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Soil Health Management Against Fusarium Asparagus Crown and Root Rot

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Introduction

In Hawaii, asparagus (*Asparagus officinalis*) can be harvested more often and over a longer period than in cold climates (Deputy, 1999). It was once considered to have minimal pest and disease problems in Hawaii until a widespread decline in 2016 and beyond (Cheng, 2017). Crown and root rot of asparagus is caused by *Fusarium oxysporium* f. sp. *asparagi* (*Foa*), a major disease of asparagus worldwide. The fungus survives in the soil indefinitely and may be seed-borne (Elmer, 2015). Infection occurs below ground colonizing the crown and roots, causing the plant to stunt and forming bright yellow ferns (Fig. 1). Besides rotting, *Foa* can also invade the xylem tissue and plants will wilt following excessive harvesting (Aegerter and Davis, 2009). Adverse environmental factors and the interactions of fusarium crown and root rot with other diseases or insects add stress to plants and further reduce plant vigor. Fig. 2 shows weakened asparagus stems that were chewed by burrowing cockroach (*Pycnoscelus indicus*). Maintaining crown vigor by proper irrigation, fertilization, or soil health management can help asparagus to withstand the damage. Asparagus can be a long-term profitable crop (up to 20-30 year from one planting) if it remains healthy. However, fusarium crown and root rot can cause the crop to senesce early without yielding or decline in spear production by 50% (Milton Agader, personal communication). Farmers would have to replant in a clean field. Long-term rotations out of asparagus or minimizing plant stress are recommended. Hybrid varieties such as ‘UC 157’, ‘Apollo’, and ‘Jersey Giant’ have increased plant vigor, which provide a degree of tolerance, but not resistant to this disease (Aegerter and Davis, 2009). Deputy (1999) also suggested that keeping the soil pH above 7 by liming could

Fig. 1. Fusarium crown and root rot on asparagus.

Fig. 2. Weakened stems of asparagus chewed by burrowing cockroaches.

suppress this disease. However, the disease is still observed despite pre-plant liming. Liming is also difficult at post-plant for a perennial crop as the planting bed is usually mulched.

Healthy soil is a soil that is capable of supporting plant life and anchorage, soil nutrient cycling, chemical and physical processes, and the biodiversity of beneficial soil organisms while suppressing pests (Doran and Zeiss, 2000). Free-living nematodes in the soil have been proven to be good soil health bioindicators (Ferris et al., 2012) as nematodes have diverse life strategies, ranging from colonizers (short life and high reproduction rate) to persisters (long life and low reproduction rate), and they have close interactions with other soil organisms. Thus, the abundance of nematodes in the soil indicate the abundance of other soil organisms they interact with, and they are playing important roles in soil nutrient cycling (Ingham et al., 1985).

Due to land limitation for a long-term rotation for asparagus production to overcome *Foa* infestation, the question that needs to be addressed is whether maintaining soil health conditions using biologically derived compounds could help the asparagus to overcome fusarium crown and root rot disease. This project examined the amendment of soil with five biologically derived compounds that have the potential to antagonize against plant pathogenic fungi (Wang et al., 2019a). Compounds tested include: 1) Actinovate® AG (Noyozyme, Milwaukee, WI) that contains a high concentration of *Streptomyces lydicus* strain WYEC 108; 2) Subtilex® NG (BASF, Research Triangle Park, NC), a biological fungicide with active ingredient *Bacillus subtilis*; 3) Shrimp Meal (Down To Earth Distributor Inc., Eugene, OR) that contains 6-6-0 and 10% Ca, 18% chitin and trace minerals derived from ground shrimp shells; 4) Crustacean Meal (PAR 4 Protein Meals, Bridgewell Agribusiness LLC, Clackamas, OR) that contains 4-0-0, 12% Ca, 23-30% chitin derived from crab and lobster shells and meal. In addition, we also included macerated brown mustard (*Brassica juncea*) 'Caliente 199' residues that release isothiocyanates upon soil incorporation as a biofumigant (Waisen and Wang, 2019; Waisen et al., 2020). Some of these biological compounds besides possessing potential antagonistic effects against soil-borne fungi might also enhance microbial activities in the soil that can improve soil health (Wang et al., 2019b).

Specific objectives of this research were to examine if 1) the above-mentioned biological compounds were able to suppress *Foa* and its disease incidence on asparagus and 2) improving soil health could contribute to the reduction of fusarium asparagus crown and root rot.

Materials and Methods

A field trial was initiated at Twin Bridge Farms, Waialua, Oahu on November 8, 2019, in a fallow land previously planted with asparagus with a history of fusarium crown and root rot disease. Twenty-four plots of 4×10 ft^2 in size on 4 inch raised bed were either amended with 1) lobster (crustacean) meal at 35 lb/1000 ft^2; 2) shrimp meal at 35 lb/1000 ft^2; 3) macerated 'Caliente 199' brown mustard shoot tissues at 3200 lb/acre dry biomass; or drenched with a water suspension of 2 gal/plot mixed with 4) Actinovate at 6 oz/acre; 5) Subtilex at 0.4 oz/acre; and 6) water only as bare ground (untreated) control. All plots were mulched with polyethylene black plastic

immediately after treatment. Asparagus seedlings were transplanted on December 11, 2019, at a 1-ft spacing between plants, thus each plot had a single row of 10 asparagus seedlings with a 10-ft buffer between plots. Each treatment was replicated 4 times and arranged in a randomized complete block design. The second application of all treatments except brown mustard was done on March 5, 2020. The treatments were delivered through the planting holes to each asparagus plant at the same application rate listed above. For treatments that required drenching, each plant was delivered 16.8 fl oz (500 ml) of the treatment solution. The second application of brown mustard occurred on May 18, 2020, due to the unavailability of materials at the earlier date. Data presented here are from the first 6 months of the study that is currently underway.

Soil health analysis: Soil was collected from 6 soil cores per plot prior to the application of treatments or from the rhizosphere of 6 asparagus plants per plot at 1, 3, and 5 month(s) after transplanting. The composite soil sample from each plot was subjected to soil respiration test using Premium Field CO_2 Test (Solvita Gel System, Solvita and Woodend Laboratory). The remaining soils were subjected to nematode community analysis. Nematodes were extracted from 250-cm^3 subsample of soil by elutriation followed by centrifugal flotation (Jenkins, 1964), identified to genus, counted, and assigned to one of the five trophic groups: bacterivores, fungivores, herbivores, omnivores or predators (Yeates et al., 1993). The nematode fauna was further analyzed by a weighting system for nematode functional guilds in relation to enrichment and structure of the soil food web and calculated into enrichment index (EI), structure index (SI), and channel index (CI) according to Ferris et al. (2001). The EI assesses soil food web responses to available nutrient resources, SI reflects the degree of trophic connection in food webs of increasing complexity as the system matures, or progressive food web simplicity as the system degrades, whereas CI depicts whether the soil is undergoing fungal or bacterial dominated decomposition pathways that would indicate if the soil is under stress conditions (Ferris et al., 2001). In addition to soil health measurement, soil pH was also monitored at 5 months after planting.

Fusarium incidence and root colonization estimation: On January 22, 2020 (1 month after transplanting), numbers of plants with yellow fern symptom (Fig. 1) per plot were recorded. Later, percentage of shoot per asparagus mat from 5 plants per plot were randomly selected to monitor for yellow fern symptom on March 5 and May 28, 2020. A total number of shoot per asparagus mat was also used as a measurement plant vigor. In addition, asparagus roots were collected on March 14, March 27, and May 20, 2020, where 9 random pieces of 1-cm long roots were subsampled and plated on 3 replicated Petri dishes of Komada medium (Komada, 1976) to monitor relative colonization of *Fusarium* on the root pieces.

Fig. 3. Asparagus root pieces on Komada medium showing initial colonization of Fusarium.

Statistical analysis: Disease incidence data were adjusted by subtracting percent of diseased plants in the bare ground from the treatment and expressed as Adjusted Disease Incidence (Fig. 3). All data were checked for normality using Proc Univariate in SAS Version 9.4 (SAS Institute Inc., Cary, NC). Nematode abundance were normalized using log10 (x + 1) wherever needed prior to analysis of variance (ANOVA) using Proc GLM. If no interaction between treatment and sampling date was detected, data from all sampling dates were combined, otherwise, data were presented by each sampling date. Means were separated using the Waller-Duncan *k*-ratio (k = 100) *t*-test.

Results and Discussion

Disease suppression: Disease incidence recorded 2, 4, and 6 months after the first treatment was consistently lowest ($P \leq 0.05$) in brown mustard biofumigation among the treatments (Fig. 4). All other treatments perform inconsistently in different dates with minimal to no suppression against the disease (Fig. 4). However, percent of asparagus root colonized by *Fusarium* on the Komada selective medium was not different among treatments but was ranked the lowest in brown mustard treatment (33%) and highest in Subtilex (47%). Previously we found that biofumigation with brown mustard though did not suppress disease incidence of Fusarium wilt on lettuce (*Lactuca sativa*) effectively, it increased the lettuce plant survival rate in a *Fusarium oxysporum* f. sp. *lactucae* field (Wang et al., 2019b).

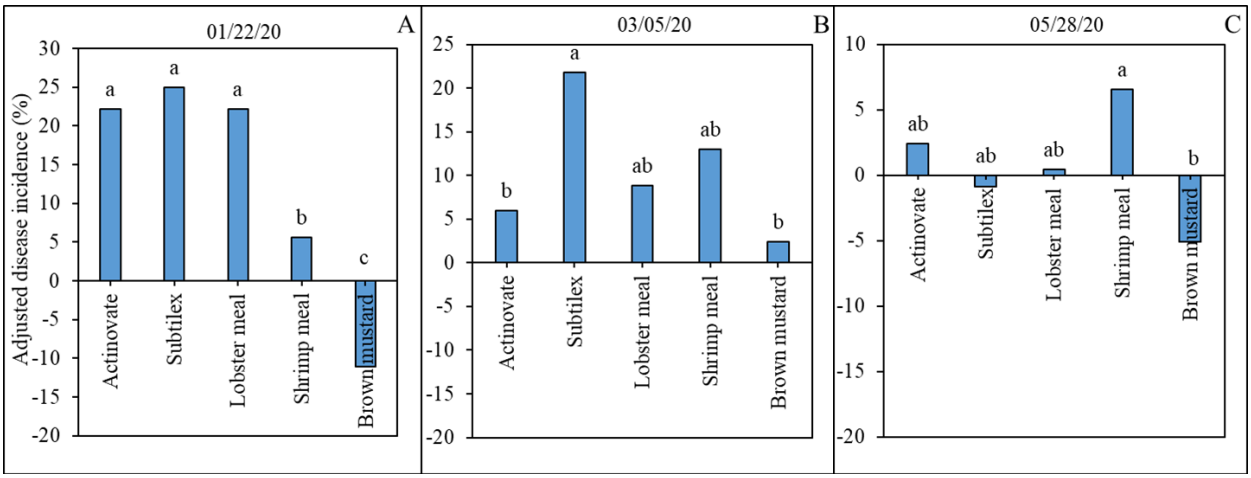

Fig. 4. Adjusted disease incidence calculated by subtracting percent of diseased plants in the bare ground control from the treatment plot at A) 2, B) 4, and C) 6 months after the first treatment. Means (n = 4) followed by the same letter(s) are not different at $P \leq 0.05$ level.

Nematode suppression and plant growth: Although it was generally reported that asparagus is not a host of root-knot nematodes, *Meloidogyne* spp. (Deputy, 1999), we observed a gradual increase in abundance of the nematode in the bare ground control treatments over the 5 months of asparagus growth. The field started with a minimal number of root-knot nematodes in the soil, but its abundance in the soil increased by 5 or 10 folds compared to the other treatments at 3 months after planting (Fig. 5A). In contrast, lobster meal and brown mustard treatments maintained root-

knot nematodes free and lower than the bare ground ($P \leq 0.05$). Effect of brown mustard as a biofumigant against root-knot nematodes is well documented (Waisen et al., 2020). Mian et al. (1982) also reported that stimulation of chitinolytic microflora following chitin soil amendments can be suppressive to plant-parasitic nematodes.

Plant Growth: While plant height was not affected by any of the soil treatments, total number of shoots per asparagus mat was 26% higher ($P \leq 0.05$) in the lobster meal compared to the bare ground, while all the other treatments were not different from the control (Fig. 5B).

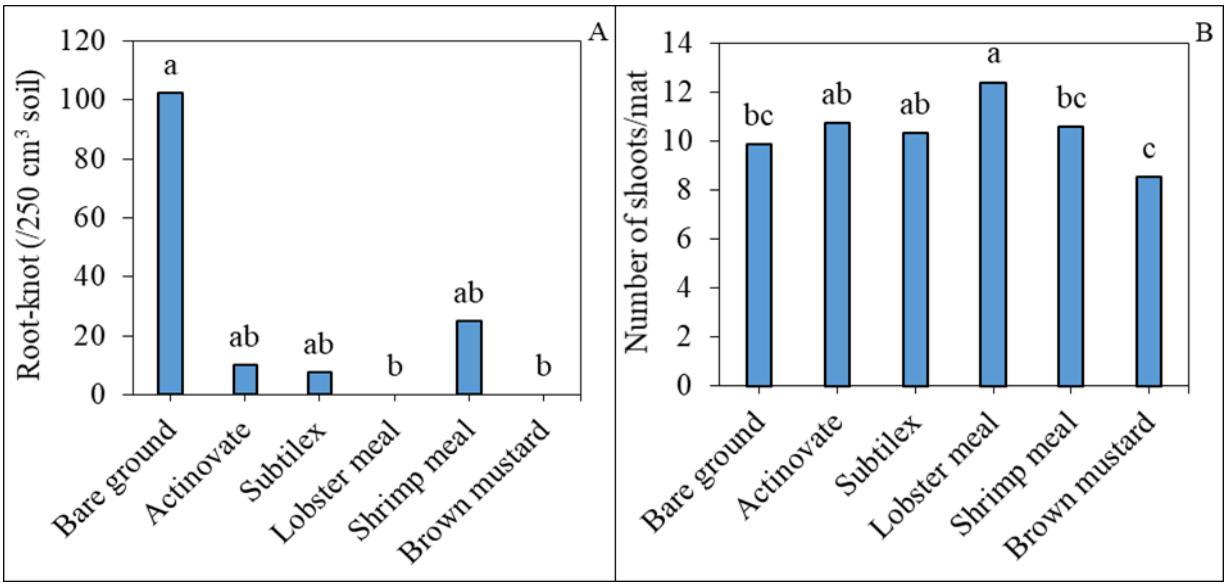

Fig. 5. A) Abundance of root-knot nematodes assessed at 3 months after planting, and B) number of shoots per mat of asparagus monitored on the 3rd and 5th month after planting. Means followed by the same letter(s) are not different at $P \leq 0.05$.

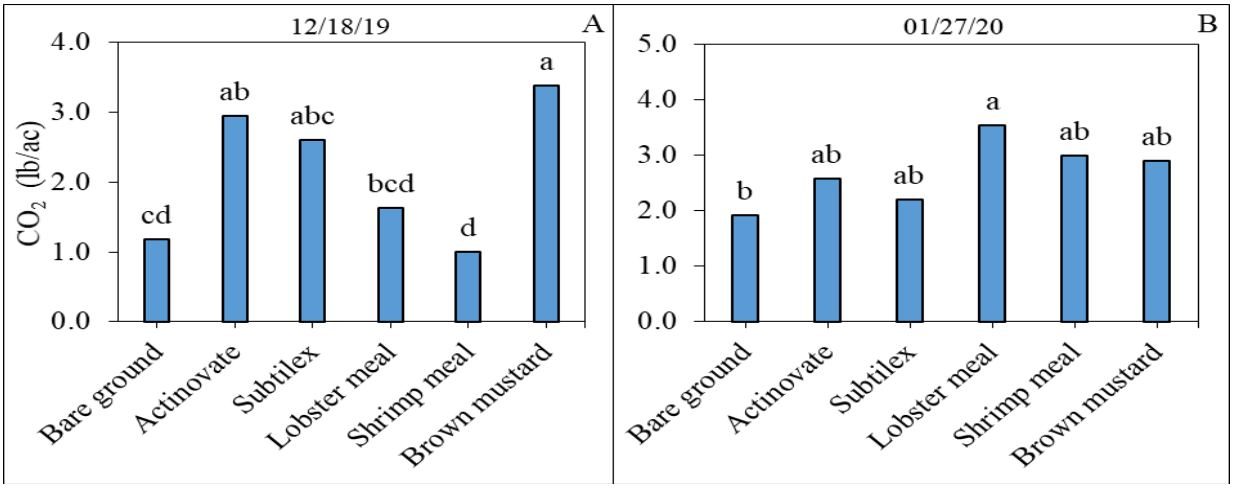

Fig. 6. Soil microbial respiration rate (carbon dioxide (lb CO_2/Acre at A) 1 and B) 2 months after treatment. Means (n = 4) followed by the same letter(s) are not different at $P \leq 0.05$.

Soil health condition: Brown mustard and Actinovate increased ($P \leq 0.05$) soil microbial respiration by 180% and 120%, respectively at 1 month after treatment (Fig. 6A), while lobster meal increased ($P \leq 0.05$) soil microbial respiration by 84% at 2 months after treatment (Fig. 6B) compared to the bare ground. This effect did not last through 3 and 5 months after planting.

A high microbial respiration rate indicates more bacterial decomposition in the soil. This coincides with the higher abundance of bacterial feeding nematodes in brown mustard and lobster meal at 3 months after planting ($P \leq 0.05$), and in the lobster meal at 5 months after planting (Fig. 7A). Enrichment index (EI), which is a weighted abundance of opportunistic bacterial feeding nematodes that is associated with soil nutrient enrichment, was significantly increased ($P \leq 0.05$) by shrimp meal and lobster meal at 5 months after asparagus planting (Fig. 7B). At the same time, lower EI in brown mustard compared to the bare ground ($P \leq 0.05$) suggested that the initial boost in soil microbial respiration by brown mustard did not last and its effect dissipated about 1 month after the treatment (Fig. 7B). Unfortunately, free-living nematodes in other trophic groups were not affected by any soil treatments tested. Thus, lobster meal and shrimp meal only enriched soil nutrients but had not improved the overall soil food web structure.

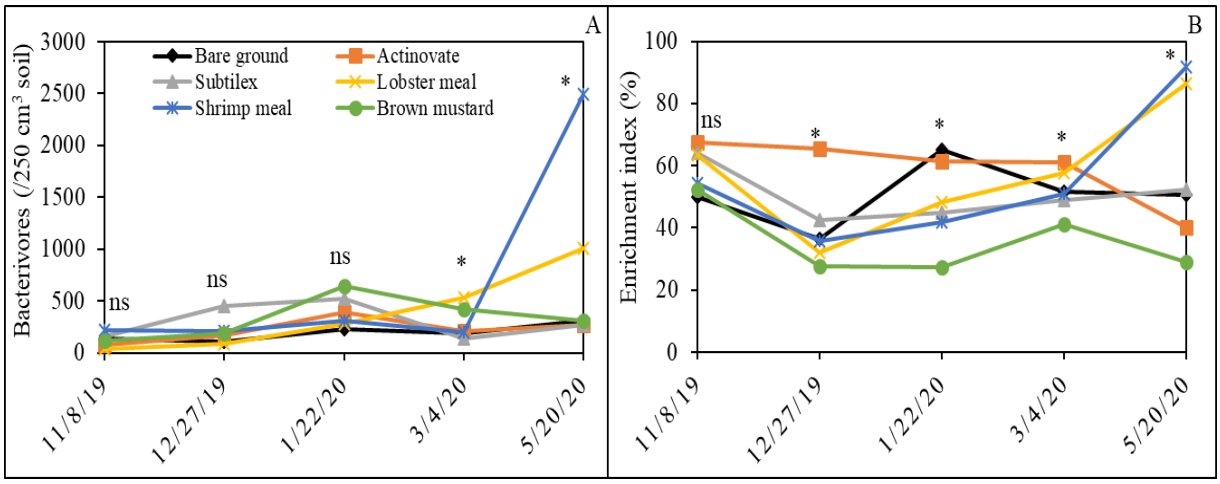

Fig. 7. Soil health condition assessed on abundance of A) bacterial feeding, and B) fungal feeding nematodes or C) enrichment index, and D) channel index during asparagus growth. Lines are means (n = 4) and those with 'ns' are not different from bare ground control and those with asterisk (*) are different at $P \leq 0.05$.

Asparagus grows best between soil pH 6.5-7.5. Soil pH from all treatment plots ranged from pH 6.4-6.6 at 5 months after planting which is within the appropriate range for asparagus growth. However, it was suggested that maintaining soil above pH 7 would reduce the disease incidence (Deputy, 2009). None of the treatments tested in this study increased soil pH above 7.

Conclusion

It is promising that amending brown mustard into the soil followed by mulching with black plastic prior to asparagus planting suppressed the disease incidence of Fusarium asparagus crown and root rot up to 5 months after asparagus planting. However, results from this study did not show that any of the biological compounds or amendment tested were able to improve soil health over the 5 months of asparagus growing period. None-the-less, lobster meal and shrimp meal increased bacterial feeding nematode abundance and resulted in higher soil nutrient enrichment at 5 months after asparagus planting. Perhaps, brown mustard as a pre-plant soil amendment should be integrated with bi-monthly or quarterly treatment of lobster or shrimp meal as a viable and proactive measure against *Foa* in an infested field. This is an ongoing experiment to further examine the long-term effects of *Foa* and soil health management effects on an asparagus crop.

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