

## RESEARCH/INVESTIGACIÓN

### POTENTIAL OF BIOFUMIGANT COVER CROPS AS OPEN-END TRAP CROPS AGAINST ROOT-KNOT AND RENIFORM NEMATODES

P. Waisen\*, B. S. Sipes, and K.-H. Wang

Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822, USA; \*Corresponding author: pwaisen@hawaii.edu

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#### ABSTRACT

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Many Brassica cover crops have the capability to perform biofumigation against plant-parasitic nematodes, but some species or cultivars are highly susceptible to the nematodes, posing an important challenge for nematode management. Current research aims to exploit the ability of nematode-susceptible Brassicas as trap crops by terminating them before targeted nematodes undergo multiple life cycles; we refer to such trap crops as “open-end trap crops”. *Meloidogyne incognita*, *M. javanica*, and *Rotylenchulus reniformis* were targeted in this research. Brown mustard cv. Caliente 199 (*Brassica juncea*) and oil radish cv. Sodbuster (*Raphanus sativus*) have been selected based on greenhouse pot experiments where brown mustard was a good host of *M. incognita* and *R. reniformis*, whereas oil radish was a poor host of *M. incognita* though susceptible to *R. reniformis*. In a separate greenhouse trial, *M. incognita* required 283 degree-days (DD) to reach egg-laying females (ELF) on both brown mustard and oil radish, but only required 266 DD on tomato. *Rotylenchulus reniformis* required 333 DD on both brown mustard and oil radish but only required 291 DD on cowpea to reach ELF. To validate greenhouse experiment results, oil radish was grown and terminated at various ages in a field trial. Regardless of the age, oil radish did not suppress soil populations of *Meloidogyne* spp. and *R. reniformis* but root-gall index on pumpkin (*Cucurbita moschata*) was reduced in 14 or 28 days after planting compared to no-trap-crop control. Additionally, three field trials were conducted to determine the open-end trap crop potentials of brown mustard and oil radish against the target nematodes. Brown mustard suppressed soil populations of *Meloidogyne* spp. in two trials and *R. reniformis* in one trial, but oil radish did not suppress the target nematodes in all trials. These results suggested that the trap crop effect may not only depend on brown mustard or oil radish susceptibility but also depends on the specific nematode DD. This is the first demonstration of the advantage of growing nematode-susceptible cover crops with biofumigation properties against plant-parasitic nematodes.

*Key words:* Biofumigation, brown mustard, degree-days, management, oil radish, trap crop

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#### RESUMEN

Waisen, P., B. S. Sipes, and K.-H. Wang. 2019. Potencial de cultivos de cobertura biofumigantes como cultivos trampa de extremo abierto contra nematodos formadores de agallas y reniformes. *Nematropica* 49:254-264.

Muchos cultivos de cobertura de Brassicas tienen la capacidad de realizar biofumigación contra nematodos parásitos de plantas, pero algunas especies o cultivares son altamente susceptibles a los nematodos, lo que representa un desafío importante para el manejo de los nematodos. La investigación actual tiene como objetivo explorar la capacidad de las Brassicas susceptibles a los nematodos como cultivos trampa al terminarlas antes de que los nematodos específicos se sometan a múltiples ciclos de vida. Nos referimos a estos cultivos trampa como "cultivos trampa abiertos de extremo". *Meloidogyne incognita*, *M. javanica*, y *Rotylenchulus reniformis* fueron objeto de esta investigación. La mostaza marrón 'Caliente 199' (mostaza; *Brassica juncea*) y el rábano oleaginoso 'Sodbuster' (rábano; *Raphanus sativus*) se seleccionaron en base a experimentos en macetas de invernadero donde mostaza era un buen hospedante de *M. incognita* y *R. reniformis*, mientras que rábano era un pobre hospedante de *M. incognita* aunque susceptible a *R. reniformis*. En un ensayo de invernadero por separado, *M. incognita* requirió 283 grados-día (DD) para llegar a las hembras ponedoras de huevos (ELF) en mostaza y rábano, pero este proceso solo necesitó 266 DD en tomate. Por otro lado, *R. reniformis* requirió 333 DD en mostaza y rábano pero solo requirió 291 DD en caupí para alcanzar ELF. Para validar los resultados del experimento de invernadero, se cultivó rábano oleaginoso y se terminó a varias edades en un ensayo de campo. Independientemente de la edad, rábano no suprimió las poblaciones de *Meloidogyne* spp. y *R. reniformis*, pero el índice de agallamiento en una calabaza (*Cucurbita moschata*) se redujo en 14 o 28 días después de la siembra en comparación con el control de cultivo sin trampa. Además, se realizaron tres ensayos de campo para determinar los potenciales de cultivo de trampa de extremo abierto de mostaza y rábano contra los nematodos objetivo. mostaza suprimió las poblaciones de suelo de *Meloidogyne* spp. en dos ensayos y *R. reniformis* en un ensayo, pero rábano no suprimió los nematodos objetivo en todos los ensayos. Estos resultados sugirieron que el efecto del cultivo trampa puede no solo depender de la susceptibilidad de mostaza o rábano sino también del nematodo DD específico. Esta es la primera demostración de la ventaja de cultivar cultivos de cobertura susceptibles a los nematodos con propiedades de biofumigación contra los nematodos fitoparásitos.

*Palabras clave:* Biofumigación, cultivo grados-días, trampa mostaza marrón, manejo, rábano oleaginoso

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## INTRODUCTION

Root-knot (*Meloidogyne* spp.) and reniform (*Rotylenchulus reniformis*) nematodes are globally important plant-parasitic nematodes, ranking first and seventh, respectively, in terms of economic and scientific importance (Jones *et al.*, 2013). These nematodes are especially damaging to many crops in Hawaii (Sipes, 1994; Sipes and Wang, 2000; Waisen, 2015). As the use of conventional nematicides becomes more restricted, cover crops with allelopathic effects are being explored as environmentally sound nematode management alternatives (Wang *et al.*, 2002; Zasada *et al.*, 2009; Hooks *et al.*, 2010; Waisen, 2019). Brassica cover crops with high concentrations of secondary metabolites, glucosinolates, have been recommended for soil-borne agricultural pest management and is known as biofumigation (Kirkegaard *et al.*, 1993). Glucosinolates in *Brassica* spp. tissues upon tissue damage are degraded by endogenous enzymes, myrosinase, to release sulfur-containing bioactive

isothiocyanates, among other products involved in biofumigation (Matthiessen and Kirkegaard, 2006).

Members of Brassicaceae have gained popularity as cover crops because of their soil ecosystem services including nutrient scavenging (Kristensen and Thorup-Kristensen, 2004), biodrilling to improve soil tilth (Chen and Weil, 2011), water conservation, weed smothering, and provision of conducive habit for natural enemies of pests and pathogens to thrive (Clark, 2008). However, most *Brassica* spp. cover crops are susceptible to key plant-parasitic nematodes such as *Meloidogyne* spp. and *R. reniformis*, posing a notable management challenge (Edwards and Ploeg, 2014; Rudolph *et al.*, 2015; Fourie *et al.*, 2016). This research explored the potential of utilizing brown mustard (*Brassica juncea*) and oil radish (*Raphanus sativus*) as "open-end trap crops" to manage *Meloidogyne* spp. and *R. reniformis*, where the nematodes are allowed to penetrate and reproduce but are anticipated to be killed through subsequent trap crop termination or biofumigation.

As opposed to dead-end trap crops where nematodes penetrate roots but do not initiate feeding sites nor support reproduction (Gardner and Caswell-Chen, 1994), open-end trap crops allow nematodes to penetrate roots, initiate feeding sites and reproduce, which is often perceived as risky for subsequent cash crops. Applications of open-end trap crops are common in entomological studies as conventional trap crops where targeted insect pests are attracted and subsequently killed with insecticides (Hokkanen, 1991; Hilje *et al.*, 2001). However, the use of an open-end trap crop strategy against plant-parasitic nematodes is negligible because of the risk of unwanted nematode reproduction and survival in the soil and treating nematodes on cover crops is considered costly. Thus, several reports have cautioned the use of oil radish, brown mustard, and white mustard in *Meloidogyne*-infested fields because of their high susceptibility to nematodes (Ploeg, 2008; Zasada *et al.*, 2009).

This research aimed to demonstrate that growing a targeted nematode-susceptible biofumigant crop as a trap crop can reduce initial soil populations of the nematode before planting a cash crop. We anticipated that *Brassica* spp. would attract target nematodes into their roots, thereby reducing soil populations of the nematodes prior to termination or biofumigation, a scenario that would otherwise be impossible had a poor or non-host cover crop been grown. Those that penetrate roots can be trapped at the time of termination or be killed by subsequent biofumigation. To ensure the efficacy of "open-end trap crop", determining when to terminate the trap crop is critical. Thus, this study compared heat units required by *Meloidogyne* spp. and *R. reniformis* to reach egg-laying females (ELF) on oil radish and brown mustard as compared to their standard susceptible hosts and determined when trap crops would reduce nematode infection rates in field settings.

Specific objectives of this project were to: 1) examine susceptibility of brown mustard cv. Caliente 199 and oil radish cv. Sodbuster to *M. incognita* or *R. reniformis*, 2) determine degree-days (DD) required by *M. incognita* and *R. reniformis* to reach ELF on brown mustard or oil radish, and 3) determine open-end trap crop potentials of brown mustard and oil radish against *Meloidogyne* spp. and *R. reniformis* in the field. The working hypotheses of this study were: 1) a biofumigant cover crop susceptible to a targeted

nematode would serve as a good open-end trap crop against the nematode, and 2) an open-end trap crop approach would work against a targeted nematode if the biofumigant cover crop was terminated before the nematode accumulated heat units required to complete a life cycle.

## MATERIALS AND METHODS

### *Nematode inocula*

*Meloidogyne incognita* and *R. reniformis* were sourced from pure cultures maintained on tomato (*Solanum lycopersicum*) cv. Orange Pixie and cowpea (*Vigna unguiculata*) cv. Iron Clay, respectively, in Magoon Research and Teaching Facility at the University of Hawaii, Honolulu, HI. Nematode eggs were extracted from roots using a 0.6% sodium hypochlorite (NaOCl) solution (Hussey, 1973), followed by centrifugal sugar flotation (Jenkins, 1964). Eggs were then hatched in Baermann trays at 24°C for 14 days before use (McSorley and Frederick, 1991). A concentration of 100 infective juveniles per milliliter of water was prepared prior to inoculation.

### *Susceptibility experiments*

Two greenhouse experiments were conducted at the Magoon Research and Teaching Facility. Brown mustard cv. Caliente 199 from Siegers Seed Company (Holland, MI) and oil radish cv. Sodbuster from Petcher Seeds (Fruitdale, AL) were seeded at 2 seeds per 200-ml Ray Leach Cone-tainers™ (Stuewe and Sons Inc., Tangent, OR) filled with sterile sand-soil mix at 1:1 ratio (v/v). Seedlings were thinned to one plant per Cone-tainer prior to inoculation. Tomato was included as *M. incognita*-susceptible control. Two-week-old seedlings were inoculated with 300 second-stage infective juveniles (J2) of *M. incognita* delivered in 3 ml of water per Cone-tainer in three 2.5-cm-deep holes surrounding each seedling. The experiment was arranged in a completely randomized design (CRD) with four replications and terminated 28 days after inoculation (DAI). At the time of termination, each potted plant was gently emptied into a sampling bag, and roots were separated. Nematodes from the soil were extracted by elutriation (Byrd *et al.*, 1976) and centrifugal flotation (Jenkins, 1964). Root systems were agitated in NaOCl to extract eggs in the same

manner as described above.

A similar experiment was conducted to examine susceptibility of brown mustard cv. Caliente 199 and oil radish cv. Sodbuster to *R. reniformis* in the greenhouse. Cowpea cv. Iron Clay was included as *R. reniformis*-susceptible control. All seedlings were inoculated at 2 wk after planting with 100 fourth-stage infective juveniles (J4) of *R. reniformis*. The experiment was arranged in CRD with four replications and terminated 28 DAI. At the time of termination, nematode eggs from entire roots or vermiform stages from the soil were extracted as described above.

#### *Nematode degree-days experiments*

Greenhouse experiments were conducted to determine DD required by *M. incognita* to reach ELF on brown mustard cv. Caliente 199 and oil radish cv. Sodbuster. Tomato cv. Orange Pixie was included as *M. incognita*-susceptible control. Brown mustard, oil radish, and tomato were seeded individually in 200-ml Ray Leach Cone-tainers™ filled with sterile sand-soil mix at 1:1 ratio (v/v). Each seedling was inoculated with 100 J2 of *M. incognita*, and the experiment was arranged in CRD with three replications of each test crop per sampling time. Seven days after inoculation, three destructive plant samples were collected every 3 days over 21 days; thus, 21 plants (i.e., 3 plant samples × 7 destructive sampling times) were planted for each test crop. At each sampling time, the whole root systems were stained and destained with acid fuchsin and acidified glycerin, respectively, according to Byrd *et al.* (1983). The number of ELF in the roots were counted at 40× magnification under a dissecting microscope (Leica Microsystems Company, Wetzlar, Germany). The experiment was terminated at the first detection of ELF, and the experiment was conducted twice.

A similar DD experiment was conducted but using *R. reniformis* where brown mustard and oil radish was inoculated with 100 J4 of *R. reniformis* per Cone-tainer. Cowpea cv. Iron Clay was included as *R. reniformis*-susceptible control. The experiment was arranged in CRD with three replications. Three destructive plant samples were collected at 3-day intervals, and roots were subjected to staining and destaining procedures in the same way as described above (Byrd *et al.*, 1983). Destained roots were examined under the

dissecting microscope at 40× magnification for ELF detection, and this experiment was conducted twice.

In each of these nematode DD trials, a WatchDog® Temperature Data Logger (Spectrum Technologies Inc., Aurora, IL) was buried 5 cm deep in a representative Cone-tainer to record soil temperatures hourly throughout the experiment. Data from the temperature loggers were downloaded, and nematode DD were calculated for each experiment using the formula,  $DD = \sum(T_e - T_b)/24 \text{ hr}$ , where DD=degree-days,  $T_e$ =hourly soil environmental temperature, and  $T_b$  is base temperature of development (9.8°C for *M. incognita* or 10°C for *R. reniformis*) (Fraisse *et al.*, 2011).

#### *Trap crop age experiment*

To determine the best age for oil radish to perform as a trap crop, a field trial was conducted at Poamoho Experiment Station (21°32'14.8"N and 158°5'20.3"W) in Waialua, HI. The soil type at the test site is a Wahiawa silty clay in the Oxisol order with Tropptic Eutruxox, clayey, kaolinitic, isohyperthermic properties, containing 18.6% sand, 37.7% silt, and 43.7% clay in the top 25 cm of soil. Soil organic matter was approximately 1%, with a pH of 6.5 (Ikawa *et al.*, 1985). The test site had mixed populations of *M. incognita*, *M. javanica*, and *R. reniformis*. Oil radish cv. Sodbuster was seeded in 1.2 × 5.5 m<sup>2</sup> plots at 22.4 kg seed/ha. Oil radish trap crop age treatments included 0, 14, 28, 42, or 56 days after planting (DAP). The experiment was arranged in a randomized complete block design (RCBD) with four replications. The treatment plots were irrigated using a sprinkler irrigation system. At the time of trap crop termination, aboveground biomass was estimated from 3 random 0.09-m<sup>2</sup> quadrants per plot and oven-dried at 105°C for 72 hr. Six soil cores from the top 10-cm soil depth were collected per plot and composited into a sampling bag immediately before trap crop termination. The soil was passed through a 4-mm<sup>2</sup> mesh sieve, and 250 cm<sup>3</sup> of the soil was subsampled for nematode extraction by elutriation (Byrd *et al.*, 1976) and centrifugal flotation (Jenkins, 1964). *Rotylenchulus reniformis* or *Meloidogyne* spp. (*M. incognita* and *M. javanica*) were enumerated using an inverted microscope (Leica Microsystems Company). Oil radish foliage

was soil incorporated to a 10-cm soil depth using a hand-held rototiller (American Honda Motor Co., Alpharetta, GA). One week after soil incorporation, pumpkin cv. Field Trip (*Cucurbita moschata*) was seeded at a 1-m spacing between seeding holes with six seeds per plot and irrigated using drip irrigation. Four months later, five pumpkin plants per plot were uprooted and rated for root-gall index (RGI) based on a 0-12 scale (Netscher and Sikora, 1990), where 0=healthy root system and 12=dead and spongy root system due to heavy galling.

#### Trap crop experiment

Two field trials using Oil radish cv. Sodbuster and three field trials using brown mustard cv. Caliente 199 were conducted at the Poamoho Experiment Station at Waialua, HI, to determine their trap crop potentials against *Meloidogyne* spp. and *R. reniformis*. Brown mustard or oil radish was seeded in  $1.2 \times 5.5$  m<sup>2</sup> plots at 22.4 kg seed/ha and compared to no-trap-crop bare ground control. The experiments were arranged in a RCBD with four replications. All treatment plots were irrigated using a sprinkler irrigation system. Six soil cores from the top 10-cm soil depth were collected per plot and composited into a sampling bag. The soil was passed through a 4-mm<sup>2</sup> mesh sieve, and a 250 cm<sup>3</sup> of the soil was subsampled for nematode extraction by elutriation (Byrd *et al.*, 1976) and centrifugal flotation (Jenkins, 1964). *Meloidogyne* spp. and *R. reniformis* were enumerated using an inverted microscope.

#### Statistical analysis

Data from the 'Susceptibility experiment', 'Trap crop age experiment', and 'Trap crop experiment' were checked for normal distribution using Proc Univariate in SAS version 9.4 (SAS Institute Inc., Cary, NC). Skewed data were logarithmically transformed by  $\log_{10}(x + 1)$  prior to a one-way analysis of variance in SAS. Means were separated using a Waller-Duncan *k*-ratio ( $k=100$ ) *t*-test, and only true means are presented. Data from the 'Nematode degree-days experiment' was based on the first detection of ELF recorded; thus, no statistical analysis was performed.

## RESULTS

#### Susceptibility experiments

Fecundity rates (eggs/g dry root) of *M. incognita* on brown mustard were not different from tomato ( $P > 0.05$ ), but fecundity was reduced ( $P \leq 0.05$ ) on oil radish compared to that on tomato (Fig. 1A). *Meloidogyne incognita* soil densities were lower ( $P \leq 0.05$ ) in both oil radish and brown mustard than tomato (Fig. 1B). Conversely, fecundity of *R. reniformis* on brown mustard was higher ( $P \leq 0.05$ ) than on cowpea but was not different ( $P > 0.05$ ) between oil radish and cowpea (Fig. 1C). No statistical difference was detected in *R. reniformis* soil population densities among the three crops tested (Fig. 1D).

#### Nematode degree-days experiments

When brown mustard, oil radish, and tomato were inoculated with J2 of *M. incognita*, the nematode required 283 DD to develop on both brown mustard and oil radish but only required 266 DD to reach ELF on tomato (Table 1). On the other hand, J4 of *R. reniformis* required 333 DD to reach ELF on both brown mustard and oil radish but only required 291 DD on cowpea (Table 1).

#### Trap crop age experiment

Aboveground biomass of oil radish was highest at 56 DAP followed by 42 and 28 DAP, which were all significantly higher ( $P \leq 0.05$ ) than terminating the cover crop 14 DAP or not planting the cover crop (Table 2). Although the heat units accumulated during these field trials varied from 0 to 834 DD among the two targeted nematodes (Table 2), regardless of the trap crop age, oil radish did not suppress ( $P > 0.05$ ) soil population densities of *Meloidogyne* spp. and *R. reniformis* compared to the no-trap-crop control (Table 2). However, *Meloidogyne* spp.-induced RGI on pumpkin was significantly reduced ( $P \leq 0.05$ ) 14 or 28 DAP compared to no-trap-crop control (Table 2).

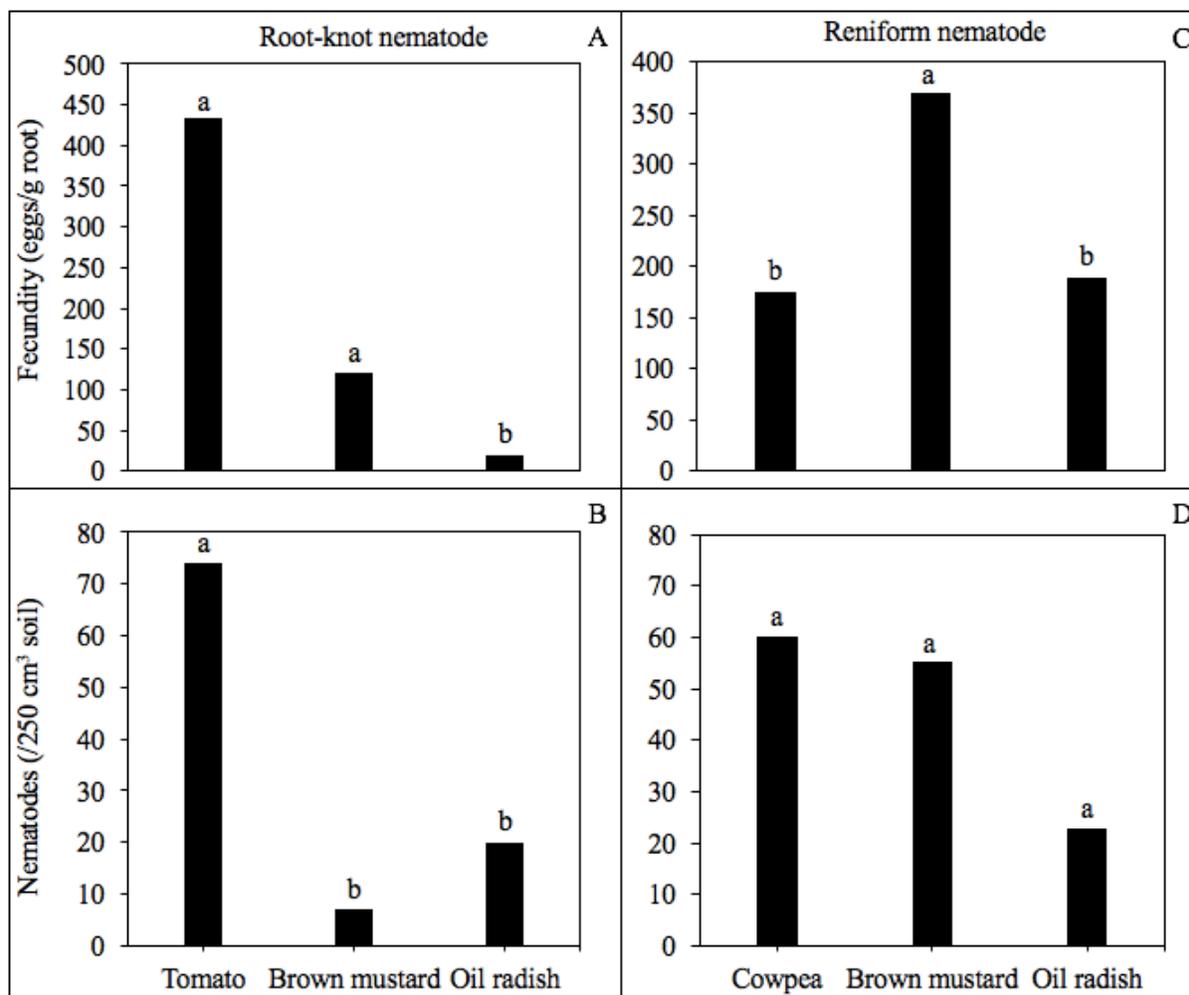


Figure 1. Fecundity (A and C) and vermiform soil populations (B and D) of *Meloidogyne incognita* or *Rotylenchulus reniformis* on brown mustard and oil radish in comparison to tomato or cowpea in greenhouse experiments. Means ( $n=4$ ) followed by the same letter(s) are not different based on Waller-Duncan  $k$ -ratio ( $k=100$ )  $t$ -test.

### Trap crop experiments

Soil populations of *Meloidogyne* spp. and *R. reniformis* were not suppressed ( $P > 0.05$ ) in any of the oil radish trap crop trials compared to no-trap crop control (Table 3). Oil radish reduced *Meloidogyne* spp. and *R. reniformis* numbers numerically when oil radish was terminated before reaching 465 and 512 DD, which are the heat units required by *M. incognita* and *R. reniformis* to reach ELF, respectively (Table 3). As a result, densities of *Meloidogyne* spp. were reduced by 46% in Trial II (Table 3). However, when oil radish was terminated beyond the DD to reach ELF of both

nematodes, no nematode suppression was detected in Trial I (Table 3).

Brown mustard reduced soil populations of *Meloidogyne* spp. in all three field trials regardless of crop termination time or heat units accumulated (420-689 DD) compared to no-trap crop control (Table 4). The effect was especially significant for *Meloidogyne* spp. in Trial I and Trial II ( $P \leq 0.05$ ). Although it was not statistically different in Trial III, in all three trials, the numbers of *Meloidogyne* spp. were reduced by  $\geq 50\%$  compared to the no-trap crop control. Brown mustard did not suppress *R. reniformis* in Trial I and II ( $P > 0.05$ ) and only

Table 1. Degree-days (DD) accumulated by infective juveniles of root-knot (Rk; *Meloidogyne incognita*) and reniform (Reni; *Rotylenchulus reniformis*) nematodes to reach egg-laying female (ELF).

Crop species	Cultivar	DAI (DD)	
		Rk <sup>y</sup>	Reni
Brown mustard	Caliente 199	24.2 (283) <sup>z</sup>	-
Oil radish	Sodbuster	24.2 (283)	29.0 (333) <sup>z</sup>
Tomato	Orange Pixie	22.7 (266)	29.0 (333)
Cowpea	Iron Clay	-	25.3 (291)

<sup>y</sup>Single-celled Rk and Reni eggs required 181.72 and 178.92 DD to hatch or 464.71 and 511.92 DD to reach ELF, respectively, at 22.8°C.

<sup>z</sup>Values outside the parenthesis are calendar days after inoculation (DAI), and those inside are DD required by infective juveniles of *M. incognita* (base temperature=9.8°C) or *R. reniformis* (base temperature=10°C) to reach ELF.

Table 2. Effects of oil radish trap crop age, on biomass production soil densities of root-knot (Rk; *Meloidogyne* spp.) and reniform (Reni; *Rotylenchulus reniformis*) nematodes, and root-gall index (RGI) on pumpkin in a field trial.

Age (DAP) <sup>x</sup>	Biomass (t/ha) <sup>y</sup>	DD Rk	DD Reni	Rk/250 cm <sup>3</sup> soil	Reni/250 cm <sup>3</sup> soil	RGI (0-12)
0	0.00 c <sup>z</sup>	0	0	0 a	43 a	9.5 a
14	0.30 c	232	229	0 a	10 a	7.7 b
28	2.78 b	417	411	0 a	45 a	7.0 b
42	3.55 b	652	643	0 a	45 a	7.8 ab
56	5.13 a	834	823	13 a	33 a	7.8 ab

<sup>x</sup>DAP=days after planting; DD=degree-days; RGI values were ranked on a 0-12 scale, where 0-12, where 0=healthy root system and 12=dead and spongy root system due to heavy galling (Netscher and Sikora, 1990).

<sup>y</sup>Amendment rate was based on dry oil radish biomass; Single-celled Rk and Reni eggs required 181.72 and 178.92 DD to hatch or 464.71 and 511.92 DD to reach ELF, respectively at 22.8°C.

<sup>z</sup>Means (n=4) in a column followed by the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) t-test.

Table 3. Trap crop effect of oil radish against root-knot (Rk; *Meloidogyne* spp.) and reniform (Reni; *Rotylenchulus reniformis*) nematodes in field trials.

Parameters	Trial I (6/16)		Trial II (12/16)	
	Rk <sup>y</sup>	Reni	Rk	Reni
DD	652 <sup>w</sup>	643	420	413
Biomass (t/ha) <sup>x</sup>	3.55		1.79	
	Nematodes/250 cm <sup>3</sup> soil			
Bare ground	0 a <sup>y</sup>	43 a	450 a	418 a
Oil radish	0 a	45 a	243 a	386 a
%Δ <sup>z</sup>	0	+4.7	-46.0	-7.6

<sup>y</sup>Single-celled Rk and Reni eggs required 181.72 and 178.92 degree-days (DD) to hatch or 464.71 and 511.92 DD to reach ELF, respectively, at 22.8°C.

<sup>w</sup>Values are DD accumulated by each nematode on oil radish at 42 and 35 days after planting in Trial I and II, respectively.

<sup>x</sup>Amendment rate was based on dry oil radish biomass.

<sup>y</sup>Means (n=4) in a column followed by the same letters are not different based on Student's t-test.

<sup>z</sup>Percent reduction by oil radish trap crop relative to bare ground control.

by 11.8% compared to the control (Table 4).

## DISCUSSION

Based on the susceptibility experiments, 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 observations were in line with previous findings that brown mustard cv. Caliente 199 was a good host of *M. incognita* (Monfort *et al.*, 2007; Edwards and Ploeg, 2014), whereas most oil radish cultivars were poor hosts of *Meloidogyne* spp. (Edwards and Ploeg, 2014). This greenhouse experiment also revealed that both brown mustard and oil radish could reduce soil population densities of *M. incognita*, indicative of a trap crop effect against *M. incognita*. However, both brown mustard and oil radish did not reduce soil population densities of *R. reniformis* compared to cowpea within 28 DAI. A longer time may be needed to trap *R. reniformis* effectively. Based on this greenhouse experiment, brown mustard cv. Caliente 199 would be a better “open-end trap crop” than oil radish cv. Sodbuster as it was susceptible to *M. incognita* and *R. reniformis* as their standard hosts. However, this did not fully accept the hypothesis that growing susceptible hosts would trap *M. incognita* and *R. reniformis* effectively from the soil. Brown mustard and oil radish being good hosts to *R. reniformis* did not

reduce *R. reniformis* in the soil, and oil radish being a poor host of *M. incognita* did reduce *M. incognita* in the soil.

Regardless of brown mustard and oil radish, J2 of *M. incognita* or J4 of *R. reniformis* required 283 or 333 DD (equivalent to 24.2 or 29.0 calendar days in the greenhouse used in this experiment), respectively, to reach ELF. Both of which were longer than on their respective standard hosts. To extrapolate this heat unit to field conditions, two more weeks of heat units accumulated under 22.8°C was added from eggs to infective juvenile stages used for our inoculum. Thus, *M. incognita* or *R. reniformis* would be estimated to require 465 or 512 DD, respectively, to reach ELF. These DD provided a comparison to the heat units accumulated during the field trials conducted at Poamoho Experiment Station.

Data generated from the first field trial conducted at Poamoho Experiment Station partially accepted the hypothesis that terminating oil radish cover crop before reaching DD to reach ELF reduced *Meloidogyne* spp. infection on the subsequent cash crop. This was reflected in lower RGI on pumpkin when terminating oil radish at a shorter period of growth (i.e., 14 or 28 DAP) than later stage (56 DAP). However, this did not suppress soil population densities of *R. reniformis*.

Both oil radish trap crop field trials suggested that regardless of its host status to the nematodes (poor host to *M. incognita* and good host to *R.*

Table 4. Trap crop effect of brown mustard against root-knot (Rk; *Meloidogyne incognita*) and reniform (Reni; *Rotylenchulus reniformis*).

Parameters	Trial I (12/16)		Trial II (8/17)		Trial III (1/18)	
	Rk <sup>w</sup>	Reni	Rk	Reni	Rk	Reni
DD	420 <sup>x</sup>	413	689	685	573	563
Biomass (t/ha)	1.22		2.08		2.86	
	Nematodes/250 cm <sup>3</sup> soil					
Bare ground	450 a <sup>y</sup>	418 a	50 a	523 a	68 a	195 a
Brown mustard	179 b	436 a	25 b	323 a	20 a	172 b
% Δ <sup>z</sup>	-60.2	+4.3	-50.0	-38.2	-70.5	-11.8

<sup>w</sup>Single-celled Rk and Reni eggs required 182 and 179 degree-days (DD) to hatch or 464.71 and 511.92 DD to reach egg-laying female, respectively, at 22.8°C.

<sup>x</sup>Values are DD accumulated by *Meloidogyne incognita* (base=9.8°C) and *Rotylenchulus reniformis* (base=10°C) in the field at termination of brown mustard at 35, 42, and 49 days after planting in Trials I, II and III, respectively.

<sup>y</sup>Means (n=4) in a column followed by the same letter(s) are not different based on Waller-Duncan *k*-ratio (*k*=100) t-test.

<sup>z</sup>Percent reduction by brown mustard trap crop relative to bare ground control.

*reniformis*), if oil radish was terminated prior to the targeted nematodes reaching ELF, nematode suppression could be achieved though at a low level (< 50%). However, oil radish as a trap crop overall did not suppress both *Meloidogyne* spp. and *R. reniformis* compared to the control. Thus, the first hypothesis that using a nematode susceptible host as an effective trap crop was only partially accepted (i.e., oil radish being a good host for *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. This finding is in line with the recommendation that oil radish could be used as a trap crop against *M. hapla* if terminated before the nematode completes a life cycle (Melakeberhan *et al.*, 2008).

Findings from the brown mustard experiment supported the first hypothesis that as a good host of *Meloidogyne* spp., brown mustard would serve as a good trap crop to reduce the soil population densities of the nematode. This hypothesis worked in our study regardless of the DD accumulated at least up to 689 DD. However, although brown mustard is 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. Besides, suppression of *R. reniformis* by brown mustard was not supportive of the second hypothesis as it did not suppress *R. reniformis* when it was terminated within one life cycle of the nematode (or less than 511 DD), but it suppressed the nematodes significantly when accumulated 563 DD (beyond one life cycle of *R. reniformis*) in Trial III. These data also suggest that DD might not be the sole factor to determine the termination date of the 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.

The variable response between these two biofumigant cover crops as trap crops could be due to their phenological variations in their root architecture. Oil radish produces a more prominent and deeper root system that reaches 1.8 m deep after 2 months of growth (Clark, 2008). This would provide the advantage of reaching more nematodes in deeper soil layers than brown mustard. Overall, brown mustard growth was slower and smaller than oil radish as reflected in its average biomass

generated in the field trials. Discrepancies in termination age, DD accumulation, targeted nematodes, and host status can affect the performance of open-end trap crop, suggesting that multiple factors should be taken into consideration to make trap crops effective against plant-parasitic nematodes. Since both brown mustard and oil radish have biofumigation properties, as long as the majority of the targeted nematodes are in their vulnerable stage, the nematodes would be prone to biofumigation treatment. These observations had been suggested in multiple review articles (Edwards and Ploeg, 2014; Fourie *et al.*, 2016). In general, the rule of thumb of the “open-end trap crop” is selecting a nematode susceptible cover crop as trap crop while terminating the trap crop before the targeted nematode reaches its ELF stage. However, this approach is complicated by a cohort of multiple stages of the targeted nematodes in the field, distribution of the nematodes in the soil profile, as well as how big the root system can grow in the field. It is amenable to allow *Meloidogyne* spp. and *R. reniformis* to go slightly beyond their first ELF stage (life cycle), which creates an opportunity for survival stages (egg or anhydrobiotic stages) to exit their dormancy (Pontif and McGawley, 2008). This is because biofumigation is more effective against vulnerable J2 than the egg stage of *M. chitwoodi* (Mojtahedi *et al.*, 1993; Ploeg, 2008) or *M. incognita* (Zasada *et al.*, 2009). In this project, we tested the trap crop effect within two life cycles of *Meloidogyne* spp. and *R. reniformis* ( $\leq 7$  wk).

In conclusion, brown mustard cv. Caliente 199 was a susceptible host to *M. incognita* and *R. reniformis* and therefore would be more effective as an open-end trap crop against these nematodes compared to oil radish cv. Sodbuster. Oil radish did not reduce population densities of both of these target nematodes in the field. However, terminating oil radish closer to DD to reach the first ELF could increase a plant-parasitic nematode problem (oil radish Trial II). However, if oil radish was planted beyond the DD for *M. incognita* and *R. reniformis* to reach ELF, then the cover crop could increase plant-parasitic nematode population densities. Brown mustard effectively served as an open-end trap crop against *Meloidogyne* spp. in all three field trials with  $\geq 50\%$  reduction, and if terminated close to 563 DD, it reduced *R. reniformis* population densities compared to the no-trap-crop control. Through a better

understanding of host plant status and the edaphic factor affecting the development of plant-parasitic nematodes, one can make better decisions when using biofumigant cover crops as open-end trap crops against nematodes.

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