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Pasteuria thornei, a novel biological seed treatment for root lesion nematode control in soybean and maize

Pedro Marcus de Souza Confort

Dissertation presented to obtain the degree of Master in Science. Area: Plant Pathology

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To Friends and Family

Close and Far

I Dedicate this work.

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"The cave you fear to enter holds the treasure you seek"

-Joseph Campbell

CONTENTS

RESUMO

Pasteuria thornei, um novo tratamento de sementes para o controle biológico de nematoides das lesões radiculares em soja e milho

O gênero *Pasteuria* compreende bactérias gram-positivas parasitas obrigatórias de artrópodes e nematoides. A distribuição das espécies deste gênero pelo mundo é ubíqua, podendo ser encontradas em ambientes aquáticos e terrestres. Este gênero foi descrito no final do século XIX e sofreu consideráveis reclassificações em relação às espécies nele compreendidos. A partir da década de 80, deu-se início a um esforço de classificação mais minucioso com relação à identificação de *Pasteuria* spp. e seus hábitos parasitários. Estes estudos foram motivados, principalmente, pela capacidade dos indivíduos deste gênero em parasitar nematoides fitoparasitas de diversas culturas. Cada espécie do gênero *Pasteuria* estabelece relações parasitárias com um gênero específico de fitonematoide. A exemplo desta interação, *Pasteuria thornei* é um parasita restrito ao gênero *Pratylenchus*, que compreende os nematoides causadores das lesões radiculares, daninhos a diversas culturas de importância agronômica. Considerando a relevância atual de estudos envolvendo o controle biológico de fitonematoides, no presente trabalho foram realizados três experimentos, cada um contendo uma réplica em época distinta, totalizando, portanto, seis experimentos. Dois experimentos tiveram por objetivo verificar a eficácia de *P. thornei* como agente de controle biológico (ACB) de *Pratylenchus brachyurus* na cultura da soja. E os demais quatro experimentos abordaram o patossistema *Pratylenchus zeae*-milho. Para esse objetivo, foram realizados dois experimentos com o intuito de verificar a eficácia de *P.thornei* como agente de controle biológico de *P. zeae* em milho, e outros dois experimentos para testar a capacidade do ACB em reduzir a perda de produtividade em plantas de milho decorrente do parasitismo do nematoide. Para os experimentos de soja, às sementes da cultivar SYN1080 foram adicionados os tratamentos como se segue: três concentrações de endósporos de *P.thornei* por semente ($5x10^6$, 10^7 e $1,5x10^7$), um grupo de controle químico comercial para comparação contendo abamectina (0,58 mg / semente) e um tratamento misto contendo abamectina (0,58 mg / semente) e 10^7 endósporos de *P.thornei*. Sementes não tratadas foram utilizadas como testemunha. As sementes tratadas foram semeadas em copos de plástico de 500 cm^3 contendo solo inoculado com 1000 nematoides (experimento 1) e 600 nematoides (experimento 2). A massa de raiz fresca e os nematoides extraídos das raízes de cada planta foram utilizados como critério de avaliação dos experimentos, a qual foi realizada aos 60 e 90 dias após a inoculação (DAI). Apenas o tratamento com a maior concentração de *P. thornei* ($1,5x10^7$) reduziu a população final de nematoides de maneira significativa atingindo 30-50% de redução, comparado àquele contendo sementes não tratadas. No entanto, os tratamentos que contém abamectina foram superiores na redução da população final de nematoides em todos os experimentos avaliados. Em relação aos experimentos de eficácia em milho, sementes do híbrido CELERON foram tratadas como explicitado: quatro concentrações de endósporos de *P.thornei* por semente ($5x10^6$, 10^7, $1,5x10^7$ e $2x10^7$), um grupo de controle comercial para comparação contendo abamectina (0,58 mg / semente) e um tratamento misto contendo abamectina (0,58 mg / semente) e 10^7 endósporos de *P.thornei*. As sementes tratadas de milho foram semeadas em copos de plástico de 500cm^3 contendo solo inoculado com 4000 e 1000 indivíduos para os experimentos de eficácia 1 e 2, respectivamente. As avaliações ocorreram aos 60 e 90 DAI. Para os estudos de produtividade, foram realizados os experimentos 3 e 4 sob um telado com parcelas experimentais constituídas por vasos de 9L preenchidos de solo infestado artificialmente. Sementes do híbrido CELERON foram utilizadas contendo os seguintes tratamentos: abamectina (0,58mg / semente), *P. thornei* (10^7 endósporos/semente) e um tratamento misto contendo abamectina (0,58mg / semente) e *P. thornei* (10^7 endósporos/semente). Dois tratamentos adicionais contendo sementes não

tratadas serviram de testemunhas, com e sem *Pratylenchus zeae*. A avaliação consistiu na medição de várias características agronômicas, como peso seco da parte aérea, massa fresca de raízes no momento da colheita e peso total dos grãos. Adicionalmente, foi mensurada a população de nematoides em raízes frescas aos 45, 90 dias e no momento da colheita. Os ensaios de eficácia mostraram que as concentrações mais elevadas de *P.thornei* ($1,5x10^7$ e $2x10^7$) possuem um potencial mensurável de controle de *P.zeae*. A redução da população de nematoides foi de 54 e 47% nos experimentos 1 e 2, respectivamente. A formulação comercial de abamectina mostrou uma redução da população de nematoides superior a 90% em ambos os experimentos. No que diz respeito aos experimentos de produtividade de milho, o potencial de controle de nematoides por *P.thornei* foi semelhante ao observado no estudo de eficácia. O tratamento com abamectina teve efeito na redução das perdas de rendimento causadas por *P.zeae* em ambos os experimentos; assim como os tratamentos misto (abamectina e *P. thornei*) e aquele contendo apenas *P.thornei* que apresentaram desempenho positivo em ambas as repetições. Em nenhum dos experimentos foi observado efeito sinérgico ou aditivo entre *P. thornei* e abamectina. Com os dados obtidos nestes experimentos, fica evidente o potencial de controle de *P.thornei* sobre *P. brachyurus* e *P.zeae* em soja e milho, respectivamente. Ainda, tanto *P. thornei* quanto abamectina apresentam o potencial de mitigar as perdas de rendimento causadas por *P.zeae* em milho através do tratamento de sementes. Isso evidencia a importância de *P.thornei* como uma ferramenta adicional para o manejo desses nematoides, e deve encorajar trabalhos subsequentes.

Palavras-chave: *Pratylenchus brachyurus*; *Pratylenchus zeae*; Controle biológico; Abamectina

ABSTRACT

Pasteuria thornei, a novel biological seed treatment for root lesion nematode control in soybean and maize

The *Pasteuria* genus comprises gram-positive bacteria that are obligate parasites of arthropods and nematodes. Species of this genus are ubiquitous, being present in both aquatic and terrestrial environments all around the world. *Pasteuria* was first described as a genus at the end of the 19th century and has undergone considerable reclassification regarding its member species. Starting in the 1980s, a more meticulous classification effort regarding the identification of *Pasteuria* spp., and its parasitic habits began. These studies were strongly motivated by the ability of individuals of this genus to parasitize phytopathogenic nematodes of several plant species. Each species of the genus *Pasteuria* establishes a strict parasitic relationship with a specific genus of phytonematode. As an example of this interaction, *Pasteuria thornei* is a parasite restricted to the genus *Pratylenchus*, which comprises the nematodes popularly known as root-lesion-nematodes, a pest of several agronomically important crops. Considering the current relevance of studies involving the biological control of phytonematodes, in the present work three experiments were carried out, each one containing a replicate, totaling, therefore, six experiments. Two experiments were intended to verify the efficacy of *P. thornei* as a biological control agent (BCA) of *Pratylenchus brachyurus* in soybean. The remaining four experiments had a similar objective in the scope of the *Pratylenchus zeae* - maize pathosystem. Two experiments were carried out to verify the efficacy of *P. thornei* as a biological control agent for *P. zeae* in maize, and afterwards, two additional experiments were performed in order to verify the capacity of the BCA to reduce productivity losses in corn plants due to the parasitism of this nematode. For the soybean experiments, the following treatments were added to the seeds of the cultivar SYN1080: three different concentrations of *P.thornei* endospores per seed ($5x10^6$, 10^7 e $1,5x10^7$), a commercial control group for comparison containing abamectin (0.58mg/seed) and a mixed treatment containing abamectin (0.58 mg / seed) and 10^7 *P. thorne*i endospores. Untreated seeds were used as a control group. The treatments were sown in 500 cm³ plastic cups containing soil inoculated with 1000 nematodes (experiment 1) and 600 nematodes (experiment 2). Fresh root mass and nematodes extracted from the roots of each plant were used as parameters of evaluation, taking place 60 and 90 days after inoculation (DAI). Only the treatment with the highest concentration of *P. thornei* ($1.5x10^7$) reduced the final population of nematodes significantly, reaching 30-50% of reduction compared to the untreated seeds. However, treatments containing the commercial control abamectin were superior in reducing the final population of nematodes in all experiments evaluated. Regarding the maize efficacy experiments, CELERON hybrid seeds were treated as described: four concentrations of *P. thornei* endospores per seed ($5x10^6$, 10^7, $1,5x10^7$, $2x10^7$), a commercial control group for comparison containing abamectin (0.58 mg / seed) and a mixed treatment containing abamectin (0.58 mg / seed) and 10^7 *P. thornei* endospores. Untreated seeds were used as a control group. The treated maize seeds were planted in 500 cm³ plastic cups containing soil inoculated with 4000 and 1000 individuals for the efficacy experiments 1 and 2, respectively. Evaluations occurred at 60 and 90 DAI. For the productivity assays, the experiments 3 and 4 were carried out under a screened greenhouse, with experimental plots consisting of 9L pots filled with artificially infested soil. Seeds of the CELERON hybrid received the following treatments: abamectin (0.58mg / seed), *P. thornei* (10^7 endospores / seed) and mixed treatment containing both abamectin (0.58mg / seed) and *P. thornei* (10^7 endospores / seed). Two additional treatments containing untreated seeds served as controls, with and without the presence of *Pratylenchus zeae.* The evaluation measured several agronomic traits, such as dry weight of the aerial parts, fresh mass of roots at harvest and total weight of grains. In addition, the nematode population was measured in fresh roots at 45, 90 days and at the time of harvest. Efficacy trials showed that the highest concentrations of *P. thornei* ($1.5x10^7$ and $2x10^7$) have

a considerable potential of *P.zeae* control. The nematode population reduction was 54 and 47% in experiments 1 and 2, respectively, for the highest *P. thornei* concentration treatment. The commercial formulation containing abamectin showed a reduction of *P. zeae* population above 90% in both experiments. Regarding the maize productivity experiments, control potential of nematodes by *P.thornei* was similar to that observed in the efficacy study. The treatments containing abamectin had an effect on the mitigation of yield losses caused by *P.zeae* in both experiments. The mixed treatment (abamectin and *P. thornei*) and the one containing exclusively *P.thornei* presented a positive performance in both replicates. In none of the experiments synergistic or additive effects were observed between *P. thornei* and abamectin. With the data obtained in these experiments, the control potential of *P.thornei* on P. *brachyurus* and *P.zeae* in soybean and corn, respectively, is evident. Additionally, *P. thornei* and abamectin in the form of seed treatment, show potential in mitigating yield losses caused by *P. zeae* in maize. This highlights the importance of *P.thornei* as an additional tool for the management of root lesion nematodes in soybean and maize, and should encourage subsequent work.

Keywords: *Pratylenchus brachyurus*; *Pratylenchus zeae*; Biological Control; Abamectin

1. *Pasteuria thornei*, A NOVEL BIOLOGICAL SEED TREATMENT FOR *Pratylenchus brachyurus* CONTROL IN SOYBEAN

ABSTRACT

The goal of this first study was to evaluate the efficiency of *Pasteuria thornei* as a biological seed treatment for *Pratylenchus brachyurus* control in soybean (*Glycine max*). Seeds of the soybean cultivar SYN1080 were treated with three concentrations of *P. thornei* endospores per seed ($5x10^6$, 10^7 and $1.5x10^7$), along with two other treatments. A commercial control group for comparison, containing abamectin (0,58mg/seed) and a mixed treatment containing abamectin (0,58mg/seed) and 10^7 endospores of *P. thornei*. These seeds were sown in plastic cups containing soil inoculated with 1000 nematodes (experiment 1) and 600 nematodes (experiment 2). The experiments were evaluated at 60 and 90 days after inoculation (DAI). Fresh root mass and total nematodes extracted from the roots of each plant were used as the assessment criteria. The treatments containing the lowest concentration of *P. thornei* showed a low degree of *P. brachyurus* control in both experiments, while the higher concentration reduced the final population of nematodes by 30-50% in comparison to the untreated seeds. However, the treatments containing abamectin were superior in reducing the nematode population in all experiments and evaluations. There was no visible synergistic effect by the combined use of abamectin and *P.thornei* in the same treatment.

Keywords: *Pratylenchus brachyurus*; *Pratylenchus zeae*; Biological control; Abamectin; *Glycine max*

1.1. INTRODUCTION

Plant parasitic nematodes, chiefly *Heterodera glycines* (soybean cyst nematode-SCN) are among the major limiting factors for soybean production in the USA (Pratt and Wrather, 1997). In Brazil, three phytonematode species are directly related to crop loses in soybean fields, the SCN, *Meloidogyne javanica* (root knot nematode-RKN) and *Pratylenchus brachyurus* (root lesion nematode-RLN) (Dias *et al.*, 2010). The RLN is nearly ubiquitous in soybean fields, occurring in 85% of the soybean fields in the State of Mato Grosso (Miranda, 2011). This is an alarming diagnosis given that Mato Grosso is responsible for 27% of the current Brazilian soybean production, which amounts to 102 million tons (CONAB, 2016).

The intense infection of soybean roots by *P. brachyurus* is reflected in above ground patches of stunted plants (Debiasi *et al.*, 2011). Such symptoms result in a yield decrease that is directly correlated with high numbers of females and juveniles feeding on the root system, which is likely to occur on a succession of susceptible

host crops, such as maize and soybean, in infested areas, as demonstrated by Ramos Junior *et al.* (2015).

To avoid a productivity deficit of up to 30% (Goulart, 2008; Schmitt and Noel, 1986). Crop rotation is often employed as a management option using *Crotalaria* spp., such as *C.spectabilis* and *C.ochroleuca* (Oliveira and Carregal, 2016). The only registered products in Brazil up to 2016 were two seed treatments: Avicta 500 FS™ (a liquid formulation containing abamectin) and Cropstar ™ (A liquid formulation containing a mixture of imidacloprid, a neonicotinoid insecticide, and thiodicarb, a carbamate insecticide/nematicide), as well as an organophosphate in furrow treatment: Rugby 200 CS™, containing cadusafos as an active ingredient (AGROFIT, 2017).

This relative scarcity of commercial formulations was likely a combination of disinterest from chemical companies in this pathosystem and the extensive usage of broad spectrum biocides to control nematodes in the past, such as methyl bromide and aldicarb (Noling and Becker, 1994; Oka *et al.*, 2000). The high efficacy of these compounds in controlling nematode populations likely led to reduced efforts in developing alternative products, but given their environmental and human toxicity (Ragoucy-Sengler *et al.*, 2000), the pressure to limit the agricultural use of these biocides has raised the importance of researching new and more environmentally friendly nematicides in the past two decades (Viaene, 2014). From 2016 onwards the number of products registered for *P. brachyurus* in soybean increased from 3 to 8, including 2 biological based products, this is evidence of the growing importance of this pathosystem. (AGROFIT, 2017).

Amidst these upcoming nematicides, biological control agents are receiving more attention, particularly fungi and bacteria, which have been shown to provide promising control potential (Freitas and Carneiro, 2000; Chen, 2004). One such agent is *Pasteuria thornei,* a gram-positive endospore forming bacterium capable of parasitizing species of the genus *Pratylenchus*. Most of the current research on *Pasteuria* spp. however, has focused on the biological control of *Meloidogyne* and *Heterodera* spp*.* (Chen and Dickson, 1998). These bacteria adhere to the nematode's cuticle while it moves through the soil or root and penetrates it, colonizing the pseudocoleom and forming microcolonies that break apart and spread the infection throughout the nematode body. This results in a loss of fertility for the female, while filling the entirety of the host's body with endospores (Starr and Sayre,

1988). Nematode control with *Pasteuria* spp. was commercially pioneered in 2013 in the United States under the seed treatment Clariva™. This treatment contains endospores of *Pasteuria nishizawae* which are released in the soil at the moment of planting, adhering to the cuticule of second stage juveniles of *H. glycines*. This interaction ultimately leads to a loss of fertility of the female host (Sayre *et al.*. 1991; Potter *et al*., 2014).

Given the relevance of *P. brachyurus* for Brazilian agriculture, and a growing necessity for more environmentally benign management options, two glasshouse experiments were carried out to evaluate the effects of the currently unregistered *Pasteuria thornei* in controlling *P. brachyurus* populations.

1.2. MATERIALS AND METHODS

1.2.1. Seed treatment

Five seed treatments were included using soybeans seeds of the cultivar SYN1080. Untreated seeds of the same cultivar were used as a control treatment and designated as treatment 1. Three of the seed treatments consisted of different concentrations of *P.thornei* per seed: $5x10^6$, $1x10^7$ and $1.5x10^7$ endospores were designated treatments 2, 3 and 4, respectively.

The two remaining treatments were carried out to evaluate and compare the current recommended formulation: 35 g of abamectin per 60 000 seeds (equaling 70 ml of Avicta 500 FS™) and 35 g of abamectin per 60 000 seeds in addition to 10^6 endospores of *P. thornei* per seed and designated treatments 5 and 6, respectively. Besides the treatments described above, all the treatments received a commercial fungicide and insecticide seed coating as well, which included 100 mL/100 kg of seeds for Maxim XL™ (metalaxyl-M at 10g/L and fludioxonil at 20g/L) and 250 mL/100 kg of seeds for Cruiser 350 FS™ (thiametoxam at 350g/L).

All *P. thornei* treatments originated from the same isolate, obtained from an unspecified site in the United States of America. The mass multiplication of this biological control agent is achieved through an *in vitro* fermentation procedure, which mimics the natural conditions necessary for its reproduction inside the nematode. Details of this *in vitro* multiplication for industrial scale are undisclosed (Daniela Ribeiro, personal communication, November 10, 2017).

The seeds for all treatments were supplied by Syngenta Brasil, and the seed coating procedures were performed individually for each treatment in November of 2014. Seeds were treated in batches of 1kg, following the order: 1) Maxim XL ™, 2) Cruiser 350 FS™, 3) Avicta 500 FS™ and 4) *P. thornei*. Double layered plastic bags were used to apply the slurries to the seeds, which were manually shaken for 2 minutes, ensuring the best homogeneity possible. For proper drying of the treatments, a 1 hour interval ensued after each coating. The seeds were stored in a refrigerator at a temperature of 16ºC upon receive.

1.2.2. Glasshouse experiments

The two glasshouse experiments were conducted at different times during the year of 2015 in the municipality of Piracicaba, Brazil. Glasshouse temperatures for the duration of the experiment were obtained from an electronic thermometer. Maximum and minimum temperature ranges were annotated daily, maximum and minimum means are described in Table 1.

Table 1. Glasshouse temperature interval means for the duration of each experiment.

	Air temperature	Evaluation period
Experiment 1	Max:37,7ºC; Min:21,3ºC	February~April (2015)
Experiment 2	Max:38,1ºC; Min:19,8ºC	September~November(2015)

The *P. brachyurus* isolate (Pb 23) was collected from cotton fields in Sapezal, Mato Grosso State, Brazil, and maintained both in soybean(cv. Pintada) and cotton(cv. Fibermax) roots in a glasshouse environment in Piracicaba, São Paulo State, Brazil. Soybean and cotton infected roots were processed in a kitchen blender for 60s, the resulting aqueous solution was sifted through 60 and 500 mesh sieves to better separate the females, juveniles and eggs from the larger pieces of root tissue and coarse sand particles. The purified aqueous solution underwent a centrifugation process in a centrifuge containing 120 cm tubes (10 cm high × 3.9 cm diam.) at 550 g (Coolen and D'Herde, 1972). Populational density quantification in the inoculum was performed by counting the number of nematodes with the aid of a Peter's slide under a light microscope 100x lens.

Plastic cups with a volume of 500 cm^3 (13.5 cm deep × 9 cm diam.) were

filled with steam-treated sandy-clay soil (121°C for 2 h), with six 2-cm-deep holes being made in the soil in each cup, in each hole a soybean seed was sowed, in a total of six seeds per cup. All 5-6 plants were maintained for the remainder of the experiment. For Experiment 1, an initial population (Pi) of 1000 specimens of *P. brachyurus* was inoculated into two oblique holes of 2 and 4 cm respectively, in Experiment 2 a Pi of 600 was established. Inoculation took place 5 days after germination. The aqueous suspension containing the infective individuals for each plot was calibrated to a maximum of 1,2ml. Nematode numbers were determined 60 and 90 days after inoculation (DAI) of the plots by extracting nematodes from 10g of homogenized roots using a kitchen blender and a centrifuge containing four 120 cm tubes (10 cm high × 3.9 cm diam.) at 550 g (Coolen and D'Herde, 1972). The nematodes were counted using light microscope at 100× magnification with the aid of a Peter's slide in order to estimate the final population (Pf) based on the total root mass of the replicate. The values of Pf were subjected to analysis of variance (ANOVA), and the means were compared using the Tukey Honestly Significant Test, performed on the R software package (R: The R Project for Statistical Comp,2012).

1.3. RESULTS

Total root weight for all treatments did not differ statistically. The overall population increase for the untreated soybean was very similar in both experiments. In Experiment 1, at 90 DAI the Pf had increased almost 10-fold with 9714 nematodes extracted from the plant roots in the control. In Experiment 2, at 90 DAI the nematode population reached 6048, also a little over 10 times the initial population. This baseline of comparison held true for all treatments across the two experiments, despite the difference in the Pi.

Table 2. Total number of *Pratylenchus brachyurus* of all stages 60 and 90 days after inoculation (DAI) of untreated seeds (control), different concentrations of *Pasteuria thornei* and abamectin

Treatments	Experiment 1 (Pi 1000)		Experiment 2 (Pi 600)	
	60 DAI	90 DAI	60 DAI	90 DAI
Untreated Control	3288 a ± 815	9714 a ± 3165	3067 a ±1070	6048 a ±1814
***P. thornei* 5x10^6 endospores/seed**	2459 b ± 610	7127 ab ± 2235	2967 a ±1606	4957 ab ± 2971
***P. thornei* 10^7 endospores/seed**	2672 b ± 550	6440 b ±1348	2610 ab ±850	3637 b ± 1942
***P. thornei* 1.5x10^7 endospores/seed**	2478 b ± 708	6286 b ±1742	1568 b ±282	2997 b ±1189
***P. thornei* 10^7 endospores/seed + Abamectin 0,583mg/seed**	223 c ± 93	530 c ±382	124 c ±63	82 c ±29
Abamectin 0,583mg/seed	423 c ± 180	703 c ±258	195 c ±88	258 c ±67

*Means followed by the standard error of six replicates for each treatment. Means within a column followed by the same letter are not significantly different at *P*= 0.05.

Regarding the *P. thornei* exclusive treatments, the higher concentration of 1.5x10^7 endospores/seed was the only treatment to consistently reduce final populations of *P. brachyurus*. In comparison to the control group, at 90 days, this treatment reduced the final nematode population by a factor of 35% in Experiment 1, and 46% in Experiment 2. The intermediate dose (10^7 endospores/seed) performed well in Experiment 1 at 60 and 90 DAI, and in Experiment 2 at 90 DAI. In the second experiment at 60 DAI however, final populations were closer to the control group and the lower *P. thornei* dose. The 10^6 endospores/seed dose had low to no effect in decreasing *P. brachyurus* population growth. Both abamectin treatments had the greatest impact on *P. brachyurus* populations, reducing the nematode infestation to levels lower than the *P. thornei* treatments and the control.

1.4. DISCUSSION

Avicta 500 FS™ was consistently the more effective treatment in reducing the final nematode population, with reduction rates of up to 95% in comparison to untreated seeds. Nematode control was greater than that reported by Bortolini *et al.* (2013) of 88% under similar experimental conditions. This high reduction rate might be the result of accumulation of the active ingredient in each pot due to overlapping areas around the seed. Predicting the accumulation and distribution of a seed treatment in small plots can prove difficult, and such reduction rates should not be expected under field conditions.

The highest concentration of *P. thornei* endospores was the only treatment, other than the abamectin treatments, to consistently reduce the nematode population compared to the untreated control. The highest spore concentration reduced the *P. brachyurus* population by 25-50%. Thus, *P. thornei* shows promise as a control agent of *P. brachyurus*, although it was not as effective in reducing the nematode population as abamectin. A spore dosage of between 10 and 15 million endospores/seed should be optimal, if it is economically feasible. It is also noteworthy that storing the seeds over a 10-month period prior to Experiment 2 did not seem to reduce the efficacy of *P. thornei.*

A synergistic effect was not observed in the treatment containing both the biological agent and abamectin. The combined treatment of abamectin and *P. thornei* was numerically lower than the abamectin alone for all sampling times and experiments, however, the differences were not significant. The absence of a synergistic or additive effect may be attributed to the fact that *P. thornei* is an obligate parasite and requires mobile nematodes for spore attachment. Abamectin paralyzes nematodes, ultimately leading to their death and resulting in fewer mobile hosts to parasitize (Faske and Starr, 2006).

The lack of an additive effect, however, does not preclude the possibility of a combined use of *Pasteuria* spp. and abamectin where there are mixed populations of plant parasitic nematodes. *Pasteuria* spp. have a strict host range, with a single species parasitizing only a single genus of plant parasitic nematodes, while abamectin is a broad spectrum nematicide, affecting several species of nematodes. Therefore, *P. thornei* showed a promising potential in reducing *P. brachyurus* population, despite being unable to reach control levels rivaling those show by the

treatments containing abamectin. Also, the storing of the seeds over the time interval from its treatment to experiment 2 showed no sign of an efficacy loss by the formulation when comparing the two experiments.

This is the first report demonstrating biological control of *P. brachyurus* populations by *P. thornei*. Previous studies on *P. thornei* have been limited to host compatibility (Starr and Sayre, 1988) and detection/occurrence (Gonzaga and Santos, 2008). The results from this study should encourage further research with *P. thornei*.

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2. *Pasteuria thornei* AS A BIOLOGICAL SEED TREATMENT: AN ALTERNATIVE CONTROL METHOD TO REDUCE YIELD LOSSES CAUSED BY *Pratylenchus zeae* IN MAIZE

ABSTRACT

Recently, several studies have pointed to the potential of biological agents for controlling phytonematodes. The experiments contained within this study had two primary objectives. First, we evaluated the efficacy of *Pasteuria thornei* as a biological seed treatment agent for *Pratylenchus zeae* control in maize, and secondly, productivity assays were carried out to observe how *P. zeae* populations impacts the yield of corn hybrids in greenhouse conditions and how biological and chemical seed treatments could mitigate these losses. Treatments consisted of: a control group of untreated seeds, four concentrations of *P. thornei* (5, 10, 15 and 20mi endospores per seed) along with two other treatments, a commercial control group containing abamectin (0,58mg/seed) and a mixed treatment containing abamectin (0,58mg/seed) and 10mi endospores of *P. thornei,* in a total of seven treatments with 6 replicates each. The experiments were carried out twice, at different time periods, and the same maize hybrid CELERON was used for both experiments. For the efficacy study, under greenhouse conditions seeds of the maize hybrid were sown in plastic cups containing soil inoculated with 4000 and 1000 individuals for experiment 1 and 2 respectively. *P. zeae* population consisted of individuals of all life stages. Evaluation of these efficacy experiments occurred 60 and 90 days after inoculation (DAI). Fresh root mass and total nematodes extracted from the root system were used as the evaluation parameter. The productivity studies were carried out in a screened greenhouse, with plots consisting of 9L pots, filled with soil inoculated artificially by the introduction of *P. zeae*-infected sugarcane roots. Treatments for this productivity study mirrored the efficacy trial, with the removal of three lower doses of the *Pasteuria* seed treatment, and the addition of a uninoculated control. Evaluation consisted of the measurement of several agronomic traits such as aerial part dry weight, fresh root mass and grain weight at the time of harvest, as well as nematode population in fresh roots at 45, 90 days and at the time of harvest. Efficacy trials showed that higher concentrations of *P. thornei* (15-20 million endospores per seed) have a measurable *P. zeae* control potential, reducing the nematode population by up to 54 and 47% in experiments 1 and 2 respectively in comparison with the untreated control treatment. The abamectin commercial formulation showed a reduction of nematode population of over 90% in both experiment installments. For the productivity assays, *P. thornei* nematode control potential was similar to what was observed in the efficacy study. However, the commercial treatment containing abamectin performed a control threshold closer to the *P. thornei*, placing them in the same tukey group. Regarding the grain weight of the uninoculated plots, those showed higher productivity in both experiments, nearly doubling the yield of the untreated control. The abamectin treatment showed effect in reducing the yield losses caused by *P. zeae* in both experiments. The treatments with both the chemical and biological control, and the treatment containing only *P. thornei* performed well in both experiments, but statistically confirmed control was only observed in experiment 2. With the data observed in both experiments it is clear that *P. thornei* has potential for controlling *P. zeae*, and can also help mitigate the yield

losses caused by this phytonematode, presenting us with yet another tool for the intricate management of this soil pathogen.

Keywords: Root lesion nematode; Biological control; Abamectin; *Zea mays*

2.1. INTRODUCTION

Second only to wheat and rice regarding worldwide consumption, maize is a highly versatile staple food serving as a rich source of carbohydrates and fibers in both human and livestock diets (Nuss and Tanumihardjo, 2010; Ranum *et al.*, 2014). The bulk of the world's production of this cereal comes from three countries, USA, China and Brazil, with a production of $361.1x10^6$ t, $216.81x10^6$ t and $79.87x10^6$ t respectively as of 2014 (FAOSTAT, 2016). Brazilian maize harvest has grown ever since, reaching the mark of $84.64x10^6$ t in 2016, being cultivated in a total area of $15.92x10^6$ ha (CONAB, 2016).

Among other factors, plant diseases contribute to a fair share of losses in maize fields, which include leaf rusts, stalk rots, ear rots and phytonematodes (Groth *et al.*, 1983; De Waele and Jordaan, 1988; Taylor and Sutton, 2009). In Brazil, considering pathogenicity and distribution, the most important nematode species for maize are *Pratylenchus zeae*, *P. brachyurus*, *Helicotylenchus dihystera, Mesocriconema* spp., *Meloidogyne incognita* and *Xiphinema* spp. (Casela *et al.*, 2006).

While aerial parts diseases are easier to spot and measure, the reflex symptoms of root diseases are often neglected or mistaken for signs of low soil fertility (Duncan and Moens, 2006; Fosu-Nyarko and Jones, 2016), this is the case for corn plants attacked by root lesion nematodes (RLN's) of the genus *Pratylenchus*, which causes the appearance of foliar chlorosis, root destruction and the formation of patches of stunted plants (Monteiro, 1963). Maize is susceptible to both *P. zeae* and *P. brachyurus*. However *P. zeae* causes more damage to maize roots, and when both nematodes are found in a mixed infestation, *P. zeae* is likely to gradually supplant *P. brachyurus* population (Olowe and Corbett, 1976). There is a scarcity of information regarding yield losses on maize in areas infested by *P. zeae*. In the municipality of Piracicaba, Brazil, yield losses of 50% were reported where this lesion nematode was present (Lordello, 1974). Symptoms such as foliage yellowing and stunted growth in patches were able to be observed in maize growing fields with

P.zeae populations of 24.2 nematodes/g soil in India (Patel *et al.*, 2002).

Losses caused by RLN's are becoming a growing concern thanks to the popularization of a no-till agriculture, which protects soil moisture by conserving the topsoil, offering a better environment for nematode survival (Jones *et al.*, 2016). Despite the widespread occurrence and the potential damage of this lesion nematode, in Brazil only abamectin is registered for *P. zeae* control in maize at a rate of 50mL/60,000 seeds for its concentrated suspension formulation (ADAPAR, 2015).

Bacterias of the gram-positive genus *Pasteuria* are obligate parasites of nematodes and crustaceans (Dickson *et al.*, 2009). Regarding nematodes, each particular strain of *Pasteuria* is attuned to a specific host species (Chen and Dickson, 1998). The genus, however, is known to parasitize all of the most notorious nematode pests in agriculture, attaching itself to the nematode's cuticle, penetrating its body and then colonizing the entirety of the host's pseudocoelom, ultimately leading to a loss of reproductive capabilities of the host (Stirling, 2014). As a biological control agent (BCA), *Pasteuria* spp. occurs naturally in nematode infested soil worldwide (Gonzaga e Santos 2008; Stirling *et al.*, 2017), circumventing some of the dangers in introducing exotic species for biological control purposes (Van Lenteren *et al.*, 2006).

Given the importance of root lesion nematodes, and the scarcity of tools for its control, the goal of this study was to evaluate the efficacy of *Pasteuria thornei* as a potential BCA for *P. zeae* control.

2.2. MATERIAL AND METHODS

2.2.1. Efficacy trials(ET)

An experiment consisting of two replicates was designed with the purpose of accessing the control efficacy of *P. thornei* as a seed treatment in controlling *P. zeae* population in maize roots. The results of this assessment would guide our treatment choices for the ensuing maize productivity assay under *P. zeae* infestation. The first experiment of the efficacy trial, and its replicate, are referred to as experiments 1 and 2, respectively.

2.2.2. ET-Seed Treatment

Seven seed treatments were included using maize seeds of the hybrid Celeron. Four of the treatments consisted of different increasing concentrations of *P.thornei* per seed: *i*) $5x10^6$ endospores, *ii*)10^7 endospores, *iii*) $1.5x10^7$ endospores *iv*) $2x10^7$ endospores. One treatment was carried out to evaluate and compare the efficacy of the current recommended abamectin formulation with that of the BCA *v*) 0.585mg of abamectin per seed (Avicta 500 FS™). Additionally, a mixed treatment was introduced verify the presence of a possible synergistic effect: *vi)* 0.585mg of abamectin per seed in addition to 10^7 endospores of *Pasteuria thornei* per seed. Untreated seeds of the same hybrid were used as a control treatment (*vii*). Besides the treatments described above, all the treatments received a commercial fungicide and insecticide seed coating as well, which included 100 mL/100 kg of seeds for Maxim XL™ (metalaxyl-M at 10g/L and fludioxonil at 20g/L) and 250 mL/100 kg of seeds for Cruiser 350 FS™ (thiametoxam at 350g/L).

The seeds for all treatments were supplied by Syngenta Brasil, and the seed coating procedures were performed individually for each treatment in november of 2014. Seeds were treated in batches of 1kg, following the order: 1) Maxim XL ™, 2) Cruiser 350 FS™, 3) Avicta 500 FS™ and 4) *P. thornei*. Double layered plastic bags were used to apply the slurries to the seeds, which were manually shaken for 2 minutes, ensuring the best homogeneity possible. For proper drying of the treatments, a 1 hour interval ensued after each coating. The seeds were stored in a refrigerator at a temperature of 16ºC upon receive.

2.2.3. ET-Glasshouse Experiments

The two glasshouse experiments were conducted at different times during the year of 2015 in the municipality of Piracicaba, Brazil. Glasshouse temperatures for the duration of the experiment were obtained from an electronic thermometer and maximum and minimum temperature ranges were annotated daily (Table 3).

Table 3. Glasshouse temperature interval means for the duration of each experiment.

	Air temperature	Evaluation period
Experiment 1	Max:37,7ºC; Min:21,3ºC	February~April (2015)
Experiment 2	Max:38,1ºC; Min:20,8ºC	September~November(2015)

The *P. zeae* isolate was collected from sugarcane roots in a field in the municipality of Jaú (São Paulo state, Brazil) by Roberto Kubo (*Instituto Biológico de São Paulo*), and maintained both in pearl millet (*Pennisetum glaucum*) and *Sorghum bicolor* roots in a glasshouse environment. Infected roots of millet and sorghum were processed in a blender for 60s, and the resulting aqueous suspension was sifted through 500 and 60 mesh sieves in order to better separate the females, juveniles and eggs from the larger pieces of root tissue and coarse sand particles. The purified aqueous solution underwent a centrifugation process in a centrifuge containing 120 cm tubes (10 cm high × 3.9 cm diam.) at 550 *g* (Coolen and D'Herde, 1972). Populational density quantification in the inoculum was performed by counting the number of nematodes with the aid of a Peter's slide under a light microscope 100x lens.

Plastic cups with a volume of 500 cm^3 (13.5 cm deep × 9 cm diam.) were filled with steam-treated sandy-clay soil (121°C for 2 h), with six 2-cm-deep holes being made in the soil in each cup. In each hole a maize seed was planted. For Experiment 1, an initial population (Pi) of 4000 specimens of *P. zeae* was inoculated into two oblique holes of 2 and 4 cm respectively, and in Experiment 2 a Pi of 1000 was established. Inoculation took place 5 days after germination. The aqueous suspension containing the infective individuals for each plot was calibrated to a maximum of 2ml. Nematode numbers were determined 60 and 90 days after the inoculation (DAI) of the plots by extracting nematodes from roots using the method described above for the inoculum. The nematodes were counted using light microscope at 100× magnification with the aid of a Peter's slide in order to estimate the final population (Pf). The values of Pf were subjected to analysis of variance (ANOVA), and the means were compared using the Tukey Honestly Significant Test, performed on the R software package (R: The R Project for Statistical Comp., 2017).

2.2.4. Productivity assays (PA)

Five treatments were established for these trials, contemplating the treatments with the most promising results in the efficacy trials. Two replicates of this experiment were carried out to evaluate the effects of the seed treatments in mitigating the yield losses caused by *P. zeae* on maize. The first experiment of the productivity assays, and its replicate, are referred to as experiments 3 and 4, respectively.

2.2.5. PA-Seed Treatment

Three seed treatments were included using maize seeds of the hybrid Celeron: *i)* 0.585mg of abamectin per seed (Avicta 500 FS™), *ii)* 0.585mg of abamectin per seed in addition to 10^7 endospores of *Pasteuria thornei* per seed and *iii)* 2x10^7 *P. thornei* endospores. Untreated seeds of the same hybrid were used as control treatments for productivity in infected soil and *P. zeae*-free soil, in a total of five treatments.

2.2.6. PA-Screened Greenhouse Experiments

The two screened greenhouse experiments were conducted at different times during the years of 2015 and 2016 in the municipality of Piracicaba, Brazil. Air temperatures for the duration of the experiment were obtained from the Esalq Meteorological Station (2017), and are depicted in Table 4.

Table 4. Air temperature interval means for the duration of each experiment.

	Air temperature	Evaluation period
Experiment 1	Max:32,9ºC; Min:18,7ºC	October~December (2015)
Experiment 2	Max:33,2ºC; Min:22,6ºC	January~April (2016)

The *P. zeae* isolate for these experiments derived from the same population used in the previous ET, and were also maintained in pearl millet and sorghum roots.

For these productivity assays, infected soil and roots were used as a source of infective individuals. Initial population was estimated through the processing of 10g

of fresh roots and 200 cm^3 of infected soil. The procedure for root processing was the same used to evaluate the ET, and the initial soil population was estimated through sieving and then by sugar flotation method (Hooper, 1986). Ceramic plant pots with 9L capacity were used as experimental plots. Nematode population for each infected plot was established through a combination of homogenized infected soil and a fresh mass of parasitized root tissue, to better emulate field conditions. The uninfected control treatment was established using steam-treated soil, similarly to the ET. The plots were filled with 8L of sandy-clay soil. Three seeds were planted in each plot after the infested vases were prepared, however, only two plants were maintained for the entire experiment and final evaluations. For experiment 3, an initial population of approximately 9,000 infective individuals (eggs, juveniles and adults) was used, consisting of 1,200 individuals in 8L of soil and 7,800 in 10g of incorporated homogenized root mass. As for the second experiment this number was approximately 11,000 per 9L vase, 2,300 in 8L of soil and 8,700 in 10g of root mass. Crop management in experiment 1 was minimal, with only a pre-sowing fertilization of 10 kg N/ha, 40kg P/ha and 30 kg K/ha, which resulted in small cobs. For experiment 4, two additional nitrogen topdressings were performed, at V3-4 and V8-9 at a dose equivalent of 80kg N/ha in urea, for each topdressing.

Non-destructive root evaluations were carried out 45 and 90 days after sowing (DAS) and consisted of the sampling of roots at 0-20cm with the aid of a plastic cylinder. Roots were separated from the soil and weighted to determine the number of individuals per gram of fresh root tissue. A final destructive evaluation was also carried out at the end of the experiment by the time of harvest, 110 DAS for experiment 3 and 101 DAS for experiment 2. Productivity was measured through the weighting of the grains of two threshed corncobs per plot, harvested at physiological maturity (R6). Additionally, fresh root weight was measured, as well as the weight of aerial parts, minus cobs, which were left to dry in a drying oven for 72 hours at 60ºC.

2.3. RESULTS

2.3.1. Efficacy trials

The nematode populational growth of both experiments were similar when comparing the 90 DAI evaluations, despite the difference in the initial population (Pi). The control treatment showed a final population 25 times larger than the initial one for the first experiment, and 26 times larger for the second experiment. This similarity is an important guideline for an accurate comparison across all treatments in both installments of the experiment.

Table 5. Total number of *Pratylenchus zeae* of all stages 60 and 90 days after sowing (DAI) of untreated seeds (Control), and different concentrations of *Pasteuria thornei* and abamectin

Treatments	Experiment 1 (Pi 4000)		Experiment 2 (Pi 1000)	
	60 DAI	90 DAI	60 DAI	90 DAI
Control	29397 a* ± 5592	100479 a ±13557	7594 a ±1538	26882 a ±4798
***P. thornei* 5x10^6 endospores/seed**	30342 a ±6254	71972 b ±7767	5890 ab ±949	21503 ab ±8609
***P. thornei* 10^7 endospores/seed**	28883 a ±5905	65973 bc ±15682	4966 bc ±769	17563 ab ±4626
***P. thornei* 1.5 x10^7 endospores/seed**	26038 ab ±5901	53106 bc ±16292	4934 bc ±1232	15261 b ±6187
***P. thornei* 2x10^7 endospores/seed**	17410 b ±2251	46025 c ±19264	3582 c ±1369	14384 b ±5986
***P. thornei* 10^{7}endospores/seed + abamectin 0,583mg/seed**	4507 c ±2960	5685 d ±2160	540 d ±142	1738 c ±1036
abamectin 0,583mg/seed	3041 c ±2476	7018 d ±5562	479 d ±332	1299 c ±661

*Means followed by the standard error of six replicates for each treatment. Means within a column followed by the same letter are not significantly different at *P*= 0.05

As expected, the control treatment showed the highest *P. zeae* population numbers over the two experiments, in both evaluation dates (60 and 90 DAI). The treatment with the lowest *P. thornei* concentration did not show a significant suppressive effect in the *P. zeae* populations in 3 out of 4 evaluations (Table 5). The exception being the first experiment's 90 DAI evaluation, in which this dose showed a control potential of nearly 30% in comparison with the untreated counterpart. This

performance was not observed consistently over other evaluations, with this lowest *P. thornei* concentration treatment showing reduction rates varying from 0-21% and no statistical significance at *P=0.05*. Intermediate *P. thornei* doses of 10^7 and $1.5x10^7$ behaved similarly. *P. thornei* at a concentration of 10^7 performed slightly better than the lowest concentration, distancing itself from the untreated control in two evaluations, 90 DAI and 60 DAI of experiments 1 and 2, respectively. The endospore concentration of $1.5x10^7$ showed positive results in all evaluations except at 60 DAI of the first experiment, in which doses ranging from 1-3 showed means very close to the untreated control group (Table 5). The highest concentration ($2x10^7$) was the only *P. thornei* exclusive treatment to demonstrate a positive effect on the reduction of *P.zeae* populations on all evaluations, with reduction rates ranging 40-55% when compared to the untreated control(Table 5)

The two treatments containing the abamectin commercial formulation showed a superior control potential over all treatments containing *P.thornei*, with reduction rates of *P. zeae* population surpassing 90%. Of those two, the treatment containing both abamectin and *P. thornei* at a concentration of $5x10^6$ endospores per seed did not differ statistically from the treatment containing solely abamectin. This treatment however showed a much higher efficacy in reducing *P. zeae* populations than its abamectin free counterpart, *P. thornei* at a rate of 10^7(Table 5).

2.3.2. Productivity assays

P. zeae populational growth followed a steeper growth curve in experiment 3 in comparison with its replicate. While the control group multiplied 32-fold from 45 DAI to the last evaluation by the time of harvest in experiment 3, in experiment 4 the same time interval between evaluations led only to 1,8 times increase. Regarding the efficacy in reducing *P. zeae* reproduction, all of the three treatments showed statistically significant results by the time of harvest, and did not differ from each other in a tukey test at *P=0.05*(Table 6). Using the control group as a base of comparison, for experiments 3 and 4 respectively, a reduction rate of 51% and 59% was observed for the treatment containing abamectin alone. The biological treatment containing *P. thornei* showed reduction rates of 44% and 46% for experiments 3 and 4 respectively. When combined, abamectin and *P. thornei* reduced *P. zeae* population by a factor of 45% in the first experiment and 31% for its replicate (Table

6).

Table 6. Total number of *Pratylenchus zeae* of all stages 45, 90 days after sowing (DAS) and by the time of harvesting, of untreated seeds (Control), different concentrations of *Pasteuria thornei* and abamectin, values in (nem/root(g)).

Treatments	Experiment 3 (Pi 9000)			Experiment 4 (Pi 11000)		
	45 DAS	**90 DAS**	**Harvest (110 DAS)**	**45 DAS**	**90 DAS**	**Harvest (101 DAS)**
Untreated Control	437 a* ± 197	6758 a ± 3253	14248 a ± 3945	3927 a ± 1252	5658 a ± 1279	7037 a ± 1084
abamectin 0,583mg/seed	123 b ± 75	5080 a ± 1728	6982 b ± 1703	1713 b ± 501	2773 b ± 719	2918 b ± 966
***P. thornei* 10^7endospores/seed + abamectin 0,583mg/seed**	172 b ± 51	3697 a ± 1245	7959 b ± 1965	1706 b ± 580	3013 b ± 789	4156 b ± 1281
***P. thornei* 2x10^7endospores/seed**	272 ab ± 142	5297 a ± 1597	8010 b ± 2222	2005 b + 834	2940 b ± 1172	3846 b ± 1073

*Means followed by the standard error of six replicates for each treatment. Means within a column followed by the same letter are not significantly different at *P*= 0.05.

The 45 DAS evaluations in experiments 3 and 4 showed a decrease in *P. zeae* population for all seed treatments containing abamectin. Also at 45 DAS, the seed treatment containing solely *P. thornei* at a rate of $2x10^7$ endospores per seed showed a substantial reduction of *P. zeae* population in experiment 4. However, in experiment 3 this same treatment did not distance itself statistically from the untreated control (Table 6).

Agronomic measurements as depicted in Table 7 shows that fresh root mass by the time of harvest did not differ statistically in the first experiment, regardless of the treatment. In experiment 4, fresh roots of the uninoculated control showed the highest mass, placing it apart from the inoculated control. The three tested seed treatments however were placed in the same tukey group as the inoculated control, with both treatments containing exclusively abamectin and *P. thornei* participating in an intermediate group with the control treatments.

Table 7. FRW: Fresh root weight, APDW: Aerial parts dry weight, TGW: Total grain weight. Values in grams (g).

Treatments	Experiment 3			Experiment 4		
	FRW	**APDW**	**TGW**	**FRW**	**APDW**	**TGW**
Control	61,88 a* ± 4,49	61,16 a ± 4,68	77,88 a ± 9,12	123,30 a ± 17,58	157,76 a ± 13,46	167,78 a ± 14,39
Inoculated control	50,93 a ± 5,44	43,04 b ± 3,47	32,08 c ± 14,77	87,90 b ± 19,38	118,10 c ± 6,91	86,32 c ± 25,36
abamectin 0,583mg/seed	55,53 a ± 16,69	44,76 b ± 11,66	52,38 b ± 12,01	101,71 ab ± 8,46	143,80 b ± 11,96	154,86 ab ± 20,69
***P. thornei* 10^7endospores/seed + abamectin 0,583mg/seed**	44,60 a ± 9,77	47,55 b ± 6,63	40,80 bc ± 17,83	75,25 b ± 15,46	134,08 b ± 8,51	147,61 ab ± 12,31
***P. thornei* *2x*10^7endospores/seed**	50,90 a ± 8,35	49,6 ab ± 5,37	47,42 bc ± 8,83	106,99 ab ± 23,34	132,65 bc ± 6,95	136,56 b ± 8,85

*Means followed by the standard error of six replicates for each treatment. Means within a column followed by the same letter are not significantly different at *P*= 0.05

In experiment 3, the aerial parts dry weight of the uninoculated control was the highest, distancing itself from all other treatments, regardless of the seed treatment applied. In experiment 4, the treatments containing abamectin and abamectin + *P. thornei* showed APDW higher than the untreated inoculated control, while the treatment containing only *P. thornei* was placed in an intermediate group together with the untreated inoculated control (Table 7).

Yield measurements reflected the effects of *P. zeae* infestation on maize and the mitigation potential of the tested treatments. In both experiments, the inoculated control showed lower yields than the uninoculated one, reducing total grain weight in the inoculated plots by over 58% for experiment 3 and 46,2% for experiment 4 (Figures 1 and 2). In experiment 3 only the treatment containing abamectin was set apart from the inoculated control, with a reduction in yield of 32% when compared to the uninoculated control. Whereas the other two seed treatments, consisting of abamectin + *P. thornei* and *P. thornei* alone did not differ statistically from the inoculated control on a tukey test at *P=0.05*. In experiment 4, however, treatments containing abamectin and abamecting+*P. thornei* performed well, with both placed in the same tukey group as the uninoculated control, with a reduction in grain weight of 10,8% and 12,3% when compared to the nematode free control. The treatment

containing *P. thornei* alone also showed positive results in experiment 4, with grain yield reduced by 18,8%. Evidencing the effects of the seed treatments when compared against the untreated control, which showed a reduction on yield of 46,2% as mentioned (Table 7).

Figure 1. Corncobs from experiment 3 (PA). **A**. Uninoculated control, **B**. Inoculated control, **C**. abamectin 0,583mg/seed, **D**. *P. thornei* 10^7endospores/seed + abamectin 0,583mg/seed **E**. *P. thornei* 2x10^7 endospores/seed.

Figure 2. Corncobs from experiment 3 (PA). **A**. Uninoculated control (2 missing plots), **B**. Inoculated control, **C**. abamectin 0,583mg/seed, **D**. *P. thornei* 10^7endospores/seed + abamectin 0,583mg/seed, **E**. *P. thornei* 2x10^7 endospores/seed.

2.4. DISCUSSION

2.4.1. Efficacy trials

Lower concentrations of *P. thornei*, $5x10^6$ and 10^7 endospores/seed, showed inconsistent results in reducing *P. zeae* population growth, often resulting in populational means for *P. zeae* very close to the untreated control. Whereas the treatments containing higher concentrations of the biological control agent, especially the dose of $2x10^7$ endospores/seed, showed more reliable results, reducing *P. zeae* populations by nearly half when compared to the untreated treatment in both experiments. The chemical treatment abamectin showed the best results in controlling *P. zeae* 7 with reduction rates of up to 95%, such rates are high but not too distant from similar works (Cabrera *et al.*, 2009; Bortolini *et al.*, 2013). However, it is important to notice that this effect is highly likely to be exacerbated by the number of seeds in one plot, due to an overlapping of the effect of each seed treatment. The treatment containing *P. thornei* + abamectin showed nearly identical results to the treatment containing abamectin alone, in all of the evaluations and experiments. This points to an absence of a synergist effect between chemical and biological treatments in this case, also likely to be related to the high effectiveness of abamectin in reducing *P. zeae* numbers, which could either mask the effects of *P. thornei* or deprive this biological control agent of its obligatory host, impeding it from reaching its full control potential. It is important to notice however, that species of the genus *Pasteuria* specialize in the parasitism of a single genus of phytonematode, and abamectin is a broad range nematicide. Therefore, the combination of both treatments might still prove beneficial in the occurrence of a multi-species infestation where *Pratylenchus* levels are high, especially if in the long run *P. thornei* establishes itself in the soil.

2.4.2. Productivity assays

Productivity assays also measured the growth of *P.zeae* population over the course of the experiments, not only to obtain more data on the efficacy of the treatments, but also to better relate yield losses and severity of infestation. The first noteworthy result of the efficacy evaluations for this experiment is how differently abamectin affected *P. thornei* populations. In the efficacy trials reduction rates of up

to 95% were observed in *P. zeae* populations, due to the treatment of the seeds with abamectin at a concentration of 0,583mg/seed. The same did not happen with *P.zeae* populations of the analog treatment in the productivity assays, with this chemical treatment showing reduction rates ranging from 51-59%, numbers that are considerably lower when compared to the previous experiment. While the treatments containing abamectin behaved differently when compared to the efficacy trials, the treatment containing *P. thornei* at a concentration of $2x10^7$ endospores/seed showed very similar results. In the efficacy trials, this same treatment presented reduction rates of 41-45% when compared to the untreated control, against 31-45% reduction in the productivity assays. This shows a consistency in *P. thornei* control potential as a biological agent, and sets a new perspective on how biological control for the genus *Pratylenchus* might be achieved through its use as a seed treatment.

Visual observation of the plots at 21 DAS revealed the first signs of the detrimental effect of the nematode, exacerbated on the earlier stages of vegetative development of the maize plants (Figure 3).

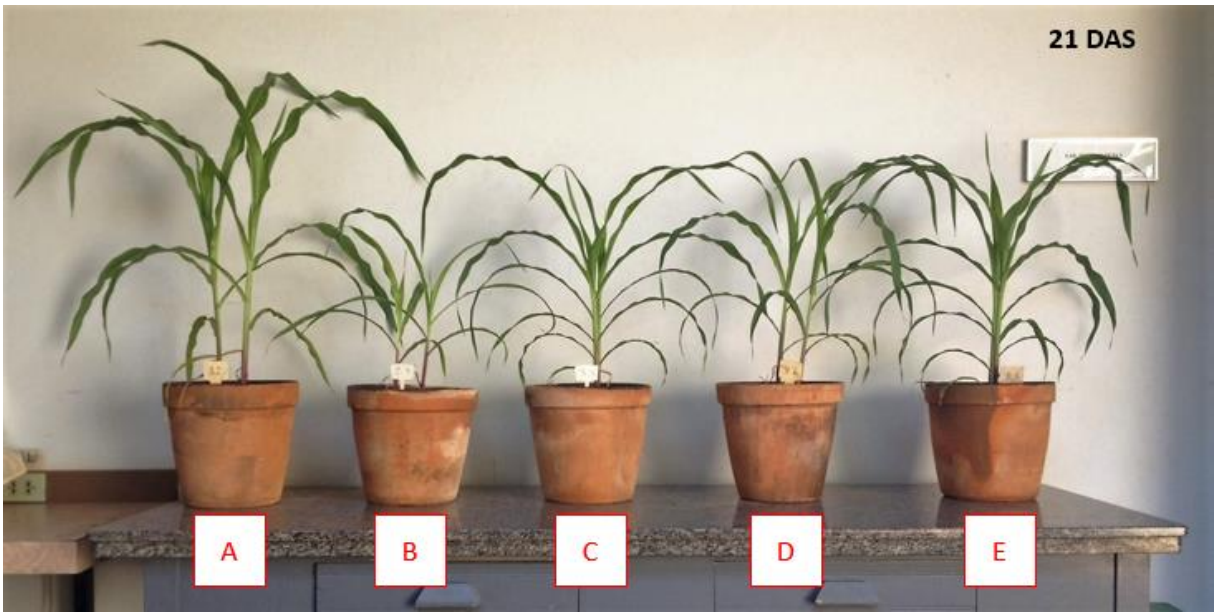

Figure 3. Maize plants at 21 DAS **A**. Uninoculated control, **B**. Inoculated control, **C**. abamectin 0,583mg/seed, **D**. *P. thornei* 10^7endospores/seed + abamectin 0,583mg/seed, **E**. *P. thornei* $2x10^7$ endospores/seed.

Pratylenchus zeae infestation had detrimental effects on most of the agronomic characteristics measured in this study, with exception of FRW for experiment 3. Despite the differences in soil fertility management for the two experiments, results showed similar trends when analyzing the effects of *P. zeae* infestation on the untreated control treatments. The reduction in productivity was around 58 and 46% when compared to the uninoculated control (TGW), for experiments 3 and 4, respectively. In general, yield losses were lower across all treatments in experiment 4 when compared to experiment 3, likely due to a better fertilizer management for this installment. This is an interesting take on the phytonematode phenomena, in which crops cultivated on soils of poor fertility are much more likely to be negatively affected by the presence of soil pathogens (Abawi and Widmer, 2000).

Experiment 4 showed a root mass weight reduction on all plants infected with *P. zeae*. Despite presenting higher root weight means, the treatment containing abamectin and the treatment containing *P. thornei* failed in distancing themselves from both controls, being placed in an intermediate group. The treatment containing both abamectin and *P. thornei* showed root mass means close to the untreated inoculated control.

All of the treatments showed positive results in reducing the negative effects of *P. zeae* parasitism on maize, presenting TGW means higher than the untreated inoculated control on both experiments. In experiment 3, only the treatment containing solely abamectin differed statically from the inoculated control regarding TGW. While in experiment 4 all the three treatments differed statistically from the inoculated control regarding TGW. The parameters FRW, APDW and TGW of the mixed treatment containing abamectin and *P. thornei* did not differ statistically from its solo counterparts in both experiments. Therefore, there is evidence to confirm an absence of synergistic or additive effects between the BCA and abamectin, not only in the efficacy trials, as state above, but also in productivity, which was reflected in the means of all agronomic traits measured.

2.4.3. Final considerations

The experiments comprised here are the first to demonstrate the control of *Pratylenchus zeae* using *P. thornei* as a biological control agent in maize roots.

Conclusions were not limited to efficacy, this work also shows the potential of *P. thornei* in preventing productivity losses in maize caused by this root lesion nematode.

In addition to the scarcity of available data on *P. thornei* control potential, not much information is available on the effects of *P. zeae* in reducing maize productivity, especially under controlled conditions. This lack of information is likely due to a combination of factors. First, the damage caused by root lesion nematodes, especially *P. zeae*, is still inadequately quantified (Goulart, 2008), and secondly, *Zea mays* is a vigorous plant, able to withstand harsh conditions, often masking its decrease in productivity due to the parasitism of nematodes (Inomoto, 2015). Most of the data regarding yield reduction caused by *P. zeae* on maize originates from observational studies or field experiments, correlating productivity with *P.zeae* population in naturally infested areas (Lordelo, 1974; Egunjobi and Bolaji, 1979; Kagoda *et al.*, 2011).

This work should give support for upcoming studies aiming to use *P.thornei* as a biological control agent for the genus *Pratylenchus*, and also stimulate colleagues in the pursue of objective results regarding the effects of root lesion nematodes on maize.

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