Best Termination Methods of Mustard (*Brassica juncea*) and Oil Radish (*Raphanus sativus*) Cover Crops for Nematode Management

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May 23rd, 2018

Economic Importance of Plant-parasitic Nematodes

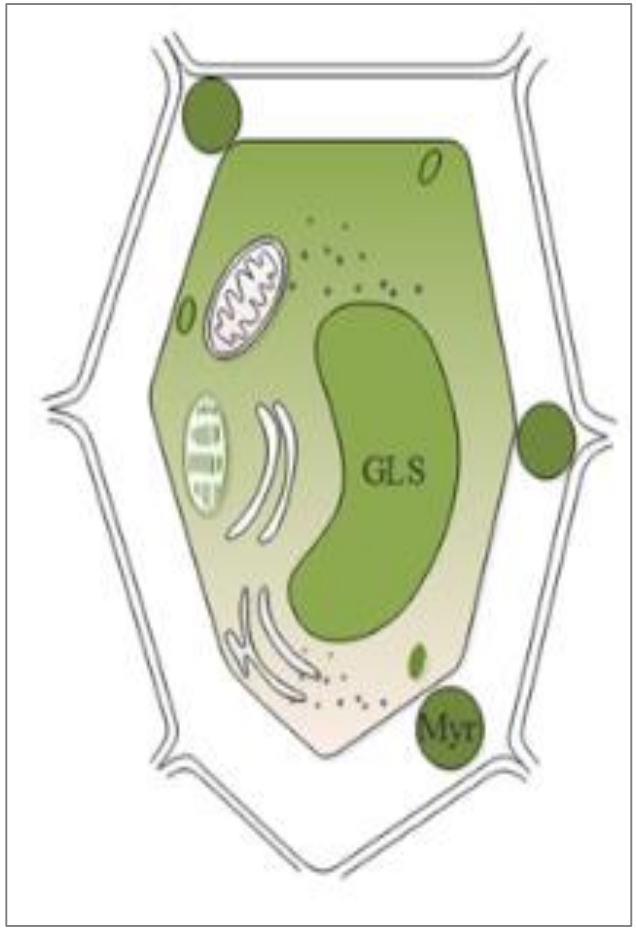
- $100 billion/yr loss worldwide ($10 billion loss in USA).
- Form disease complex with other opportunistic soil-borne pathogens.

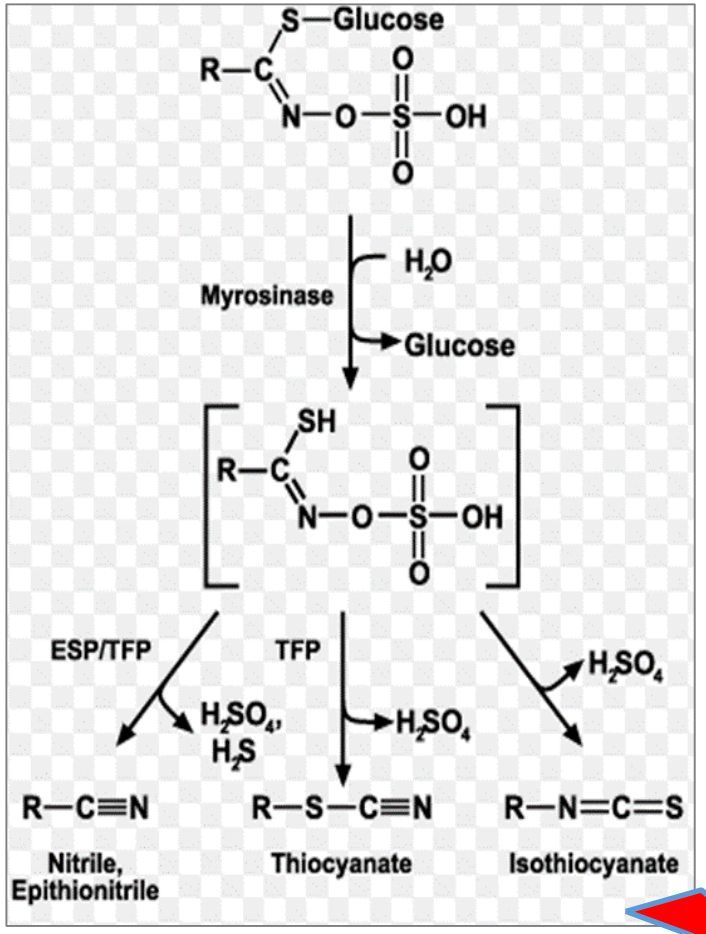


Cover Crops with Allelopathic Effects: Natural Nematicides

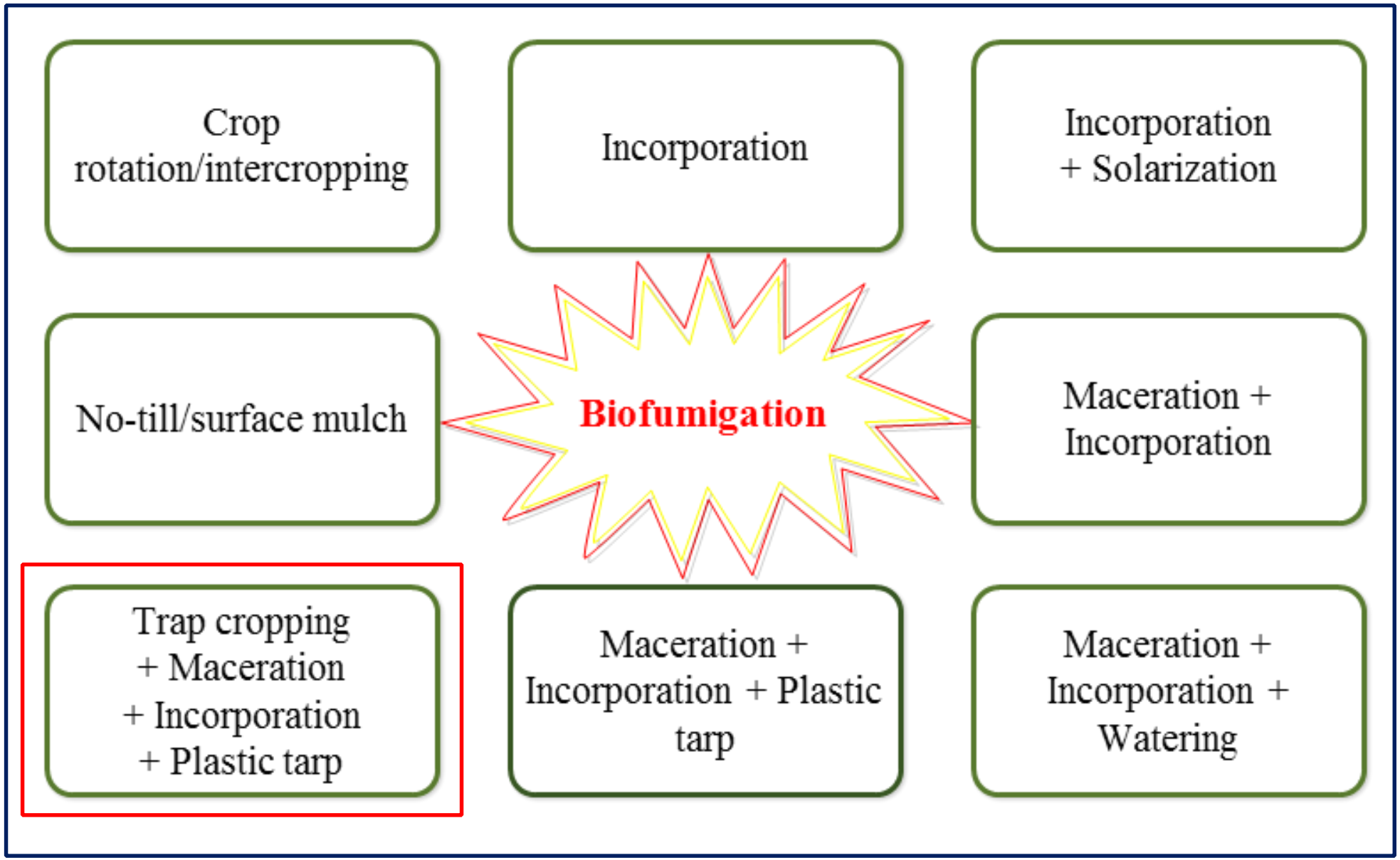
Biofumigation

Soil fumigation with plant-derived allelopathic compounds (isothiocyanates)

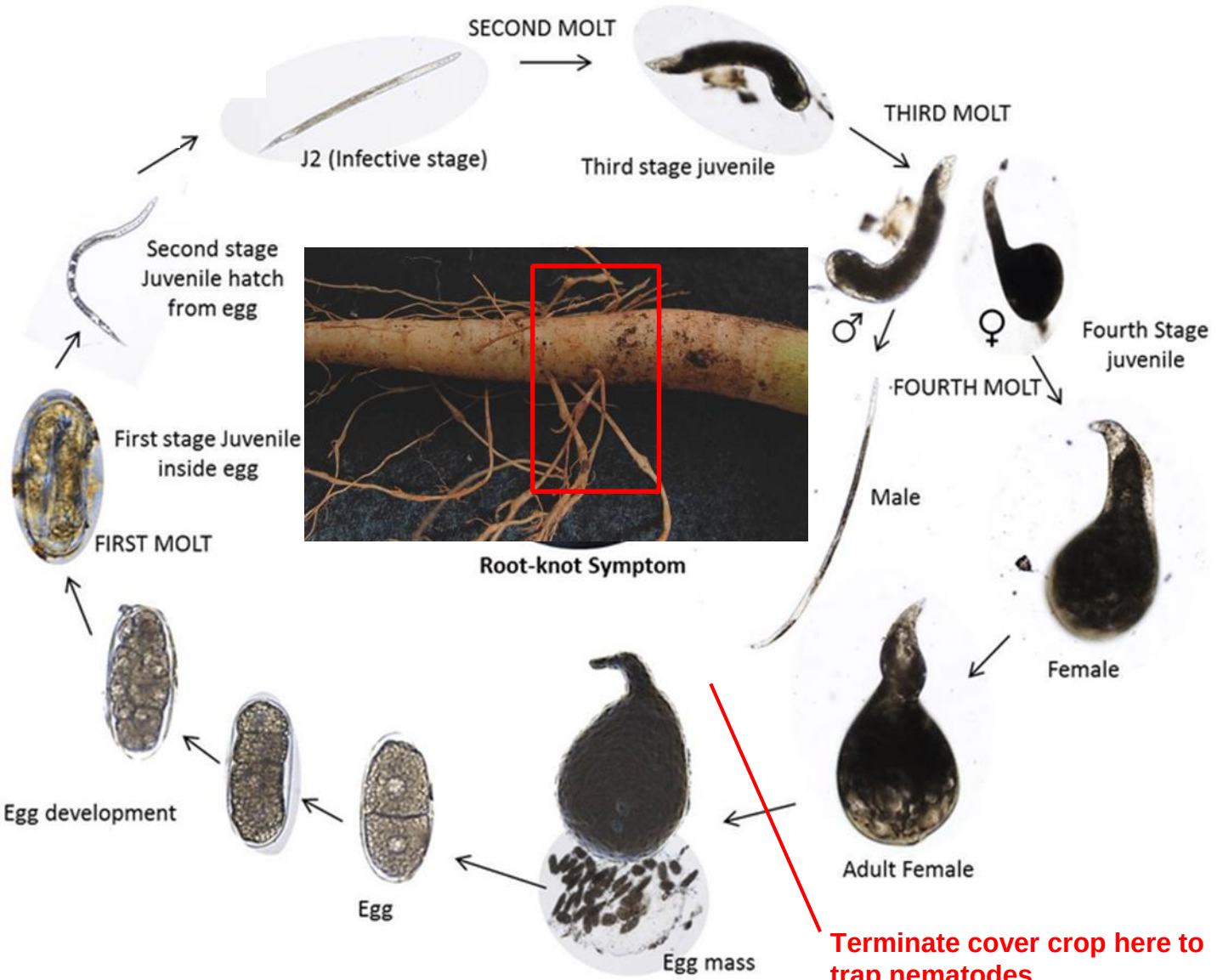


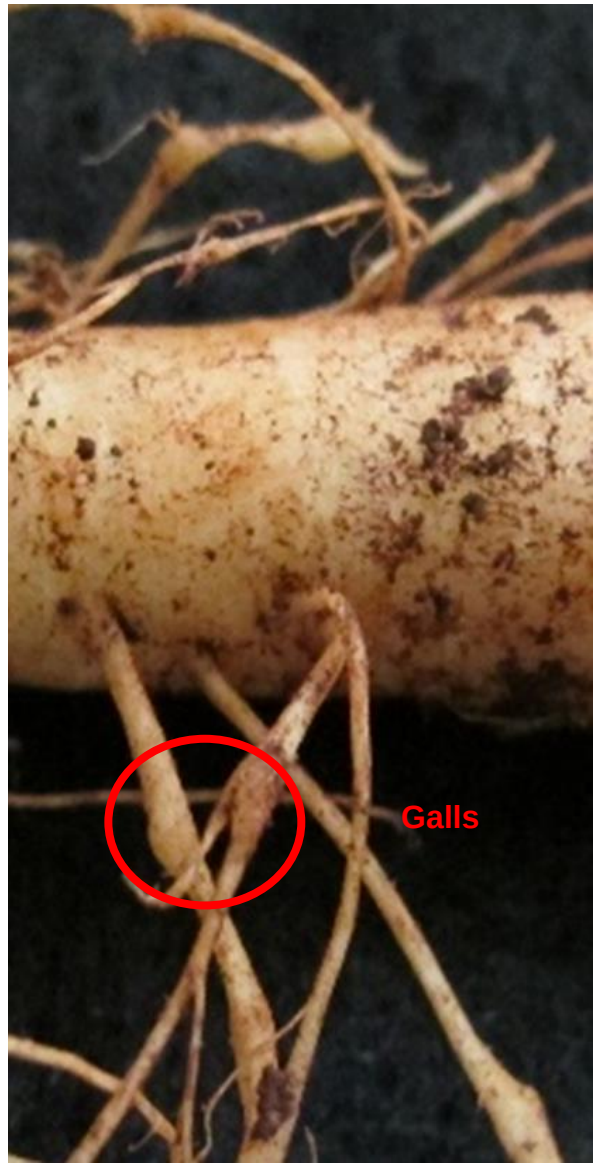


Biofumigation Methods



Trap Cropping Effect





Objectives

Overall Goal: To develop best termination methods of brown mustard (*Brassica juncea*) and oil radish (*Raphanus sativus*) cover crops for nematode management.

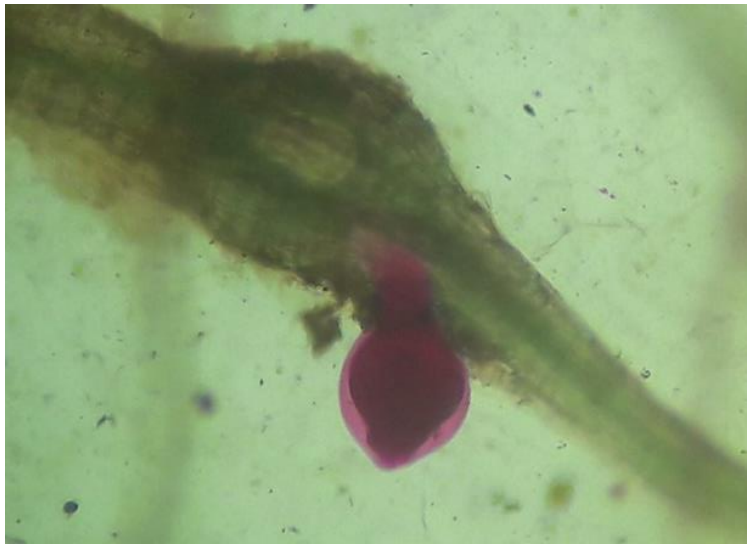
✓ 1. Determine the best time to terminate the cover crop for trap cropping effect: Avoiding root-knot nematode *(Meloidogyne incognita)* to reach the heat units of egg-laying females.

2. Determine the best termination method for brassicaceous cover crops to maximize biofumigation effect.

Determine Heat Units of Root-knot Nematodes on Mustard

Experimental Design:

- Treatments: Mustard and tomato seedlings were inoculated with 100 juveniles of *M. incognita*.
- Plants were destructively sampled at 3 days interval beginning at 9 days after inoculation (3 reps) until day 24.
- Roots were stained with acid fuchsin.



Heat units accumulated by *Meloidogyne incognita* to reach egg-laying female

Heat units to reach egg-laying female = 274.5 DD (3.5 weeks)

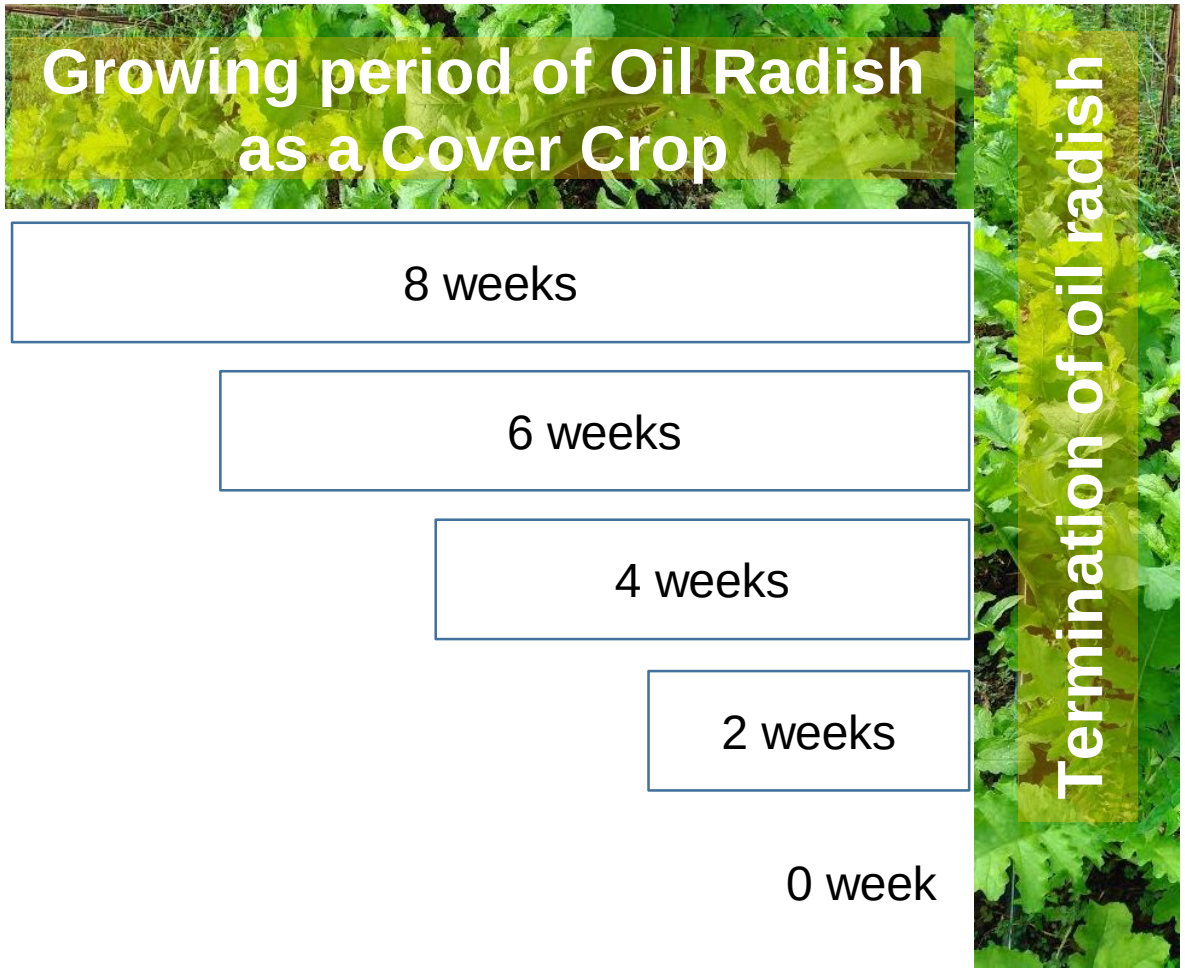
Heat unit (degree days, DD) = [∑(T– T*base*)] × total hour/24hr

T = soil temperature for each hour

T*base* = base temperature of *M. incognita*, 9.8°C

----------(modified from Fraisse et al., 2011)

Field Trial I: Field Trial Verification on Termination Time



Treatments: Oil radish was planted for different length of time (0, 2, 4, 6 and 8 weeks).

Conventional Termination Method of Biofumigant Crops = No tissue maceration

Experiment was arranged in RCBD with 4 replications. Pumpkin was planted after oil radish (OR) termination and incorporation, nematodes were sampled at OR termination and at 4-week interval over a 4-month period during pumpkin growth.

When is the best time to terminate oil radish?

RGI = 0

Repeated measure over 3 sampling dates at monthly interval

	Nematodes/250 cm^3 soil				
Herbivores	0	2	4	6	8
Root-knot nemamtode	178 A	140 A	213 A	160 A	467 A
Reniform nematode	371 A	256 A	874 A	168 A	312 A
Stubby root nematode	36 A	20 A	32 A	22 A	33 A

Root Gall Index on pumpkin at 4 months after planting

RGI = 12

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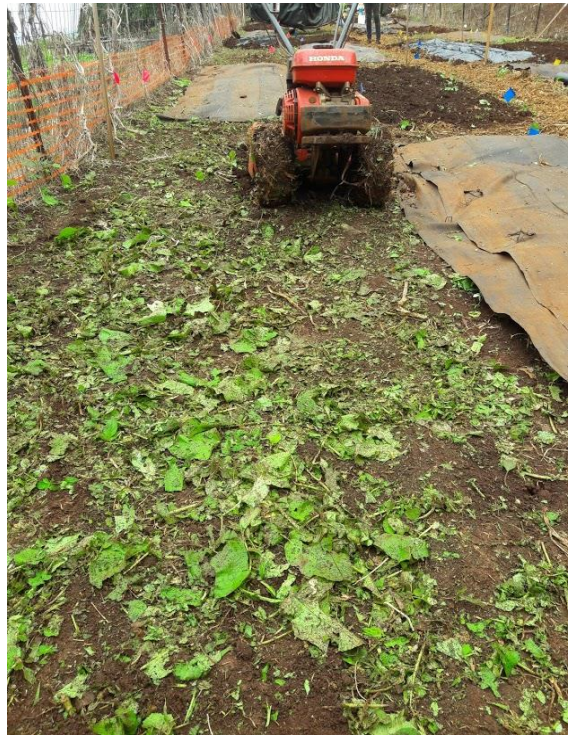
✓ 2. Determine the best termination method for brassicaceous cover crops to maximize **biofumigation effect**.

Determine the Best Termination Method of Cover Crop for Biofumigation Effect

Seeding: 10 lb/acre

Experimental design: 7 treatments with 4 replications in RCBD.



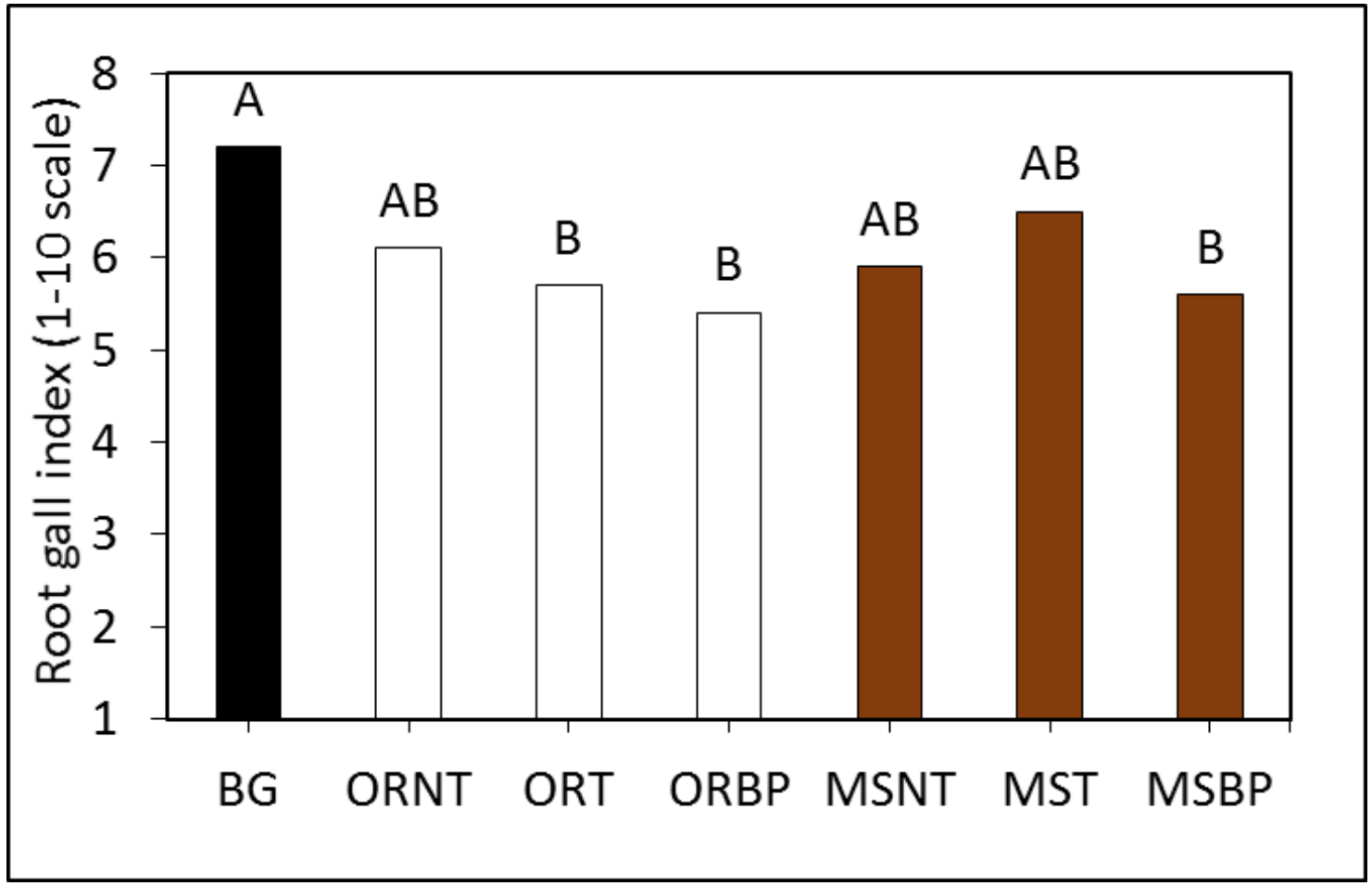
Treatments:

ORNT = oil radish + sickle + weed mat;
ORT = oil radish + line trim + till;
ORBP = oil radish + line trim + till + black plastic;

MSNT = mustard + sickle + weed mat;
MST = mustard + line trim + till;
MSBP = mustard + line trim + till + black plastic;
BG = bare ground fallow

Results (Trial II)

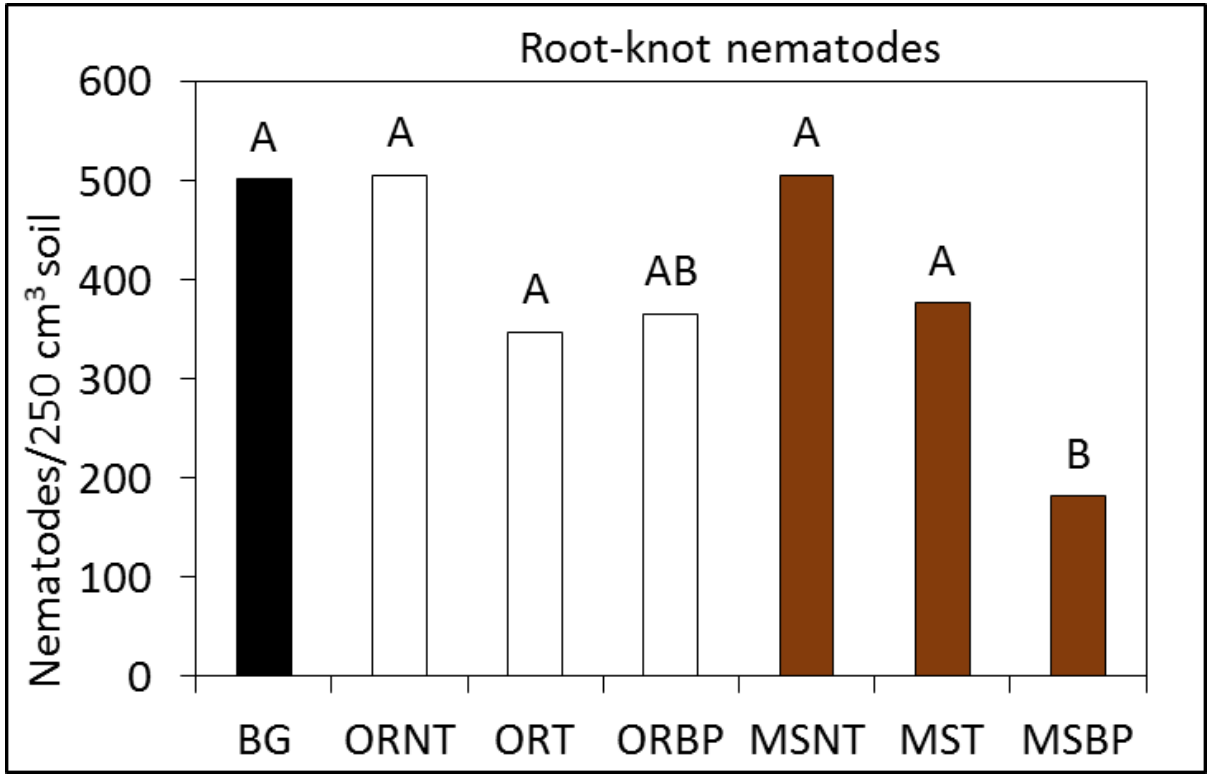
Root Gall Index (n = 4, *P* = 0.0403)



Results

Field Trial II: Biofumigation against Plant-parasitic Nematodes

(repeated measures over 3 months; n = 84)



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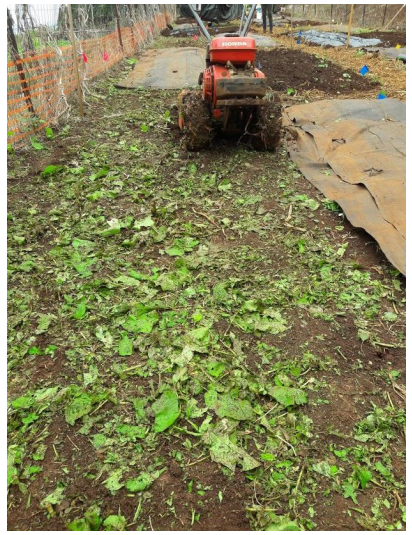
MSNT = mustard + sickle + weed mat;
MST = mustard + line trim + till;
MSBP = mustard + line trim + till + black plastic;
BG = bare ground fallow

Field Trial II – Brown mustard only

Experimental design: 7 treatments with 4 replications in RCBD.

Seeding: 10 lb/acre (brown mustard)

T



MT

MTBP

Treatments:

BG = bare ground fallow;
NT = sickle + weed mat;
MNT = maceration + no-till;
NTBP = maceration + no-till + black plastic tarp;

T = till without maceration;
MT = macerate + till
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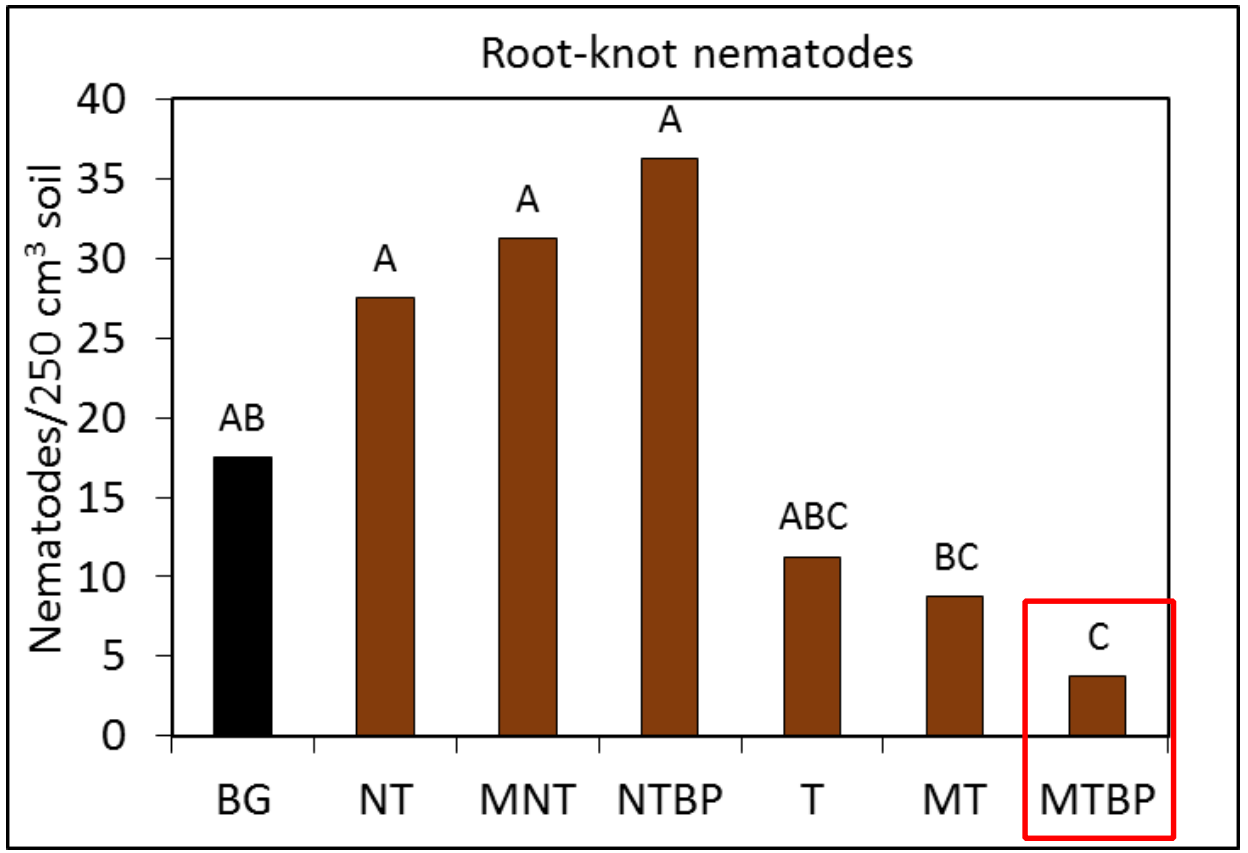
Plant-parasitic Nematodes



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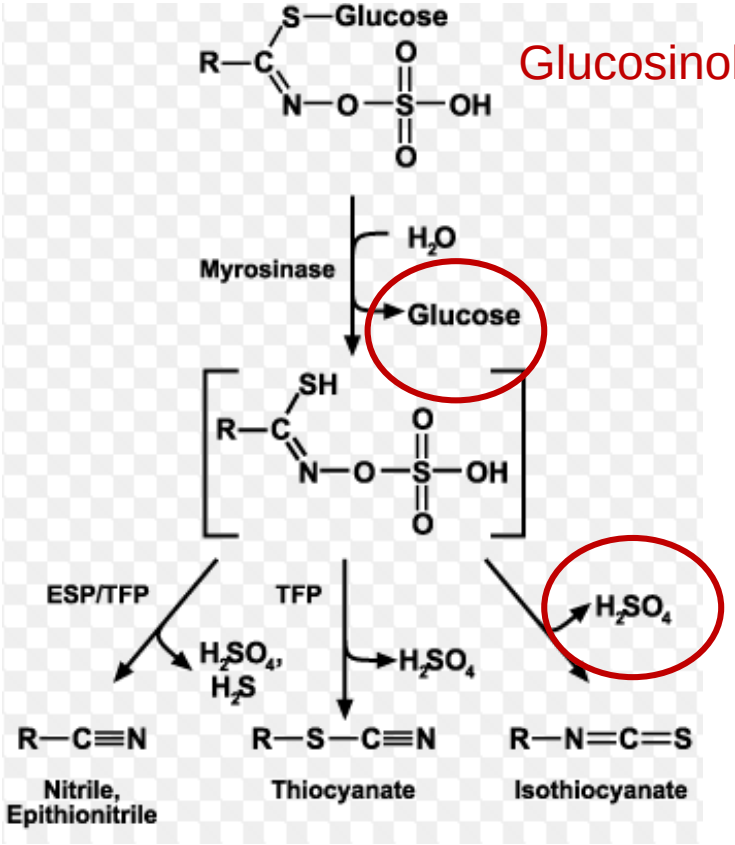
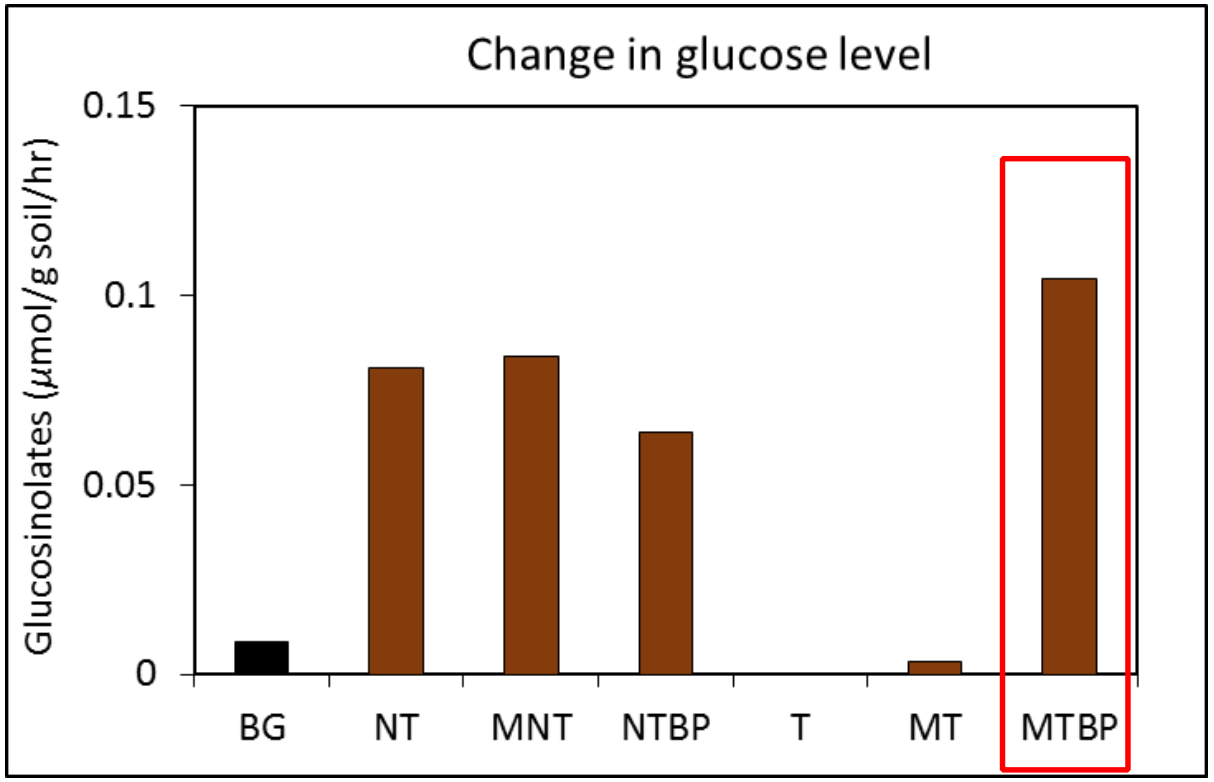
Field Trial II

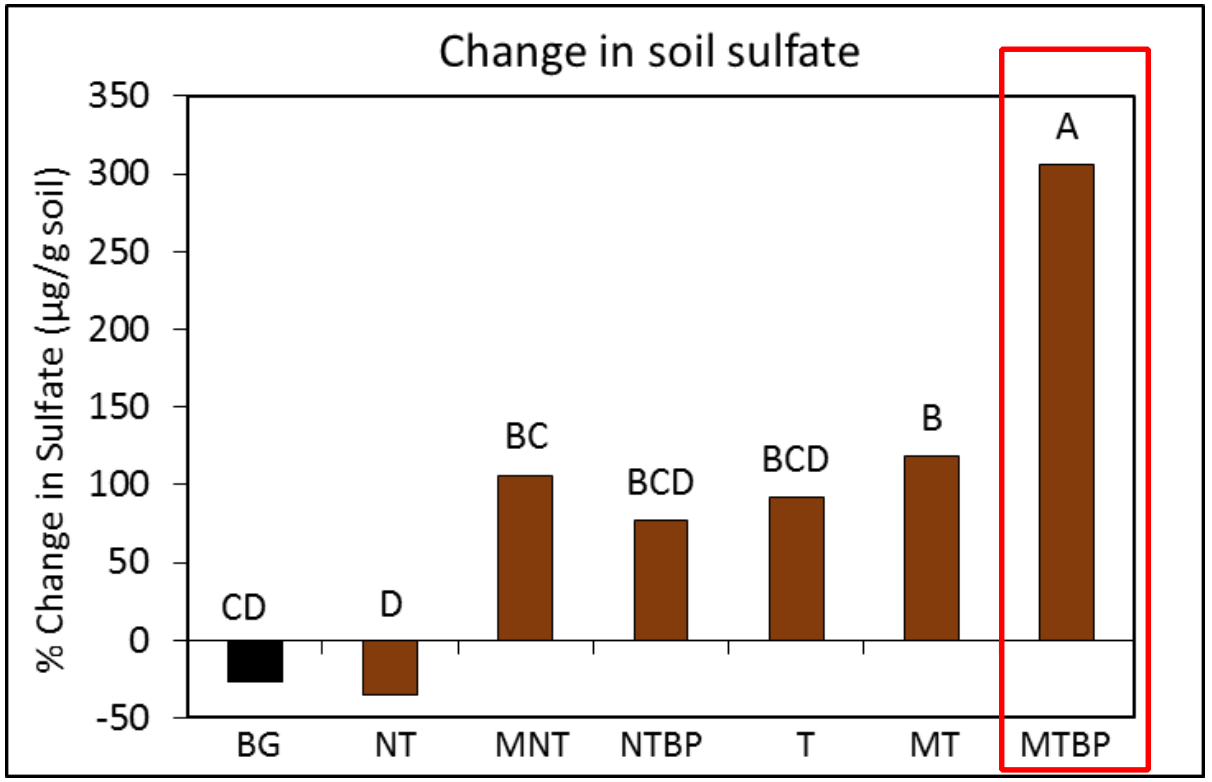
At 1 week after biofumigation:

1) Quantify **myrosinase activity – glucose diagnostic kit** (Al-Turki and Dick, 2003).
2) Quantify **sulfate** (Fahey et al., 2001).



Myrosinase Activity





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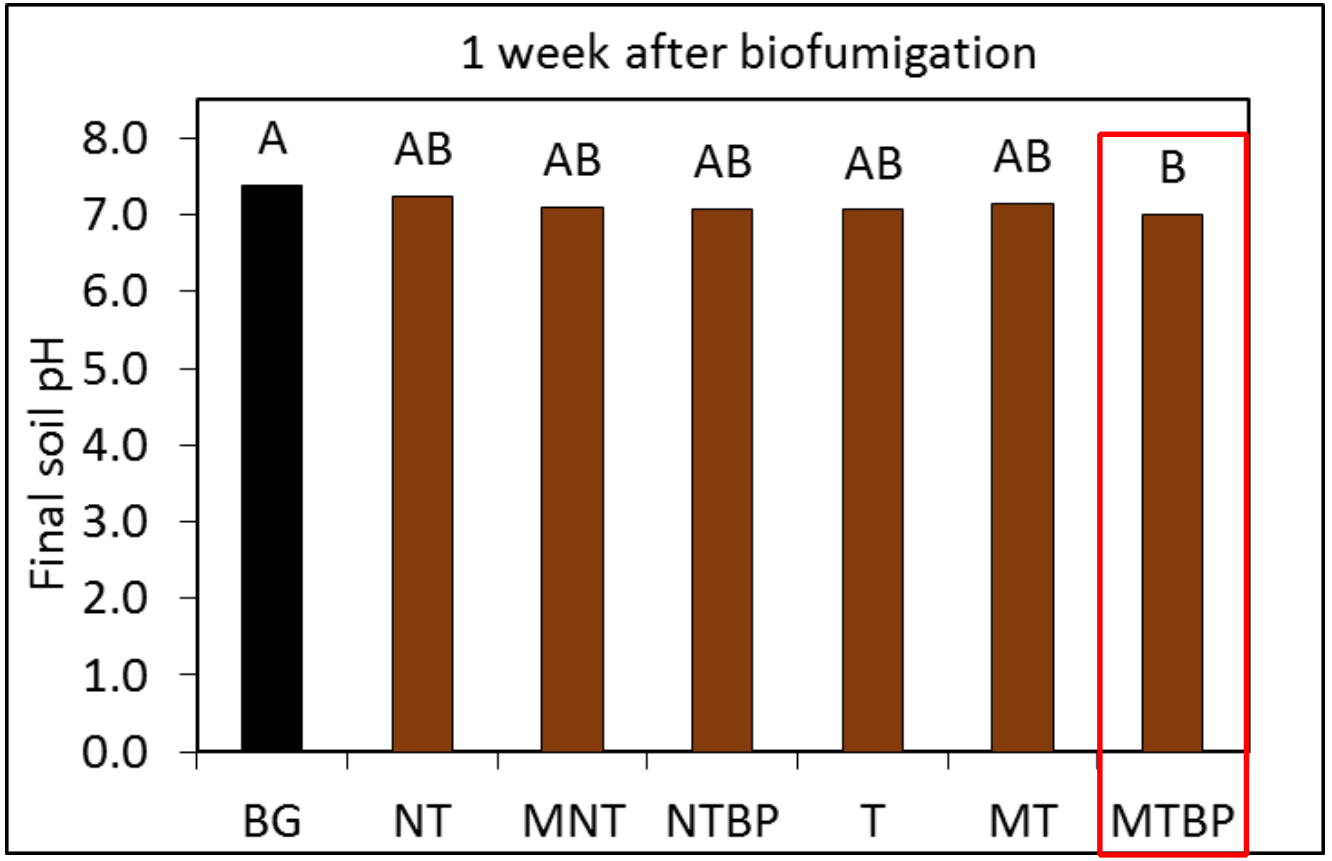

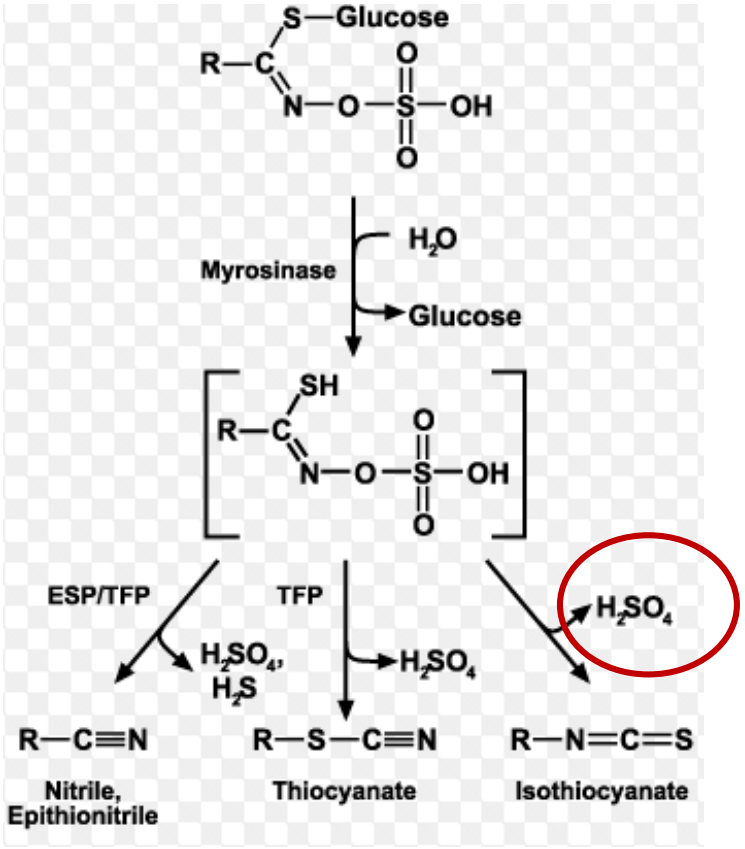

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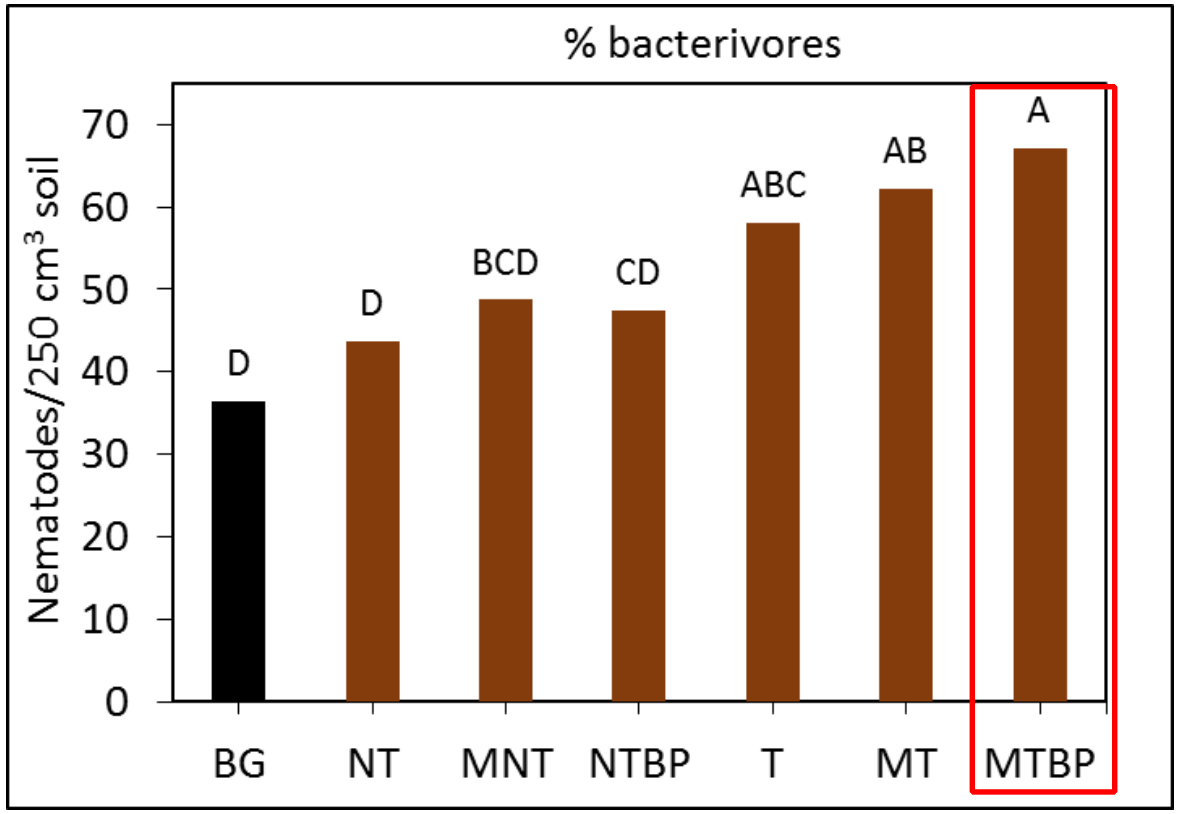
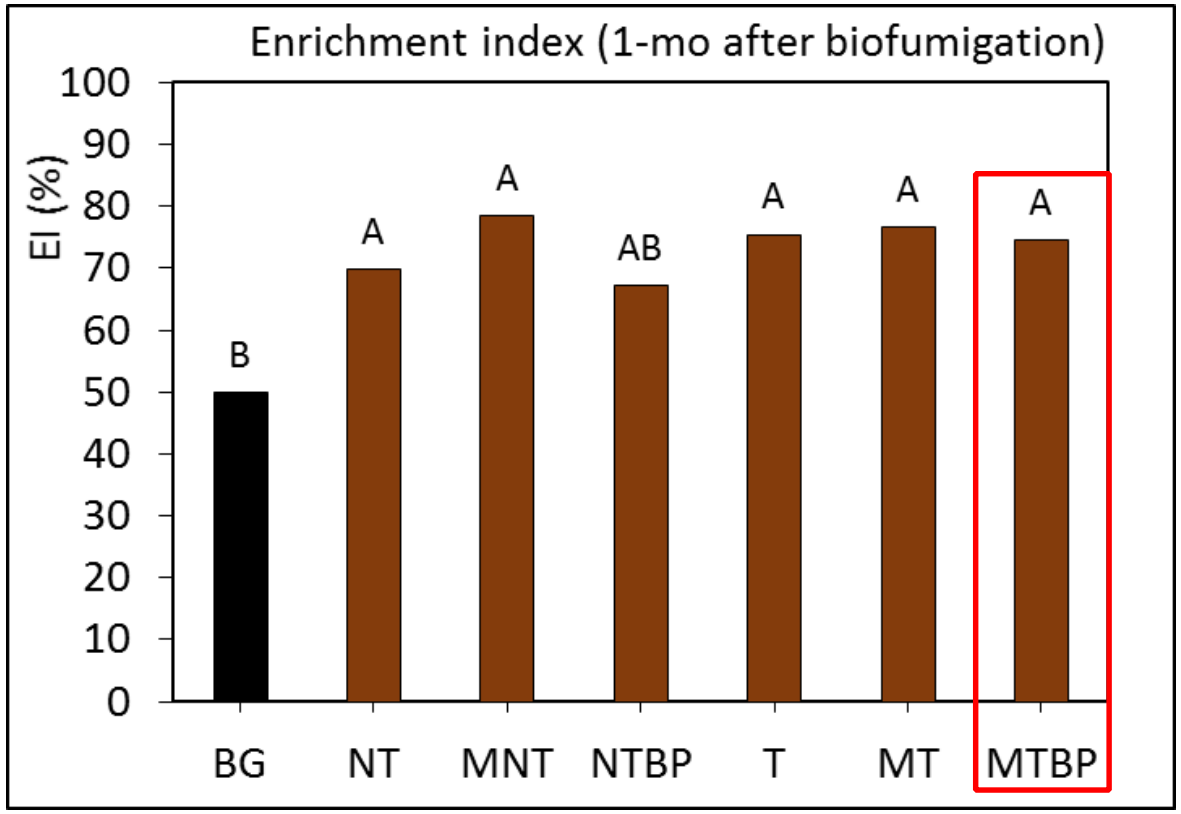
Soil Health Benefits of Biofumigation





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Conclusions

- Root-knot nematodes reach egg-laying female stage in 3.5 weeks (274.5 DD).
- The most effective biofumigation method against root-knot nematodes was by growing brown mustard for around 4 weeks followed by termination with tissue maceration, till and covering with black plastic for 1 week (MTBP).
- Glucose and soil sulfate levels were both good indicators of biofumigation effect, however, sulfate was more stable in soil and was significantly higher in MTBP than other biofumigation methods tested.

Summary

Step-by-step protocol for effective biofumigation against plant-parasitic nematodes:

1. Grow brown mustard or oil radish as cover crop at a seeding rate of 10 lb/acre (11 kg/ha) for **4-5 weeks** (**trap cropping** is served at this time), producing dry biomass equivalent to 0.5-1.5 t/acre (1.2-3.7 t/ha).
2. **Macerate tissues** using line trimmer or flail mower to enhance conversion of glucosinolates to isothiocyanates (Fig. 4A).
3. **Incorporate** macerated tissues using roto-tiller to 4-6 inches (10-15 cm) soil depth (shallow till minimize soil disturbance).
4. Cover black **plastic mulch** to contain isothiocyanates from escaping into the air.
5. Uncover the plastic mulch **7 days** after tarping then transplant cash crop seedlings immediately (avoid direct seeding of small seeded crops).

Oil Radish vs Brown Mustard

Brown mustard (tilled and covered with black plastic) produced more biofumigants against root-knot nematodes

Oil radish (tilled and covered with black plastic) is better for soil health improvement (more bacteria decomposition)

Fig. 5. Abundance of A) root-knot and B) bacterivorous nematodes affected by oil radish (OR) and mustard (MS) terminated by no-till (NT), macerated and tilled (T) or macerated, tilled and covered with black plastic (BP) for 1 week as compared to the bare ground (BG) control.

Acknowledgements

This project is in part supported by WSARE (project number GW18-926) and in part supported by CTAHR Hatch 9034R.

What is biofumigation?

Biofumigation is the use of brassica plant-derived chemicals to manage soil-borne pests and pathogens in agroecosystems. Plants in Brassicaceae family such as brown mustard, *Brassica juncea* and oil radish, *Raphanus sativus* contain glucosinolates (GLS) and myrosinase (Myr) enzyme. Upon maceration of tissue and disruption of the plant cell, Myr comes in contact with GLS and breaks it down into sulfate and isothiocyanates (ITC) among other volatile compounds (Fig. 1). Of these compounds, especially ITC, is toxic to soil-borne pathogens and pests including plant-parasitic nematodes. However, suppression of plant-parasitic nematodes using biofumigation has been inconsistent. Current research at the College of Tropical Agriculture and Human Resources is developing a step-by-step protocol to help farmers to perform biofumigation against nematode pests.

To enhance the nematode suppressive effects of biofumigation, researchers are integrating ***trap cropping*** approach to this ecological based nematode management strategy.

Fig 1. A) Intact plant cell of biofumigant crop showing distribution of glucosinolate (GLS) and myrosinase (Myr); B) GLS hydrolysis pathway to form isothiocyanate (ITC), sulfate and other products.

Soil health-friendly

Biofumigation

Ability of oil radish to improve soil health has previously been documented due to its abilities to scavenge nutrients, smother weeds, enhance antagonists of nematodes, alleviate soil compaction through bio-drilling and improve water infiltration while brown mustard releases potent ITC suppressive to plant-parasitic nematodes (Clark, 2008). In our study, following the biofumigation protocol described, brown mustard suppressed soil populations of root-nematodes more efficiently while oil radish increased abundance of bacterivorous nematodes than the BG control (Fig. 5).

Therefore, future research should look into examining Soil Health-friendly biofumigation approach using mixed cover cropping of mustard and oil radish.

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Last edit on May 23, 2018

When to terminate the trap crop?

Conventional trap crop of plant-parasitic nematodes is a susceptible host that allow the infection of the nematodes, but if terminated prior to the nematode mass reproduction, it would trap the nematodes. When the trap crop is a biofumigant crop, the trapped nematodes would then be fumigated at crop termination and hence reduce the nematode pressure in the field. Therefore, time of termination of biofumigant crops for biofumigation is critical. Results from a field trial in Hawaii showed that terminating oil radish at 4 weeks after planting provided sufficient biomass (Fig. 2) to stimulate the subsequent pumpkin plant growth while reducing root-gall index on the crop (Fig. 3).

Fig. 2. 'Sodbuster' oil radish terminated at different time of growth.

Fig. 3. Effect of oil radish growing time on A) pumpkin root gall index (1-10 scale) caused by root-knot nematodes, and B) pumpkin fruit weight per plot.

Trap Cropping and Biofumigation for Plant-parasitic Nematode Management

'Caliente 199' Brown mustard (*Brassica juncea*)

'Sodbuster' Oil radish (*Raphanus sativus*)

Terminate biofumigant crops

A step-by-step method to conduct biofumigation for best nematode management practice is developed based on the understanding that oil radish and brown mustard are hosts of root-knot nematodes, tissue maceration is required for GLC to be converted to ITC, and ITC is volatile.

Flail mower

Line trimmer

Fig. 4. A) Flail mower and line trimmer; B) hand-held rototiller incorporating macerated tissues into soil; C) cover the soil with black plastic mulch.

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Acknowledgements

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