance inducer; Culture filtrates

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1. INTRODUCTION

Brazil has started melon crop (*Cucumis melo* L.) production on the 60s, mainly on the South region of the country. Nowadays, the main producers are located on the Northeastern region; with Rio Grande do Norte and Ceará as the major producing states. Melon is currently the most exported fruit of the country, with 224,688,423 tonne of fresh fruits exported annually, generating US$148,741,470 (Barbieri, 2018). Not just the economic, but melon crop also represents a great social relevance, mainly due to its adaptation to the environmental conditions of the semiarid regions of Northeastern, providing gains to the producers and allowing the development of these less fortunate areas.

Despite these numbers, Brazilian northeast melon producers are facing some challenges. Currently, the total cultivated area is around 13,700 ha, representing a reduction of 6.3% when compared to 2016/2017 season (Costa and Souza, 2017). According to these authors, this reduction must be attributed mostly to drought on the main producing areas. On this context, root diseases, as the ones caused by plant parasitic nematodes, becomes even more important, because they may affect for example the water/nutrients uptake (Ferraz and Brown, 2016; Bedendo, 2018). As a result, the diseased plants are less resistant to the harsh environmental conditions, and also represent a potential loss to the farmers.

Among plant parasitic nematodes, the root-knot nematodes (*Meloidogyne* spp.) are the major threats to melon crop (Moura et al., 2002). Severed attacked plants present shorter root system and large amount of galls, which is the main symptom of root-knot disease. Also, plants often present malnourishment and poor development of aerial portion (Moura et al., 2002; Pinheiro and Amaro, 2010). Besides its directly relevance, the root-knot nematode may predispose plant tissues to other important soil-borne pathogens (Pinheiro; Amaro, 2010), like *Didymella bryoniae*, which is the causal agent of gummy stem blight, one of the most important melon diseases worldwide (Dalcin et al., 2017). This synergic effect is not only able to result in greater losses to the farmer, but it also raises the difficulty to execute a properl disease management.

Regarding the management of plant parasitic nematodes, there are just few tools available to control these organisms. A great number of researchers are now dedicating in studying alternative management methods, mainly because cultural practices and resistant cultivars are not always available (De Souza et al., 2015). Currently, there are not commercial resistant hybrids or cultivars of *C. melo* to *Meloidogyne* species nowadays. Also, there are just a few resistant genotypes for *M. incognita* and *M. javanica*, but the same cultivar does not

showed resistance for both root-knot species (Pinheiro and Amaro, 2010; Ferraz et al., 2012; Diniz et al., 2016a; Diniz et al., 2016b). Furthermore, there are some efforts to understand *Cucumis metuliferus* (E. Mey) resistance mechanism against root-knot nematode and use it for breeding programs (Ling et al., 2017; Ito et al., 2014; Pinheiro and Amaro, 2010). It is relevant to mention that most chemical nematicides have been removed from the market, mainly due to the environmental contamination risks and high toxicity of compounds to human health (Kerry, 1992). Thus, some alternative tools may be used to reduce losses caused by root-knot species and may be integrated on management practices.

Biological control and induced resistance are already known in the scientific literature, with some promising results on reducing the root-knot nematode reproduction and its symptoms on several crops (Zinovieva et al., 2013; Owen et al., 2002; Chinnasri et al., 2003), including *C. melo*. Regarding biological control agents, several commercial products are available on the Brazilian market, based upon, for example, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Bacillus* spp., (AGROFIT, 2018). Furthermore, it is necessary to research their effectiveness against *Meloidogyne* species on melon, mainly due some inconsistences of control (Medeiros et al., 2009; Abdeldaym et al., 2014) or lack of information.

Regarding the resistance inducers, there are some information available, including commercial uses of acibenzolar-S-methyl (ASM) (CAS 135158-54-2) on melon plants against *Acidovorax citrulli*, the causal agent of bacterial fruit blotch (Di Piero, 2005; Cabral et al., 2010). Despite this fact, for some *C. melo* pathogens, like plant parasitic nematodes, there are no information available about the effects of resistance inducers on the control of these organisms.

In this context, the main objective of the present work was to evaluate the influence of resistance inducers and biological control agents on *M. incognita* control at melon plants. Also, evaluate the effects of these agents on the development of melon plants and its effects on different *M. incognita* stages.

2. LITERATURE REVIEW

2.1. *Cucumis melo*: the host and its production

The Curcubitaceae family encompasses over 100 genera, with more than 900 species of plants, collectively known as cucurbits or gourd (Schaffer and Paris 2016). The plants from this family are distributed in tropical, subtropical and temperate regions and at least 20 genera are used for culinary purposes, which include mature fruit flesh, whole immature fruits and/or seeds consumption (Schaffer and Paris 2016; Pinheiro and Amaro, 2010). Many cucurbits presents great economic relevance worldwide. In Brazil, the most cultivated species are squash *Cucurbita moschata* Duchesne ex Poiret) and zucchini (*Cucumis pepo* L. Dumort) (, pumpkin (*Curcubita maxima* Duschesne), watermelon (*Citrullus lanatus* (Thunb) Matsum. and Nakai), cucumber (*Cucumis sativus* L.), west indian gherkin (*Cucumis anguria* L.) and melons (*Cucumis melo* L.) (Pinheiro and Amaro 2010).

It is not totally clear, but research points that the Southwest African continent and Indian peninsula are probably the origin center of *Cucumis* species (Mallick and Massui 1986). According to this authors, when the continent has been divided, the vegetation at those places has been separated. In addition to that, the occurrence of those plants on different shapes and on different parts of the modern word are a result of the dissemination by animals and mankind.

Melons are considered to be part of two subspecies, *C. melo* ssp. *melo* and *C. melo* ssp. *Agrestis*. Those subspecies may be recognized by long, spreading hairs and short hairs on ovaries (Schaffer and Paris, 2016). Furthermore, Pitrat et al. (2000) recognized and classified 16 cultivar-groups of melon. Inodorus group is composed of winter or casaba melons, ‘honeydew’, ‘Jaune Canary’, and ‘Piel de Sapo” (Also referred as ‘Santa Claus melon’) melons. The muskmelons, or Reticulatus Group, present netted rind and include cultivars known as ‘cantaloupes’, which include ‘PMR45’, ‘Hale’s Best Jumbo’, and ‘Top Mark’ as the lightly netted, unfurrowed varieties, such as ‘Galia’ and ‘Persian’ melons (Schaffer and Paris 2016). The French ‘Charentais’ melon is classified under the Cantalupensis group. The distinction between groups is becoming blurred due to breeding, since groups are easily hybridized one to another.

Regarding *C. melo* crop in Brazil, it was first introduced on the 60s. Until that moment, the national consumption had been supplied by Chile and Spain importation (Araújo and Vilela 2003). The major producer areas at that period were Rio Grande do Sul and São

Paulo states, however, climatic factors were an obstacle to both production and yield (Dias et al. 1998).

With commercial cultivation emergence in the Northeastern region of Brazil, melon production has rapidly expanded, achieving an increase of 366% on total production in the 1987-1996 period. This increase was caused mainly by larger cultivated farms, which were 182% over the previously dedicated areas to melon crop. Thereby, this crop has been gaining more notoriety in and out of Brazil and nowadays consists in an important agribusiness to the Northeastern region of the country (Nagai 1990; Dias et al. 1998; Barbieri 2018). Currently, melon is cultivated in Bahia, Pernambuco, Ceará and Rio Grande do Norte states, with the last two representing the top producers of Brazil (Resende and Costa 2010; IBGE 2012).

Regarding the top producers, melon belt region is concentrated in two important zones of Ceará and Rio Grande do Norte states: the Mossoró-Assú (RN) and Baixo Jaguaribe (CE). Together, in 2016/2017 season, the cultivated area represented 12,545 ha and production has accounted for 227 thousand tonne of fresh fruit, which corresponded to US$ 148 million (Barbieri 2018).

Besides its undeniable economic relevance, *C. melo* crop has a strong social component, due to the possibility of economic exploitation of the Semi-arid Region in Brazilian Northeast (Buso et al. 2004). Melon is a specie with specific requirements of temperature: 28°C – 32°C for germination, 20°C – 32°C for flowering and 25°C – 30°C for a good vegetative development (Nicolas et al. 1989; Silva et al. 2003). Those requirements make the Brazilian Northeast region an ideal place for its cultivation.

Nowadays, the Brazilian northeast melon producers are facing some challenges. Currently, both cultivated area and total production decreased in 6.3% and 8%, respectively, when comparing 2016/2017 season (Barbieri 2018; Costa and Souza 2017). The main regions have been suffering with drought, which among others factors, implies in water salinization and higher incidence of pests, which reduces °brix and affects the appearance of fruits. Drought aggravates the incidence of pests, notably *Liriomyza* spp., because it migrates from dry vegetation to the crops, which stay green due to irrigation (Barbieri 2018; Nascimento and Reis 2015). Furthermore, root diseases, as the ones caused by plant parasitic nematodes, becomes even more important, because they are able to affect, among other processes, the water/nutrient uptake (Ferraz and Brown 2016; Bedendo 2018). As a result, diseased plants are less resilient to the harsh environmental conditions and also represent a potential loss to the farmers.

2.2. Plant Parasitic nematodes, root-knot and *Meloidogyne incognita* on melon: The pathogen

With the intensive cultivation in the currently agricultural system, it has been benefiting the development of several diseases, notably plant parasitic nematodes that are able to cause significant losses in areas with highly nematodes' density (Pinheiro and Amaro 2010). The host can be damaged in different intensity, ranging from tiny injuries to destruction of the root-system, with consequent senescence of the plant (Souza et al., 2015). Regarding *C. melo*, the plant parasitic nematodes consist in serious treats. For example, in United States of America, on 1964, plant parasitic nematodes were responsible for losses of two millions dollars in this crop (annually) (Leclerg 1964). In addition to that, there are several records of plant parasitic nematodes infecting melon plants in Oregon State (Merrifield 2000). Additionally, in North Caroline, 10% losses caused by *Meloidogyne* spp. in cantaloupe was registered (McSorley et al. 1987). In Indiana State, losses my hit 50% in fields that are heavily infested with root-knot nematodes, which are more severe in hot dry summers (Latin 1993).

In Brazil, Moura et al. (2002) registered increasing reductions in yield due to high densities of plant parasitic nematodes. Losses of 100% were registered in some farms in RN (Pinheiro and Amaro 2010). Both works reported *Meloidogyne* spp. and *R. reniformis* as the most important species to melon crop in Brazil.

Moura et al. (2002) reported two syndromes caused by plant parasitic nematodes. The first one, the root-knot disease, consisted in plants with stunted and severed galled root systems (Figure 1, Figure 2). Inside this galls, which consist in the main symptom of the disease, there were observed *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. The second one, consisted in plants with their root system stunted, but, instead of galls, there were several localized necrosis, which were found to be caused by *R. reniformis* crop. Torres et al. (2004) has published the first report of *Pratylenchus brachyurus* infesting melons plants in field conditions in Brazil. Conversely, its host status had been already verified in laboratory conditions, but not in field until this work (Machado and Inomoto 1999). However, damage/losses were not reported, despite the lesion nematode association with *C. melo* has been already reported in other countries (Naveda et al. 1999).

The root-knot nematode (*Meloidogyne* spp.) is among the most economically important group of plant parasitic nematodes (Jones et al. 2013). They are obligated plant

parasites that are distributed around the world and heavily polyphagous, which means that they have the ability to parasites almost every species of vascular plant (Jones et al. 2013; Ferraz, 2018). Furthermore, their name comes from the galls (root-knots), induced by this nematodes on the roots of their hosts plants.

Mature females of *Meloidogyne* sp. lay eggs in a protective gelatinous matrix, originating an egg mass (Jones et al. 2013). This matrix is produced by the females and it is secreted through the anus during oviposition with adherent properties, keeping the eggs agglomerated and protected (Ferraz et al. 2018). Those masses are often found on root surface, but they may be immersed in the galls or plant tissue, with probably 400 eggs, but there are reports of egg masses with more than 1000 eggs (Jones et al. 2013; Ferraz 2018).

After embryogenesis, the first-stage juveniles (J1) moults inside the egg to the infective second-stage juvenile (J2), which presents filiform shape and hatches from the egg to search plant roots. The hatching is only dependent of suitable temperature and moisture conditions. Plant stimulus are not required for the process. However, root diffusates and generation number within a season must influence the hatching response (Jone et al. 2013; Ferraz et al 2018; Curtis et al. 2009; Magnusson 1986).

J2s penetrate roots directly, near the root tips, but they present the ability to penetrate at any site. Usually, they use a combination of physical damage through trusting of the stylet and enzymatic breakdown with cellulolytic and pectolytic enzymes. After penetration, the J2s migrate intercellularly, through cortical parenchyma, moving upwards and positioning in the vascular cylinder, towards the endodermis and pericycle (Jones et al. 2013; Ferraz et al 2018). The J2 initiates the formation of a permanent feeding site, which consists of several giant cells, mainly through secretion of several effectors, produced in the pharyngeal glands and delivered into the plant cell cytoplasm by the stylet (Davis et al. 2008). This nematodes' effectors manipulate the plant response to infection, though only few of them have been characterized (Gheysen and Mitchum 2011). The J2s stimulates half a dozen cells in the vascular parenchyma to undergo repeated mitosis without intermittent cytokinesis. The resulting giant-cells contain multiple enlarged nuclei (up to > 100) and are embedded in proliferated tissue that forms the gall (Gheysen and Mitchum 2011). This cells act as specialized sinks, supplying nutrients to the sedentary J2 (Jones et al. 2013). After feeding, the J2 swells and moults additional three times to reach the reproductive stage (Figure 1).

The J3 and J4 stages lack a functional stylet and do not feed. The males are mobile and leave the roots, being more common under special conditions, such as nutritional stress. The adult females continue to feed and enlarge, becoming a pear-shaped nematode, which is

visible at naked eye when removed of the galls (Jones et al. 2013; Ferraz 2018). The life cycle is heavily influenced by some factors, such as temperature humidity and, of course, the plant host. Generally, the cycle is completed in 3 – 4 weeks and the optimal temperature for *M. incognita* and *M. javanica* ranges from 25 to 30 °C (Ferraz 2018). As the nematode develops, the symptoms appear and the severity depends on the plant species and cultivar. Also, this may be influenced by the nematodes' population density and soil type influence the severity.

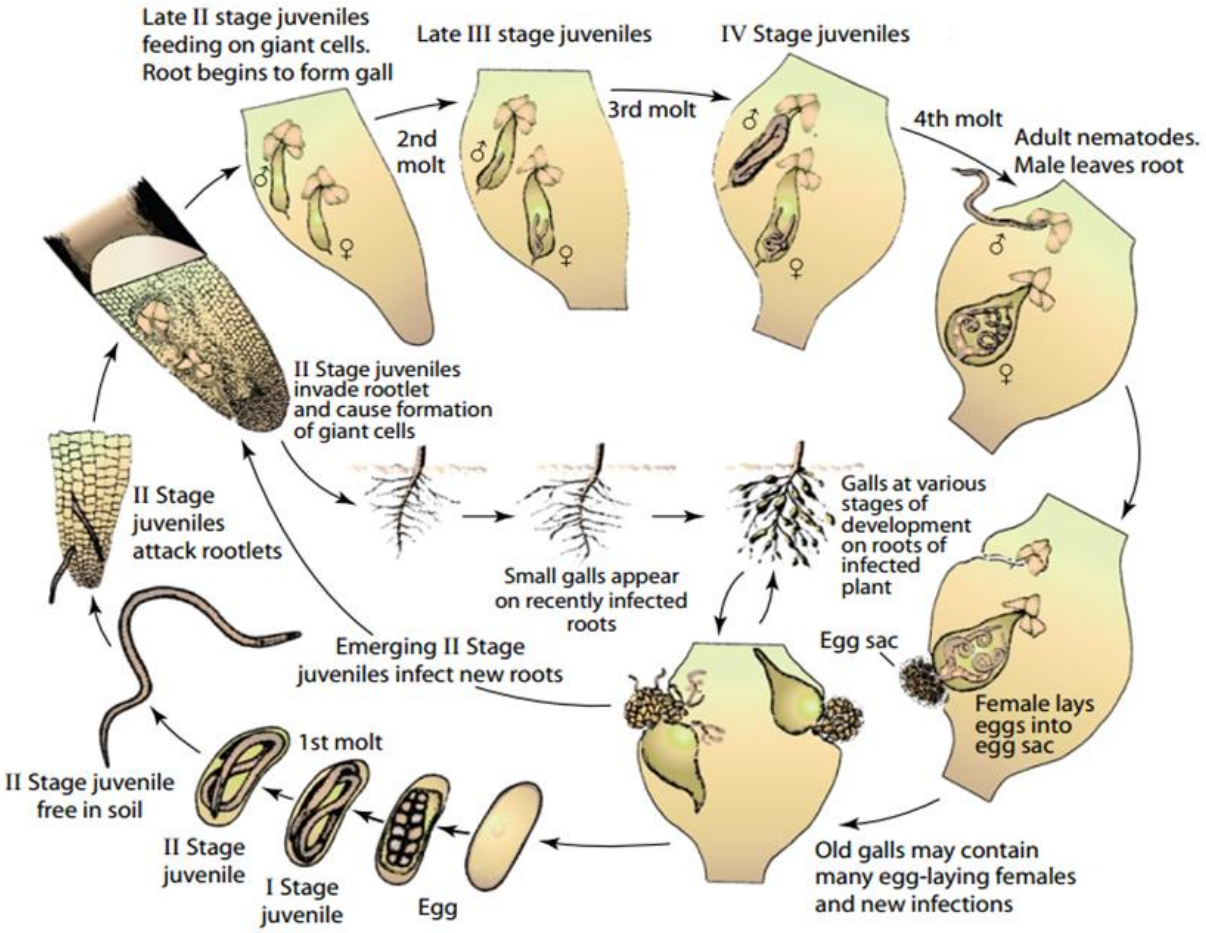

Figure 1. Root-knot nematode life cycle (Adapted from Agrios 2005)

Vegetable crops, like *C. melo*, often present high susceptibility to root-knot nematode (Huang 1992). Regarding melon and root-knot nematode, the pathosystem was first reported in Brazil by Mello (1958). In addition, its economic relevance in Assú, one of the main producers (RN state, Brazil) was reported in 1993 by Tihohod et al. (1993). Lordello (1976) summarized data about the susceptibility of several crops to the four major root-knot species (*M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria*). At his analyses, the author gave ratings for each crop accessed, based on the infestation and the presence of mature females and egg masses. When tested against the four species, melon presented #4 rating, which corresponded to "severed infestation, being abundant not only the mature females, but also egg masses".

In the same period, Ponte et al. (1977) presented a list with 39 plants that were cultivated in Rio Grande do Norte State and the species of root-knot associated with them, in field conditions. This work reported the prevalence of *M. incognita*, which was associated with 30 species of the 39 analyzed. Included on this, there were the *C. anguria*, *Cucumis africanus* Lindll and *C. melo*. Torres et al. (2007) reported prevalence of *Helicotylenchus* sp. and *R. reniformis* in the producing areas in 2006, with the root-knot present in 17% of the surveyed areas. Unfortunately, the *Meloidogyne* species were not identified.

Nowadays, there is no updated field survey about occurrence of plant parasitic nematodes, but root-knot species stands out as the most important one. Furthermore, there is no updated survey to verify which root-knot species is the most prevalent. However, it is known that *M. incognita* (Pontes et al. 1977; Moura et al. 2002), *M. javanica* (Pinheiro and Amaro 2010) and *M. enterolobii* (Torres and Medeiros 2007) were reported in melon fields.

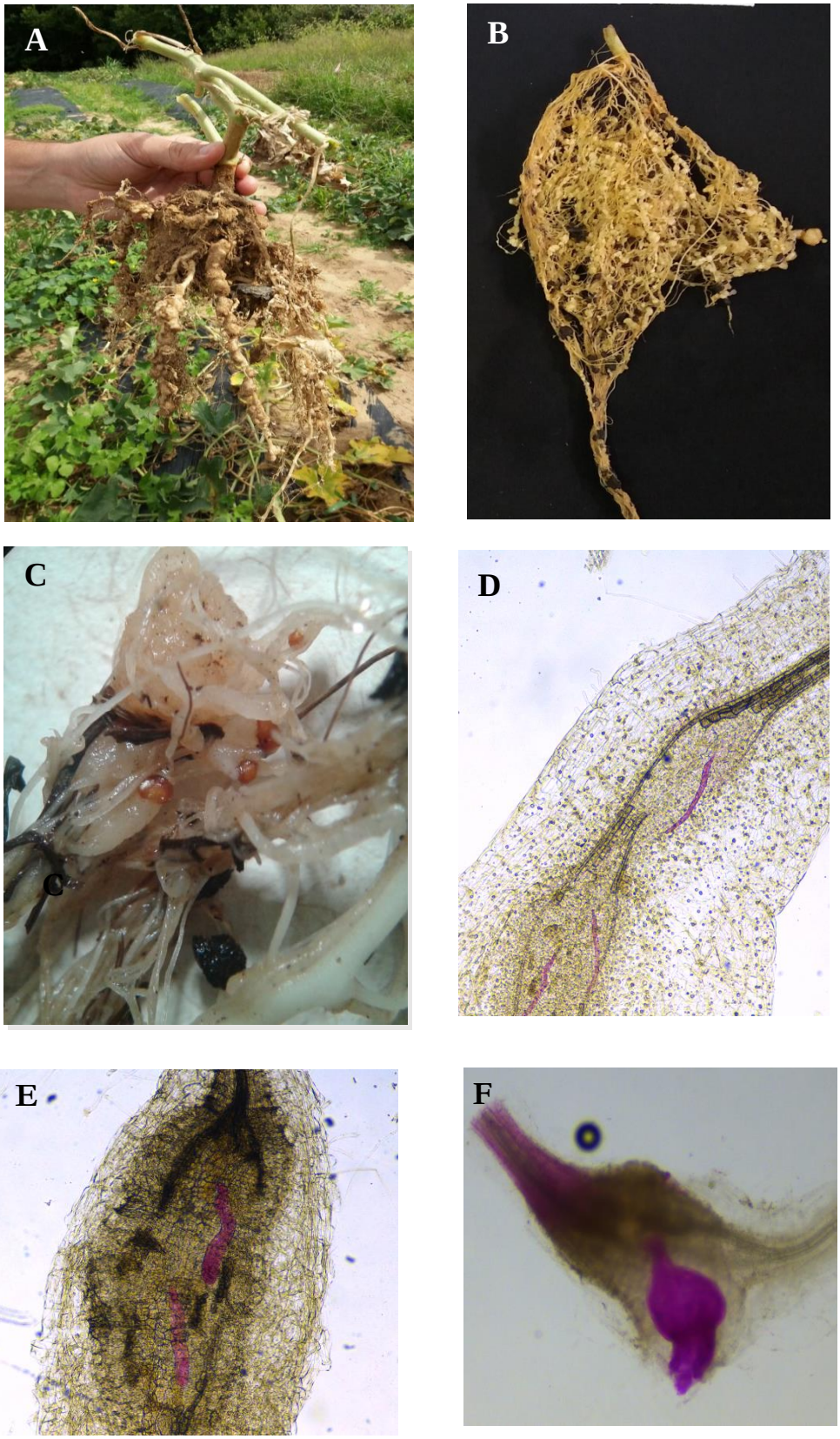

Figure 2. A-B) Symptoms caused by *Meloidogyne incognita* in melon roots in infested areas (Vincennes, IN, USA) (A) and under laboratory conditions (B); **C)** Detail of small size galls and the egg masses (brownish spots). **D-F)** Second stages juveniles (J2) (D) and swelled juveniles (E) at 7 and 10 days after inoculation. Mature female inside severed attacked melon roots (F). Staining: acid fuchsin.

2.3. The alternative control of plant parasitic nematodes

The management of plant parasitic nematodes has been a challenge to the agriculture worldwide. For decades, the main resources to control those pests have been crop rotation/succession and the use of resistant plants or cultivars. The first one is recommended to phytonematodes control at short cycle annual and perennial crops (Halbrendt and LaMondia 2004). If a susceptible genotype is planted over and over at the same area, nematodes' population will increase (Ferraz et al. 2012), leading to increasing losses. The rotation with a non-host/poor host plant limit the population increase, which declines naturally due to natural mortality. With careful planning, it is possible to keep nematodes' population below the damage threshold and avoid losses in the more profitable crop (Ferraz et al. 1999; Halbrendt and LaMondia 2004). In addition, the use of resistant plants consist in one of the most efficient and economic methods to avoid losses caused by plant parasitic nematodes (Roberts 2002). At this work, resistance is defined as the capacity of the plant on reducing or preventing nematode reproduction, through the resistance genes, present on its genome (Trudgill 1991). Also, according to the same author, the capacity of withstand the damage caused by the nematode is defined as tolerance. Additionally, the chemical control of plant parasitic nematode is not viable, because most nematicides are not effective and present a high cost. Also, most nematicides were removed from market due to toxic effects and environmental problems (Kerry 1992).

The problem around the plant parasitic nematode management is the use of both methods. In Spain, it is common to use vegetable crops in rotation/succession with solanaceous crops, like tomato – melon (Meneses and Castilla 2009). Those crops are widely known for their susceptibility to root-knot nematodes (Inoue et al. 2016, Pavan et al. 2016; Perry et al. 2009; Devran et al. 2011; Jones et al. 2013). Furthermore, several nematodes, like *Meloidogyne* spp., present a wide spectrum of hosts, which ranges from grasses to trees, limiting crop rotation options (Oka et al. 2000). Besides, sources of genetic resistance are scarce for melon. Conversely, there are some species that shows resistance to some root-knot species, notably *C. metuliferus*, which has been attracting attention of several researchers for its resistance potential. There are some efforts to understand the mechanisms involved in that resistance phenomena and to use it to breed resistant melon plants (Ling et al. 2017; Ito et al. 2014; Pinheiro and Amaro 2010).

On this scenario, alternative tools are needed. The biological control and induced resistance are already known in the scientific literature, with some promisors results on

reducing the root-knot nematode reproduction and its symptoms on several crops (Zinovieva et al. 2013; Owen et al. 2002; Chinnasri et al. 2003), including *C. melo*. Furthermore, both tools may be combined with other management techniques, such as crop rotation.

2.3.1. Biological control agents

Biological control consists in reducing plant parasitic nematodes population using a living organism that is not the host itself, but usually is an antagonist (Ferraz et al. 2012). In nature, those natural antagonists suppress the nematode development, keeping its population below than would happen with their absence (Stirling 1991). The microorganisms present in the soil in different agriculture zones are too abundant and in constant association, which results in a balance. The parasitism of nematodes are affected in different intensities by the microbiota present in soil and rhizosphere. The mechanisms of biological control agents can be parasitism, predation, competition and antibiosis (Ferraz et al. 2012).

Fungi, predators nematodes, tardigrades, turbellaria, collembolan, mites, protozoan and bacteria interacts with nematodes in soil and, in some cases, they drastically reduce the population of this plant pathogens (Jatala 1986; Stirling 1991; Oka et al. 2000). However, not all antagonist are suitable for commercial use, with fungi and bacteria being the most promisors. There are several commercial products available at Brazilian market, composed by *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Bacillus* spp. and so on (AGROFIT, 2018). Furthermore, it is necessary to research their effectiveness against *Meloidogyne* species on melon, mainly due some inconsistences of control (Medeiros et al. 2009; Abdeldaym et al. 2014); or lack of information.

2.3.1.1. *Bacillus* spp.

Several bacteria are utilized on biological control against plant pathogens. The main groups are *Bacillus*, *Pseudomonas*, *Rhizobium*, *Pasteuria* and *Enterobacter* (Medeiros et al. 2018). Furthermore, the bacteria found on the rhizoplane, the rhizobacteria (often referred as PGRP - plant growth-promoting rhizobacteria) are widely known for its antagonistic effect against plant parasitic nematodes (Kloepper et al. 1990; Siddiqui and Mahmood 1999). Also, as the name suggests, those PGRP may improve the plants' growth through the increasing of nutrients available for the root system and due to the synthesis of growth-promoting

substances (Ferraz et al. 2012). Additionally, those rhizobacteria may induce resistance to diseases and on different parts of the plant (Vieira Junior and Romeiro 2007).

Bacillus spp. are among the most studied and used species, presenting suppressive effects against post-harvest, foliar and soil pathogens, including phytonematodes (Medeiros et al. 2018). Its *in vitro* efficacy has been already reported for *M. incognita* and for other plant parasitic nematodes (Mendoza et al. 2008; De Souza et al. 2015). The addition of *Bacillus* sp. suppressed the population of some phytonematodes on cotton fields to the same level as nematicide treatment (Anter et al. 1995). Similarly, Araújo and Marchesi (2009) reported growth promotion on tomato plants and reduction of egg masses of *Meloidogyne* spp on plants treated with *B. subitilis*, which performed better than the nematicide carbofuran. Also, PGRP increased some parameters of tomato plants, such as dry shoot weight (*B. amyloliquefaciens*, *B. subtilis* and *B. cereus*), plant height (*B. amyloliquefaciens*) and fruit per plant (*B. amyloliquefaciens* and *B. cereus*). Furthermore, those bacteria have reduced the galls, number of egg masses per root system and number of eggs per egg mass of *M. incognita* on tomato plants (Almaghrabi et al. 2013).

Despite those results, inconsistency on *M. incognita* control on melon AF 682 was found by Medeiros et al. (2009). Among the 117 strains accessed, the *Bacillus* ENM7, ENM10 and ENM51 have significantly reduced egg mass and/or gall index. However, when tested again, isolate and mixed, they did not confirm their efficiency *in vivo*. More information about the efficiency of *Bacillus* spp. on root-knot nematode control on melon is needed.

2.3.1.2. *Pochonia chlamydosporia*

Among promising biological control agents, *P. chlamydosporia* stands out as one of the most researched (Manzanilla-López et al. 2013). This fungus act as a eggs and females parasite of root-knot and cyst nematodes. Additionally, it is able to parasite eggs within females, roots and cysts or laid on gelatinous masses, as the produced by *Meloidogyne* spp (Kerry 2001). *P. chlamydosporia* presents the advantage of being easily produced, being polyphagous and being used on propagative plant material (Kerry, 1992). Furthermore, this species can survive in soil due to its saprophytic ability, despite the absence of its host. Additionally, it presents the ability of colonizing the rhizosphere of some plants, allowing the

inoculum maintenance in soil, with it more abundant on infected roots than healthy roots (Kerry, 2001; Bourne et al. 1996).

As already stated, *P. chlamydosporia* is a parasite of eggs and females. Due to that fact, *P. chlamydosporia* produces a huge amount of extracellular enzymes, mainly chitinases and proteases, like collagenase. This characteristic plays an important role on eggs infection (Huang et al. 2004). Despite this, some enzymes are just produced when the fungus is in close contact with the egg shell, like the VCP1, a serine protease of the subtilisin-like enzymes family (Ward et al. 2012; Esteves et al. 2009).

Viggiano et al. (2014) reported the suppression of *M. javanica* on cucumber, due to treatment with *P. chlamydosporia* (Isolate Pc-10, 18g/L). It has resulted on a reduction of 46% and 49.4% on number of galls on the first and second assays, respectively. Furthermore, the use of this strain at same dose on substrate was sufficient to control *M. javanica*, without any additional application. The same strain of *P. chlamydosporia*, in field conditions, has reduced *M. incognita* population and improved carrot quality and yield (Bontempo et al., 2017). The concentration used was 3kg per ha. Additional results, with the Pc10 have already been reported on tomatoes and sugarbeet (Podestá et al., 2009; Dallemole Giaretta et al., 2010).

Besides its effects on root-knot species, it has also benefited plant growth promotion in some plants, such as tomato, lettuce sweet pepper, cucumber and melon (Dallemole-Giaretta et al. 2015; Ceiro 2015 *apud* Hidalgo-Díaz et al. 2017). Although, no root-knot control has been accessed for *C. melo* and there is no recommended dose for this crop. Further investigation is needed.

2.3.1.3. *Paecilomyces lilacinus*

Similarly to *P. chlamydosporia*, *P. lilacinus* is a parasite of eggs, females and cysts, being one of the most studied hyphomycetes to biological control use at plant parasitic nematodes, presenting low specificity to the host and some differences of effectiveness depending of the strain (Atkins et al. 2005; Medeiros et al. 2018). Despite its classification, it presents the ability of antagonizes all the nematodes stages, although it is especially effective against eggs (Tranier et al. 2014). *P. lilacinus* is primarily saprophyte, living in different substrates of the soil (Ferraz et al. 2012), and eventually colonizes the eggs, through direct penetration of the egg shell by an individual hyphae. In addition to that, several substances are

related to its action mode, as leukotoxins, chitinases, proteases and acid acetic (De Souza et al. 2015).

Lara et al. (1996) showed that *P. lilacinus* is able to reduce *M. incognita* population, on soil and roots, and increase tomato's yield. Kiewnick and Sikora (2006) reported that a single pre-planting application of *P. lilacinus* strain PL251 (concentration: 1 x 106 CFU/g of soil) is sufficient to control *M. incognita*. Perveen et al. (1998) showed that the use of *P. lilacinus has* reduced the gall index of several plants (*Curcubita pepo* L., *C. lanatus*, *Cyamopsis tetragonoloba* (L.) Taub and *Capsicum annuum* L.) infested with *M. javanica*. However, despite its efficacy, there are some concerns around healthy safety. Some strains have been reported causing ocular infection and facial lesions on humans. That is an important factor that heavily influences its registration as a biopesticide (Ferraz et al. 2012).

2.3.2. Resistance inducers: Acibenzolar-S-methyl, thaxtomin A and filtrates

The induced resistance as a management tool against plant parasitic nematodes is fairly rare, even rarer when we consider this phenomenon and root-knot x melon pathosystem. However, promising results have already been reported, with resistance inducers reducing not only the reproduction but also the symptoms caused by this pests in several crops (Owen et al. 2002; Chinnasri et al. 2003; De Souza et al. 2015). The induced resistance consist in the activation of latent mechanisms of resistance within the plant, through stimuli of an elicitor, which must be of different nature, such as biotic and abiotic ones (Pascholati and Dalio 2018). After elicitation, there is the activation of the systemic acquired resistance (SAR) or induced systemic resistance (ISR), which are mediated by the salicylic acid and jasmonate acid/ethylene, respectively (Pascholati and Dalio 2018). Plants are able to defend themselves against a widely broad of pathogens and this phenomenon has been known for more than 100 years (Hammerschmidt 2007). This resistance may involve structural and biochemical pre and post formed mechanisms (Pascholati and Dalio 2018). The induced resistance may be used at integrated pest management programs (Pascholati and Cia 2009).

Among the agents used to induce resistance, acibenzolar-S-methyl stands out as the first "plant activator" available at Brazilian market (Silva Junior and Behlau 2018). It has already reduced the number of galls caused by *M. javanica* in soybean (*Glycne max* (L.) Merr.) and has not interfered in vegetative parameters of the plants (Brito et al. 2016). Chinnasri et al. (2003) reported that one single foliar spray of this inducer was sufficient to

decrease the reproduction of *R. reniformis* and *M. javanica* on soybean and Cowpea (*Vigna unguiculata* (L.) Walp.). Other works presented similar results (Silva et al. 2002a; Silva et al. 2002b).

Additionally, filtrates and fermented obtained from biological control organisms in culture medium and their metabolites have been highlighted, because they show a complex interaction with plants, acting as plant growth promoters and resistance inducers (Vinale et al. 2008). Furthermore, this filtrates have nematotoxic proprieties against plant parasitic nematode, influencing their mobility and/or egg hatching (Dong et al. 2006; Cayrol et al. 1989). These culture filtrates presents a wide range toxicity. For example, filtrates obtained from *B. firmus* have showed deleterious effects against *M. incognita* and against two migratory endoparasites, the burrowing nematode *Radophollus similis* and the stem nematode *Ditylenchus dipsaci* (Mendoza et al. 2008).

The thaxtomin A is a toxin (or effector) produced by *Streptomyces scabies*. It acts as a cellulose biosynthetic inhibitor, which promotes plant cell hypertrophy and hypersensitive reaction (Tegg et al. 2016). Also, it was found that this effector shows the ability of inducing resistance in several plants and against several plant pathogens (Garcia et al. 2008a; Garcia et al. 2008b). Despite this, there is no evidence against plant parasitic nematodes, such as root-knot. Furthermore, the preparations with partially purified thaxtomin have showed that those may enhance some plants characteristics, such as more chlorophyll content (Garcia et al. 2008a; Garcia et al. 2008b).

There are other resistance inducers that have already showed similar effects against several phytonematodes in several crops, such as potassium phosphide (Oka et al. 2007; Dias-Arieira et al. 2012); the β-amynobutyric (Oka et al. 1999; Sahebani and Hadavi 2009; Chinnasri et al. 2006), several rhizobacteria (Siddiqui and Shaukat 2002a; Siddiqui and Shaukat 2002b); *Trichoderma* spp. (Tranier et al. 2014), and arbuscular mycorrhizic fungus (Vos et al. 2012; Daneshkah et al. 2013).

3. MATERIAL AND METHODS

3.1. Assessing the host status of melon hybrids

The objective of the present experiment was to access the host status of some melon hybrids.

3.1.1. Melon seed acquisition, plant material and experimental design

The seeds were kindly donated by Sakata Seed Sudamerica®. In total, were obtained ten hybrids to access their host status to *M. incognita* and they are specified on the table below (Table 1). Among them, there are commercial and experimental genotypes.

To obtain the melon plants, seeds of each hybrid were sown on plastic pots of 2l capacity, containing autoclaved soil. After seven days, the seeds germinated and the seedlings were thinned, leaving two per pot. In the present experiment, the ten melon hybrids were used, and for each genotype five repetitions were added. Also, cucumber (*C. sativus*) was used as a standard to assess the inoculum infectiveness. The experiment was completely randomized, with the experimental unit consisting of one pot containing two plants.

Table 1. Hybrids, melon types, status and production area

Hybrid	Melon type	Status*	Cropped area*
'AF 4945'	Canary melon	Commercial	RN/CE**
'AF 6768'	Canary melon	Experimental	RN/CE/France/ Spain
'AF 7607'	Smell melon	Commercial	South of Brazil
'AF 9136'	Canary melon	Experimental	RN/CE
'AF 9763'	Canary melon	Experimental	RN/CE
'AF 11682'	Canary melon	Experimental	RN/CE
'Asturia'	Piel de sapo melon	Commercial	RN/CE
'Grand Prix'	Piel de sapo melon	Commercial	RN/CE
'Iracema'	Canary melon	Commercial	RN/CE/France/ Spain
'Premier'	Canary melon	Commercial	RN/CE

*Information provided by Sakata Seed Sudamerica in 2015.** RN: Rio Grande do Norte State; CE: Ceará State.

3.1.2. Inoculum acquisition, inoculation and nematode extraction

In the present experiment, cotton and melon roots, previously infested, were used as inoculum source. The plants were removed from the pots, had the roots washed with tap water, cut in 1cm pieces and blended with commercial sodium hypochlorite 0.5%, used to dissolve the egg masses. After that, the resultant suspension was poured through a 60 and 500 mesh sieve (60 mesh – 0.260mm aperture – to retain the coarse root particles; and 500 mesh – 0.025mm aperture – to retain fine particles, like tiny roots pieces and nematodes), being collected on 250ml beaker. After that, the suspension was submitted to the centrifugal-flotation technique for the isolation and concentration of nematodes, with sucrose solution (Coolen and D'Herde 1972). After this process, the nematodes were estimated based upon two 0.5ml counts on Peters' counting slide, under light microscope.

Before perform the inoculation process, two holes (2cm and 4cm depth) were made close to the plant stem, and vermiculite was added inside the holes. After homogenization, inoculum droplets were poured on both holes, with the use of an automatic pipette, followed by covering with vermiculite. The initial population (Pi) consisted on 400 nematodes (eggs and second stage juveniles). After the inoculation procedures, the plants were transferred to a greenhouse, located on the Department of Plant Pathology and Nematology (ESALQ/USP), and maintained there until the analysis.

3.1.3. Assay evaluation and statistical analysis

The evaluation was performed 60 days after the inoculation (DAI). The plants had their aerial portion removed and the roots were washed and stored on plastic bags. After that, the material was transported to the Laboratory of Nematology, located at ESALQ/USP, where the analysis was carried out. The roots were quickly inspected, with a dissecting microscope, to count the number of galls on the root system and access the gall index (Gi). According to the obtained number, the plant received a score, which ranged from 0 to 5, as described by Taylor and Sasser (1978). After this process, the nematodes were extracted following the method of Coolen and D'Herde (1972), as described on item 3.1.2., being killed under low heat and stored on flaks for counting. To this step, two counts of 0.5mL were made, by using a Peters' slide.

At the end of the evaluation, the variables Final population (Pf), Reproduction Factor (R value), Nematodes per gram of root (Nem/g) and Gi was obtained. The R value

(Oostenbrink 1966) is calculated by Pf/Pi and it is widely accepted, on the nematology, that if this value is below 1, the plant is classified as resistant (R<1) and, otherwise, if the R value is equal or above 1 (R≥1), the plant is classified as susceptible. Furthermore, the obtained data were submitted to analysis of variance (Anova) by using the R statistical software (R Core Team 2013).

3.2. Effect of resistance inducers and biological control agents on root-knot nematode control and melon plants development

3.2.1. Trial #1

The objective of the trial #1 was to verify the influence of resistance inducers and biological control agents on root-knot nematode control and melon plants development. Thus, the used biological agents *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* and the resistance inducers PPT (partially purified thaxtomin A) and ASM were investigated.

3.2.1.1. Plant material acquisition

The present experiment was carried out in a greenhouse, located in the Department of Plant Pathology and Nematology at ESALQ/USP. Based on the previous experiment (item 3.1), the cultivar ‘Asturia’ was chosen to be used on further experiments, mainly for being susceptible to *M. incognita* and due to its good development on greenhouse , besides for being a commercial cultivar.

To obtain the melon plants, ‘Asturia’ seeds were sown on 2L capacity pots, containing autoclaved sifted soil. After that, the pots were kept on glasshouse, until seed germination had occurred. After this, plants were thinned to keep one plant per pot. The plants were treated and used on the present experiment approximately seven days after the germination process, when they were showing the first true leaf.

3.2.1.2. Biological control agents and the resistance inducers

For this experiment, three biological control agents were used *Paecilomyces lilacinus*, *Bacillus amyloliquefaciens* and *Pochonia chlamydosporia*. The first and second

ones were isolated, identified and characterized by Brandão (2018), being grown on Potato-Dextrose-Agar medium (PDA) and stored inside a growth chamber (BOD), on dark (25°C). Regarding the identification of this bacteria, Brandão (2018) explained that due to similarity in the gDNA, the bacteria specie is either *B. amyloliquefaciens* or *B. methylotrophicus*. In our work, the first specie will be adopted. For plant treatment, *P. lilacinus* mycelium was used. For this purpose, five discs containing mycelium were transferred for Erlenmeyer flasks (250ml volume capacity), containing 100ml of Potato-dextrose broth medium (PD). Then, the flaks were sealed and kept in a shaker, under constant shaking at 225 rpm (in dark, 25°C) for 10 days. When it was needed, the mycelium was removed from the liquid media and weighted. After that, the total weight was divided by the number of plants

For *B. amyloliquefaciens*, two Petri dishes containing the bacteria on PDA medium were washed with deionized water. To help this process, the colonies were gently rubbed with a Drigalski loop. The final suspension was poured on a Beaker and stored until plant treatment. Also, 1ml was used in a spectrophotometer at 600nm to access the bacterial optical density of the suspension.

For *P. chlamydosporia*, was used the Rizotec®, which is a commercial biological nematicide that consists on the fungus chlamydospores (Strain P11). This product was kindly donated by Stoller® and the strain P11 was previously characterized by Dallemole-Giaretta (2008).

Also, on this experiment, two resistance inducers were utilized: the acibenzolar-S-methyl (the ASM, also referred as Bth) and partially purified thaxtomin (PPT). For the first resistance inducer, 0.05g of ASM was added to 1L of deionized water and kept on stirring, until it complete dissolves. The ASM was used for being a salicylic acid analogue, widely adopted as a standard for resistance inducers experiments.

Regarding the PPT, it was used partially purified thaxtomin obtained from preparations of *Streptomyces scabiens*, grown on oatmeal broth medium, maintained on shaker for five days (Dark, 28°C and at 150rpm). After cultivation, the suspension was filtered using a sieve to remove the mycelium and transferred to a separating flask, being added ethyl acetate at 3:5 proportion (v/v). The final suspension was manually shaken until phase separation occur. After this process, the bottom phase was discarded and the suspension was filtered by using Whatman filter paper (n°40) under vacuum. At the end of the process, sodium sulfate was added (0.2g per 100ml) and the solution was shaken and kept overnight until the ethyl acetate completely volatilized. After this, methanol was added to resuspend the thaxtomine A and centrifuged at 6000 rpm for 5 minutes. The supernatant was collected and

the thaxtomine A preparation was maintained on methanol and stored on freezer (at -20°C) until usage.

Before the plant treatment, the partially purified solution (PPT) was diluted on deionized water (1:9) (v/v) to create a spray volume. The concentration of thaxtomin A after this dilution was ~81µg equivalent of thaxtomin per ml and was measured in a spectrophotometer at 400nm. The used concentration was based on Garcia et al. (2008a), which used similar concentration to study the control of anthracnose on *C. sativus*.

3.2.1.3. Trial design, plant treatment, inoculum acquisition and inoculation procedures

The present experiment was had seven treatments: Water (control), Water +N (control) (+N: inoculated with nematodes), *P. chlamydosporia* +N, *P. lilacinus* +N, *Bacillus* sp. +N, TPP A+N and ASM+N. Each one had five repetitions and the experimental unit consisted of one plant per pot. Also, a non-inoculated treatment was included for each of them, for comparison purposes and aiming to access the treatment effects on the development of the melon plants.

Regarding plant treatment, the biological control agents and the resistance inducers were applied 24 hours before the inoculation process. On the moment of the plant treatment, five holes (2cm depth) were performed around the plants and all microorganisms were applied as soil drench. *P. lilacinus* mycelium (~4g for each plant) and *Bacillus* sp. suspension (5 ml of a 500ml suspension, with absorbance of 0.053), were used, whereas *P.* chlamydosporia was incorporated following the manufacturer's instructions (0.3g per pot). After this process, the holes were covered with soil.

For the ASM and PPT, both resistance inducers were sprayed on the aerial portion to the point of run-off. Three applications were performed, with seven days of interval, following the ASM spray schedule registered for *C. melo*. Also, water treated plants (inoculated and non-inoculated) were included as negative control.

The inoculum was obtained from cotton roots, previously infected with *M. incognita*. The nematodes were extracted following the same procedures described on item 3.1.2. and counted on Peters' slide. For the present experiment, 1000 nematodes (eggs and juveniles J2) were used as an initial population (Pi) and the inoculation process was carry out following

similar procedures, as described on item 3.1.2. After the inoculation, plants were completely randomized and maintained in a glasshouse until the moment of analysis.

3.2.1.4. Trial evaluation and statistical analysis

The evaluation was performed at 40 DAI due to a whitefly outbreak (*Bemisia tabaci*). For this purpose, plants were transferred to the Laboratory of Post-Harvest, located in the Department of Plant Pathology and Nematology, ESALQ/USP. For the analyses, plants were removed from the pots, followed by section of the aerial portion and removal of the soil attached to the roots. Then, it was assessed the fresh and dry weight of the aerial portion (g), root weight (g), fruit weight (g), diameter of three portions of the plant stem (base, middle and apex)(mm) and plant height (cm). Also, chlorophyll content of the leaves was measured by using a SPAD equipament (SPAD-502 model, Konica Minolta®). For this purpose, two leaves were picked and five reads were performed for each of them. It was standardized to select the 5th leaf, from the base to apex (young leaf), and 5th leaf, from the apex to base (old leaf). After being weighed, the roots of the inoculated plants were transported on plastic bags to the laboratory of nematology, where they were stored on fridge, until the nematode extraction.

The nematode extraction was conducted according to the procedures described at the item 2.1.2. (Coolen and D' Herde 1972). After this, the obtained suspension was poured on two sieves (60 and 500 mesh

The same variables regarding the nematode reproduction were obtained: Final population (Pf), Reproduction Factor (R value) and Nematodes per gram of root (Nem/g). The R value (Oostenbrink 1966) was calculated as already described. Thus, it was adopted that the plant was resistant if $R<1$ and susceptible if $R\geq1$. As the previous experiment, the obtained data was submitted to analysis of variance (Anova) by using the R statistical software (R Core Team 2013).

3.2.2. Trial #2

On trial #2, none of the resistance inducers was included and just the biological control organisms were tested. We decided to carry out additional assays to test them

separately, to facilitate the execution of the experiments. Thus, the trial #2 followed similar procedures as the described on trial #1, with few modifications, which are described below.

3.2.2.1. Plant material acquisition

To obtain the melon plants for experiment 2, 'Asturia' melon seeds were sown on Petri dishes containing sterilized filter paper (Whatman® Filter, n°40), previously wet in sterilized deionized water. After this, seeds were stored on growth chamber (dark at 28°C). After the germination, the pre-germinated seeds were transferred to the 2l pots, containing autoclaved sifted soil. The plants were maintained on greenhouse and, like the experiment 1, were treated and inoculated after the growth of the first true leaf.

3.2.2.2. Biological control agents

For the trial #2, were adopted the same procedures as already described on item 3.2.1.2. Similarly, three agents were used: *B. amyloliquefaciens*., *P. lilacinus* and *P. chlamydosporia* and they were used on the same way, as a bacterial suspension, mycelium and Rizotec® formulation, respectively. For *B. amyloliquefaciens*, two Petri dishes containing 24h colonies (PDA medium) were washed with deionized water with the help of a Drigalski loop. The obtained suspension was poured on a Beaker and a 1ml aliquot was taken to a spectrophotometer for reading at 600nm. For *P. lilacinus*, five mycelial discs were transferred to an Erlenmeyer flask (250ml), containing PD broth medium. After ten days, the mycelium was retrieved from the media, being weighted, and distributed on equal portions to the plants. For *P. chlamydosporia*, a stock solution was made by adding 6g of Rizotec® on 120ml of deionized water, followed by homogenization in a magnetic stirrer. All these materials were stored on refrigerator until the moment of usage.

3.2.2.3. Trial #3 design, plant treatment, inoculum acquisition and inoculation procedures

On this experiment, the plant treatments were: Water (Standard), Water +N (Standard), *B. amyloliquefaciens* +N, *P. lilacinus* +N, *P. chlamydosporia* +N. For each treatment, it was adopted five repetitions and the experimental unit was one plant per pot and

an additional non inoculated treatment was included for each of them, to check the treatments' effects on the melon and for comparison purpose.

Following the procedures adopted on item 3.2.1.3., regarding plant treatment, the biological control agents were used 24 hours before the inoculation of the nematodes. For this purpose, five holes (2cm depth) were made around the plant basis and the microorganisms were applied as soil drench. Moreover, *P. lilacinus* mycelium (~.0.8 g) and the *B. amyloliquefaciens* suspension (10 ml of a 500ml suspension, with absorbance of 0.337) were used. For *P. chlamydosporia*, the stock solution was manually homogenized and a 10ml aliquot (containing ~0.5g of Rizotec® per pot) was poured equally inside the holes. After the plant treatment, the holes were covered with the pot soil and the plants were distributed randomized.

The *M. incognita* inoculum was obtained from cotton roots, previously infected and kept inside a greenhouse until nematode population increased. The nematodes were extracted following the Coolen and D' Herde technique (1972), as already described on item 3.1.2. Also, two 0.5ml aliquots were counted on Peters' slide to estimate the nematode population. As in the trial #1, the Pi was 1000 nematodes (eggs and second stage juveniles) and the inoculation procedures adopted were similar to the described on item 3.1.2, being made two holes (2cm and 4cm depth) close to the plant stem. After that, the suspension was poured on the two holes and covered with vermiculite. After this process, plants were maintained on greenhouse, located on the experimental field of the Department of Plant Pathology and Nematology (ESALQ/USP), until the time of analysis.

3.2.2.4. Trial evaluation and statistical analysis

The trial #2 was evaluated 60 DAI and followed similar procedures as described on item 3.2.1.4. The plants were transferred to the Laboratory of Post-Harvest and were removed from their pots and the aerial portion removed. Similar to the trial #1, the fresh and dry weight of the aerial portion (g), root weight (g), fruit weight (g), diameter of three portions of the plant stem (base, middle and apex)(mm) and plant height (cm) were evaluated. Moreover, it was carried out the SPAD analyses to measure the chlorophyll content of the leaves. The roots were transferred to the Laboratory of Nematology and stored on refrigerator until nematode extraction was performed.

For this step, similar procedures as described at the item 3.1.2 were used. (Coolen and D' Herde 1972). At the end of the process, nematodes were killed on low heat and stored inside flaks until further analyses. Nematodes were counted on Peters' slide by two counts of 0.5ml, taken from the homogenized suspension. After this, Pf, R value and Nem/g were estimated and all data were submitted to Anova on R statistical software (R Core Team 2013).

3.2.3. Trial #3

On the Trial #3, only the resistance inducers were tested, although it was carried out along with the experiment 3.3.4. The present experiment followed similar procedures as already described for experiment 1 and 2.

3.2.3.1. Plant material and resistance inducers acquisition

Similarly, to obtain the melon plants for experiment 3, the 'Asturia' melon seeds were sowed on Petri dishes containing sterilized filter paper (Whatman® Filter, n°40), previously wet in sterilized deionized water. After this, seeds were stored on growth chamber for three days (dark at 28°C). After the germination, the pre-germinated seeds were transferred to the 2l pots, containing autoclaved sifted soil. The plants were kept on greenhouse and, like the experiment 1 and 2, were treated and inoculated after the growth of the first true leaf. As the melon plants grew and start to reach certain height, their stems were carefully tied on screens to conduct the plants. Also, when fruits appeared, they were tied in to make sure that the weight of them did not break the plants.

Regarding the resistance inducers, similar procedures were adopted for both ASM and PPT. To obtain the first resistance inducer, 0.05g of ASM was added on one liter of deionized water and dissolved using a magnetic stirrer. After this, the solution was stored on refrigerator until use. For TPP, similar procedures were adopted, but no dilution was made, since this was store using deionized water. The concentration equivalent of thaxtomin A was ~100µg/ml, measured by using a spectrophotometer with absorbance at 400nm.

3.2.3.2. Experimental design, plant treatment, inoculum acquisition and inoculation procedures

For the present experiment, the treatments were: Water +N (control) (+N: plants inoculated with nematodes), ASM +N, TPP +N. For each treatment, five repetitions were used and the experimental unit was one plant per pot randomly placed in the greenhouse. Additionally, a non-inoculated treatment was included for each of them, to verify the treatments effects on the melon development and for comparison purpose.

Following the procedures adopted on item 3.2.1.3., the resistance inducers were applied 24 hours before the inoculation process. As adopted on trial #1, both inducers were sprayed onto the aerial portion of the plants until the runoff point. Three application were performed with seven days of interval and inoculation was carried out after the first application. To perform this process, plants were transferred to the Laboratory of Post-Harvest, located in the Department of Plant Pathology and Nematology (ESALQ/USP), where the inoculation took place.

The *M. incognita* inoculum was acquired from cotton roots, previously infected and kept on greenhouse until nematode population increased. This *M. incognita* population was donated by Sakata Seed and was characterized by the own company. As already mentioned, the nematodes were extracted following the Coolen and D' Herde technique (1972), which was already described on item 3.1.2. Furthermore, the nematode density was estimated by two counts of 0.5ml, using a Peters' slide under light microscope. Similarly to the other experiments, the Pi consisted of 1000 nematodes (eggs and second stage juveniles) and the inoculation procedures adopted were similar to the described on item 3.1.2, being made two holes (2cm and 4cm depth) close to the plant stem. After that, a 0.15ml suspension was poured on each hole and covered with vermiculite. Then, plants were maintained on a greenhouse located in the experimental field of the Department of Plant Pathology and Nematology (ESALQ/USP) for approximately 60 days until the time of analysis.

3.2.3.3. Experiment evaluation and statistical analysis

The evaluation took place on the Laboratory of Post-Harvest and followed similar procedures as the described on item 3.2.1.4. Plants were collected and were removed from their pots and the aerial portion sectioned. Similarly to the previous experiments, the obtained measures were: the fresh and dry weight of the aerial portion (g), root weight (g), fruit weight

(g), diameter of three portions of the plant stem (base, middle and apex)(mm) and plant height (cm). Additionally, the SPAD analyses was performed. The roots were transferred to the Laboratory of Nematology and stored inside a refrigerator until nematode extraction.

For this step, similar procedures were adopted, as already described at the item 3.1.2. (Coolen and D' Herde 1972). At the end of the process, nematodes were killed on low heat and stored on flaks until further analyses. Nematodes were counted on Peters' slide by two counts of 0.5ml, taken from the homogenized suspension. After this, the Pf, R value and Nem/g were estimated and all data was submitted to Anova by using the R statistical software (R Core Team 2013).

3.2.4. Trial #4

Due to the differences in the obtained data for *P. chlamydosporia* on trials #1 and trial #2, an additional assay was carried out to verify the effectiveness of crescent doses of Rizotec® on the control of *M. incognita* on melon.

3.2.4.1. Plant material and nematode acquisition

Similarly to previous experiments, 'Asturia' seeds were previously germinated by using Whatman filters (n°40) within Petri dishes, which were stored on growth chamber at dark (28°C). After three days, these pre-germinated seeds were planted in pots of 2l capacity and maintained in greenhouse. After the growth of the true leaf, plants were submitted to the treatments and inoculated with the nematodes.

Regarding *M. incognita*, the population used was the same described in the item 3.2.3.2. The inoculum was obtained from cotton roots, previously infested and maintained in greenhouse to allow nematode increased. Nematodes were extracted following the Coolen and D'Herde method (1972) and the nematodes were counted by using Peters' slide by two counts of 0.5ml. After calibration, the inoculum was used in the inoculation process.

3.2.4.2. Plant treatment, inoculation and experimental design

To treat the plants, stock solutions (60ml volume) were prepared for each dose and stored in separate labelled flasks. The used doses (treatments) were dose 0 (control treatment),

dose 1 (0.1 g of Rizotec® per plant), dose 2 (0.25 of Rizotec® per plant), dose 3 (0.5 of Rizotec® per plant), dose 4 (1g of Rizotec® per plant) and dose 5 (2g of Rizotec® per plant). After manual shaking, a 5 ml aliquot was equally poured in four holes (2cm depth), previously made around the plant stem. After this, the holes were carefully covered with soil of the own pot and the plants were randomly placed in the greenhouse, until inoculation.

Regarding this process, two holes (2cm and 4cm depth) were made close to the plant stem. After this, an aliquot of 0.42 ml was poured on each hole, followed by the addition of vermiculite to close them.

Plants were maintained on greenhouse for approximately 60 days until the moment of analysis. Similarly to the previous experiments, each treatment had five repetitions and the experimental unit was one plant per 2l pot. Additionally, non-inoculated plants were included for each treatment.

3.2.4.3. Trial evaluation and statistical analyses

The evaluation occurred once again on the Laboratory of Post-Harvest and followed similar procedures, as the described on item 3.2.1.4. Plants were collected and were removed from their pots and the aerial portion sectioned. The same variables were measured: the fresh and dry weight of the aerial portion (g), root weight (g), fruit weight (g), diameter of three portions of the plant stem (base, middle and apex)(mm), plant height (cm) and chlorophyll content (SPAD units). The roots were transferred to the Laboratory of Nematology and stored inside a refrigeratoruntil nematode extraction.

The extraction was performed similarly as already described in the item 3.1.2. (Coolen and D' Herde 1972). On the end of the process, nematodes were killed on low heat and stored on flaks until further analyses. Nematodes were counted using a Peters' slide by performing two counts of 0.5ml, taken from the homogenized suspension. After this the Pf, R value and Nem/g were estimated and all data were submitted to Anova on R statistical software (R Core Team 2013).

3.3. Effect of culture filtrates obtained from biological control agents

The experiments detailed below aimed to verify the effect of culture filtrates, obtained from the biological control agents' growth in media, on the root-knot nematode's

egg hatching, on melon seeds, on melon development and on the control of *M. incognita* in melon plants.

3.3.1. Effect of culture filtrates and resistance inducers on egg hatching

To verify the effect of culture filtrates on the egg hatching, three experiments were performed, and, on two of them, partially purified PPT was also included. The methodology adopted is described below.

3.3.1.1. Acquisition of culture filtrates

For culture filtrate preparations, five media discs having the microorganisms, collected from the colony edges, were transferred to Erlenmeyer flaks (250mL capacity), containing 100ml of PD broth media, previously autoclaved (121°C at 1ATM, for 20 minutes) for sterilization. After transferring the microorganisms, the flasks were stored on shaker for ten days, at 25°C, 225 rpm and in the dark. Only on experiment 2, for *P. chlamydosporia* cultivation, instead of discs, 0.8g of Rizotec® was collected in an aseptic flow chamber and added on the PD broth media and stored on shaker, at the same conditions described.

After ten days, the filtration process was carried out. For experiment 1 and 2, *P. lilacinus* and *P. chlamydosporia* had the mycelium removed by filtration on Kitasato flask with Whatman® filter (n°40), by using a vacuum pump. After this process, all filtrates were centrifuged at 12,352 *g* and the supernatants were transported to a flow chamber, being filtered through Millipore filter 0, 22µm (Merck®) to remove the reminiscent cells. For experiment 3, *P. chlamydosporia* and *P. lilacinus* were transferred for aseptic flow cabinet and were filtered on sterilized kitasato flask/vacuum bomb with filter paper n°40. After this, the filtrates were immediately transferred for sterilized Falcon tubes (50ml). The *B. amyloliquefaciens* filtrate was obtained after centrifugation at 12,352 *g* and filtration of the supernatant was performed through Millipore filter (pore diameter: 0. 22µm).

After this step, all filtrates were stored inside sterilized Falcon tubes (50ml capacity) on fridge at -10°C until usage.

3.3.1.2. Inoculum acquisition and trial design

For the experiments, the inoculum was obtained by using the Coolen and D' Herde method (1972) from cotton roots previously infested with *M. incognita* and the number of eggs was estimated by performing two counts of 0.5ml on Peters' slide. After this, on a flow chamber, 500 µl of inoculum was poured inside sterilized Eppendorf microtubes, with 2ml capacity on experiment 1 and 1.5ml capacity on experiment 2 and 3. After adding the inoculum, 500 µl of the treatment was added. Then, the microtubes were sealed, shaken and stored on a growth chamber, in the dark and at 28°C. Moreover, the experiment 3 was the only one kept on a shaker, to aerate the nematode suspension, on dark and maintained at 25°C. Despite that, in all three experiments, the number of eggs and J2 were counted after seven days and the hatching percentage and inhibition percentage were estimated. Also, on experiment 2 and 3, after nematode counting, the eggs were washed with tap water, by using a 500mesh sieve, and submitted to a hatching chamber to verify the egg viability. For this purpose, a nylon screen covered with a wet napkin was placed on top of a Petri dish, filled with deionized water. The nematodes, after being washed, were gently poured on top of the paper and the Petri dishes were placed randomized on growth chamber, in the dark and at 28°C for additional seven days and the J2 were counted.

All experiments followed the same procedures, but some differences are noteworthy. On experiment 1, the filtrates of *B. amyloliquefaciens* and *P. lilacinus* and water (Standard) were tested; on experiment 2 the filtrates of *B. amyloliquefaciens*, *P. chlamydosporia*, *P. lilacinus* and water (Standard) were tested. Also, PPT (methanol) and methanol (Standard for PPT) were included; on experiment 3, the filtrates of *B. amyloliquefaciens*, *P. chlamydosporia* and *P. lilacinus* and water (standard) were tested. Also, PPT (on water) was included. Regarding the water treatment, it is noteworthy that this control treatment was adopted as 100% hatching.

At the end of the experiments, the egg hatching percentage and inhibition percentage were obtained. For experiments 2 and 3, the number of J2, after washing, was also obtained. All data were submitted to Anova on the R statistical software (R Core Team, 2013).

3.3.2. Effect of culture filtrates on melon seeds

The present assay aimed to verify the effect of culture filtrates obtained from the biological control agents on melon seeds.

3.3.2.1. Performing experiments and acquisition of filtrates and seeds

The present experiment was carried out at the Biochemical and Physiological Plant Pathology Laboratory, located on Department of Plant Pathology and Nematology, ESALQ/USP. For the present experiment, 'Asturia' melon hybrid was used. The seeds were donated by Sakata Seed Sudamerica. Regarding the filtrates, they were obtained from cultivation on PD broth media and followed the same steps as described on item 3.3.1.1., by transferring colony discs collected from the edge to Erlenmeyer flasks, containing 100ml of this medium. After that, the same procedures adopted for experiment 3 (item 3.3.1.1) were carried out and the filtrates were obtained and stored on freezer until use.

3.3.2.2. Trial procedures

Before the treatment, seeds were transported to an aseptic flow chamber and washed for 1 minute in commercial sodium hypochlorite (diluted to 1%) and rinse three times on deionized water. After that, the filtrates were poured inside a sterilized Beakers and the seeds were treated by immersing them in the filtrates, and maintained for one minute under constant shaking, being transferred to Petri dishes, containing filter paper previously wet on deionized sterilized water. With that, the experiments were carried out with water (standard), PD broth media (standard), *B. amyloliquefaciens*, *P. chlamydosporia* and *P. lilacinus*. For each treatment, five Petri dishes were used and each one contained ten seeds that were carefully placed on top of the paper, at 2 cm apart from each other. The seeds were evaluated 2, 3 and 4 days after the treatment (DAT). The obtained results were submitted to Anova by using the R statistical software (R Core Team, 2013).

3.3.3. Effect of culture filtrates on the control of root-knot nematode on melon plants

The experiments aimed to verify the effect of culture filtrates obtained from *Bacillus amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* on the control of root-knot disease on melon. Additionally, verify the effect of this culture filtrates on melon growth and development. For these purpose, two experiments were carried out and the first and second one were carried out in parallel with the experiment 1 (item 3.2.1) and experiment 3 (item 3.2.3), respectively. Both assays followed the same procedures of plant material acquisition

(item 3.2.1.1 and item 3.2.3.1, respectively), experimental design, inoculum acquisition and inoculation procedures (item 3.2.1.3 and item 3.2.3.2, respectively), experimental evaluation and statistical analysis (item 3.2.1.4 and item 3.2.3.3). A brief summary is presented below, highlighting the major differences and particularities of both experiments.

3.3.3.1. Filtrate acquisition, trial procedures and statistical analysis

For the first experiment, the treatments consisted of Water (control) +N, *B. amyloliquefaciens* filtrate +N and *P. lilacinus* filtrate +N (+N: plants inoculated with nematodes). For the second experiment, the treatments were Water (control) +N, PD media +N, *B. amyloliquefaciens* +N, *P. lilacinus* +N and *P. chlamydosporia* +N. An additional non-inoculated treatment was also included for each of them.

For both experiments, the same method was used, in order to obtain the filtrates, which was described in item 3.3.1.1. Briefly, five mycelium/colony discs were collected from the colony edges and transferred into Erlenmeyer flasks, containing 100 ml of PD broth media. After ten days, the cells were removed from the media. For *P. lilacinus* and *P. chlamydosporia*, the mycelium was removed by centrifugation (20,000 *g* for 15 minutes) and the supernatants were collected and filtrated by using filter n° 40. For *B. amyloliquefaciens*, the medium was centrifuged (20,000 *g* for 15 minutes) and the supernatant was collected. Afterwards, an additional filtration was performed for each of them, using Millipore filters (0.22 µm). The obtained filtrates were stored inside Falcon tubes in the freezer.

For the first experiment, before the use, the filtrates were diluted in water, being added 50 ml of bacterial filtrate in 150ml of water and 45 ml of the fungal filtrate in 180 ml of water. After this, a 10 ml aliquot was used to treat the plants. Similarly, on the second experiment, all filtrates were diluted in water (1:3) (v/v) before the use and a 40ml aliquot was used to treat the plants. These dilutions were performed to lower their viscosity and to achieve better distribution in the soil.

At the time of treatment, 24 hours before the inoculation, four holes (2 cm depth) were made close to the plant stem. The diluted filtrates were poured in each hole, being covered with soil afterwards.

On both experiments, the melon plants were inoculated with 1000 nematodes (Eggs and J2) and were maintained in a greenhouse for 40 DAI and 60 DAI, respectively. After this period, plants were analyzed and nematodes were extracted. The same reproductive variables

were obtained (Pf, R value and Nem/g) and the growth/development parameters were accessed (the fresh and dry weight of the aerial parts, root weight, fruit weight, diameter of the plant stem (base, middle and apex), plant height and chlorophyll amount (SPAD)). All data were submitted to Anova analysis by using the R statistical software (R Core Team 2013).

3.4. Effect of resistance inducers on root-knot nematode penetration and post-penetration on melon seedlings

The present experiments aim to verify the effects of resistance inducers on penetration and post-penetration of *M. incognita* on melon seedlings. For this purpose, three experiments, two for benzothiadiazole (Bth) and one for TPP, were performed and are further detailed bellow. Additionally, the Bth (CAS 273-13-2, Synonymous: ASM) is an analogue of salicylic acid, referred in Brazil as the ASM. With this, in the present work, Bth will be referred as ASM.

3.4.1. ASM experiments

3.4.1.1. Assay execution, plant material attainment and the resistance inducer

The experiments were carried out in association with the laboratory of Dr. Tesfaye Mengiste and the Laboratory of Nematology, headed by Dr. Jamal Faghihi. The experiments were carried out in a greenhouse, located on Botany and Plant Pathology Department at Purdue University, in West Lafayette (IN, USA).

To verify the effects of ASM on penetration and post-penetration of *M. incognita* on melon seedlings, the melon ‘Aphrodite’ F1 was acquired commercially and used on two experiments. The seeds were sowed and pre-germinated on Petri dishes, containing Whatman Filter paper (n°40), previously immerged in distilled water. After this, the seeds were maintained for two days inside an incubator, in the dark, at 28°C. With the germination, the pre-germinated seeds were planted on substrate (Sand, SunGro® propagation mix and Turface® clay, at 5 – 5 – 2 proportion (v/v/v)), which was autoclaved for 40 min at 121°C and 1 ATM. On both ASM experiments, the experimental unit consisted of one plant per pot (250cm³). Approximately ten days after the sown, after the presence of the first true leaf, plants were used on the experiment.

To prepare the ASM solution, .0.05g of the ASM was added in 1l of distilled autoclaved water. The solution was kept on a magnetic stirrer until it complete dissolution, being stored on a plastic bottle with a trigger sprayer for plant treatment.

3.4.1.2. Plant treatment, inoculum acquisition and inoculation process

For the treatment, plants were treated 24 hours before the inoculation process, being sprayed with ASM until the runoff point. Also, a control treatment was included, with plants sprayed with distilled water.

For these experiments, second stages juveniles were used as inoculum, mainly, to uniform the penetration rate. The inoculum was obtained from eggplant (*Solanum melongena* L.) and tomato (*Solanum lycopersicum* L.) roots, previously infested with *M. incognita*, which was isolated originally from infested melon roots, collected in Vincennes (IN, USA). The severed galled roots were blended with commercial sodium hypochlorite (0.5%) and the resultant suspension was submitted to a modified Baermann method (Hooper 1986). The second stage juveniles were recovered three days after this, being estimated by counting 0.5ml twice on a nematode counting dish. After this process, nematodes were stored in refrigeratoruntil the moment of use.

On the moment of inoculation, two holes (2cm and 4cm depth) were made close to the plant basis. After this step, an aliquot of 0.77µl of inoculum was poured on each hole, being covered with soil, after this process. During this process, the inoculum was kept on a magnetic stirrer for homogenization. The experimental units were inoculated with 150 J2 and ordered completely in a randomized way. Plants were maintained in a greenhouse until the sampling moment.

3.4.1.3. Sample collection, root staining and data analysis

On both ASM experiments, four plants of each treatment were collected at 3, 5 and 7 DAI and submitted to fuchsin acid staining (Byrd et al. 1983; Daykin and Hussey 1985). For this purpose, plants were transported to the Nematology Laboratory at Purdue University and carefully removed from their pots, followed by soil removal in tap water and section of the aerial portion. After this, plants were immersed in sodium hypochlorite solution (80ml of commercial NaOCl and 200ml of water) for 30 minutes until the root completely destained.

Then, roots were rinsed and soaked in tap water for 15 minutes to remove residual NaOCl. Afterwards, roots were transferred to another Beaker containing 50ml of tap water and 1ml of fuchsin acid stain solution (3.5g of fuchsin acid in 250ml of acetic acid and 750ml of distilled water) being boiled on microwave oven for approximately one minute and then cooled off under room temperature, followed by drain and rinse in running tap water. Finally, roots were placed inside a Beaker containing acidified glycerin (1ml of HCl for 1l of glycerin) and boiled over again to destaining.

Roots were stored on room temperature on this glycerin solution and were analyzed under a dissection microscope. During the analyses, it was counted the number of nematodes (Total and per stage) and the amount of galls (Gt). Also, it was counted the number of empty galls (Eg) and the percentage of Eg. The results were submitted to Anova on the R statistical software (R Core Team 2013).

3.4.2. Partially purified thaxtomin A assay

3.4.2.1. Assay execution, plant material attainment and the resistance inducer

The present assay was carried out under greenhouse conditions, in the Department of Plant Pathology and Nematology at ESALQ/USP. Following similar procedures of the item 3.4.1.1, the hybrid ‘Asturia’ was sown on Petri dishes, containing Whatman Filter paper (n°40), previously immerged in sterilized deionized water. Then, the Petri dishes were stored inside a growth chamber in the dark at 28°C, until germination had occurred. After this, the germinated seeds were transferred to 500ml pots, containing autoclaved sifted soil. Plants were maintained in the greenhouse and treatment was performed after ten days, with the growth of the true leaf.

3.4.2.2. Plant treatment, inoculum acquisition and inoculation process

As adopted for the ASM experiments (item 3.4.1.), the melon seedlings were treated with PPT 24 hours before the inoculation process. To obtain this resistance inducer, *S. scabies* was cultivated on oatmeal broth medium for seven days, as already described in item 3.2.1.2. After obtaining the preparations, the solution was maintained on methanol and stored in freezer until use. Before the plant treatment, the tubes containing the resistance inducer were maintained open under room temperature inside a hood for 24 hours, until methanol was

completely volatilized. Then, deionized water was added and the solution was shaken on a Vortex mixer, until it presented a yellowish color. The equivalent concentration of thaxtomin A was 248.13 µg per ml, which was measured in a spectrophotometer based upon absorbance at 400nm. Thus, the PPT was diluted in deionized water (1:9 (v/v)) to create a spray volume, being applied onto the seedlings aerial portion until runoff point. Also, control plants were treated with deionized water.

To inoculum acquisition, previously infested cotton roots were blended in a blender with commercial bleach 0.5%, to dissolve the egg masses. After that, the suspension was submitted to the Baermann modified to shallow plate (Hooper 1986) and stored on 27°C. Four days later, the J2 were collected on a Beaker and two aliquots of 0.5ml were used for counting the number of J2 on a Peters' slide. The inoculum was stored inside a refrigerator until it was used in the experiment.

Before the inoculation process, as already described on item 3.4.1.2, two holes (2cm and 4cm depth) were made close to the plant stem. Then, two inoculum droplets (0.8ml) were poured in, being covered with vermiculite after this process. For this experiment, 120 second stage juveniles were used. After this process, plants were maintained in a greenhouse until sampling was performed.

3.4.2.3. Sample collection, root staining and data analysis

For the PPT experiment, the plant material was collected at 3, 5, 7, 10 and 14 DAI. Four plants of each treatment were randomly picked and transported to the Biochemical and Physiological Plant Pathology Laboratory. Then, the melon seedlings were removed from the pots, followed by soil removal in tap water and section of the aerial portion. After this process, plants were submitted to similar procedures as described in item 3.4.1.3, being stained with fuchsin acid and stored in acidified glycerin. The number of nematodes (Total and per stage), the number of galls (Gt) and empty galls (Eg) and percentage of Eg were obtained at the end of the assay. The results were submitted to Anova by using the R statistical software (R Core Team 2013).

4. RESULTS AND DISCUSSION

4.1. Host status of *M. incognita* on melon hybrids

The data obtained for the host status experiment, described on item 3.1, are detailed on Table 2.

The cucumber was included to access the inoculum infectivity and the obtained values for R value, Nem/g and Pf indicates a successful inoculation, since *M. incognita* was able to infect and develop properly. The obtained values for Pf, R value and Nem/g on cucumber were 4,138.6 10.3 and 368, respectively.

The resistance, in Plant Nematology is the ability of the plant in suppresses the nematode development and reproduction (Roberts 2002). On the other hand, regarding *C. melo*, all tested melon genotypes showed susceptibility reaction to *M. incognita* (R value ≥ 1). The root-knot nematode density increased in the roots of cucumber and in all melon hybrids at the same rate, what is indicated by the R value, which did not present statisticall differences. Additionally, the R values ranged from 5.7 on 'Af 9723' to 19.51 on 'Af 11838'.

Despite that, the Nem/g values showed significant differences (Tukey honest test, p ≥ 5%), being the lowest 271 and the highest 1791, obtained for ''Af11682' and 'AF 4345', respectively.

Regarding the reaction, all melon plants were classified as susceptible and exhibited galls, the typical symptom caused by root-knot species, which occurred both in isolated and concatenated arrangements, and in different sizes. In addition to that, the egg masses presented a brownish color and were visible, numerous and easily spotted. Furthermore, the Gi presented no differences among the genotypes due the large number of galls and was not included in Table 2.

The presented results are in agreement with the literature available about *M. incognita* and melon plants. Most vegetable crops presents high susceptibility to *Meloidogyne* species (Huang 1992). Diniz et al. (2016b) investigated the host status of 15 melon genotypes against *M. incognita* and *M. javanica* and found no resistant hybrids to the first species among them. Furthermore, despite the genotypes had exhibited differences on the R value (RF on that paper), all melons were classified susceptible to *M. incognita* and the R value ranged from 2.52 to 9.22. Ito et al. (2014) was accessing different rootstocks with resistance to *M. incognita* and *M.* javanica and, among them, ten melon genotypes were tested. From them, three genotypes ('CNPH 01-962', 'CNPH 01-963' and 'Gaúcho Redondo') presented

resistance to *M. incognita*, when accessed in the years 2010 and 2011 (2010: R values 0, 0 and 0, respectively; 2011: R values 0.3, 0.2 and 0.7, respectively).

Table 2. Reproductive variables (Pf, R value and Nem/g) and host reaction in melon plants 60 days after inoculation with *Meloidogyne incognita*.

Genotype	Pf	R value	Nem/g	Reaction
Cucumber	4138.6	10.3 A*	368 c*	**Standard****
'Asturia'	4854.7	12.1 A	339 c	**S**
'Grandprix'	2981	7.4 A	280 c	**S**
'AF 4345'	5463.7	13.7 A	1791 a	**S**
'AF 11682'	2381.1	5.9 A	271 c	**S**
'AF 11838'	7806.1	19.5 A	1276 ab	**S**
'Iracema'	3259.2	8.1 A	509 bc	**S**
'AF 9723'	2292.2	5.7 A	486 bc	**S**
'AF 7607'	2453.6	6.1 A	389 bc	**S**
'AF 6768'	4318.4	10.8 A	415 bc	**S**
'AF 9136'	5211.2	13 A	801 abc	**S**

*Numbers followed by same letters did not differ statistically at Tukey honest test ($p \geq 5\%$). **Cucumber was included as a susceptible control for comparison purpose. Variables: Pf: Final population, R value: reproductive factor, Nem/g: nematode per gram of root, reaction: Susceptible (S) or resistant (R).

Paiva et al. (2004) identified 8 genotypes with resistance to *M. incognita*, but the researchers classified the genotypes solely based on the Gi value. It is important to mention that gall development is not essential for normal nematode growth and development (Hussey 1985), but can be an indicative of resistance. Dong et al. (2007), when comparing methods for assessing resistance in peanut to *M. arenaria*, found out that the Gi value based on percentage of infested roots was more reliable than Gi based on number of galls to separate genotypes into appropriate resistance categories. Also, we agree with the authors, who stated the difficulties of counting the galls on later harvest dates, since galls are usually conjunct, and pointed out the time-consuming effort to do so. We would like to add the number and size of galls also as a difficulty, which made the process not practicable.

It is interesting to mention that, in Brazil, there are no melon genotypes with resistances to both *M. javanica* and *M. incognita* (Diniz et al. 2016b; Ito et al. 2014). Moreover, Ogallo and McClure (1995) stated that inoculations with an incompatible nematode, which the plant shows resistance, can induce resistance against the compatible species. Thus, with the occurrence of *M. incognita* and *M. javanica* on the same area, the use

of genotypes that present resistance to one of them can affect the other (De Souza et al. 2015). Plant parasitic nematode populations interaction and dynamics are not the focus of the present research, but we would like to mention this for discussion purposes. Also, this motivates us to verify the reaction of the tested hybrids in the present work against *M. javanica*.

The interaction of root-knot nematode and melon plants was first reported in Brazil in 1958 and *Meloidogyne* species still are a major threat to melon cultivation on the main producing areas (Huang 1992; Mello 1958; Moura et al. 2002; Pinheiro and Amaro 2010). The use of resistant genotypes to plant parasitic nematodes is one of the most efficient and economic methods to avoid losses (Roberts 2002), but is not always available (Ferraz et al. 2012). Furthermore, their importance are even higher, due to their interaction with several other pathogens, for example, the gummy stem blight causal agent *D. bryoniae* (Dalcin et al. 2017). With few tools available, other management strategies like biological control agents and resistance inducers are needed and will be discussed below.

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4.2. Effect of resistance inducers and biological control agents on root-knot nematode control and melon plants development

The obtained data of the trials #1, #2, #3 and #4 (item 3.2) are summarized on Tables 3 – 8.

In trial #1, statistically differences were not found for fresh and dry weight, shoot diameter and SPAD values (Table 3). On the other hand, significative statistical differences (Scott-Knot 5%) were found for plants' height and fruit weight (Table 3).

In trial #2, treatments did not differ statistically for fresh and dry weight, shoot diameter and SPAD value (old leaf), but differed (Scott-Knot 5%) for root weight and SPAD (young leaf) values (Table 4).

In trial #3, the treatments did not differed statistically in fresh and dry weight, root weight, fruit weight, shoot diameter (basis and apex) and chlorophyll content on young and old leaves (Table 5). Statistical differences (Scott-Knot 5%) were found for plant's height and shoot diameter (middle) values (Table 5).

In trial #4, no differences were found for fresh and dry weight, plant's height, root weight, shoot diameter and chlorophyll content (old) values (Table 6). Significative statistical differences (Scott-Knott at 5%) were found for fruit weight and chlorophyll content (young) values (Table 6).

Regarding the differences in plants height in trial #1, melon plants treated with the resistance inducers (inoculated and non-inoculated) were shorter in comparison to the other treatments. Similarly, in trial #3, plants treated with both resistance inducers were shorter, but only when inoculated. This can be an indicative of the physiological cost of resistance, which is an explanation regarding negative correlations between maximum plant growth rate and synthesis of defense-related structural compounds, for example cellulose hemicellulose, etc. (Hoffland et al. 1996). Barbosa et al. (2008) reported that cotton plants treated with ASM presented lower height, differing from the control. Furthermore, Gondim et al. (2008) showed that all ASM concentrations caused a reduction on the growth of melon seedlings, especially at day 6. This effect was more pronounced in the shoot height and the size of secondary leaves. Additionally, Bth concentrations above 100mg/l had a negative effect on plant height, flower and fruit numbers in bean and cucumber plants under free-pathogen conditions (Azami-Sardooei et al. 2013).

It is interesting to mention that, despite this height loss observed in our results, no significant differences were found for fresh and dry weight, resulting in shorter, but thicker melon plants. This loss in the height, but with no weight penalties was reported and related to an increased synthesis of structural compounds, such as lignin, due to the treatment with the resistance inducer. These structural compounds restrict the meristematic cell division and elongation (Gondim et al. 2008). This process involves the enzyme peroxidase and its increased activity induced by ASM is widely documented for several plants, including melon (Gondim et al. 2008; Iriti and Faoro 2003; Soylu et al. 2003).

The same reasoning can be used to explain the same effects caused by the PPT. The thaxtomin A, one of the main components of the PPT is a cellulose biosynthesis inhibitor, which can also alter the flux of Ca^{2+} and H^{+} in the cytoplasmic membrane and cause programed cell death in plants (Duval et al. 2005; Scheible et al. 2003). Additionally, the PPT was reported inducing resistance in several plants against several pathogens, increasing the activity of chitinases, phenylalanine ammonia-lyase (PAL) and peroxidase (Garcia et al. 2008a; Garcia et al. 2008b.). Based upon these data, it is reasonable to attribute its effect to the similarities with ASM. Besides, it is noteworthy that on experiment 3, non-inoculated TPP treated plants presented the highest height, despite no statistical differences. Additionally, these plants exhibited the thickest shoot diameter (middle), which could be related to structural compounds.

Concerning the fruit weight, in trial #1, plants treated with ASM, ASM +N and *P. lilacinus* had heavier fruits than in the other treatments. The effect of ASM in activating SAR

on melon fruits is already known (Huang et al., 2000). Furthermore, on melon fruits, ASM applications can enhance respiration rate, ethylene production and also lower the declining of fruit firmness (Li et al., 2015). It is reasonable to attribute the increased weight of the fruits to the increased ethylene production, which plays a key regulatory role in fruit ripening (Barry and Giovannoni 2007). Thus, due to its ability to speed up this process, consequently, it is able to increase the fruit weight.

Regarding *P. lilacinus*, not only its ability to suppress root-knot nematode population is remarkable. It was already reported increasing yields in tomatoes (Lara 1996; Kiewnick; Sikora 2004), pointed gourd (*Trichosanthes dioica* Roxb.) (Reddy 2014) and okra (*Abelmoschus eschulentus* (L.) Moench) (Kannan and Veeravel 2012), but no reports were found for melon plants. In fact, *P. lilacinus* produces toxic compounds and enzymes against nematodes, but also can act indirectly, promoting plant growth and enhancing yield of the parasitized plants (Mahapatra and Sahani 2007; De Souza et al. 2015). On the other hand, on trial #1, the melon plants treated with *P. lilacinus* had heavier fruits in a free-pathogen situation, which did not occur in trial #2, although non-inoculated plants presented 65.58g of fruits per plant and inoculated plants presented 112.48 g. Additionally, the treatments with its culture filtrates seems to have the same proprieties as an inducer of fructification, but this will be discussed further below.

On all these three trials, the lack of severe above-ground symptoms caused by *M. incognita*, such as the loss in fresh and dry weight, may be due to the age of the seedlings used. On all these trials, the lack of severe above-ground symptoms caused by *M. incognita*, such as the loss in fresh and dry weight, may be due to the age of the seedlings used. Ploeg and Phillips (2001) reported that severe damage caused by root-knot nematode could be achieved due to the infection on very young seedlings. Otherwise, delay in exposure of melon seedlings (14 days) to root-knot nematode could increase yield, in comparison, but may result in higher populations at harvest. These observations are in agreement with our data.

Regarding root weight, in trial #2, non-inoculated and inoculated plants were statistically grouped separately (Table 4). Plants treated with water, *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* presented lighter roots than the inoculated ones, and the obtained values were 9.31g, 5.31g, 7.38g and 5.07g, respectively. Furthermore, plants treated with water + N, *Bacillus* sp. + N, *P. lilacinus* + N and *P. chlamydosporia* + N presented heavier roots, being the obtained values around 17.62, 13.89, 12.35 and 14.29, respectively. In agreement with our results, Ploeg and Phillips (2001) reported a dramatic increase in root weight of inoculated melon plants. On their experiments, increasing Pi resulted in heavier

roots, but severely galled root system, what was also observed in the present work (Figure 3). Similarly, increased root weight was obtained for watermelon (*Citrullus lanatus* (Thunb.) Matsumura & Nakai) infested with *M.* incognita (Xing and Westphal 2012). Moreover, this phenomenon seems to vary from species to species, since the root growth was absent in *M. incognita*/*M. javanica* infesting wheat (*Triticum aestivum* (L.) Thell) (Roberts and Van Gundy 1981). Additionally, this was observed only in Trial #2, which is the assay where the highest nematode reproduction was achieved.

Regarding the SPAD values for chlorophyll content, no statistical differences were observed in trial #1, for old or young leaves. However, if we compare the SPAD values obtained from old leaves of only inoculated plants (+N), all plants that received the biological control agent or resistance inducer were grouped together, differing statistically of the water treated plants (+N) (Scott-Knott at 5%). This was only obtained for trial #1. Regarding trial #2, all treatments (non-inoculated or inoculated) presented higher chlorophyll content in young leaves (Scott-Knott at 5%) when compared to the control plants.

Regarding trial #3, no differences were found, however we would like to mention that the ASM and PPT inoculated treatments (+N) showed higher values than the water treated plants (Obtained values were 14.2, 21.7 and 21.4 for Water +N, ASM +N and TPP +N, respectively). Similarly to trial # 2, in trial #4, statistical difference was found only for SPAD values obtained for young leaves. The treatments “Dose 0” (control), “Dose 1”, “Dose 5” and “Dose 5+N” present higher chlorophyll content, when compared to the other treatments. Additionally, regarding the non-inoculated plants, it is interesting to point out that the only treatment that presented high SPAD values on either inoculated or non-inoculated plants was the maximum dose (Dose 5).

The chlorophyll meter “Soil Plant Analysis Development” (SPAD) can provide values related to the chlorophyll content on plants’ leaves without destroying them (Jesus and Marenco 2008) and can be used to access severity of plant diseases (Jesus and Marenco 2008; Rosyara et al. 2007; Jiang et al. 2018). The root-knot species can reduce the photosynthetic rate in some plants, but also cause nutritional deficiency. Additionally, chlorophyll content can be used as an indicator to evaluate nematode damage (Loveys and Bird, 1973; Giné et al. 2014; López and Verdejo-Lucas 2017; Jiang et al. 2018), although the chlorophyll content might be influenced by several factors, such as luminosity, nourishment and also can decline due to natural senescence, being an interesting tool for analyses, but not reliable as a sole parameter (Li et al. 2018; Freeden et al. 1990).

Our results indicate that the biological control organisms and the resistance inducers had the ability to increase chlorophyll content of melon plants in the presence of *M. incognita*, but the first group of agents can also increase in a free-pathogen situation. Most biological control organisms could help the plant to avoid abiotic stress (Medeiros et al. 2018; Ferraz et al. 2012; Gamliel and Katan 1993), thus improving overall yield of the plants (De Souza et al. 2015). Whereas resistance inducer can affect several growth parameters, such as plant growth, defense compounds and chlorophyll content (Hassan et al. 2006), no significative increases were showed under a free-pathogen scenario.

Regarding nematode development, *M. incognita* did not reproduce properly on experiment 1, but good reproduction was achieved in trial #2 (Table 5). In fact, the obtained Pf for the standard treatment on trial #1 was only 5%, of the value obtained for the same treatment in trial #2. The low reproduction can be attributed to poor inoculum infectiveness. Furthermore, the inoculated plants presented small young galls and the egg masses were not abundant, as on trial #2. Taking this into account, longer period was necessary to allow properly nematode development .Moreover, regarding the evaluation at 40 DAI, similar reproduction was achieved on tomatoes evaluated 45 days after transplanting to pots, containing soil infested with 3000 eggs (Xavier et al. 2017). Despite the reason for the poor development, the nematode reproduction parameters of the trial #1 and #2 will be further discussed below.

Table 3. Trial #1: Effect of resistance inducers and biological control agents on *Cucumis melo* plants non-inoculated or inoculated (+N) with *Meloidogyne incognita* (1000 individuals). Evaluations carried out at 40 days after the inoculation

Treatment**	Fresh weight (g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	Stem diameter (mm)			Chlorophyll content in the leaves***	
						Basis	Middle	Apex	Old	Young
Water	89.3 A*	11.4 A	119.8 A	15.1 A	2.1 B	5.0 A	4.6 A	1.8 A	31.7 A	31.3 A
Water + N	86.7 A	11.9 A	120.7 A	17.5 A	0.0 B	5.8 A	5.2 A	1.9 A	23.6 A	32.8 A
Acibenzolar-S-methyl	65.9 A	9.0 A	67.7 B	12.7 A	29.5 A	5.5 A	4.6 A	1.9 A	34.3 A	29.2 A
Acibenzolar-S-methyl + N	73.2 A	9.7 A	91.9 B	14.1 A	34.5 A	5.3 A	4.1 A	1.7 A	38.6 A	33.1 A
Bacillus amyloliquefaciens	103.1 A	12.9 A	123 A	21.8 A	6.5 B	6.2 A	5.2 A	2.1 A	43.3 A	37.6 A
Bacillus amyloliquefaciens + N	99.1 A	12.4 A	124.1 A	18.6 A	11.3 B	5.6 A	4.9 A	2.3 A	40.6 A	32.0 A
Paecilomyces lilacinus	80.8 A	10.2 A	114.6 A	24.6 A	23.4 A	5.2 A	5.0 A	2.3 A	34.7 A	37.8 A
Paecilomyces lilacinus + N	106.8 A	14.7 A	124.9 A	24.0 A	0.15 B	6.2 A	4.9 A	1.8 A	44.0 A	39.3 A
Pochonia chlamydosporia	85.2 A	11.9 A	112.3 A	22.0 A	0.0 B	5.4 A	4.8 A	2.9 A	36.5 A	38.9 A
Pochonia chlamydosporia + N	81.9 A	11.9 A	121.9 A	17.5 A	7.6 A	5.4 A	4.8 A	2.3 A	37.1 A	35.4 A
PPT †	100.2 A	14 A	96.3 B	22 .0 A	0.0 B	5.7 A	4.8 A	2.5 A	37.8 A	41.1 A
PPT † + N	85.8 A	11.1 A	83.4 B	19.0 A	0.2 B	6.3 A	5.0 A	2.4 A	36.3 A	39.9 A

*Numbers followed by the same letters did not differ statistically (Scott-Knot at 5%). **Resistance inducers and biological control agents applied 24 hours before the inoculation process *** Chlorophyll content of the leaves expressed in SPAD units; †Partially purified thaxtomin;

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Table 4. Trial #2: Effect of biological control agents on *Cucumis melo* plants non-inoculated or inoculated (+N) with *Meloidogyne incognita* (1000 individuals). Evaluations were carried out at 60 days after the procedure.

Treatment**	Fresh weight(g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	Stem diameter (mm)			Chlorophyll content in the leaves***	
						Basis	Middle	Apex	Old	Young
Water	63.2 A*	9.7 A	161.4 A	9.3 B	52.8 A	5.05 A	4.8 A	1.4 A	25.3 A	19.3 B
Water + N	55.7 A	9.6 A	161.8 A	17.6 A	84.7 A	4.66 A	4.2 A	1.5 A	28.6 A	21.9 B
Bacillus amyloliquefaciens.	43.2 A	8.7 A	168.3 A	5.3 B	56.3 A	4.54 A	3.9 A	1.3 A	29.9 A	37.2 A
Bacillus amyloliquefaciens + N	64.0 A	9.9 A	189.9 A	13.9 A	41.8 A	5.04 A	4.8 A	1.2 A	29.1 A	33.1 A
Paecilomyces lilacinus	52.3 A	9.7 A	184.8 A	7.4 B	65.6 A	4.97 A	5.1 A	1.5 A	32.7 A	33.2 A
Paecilomyces lilacinus + N	72.9 A	11.2 A	196.9 A	12.3 A	112.5 A	5.13 A	5.0 A	1.6 A	25.7 A	36.0 A
Pochonia chlamydosporia	48.5 A	9.2 A	172.5 A	5.1 B	63.1 A	5.81 A	4.9 A	1.4 A	28.1 A	30.0 A
Pochonia chlamydosporia + N	65.7 A	9.8 A	196.2 A	14.3 A	73.7 A	5.1 A	4.2 A	1.4 A	31.0 A	41.3 A

*Numbers followed by the same letters did not differ statistically (Scott-Knott at 5%). %).**biological control agents applied 24hours before the inoculation procedure ***Chlorophyll content of the leaves expressed in SPAD units.

Table 5. Trial#3: Effect of resistance inducers on *Cucumis melo* non-inoculated and inoculated (+N) with *Meloidogyne incognita* (1000 individuals). Evaluation carried out at 60 days after the inoculation procedure.

Treatment**	Fresh weight (g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	shoot diameter (mm)			Chlorophyll content in the leaves***	
						Basis	Middle	Apex	Old	Young
Water	62.0 A*	8.3 A	101.7 A	12.1 A	158.4 A	5.1 A	4.3 B	2.0 A	13.2 A	20.8 A
Water + N	71.4 A	8.4 A	102.4 A	13.5 A	148.4 A	4.7 A	4.1 B	1.9 A	14.2 A	25.4 A
Acibenzolar-S-methyl	59.0 A	9.0 A	103.0 A	12.5 A	144.9 A	5.2 A	4.3 B	1.3 A	24.6 A	24.8 A
Acibenzolar-S-methyl + N	54.8 A	8.1 A	88.2 B	9.6 A	133.5 A	5.0 A	3.8 B	1.4 A	21.7 A	26.8 A
PPT†	76.5 A	9.1 A	119.2 A	12.2 A	160.4 A	5.2 A	4.7 A	1.5 A	17.0 A	28.1 A
PPT† + N	58.7 A	7.7 A	84.8 B	14.6 A	176.7 A	5.2 A	4.2 B	1.5 A	21.4 A	24.1 A

*Numbers followed by the same letters did not differ statistically (Scott-Knott at 5%). **Resistance inducers applied 24 hours before the inoculation procedure ***Chlorophyll content of the leaves expressed in SPAD units. †Partially purified thaxtomin A

Table 6. Effect of different doses of Rizotec® (*Pochonia chlamydosporia*) in the *Cucumis melo* plants non-inoculated or inoculated (+N) with *Meloidogyne incognita* (1000 individuals). Evaluation carried out at 60 days after the inoculation procedure.

Treatment**	Fresh weight (g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	Stem diameter (mm)			Chlorophyll content in the leaves	
						Basis	Middle	Apex	Old	Young
Dose 0	46.8 A*	5.0 A	78.6 A	16.0 A	136.1 A	4.9 A	4.4 A	1.5 A	18.2 A	30.3 A
Dose 0 + N	44.5 A	6 .0A	99.2 A	10.2 A	97.0 B	4.7 A	4.2 A	1.2 A	8.0 A	25.9 B
Dose 1	52.7 A	5.1 A	88 A	19.0 A	145.9 A	4.9 A	4.2 A	1.6 A	17.3 A	32.5 A
Dose 1 + N	40.5 A	5.0 A	83.9 A	19.0 A	117.7 B	4.1 A	4.1 A	1.6 A	13.6 A	27.5 B
Dose 2	42.4 A	4.8 A	89.6 A	14.3 A	127.0 A	4.4 A	4.1 A	1.5 A	19.5 A	28.4 B
Dose 2+ N	53.7 A	6.1 A	93.5 A	20.8 A	130.0 A	4.9 A	4.2 A	1.5 A	16.0 A	28.9 B
Dose 3	38.8 A	4.3 A	87.1 A	17.3 A	108.8 B	4.2 A	4.0 A	1.5 A	17.4 A	27.4 B
Dose 3 + N	39.9 A	4.6 A	85.9 A	14.4 A	103.1 B	4.3 A	4.0 A	1.4 A	14.8 A	27.1 B
Dose 4	39.5 A	5.2 A	84.9 A	17.8 A	101.4 B	4.5 A	4.0 A	1.8 A	13.8 A	27.5 B
Dose 4 + N	54.7 A	5.7 A	96.9 A	17.1 A	141.2 A	5.1 A	4.5 A	1.5 A	15.2 A	28.7 B
Dose 5	53.4 A	6.1 A	92.1 A	13.6 A	144.7 A	4.7 A	4.3 A	1.5 A	14.8 A	31.5 A
Dose 5 +N	51.3 A	5.4 A	89.6 A	17.1 A	144.8 A	4.9 A	4.6 A	1.7 A	11.0 A	30.4 A

*Numbers followed by the same letters did not differ statistically (Scott-Knott at 5%). **Rizotec® doses applied 24 hours before the inoculation procedure. Doses: dose 0 (control treatment), dose 1 (0.1 g of Rizotec® per plant), dose 2 (0.25 of Rizotec® per plant), dose 3 (0.5 of Rizotec® per plant), dose 4 (1g of Rizotec® per plant) and dose 5 (2g of Rizotec® per plant) ***Chlorophyll content of the leaves expressed in SPAD units.

As discussed above, *M. incognita* did not reproduce properly in plants in trial #1. However, better reproduction was achieved in the other experiments, notably in trial #2, which presented the highest populations (Table 7). In fact, the obtained Pf for the control treatment in trial #1 was only 5%, of the value obtained for the same treatment in experiment 2. When compared with the trial # 3 and #4, the reproduction were 18% and 21%, respectively. Furthermore, especially in trial #1, the inoculated plants presented small sized galls and the egg masses were not abundant, as in trial #2, #3 and #4. The lower reproduction rate can be attributed to either poor inoculum infectiveness, heat-stress and/or different temperatures when the experiments took place. However, if we take into account the duration of the trial #1 (40 days), the longer rate might have allowed a better nematode development. In agreement with this, similar reproduction was achieved in tomatoes evaluated 45 days after transplanting to pots, containing soil infested with 3000 eggs (Xavier et al. 2017). Despite this reason, the low *M. incognita* development on the trial #1 did not invalidate or disqualify our data, since most results showed consistence in the other trials, except for *P. chlamydosporia*, which will be discussed separately.

Regarding trial #1, 2 and 3, all treatments reduced the Pf when compared to the control treatment (Table 7), except by *P. chlamydosporia* on trial #1. Moreover, except by *P. chlamydosporia*, all control agents presented lower R value, but only *B. amyloliquefaciens* treatment statistically differed from the control (Duncan at 5%) in both experiments that was carried out. Regarding trial #2, despite the lack of statistical difference, it is noteworthy that all treated plants (+N), including *P. lilacinus* and *P. chlamydosporia*, showed fewer symptoms than water treated plants (Figure 3), presenting fewer galls and cleaner roots. In fact, the Pf reduction was 58.24%, 33.1% and 50.45%, for *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia*, respectively. Interesting, *P. chlamydosporia* presented fewer Nem/g, differing statistically from the control plants (Duncan at 5%). Such symptom difference could not be confirmed on trial #1, 3 and 4 due to small size of the galls and the lower nematode reproduction.

B. amyloliquefaciens treated plants presented a reduction of 76.74% and 58.24% in trial #1 and 2, respectively. Also, in trial #1, treated plants presented lower R value and differed statistically from the control. *Bacillus* spp. are plant growth-promoting rhizobacteria and are well-known for their effect against plant parasitic nematode (De Souza et al. 2015; Medeiros et al. 2018; Anter et al. 1995). The rhizobacteria have a wide variety of suppression mechanisms and can act in several stages of the plant parasitic nematode life cycle, being able

of suppress egg hatching, juveniles mobility, nematodes feeding and reproduction (Ferraz et al. 2012). Additionally, they can promote growth and improve general plant yield.

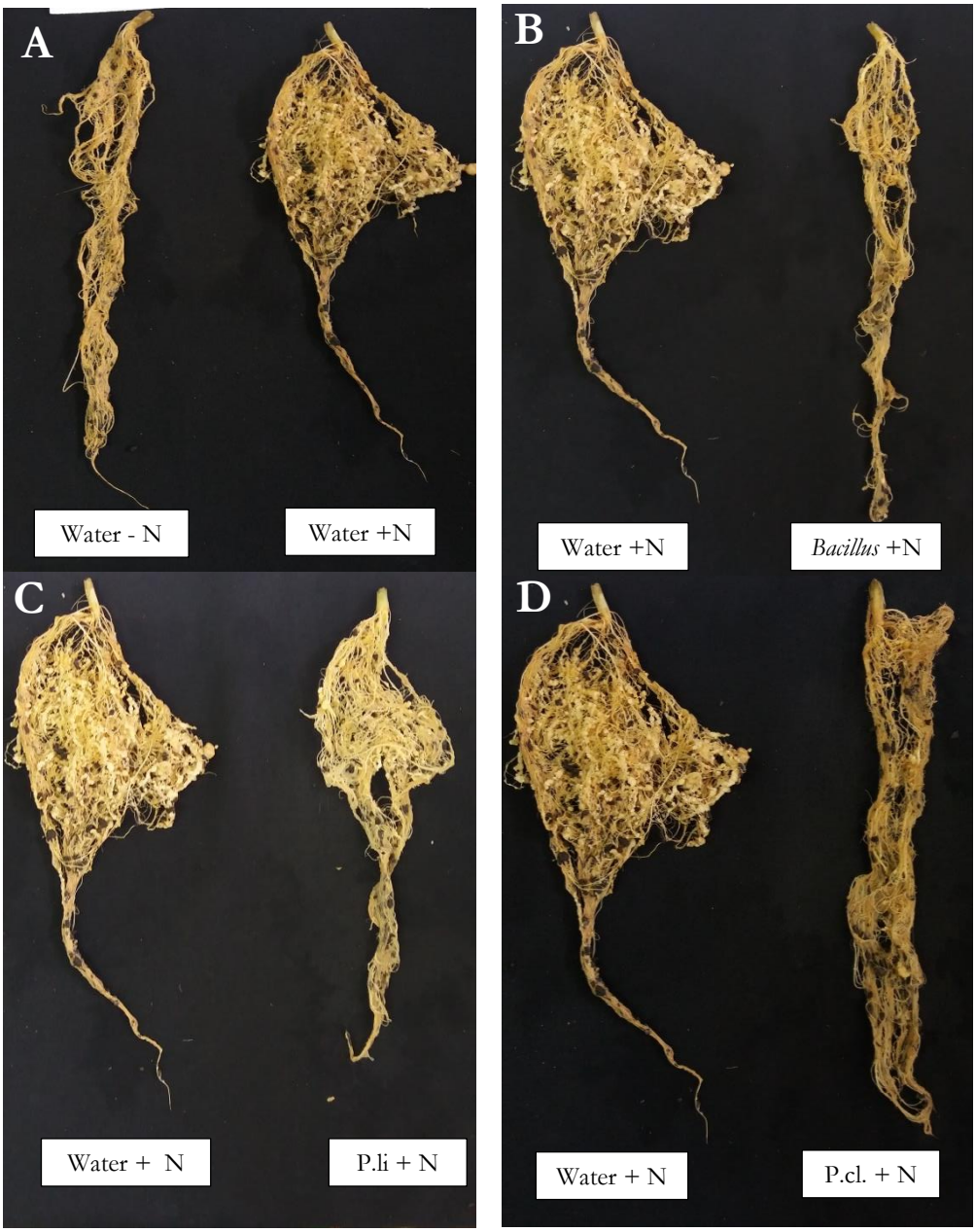

Figure 3. Roots of *Cucumis melo* plants ~60 days after treatment and inoculation with *Meloidogyne incognita* (1000 individuals). A) Water treated (Control) non-inoculated (-N) and water treated inoculated (+N); B) Water treated (Control) +N and *Bacillus amyloliquefaciens*. +N; C) Water treated (Control) +N and *Paecilomyces lilacinus* (P.li) + N; D) Water treated (Control) +N and *Pochonia chlamydosporia* (P.cl.) + N; Plants treated with the control agents exhibit fewer galls on the roots in comparison to the water treated and inoculated plants.

Table 7. Final population (Pf), reproduction factor (R value), nematodes per gram of root (Nem/g) and Pf reduction obtained for melon plants infested with 1000 eggs of *Meloidogyne incognita* and treated with different biological control agents and resistance inducers. Data obtained for trial # 1 (Exp. 1, 40 DAI**), #2 (Exp. 2, 60 DAI**) and #3 (Exp. 3, 60 DAI**)

Treatment****	Pf			R value			Nem/g			% Pf reduction**		
	Exp. 1	Exp. 2	Exp.3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Water + N (Control)	1,463.7	27,703.9	4,981.4	1.5 a*	27.7 a	5.0 a	61 ab	1,444.4 a	575 a	0.0	0.0	0.0
Acibenzolar-S-methyl + N	501.5	-	1,960.9	0.5 ab	-	2.0 b	22 ab	-	421 ab	65.7	-	60.6
B. amyloliquefaciens + N	394.5	11,568.9	-	0.3 b	11.6 b	-	31.4 ab	1,351.2 ab	-	76.1	58.2	-
Paecilomyces lilacinus + N	526.4	18,540.3	-	0.5 ab	18. ab	-	22.0 b	1,645.6 ab	-	64.0	33.1	-
Pochonia chlamydosporia + N	1,309.1	13,725.4	-	1.3 ab	13.7 ab	-	80.3 a	894.5 b	-	10.6	50.4	-
TPP† + N	581.5	-	2966	0.6 ab	-	3.0 ab	37.4 ab	-	416 b	60.3	-	40.4

*Numbers followed by the same letters did not differ statistically (Duncan at 5%). **Days after inoculation. ***Control taken as 100%. **** Treatments applied 24 hours before the inoculation procedure. - It was not tested in the experiment.

The *B. subtilis* treatment in tomato plants inoculated with root-knot nematode resulted in growth promotion and reduction of egg masses, being more efficient than the nematicide treatment. Medeiros et al. (2009) screened the efficiency of 117 bacterial strains against *M. incognita* race 2 in melon plants and found three endophytic *Bacillus* (ENM7, ENM10 and ENM51) that significantly reduced egg mass and/or gall index. Moreover, the *Bacillus* ENM51 treatment in melon 'Golden mine' infested with *M. incognita* resulted in fewer galls and lower nematode reproduction (Pereira et al. 2016), which is in agreement with our results. Furthermore, the authors reported that *Bacillus* spp. could enhance the plant resistance to water stress and also induce the activity of some stress-associated enzymes, like peroxidase.

P. lilacinus is one of the most studied biological control organisms against plant parasitic nematodes (Atkins et al. 2005), but there is a scarcity of reports concerning *M. incognita* and melon plants. *P. lilacinus* was already isolated from the rhizosphere of melon plants 'Golden Mine' cultivated in the Vale do São Francisco region (Coutinho et al. 2009). On tomato plants, it not only suppressed *M. incognita* population in soil and roots, but also increased the fruit yield (Lara et al. 1996). One single application of *P. lilacinus* at a concentration of 1 x 10^6 CFU/g soil was sufficient to control *M. incognita* in tomato plants. Additionally, soil previously treated with this fungus reduced the amount of galls in the root system and the number of egg masses by 66% and 74%, respectively, when compared to the control treatment (Kiewnick and Sikora 2006). Besides, the effectiveness of *P. lilacinus* against *Rotylenchulus reniformis* in *Cicer arietinum* L. was reported (Ashraf and Khan 2008). This plant parasitic nematode is also an important pathogen to melon crops in Brazil. Thus, this allows the management of two important diseases on *C. melo* with the same biological control agent.

The trial #4 was carried out to clarify the discrepancy obtained for *P. chlamydosporia* in trial #1 and trial #2 (Pf reduction for experiments 1 and 3 were 10.6 and 50.4, respectively) (Table 7). *P. chlamydosporia* was effective in reducing *M. incognita* population in trial #2 and 4 (Table 7 and 8). For the first one, plants treated with Rizotec® presented ~50% less nematodes in the root system and showed less symptoms. Furthermore, regarding trial #4, the doses and *M. incognita* Pf/Fr were negatively correlated (-0.88%). Additionally, these factors were better adjusted by a polynomial trendline (R^2=0.92) (Figure 4), which indicates that crescent doses of Rizotec® resulted in linear decreasing of *M. incognita* Pf (dose 1 – 4), that stabilize (dose 4 and 5), forming a polynomial shaped trendline (Figure 4). Additionally, the linear trendline was verified and presented a R^2 = 0.78. The used

doses reduced the Pf in 7, 33, 26, 60.6 and 65.5%, respectively, when compared to dose 0 (Table 8). However, regarding the R value, the only dose that differed statistically was dose 5 (2g/plant) (Duncan at 5%) and, on the other hand, the dose 4 (1g/plant) presented the lowest value for Nem/g and differend statiscally from dose 3, which presented the highest value (345 Nem/g compared to 612.5 and 464 for Dose 3 and control plants, respectively) (Duncan at 5%).

The dose 2 (0.25g/plant) showed higher population reduction than the dose used in trial #1 (0.3g/plant), which reduced in 33% and 10.6% the Pf, respectively. Taking this in account, some explanations was though out: (1) problem in the moment of application (Soil-drench); (2) product was out of expiration date; (3) product was not correctly prepared/homogenized. Furthermore, the highest doses (dose 4 and 5) were the most effective on reducing *M. incognita* development. However, increasing the dose may be costly under field conditions (Bontempo et al 2017). The melon crop is a high investment culture and a costly control measure such as high Rizotec® doses may be practicable in some situations, for example, under high root-knot nematodes densities that would justify the investment. As suggested by Bontempo et al. (2017) regarding carrot crop and high doses of *P. chlamydosporia* to control *M. incognita*, this cost-benefit should be taken into consideration and field analyses might be necessary.

P. chlamydosporia was first reported as a parasite of nematode eggs by Wilcox and Tribe (1974) and now consist in one of the most studied biological control agents of nematodes (Manzanilla-López et al. 2013). Despite this, few information are available regarding its effect on *C. melo* and the root-knot nematode pathosystem. However, it is reported the growth promotion ability of *P. chlamydosporia* for sweet pepper, cucumber, tomato and melon (Ceiro 2015 *apud* Hidalgo-Díaz et al. 2017), which was not observed in the present work due to statistically similarity (Table 3, 4 and 6), but could explain the fewer Nem/g obtained for inoculated plants on trial #2. For *C. sativus* infested with *M. javanica*, *P. chlamydosporia* reduced in 46% and 49.4% the number of galls per gram of root system, by applying 18g/L in cucumber seedlings (Viggiano et al. 2014). Promising results were also reported for *M. jacanica* on tomato, carrot (*Daucus carota* L.) and sugar beet (*Beta vulgaris* L.) plants (Podestá et al. 2009; Coutinho et al. 2009; Dallemole-Giaretta et al. 2010).

Table 8. Trial #4: Final population (Pf), reproduction factor (R value) and nematodes per gram of root (Nem/g) obtained for melon plants treated with different doses of Rizotec® (*Pochonia chlamydosporia*) and inoculated with 1000 eggs of *M. incognita*. Evaluations were carried out at 60 days after the inoculation procedure

Treatment**	Pf	R value	Nem/g	% Pf reduction**
Dose 0 + N	6,028.5	6.0 A*	465 ab	0
Dose 1 + N	5,606.7	5.6 AB	574 ab	7.0
Dose 2+ N	4,037.8	4.0 AB	346 ab	33.0
Dose 3 + N	4,462.9	4.5 AB	612.5 a	26.0
Dose 4 + N	2,375.4	2.4 AB	345 b	60.6
Dose 5 +N	2,077.2	2.1 B	516 ab	65.5

*Numbers followed by the same letters did not differ statistically (Duncant at 5%). **Rizotec® doses applied 24 hours before the inoculation procedure. Doses: dose 0 (control treatment), dose 1 (0.1 g of Rizotec® per plant), dose 2 (0.25 of Rizotec® per plant), dose 3 (0.5 of Rizotec® per plant), dose 4 (1g of Rizotec® per plant) and dose 5 (2g of Rizotec® per plant). ***Control taken as 100%

Also, the resistance inducer ASM reduced the Pf by 65.74% and 60.6%, for trials #1 and 3, respectively. The ASM is widely used as a resistance inducer against many pathogens (Ge et al. 2008; Iriti et al. 2007), including some plant parasitic nematodes, such as *M. javanica* on cowpea (*Vigna unguiculata* L. Walp.) and soybean plants (*Glycine max* (L.) Merr.) (Chinnasri et al. 2003), *M. incognita* in tomato plants (Zinovieva et al. 2013) and *M. arenaria* in grapevine plants (*Vitis vinifera* L.) (Owen et al. 2002). Regarding melon crop, the majority of reports focus on post-harvest diseases (Li et al. 2015; Ge et al. 2008; Wang et al. 2008; Zhang et al. 2011), and the present work is one of the few works regarding ASM and *M. incognita* in melon plants. Zinovieva et al. (2013) reported the increased resistance of tomatoes treated with ASM against *M. incognita*, even with the resistance gene (Mi gene)

deactivated. Furthermore, Chinnasri et al. (2016) reported the induction of pathogenesis-related gene 1 (PR-1) by ASM in pineapple against the plant parasitic nematode *R. reniformis*.

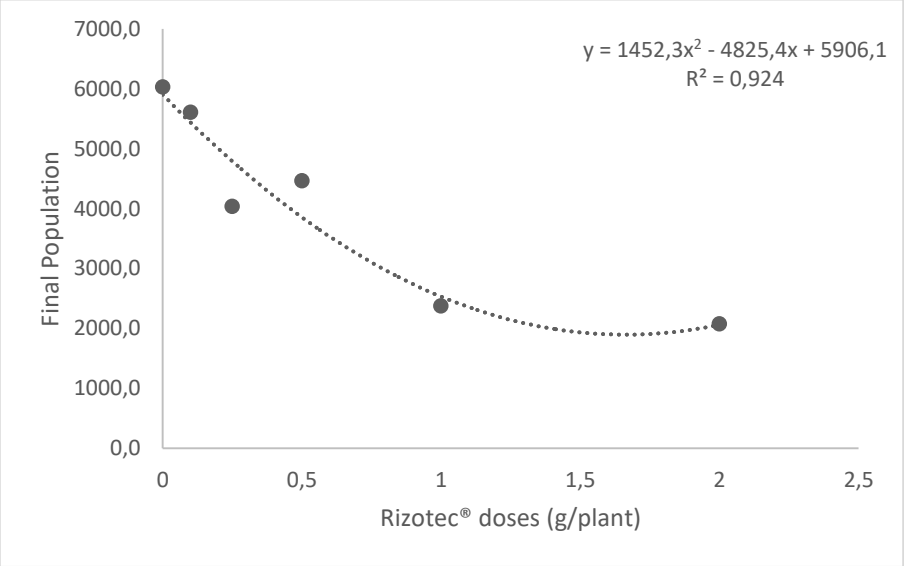

Figure 4. Relation between Rizotec® crescent doses and the final population obtained from melon infested with 1000 eggs of *Meloidogyne incognita* Evaluation carried out at 60 days after inoculation procedure.

As already stated, the thaxtomin A, the main component of the PPT, was found to induce resistance against pathogens and activate several defense-related enzymes, such as peroxidase (Garcia et al. 2008a). Thus, the present work was the first attempt to verify the effect of these preparations in the control of a plant parasitic nematode. The PPT applications reduced *M. incognita* population in 60.2 and 40.4, in trials #1 and 3, respectively. Furthermore, the PPT treated plants presented the lowest Nem/g value and differed statistically from the control (Duncan at 5%). It is possible that the presence of methanol increased the PPT effect in the trial #1. It was found that methanol elicits accumulation of glycosides and phenolic compounds in *Stevia rebaudiana* Bertoni (Álvarez-Robles et al. 2016), but further experiments are necessary in the case of melon plants. Despite this, our work presents the PPT as a possible management tool for *M. incognita* in melon.

4.3. Effect of culture filtrates on melon seeds, *M. incognita* egg hatching and in the control of root-knot nematode in melon plants

The obtained data of the filtrates assays are summarized in Tables 9 - 13.

Regarding seeds, PD broth medium had a negative effect on germination, which made the process slower in comparison to the other treatments (Table 9). In addition to that, all filtrates differed statistically from the PD broth medium (Scott-Knott at 5%), except by *P. lilacinus* filtrate at the first evaluation (Table9). However, at the second and third evaluation times, it was grouped statistically with the other treatments (Table 9). At the third evaluation time, 60% of the seeds treated with PD medium germinated, but radicles were smaller, in comparison to the other treatments (Figure 5). Additionally, melon seeds treated with *P. chlamydosporia* filtrates exhibited hairy radicles (Figure 5 F), which was not observed in any other treatment.

Concerning the effects of the culture filtrates on egg hatching, both *B. amyloliquefaciens* and *P. lilacinus* filtrates differed statistically from the water treatment in experiment 1 (Scott-Knot at 5%) (Table 10). The treatment presented a percentage of inhibition of 82.9 and 87.9, respectively. Regarding experiment 2, all treatments differed statistically from the control treatment, including the PD broth media (Scott-Knot at 5%). This inhibition observed in the PD broth media might be related to contamination, even with the addition of antibiotic/fungicide. Regardless, the percentage of inhibition obtained for *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* were 84.3, 68.2 and 83.5, respectively. Furthermore, PPT did not differed from methanol on experiment 2, presenting a percentage of inhibition of 80.6 and 83.3, respectively. However, when tested in water, PPT does not inhibited the egg hatching, being grouped statistically with the control treatment. Besides, the other treatments differed statistically from both TPP and control treatment, with *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* presenting 50.2, 94.3 and 78.5 of percentage of inhibition, respectively (Table 10).

Regarding the effect of the filtrates in melon plants (greenhouse assay), no statistical differences were found regarding the plants fresh and dry weight, height, diameter and chlorophyll content. However, melon plants treated with *B. amyloliquefaciens* filtrate, both the inoculated or non-inoculated with *M. incognita*, presented heavier roots, differing statistically from the other treatments (Table 11). Additionally, plants treated with *P. lilacinus* filtrate produced more grams of fruits per plant and differed statistically from the others (Table 11). However, on the second experiment no statistical differences were found in the accessed parameters, except for shoot diameter (apex), which were thicker in control melon plants (-N and +N) and in melon plants treated with *P. chlamydosporia* filtrate (+N).

Regarding the effects of filtrates on *M. incognita* development, the obtained data is summarized at Table 13. In the greenhouse assay, *B. amyloliquefaciens* and *P. lilacinus*

filtrates presented a Pf of 349.5 and 1008, which correspond in a reduction of 76.1% and 31.1% when compared to the control plants (Pf 1,463.7), respectively (Table 13). Additionally, the treatments presented lower R value than the water-treated plants, but only the *B. amyloliquefaciens* filtrate statistically differed from the control (Duncan at 5%). However, the melon plants treated with *P. lilacinus* filtrate had more nematodes per gram of root and differed statistically from the control in the experiment 1 (Duncan at 5%), despite presented a 31.1% reduction in comparison to the control plants

The data obtained for the second assay followed a similar pattern. All treatments presented lower population, R value and Nem/g, however, only *B. amyloliquefaciens* filtrate was statistically grouped apart from the control in these parameters (Duncan at 5%). The treatments reduced the population in 47.6%, 40.3% and 40% (*B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* filtrates, respectively). Additionally, *P. lilacinus* filtrate treatment does not showed more Nem/g values as the previously assay. Interesting, PD medium presented lower R value than water treated plants, reducing the population in 47.1%.

Ferraz et al. (2012) lists the possible mechanisms involved in the antagonisms of plant parasitic nematodes. The PGPR can produce and release a wide variety of secondary metabolites that can diffuse in the soil and be absorbed by the nematodes eggs. These metabolites can kill their cells and stop the development of the juveniles or make them less mobile in soil. Furthermore, these metabolites can be absorbed by the plant and change its relation with plant parasitic nematodes by altering the plant chemical composition, making it less attractable to the nematodes and by inducing cell death (hypersensitivity reaction) of the feeding cells, major food source to sedentary nematodes. Since the PGPR are more effective on reducing egg production than galls, it is possible that this is one of the mechanism involved, since poor feeding leads to less reproductive females.

Regarding *P. lilacinus* and *P. chlamydosporia*, those antagonistic fungi parasitize root-knot nematodes through secretion of extracellular hydrolytic enzymes and other secondary metabolites (Siddiquee et al. 2012). For *P. lilacinus*, culture filtrates obtained from strain 6029 exhibited toxicity against *M. incognita* and no significant differences in nematicidal activity were observed between boiled and unboiled culture filtrates (Sharma et al., 2014). Further analysis by the authors suggested the presence of phenolic and alcohol compounds that could be secreted with time in the culture broths. However, Cayrol et al. (1989) tested different liquid media to obtain cultures filtrates, under different conditions and was found inconsistence in toxicity towards nematodes. The best nematicidal condition was obtained by using malt medium with stationary aerated cultures. Also, light had no effect on

toxic stability. Based on this, it is possible that different liquid media used for *P. lilacinus* and *P. chlamydosporia* growth may have different efficiency than what was presented in our work.

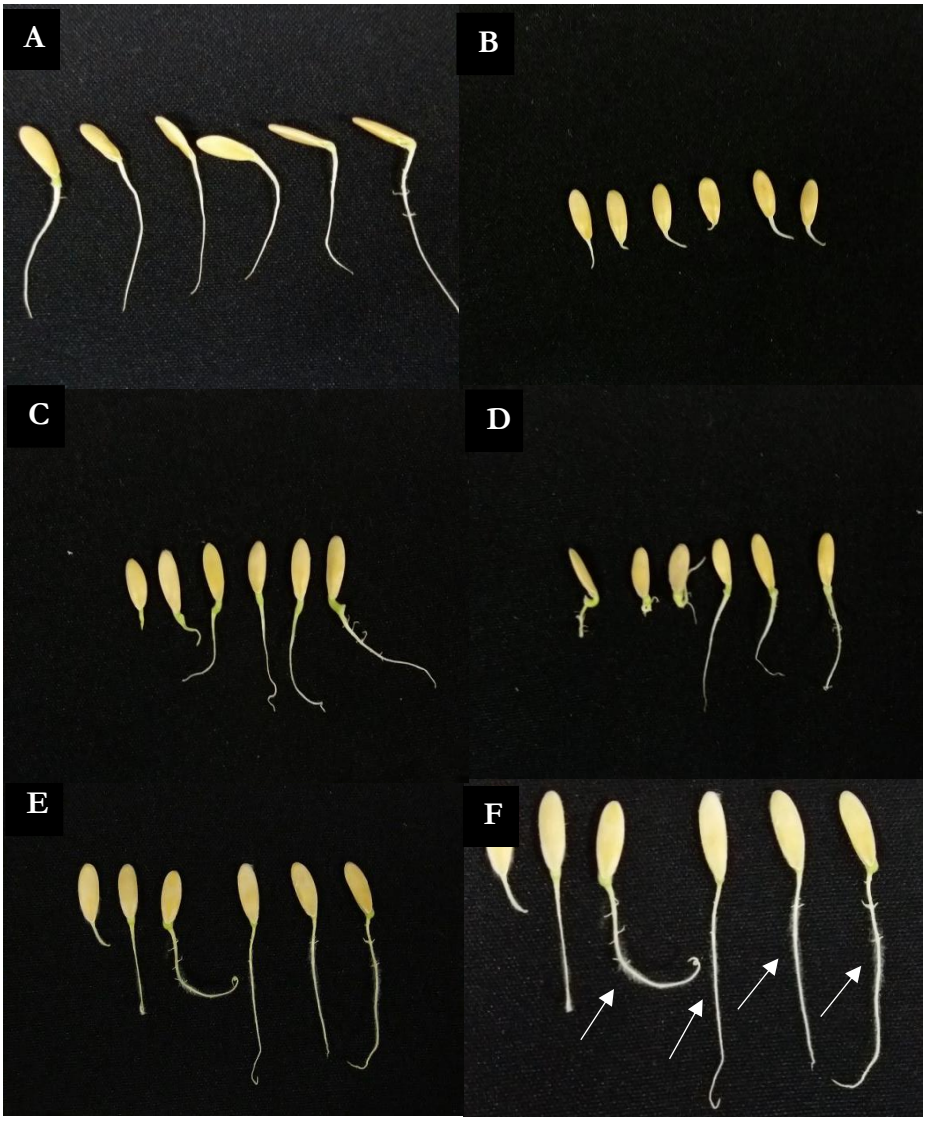

Figure 5. *Cucumis melo* seeds treated with filtrates obtained from biological control organisms 4 days after sowing. A) Water treated; B) Potato-Dextrose broth medium; C)*Bacillus amyloliquefaciens*.; D) *Paecilomyces lilacinus*; E) *Pochonia chlamydosporia*. F) Detail of the hairy roots of the seeds treated with *Pochonia chlamydosporia* filtrate (Arrows)

Vinale et al. (2008) investigated the role of *Trichoderma* secondary metabolites in the interaction with plants. Not only its metabolites induced resistance, but also an auxin-like activity was observed, which also affected the growth of *Pisum sativum* L. and *Brassica napus* L. seedlings. In the present work, both *P. lilacinus* and its culture filtrate increased fruit

weight (Table 3 and Table 12, respectively.). This is a strong indicative that some compounds, maybe an effector with hormone-like activity is present on the cell-free filtrates.

Regarding the inhibition of egg hatching by *P. chlamydosporia* some extracellular enzymes secreted by this fungus may play an important role in the infection of eggs (Huang et al. 2004). Also, specific chitinases and proteases have been isolated and their activities against nematode eggshell was reported (Segers 1996; Tikhonov et al. 2002). Besides, its parasitism on eggs and females, the fungus *P. chlamydosporia* also promoted growth of tomato and lettuce seedlings (Dallemole-Giaretta et al. 2015) by colonization of the rhizoplane. Furthermore, as a result of the interaction with the plant, it enhanced tolerance to biotic and abiotic stresses, and also increased plant growth (Escudero and Lopez-LLorca 2012; Dallemole-Giearetta et al. 2015; De Souza et al. 2015). Despite this, no currentling report involving *P. chlamydosporia* filtrates and growth promotion or control of root-knot disease is available. Our work showed that this filtrate stimulate hairy roots in melon plantule by seed treatment and did not affect the germination. This is in agreement with the behavior of *P. chlamydosporia*, which is a fungus that colonizes the rhizoplane and the root tissues in nature (Dallemole-Giaretta et al. 2015; Bordallo et al. 2002). As a result, this hairy root stimulation might be able to help plants to avoid stresses induced by plant parasitic nematode.

Culture filtrates constitute an interesting management tool for plant parasitic nematode control. They can exhibit desirable effects, such as the nematicidal effect and the influence in some plants characteristics. Here, we have found that one single application of the tested filtrates not only could reduce *M. incognita* reproduction, but they also showed effects on egg hatching, seed germination, and in some plant traits. The majority of reports regarding culture filtrates focus on their effects in nematodes egg hatching and in juveniles mortality after the exposure. These deleterious effects were not only reported for plant parasitic nematodes (Meyer et al. 2004; Sharma and Sharma 2017; Zaki 1999), but also for zoo parasitic nematodes (Braga et al. 2011a; Braga et al. 2011b). In addition to that, few reports are available regarding the influence of these culture filtrates on plant development or in the control of root-knot nematode. Our results showed that filtrates can be also used as seed treatment, since they enhanced the germination in comparison to the PD broth medium. Additionally, further investigation is needed aiming to verify if the treated seeds can exhibit some effect against *M. incognita* and/or if the treatment can improve the overall plant yield.

Table 9. Effect of filtrates from biological agent on melon seed germination at different periods.

Treatment	% of germinated seeds		
	2 days after sowing	3 days after sowing	4 days after sowing
Water (control)	74 A*	96 A	94 A
Potato – Dextrose medium (control)	16 B	35 B	60 B
Bacillus sp.	62 A	75 A	88 A
Paecilomyces lilacinus	42 B	68. A	90 A
Pochonia chlamydosporia	62 A	86 A	90 A

*Numbers followed by the same letters did not differ statistically (Scott-Knott at 5%).

Table 10. Effects of filtrates obtained from biological control agents on egg hatching, inhibition and number of second stages juveniles, seven days after washing followed by hatching chamber

Treatment	Experiment 1		Experiment 2			Experiment 3		
	%Hatching	%Inhibition	%Hatching	%Inhibition	N° of J2 - after hatching chamber	%Hatching	%Inhibition	N° of J2 - after hatching chamber
Water (Control**)	100.0 A*	0.0 A*	100.0 A*	0.0 A	33	100.0 A*	0.0 A	
Potato-Dextrose medium (Control)	-	-	16.7 B	83.3 B	0	54.9 B	45.1 B	
Methanol	-	-	13.6 B	86.4 B	0	-	-	
B. amyloliquefaciens filtrate	17.1 B	82.9 B	15.7 B	84.3 B	0	49.8 B	50.2 B	Not observed
P. lilacinus filtrate	12.1 B	87.9 B	31.8 B	68.2 B	0	5.7 B	94.3 B	
P. chlamydosporia filtrate	-	-	16.5 B	83.5 B	0	21.5 B	78.5 B	
PPT	-	-	19.4 B ^{meth}	80.6 B ^{meth}	0	126.9 A ^{water}	-26.9 A ^{water}	

*Numbers followed by the same letters did not differ statistically (Scott-Knott at 5%). ^{meth}: PPT in methanol; ^{water}: PPT in water; **Control taken as 100% hatching. - It was not tested in the experiment.

Table 11. Effect of culture filtrate on *Cucumis melo* plants non-inoculated or inoculated (+N) with *Meloidogyne incognita* (1000 individuals). Evaluations carried out at 40 days after the inoculation procedure (Experiment 1).

Treatment**	Fresh weight (g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	Stem diameter (mm)			Chlorophyll content in the leaves***	
						Basis	Middle	Apex	Old	Young
Water	89.3 A*	11.4 A	119.9 A	15.1 B	2.1 B	5.0 A	4.5 A	1.8 A	31.7 A	31.2 A
Water + N	86.7 A	12.0 A	120.7 A	17.5 B	0.0 B	5.8 A	5.1 A	1.9 A	23.6 A	32.7 A
Bacillus filtrate	105.6 A	15.8 A	140.1 A	25.7 A	0.5 B	5.8 A	5.21 A	2.3 A	32.1 A	33.6 A
Bacillus filtrate + N	102.3 A	12.1 A	139.7 A	21.9 A	0.0 B	5.9 A	5.3 A	2.3 A	37.3 A	40.1 A
P. lilacinus filtrate	87.3 A	11.6 A	128.8 A	16.5 B	50.6 A	6.2 A	5.1 A	2.4 A	29.9 A	36.2 A
P. lilacinus filtrate + N	73.9 A	10.8 A	123.5 A	15.4 B	27.6 B	5.6 A	4.8 A	2.0 A	27.8 A	33.7 A

*Numbers followed by the same letters did not differ statistically (Scott-Knot at 5%). ** Treatments applied 24 hours before the inoculation procedure ***Chlorophyll content of the leaves expressed in SPAD units.

4.1. Effect of resistance inducers on root-knot nematode penetration and post-penetration on melon seedlings

The obtained data of the ASM and PPT experiment are summarized in Figure 6 and Table 14. The penetration occurred on all experiments and juveniles developed properly. However, the numbers of nematodes inside roots, at the end of each experiment were 13.8%, 9.3% and 30% of the inoculated amount, for the Bth experiments (1 and 2) and PPT experiment, respectively.

Regarding ASM, no statistical differences were found in the number of nematodes per root system, total of galls (Gt) and proportion of nematodes per stage (J2 and swollen) on both experiments (Figure 4). Also, no juveniles were observed at 3 DAI in both experiments. Empty galls (Eg) were observed and counted in both ASM experiments, on untreated and ASM treated melon plants, and did not differed statistically. In experiment 1, the percentage of Eg was 12.5% and 16.8%, at 5 DAI, and 11% and 20%, at 7 DAI, for non-treated and Bth treated plants, respectively (Figure 4). In experiment 2, untreated plants and ASM treated plants presented 0% and 12%, at 5 DAI, and 14.2% and 20.2%, at 7 DAI (Figure 4). Furthermore, under microscopy observation, Eg presented darker color in comparison to normal galls, and reminiscences of the nematodes could be observed (Figure 7A and 7B). No differences were found on Gt and Eg among the treatments, but as expected the number of Gt and Eg increased, as further assessments were carried out. At 14 DAI, the percentage of Eg obtained for non-treated and thaxtomine treated plants were 17.8 and 19.3, respectively.

Table 12. Effect of culture filtrate on *Cucumis melo* plants non-inoculated and inoculated (+N) with *M. incognita* (1000 individuals) of *Meloidogyne incognita*. Evaluation carried out at 60 days after the inoculation procedure (Experiment 2).

Treatment**	Fresh weight (g)	Dry weight (g)	Height (cm)	Root weight (g)	Fruit weight (g)	Stem diameter (mm)			Chlorophyll content in the leaves***	
						Basis	Middle	Apex	Old	Young
Water	62 A*	8.3 A	101.7 A	12.1 A	158.4 A	5.1 A	4.3 A	2 A	13.2 A	20.8 A
Water + N	71.4 A	8.4 A	102.4 A	13.5 A	148.4 A	4.7 A	4.1 A	1.9 A	14.2 A	25.4 A
Potato dextrose medium	73.3 A	9.3 A	100.8 A	13.7 A	151.9 A	4.6 A	4.3 A	1.5 B	19.2 A	25.9 A
Potato dextrose medium+ N	59.6 A	7.6 A	98 A	11 A	132.9 A	4.6 A	4.1 A	1.5 B	15.2 A	26.2 A
B. amyloliquefaciens filtrate	62.6 A	8.4 A	94 A	13.5 A	170.4 A	5.1 A	4.2 A	1.5 B	16 A	23.5 A
B. amyloliquefaciens filtrate + N	80.3 A	9.8 A	98.1 A	15.2 A	166.5 A	5.4 A	4.4 A	1.5 B	17.8 A	25.3 A
P. lilacinus filtrate	69.1 A	8.3 A	99.8 A	10.8 A	188.8 A	4.8 A	4.3 A	1.4 B	13.4 A	25.3 A
P. lilacinus filtrate + N	63.9 A	7.7 A	89.7 A	13.8 A	134.4 A	4.8 A	4 A	1.5 B	22.8 A	27.9 A
P. chlamydosporia filtrate	60.7 A	8.1 A	97.8 A	14.3 A	161.7 A	4.7 A	4.2 A	1.1 B	17.7 A	24.4 A
P. chlamydosporia filtrate +N	56.8 A	7.8 A	85.7 A	11.9 A	109.1 A	5 A	4.6 A	1.7 A	19.8 A	24.5 A

*Numbers followed by the same letters did not differ statistically (Scott-Knot at 5%). ** Treatments applied 24 hours before the inoculation procedure ***Chlorophyll content of the leaves expressed in SPAD units.

Table 13. Final population (Pf), Reproduction factor (R value), number of nematodes per gram of root (Nem/g) and percentage of reduction (%reduction) obtained from *Cucumis melo* plants treated with filtrates from biological control organism and inoculated with *Meloidogyne. Incognita* (+N) (1000 individuals). Evaluations were carried out at 40 days (Exp.1) and 60 days (Exp.2) after the inoculation procedure.

Treatment**	**Pf**		**R value**		**Nem/g**		**% Pf reduction****	
	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
Water + N	1,463.7	4,981.4	1.5 a	5.0 a	61 ab	575 a	0	0
Potato Dextrose medium	-	2,635.4	-	2.6 ab	-	356 ab	-	47.1
Bacillus amyloliquefaciens filtrate + N	349.54	2,611.2	0.3 b	2.6 b	31 b	303 b	76.1	47.6
Paecilomyces lilacinus filtrate+ N	1,008.07	2,971.8	1.0 ab	3.0 ab	106 a	421 ab	31.1	40.3
Pochonia chlamydosporia filtrate +N	-	2,988.3	-	3.0 ab	-	309 ab	-	40.0

*Numbers followed from the same letters did not differ statistically (Duncan at 5%). ** Treatments applied 24 hours before the inoculation procedure ***Control taken as 100%.

The empty galls observed in our work were also reported for resistant and susceptible melon plants infested with *M. incognita* (Faske 2013). Additionally, Mc Clure et al. (1974a) reported galls frequently found "empty", which did not appear to contain any nematode, in cotton roots infested with *M. incognita*, but no attempt was made to determine the relative number of these structures. Eg were observed as a biological response of *C. melo* var *texanus* and *C. metuliferus* against *M. incognita*, and they may have contained males that eventually left the root (Faske 2013). Moreover, *C. metuliferus* resistance is related to an increased stimulation of juveniles toward maleness (Fassuliotis 1970). Other explanation is the possibility of juveniles emigrate from the roots, as they fail to establish a properly feeding site (Faske 2013). Furthermore, our results are in agreement with Mc Clure et al. (1974b), which showed remnants of the nematode, associated to Eg. Moreover, it was reported that Eg contains senescent syncytia that often left large cavities in the roots, upon collapse.

On PPT experiment, statistical difference was found at 10 DAI, on number of nematodes, but not at 14 DAI (Table 14). In fact, on TPP treated plants, a dramatic increase of the amount of nematodes was found from 7 to 10 DAI, which was 12 and increase to 31 nematodes, respectively. This value represents an increase of 158%. Furthermore, this increase also happened in non-treated plants, but occurred from 10 to 14 DAI, and the values went from 12 to 33.5, respectively, which is an increment of 179%. Additionally, the number of galls followed the same pattern, differing statistically only at 10 DAI. Faske (2013) reported differences on penetration, post-penetration and reproduction of *M. incognita* in susceptible and resistant melon plants. Interesting, more juveniles penetrated the resistant species (*C, metuliferus*) rather than the susceptible melon (*C. melo* 'Hales Best Jumbo') at 3 DAI, but fewer J2 were found in the root system of the resistant genotypes. Furthermore, a delayed rate of nematode development was observed at 7, 14 and 21 DAI, which contributed to increased egg production. The author further discussed that the mechanisms of resistance was related to three different effects on *M. incognita*: 1) a reduction in root penetration; 2) Most of the J2 failed to establish a feeding site; 3) A delayed rate in the development to reach maturity. Unfortunately, none of these points were observed in our work. However, they were confirmed by other researchers using resistance inducers against several species of plant parasitic nematodes (Daneshkhah et al. 2013; Oka et al. 1990, Herman et al. 1991; Fassuliotis 1970; Walters et al. 2006).

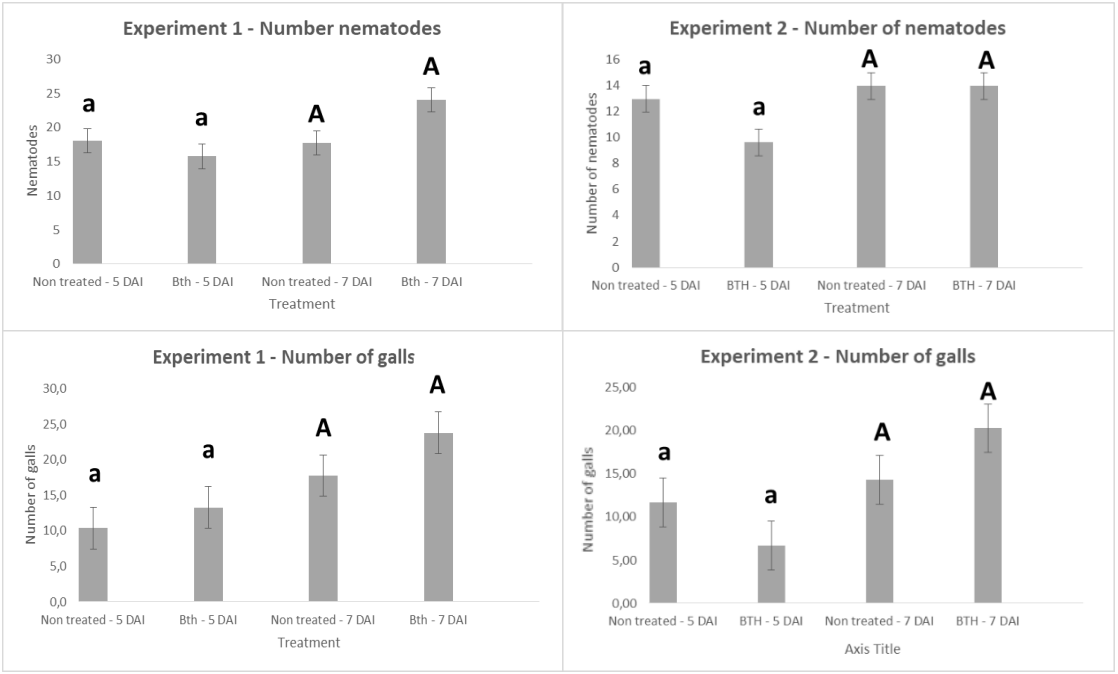

Figure 6. Number of nematodes and number of galls obtained from benzothiadiazole (Syn.: acibenzolar-S-methyl) treated and non-treated *Cucumis melo* plants at 5 and 7 days after inoculation (DAI) with second stage juveniles of *Meloidogyne incognita*. Bars with the same letter (lowercase for 5 DAI and uppercase for 7 DAI) did not differ statistically (Scott-Knott at 5%).

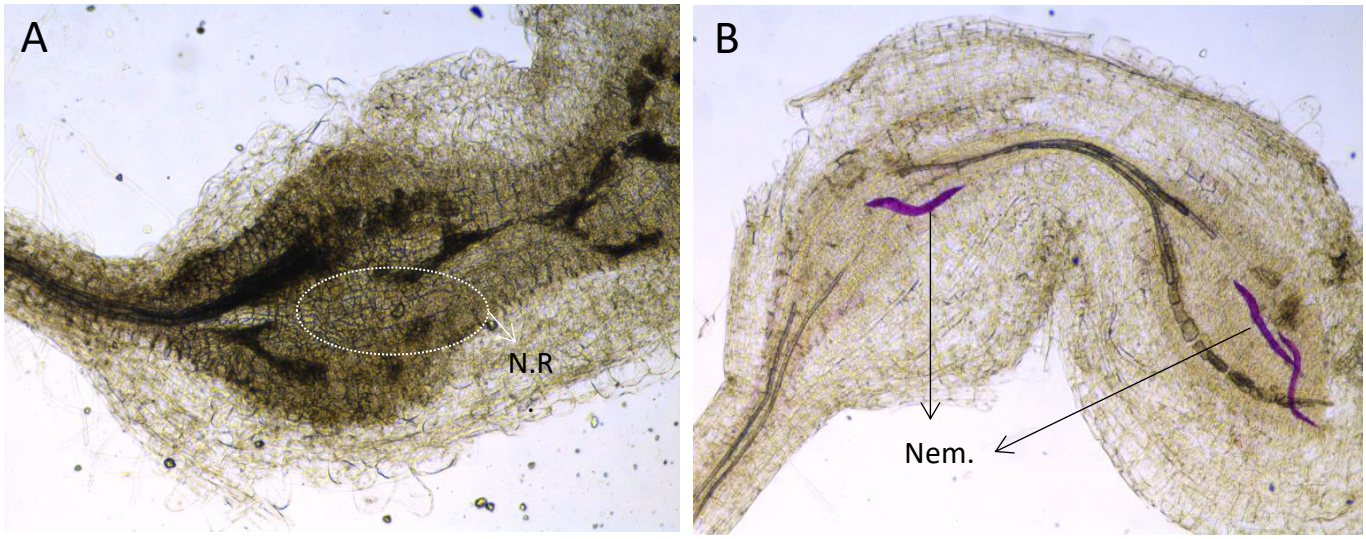

Figure 7. Galls observed under light microscopy 5 days after inoculation (x1000 magnification). A) Empty galls containing nematode reminiscence; B) Galls containing nematodes; Nem: Nematodes; N.R.: Nematode reminiscence; Staining: fuchsin acid.

Table 14. Total of nematodes, number of galls and percentage of empty galls observed in non-treated (NT) or partially purified thaxtomine A (T) treated *C. melo* plants at different days after inoculation (DAI).

DAI	Total of nematodes (average)		Number of galls		%Empty galls	
	NT	T	NT	T	NT	T
3 DAI	1.5 a A	1.25 a A	0.0 a A*	0.0 a A	0.0	0.0
5 DAI	6.25 a A	6.0 a A	4.0 a A	5.0 a A	22.5	3.0
7 DAI	9.0 a A	12.0 a A	7.0 a A	10.0 a A	13.8	4.1
10 DAI	10.0 a A	31.0 b B	12.0 a A	33.0 b B	22.7	14.5
14 DAI	34.0 b A	26.0 b A	40.0 bA	33.0 bA	17.8	19.2

*Numbers followed by same letters did not differ statistically. Uppercase: only comparison at the same DAI; lower case: entire column.

In our greenhouse assays, ASM and PPT were able to reduce the Pf of *M. incognita* twice, but no evidence of which stages these elicitors affects was found. The establishment of the interaction nematode-plant involves several phases: (1) Attraction, recognition and penetration; (2) Movement in the axial region; (3) Definition of plant tissue for feeding; (4) Giant cells induction; (5) Feeding from these specialized cells and ontogenesis (Salgado and Silva 2005). Between each of these phases of root-knot parasitism, the activation of defense mechanism may occur (Faria et al. 2003).

Regarding ASM, as already stated, no differences were found in the accessed periods, which points that ASM has a delayed effect in the melon plants and *M. incognita* interaction, since alterations in penetration and/or development of the nematode were not observed. Oka et al. (1999) reported that foliar spray and soil-drenching with DL – β-amino-*n*-butyric acid (BABA) on tomato plants, infested with *M. javanica*, reduced both root-galling 7 days after inoculation and the number of eggs 30 days after inoculation. Also, on BABA treated plants it was observed lower penetration and development of the juveniles. Other chemicals, as salicylic acid, were inefficient or phytotoxic to tomato plants. Besides, Owen et al. (1998) concluded that the induced resistance by ASM affected the physiology of the root-

knot feeding cells and, consequently, this lead to poor feeding and development. On this regard, Veronico et al. (2018) revealed based upon histochemical analysis the accumulation of high lignin levels at later infection stages in ASM-treated galls in comparison to untreated ones. Taking our results and the literature available, further investigations should be performed by using ASM, but longer time periods (>7 DAI) should be sampled.

Regarding PPT, our greenhouse experiments and the penetration assay clashes in the obtained data. The increase of nematodes first observed in PPT treated plants and then observed in untreated plants seems to indicate that our treatment induced susceptibility. However, as already stated, in two greenhouse assays, PPT presented lower Pf in comparison to water treated plants. Three hypothesis were build up to explain the discrepancy in the obtained data. The first one concerns the spray procedure in this experiment. In the greenhouse assays, plants were treated with PPTs by foliar sprays, with seven days of interval between each of them. In comparison, in this penetration assay, a single foliar spray was performed 24 hours before the inoculation procedure. Additionally, the increase of nematodes in treated plants occurred between 7 – 10 DAI, which coincide with the second spraying. The induced resistance has several advantages, such as effectiveness against a broad range of pathogens, systemicity and stability of the resistance. However, in some cases, the resistance can be incomplete, needing to be reactivated (Silva and Resende 2001). A classic experiment carried out by Underwood (1997) showed the importance of the timing of induced resistance for herbivores. It was verified that all four soybean genotypes showed resistance against beetles up to 3 days after induction. However, after 15 days, the resistance decreased and all genotypes showed high susceptibility to the herbivores. It is possible in our case, that another stimulus or reinforcement could have improved the melon resistance against the second stage juveniles.

The second hypothesis can be related to the different equivalent concentrations of thaxtomin A. The concentration equivalent of thaxtomin A in trials #1 and #3 were ~81µg and ~100 µg, respectively. In the penetration experiment, the concentration equivalent of thaxtomin A was ~248µg. Different concentrations can lead to different reactions by the plant. Garcia et al. (2008b) showed that high concentrations of thaxtomin A decreased the amount of proteins per gram in cucumber plants. Also, concentration equivalent of 100µg of thaxtomin A decreased the chlorophyll content in sorghum (*Sorghum bicolor* (L.) Moench), when compared to the 50 µg dose. The same pattern was observed for phenylalanine ammonia-lyase (PAL) activity, which was higher when the 50 µg concentration was used. It is important to mention that no example was found in literature of an inducer, which

depending of the concentration, cause a resistance/susceptibility phenotype. Most of the reports in literature concerns the fact that the same resistance inducer can have different effects against different plant pathogens or in different cultivars. For example, Cordova-Campos et al. (2012) reported that ASM treatment induced resistance in wild accessions of common bean against *Enterobacter* sp. (Strain: FCB1), but induced susceptibility in yield-improved cultivars against the same pathogen. Casarrubias-Castillo (2014) showed that different inducers can have different effects, for example, whereas an incompatible pathogen (*Pseudominas syrangae* pv. *syrangae*) induced resistance in *Amaranthus cruentus* against *Clavibacter michiganensis* subsp. *michiganensis*, the ASM treatment induced susceptibility. On the other hand, pathogens that can predispose plant tissues to other pathogens are less reported, such as *Helminthosporium maydis* (susceptible interaction) that could alter the host plant and make it susceptible to *H. carbonum* (resistant interaction), which was attributed to the lower anthocyanin content in maize mesocotyls (Pascholati and Nicholson 1983).

A third hypothesis is also possible. The apparent faster cycle or the lack of resistance mechanisms did not reflect the egg production of the females. As previously explained, the PGPR can interfere in *Meloidogyne* spp. reproduction trough poor development of the giant cells that provide poor amount of food, which is not enough for a massive egg production (Ferraz et al. 2012). In accordance to this, treatment with the resistance inducers salicylic and jasmonic acid on tomato plants (Mi+ inactivated) lead to less fertile females of *M. incognita* (Zinovieva et al., 2013).

5. FINAL CONSIDERATIONS

All accessed melon cultivars were classified as susceptible when infested with *M. incognita*. No statistical difference was found for the R value. On the other hand, statistical difference was obtained from Nem/g, with the lowest and the highest being 'AF 11682' and 'AF 4345', respectively.

A summary of the effect of resistance inducers, biological control agents and their filtrates is presented below (Table 15).

Regarding the trial #1 – #4 (item 3.2), severe above-ground symptoms were not observed on the present work, probably due to the seedlings age at the inoculation moment.

Few information are available about alternative control of *M. incognita* on melon. Regarding the greenhouse experiments, both resistance inducers and biological control agents exhibited influence on some characteristics of *C. melo* development, and showed potential on the control of the root-knot nematode. On trial #1, melon plants treated with resistance inducers presented lower height; despite no statistical differences were found for fresh and dry weight. Also, ASM (inoculated and non-inoculated), *P. lilacinus* (non-inoculated) and *P. chlamydosporia* (inoculated) treated plants produced more gram of fruits per plant. On trial #2, all inoculated melon plants differed statistically and presented heavier roots than non-inoculated plants and this can be attributed to the root-knot nematode development. Despite that, plants treated with biological control agents presented fewer symptoms when compared to control plants. Also, all treated plants exhibited higher chlorophyll content on young leaves than both controls. Moreover, in both trials #1 and #2, the *M. incognita* population was reduced, except by *P. chlamydosporia* on the trial #1. In trial #3, PPT and ASM treatments (+N) presented shorter plants, but only TPP (-N) presented higher shoot diameter (middle). Regarding trial #4, dose 0, dose 1 and dose 5 (-N and +N) presented more chlorophyll content on young leaves.

Regarding control, from all treatments *B. amyloliquefaciens* was the most efficient treatment and reduced the *M. incognita* population twice and differed statistically from the control. Additionally, *P. lilacinus* and *P. chlamydosporia* presented less Nem/g values in trial #1 and #2, respectively. Regarding the resistance inducers, both ASM and PPT treatments reduced the Pf of the treated plants, presenting lower R value and Nem/g in trial #3, respectively.. The TPP treated plants presented lower Nem/g, however, even presenting lower R value and Pf, it did not differed statistically from the control.

Concerning trial #4, Rizotec® doses and *M. incognita* population showed negative correlation (-0.88). The doses 4 (1g per plant) and 5 (2g per plant) differed statistically in Nem/g and R value, respectively. Only the dose 5 presented high chlorophyll content in both inoculated and non-inoculated plants.

Regarding culture filtrates, promising results were obtained. Both *P. lilacinus* and *B. amyloliquefaciens* reduced the *M. incognita* population, in greenhouse experiment. In addition, filtrates obtained from *P. lilacinus* and *B. amyloliquefaciens* increased the fruit weight per plant and root weight, respectively, despite the melon plants being inoculated or not with *M. incognita*. Furthermore, the tested filtrates presented suppressive effect on *M. incognita* egg hatching, but further evidence is necessary due to lack of statistical differences with the potato-dextrose broth medium. Additionally, the tested filtrates improved the germination of melon seeds, despite the suppressed effect of PD broth medium on them. Also, *P. chlamydosporia* treatment induced hairy roots, which were not observed on the other treatments.

Regarding the *in vitro* experiments, all treatments affected the egg hatching, except the PPT in water. The filtrates obtained from *B. amyloliquefaciens*, *P. lilacinus* and *P. chlamydosporia* suppressed the egg hatching in the three experiments.

Regarding the penetration assays, no effect of ASM on penetration and post-penetration of *M. incognita* J2 was observed, needing additional assays with longer sampling times. Concerning PPT, our data can indicate a susceptible induction on melon, but further evidence is needed.

In conclusion, the obtained data point out the potential of the resistance inducers, biological control organisms and their culture filtrate to control *M. incognita* on melon. With little information available, our results are a milestone for the alternative control of *M. incognita* on *C. melo*. Here, we showed that resistance inducers, biological control agents and the filtrates could improve some of *C. melo* characteristics and suppressed root-knot nematode population. Moreover, this is the first work showing the effect of PPT on plant parasitic nematodes.

Table 15. Summary of the obtained data regarding resistance inducers, biological control agents and their filtrates in several parameters of melon plants and *Meloidogyne incognita*.

Treatment	Fresh/ dry weight	Height	Root weight	Fruit weight	Shoot diameter		Chlorophyll content			*Meloidogyne incognita*		
					Basis	Middle	Apex	Old	young	Egg hatching	Final Population	Symptom (galls)
Control	The control plants were the standard for comparison											
ASM	- -	Reduced (-N)(+N)	-	Increased (+N)	-	-	-	Increased **(+N)	-	Not tested	Reduced	-
Bacillus amyloliquefaciens	- -	-	Increased (-N)(+N)	-	-	-	-	Increased **(+N)	Increased (-N)(+N)	inhibited	Reduced	Reduced
Filtrate *B. amyloliquefaciens*	- -	-	Increased (-N)(+N)	-	-	-	Reduced (-N)(+N)	Increased **(+N)	-	inhibited	Reduced	-
Paecilomyces lilacinus	- -	-	-	Increased (-N)	-	-	-	Increased **(+N)	Increased (-N)(+N)	inhibited	Reduced	Reduced
Filtrate *P. lilacinus*	- -	-	-	Increased (-N)	-	-	Reduced (-N)(+N)	Increased **(+N)	-	inhibited	Reduced	-
Pochonia chlamydosporia	- -	-	-	Increased (-N)(+N)	-	-	-	Increased **(+N)	Increased (-N)(+N)	inhibited	Reduced	Reduced
Filtrate *P. chlamydosporia*	- -	-	-	-	-	-	Reduced (-N)	Increased **(+N)	-	inhibited	Reduced	-
PPT	- -	Reduced (-N)(+N)	-	-	-	Increased (+N)	-	Increased **(+N)	-	No effect	Reduced	-

Legend: "-"No differences were observed. Reduced/increased: treatments showed statistical difference at least in one experiment. ASM: acibenzolar-S-methyl. PPT: partially purified thaxtomin A. Differences observed in non-inoculated plants (-N) and inoculated plants (+N). ** (+N) when only compared to inoculated control plants in trial #1.

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