

What plastic mulch can help biofumigation to better manage nematodes?

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## Introduction

Brassica cover crops such as oil radish (Raphanus sativus) and brown mustard (Brassica juncea) (Fig. 1) produce naturally occurring compounds (glucosinolates), which upon tissue damage through soil incorporation, herbivory, or other forms of mechanical damage, break into down a characteristic pungent gas (allyl isothiocyanates). The gas is volatile and toxic, similar to chemical properties of the active ingredient in Vapam® (methyl isothiocyanate), a synthetic soil fumigant. For that reason, the natural compounds in Brassicas can be used as alternative soil fumigants for managing soil-borne pests and pathogens including nematodes. The use of these plantderived compounds for the management of soil-borne pests and pathogens in agroecosystems is called biofumigation (Kirkegaard et al., 1993). Biofumigation involves soil incorporation of Brassica tissues, residues, seed meals or seed extracts, and because the gas is volatile, covering a tarp



Fig. 1. A) Oil radish and B) brown mustard

(Gimsing and Kirkegaard, 2009) or compacting the soil with a roller (Riga, 2011) reduced volatilization loss and enhanced biofumigation effects. Thus, effects of biofumigation depend on method of terminating Brassica cover crops (biofumigation methods). A recent study conducted at the University of Hawai'i showed that macerating aboveground brown mustard tissues and incorporating into 4-inch soil depth followed by covering polythene black plastic mulch for 1 week suppressed plant-parasitic nematodes and improved soil health (Waisen, 2019). This study compared clear solarization plastic mulch to black plastic mulch on their ability to enhance biofumigation effects against plant-parasitic nematodes.

On the other hand, one common issue with practicing biofumigation is its non-target impacts on beneficial soil organisms. Brown mustard is more suitable for biofumigation because it is more pungent due to its higher concentration of glucosinolate than oil radish (Ngala et al., 2015; Rudolph et al., 2015). However, oil radish has better soil health improvement properties than brown mustard (Clark, 2008) due to its swollen and long taproot. Therefore, a field trial was conducted to examine if cultivating oil radish and brown mustard cover crops together could enhance biofumigation effects as well as improving soil health using two different plastic mulch. Soil health was measured in terms of abundance of free-living nematodes important in plant nutrient cycling (Fig. 2).



Fig. 2. Free-living nematodes from left to right are predatory, omnivorous, fungal and bacteria feeding nematodes that play important roles in soil nutrient cycling (picture: Wang, K.-H.).

### Materials and methods

#### Trial I - Brown mustard biofumigation using clear and black plastic mulch:

A field trial was conducted on May 17, 2018, at Poamoho Experiment Station, Wahiawa, HI, to compare the ability of clear and black plastic mulch to enhance brown mustard biofumigation effects using brown mustard against plant-parasitic nematodes. 'Caliente 199' brown mustard from Siegers Seed Co. (Holland, MI) was seeded at 10 lb/acre (1:1, w/w) in  $4 \times 14$  ft<sup>2</sup> treatment plots. Five weeks later, the brown mustard was subjected to various termination methods (Fig. 3). These included 1) macerating aboveground tissues using a line trimmer followed by incorporating the tissues to 4-inch soil depth (MT) and; 2) MT followed by either covering black plastic mulch (MTBP); or 3) covering clear solarization plastic mulch (MTS). The brown mustard was amended at 0.51 t/acre (dry wt). A bare ground (BG) control was included and the experiment was arranged in a randomized complete block design with 4 replications. One week after the biofumigation, plastic mulches were uncovered and 2-week-old 'Parthenon' zucchini (*Cucurbita pepo*) seedlings

were transplanted individually per planting hole at a density of 5 plants per plot. Soil samples, 4 soil cores per plot from 4-inch soil depth, were collected 1 week after biofumigation and at monthly interval during the zucchini growth. Nematodes were extracted from 250-cm<sup>3</sup> soil subsample by elutriation and centrifugal sugar flotation method (Byrd et al., 1976; Jenkins, 1964). All nematodes were morphologically identified to genus except for Rhabditidae identified to family level using a Leica<sup>™</sup> Inverted Microscope (Leica Microsystems Co, Wetzlar, Germany). Nematode data were subjected to nematode community analysis (Ferris et al., 2001).



Fig. 3. Biofumigation methods where A) Brassica tissues are macerated, B) incorporated into 4-inch soil depth, and covered with C) black plastic mulch or D) clear solarization mulch.

#### Trial II - Brown mustard and oil radish biofumigation using clear and black plastic mulch:

A second field trial was initiated on September 27, 2018, at the Poamoho Experiment Station to examine if cultivating both oil radish and brown mustard together would enhance biofumigation effects against plant-parasitic nematodes and improve soil health. 'Sodbuster' oil radish from Petcher Seeds (Fruitdale, AL) and the 'Caliente 199' brown mustard were seeded together at 10 lb/acre (1:1, w/w) in  $4 \times 14$  ft<sup>2</sup>. Five weeks later, the Brassicas were subjected to the same biofumigation methods (BG, MT, MTBP, and MTS) and as in Trial I. The oil radish and brown mustard mix was amended at 1.00 t/acre (dry wt). One week after the biofumigation, treatment plots with the plastic mulch were uncovered and 2-week-old 'Parthenon' zucchini (*Cucurbita pepo*) seedlings were transplanted at the same planting density as in Trial I. Soil sampling and nematode extraction procedures were same as in Trial I. In addition, a Premium Field CO<sub>2</sub> Test (Solvita gel system, Solvita and Woodend Laboratory) was done to test for soil respiration rate at 1 week after biofumigation.

All data 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) transformation prior to analysis of variance (ANOVA) using Proc GLM in the SAS. Means were separated using

Waller-Duncan k-ratio (k=100) t-test and only the true means were presented.

### **Results and discussion**

#### Effects of brown mustard biofumigation using plastic mulch on nematode:

Both biofumigation methods that involved plastic mulch (MTBP or MTS) suppressed rootknot and reniform nematodes (Fig. 4), two of the economically important plant-parasitic nematodes commonly found in agroecosystems in Hawai'i. Covering black plastic (MTBP) improved root-knot nematode suppression (Fig. 4A) compared to biofumigation without covering plastic mulch (MT) or bare ground control (BG) while covering clear solarization mulch (MTS)

improved reniform nematode suppression better than MT or BG (Fig. 4B). These results supported our hypothesis that covering soil incorporated with biofumigant crop residues improved biofumigation effect against plant-parasitic nematodes. This is encouraging because several reports indicated that terminating biofumigant crops without tillage failed to suppress plant-parasitic nematodes (Gruver et al., 2010). Riga (2011) also concluded that when terminating arugula (Eruca sativa) only by soil tillage and compacting the soil using a roller did not suppress Northern root-knot nematode (Meloidogyne hapla). Similarly, Vervoort et al. (2014) reported that brown mustard also did not suppress stubby root (Trichodorus spp.) and stunt (Tylenchorhynchus spp.) nematodes when simply incorporating into the topsoil. It is also very encouraging to see that while MTBP could not suppress a more difficult to manage plant-parasitic nematode, the reniform nematodes, MTS was able to overcome this challenge. This is probably due to the higher heat generated in MTS to kill more nematodes.



Fig. 4. Biofumigation effects of brown mustard using black and clear plastic mulch on A) root-knot and B) reniform nematodes.

Effects of brown mustard biofumigation using plastic mulch on soil health:

When brown mustard biofumigation was conducted by using black plastic (MTBP), it

consistently increased soil nutrient enrichment (EI) as indicated by a higher abundance of bacterial (Fig. 5A) and fungal (Fig. 5B) feeding nematodes than the bare ground control (BG). This trend is also often seen in previous field trials using either brown mustard or oil radish and terminated by the MTBP method (Waisen, 2019). However, biofumigation with solarization mulch (MTS) did not increase both of these free-living nematodes that play important roles in soil nutrient cycling (Fig. 5).

In general, Brassica cover crops are good green manure crops for soil health management. This benefit of biofumigation cannot be found in conventional fumigation using metam sodium or 1, 3-dichloropropene (Collins et al., 2006). Besides oil radish and brown mustard, rapeseed (*Brassica napus*) is another glucosinolate cover crop that stimulated bacterial and fungal feeding nematodes (Gruver et al., 2010). Unlike MTS, MTBP seemed to provide dual benefits of root-knot nematode suppression and green manure effects. However, if farmers' interest was to manage plant-parasitic nematodes, MTS is recommended, as it did not



Fig. 5. Biofumigation effects of brown mustard using black and clear plastic mulch on abundance of A) bacterial and B) fungal feeding nematodes.

reduce the numbers of free-living nematodes compared to the untreated control (BG; Fig. 5).

# Effects of Brassica mix biofumigation using plastic mulch on nematode and soil health:

Combining oil radish and brown mustard (1:1, w/w) by broadcasting the seed mixture followed by MTBP or MTS failed to suppress root-knot nematodes (Fig. 6A) and instead increased population densities of reniform nematode (Fig. 6B). These unexpected findings could be explained by the fact that oil radish outgrown brown mustard and resulted in biomass dominated by oil radish leaves which has a lower concentration of glucosinolate than brown mustard (Ngala et al., 2015; Rudolph et al., 2015). Future research on oil radish and brown mustard mix could consider higher seeding rate of brown mustard vs oil radish or separating oil radish and brown mustard planting rows to avoid smothering effects from oil radish.

On the other hand,

since oil radish biomass



Fig. 6 Biofumigation effects of oil radish-brown mustard mix using black and clear plastic mulch on A) root-knot and B) reniform nematodes

dominated brown mustard biomass in Trial II, the abundance of bacterial and fungal (Fig. 7A-B) feeding nematodes were numerically higher in MTS and MTBP than BG and MT alone. This led to an increase in nematode EI (Fig. 7C) in treatments with plastic mulch (MTBP and MTS) than BG control. Even the biofumigation without plastic mulch increased EI, indicating improved soil health condition by oil radish-brown mustard mix.



Fig. 7. Biofumigation effects of oil radish-brown mustard mix using black and clear plastic mulch on abundance of A) bacterial and B) fungal feeding nematodes or C) nematode enrichment index.

When using Premium Field CO<sub>2</sub> Test for soil respiration rate at 1 week after biofumigation, higher soil microbial activities were detected in MTBP and MTS compared to the BG control (Fig. 8). This suggested that biofumigation by MTBP or MTS using oil radish-brown mustard mix did enhance soil microbial activities.

## Summary

Brown mustard biofumigation using black plastic mulch suppressed only root-knot nematodes and improved soil health in terms of enhancing bacterial and fungal decomposition. However, brown mustard biofumigation with solarization mulch suppressed both root-knot and reniform nematodes with no impact on free-living nematodes. In a field infested with both rootknot and reniform nematodes, biofumigation with solarization mulch is recommended. The biofumigation effects of mix oil radish-brown mustard mix trial conducted here are inconclusive due to the smothering effect of oil radish over brown mustard. None-the-less, biofumigation on oil radish dominated crop residues significantly enhanced bacterial and fungal decomposition as well as overall soil microbial activities regardless of using black plastic or solarization mulch. Future research needs to focus on fine-tuning seeding rates and patterns of oil radish and brown mustard mix without smothering effect.



Fig. 8. A) Solvita<sup>®</sup> gel color and carbon dioxide concentration affected by oil radish-brown mustard mix biofumigation using black and clear plastic mulch.

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