Detecting Nematode-trapping Fungi in a No-till Mixed Cover Cropping System

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Introduction

Nematode-trapping fungi (NTF) are a category of natural enemies of nematodes that form trapping structures to capture nematodes in the soil. This group of fungi can be found in nearly all soils, but usually present in low abundance in agricultural soil that has been frequently tilled. We are proposing to augment indigenous NTF in field soil through conservation tillage and cover cropping. This is known as conservation biological control. Mounting evidence suggests that NTF are responsive to organic amendments and their nematode prey densities. However, a previous soil dilution plating method failed to detect NTF effectively. The objective of this experiment was to develop a more efficient NTF assay method. We hypothesized that NTF is more abundant in the rhizosphere than in the soil further from the roots. Thus, our approach is to quantify NTF in the rhizosphere to evaluate the enhancement of NTF by cover crop conservation tillage.

Materials and Methods

A field experiment was established in 2012 at the Poamoho Experimental Station. Field plots were planted with either 1) sunn hemp (SH, *Crotalaria juncea*), 2) oats (O, *Avena sativa*), 3) sunn hemp and oats (SHO), or 4) left fallow with weeds as control (Control). All plots were then no-till planted with eggplant 3 months later. The same cover crop was then inter-planted with the eggplant crop again, and then periodically clipped to add as organic surface mulch under the eggplant canopy (Fig. 1). The experiment was in a randomized complete block design with 4 replications. Soil samples were collected at 2-month intervals from each plot over a 10-month eggplant cropping period. Soil samples were subjected to cowpea bioassay.

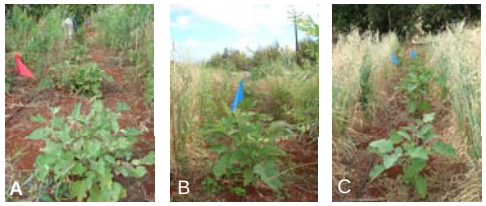

Fig 1. Eggplant planted into A) sunn hemp (SH); B) sunn hemp + oat (SHO); and C) oat (O) plots in a no-till system.

Cowpea bioassay: Due to lack of eggplant roots to sample initially, rhizosphere NTF assay was performed on a cowpea seed planted into each soil sample (Fig. 2). At 10 weeks after cowpea planting, roots were taken out of the soil and agitated off.

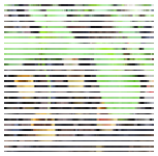

Fig 2. Cowpea bioassay

Subsamples of the roots were diluted into 0.05, 0.005, 0.0005 dilution. 100µl of each dilution were plated into quarter strength corn meal agar (CMA/4) petri dish (Fig. 3). Each dilution was repeated 5 times.

Eggplant root assay: At the end of the experiment, eggplant roots were taken from 8 plants in each experimental plot and assayed for NTF using serial dilution plating method as described above.

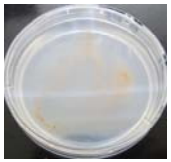

Fig 3. Eggplant roots

Quantification of NTF: At 3 weeks after plating, CMA/4 plates were observed under an inverted microscope (Fig. 4). NTF were identified using Cooke & Godfrey's key (1964). Numbers of positive plates were counted and colony forming units (cfu) of NTF were estimated by Most Probable Number program (Woomer, et al., 1990).

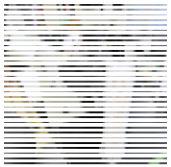

Fig 4. Soil dilution plating

Results and Discussion

- Three NTF species most commonly found in this field were *Arthrobotrys dactyloides* that formed nematode constricting rings, *Arthrobotrys oligospora* and *Dactylaria eudermata* that formed 3-dimension adhesive nets (Fig. 5).
- NTF that form constricting rings are parasitic NTF (pNTF). They form traps spontaneously, and are efficient "nematode trappers" (Jasson, 1982).
- NTF that form 3-dimensional nets are saprophytic NTF (sNTF). Under low nematode population densities, sNTF remain saprophytic (feeds on organic matter), thus inefficient at trapping nematodes.
- Ideally, we want to enhance both sNTF and pNTF concurrently.

Fig 5. Three of the most commonly found NTF A) *Arthrobotrys dactyloides*; B) *A. oligospor*a, and C) *Dactylaria eudermata.*

Results and Discussion (continue)

Table 1. Analysis of variance of pNTF (*Arthrobotrys dactyloides*), sNTF, and total NTF throughout five sampling dates from cowpea bioassay and eggplant assay.

	pNTF		sNTF		NTF	
Factors	df	*P*	df	*P*	df	*P*
rep	3	NS	3	*	3	@
Date	3	**	3	**	3	**
Cover crop (CC)	3	NS	3	@	3	@
Assay	1	*	1	NS	1	NS
CC × Assay	3	NS	3	NS	3	NS
Date × CC × Assay	9	NS	9	NS	9	NS

Table 2. Contrast analysis of colony forming units of pNTF, sNTF, and total NTF.

	pNTF	sNTF	NTF
SH	0.9975[z]	4.807	5.8045
SHO	3.2075	3.6295	6.837
O	1.8685	2.3945	4.263
BG	0.645	2.477	3.122
	Contrast	analysis	
SH vs no SH	NS	*	*
O vs no O	NS	NS	NS
CC vs no CC	NS	NS	NS

[z] Means are average of __ observations.
[y] CC = cover crop

@, *, **, indicate significantly different at $P \leq 0.10$, 0.05, and 0.01, respectively.

- ANOVA (Table 1) indicated that cfu of NTF changes over time. Recovery of NTF from eggplant root assay in the last two sampling dates was higher ($P < 0.05$) than that in the earlier cowpea bioassay (data not shown).
- However, no significant interaction occurred between CC x Assay, or sampling x CC x Assay. Thus, data were combined for contrast analysis (Table 2).
- Contrast analysis indicated that NTF especially sNTF were more abundant in sunn hemp plots than those without sunn hemp (oat and BG).

Conclusion

- This result revealed that cowpea bioassay or eggplant root assay provided efficient assay methods to detect differences in NTF abundance among cover crop treatments. Previously, soil dilution method failed to detect this difference in the same field site (Wang et al., unpublished).
- Successful detection of NTF using root assays also suggested that NTF was abundant in the root zone.
- Planting of sunn hemp in no-till system enhanced NTF better than no-sunn hemp treatments.

Acknowledgements

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