Available Online at ESci Journals
International Journal of Phytopathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/phytopathology

IMPACTS OF SUNNHEMP AND PIEGON PEA ON PLANT-PARASITIC NEMATODES, *RADOPHOLUS SIMILIS* AND *MELOIDOGYNE* SPP. AND BENEFICIAL BACTERIVOROUS NEMATODES

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ABSTRACT

Plant-parasitic nematodes such as burrowing nematode (*Radopholus similis*) and root-knot nematode (*Meloidogyne* spp.) are dominant in the banana, Musa spp., ecosystem. Beneficial nematodes such as bacterivores are also found in banana fields. A tropical cover crop, sunn hemp (*Crotalaria juncea*) (SH), can be used to suppress plant-parasitic nematodes and enhance beneficial bacterivorous nematodes. However, SH cultivation in Hawaii is under the threat of the flour beetle. Thus, two experiments: Trial-I and Trial-II were conducted to compare the effects of another tropical cover crop, pigeon pea (*Cajanus cajan*) (PP) with SH and no-cover crop control (CC) on *R. similis* and *Meloidogyne* spp. suppression and beneficial bacterivorous nematodes enhancement. In both experiments soils infested with *R. similis* and *Meloidogyne* were sampled and amended with cover crop treatments (SH or PP) or CC and kept for two weeks. At the end of each experiment, nematodes were extracted through the Baermann funnel technique. The results of Trial-I and Trial- II showed that SH and PP did not reduce *R. similis* number ($P > 0.05$). However, *Meloidogyne* numbers were reduced by SH and PP in Trial-I ($P < 0.05$). In Trial II, *Meloidogyne* was not found in SH and PP. In both experiments, SH consistently increased beneficial bacterivorous nematodes number ($P < 0.05$). Cover crop PP increased beneficial bacterivorous nematode numbers in Trial -I ($P < 0.05$), but not in Trial –II ($P > 0.05$). However, the trends associated with the numbers of beneficial nematodes were consistently higher in PP compared to CC. Farmers could choose PP as an alternate to SH, as a cover crop for *Meloidogyne* suppression and beneficial nematode enhancement.

Keywords: *Cajanus cajan, Crotalaria juncea,* free-living nematodes*, Radopholus similis,* root-knot nematode.

INTRODUCTION

The banana, *Musa* spp., is an important food crop in the world and a staple crop in many tropical regions (Sharrnock and Frison, 1999). Within the US, Hawaii ranks number one in banana production. However, the banana production of Hawaii has been declined since 2000 mainly because of the attack of *Banana bunchy top virus* (BBTV) and plant-parasitic nematodes (Wang and Hooks, 2009). Multiple plant-parasitic nematodes such as root-knot nematode, *Meloidogyne* spp. (Wang and Hooks, 2009) and burrowing nematode, *Radopholus similis* (Cobb) Thorne (McIntyre *et al.,* 2000; Wang and Hooks, 2009) are associated with the production decline of banana.

Plant-parasitic and beneficial nematodes such as bacterivores are closely associated on banana fields. In contrast to plant-parasitic nematodes, beneficial nematodes such as bacterivores and fungivores have a role in soil nutrient cycling and soil health improvement (Wang and McSorley, 2005). Higher number of bacterivorous nematodes is an indicator of a healthy soil (Wang and McSorley, 2005; Marahatta *et al.,* 2010). Nematode management for Hawaiian banana should include a strategy for suppressing *R. similis* and *Meloidogyne*, and improving the bacterivorous nematode population.

Tropical cover crop sunn hemp, *Crotalaria juncea* L., (SH) is a poor host or non-host of multiple plant-parasitic nematodes (Wang *et al.,* 2002) and SH helps to enhance beneficial bacterivorous nematodes (Wang *et al.,* 2002; Wang *et al.,* 2011; Marahatta *et al.,* 2012a). Furthermore, SH produces an allelopathic compound, monocrotaline, after incorporating its foliage in soil. This

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allelopathic compound, monocrotaline, is responsible for killing *Meloidogyne* and *R. similis* (Wang *et al.,* 2002). Thus, SH has been used in suppressing the plant-parasitic nematodes of banana and several other crops such as bitter melon (Marahatta *et al.,* 2010), pumpkin (Wang *et al.,* 2011) and pineapple (Marahatta *et al.,* 2012b) and enhance beneficial bacterivorous nematodes (Wang *et al.,* 2002; Wang *et al.,* 2011). However, a pest insect, flour beetle, can cause SH wilting and is a challenging problem of SH, if multiple cycles of SH are grown on the same field (K.-H. Wang, personal communication, 2012). Therefore, researchers have initiated to evaluate other leguminous cover crops such as pigeon pea, *Cajanus cajan* (L.) Millsp., (PP) for managing multiple plant-parasitic nematodes in the tropics. The cover crop PP is commonly used for soil conservation and is popular among organic farmers of Hawaii. Furthermore, PP has been documented as *Meloidogyne* suppressive plant (Reddy *et al.,* 1986). As a leguminous cover crop, PP produces a lot of foliage, which can be used as a soil amendment material and could enhance beneficial nematodes. Therefore, current research compares the effects of SH and PP on 1) *R. similis* and *Meloidogyne* spp. suppression and 2) beneficial bacterivorous nematode enhancement.

MATERIALS AND METHODS

Two laboratory experiments were conducted at Kauai Community College, Lihue, HI in Spring 2013 (Trial – I) and in Summer 2013 (Trial- II). In both experiments, Trial I and Trial II, soil infested with *Radopholus similis and Meloidogyne* spp. was collected from an established orchard planted with the tall variety of banana at S & F Takahashi Farm, Kalaheo, Kauai, HI, USA. The field soil collected from 15cm.-depth was composited and well-mixed in a large plastic bag. In each experiment, the final weight of composite soil sample was about 5 kg.

At starting of Trial I, soil nematodes were extracted using Baermann funnels (Walker and Wilson, 1960) and determined the initial nematode population (Pi). The Pi for *R. similis* and *Meloidogyne*/100 cm^3 soil, were 64 and 16 second stage juveniles (J2), respectively. After knowing the Pi, effects of sunn hemp (SH) and pigeon pea (PP) on *R. similis* and *Meloidogyne,* and bacterivorous nematodes were tested and results were evaluated in Trial I and Trial II.

In each experiment, *R. similis* and *Meloidogyne* infested field soil was potted into fifteen 7.62 cm-diameter planter pots. Each pot was filled with 300 cm^3 soil and immediately incorporated with (SH or PP) or without (CC) dry cover crop foliage powder at 1.0% (w/w). Cover crop foliage powder used for soil amendment was prepared with SH or PP plants grown at Kauai Community College Farm. These SH and PP were oven-dried at 72°C for three days to a constant weight and ground with a commercial blender (Winsted Conn, Waring Products Co., CT) into powder form (Marahatta *et al.,* 2012b). Each potted soil weight averaged 178.95g /pot. In each experiment, of the fifteen total pots, there were 5 of CC, SH and PP, respectively. Thus, the experiments were replicated five times and arranged in the randomized complete block design (RCBD).

The pots were left to incubate for 7 days before the Baermann funnel technique was used for nematode extraction. Fifty mL of water was used per Baermann funnel and left for additional 7 days to extract nematodes (Walker and Wilson, 1960). The extracted dominant plant-parasitic nematodes, *Meloidogyne* and *R. similis* and beneficial bacterivorous nematodes (Yeates *et al.,* 1993) such as Rhabditidae, *Cephalobus*, and *Eucephalobus* were identified whenever possible and counted using an inverted microscope (Fluovert, Leitz Wetzlar, Germany).

Statistical Analysis: Data were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure in Statistical Analysis System (SAS Institute, Cary, NC). Nematode abundance were log (x +1) -transformed accordingly to PROC UNIVARIATE in SAS prior to ANOVA. Untransformed arithmetic means are presented. Means were separated by Waller-Duncan *k*-ratio (*k*=100) *t*-test wherever appropriate.

RESULTS AND DISCUSSION

In Trial I and Trial II, the following effects of SH and PP were found at the termination of experiment (after 14 days from starting the experiment).

Effects on *Meloidogyne:* *Meloidogyne* was the most prevalent and damaging nematode at the soil sampled site. Before the beginning of the experiment, nematode population densities were not different among treatments (data not shown). In Trial I, compared to CC, SH and PP reduced *Meloidogyne* number (P < 0.05). In Trial II, *Meloidogyne* was not found in SH and PP (Figure 1).

In current experiment, compared to no cover crop amended treatment CC, SH and PP consistently reduced or showed a lower population trend of *Meloidogyne*. These results are consistent with the earlier findings where soil incorporated with SH 'Tropic Sun' (Wang *et al.,* 2011) and

PP 'FL81D' and 'Norman' (Reddy *et al.,* 1986) reduced soil population of *Meloidogyne* in comparison to bare ground fallow plots in Hawaii and Florida, respectively. *Meloidogyne* suppression results of SH and PP of current experiment shows the importance of allelopathic properties: yellow resinous microscopic secretions of PP (Rizvi and Rizvi, 1992) and monocrotaline of SH (Rodriguez-Kabana *et al.,* 1992; Wang *et al.,* 2001; Jourand *et al*., 2004) in the management of the most prevalent plant-parasitic nematode. Current results validate the *Meloidogyne* suppressive characteristics of SH and PP in banana grown soil. Furthermore, this result has demonstrated that powdered form of SH or PP could be used and effectively suppressed *Meloidogyne* population in absence of field grown cover crops.

Effects on *Radopholus similis:* At the soil sampled site, *R. similis* was the second most prevalent plant-parasitic nematode. Compared to no cover crop treatment CC, both cover crops, SH and PP, consistently did not reduce *R. similis* number ($P > 0.05$) at termination of Trial I and Trial II (Figure 2).

In contrast to the suppressive effects of SH and PP on *Meloidogyne*, there was no significant results in reducing the *R. similis* number in either of the two trials. However, the trends of *R. similis* number found in SH and PP in Trial I and Trial II are consistent with the findings of Chitamba et al. (2014) where banana and SH intercropped treatment suppressed *R. similis* in a glasshouse experiment.

Effects on Beneficial Bacterivorous Nematodes: In Trial I, higher numbers of bacterivorous nematodes were found in SH followed by PP ($P < 0.05$). This effect of SH ($P < 0.05$), not PP ($P > 0.05$), on bacterivorous nematodes number was consistent in Trial II (Figure 3). Rhabditidae was the most dominant bacterivorous nematode consistently found in Trial I and Trial II. Other bacterivorous nematodes found in both experiments were *Cephalobus*, and *Eucephalobus* (data not presented).

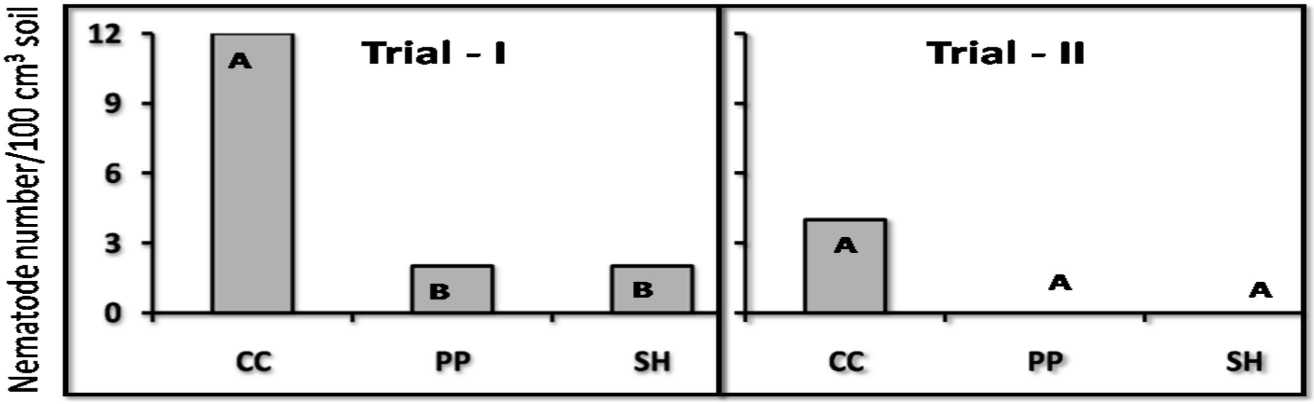

Figure 1. Effects of soil incorporated cover crops, sunn hemp (SH) and pigeon pea (PP), and no cover crop control (CC) on abundance of *Meloidogyne* spp. at termination of the experiment. Means are average of 5 replications. Means followed by same letter(s) do not differ according to Waller-Duncan *k*-ratio ($k = 100$) *t*-test based on log (x+1) transformed values.

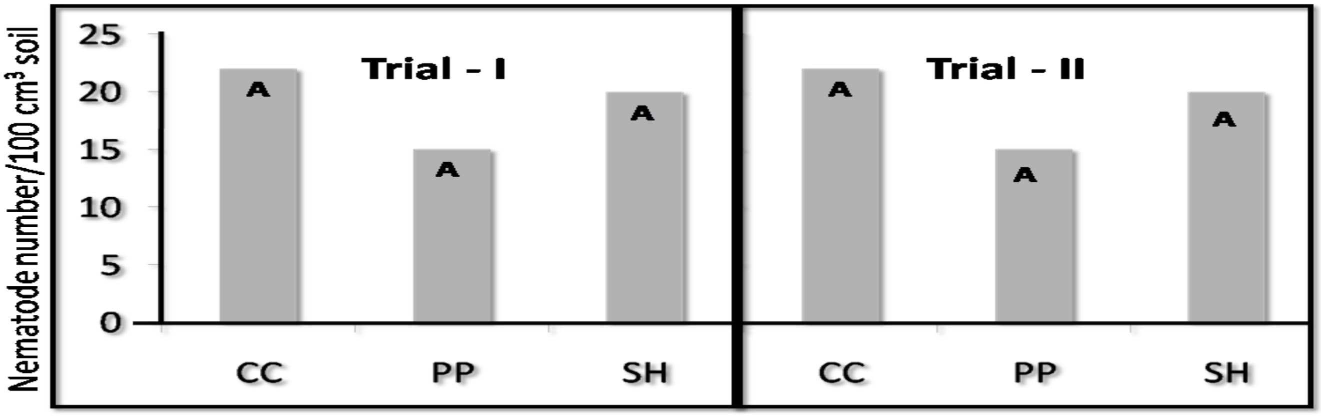

Figure 2. Effects of soil incorporated cover crops, sunn hemp (SH) and pigeon pea (PP), and no cover crop control (CC) on abundance of *Radopholus similis* at termination of the experiment. Means are average of 5 replications. Means followed by same letter(s) do not differ according to Waller-Duncan *k*-ratio ($k = 100$) *t*-test based on log (x+1) transformed values.

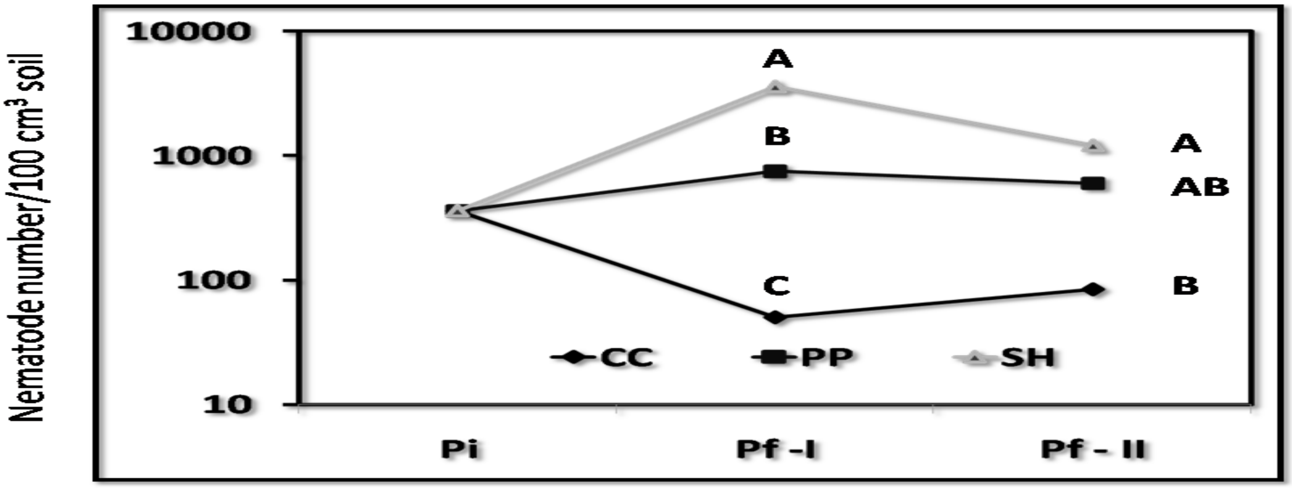

Figure 3. Effects of soil incorporated cover crops, sunn hemp (SH) and pigeon pea (PP), and no cover crop control (CC) on abundance of beneficial bacterivorous nematodes at starting (Pi) and termination (Pf- I and Pf- II for Trial-I and Trial-II, respectively) of the experiment. Means are average of 5 replications. Means followed by same letter(s) in each soil sampling date do not differ according to Waller-Duncan k-ratio (k=100) t-test based on log (x+1) transformed values.

Abundances of beneficial bacterivorous nematodes is often used as an indicator of soil nutrient enrichment (Bongers and Bongers, 1998; Ferris *et al.,* 2001). In the current experiment, SH followed by PP consistently increased the abundances or population trend of bacterivorous nematodes and demonstrated that cover crop incorporated treatments were enriched with nutrients. This result of SH is consistent with the previous findings where SH incorporated field plots consistently enhanced bacterivorous nematodes in strip-tilled SH cover cropping system (Marahatta *et al.,* 2010; Wang *et al.,* 2011). Additionally, the current experiment had demonstrated PP as a potential cover crop for enhancing soil nutritional status after SH, as indicated by beneficial nematode numbers. Thus, a higher population of bacterivorous nematodes, and a lower population of *Meloidogyne* on SH followed by PP incorporated pots indicate two simultaneous benefits of both tropical cover crops.

CONCLUSION

Both cover crops, SH and PP, did not suppress *Radopholus similis*, but suppress or show a trend of lower *Meloidogyne* number compared to CC. Compared to CC, PP enhanced beneficial nematodes in Trial I, and show a trend of higher number of beneficial nematodes in Trial II. Although SH had a greater effect in increasing beneficial nematode populations in both trials, farmers are recommended to use PP and SH alternately to protect the crop from flour beetles.

ACKNOWLEDGEMENTS

This project was supported by grants from the United States Department of Agriculture/National Institute of Food and Agriculture (award number 2012-38426-19624), and the National Center for Research Resources (5P20RR016467-11) and the National Institute of General Medical Sciences (8P20GM103466-11) from the National Institutes of Health. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. Special thanks to S & F Takahashi Farm, Kalaheo, Kauai, HI.

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