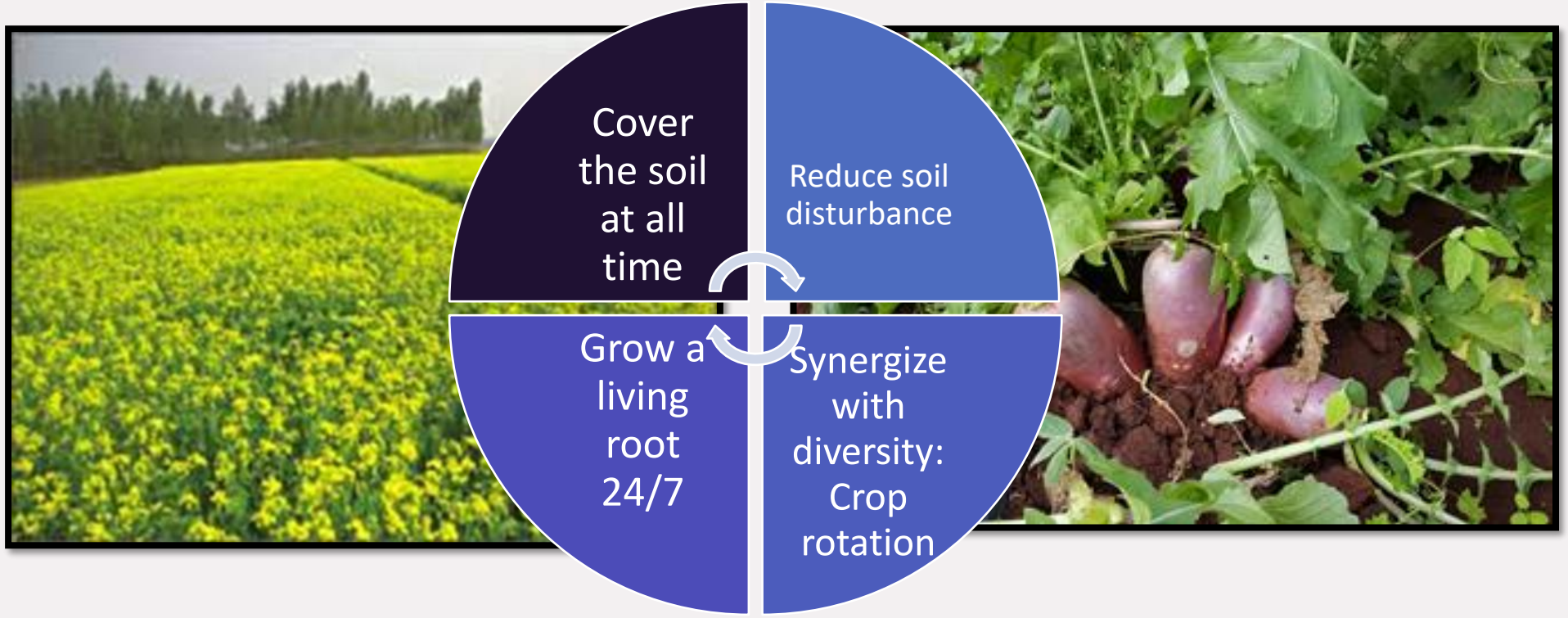
Biofumigation Approaches to help Farmers Reduce Soil-borne Disease Pathogen Problems in Agroecosystems



Koon-Hui Wang, Phillip Waisen, Roshan Paudel, Lauren Braley, J. Silva, J. Uyeda
CTAHR, University of Hawaii at Manoa

Meloidogyne spp.

Rotylenchulus reniformis

Reniform and Root-knot Nematodes are two common Plant-parasitic Nematodes found on wide range of crops in Hawaii

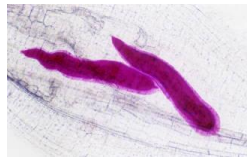
Root-knot Nematode Damage on Tomato & Zucchini in Hawaii

	Healthy roots	Infected by root-knot nematodes	Yield loss (%)
Tomato (Komohana, root-knot resistant var)			53
Zucchini			72

(Wang et al., 2017)

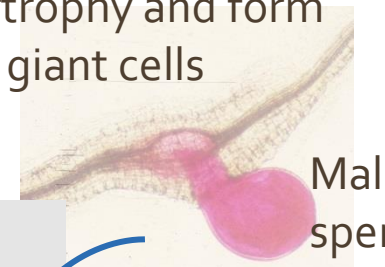
Root-knot nematode (*Meloidogyne* spp.)

J2
(move inside root tissues until a feeding site is found)

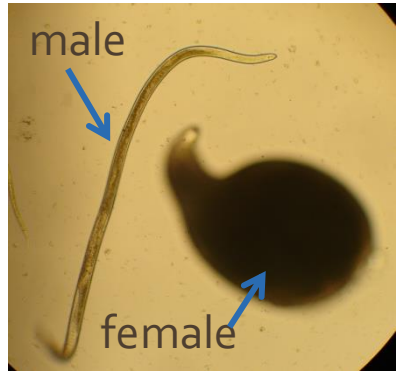


J3-J4
(sausage shape)

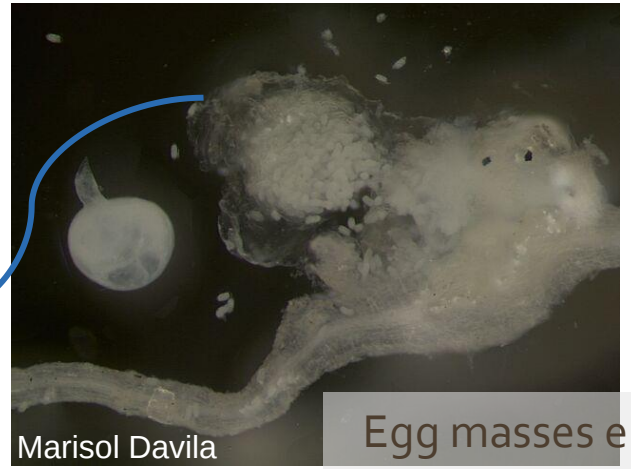
Feeding site undergoes hypertrophy and form giant cells



Male spends most time outside of the roots



Root-knot nematodes can produce eggs parthenogenetically (without sexual reproduction)



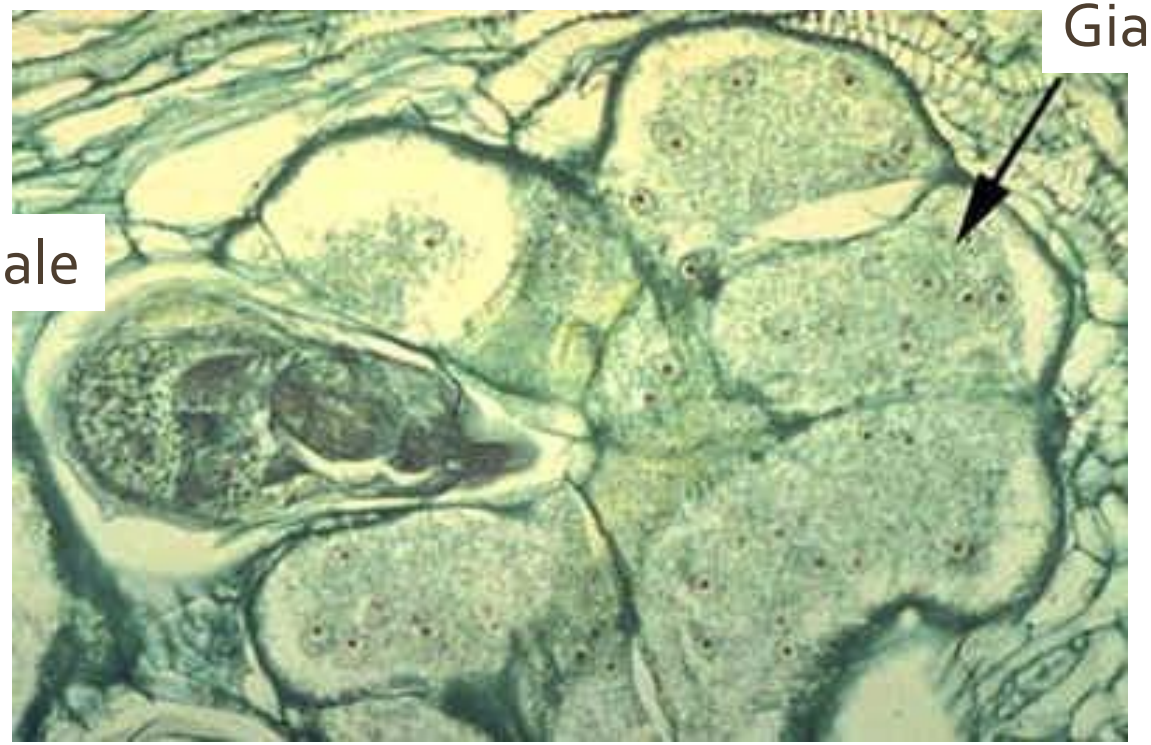
Egg masses erupt from root galls

egg

J2
(infective stage)

Giant cells

= enlarged, multinucleate cell formed in roots by repeated nuclear divisions without cell wall formation, this phenomenon is known as hypertrophy. It is induced by secretions of root-knot nematodes.



This lead to swollen of roots, thus forming root galls or root knots.

Picture: Society of Nematologists

Reniform nematode has a broad host range

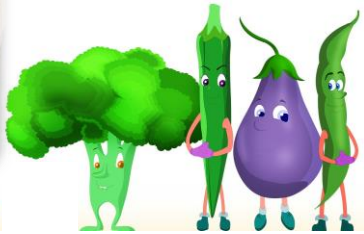
Pineapple

Papaya

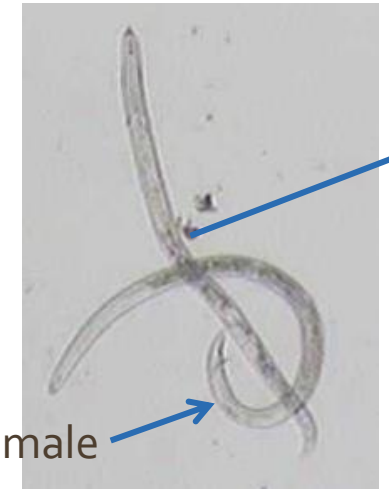
Cowpea

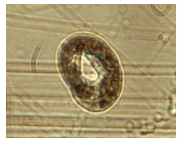
Sweet potato

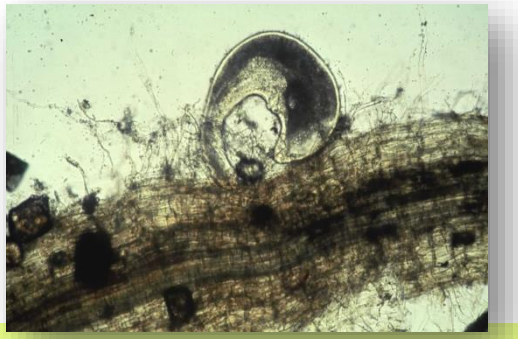
...and wide range of vegetable crops



Reniform Nematode (Rotylenchulus reniformis)







Pre-adult female

slightly swollen

Kidney-shape female

male

anhydrobiosis

Difficult to manage (can survive in dry fallow soil for years)

J_4

J_3

Vermiform stages stay outside of roots

J_2

egg

Easy to manage

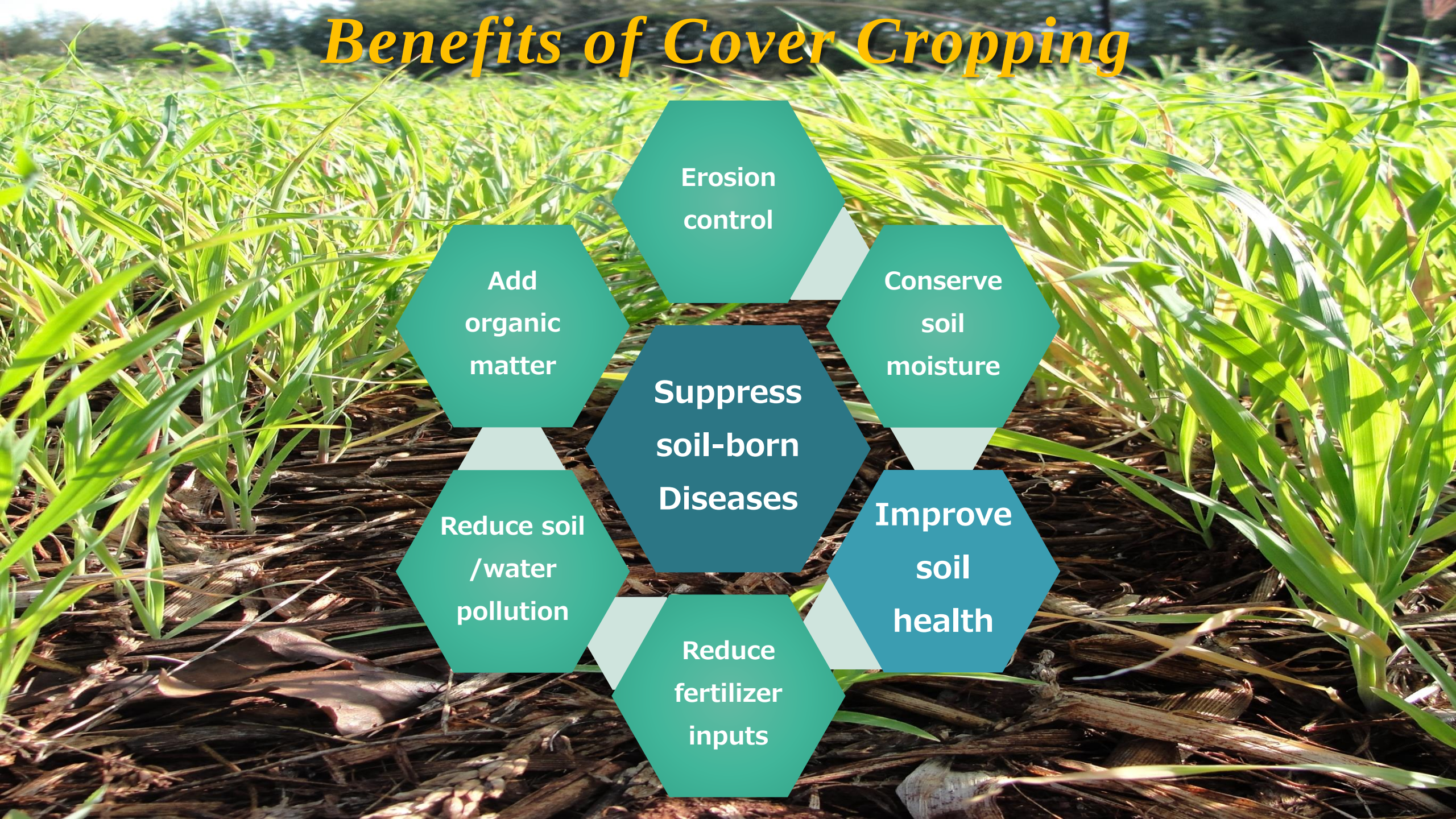
Anhydrobiosis

= Some nematodes can survive the loss of all their body water and enter a state of anhydrobiosis in which their metabolism comes reversibly to a standstill.

Scanning electron micrography of a nematode after dehydration.
(Sugar Team, http://coursewares.mju.ac.th:81/e-learning47/PP300/0016 sugarteam1014/5605nematode/004%20under%20microscope/page_01.htm)

This is making reniform nematode very difficult to manage.

Benefits of Cover Cropping



Cover Crops with Allelopathic Compounds against PPN

Sunn hemp
Crotalaria juncea
-- monocrotarine

T. erecta and *T. polynema* are resistant to root-knot but very susceptible to reniform nematodes.

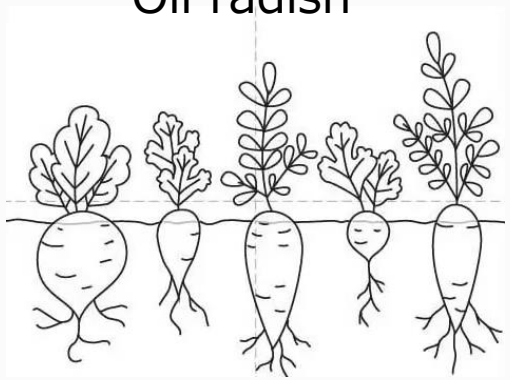
French Marigold
Tagetes patula
-- α-terthinyl

Brown mustard
(*Brassica juncea*)
-- glucosinolate

Sorghum-sudangrass
-- Dhurrin

Brasicaceous Cover Crops

Oil radish



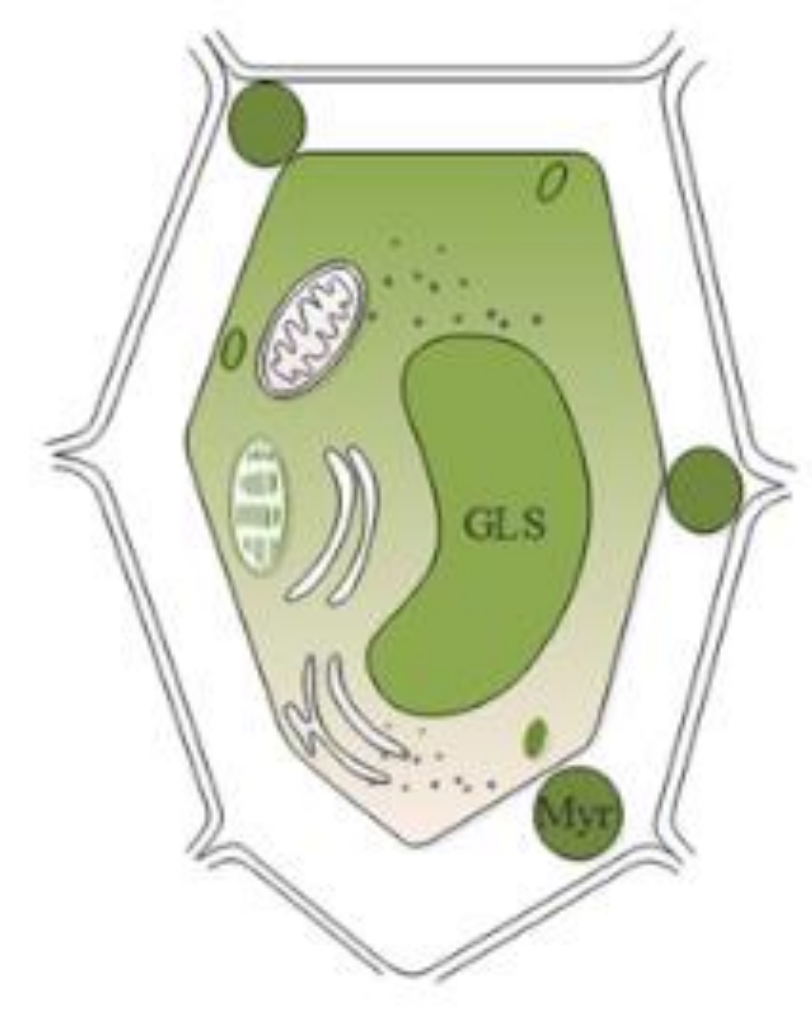
Brown mustard

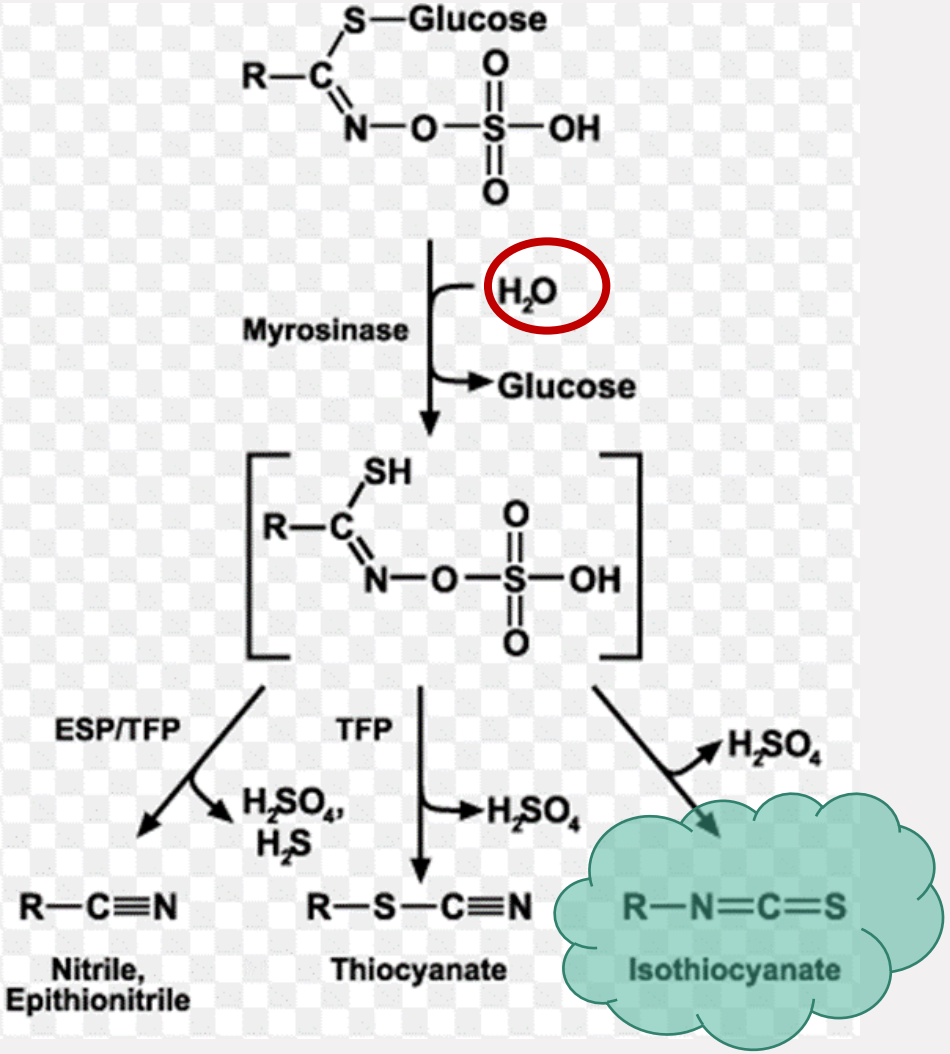
Picture Credit: Joel Gruver

Benefits of Brasicaceous cover crops

- Biodrill
- Nutrient scavenging
- Nematode trap crop
- Biofumigation

Biofumigation





The use of glucosinolate (GL)-derived isothiocyanate (ITC) from brassica cover crops is known to suppress soil-borne pests and pathogens (Kirkegaard et al., 1993).

Root-knot nematode

Panama wilt

Fusarium wilt

Rhizoctonia bottom rot

Asparagus crown and root rot

Targeted Soil-Born Diseases

- ✓ Zucchini nematodes
- Lettuce Fusarium Wilt
- Banana Fusarium Wilt (Panama Wilt)
- Asparagus Crown and Root Rot

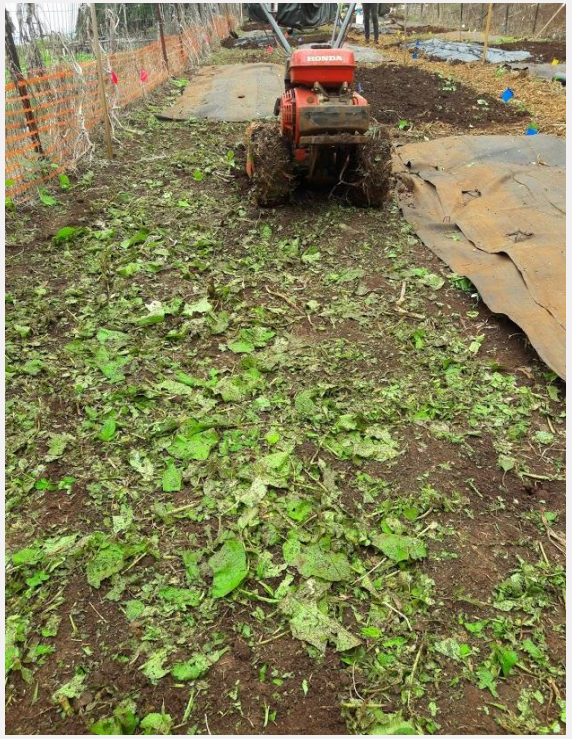
Biofumigation by MTBP

(= Macerated, Till, cover with Black Plastic)

Seeding: 10 lb/acre

5 weeks

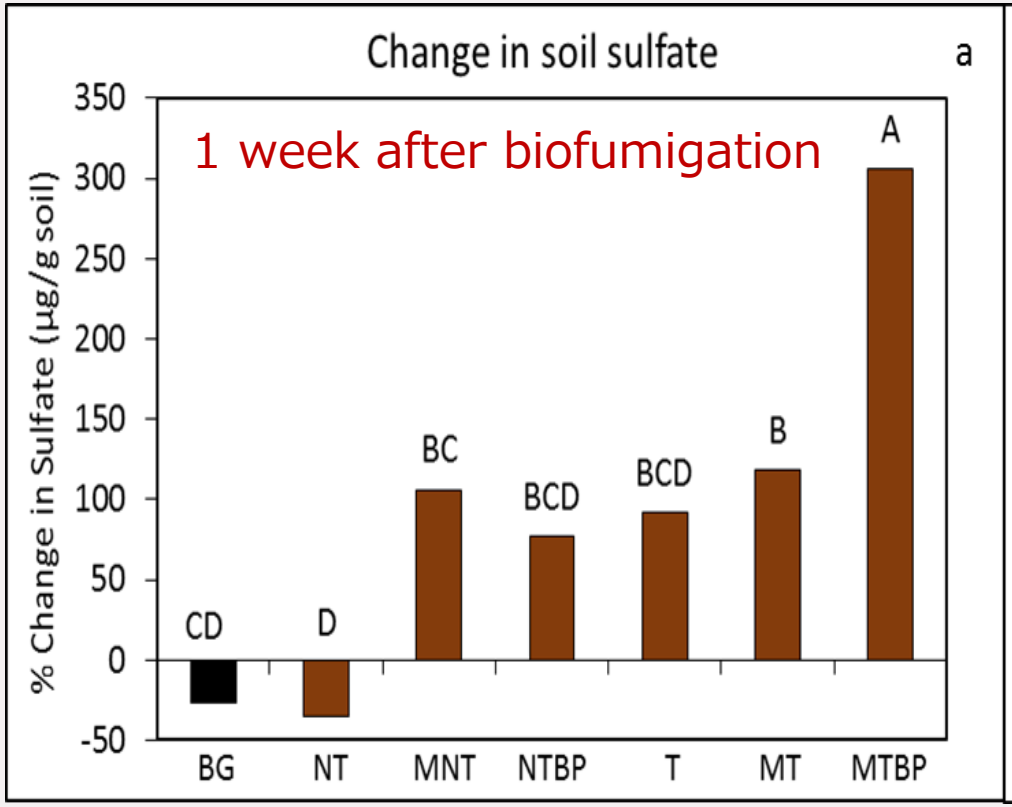
Flail mower

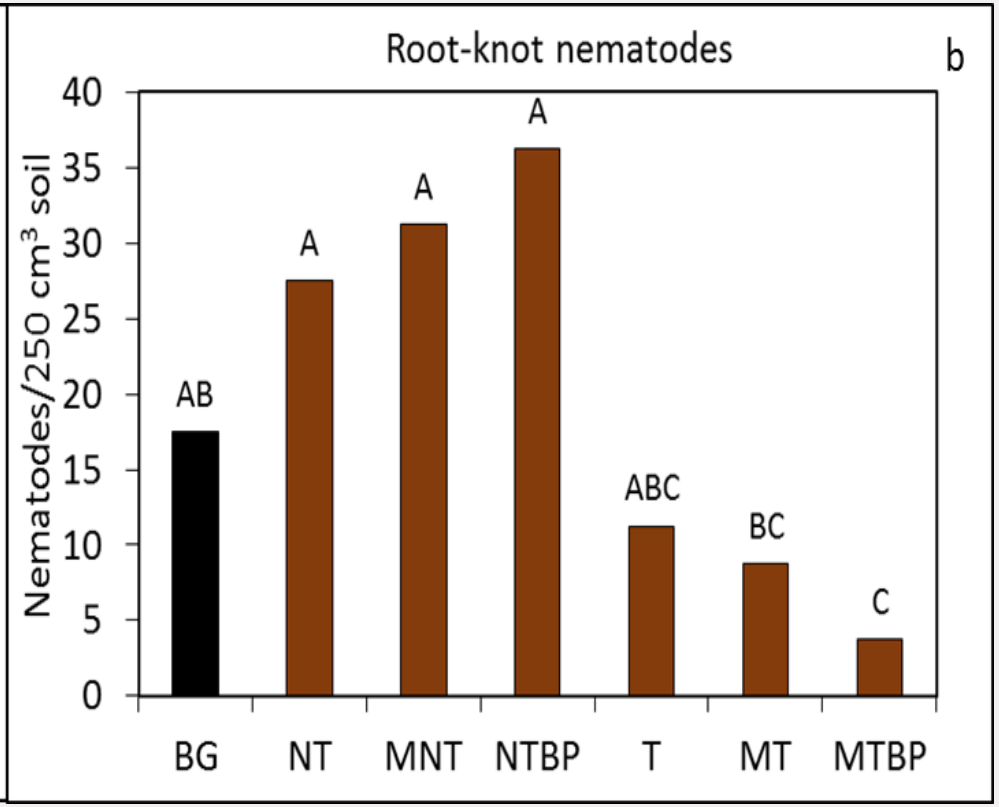


Till

Cover with plastic
(1 week)

Suppression of Root-knot Nematodes by MTBP-Biofumigation



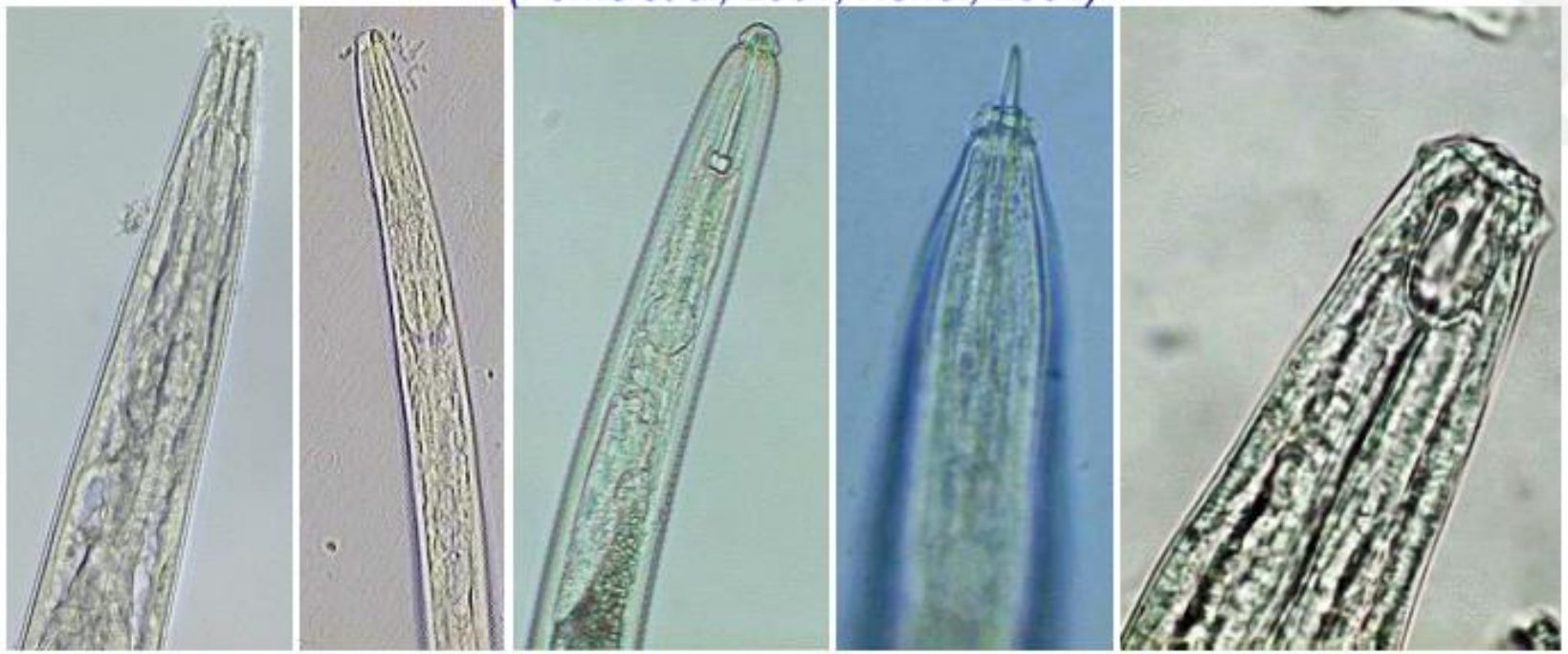


- Higher soil sulfate concentration in MTBP indicated higher efficacy of biofumigation than bareground (BG), no-till (NT), macerated no-till (MNT), no-till with black plastic (NTBP), till (T), and (macerated till).
- Thus, MTBP resulted in lowest population of root-knot nematodes after a zucchini crops.

(Waisen et al., 2020 Applied Soil Ecology 154)

Using nematodes as soil health indicators

(Ferris et al, 2001; Neher, 2001)



Bacterivore **Fungivore** **Herbivore** **Omnivore** **Predator**

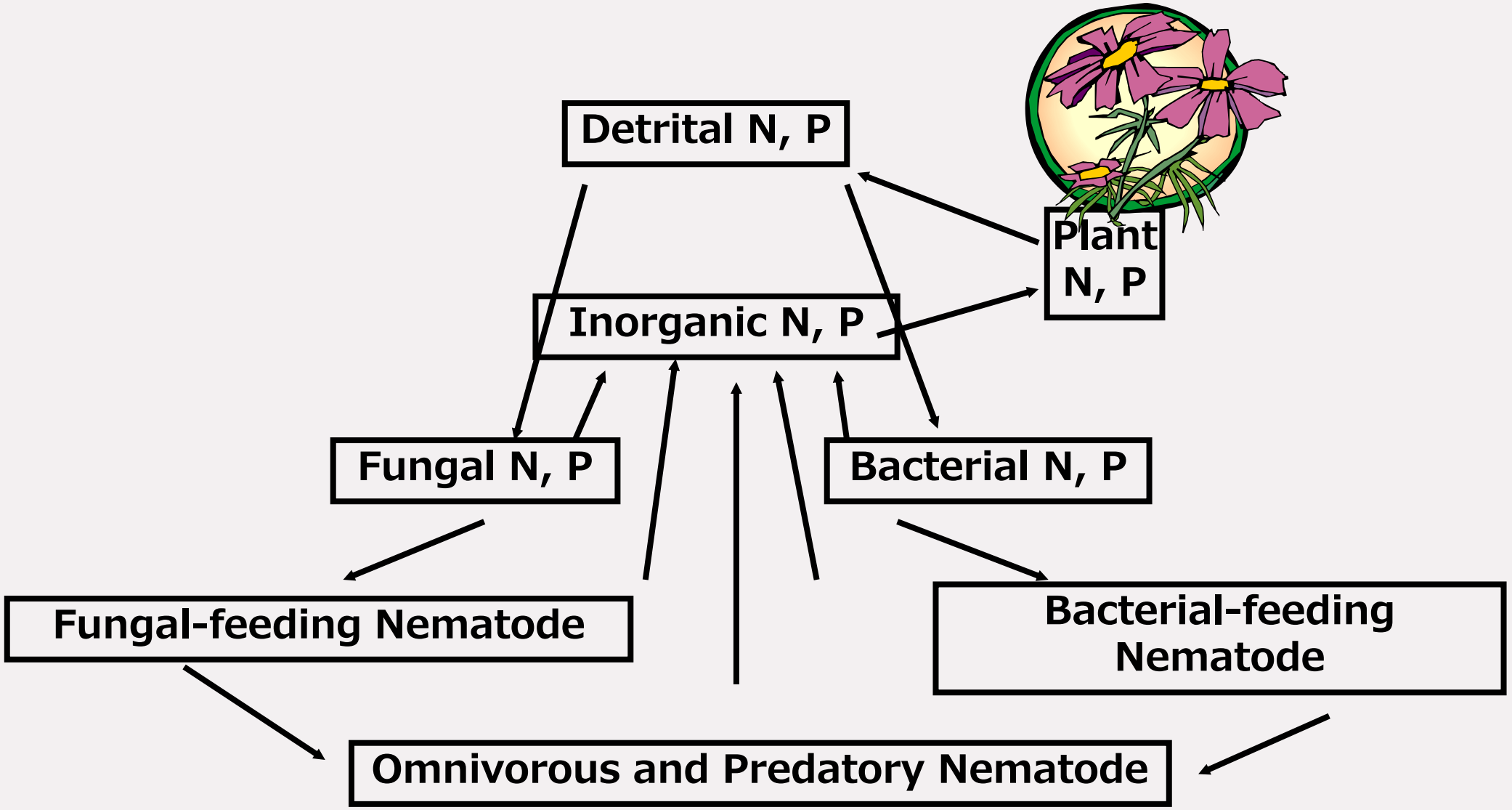
EI=Enrichment index

CI=Channel index

SI=Structure index

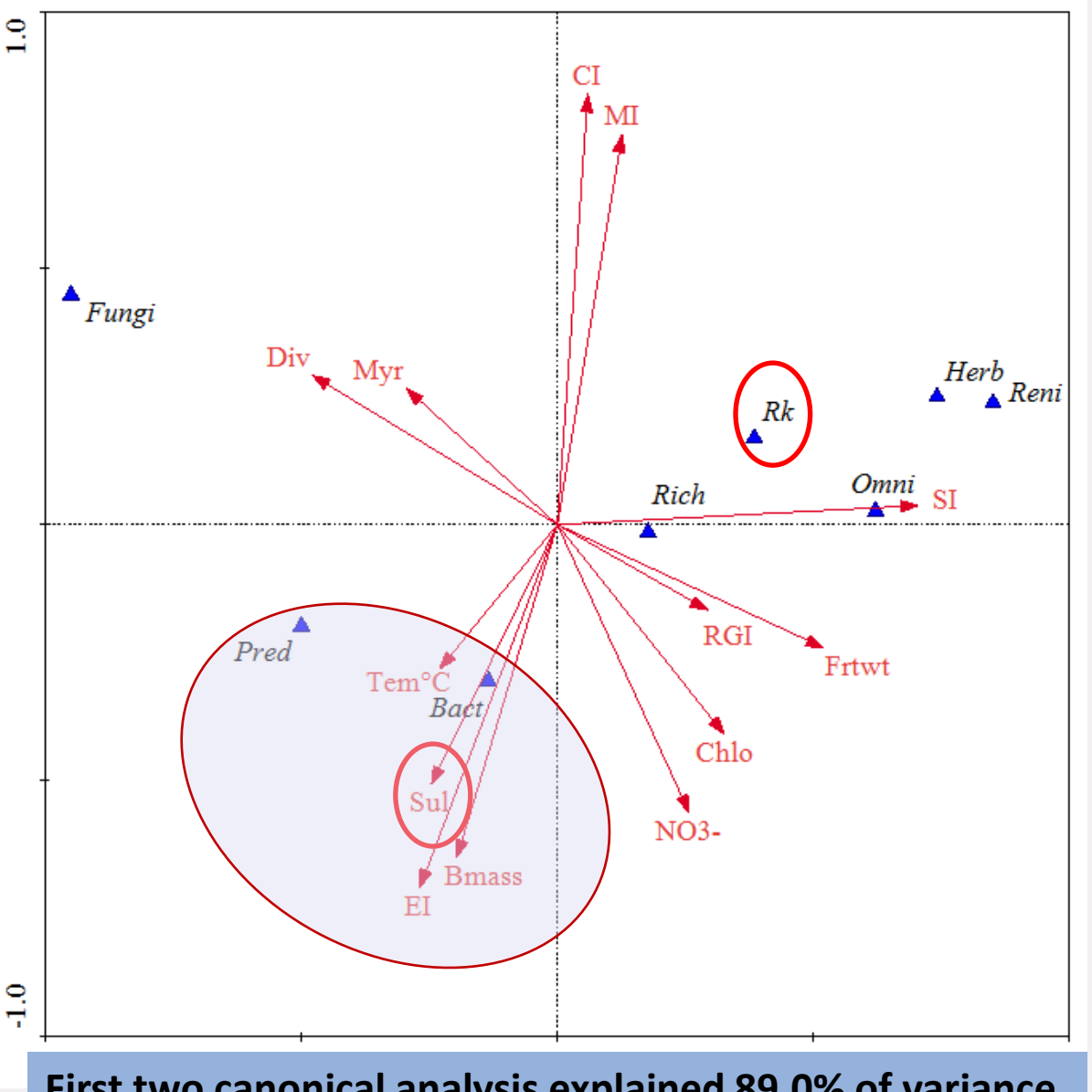
+ richness, diversity

Nematodes and Soil Nutrient Cycling



(modified from Ingham *et al.*, 1985)

Relationship between Biofumigation indicator to Nematodes



First two canonical analysis explained 89.0% of variance

- Efficacy of biofumigation (indicated by [Sulfate] (Sul) was negatively related to abundance of plant-parasitic nematodes (Rk = root-knot, Reni = reniform, Herb = combination of plant-parasitic nematodes) but positively related to abundance of bacterivores (Bact), predatory nematodes (Pred) and Enrichment index (EI = indicate soil is enriched with nutrients).
- Thus, **biofumigation did not compromise bacterial decomposition and soil health conditions.**

(Waisen, Wang et al., 2021 Pedosphere)

Biofumigation on Zucchini is Affordable and Profitable

Biofumigation	Plastic cost/row	Plastic/ft²	Plastic cost/ acre	Seed+ plastic cost/acre	Yield loss saved from nematode control	Source
Solarization[z]	$40.96	$0.0171	$743.42	$804	$11,021	Hardware World
Black Plastic[z]	$448.86	$0.0224	$977.62	$1,038	$14,327	Farm Plastic Supply

Biofumigation	Zucchini yield
Solarization	20%
Black plastic	26%
Ideal condition	33,600 lb/acre

Compared to BG

COOPERATIVE EXTENSION
UNIVERSITY OF HAWAI'I AT MANOA
COLLEGE OF TROPICAL AGRICULTURE AND HUMAN RESOURCES

Root-knot nematode

Panama wilt

Fusarium wilt

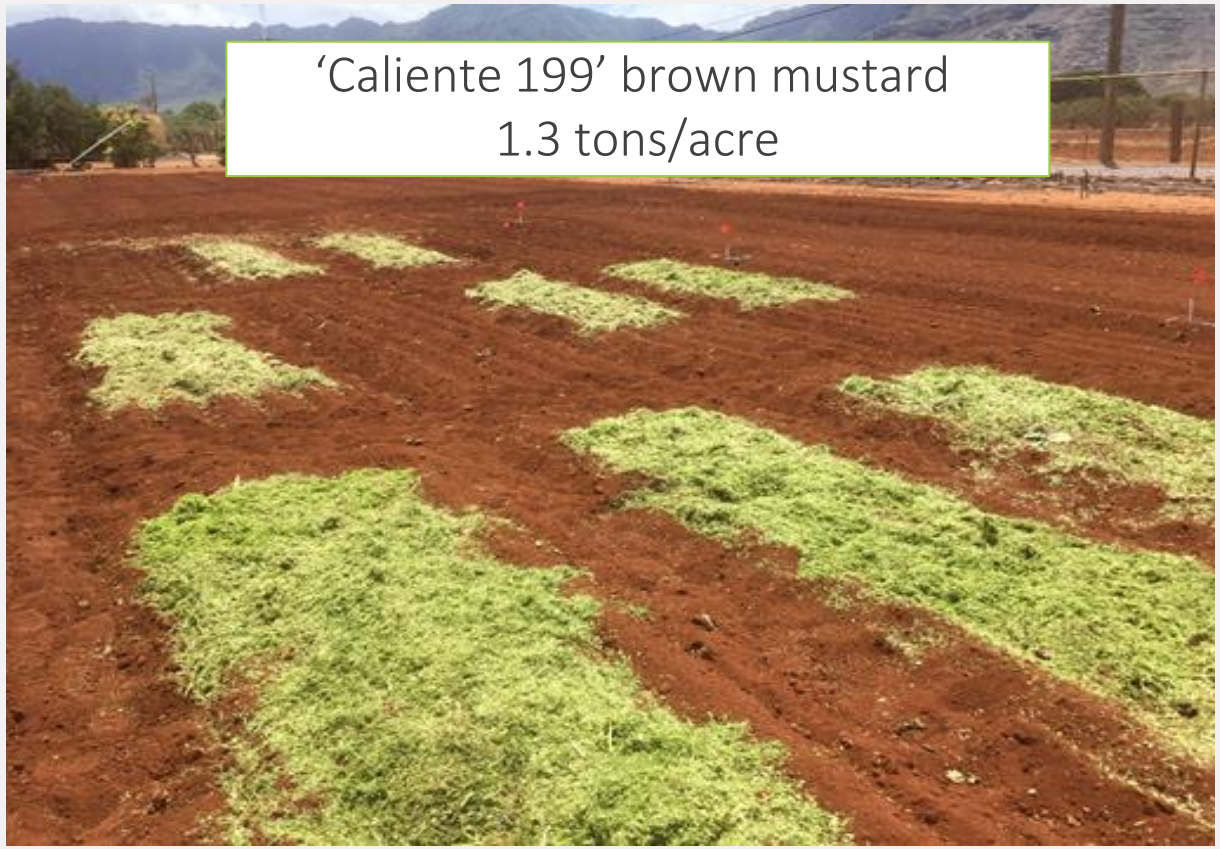
Fusarium oxysporum f. sp. lactuca

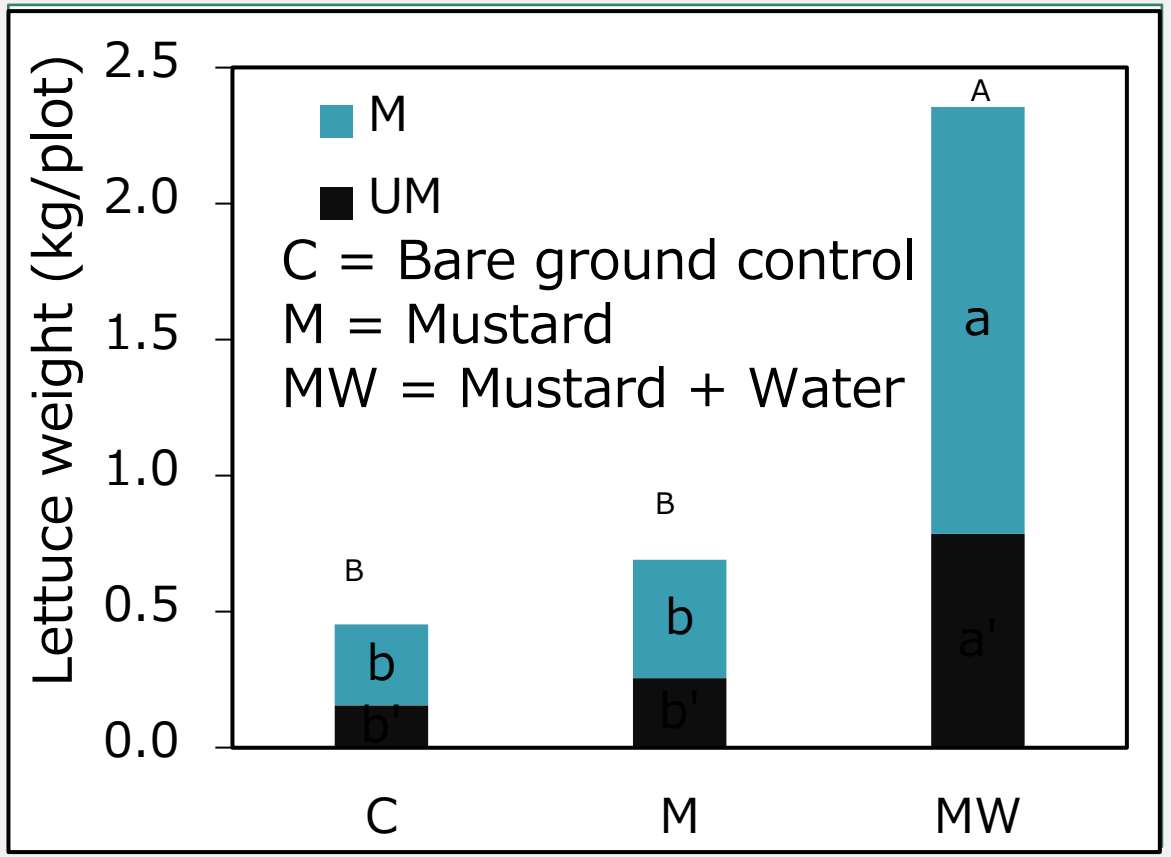
Rhizoctonia bottom rot

Targeted Soil-Born Diseases

- Zucchini nematodes
- ✓ Lettuce Fusarium Wilt
- Banana Fusarium Wilt (Panama Wilt)
- Asparagus Crown and Root Rot

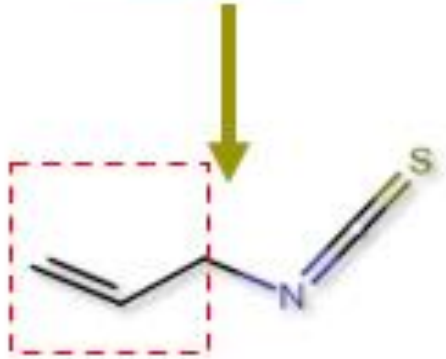
Lettuce Fusarium Wilt





Biofumigation with macerated brown mustard, soil incorporated + water to reach ~40% soil moisture and tarp with **solarization mulch** for 1 week prior to lettuce planting increased lettuce marketable yield by **5 folds** compared to the C and M only.

Brassica spp.
(e.g. *Brassica juncea*, brown mustard)



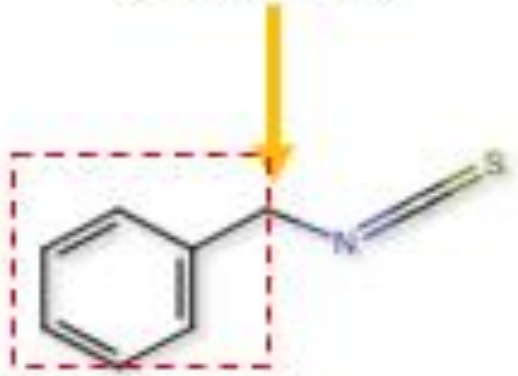
allyl isothiocyanate
(AITC)

methyl isothiocyanate
(MITC)

Alternative Biofumgation: Papaya Ground Seeds (PGS)

(Braley, 2022)

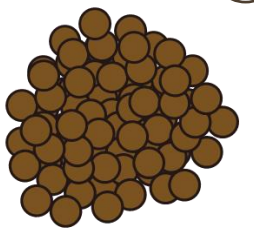
Carica papaya
(Papaya fruit)



benzyl isothiocyanate
(BITC)

PGS Preparation

1 Extract seed



Papaya seed

2 Dry @ 50℃ 48hrs

Grind in mill

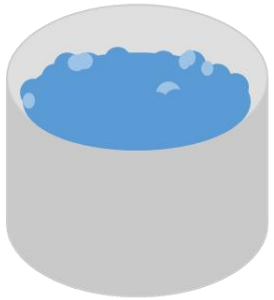
3

Ground papaya seed (PGS)

Crude Extract (CE) Preparation

PGS

1

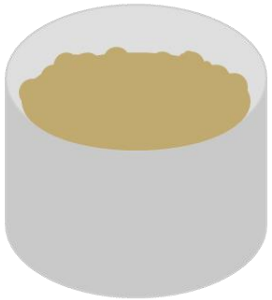


Boiling water (100 ˚C)

10 min.

stir

2

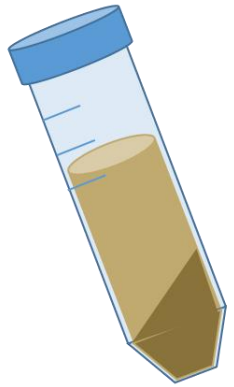


Decrease to 70 ˚C

4 hrs

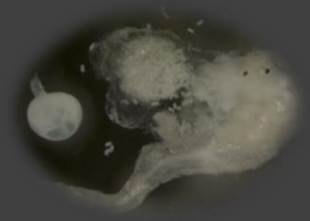
stir

3

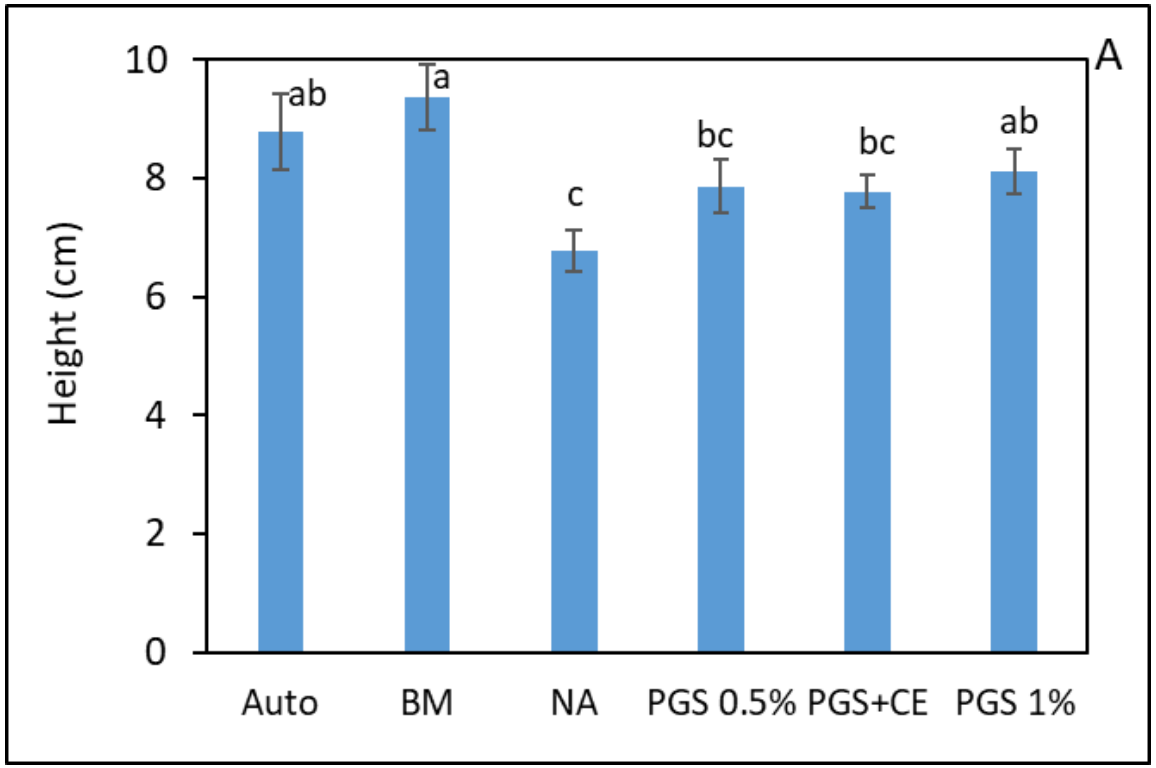


Centrifugation

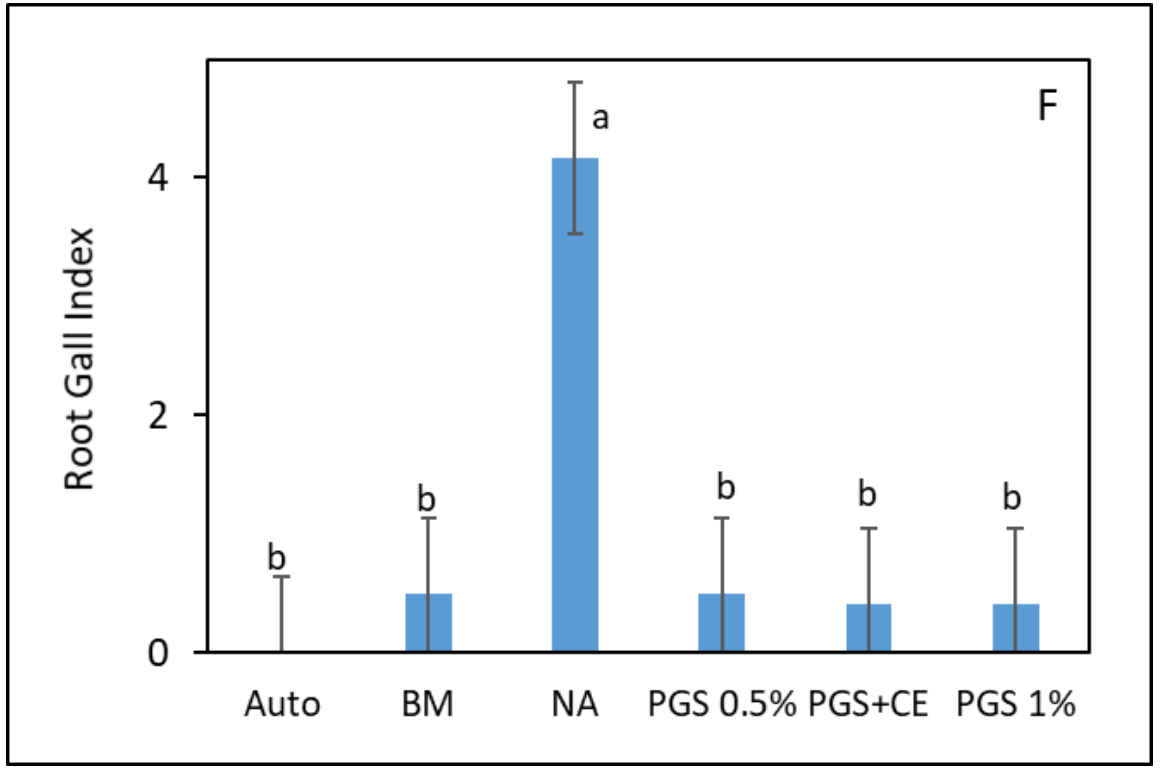
Effects of Biofumigation on Lettuce



Nematodes



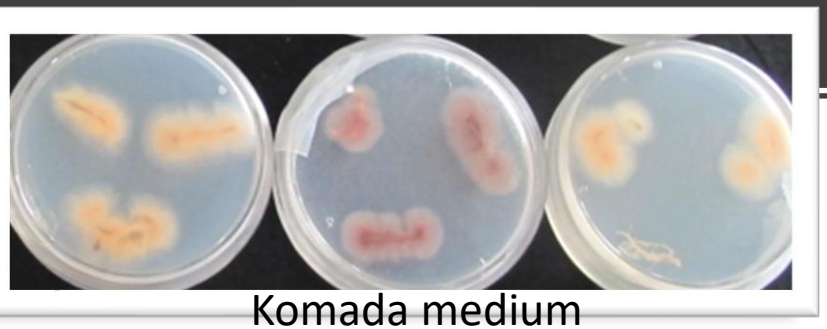
PGS 1% and brown mustard (BM) biofumigation increased lettuce growth.



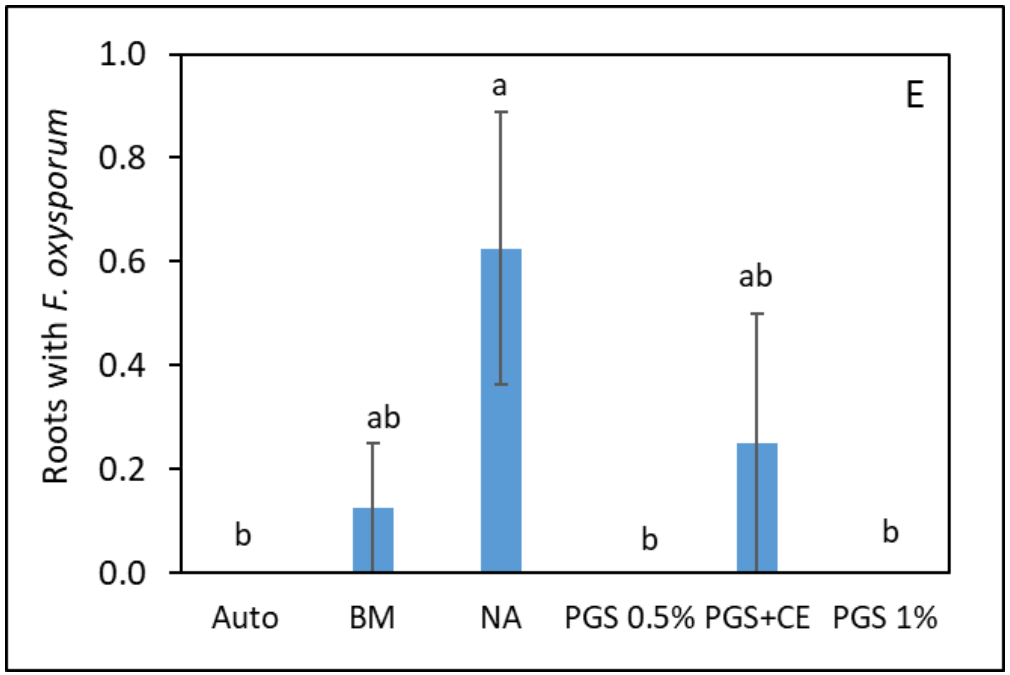
All biofumigation reduced root gall formation compared to no amendment (NA) control.

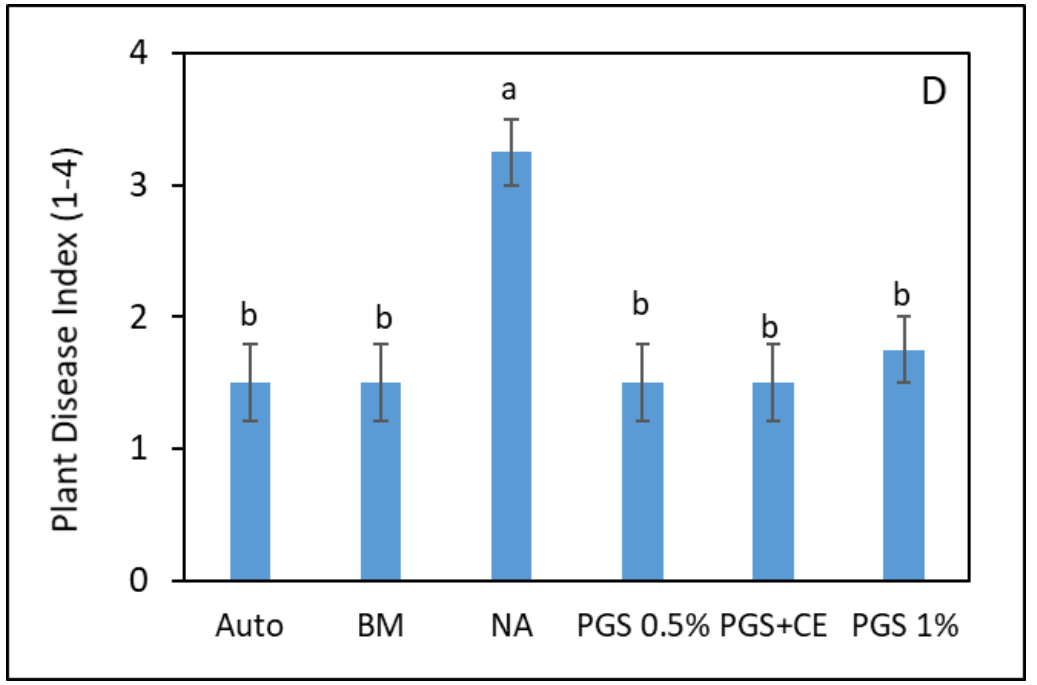
Biofumigation reduced colonization of Manoa lettuce root pieces by *F. oxysporum*

Fungus



Komada medium





Biofumigation for Lettuce is profitable for small-scale production provides an alternative to fumigation on infested soil

Materials	Dry Amendment (lb)/acre	Price ($)	Seed (lb)/acre of amendment	Cost ($)/acre	Source
Brown mustard	4453.5	6.1/lb seed	16.63	101.44	Siegers Seed Company
Solarization mulch	-	0.0171/ft^2	-	744.88	Hardware World (include shipping cost)
Total cost				846.32	

- Commercial Manoa lettuce yields: **15,692 lb/acre**.
- Farm gate value (NASS, 2020) of head lettuce in HI is only **$2.03/lb** or **$32,403/acre**.
- Biofumigation can be profitable for Manoa lettuce when needed.
- Once introduced into a field, *Fusarium oxysporum* f. sp. *lactucae* will probably remain indefinitely. This remains a viable option for farmers once in a while when needed.

Root-knot nematode

Panama wilt

Fusarium wilt

Targeted Soil-Born Diseases

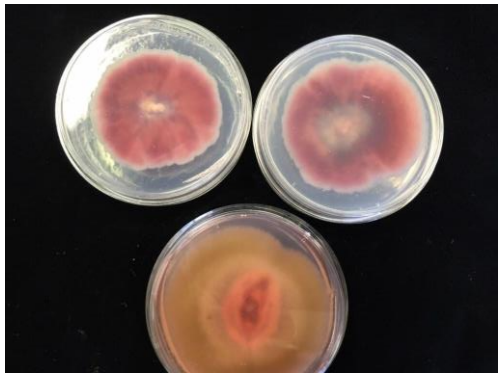
- Zucchini nematodes
- Lettuce Fusarium Wilt
- ✓ Banana Fusarium Wilt (Panama Wilt)
- Asparagus Crown and Root Rot

Fusarium oxysporum f. sp. cubensis

Soil Drenching Solution of Organic Compounds against Panama Wilt

Treatments: (5 gal water / plant)

- A = Actinovate (*Steptomyces lydicus*),
- L = Lobster meal,
- M = Mustard (ground),
- Sb = Subtilex (*Bacillus subtilis*),
- Sh = Shrimp shell meal,
- V = Vermicompost tea,
- W = Water



Banana root samples on Komada selective medium

Mustard (macerated)

Soil Drenching Solution of Organic Compounds against Panama Wilt

- A = Actinovate (*Steptomyces lydicus*),
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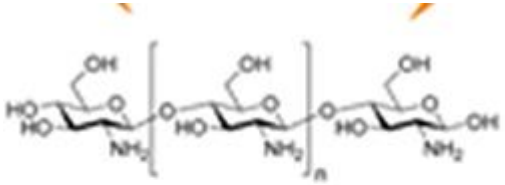
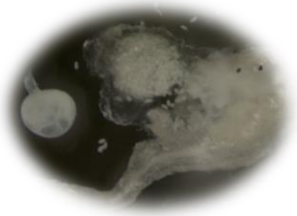
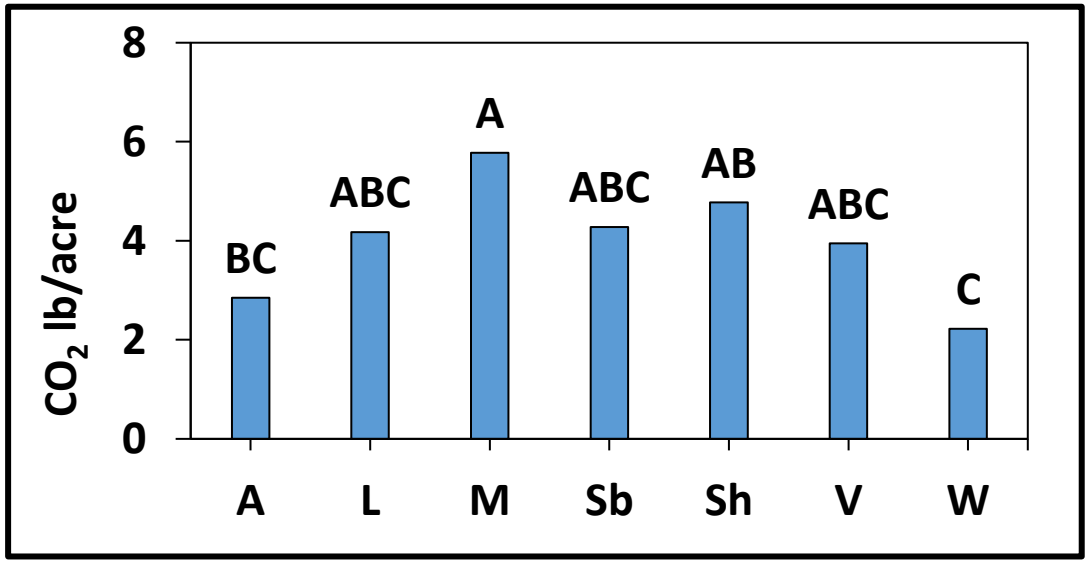
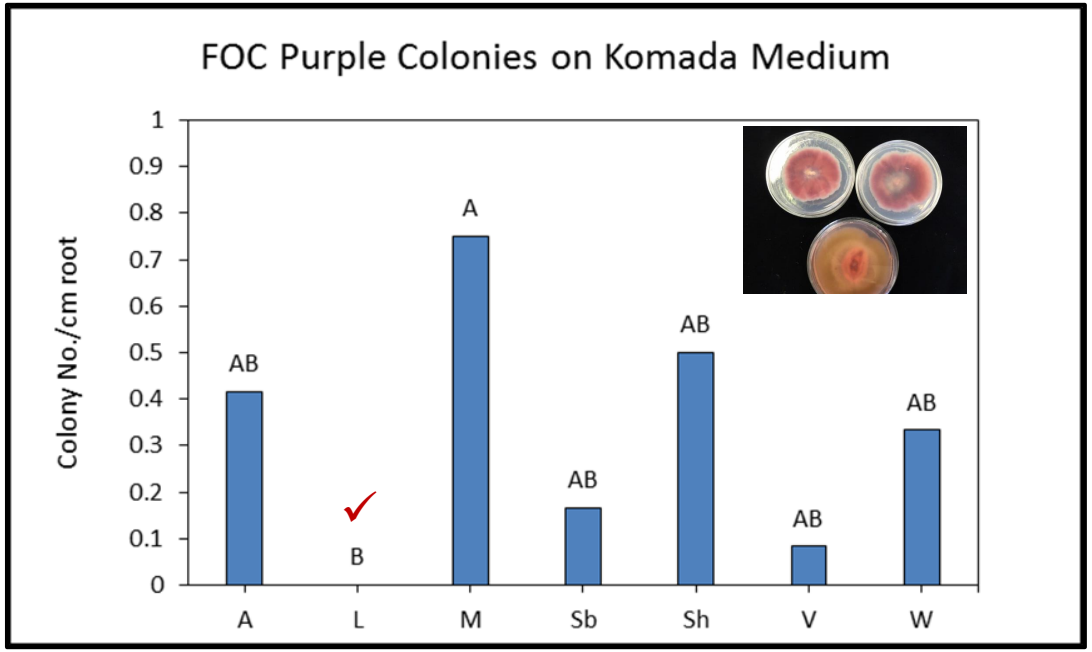




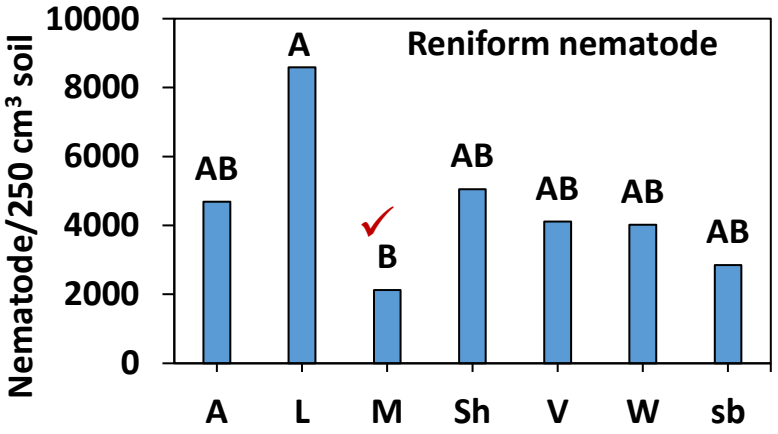
- Lobster meal suppressed Foc, mustard meal increased soil microbial activities.

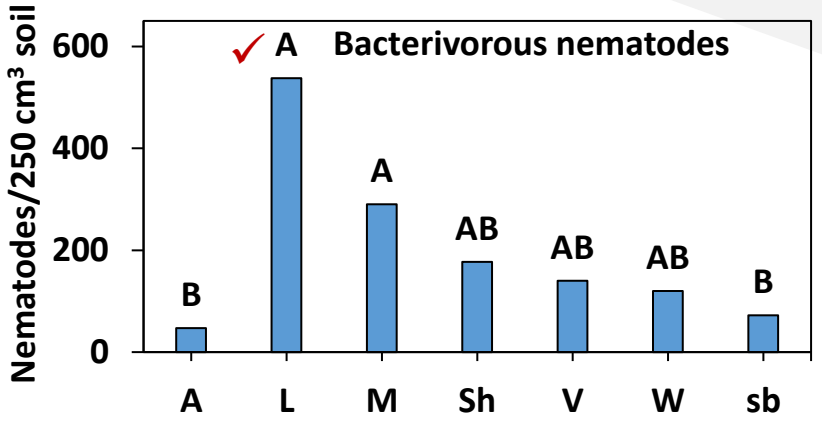


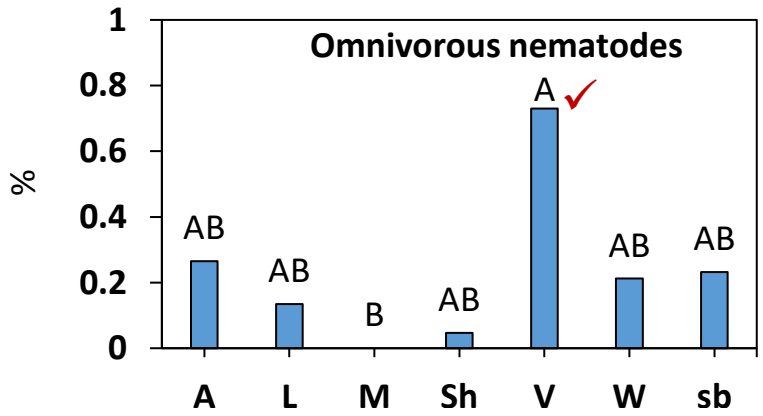


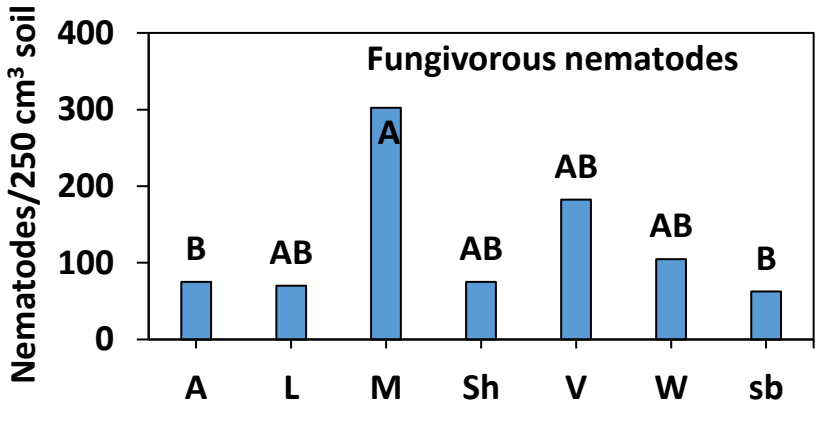
Organic Soil Drench on nematodes and soil health

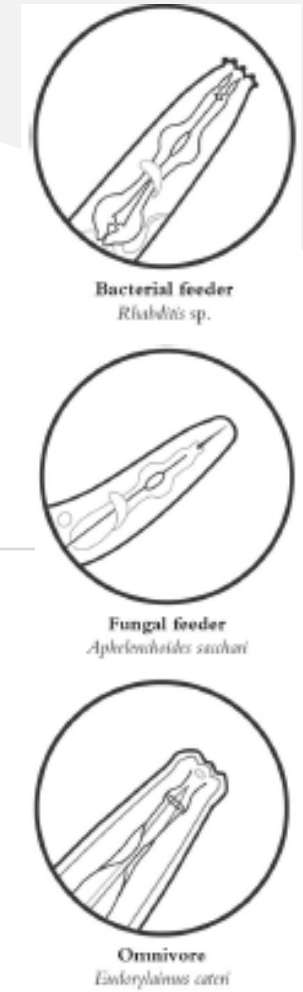
(8 weeks after treatment)





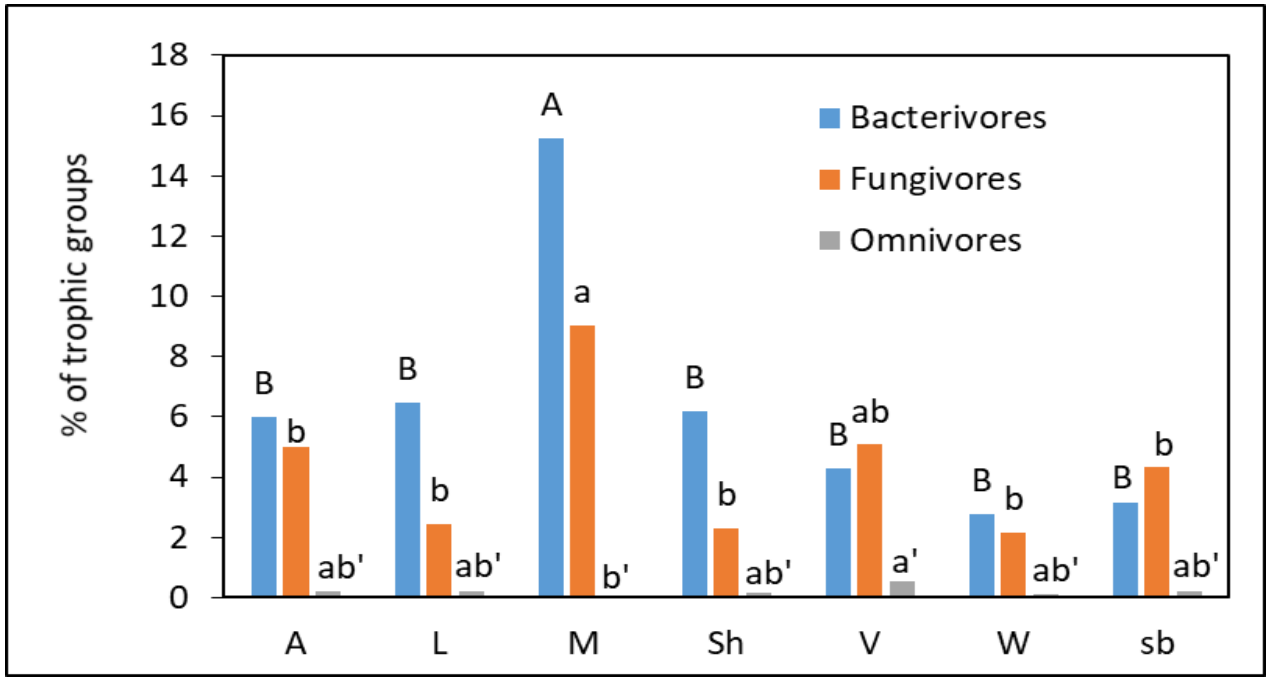






- Mustard resulted in lowest abundance of reniform nematodes.
- Lobster and mustard increased bacterivorous and fungivorous nematodes.
- Vermicompost tea increase % omnivorous nematodes.
- Lobster enhanced bacteria decomposition, mustard enhanced fungal decomposition.

Banana Fusarium Wilt (*Fusarium oxysporum f. sp. cubense, Foc*)



- Biofumigation with brown mustard + soil drenching enhance bacterial and fungal decomposition at 2 months after treatment. Thus, improving soil nutrient cycling.
  - At the standard banana yield of 22,000-30,000 lb/acre/yr and an elected price of $1.104/lb (~$24,288-$33,120/yr), combination of both crustacean meal and brown mustard amendment can still be affordable and worthwhile.

Treatment	Rate		Unit cost ($)	$/acre
Actinovate AG	6.0	oz/acre	117/18 oz	3.34
Subtilex® NG	0.4	oz/acre	120/2 oz	2.06
Shrimp shell meal	35.0	lb/1000 ft^2	37.81/15 lb	329.40
Crustacean meal	35.0	lb/1000 ft^2	52/40 lb	169.88
Brown mustard	1.7	lb/plant	6.1/lb seed	16.07

http://www.hawaiitropicalfruitgrowers.org/conferences/2019/2019_Crop_Insurance_for_Banana_Flyer.pdf

Targeted Soil-Born Diseases

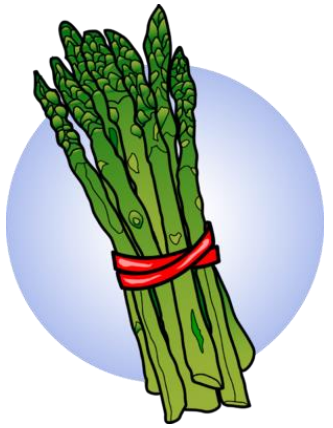
- Zucchini nematodes
- Lettuce Fusarium Wilt
- Banana Fusarium Wilt (Panama Wilt)
- ✓ Asparagus Crown and Root Rot


Fusarium wilt


Rhizoctonia bottom rot

Fusarium oxysporum f. sp. *asparagi* (*FOA*)

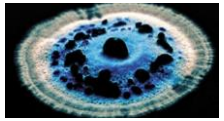
Asparagus crown and root rot

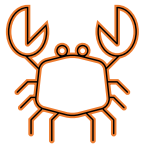


Biological Stimulants

Trial I (2019 Nov – 2020 Oct)

- Actinovate® AG (Noyozyme, Milwaukee, WI) - *Streptomyces lydicus* WYEC 108 (AG)
- Subtilex® NG (BASF, Research Triangle Park, NC) - *Bacillus subtilis* (Sb)
- Shrimp Meal contains 6-6-0 and 10% Ca, 18% chitin at 0.17 kg/m^2 (Sh)
- Crustacean Meal (PAR 4 Protein Meals, Bridgewell Agribusiness LLC, Clackamas, OR): 4-0-0, 12% Ca, 23-30% chitin from crab and lobster shells and meal at 0.17 kg/m^2 (L)
- macerated brown mustard (*Brassica juncea*) 'Caliente 199' 3.6 tons/ha - release isothio-cyanates upon soil incorporation as a biofumigant (M)
- Unamended control (C)



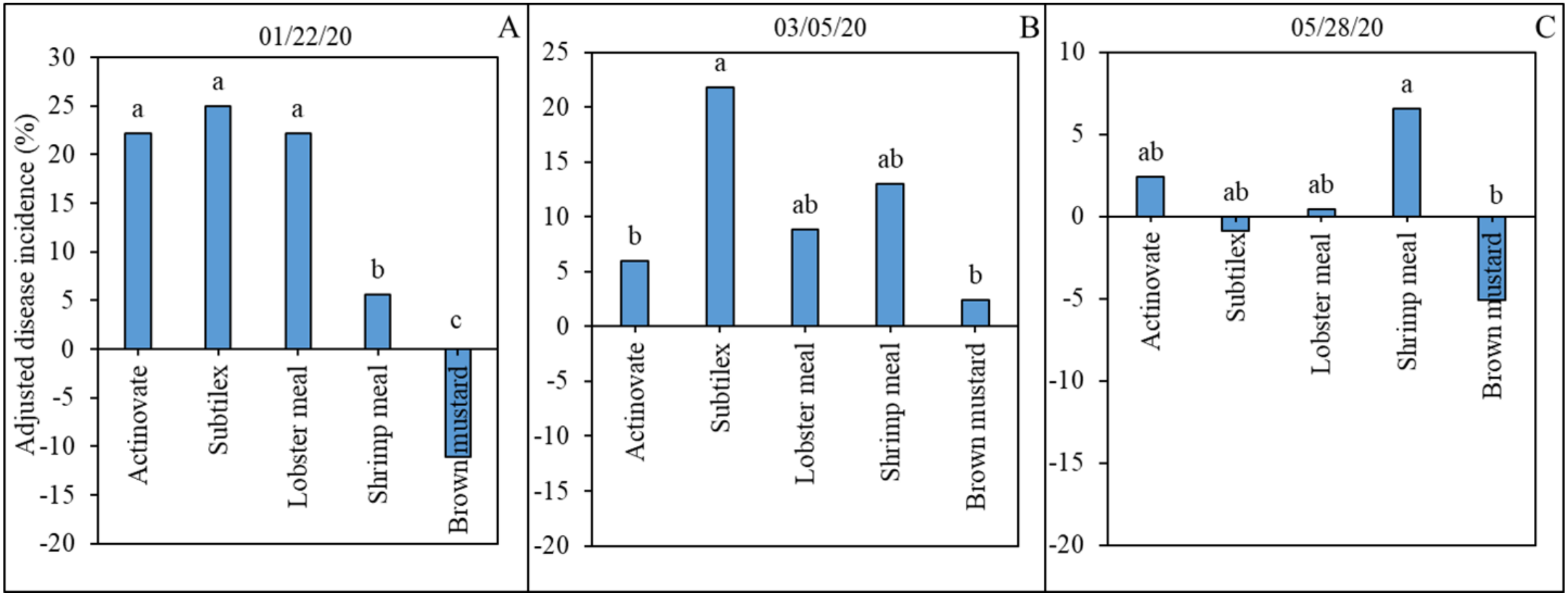


Trial II (2020 Oct – 2021 Feb)

Chitinolytic product, Armour-Zen (a.i. 15% chitosan, Botry-Zen, Dunedin, New Zealand) applied at 1% concentration by:

- foliar application (CF)
- soil drenching (CD)
- foliar+soil drenching (CFD)
- M
- L
- C

Trial I. Asparagus crown rot Disease incidence

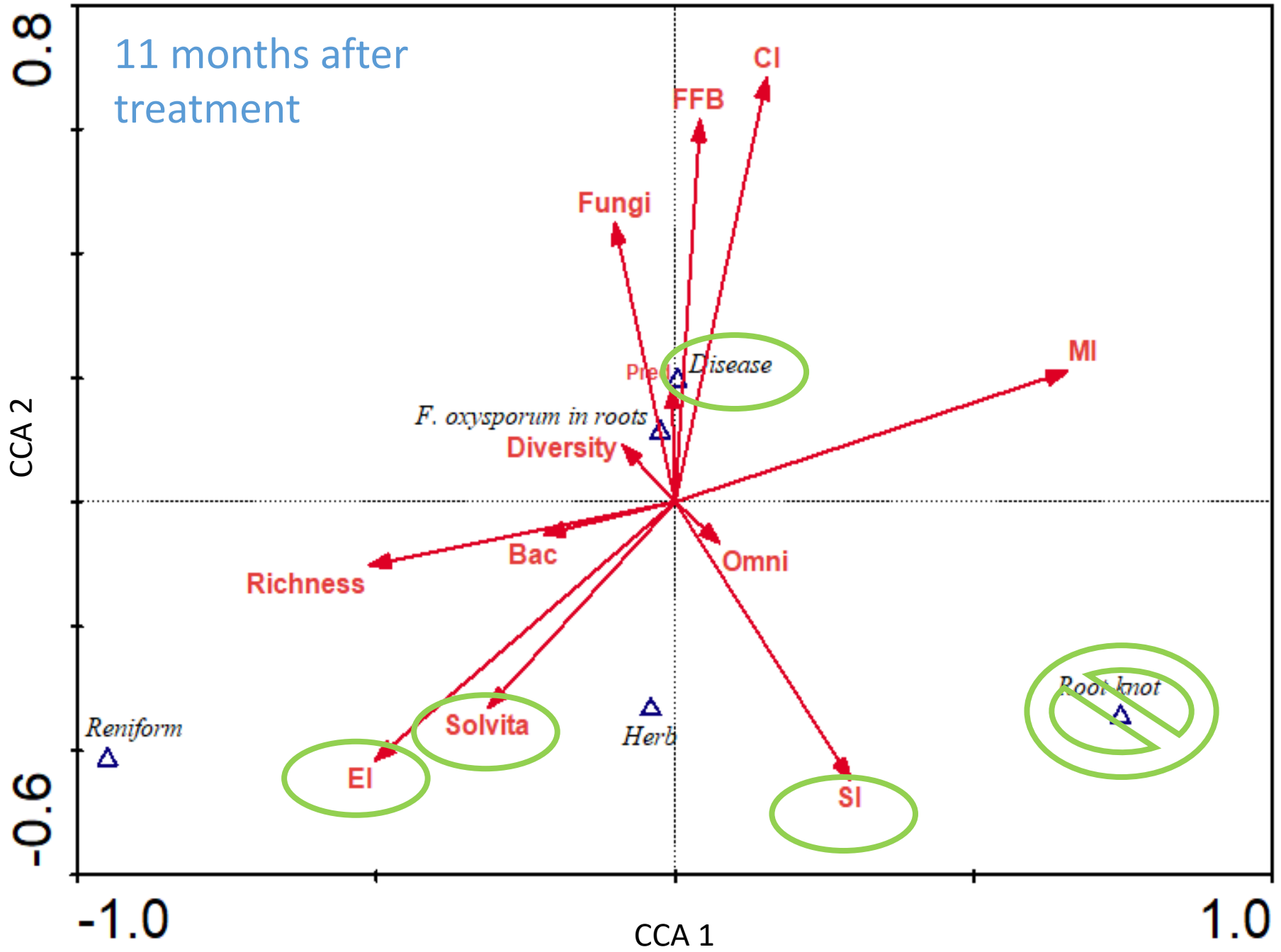


Relative to untreated control, only brown mustard amendment reduced disease incidence of Foa in Trial I in two out of the 3 sampling dates.

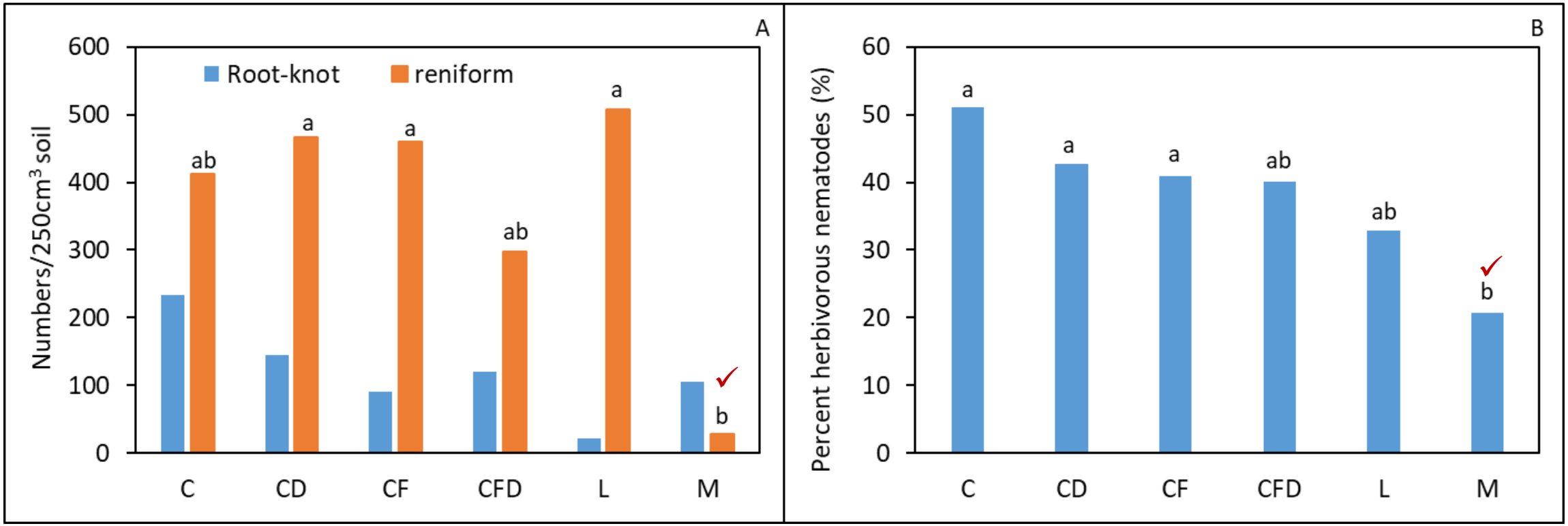
Trial I

- First two axes explained 91.1% of the variables

SI = Structure index
EI = Enrichment index
CI = Channel index
MI = Maturity index
FFB = Fungivores/ Bacterivore + Fungivores
Bac = bacterivores
Fungi = Fungivores
Omni= Omnivores
Pred = predatory nematodes
Herb =herbivores



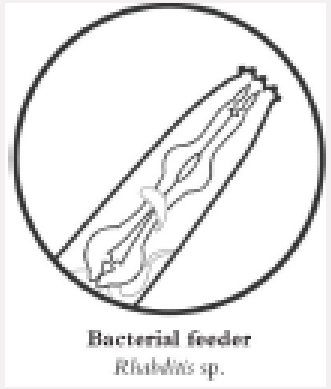
Trial II. Plant-parasitic nematodes on asparagus rhizosphere



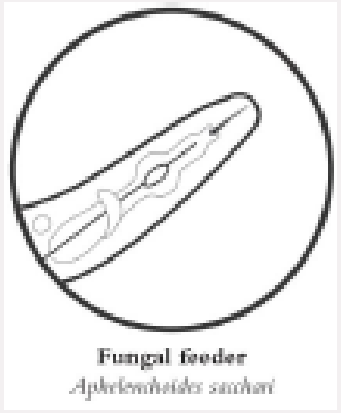
C=control; CD=Chitosan drench; CF=Chitozan foliar; CFD=Chitosan foliar & drench, L = crustacean meal; M=mustard

- No difference for root-knot nematodes in the soil; reniform and over all abundance of plant-parasitic nematodes were lowest in M.

Summary



EI = Enrichment index



CI = Channel index

SI = Structure index

- **Soil treatments that increased nutrient enrichment and improved soil food web structure also lead to less diseases.**
- **Enhancement of soil health can reduce Nematode and Fusarium diseases on** various crops.

Cover Crops with Allelopathic Compounds against PPN

Sunn hemp
Crotalaria juncea
-- monocrotarine

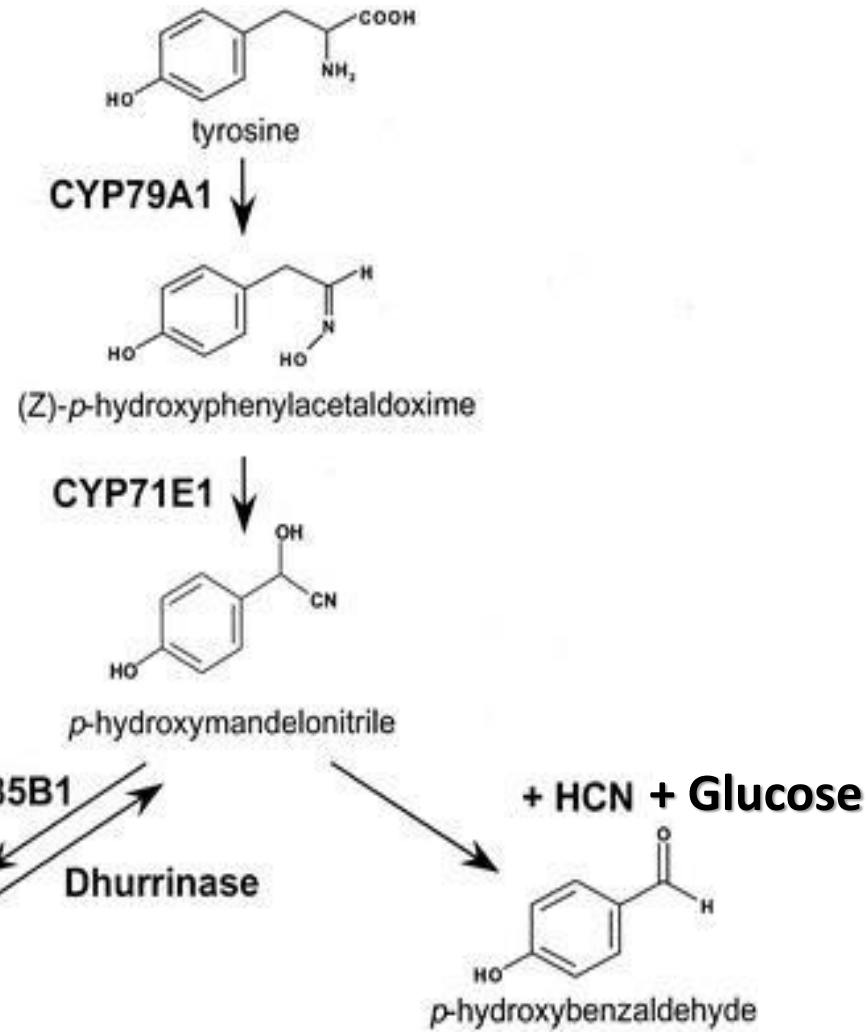
T. erecta and *T. polynema* are resistant to root-knot but very susceptible to reniform nematodes.

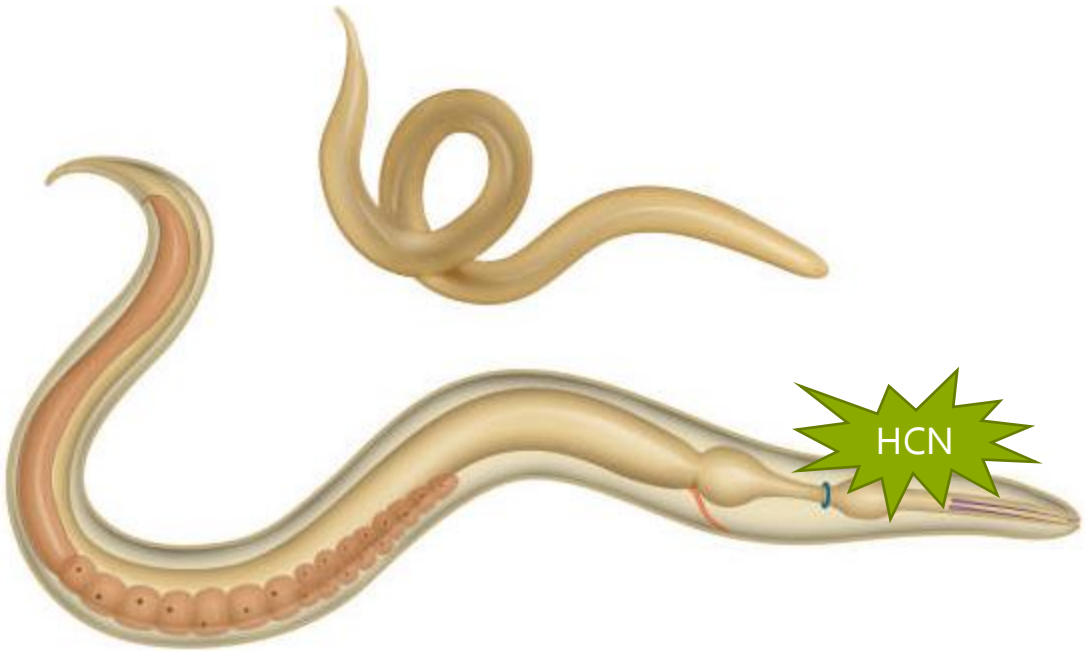
French Marigold
Tagetes patula
-- α-terthinyl

Brown mustard
(*Brassica juncea*)
-- glucosinolate

Sorghum-sudangrass
-- Dhurrin

Biofumigant from Sorghum/Sorghum-Sudangrass

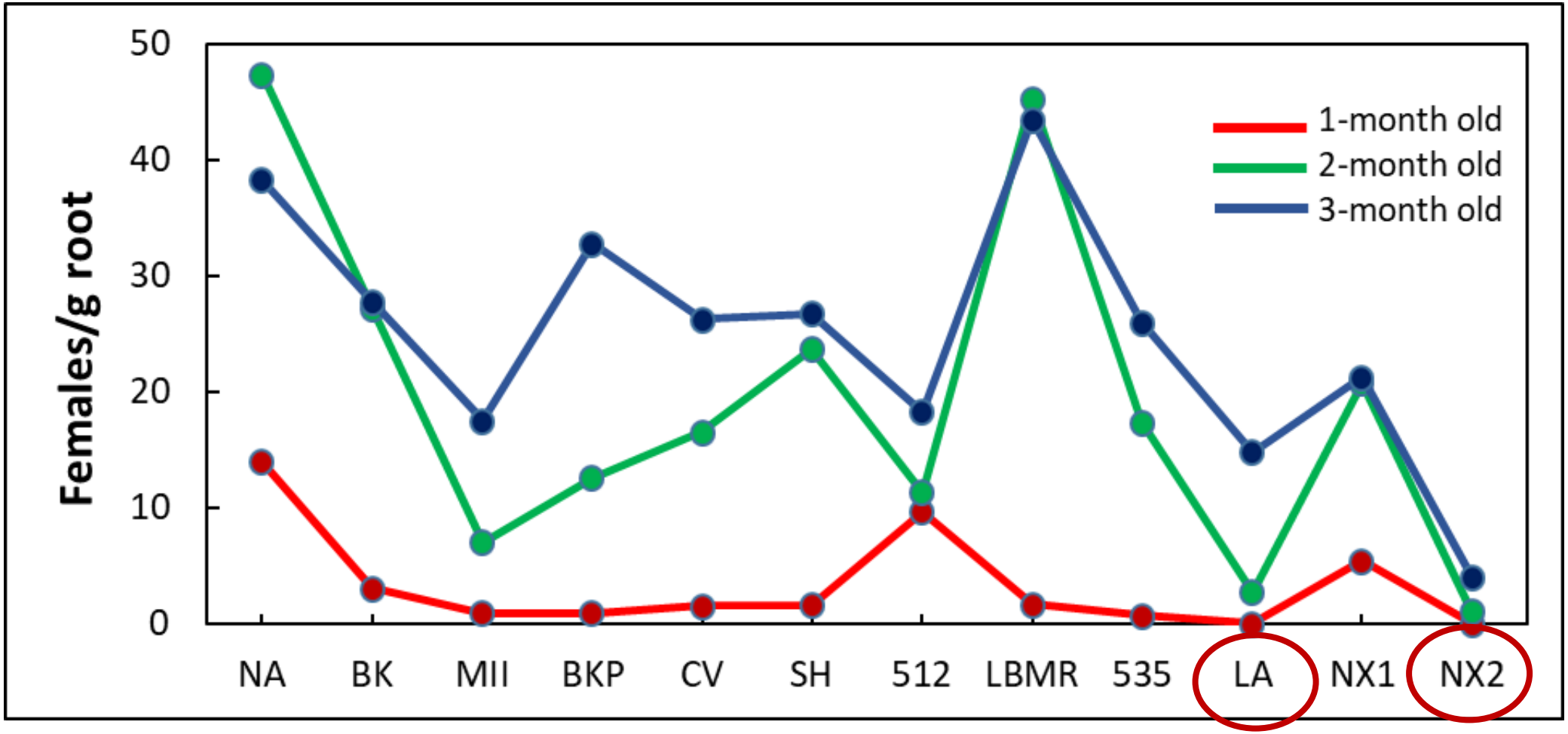




Leaf tissues release HCN (nematicidal) upon hydrolysis of dhurrin (= Biofumigation).

Busk and Moller, 2002

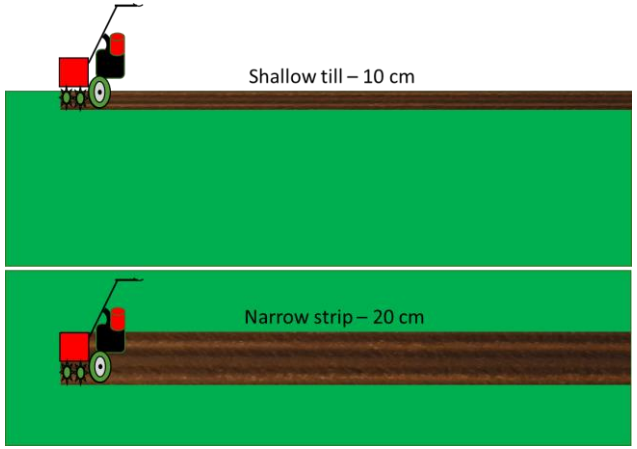
Allelopathic Effects of SSgH against Root-knot nematodes is age dependent for most var except for NX2



Implication: Allelopathic effects of SSgH against RKN decreased as the plant aged, but biomass production was 4 times higher at 2 months vs 1 month after planting.

SSgH Cover Crop in a Strip-till system

SSgH in a no-till system did not improve water infiltration or soil organic matter at the end of an eggplant crop. Thus, we test SSgH in a low till system.

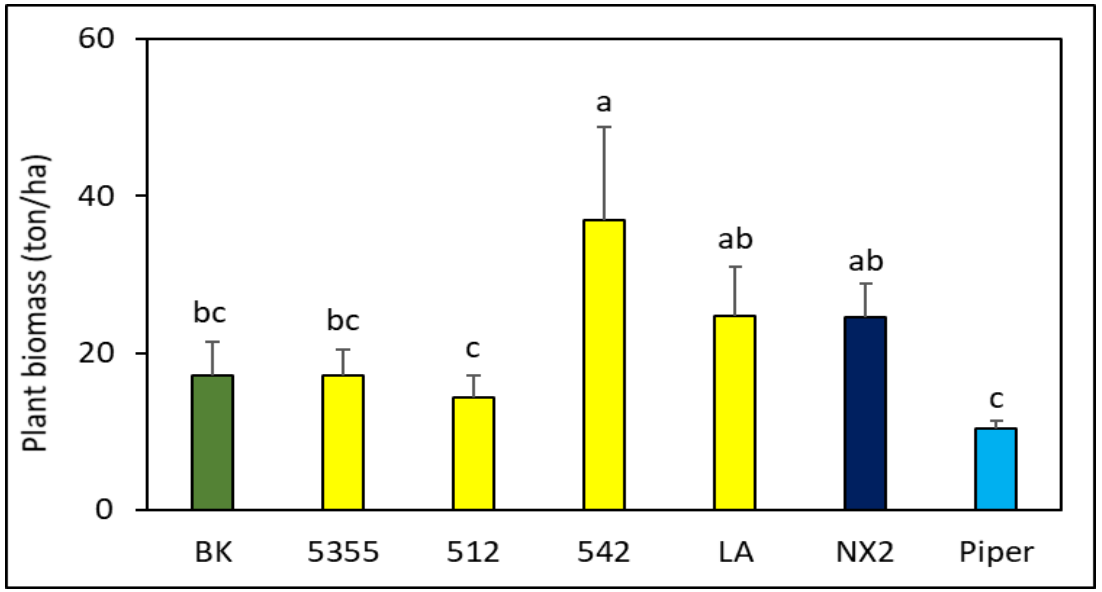


Field Trial at Poamoho Station

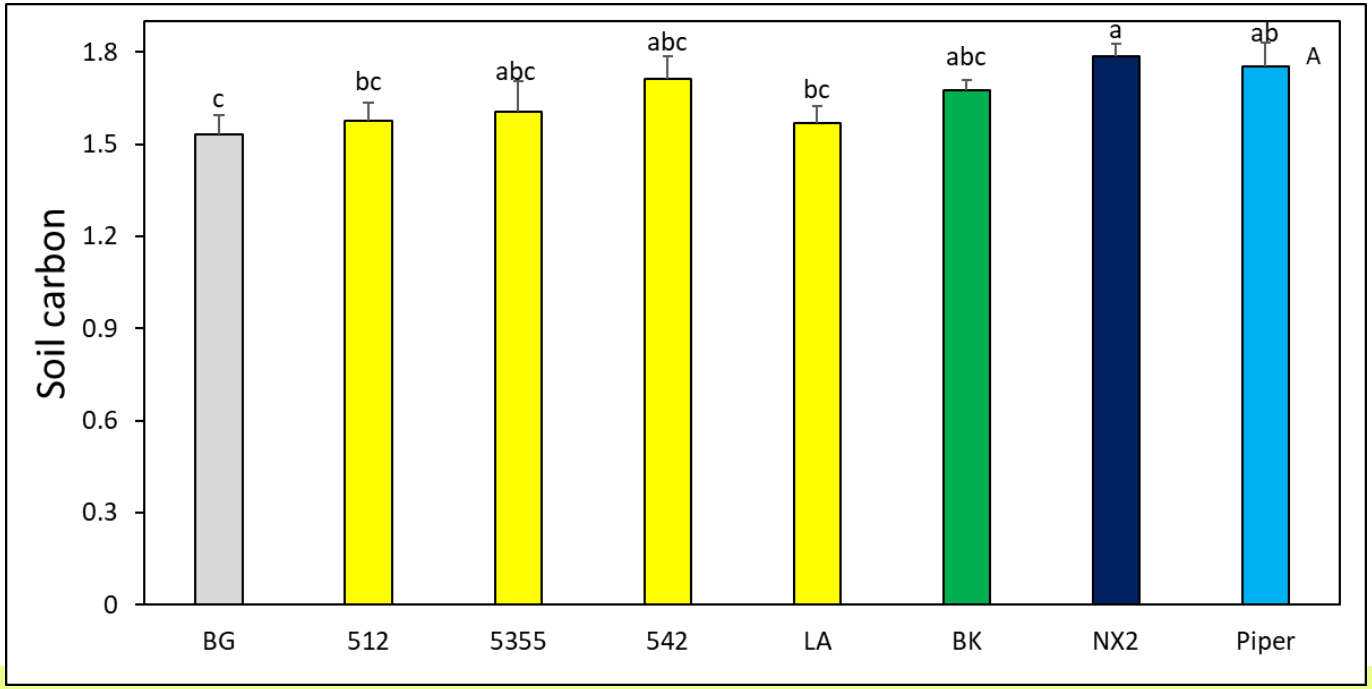
- Treatments – 7 SSgH varieties and one bare ground (BG) control.
- Terminated with a flail mower at 2.5 months.
- Strip till of 20-cm wide and 10-cm deep strip for all SSgH plots. Till BG.
- Planted eggplant for 6 months.

SSgH's Soil Building Abilities

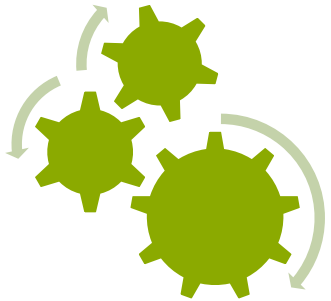
SSgH biomass in 2.5 months

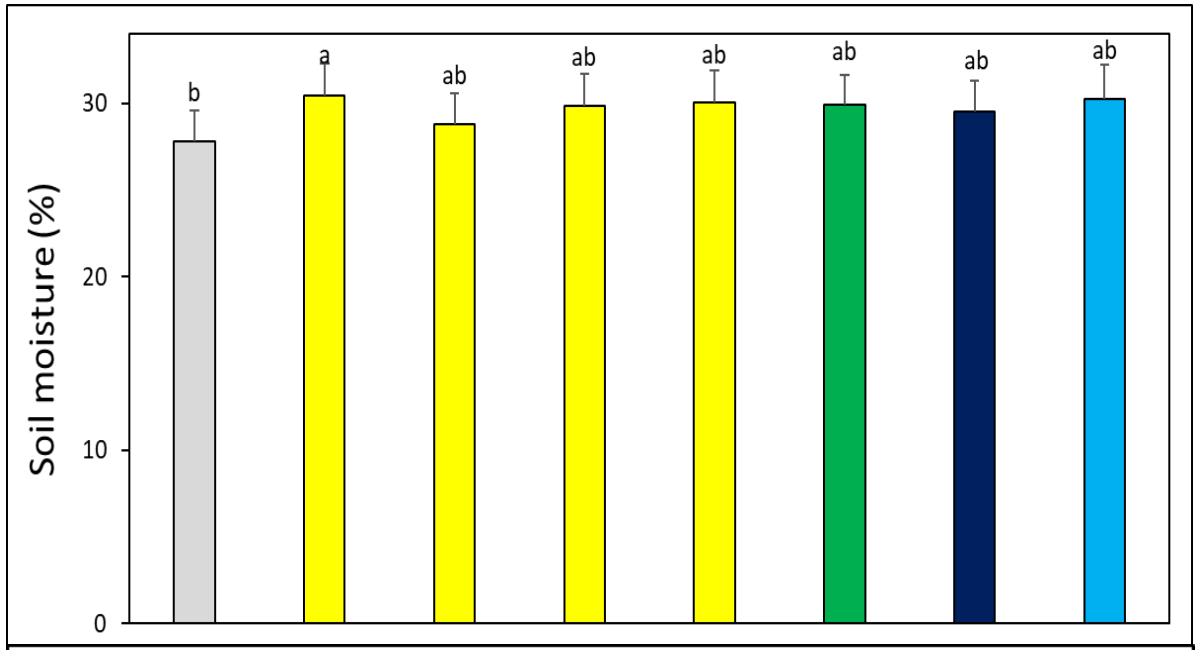


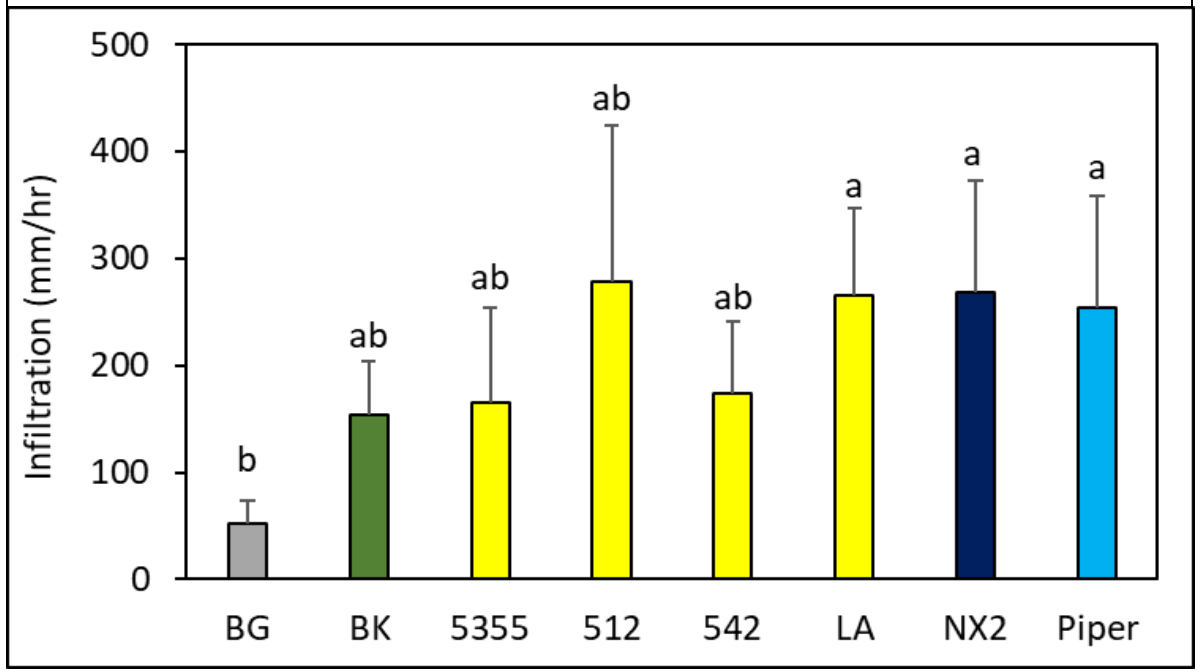
NX2 and Piper increased soil C throughout the SSgH-eggplant cropping cycle.

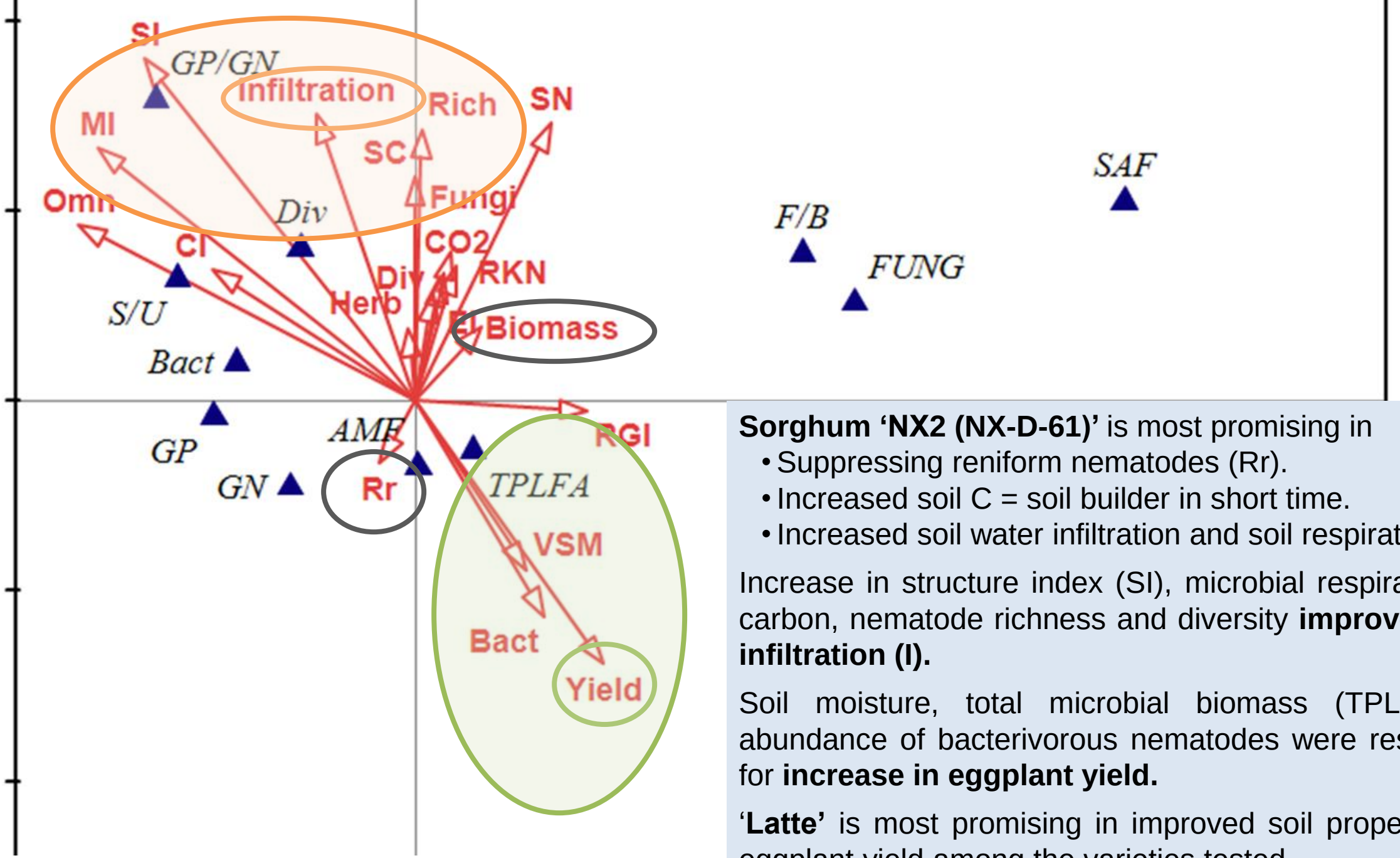


SSgH's Water Conservation Abilities









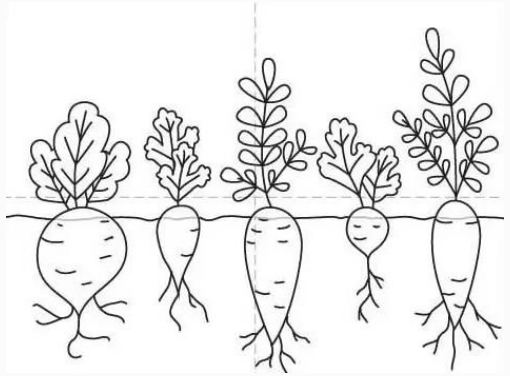
Sorghum 'NX2 (NX-D-61)' is most promising in

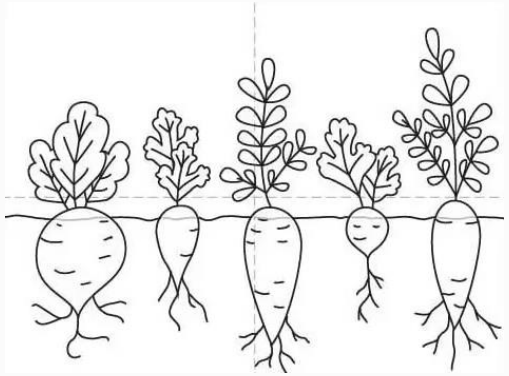
- Suppressing reniform nematodes (Rr).
- Increased soil C = soil builder in short time.
- Increased soil water infiltration and soil respiration.

Increase in structure index (SI), microbial respiration, soil carbon, nematode richness and diversity **improved water infiltration (I).**

Soil moisture, total microbial biomass (TPLFA) and abundance of bacterivorous nematodes were responsible for **increase in eggplant yield.**

'**Latte'** is most promising in improved soil properties and eggplant yield among the varieties tested.





- Biofumigation with Brassica cover crops required MTBP procedures to be more effective against plant-parasitic nematodes and Fusarium pathogens.

- Biofumigation with sorghum might also required MTBP procedure, but since it generated great amount of biomass, strip-till cover cropping might be more efficient to improve soil health while suppressing soil-borne diseases.

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University of Hawaii at Manoa

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Phillip Waisen, Roshan Paudel, Lauren Braley, Justin Mew, J. Silva, J. Uyeda.

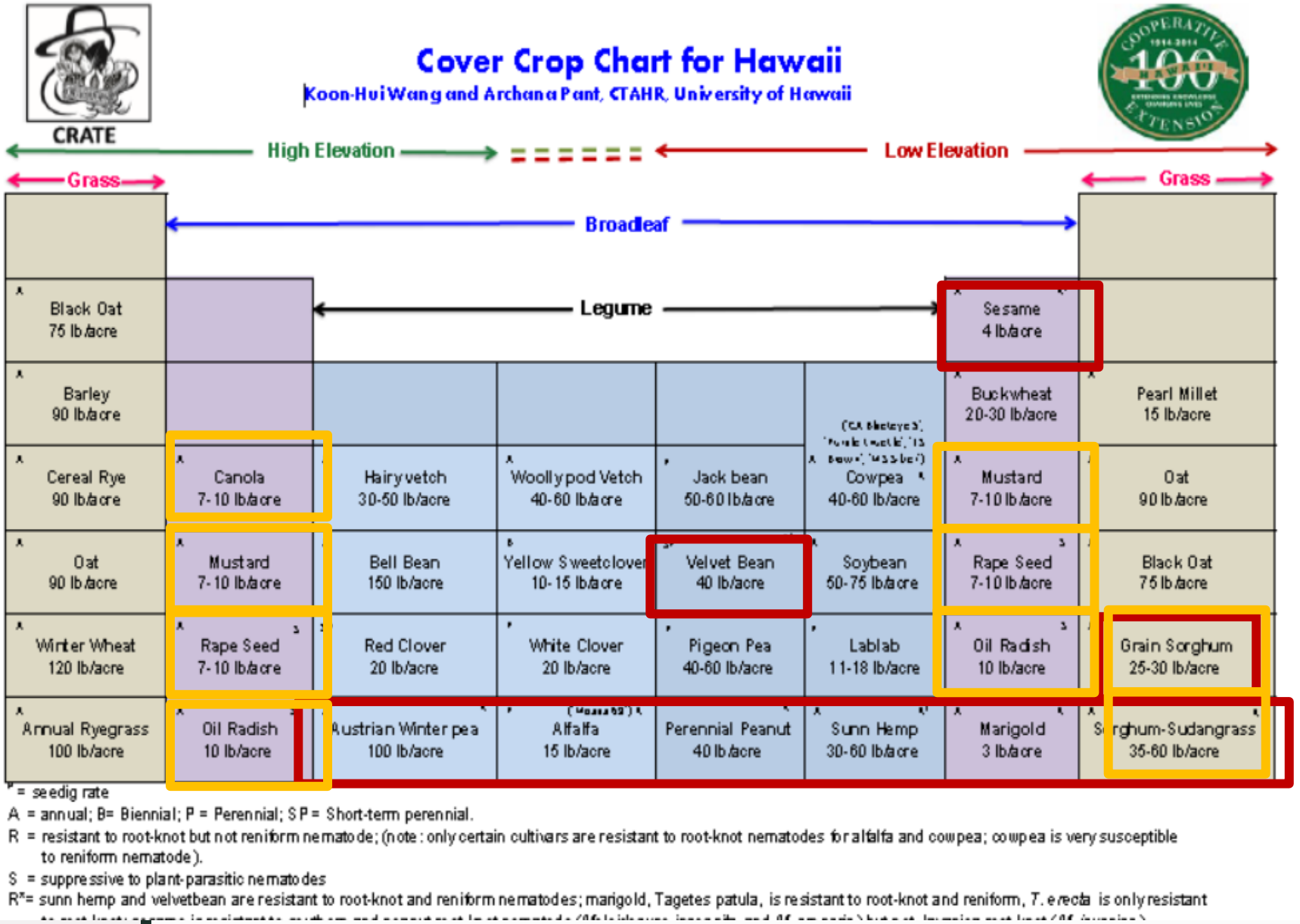
Poamoho Station

Kahuku Farm
Owen Kaneshiro Farm
Twin Bridge Farms

https://cms.ctahr.hawaii.edu/wangkh/

Cover Crop Selection

R= resistant to root-knot



Cover Crop Chart for Hawaii

Koon-Hui Wang and Archana Pant, CTAHR, University of Hawaii

CRATE

High Elevation ← → Low Elevation

Grass	Broadleaf	Legume	Broadleaf	Grass
Black Oat 75 lb/acre			Sesame 4 lb/acre	
Barley 90 lb/acre			Buckwheat 20-30 lb/acre	Pearl Millet 15 lb/acre
Cereal Rye 90 lb/acre	Canola 7-10 lb/acre	Hairy vetch 30-50 lb/acre; Woolly pod Vetch 40-60 lb/acre; Jack bean 50-60 lb/acre; Cowpea 40-60 lb/acre	Mustard 7-10 lb/acre	Oat 90 lb/acre
Oat 90 lb/acre	Mustard 7-10 lb/acre	Bell Bean 150 lb/acre; Yellow Sweetclover 10-15 lb/acre; Velvet Bean 40 lb/acre; Soybean 50-75 lb/acre	Rape Seed 7-10 lb/acre	Black Oat 75 lb/acre
Winter Wheat 120 lb/acre	Rape Seed 7-10 lb/acre	Red Clover 20 lb/acre; White Clover 20 lb/acre; Pigeon Pea 40-60 lb/acre; Lablab 11-18 lb/acre	Oil Radish 10 lb/acre	Grain Sorghum 25-30 lb/acre
Annual Ryegrass 100 lb/acre	Oil Radish 10 lb/acre	Austrian Winter pea 100 lb/acre; Alfalfa 15 lb/acre; Perennial Peanut 40 lb/acre; Sunn Hemp 30-60 lb/acre	Marigold 3 lb/acre	Sorghum-Sudangrass 35-60 lb/acre

= seedig rate

A = annual; B= Biennial; P = Perennial; SP = Short-term perennial.

R = resistant to root-knot but not reniform nematode; (note: only certain cultivars are resistant to root-knot nematodes for alfalfa and cowpea; cowpea is very susceptible to reniform nematode).

S = suppressive to plant-parasitic nematodes

R*= sunn hemp and velvetbean are resistant to root-knot and reniform nematodes; marigold, Tagetes patula, is resistant to root-knot and reniform, T. erecta is only resistant

https://gms.ctahr.hawaii.edu/gs/handler/getmedia.ashx?moid=3982&dt=3&g=12

Cover Crop Prescription

	Sorghum /SSgH	Marigold	Sunn hemp	Velvet bean	Brown mustard	Sunflower	Alfalfa	Cowpea
Root-knot and reniform resistant	Allelo-pathic	Allelo-pathic	Allelo-pathic	Allelo-pathic	Allelo-pathic	No Allelo-pathic, Resistant ?	No Allelo-pathic, R to root-knot	No Allelo-pathic, susceptible to reniform
High salinity	Tolerant	Moderately tolerant	x	x	x	Tolerant	Tolerant	X when young, Tolerant when old
Day length sensitive	yes	yes	yes	yes	no	yes	no	no