Cover Crop "5-in-1 Approach" for Nematode Management Using Mustard and Oil Radish

Final report for GW18-026

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Project Information

Summary:

Abstract:

Root-knot (Meloidogyne incognita and M. javanica) and reniform (Rotylenchulus reniformis) nematodes are major plant parasites in cucurbit cropping systems in Hawaii. This "Cover crop 5-in-1 Approach" project, as the name suggests focused on utilizing 5 mechanisms that Brassica cover crops (Brassicaceae) employ to combat the plant-parasitic nematodes. The mechanisms included poor or non-host effect, trap crop effect, biofumigation effect, host plant tolerance due to green manure effect, and enhancement of soil-borne nematode antagonistic microorganisms. During this 2-year project, 4 greenhouse and 7 field experiments were conducted. Two greenhouse experiments examined susceptibility of ‘Caliente 199’ brown mustard (Brassica juncea) and ‘Sodbuster’ oil radish (Raphanus sativus) to the nematodes. Brown mustard was a good host of M. incognita and R. reniformis whereas oil radish was a poor host of M. incognita but good host of R. reniformis. Based on greenhouse trials, M. incognita or R. reniformis eggs required 465 degree-days (DD) or 512 DD, respectively to reach egg-laying females on brown mustard and oil radish. With a daily average of 22°C at 4-inch soil depth in Hawaii, these results could mean M. incognita or R. reniformis eggs can complete a life cycle on the Brassicas in 5 or 6 weeks, respectively. Thus, Brassica cover crop should be terminated 5-6 weeks after planting to avoid further nematode reproduction. In the field, when oil radish was grown for 6 weeks, it did not reduce soil populations of both root-knot and reniform nematodes. However, when brown mustard was grown for 5 and 6 weeks, it reduced numbers of root-knot nematodes but not when grown from 7 weeks. Conversely, reniform nematode was reduced when brown mustard...
was grown for 7 weeks. These results indicated that brown mustard was a good trap crop of both nematodes but the trap crop effect cannot be predicted by DD, unlike what we had hypothesized.

Biofumigation effects of the Brassicas on the nematodes were assessed 1 week after soil incorporation of above-ground biomass and at monthly interval after a zucchini (Cucurbita pepo) crop was planted in multiple field trials. Overall, oil radish failed to suppress both root-knot and reniform nematodes but increased bacterial feeding nematodes and nematode enrichment index (EI) indicative of nutrient enrichment, a clear demonstration of green manure effect. Whereas biofumigation with brown mustard suppressed root-knot nematodes in three replicated trials and suppressed reniform nematode in one of the three trials. Bacterial feeding nematode population and EI were transiently increased for up to 1 month after brown mustard biofumigation. Among all these field trials, we consistently found that biofumigation against plant-parasitic nematodes was most effective when aerial tissues were macerated and tilled followed by covering the soil with black plastic mulch. The results were confirmed by significant increase in soil glucose and sulfate concentrations, the by-products of biofumigation besides isothiocyanates. During these studies, we also found that although both sulfate and glucose in the soil were negatively related to the targeted nematodes based on canonical correspondence analysis, sulfate was a better indicator of biofumigation effect as it had a stronger negative relationship with plant-parasitic nematodes and it is more stable in the soil for analysis.

Introduction:

This project examined the mechanisms of Brassica cover crops employed to manage plant-parasitic nematodes including 1) biofumigation effects that is toxic to plant-parasitic nematodes; 2) trap cropping effect that allows nematode infection but also making them more vulnerable to allelopathic effect of biofumigant; and 3) green manure effect that improves plant tolerance to nematode damage. Our goal was to maximize the benefits of Brassica cover crops by integrating biofumigation with different plastics or through mixed planting of brown mustard (Brassica juncea) and oil radish (Raphanus sativus) cover crops. Plant-parasitic nematodes targeted in this project were root-knot nematodes (Meloidogyne incognita and M. javanica) and reniform nematode (Rotylenchulus reniformis).

Project Objectives:

The overarching goal was to develop biofumigation method suppressive to plant-parasitic nematodes and yet beneficial to soil health for cucurbit crop production.

Specific objectives of this project were to:

1. Examine susceptibility of ‘Caliente 199’ brown mustard and ‘Sodbuster’ oil radish to root-knot (Meloidogyne incognita) and reniform (Rotylenchulus reniformis) nematodes.
2. Determine heat-units required by incognita and R. reniformis to reach egg-laying females on ‘Caliente 199’ brown mustard and ‘Sodbuster’ oil radish;
3. Compare ‘Caliente 199’ brown mustard and ‘Sodbuster’ oil radish for their biofumigation effects against plant-parasitic nematodes and soil health improvement benefits;
4. Determine best biofumigation method suppressive to targeted plant-parasitic
nematodes;
5. Determine best biofumigation indicator for assessing suppressive activities on plant-parasitic nematodes;
6. Compare black plastic and clear solarization mulch for their ability to enhance biofumigation effects on plant-parasitic nematodes;
7. Examine biofumigation effects on free-living nematodes as indicators of soil health.

Cooperators

- **Owen Kaneshiro**
  Farm manager/owner
  Owen Kaneshiro Farm LLC (Commercial (farm/ranch/business))

- **Rose Mathews**
  Farm manager/owner
  Bear Claw Farm (Commercial (farm/ranch/business))

Research

Materials and methods:

**Objective 1 - Brassica susceptibility experiments**

Greenhouse experiments were conducted to compare susceptibility of ‘Caliente 199’ brown mustard (*Brassica juncea*) and ‘Sodbuster’ oil radish (*Raphanus sativus*) to root-knot nematode (*Meloidogyne incognita*) as compared to a known susceptible host, ‘Orange Pixie’ tomato (*Solanum lycopersicum*) grown in a sterile sand: soil mix. A similar experiment was conducted to examine susceptibility of these two Brassica crops to reniform nematode (*Rotylenchulus reniformis*) and compared to ‘Iron Clay’ cowpea (*Vigna unguiculata*) serving as the nematode-susceptible control. Seedlings were inoculated 2 weeks after planting with the designated nematodes prepared from pure cultures. The experiments were terminated 28 days after inoculation. At termination of each experiment, the nematode eggs from entire roots or vermiform stages from the entire pot of soil were extracted and enumerated.

**Objective 2 - Degree-days, termination age, and trap crop experiments**

**Degree-days experiments:**

Two greenhouse experiments were conducted to determine degree-days (DD) required by *M. incognita* to reach egg-laying female (ELF) on ‘Caliente 199’ brown mustard and ‘Sodbuster’ oil radish. ‘Orange Pixie’ tomato was included as *M. incognita*-susceptible control. Destructive plant samples were stained with acid fuchsin every 3 days over 21 days, and examined for ELF. A temperature data
Logger was used to record temperature and calculated for DD to reach ELF. A similar experiment was conducted using *R. reniformis*. ‘Iron Clay’ cowpea was included as *R. reniformis*-susceptible control. Heat units were calculated using the formula, $DD = \Sigma(T_e - T_b)/24$ hours, where $DD$=degree-days, $T_e$=hourly soil environmental temperature, and $T_b$ is a base temperature of development (9.8°C for *M. incognita* or 10°C for *R. reniformis*).

**Termination age experiment:**

To determine the best termination time for oil radish as a trap crop in the field, ‘Sodbuster’ oil radish was grown for 0, 14, 28, 42 or 56 days. At trap crop termination, oil radish foliage was soil incorporated using a hand-held rototiller. One week after soil incorporation, a ‘Field Trip’ pumpkin (*Cucurbita moschata*) was seeded. Four months later, five pumpkin plants per plot were uprooted and rated for 0-12 root-gall index (RGI) (Netcher and Sikora, 1990).

**Trap crop experiments:**

Two field trials using ‘Sodbuster’ oil radish and three field trials using ‘Caliente 199’ brown mustard were conducted to determine their trap crop potentials against *Meloidogyne* spp. and *R. reniformis*. Oil radish or brown mustard was seeded 10 lb/ac and compared to no-trap crop bare ground control. Nematodes were extracted by elutriation (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964), and *Meloidogyne* spp. and *R. reniformis* were enumerated using an inverted microscope.

**Objective 3 and 4 - Effects of brown mustard and oil radish termination methods**

**Trial I - Brown mustard vs oil radish termination methods:**

A field trial was conducted to compare effects of ‘Sodbuster’ oil radish and ‘Caliente 199’ brown mustard termination methods in a field naturally infested with *Meloidogyne* spp. and *R. reniformis*. Six-week-old oil radish and brown mustard were terminated by 1) no-till (NT) where aboveground cover crop tissues were sickled off at soil line with minimal tissue damage and the residues covered with a light exclusion woven weed mat; 2) tissue maceration using a line trimmer (MT), or 3) MT followed by covering soil with an impermeable black plastic (MTBP). A bare ground (BG) was included as a no-Brassica treatment control. One week after the termination of the biofumigant crops, weed mat and black plastic were uncovered, and 2-week-old ‘Felix’ zucchini (*Cucurbita pepo*) seedlings were transplanted.

**Trials II and III - Brown mustard termination methods:**

Only brown mustard was tested in Trial II but with additional biofumigation methods. The brown mustard was grown for 5 weeks and subjected to 1) no-till (NT) where aboveground cover crop tissues were sickled off at soil line with minimal tissue damage and the residues covered with the woven weed mat; 2) NT followed by tissue maceration using a line trimmer (MNT); 3) MNT followed by covering soil with impermeable black plastic (NTBP); 4) tillage without tissue maceration (T); 5) tissue maceration followed by soil tillage (MT); and 6) MT followed by covering soil with impermeable black plastic (MTBP). A tilled bare ground (BG) soil treatment without biofumigation was included as a control. One week after the termination of the biofumigant crops, weed mat and black plastic were uncovered, and 2-week-old ‘Felix’ zucchini seedlings were transplanted. Trial III was initiated in the same field, repeating all the treatments in Trial II except that the ‘Caliente 199’ brown mustard was grown for 7 weeks due to slower growth in December and the rainy weather that hindered tillage operation.
Objective 5 - Biofumigation indicators

Soil glucose analysis:
Soil subsample was drawn from each soil sample in Trial I, II and III before and 1 week after biofumigant crop termination to quantify glucose concentrations. In Trials I and III, the soil subsamples were transferred onto dry ice in an attempt to arrest microbial degradation of glucose. However, in Trial II, 2 ml of toluene was added to soil subsamples immediately after soil sampling in the field to deactivate microbial activities prior to transportation to the laboratory. At the laboratory, glucose concentration in each soil sample was enzymatically determined using a Glucose (HK) Assay Kit (Sigma-Aldrich Chemical Co., St. Louis, MO) according to the manufacturer’s instructions. Based on color change at 340 nm wavelength, myrosinase (Myr) enzyme activity was estimated according to Palmieri et al. (1987).

Soil sulfate analysis:
Soil subsamples from Trial III were submitted to Agricultural Diagnostic Services Center of the University of Hawaii, Honolulu, HI to analyze soil sulfate concentration. The difference in soil sulfate concentration between termination date and one week after termination was calculated.

Objective 6 - Black plastic vs clear solarization mulch

Trial I - Brown mustard biofumigation:
A field trial was conducted to compare the ability of black plastic and clear solarization mulch to enhance biofumigation effects against plant-parasitic nematodes. A 5-week-old brown mustard was terminated by 1) macerating aboveground tissues using a line trimmer followed by soil incorporation (MT); 2) MT followed by either covering black plastic mulch (MTBP); or 3) covering clear solarization plastic mulch (MTS). A bare ground (BG) control with no biofumigant crop was included. One week after the biofumigation, the treatments were uncovered and 2-week-old ‘Parthenon’ zucchini seedlings were transplanted. Zucchini fruits were harvested and canopy width was measured monthly until zucchini crop termination. At termination of the zucchini crop, roots weighed and the root-gall index was rated based on a 0-10 scale (Netscher and Sikora, 1990).

Trial II - Brown mustard and oil radish mix biofumigation:
A second field trial was initiated on September 27, 2018, at the same experiment site as in Trial I except that a cover crop mix of ‘Sodbuster’ oil radish and ‘Caliente 199’ brown mustard was seeded at 10 lb/ac. A 5-week old cover crop mix was subjected to the same biofumigation methods (BG, MT, MTBP, and MTS) as in Trial I. One week after the biofumigation, treatment plots with the plastic mulch were uncovered and 2-week-old ‘Parthenon’ zucchini seedlings were transplanted and data collection the same way as in Trial I.

Objective 7 - Biofumigation effects on soil health

Nematode community analysis:
Nematodes extracted from all experiments in obj. 3 & 4 (Trials I, II and III) or obj. 5 (Trials I and II) were identified to genus under an inverted microscope. Nematode data were subjected to nematode community analysis in which every nematode in a sample was assigned to one of the five trophic groups either bacterivores, fungivores, herbivores, omnivores or predators (Yeates et al., 1993) and abundance of each trophic group was enumerated. Nematode richness, diversity, fungivore to fungivore plus bacterivore ratio (F/F+B) (Freckman and Ettema, 1993), Maturity
index (MI) (Yeates and Bird, 1994), were calculated. The nematode fauna was also analyzed by a weighting system of the nematode functional guilds in relation to enrichment and structure of the soil food web (Ferris et al., 2001). These indices include Enrichment index (EI), structure index (SI), and channel index (CI).

*Solvita® test - Respiration:*

Solvita® test was only conducted in obj. 6 using soil subsamples collected 1 week after biofumigation in each trial to estimate the amount of respired carbon dioxide generated by microbial biomass. Color of gel test strip was read using a Solvita® reader to estimate the amount of carbon dioxide.

*Statistical/ Canonical correspondence analyses:*

Data in all experiments (obj. 1-7) were checked for normality using Proc Univariate in SAS Version 9.4 (SAS Institute Inc., Cary, NC). Wherever necessary data were normalized using log10 (x + 1) or square root transformation prior to analysis of variance (ANOVA) using Proc GLM in the SAS. Where necessary, nematode data were subjected to repeated-measures ANOVA to detect any interaction between treatment and date of sampling. If significant interaction between treatment and date occurred, data were subjected to one-way ANOVA by date. Means were separated using Waller-Duncan k-ratio (k = 100) t-test and only the true means were presented. Canonical correspondence analysis (CCA) was performed for data in Trials I, II and III (obj. 3 & 4) to detect relationships between environmental and species variables using CANOCO 4.5 for Windows. Species variables included richness, abundance of nematode trophic groups (bacterivores, fungivores, herbivores, omnivores, and predators) including root-knot and reniform nematodes in the soil. Environmental variables included nematode community indices [EI, F/(F+B), CI, MI, SI and richness], indicators of biofumigation (Myr activity and soil sulfate), soil nitrate and temperature, cover crop biomass, zucchini yield (fruit weight), plant growth (chlorophyll content and canopy width) and RGI.

Research results and discussion:

*Objectives 1 and 2*

*Susceptibility experiments:*

Fecundity of *M. incognita* on brown mustard was not different from tomato (*P > 0.05*) but it was reduced (*P ≤ 0.05*) on oil radish compared to tomato. On the other hand, fecundity of *R. reniformis* on brown mustard was higher (*P ≤ 0.05*) than cowpea but was not different (*P > 0.05*) between oil radish and cowpea. Based on these results, brown mustard was a susceptible host of both *M. incognita* and *R. reniformis* whereas oil radish was a good host of *R. reniformis* but a poor host of *M. incognita*. These results revealed that both oil radish and brown mustard tested could serve as trap crops of *R. reniformis*. ‘Caliente 199’ brown mustard would be a better “open-end trap crop” than ‘Sodbuster’ oil radish since it is as susceptible to *M. incognita* and *R. reniformis* as their standard hosts. However, growing susceptible hosts did not trap *M. incognita* and *R. reniformis* effectively from the soil as brown mustard and oil radish being good hosts to *R. reniformis* did not reduce *R. reniformis* population in the soil, and oil radish being a poor host of *M. incognita* also did reduce *M. incognita* in the soil.
Degree-days experiments:

When brown mustard, oil radish, and tomato were inoculated with infective juveniles of *M. incognita*, the nematode required 283 degree-days (DD) to reach egg-laying female (ELF) on both brown mustard and oil radish but only required 266 DD on tomato. On the other hand, infective juveniles of *R. reniformis* required 333 DD to reach ELF on both brown mustard and oil radish but only required 291 DD on cowpea. Regardless of brown mustard and oil radish, infective juveniles of *M. incognita* or *R. reniformis* required 283 or 333 DD (equivalent to 24.2 or 29.0 calendar days in the greenhouse), respectively to reach ELF. Both of which were longer than when the standard hosts were planted. Taking into account DD during two more weeks of hatching eggs at 22.8°C, *M. incognita* or *R. reniformis* would estimate to require 465 and 512 DD, respectively to reach ELF in field conditions. With a daily average of 22°C at 4-inch soil depth in Hawaii, these results could mean *M. incognita* or *R. reniformis* eggs can complete a life cycle on the Brassicas in 5 or 6 weeks, respectively. Thus, Brassica cover crops should be terminated 5-6 weeks after seeding to avoid further nematode reproduction.

Termination age experiment:

The oil radish biomass production was highest at 56 days after planting (DAP), followed by 42 and 28 DAP, which were all significantly more biomass than terminating the cover crop at 14 DAP or not planting the cover crop (*P* ≤ 0.05). Although the DD accumulated during these field trials varied from 0 to 834 DD among the two targeted nematodes, regardless of the trap crop age, oil radish did not suppress (*P* > 0.05) soil populations of *Meloidogyne* spp. and *R. reniformis* compared to the no-trap-crop control. None-the-less, root-gall index (RGI) on pumpkin was significantly reduced (*P* ≤ 0.05) in 14 or 28 DAP compared to the control and 56 DAP. However, this did not suppress soil populations of *R. reniformis*.

Trap crop experiments:

Soil populations of *Meloidogyne* spp. and *R. reniformis* were not suppressed (*P* > 0.05) in any of the two oil radish trap crop trials compared to no-trap-crop control. Interestingly, oil radish reduced *Meloidogyne* spp. and *R. reniformis* numbers numerically when terminated before reaching 465 and 512 DD, the heat units required by *M. incognita* and *R. reniformis* to reach ELF, respectively. This actually resulted in 46% reduction of *Meloidogyne* spp. However, when oil radish was terminated beyond the DD to reach ELF of both nematodes, no nematode suppression was detected. On the other hand, brown mustard reduced soil populations of *Meloidogyne* spp. in all three field trials regardless of termination age or heat units accumulated (420-689 DD) compared to the control. The effect was especially significant in Trials I and II where *Meloidogyne* numbers were significantly suppressed (*P* ≤ 0.05). Although it was not statistically different in Trial III, in all three trials, the numbers of *Meloidogyne* spp. were reduced by ≥ 50% compared to the control. On the other hand, brown mustard did not suppress *R. reniformis* in Trial I and II (*P* > 0.05) but only suppressed in Trial III (*P* ≤ 0.05) by 11.8% compared to the control.

Both oil radish trap crop field trials suggested that regardless of the host status of oil radish to the nematodes (poor host to *M. incognita* and good host to *R. reniformis*), if oil radish was terminated before the targeted nematodes reaching ELF, nematode suppression could be achieved though at a low level (< 50%). However, overall oil radish as a trap crop did not suppress both *Meloidogyne* spp. and *R. reniformis* significantly compared to the control. Thus, the first hypothesis that using nematode susceptible host as an effective trap crop was only partially accepted (i.e. oil radish being a good host of *R. reniformis* still did not suppress the nematode). However, the second hypothesis that terminating trap crops before the
nematodes reaching ELF would reduce the targeted nematode soil populations was accepted in this experiment.

Findings from the brown mustard experiment supported the first hypothesis that a good host of *Meloidogyne* spp., brown mustard, would serve as a good trap crop to reduce soil population of the nematode. This hypothesis worked in our study regardless of the DD accumulated at least up to 689 DD. However, although brown mustard was also a good host of *R. reniformis*, it only reduced population densities of the nematodes in one out of the three trials, thus rejecting the first hypothesis. In addition, suppression of *R. reniformis* by brown mustard was not in favor of hypothesis 2 as it did not suppress *R. reniformis* when it was terminated within one life cycle of the nematode (or less than 512 DD), but it suppressed the nematodes significantly when accumulated 563 DD (beyond one life cycle of *R. reniformis*) in Trial III. These data also suggested that DD might not be a sole factor to determine termination date of open-end trap crop against plant-parasitic nematodes. This might be because the longer the trap crop is growing, the larger the biomass, hence a bigger root system would be generated that can help to trap more nematodes.

**Objectives 3 and 4**

**Brown mustard vs oil radish termination methods:**

The biofumigation effect was stronger with brown mustard than oil radish. *Meloidogyne* spp. were suppressed if brown mustard tissues were macerated and tilled into the soil followed by covering black plastic (MTBP) in all 3 trials, and reduced *Meloidogyne*-induced root galling on zucchini crop in Trials I and II. Brown mustard terminated by MTBP suppressed *R. reniformis* in Trial I and trends showed that 33.9 and 54.9% of the nematode were reduced in Trials II and III, respectively. Regardless of the termination methods, oil radish did not suppress *R. reniformis*. Soil glucose and sulfate were analyzed as indicators of biofumigation. Soil sulfate was more stable in the soil than glucose, and clearly depicted biofumigation efficacy ranking in the order of MTBP > MT > T, all of which were higher than that in no-till treatments and the BG. A similar result was obtained in the glucose analysis when toluene (methylbenzene) was added immediately to the soil samples at the time of sampling to arrest microbial activities. Suppression of plant-parasitic nematodes in MTBP was corresponding to zucchini growth in Trials I and III.

**Objective 5**

**Biofumigation indicators:**

Percent increase in myrosinase (Myr) activity determined from soil glucose concentrations before and 1 week after biofumigant crop termination was highest in brown mustard terminated by MTBP and T in Trial II when toluene was added immediately at soil sampling but not in Trials I and III when toluene was not added until samples were brought to laboratory prior to glucose analysis. Although there was no statistical difference detected, on average brown mustard (22.9%) had a higher percent increase in Myr activity compared to oil radish (5.7%). When soil sulfate was measured in Trial III, MTBP and MT had the highest increase in soil sulfate concentration, significantly higher ($P \leq 0.05$) than all no-till treatments as well as the control. Even MNT and NTBP had a higher increase in soil sulfate than the BG control ($P \leq 0.05$).

Although glucose analysis worked well to indicate the performance of biofumigation in Trial II, it did not work in Trial I and III when toluene was not added immediately at soil sampling time to arrest further glucose degradation from soil.
microbial activities. This has also been observed by Al-Turki and Dick (2003). However, soil sulfate assay appeared to be a better indicator of biofumigation than soil glucose assay because it is stable in the soil, not easily degraded by soil microorganisms, and it is not volatile like ITC. Increase in soil sulfate content in MTBP, MT or T compared to all no-till treatments and BG control supported the importance of incorporating brown mustard tissue into the soil for better biofumigation effect. Covering the soil with black plastic and macerating the tissues (MTBP and MT) did reduce the variation of sulfate content in Trial III, indicating a more stable biofumigation effect.

**Objective 6**

In additional field trials, brown mustard alone or in combination with oil radish cultivated for 5 weeks were evaluated for their biofumigation effects with no plastic, black plastic or solarization plastic. Brown mustard biofumigation along with solarization plastic suppressed both root-knot and reniform nematodes, but that with black plastic only suppressed root-knot nematodes. On the other hand, while biofumigation with black plastic increased abundance of bacterial and fungal feeding nematodes, that with solarization did not. Thus, the choice of plastic affected biofumigation and green manure effects of Brassica crops. We also concluded that combining oil radish and brown mustard for biofumigation was not recommended as oil radish outgrew brown mustard. None-the-less, our results also verified that biofumigation dominated by oil radish cover crop improved soil nutrient cycling in the soil regardless of plastic used.

**Objective 7**

**Biofumigation effects on soil health:**

Interestingly, all biofumigation methods did not compromise soil health. Instead, biofumigation with oil radish enhanced nutrient enrichment as indicated by higher abundance of bacterivorous nematodes and enrichment index (EI) calculated from these groups of nematodes throughout the zucchini crop compared to the untreated control. Whereas biofumigation with brown mustard, regardless of termination methods, enhanced bacterial decomposition and EI for up to 1 month after biofumigation in one of the three trials. Though no significant effect was detected in the Trial II, terminating brown mustard by tissue maceration and tillage also increased abundance of bacterivores throughout the zucchini crop. Biofumigation efficiency measured by glucose analysis (with toluene added immediately after soil sampling to arrest soil microbial degradation of glucose) had a strong positive relationship with abundance of all free-living nematodes including bacteriovorous, fungivorous, omnivorous and predatory nematodes. When using sulfate as an indicator of biofumigation, it was again positively related with abundance of bacterivorous and predatory nematodes, as well as EI. Multivariate canonical correspondence analysis clearly depicted that increasing soil temperature by covering soil with black plastic, and high brown mustard biomass enhanced biofumigation. In conclusion, brown mustard biofumigation was consistently suppressive to plant-parasitic nematodes and enriched nutrients transiently up to 1 month after biofumigation whereas oil radish did not suppress plant-parasitic nematodes but consistently enriched nutrients throughout zucchini crop.

**Participation Summary**

2 Farmers participating in research
Educational & Outreach Activities

25 Consultations
4 Journal articles
3 On-farm demonstrations
3 Published press articles, newsletters
9 Webinars / talks / presentations
6 Workshop field days
1 Other educational activities: none

PARTICIPATION SUMMARY:

113 Farmers
25 Ag professionals participated

Education/outreach description:

Workshops/field days
There were four field days and two workshops conducted during the course of this project period. Field demonstrations were conducted at the experiment site so that farmers were able to see biofumigation effects at first hand. In addition, a presentation on biofumigation for nematode management was shared with farmers during a “WSARE Hot Shot IPM” program at Kahului, Maui to reach out to wider audience away from Oahu.

5. Waisen, P. and Wang, K.-H. 2018. Best termination methods of mustard and
https://www.flickr.com/photos/150583970@N07/with/42316804051/


Journal articles:


Extension publications


Presentations


free-living nematodes? 31st Annual CTAHR Student Research Symposium, University of Hawaii at Manoa, Honolulu, HI. April 15, 2019 (Abstract 32).


Outreach online


Project Outcomes

113 Farmers reporting change in knowledge, attitudes, skills and/or awareness

2 Farmers changed or adopted a practice

5 Farmers intend/plan to change their practice(s)

2 Grants received that built upon this project

Did this project contribute to a larger project?:

Yes
New working collaborations

Project outcomes:

The outcome of developing this improved biofumigation method through the determination of best cover crop termination age (5-6 weeks) and best termination method (MTBP) would provide farmers with plant-parasitic nematodes problem a viable cover crop-based nematode management approach. This is the first comprehensive study using biofumigation that simultaneously examined the management of plant-parasitic nematode and soil health. While we determined that brown mustard MTBP did not compromise soil health, biofumigation with oil radish consistently improved soil nutrient enrichment and fungal decomposition in the soil. From an economic standpoint, farmers with plant-parasitic nematode problems should cultivate brown mustard to manage nematode problems through biofumigation. Whereas farmers that don’t have plant-parasitic nematode problem should grow oil radish cover crop for soil health benefits and cut cost on fertilizer inputs.

The short-term outcome of this project was that 113 farmer participants that attended various workshops and field days gained knowledge on the benefits of biofumigation. This was the first step forward towards the adoption of this nematode and soil health management approach. Through these results and outcomes, the PIs had since received further funding on biofumigation targeting other soil-borne pathogens in other cropping systems. The long-term success of this information transfer would lead to less synthetic nutrients input, improve in soil nutrient cycling, minimizing synthetic nematicide use. Thus, it will have a long-term impact on a healthy environment that will lead to social well-being for the farming community.

Knowledge Gained:

*Trap crop effect was based on Brassica susceptibility and nematode sensitivity:*

This is the first field study that demonstrated that nematode-susceptible Brassica cover crops can be used as ‘open-end trap crop’ against the nematode in contrast to well known ‘dead-end trap crop’. These trap crop studies elucidated that trap crop effect of Brassicas was based on their susceptibility to targeted nematodes and the sensitivity of the nematode species to the trap crops but not based on nematode degree-days as originally hypothesized. This was because if trap crop effect was only based on high susceptibility, brown mustard being a good host of both *M. incognita* and *R. reniformis* would reduce both nematodes in the soil but instead only consistently suppressed root-knot nematodes. This also indicated that root-knot nematodes were more sensitive to trap crops than reniform nematode.

*Brown mustard biofumigation covering plastic mulch achieved efficacy:*

Brown mustard was a better biofumigant crop than oil radish in terms of suppressing plant-parasitic nematodes. We learned that method of biofumigation was critical for suppression of plant-parasitic nematodes because mere soil incorporation of Brassica tissues with or without tissue maceration did not suppress the targeted nematodes. Biofumigation effect was only apparent when aerial tissues were macerated and soil incorporated followed by covering plastic mulch. It was in logical sequence that tissue maceration would enhance myrosinase activity, soil incorporation would maximize biofumigant (isothiocyanates) contact with
nematodes, and covering plastic mulch would minimize volatilization loss of isothiocyanates, together maximizing biofumigation effect.

**Oil radish enriched plant nutrients but brown mustard did transiently:**

Oil radish biofumigation was not suppressive to plant-parasitic nematodes but consistently increased bacterial feeding nematodes and nematode enrichment index (EI) indicating nutrient enrichment whereas brown mustard was suppressive to plant-parasitic nematodes and increased bacterial feeding nematodes and EI for up to one month after biofumigation.

**Sulfate was the best biofumigation indicator than glucose:**

Glucose and sulfate were known to be released along with isothiocyanates (biofumigant gas) during glucosinolate hydrolysis. Because isothiocyanates are volatile and impractical to accurately quantify in field conditions, soil glucose and sulfate concentrations were measured instead right before biofumigation and 1 week after biofumigation. Change in concentrations of glucose and sulfate had negative relationships with suppression of targeted nematodes when plotted in multivariate Canonical Correspondence Analysis (CCA). However, sulfate had a stronger negative relationship and did not require toluene (methyl benzene), in its sample preparations compared to glucose, suggesting that sulfate was a good biofumigation indicator than glucose. This was the first report of using soil sulfate as an indicator of biofumigation. At the same time, sulfate had a strong positive relationship with the abundance of bacterivores, enrichment index (EI), soil temperature during the biofumigation period, and biomass of brown mustard.

**Black plastic vs clear plastic mulch on biofumigation effect:**

The use of black plastic mulch in brown mustard biofumigation was only suppressive to root-knot but not reniform nematodes. In addition, black plastic mulch improved soil health in terms of increasing abundance of bacterial and fungal feeding nematodes and enrichment index, indicative of nutrient enrichment. However, solarization mulch was suppressive to both root-knot and reniform nematodes. In doing so, solarization mulch neither improved nor compromised soil health.

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