EFFECTS OF *CROTALARIA JUNCEA* ON THE ANHYDROBIOTIC STATE OF *ROTYLENCHULUS RENIFORMIS*

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ABSTRACT

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Although *Crotalaria juncea* is known to produce allelopathic compounds against many plant-parasitic nematodes, it is unclear if this allelopathic effect suppresses the anhydrobiotic state of reniform nematode, *Rotylenchulus reniformis*. Two greenhouse experiments were conducted where soil from a fallow pineapple field with a history of reniform nematode infestation was preconditioned by 1) keeping the soil dry (DRY), 2) irrigating (IRR), 3) growing sunn hemp (SH), or 4) growing cowpea (CP) for 3 mo. The conditioning mimicked four different schemes of field conditions that stimulate different reniform nematode survival states. DRY was expected to encourage a non-active state, whereas IRR, SH and CP were expected to encourage active states. At the end of conditioning, soils were either incorporated or not incorporated with sunn hemp foliage. Cowpeas were planted for bioassay. At termination of conditioning, DRY resulted in higher numbers of non-active (anhydrobiotic) nematodes than IRR, SH, and CP ($P < 0.05$). At termination of the cowpea bioassay, soil incorporation of SH suppressed numbers of reniform nematodes in soils and in bioassay cowpea roots when soil was conditioned with CP, but not in DRY conditioning. Soil incorporation of SH also reduced numbers of reniform nematodes in bioassay cowpea roots when the soil was conditioned with SH. Thus, soil incorporation of SH did not effectively suppress reniform nematodes in their anhydrobiotic state.

Key words: allelopathic, reniform nematode, sunn hemp, survival stage.

RESUMEN

Marahatta, S. P., K.-H. Wang, B. S. Sipes, and C. R. R. Hooks. 2012. Efectos de *Crotalaria juncea* sobre el estado anhidrobiótico de *Rotylenchulus reniformis*. Nematropica 42:34-40.

Aunque se conoce que *Crotalaria juncea* produce compuestos alelopáticos contra muchos nematodos fitoparásitos, no es claro si este efecto alelopático afecta al estado anhidrobiótico del nematodo reniforme, *Rotylenchulus reniformis*. Se llevaron a cabo dos experimentos de invernadero en donde se utilizó suelo de un campo de piña infestado con nematodo reniforme y se precondicionó el suelo en una de las siguientes maneras: 1) manteniendo el suelo seco (DRY), 2) irrigando (IRR), 3) cultivando crotalaria (SH), ó 4) cultivando caupí (CP) por tres meses. El acondicionamiento simula cuatro condiciones de campo que estimulan diferentes estados de supervivencia en el nematodo reniforme. Esperábamos que el suelo seco causara estados inactivos, mientras que los irrigados y sembrados con crotalaria y caupí causaran estados activos. Al final del condicionamiento, se separó el suelo en dos tratamientos, uno con y otro sin incorporación del follaje de crotalaria. Se utilizó el cultivo de caupí para bioensayo. El condicionamiento de suelo seco resultó en la mayor cantidad de estado inactivos (anhidrobióticos) del nematodo ($P < 0.05$). Al final del bioensayo con caupí, la incorporación del follaje de crotalaria causó supresión de las densidades del nematode en los suelos acondicionados con caupí (CP), pero no en los secos (DRY). También se observó supresión en los bioensayos con caupí en los suelos acondicionados con crotalaria. Por tanto, concluimos que la incorporación de cortalaria no afecta efectivamanete a los estados anhidrobióticos del nematodo reniforme.

Palabras clave: alelopático, crotalaria, nematodo reniforme, estado de supervivencia.

INTRODUCTION

Reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is a major pest on many crops in Hawaii including pineapple, *Ananas comosus* (Caswell *et al.*, 1991; Robinson *et al.*, 1998; Wang *et al.*, 2001; 2002a; Wang and Hooks, 2009). Pineapple producers in Hawaii typically deep plow their fields and allow them to remain fallow for 3 to 12 mo prior to planting a new crop (Rohrbach and Schmitt, 2003). Keeping the field fallow without a susceptible host for plant-parasitic nematodes is one nematode management approach (Viaene *et al.*, 2006). However, dry fallow conditions often cause *R. reniformis* to enter an anhydrobiotic state (Gresham, 1992; Sehgal and Gaur, 1989; Tsai and Apt, 1979). Anhydrobiosis is the ability of organisms to survive extreme dehydration (Giard, 1894; McSorley, 2003). Specifically, anhydrobiotic nematodes can survive a variety of extreme environmental conditions, including dry fallow soils without a host and plant debris (McSorley, 2003). Thus, their ability to enter an anhydrobiotic state makes managing *R. reniformis* in agricultural fields a more challenging task (Womersley and Ching, 1989).

Sunn hemp, *Crotalaria juncea* L., is a tropical cover crop known for its ability to effectively suppress *R. reniformis* (Caswell *et al.*, 1991; Marla *et al.*, 2008; Wang *et al.*, 2001). Sunn hemp serves as a poor host for *R. reniformis* and releases an allelopathic compound toxic to this nematode when incorporated into the soil (Marla *et al.*, 2008; Wang *et al.* 2001; 2002b). Monocrotaline, an allelopathic compound found in *Crotalaria* spp., is toxic to many plant-parasitic nematodes (Crout, 1968; Jourand *et al.*, 2004; Rich and Rahi, 1995; Rodriguez-Kabana *et al.*, 1992; Wang *et al.*, 2001; 2002a). When sunn hemp was planted as a cover crop in rotation with pineapple, it suppressed *R. reniformis* populations compared to fallow with weeds prior to pineapple planting (Caswell *et al.*, 1991) and 6 mo after pineapple planting (Wang *et al.*, 2002b).

Objectives of this research were to compare the effects of sunn hemp amendment on *R. reniformis* under various soil conditions that either stimulate or suppress the anhydrobiotic state of *R. reniformis*. The working hypothesis is that sunn hemp will suppress *R. reniformis* more effectively in their active than their anhydrobiotic state.

MATERIALS AND METHODS

Two greenhouse experiments were conducted at the University of Hawaii Magoon Greenhouse Facility (Trial I) and Gilmore Greenhouse Facility (Trial II) in 2010. Field soil was collected from pineapple fields with a history of *R. reniformis* infestation. The soil collected was Wahiawa silty clay (Wahiawa series; clayey, kaolinitic, isohyperthermic, Tropeptic, Eutrustox; Oxisol) with a pH of approximately 7.

Trial I

Soil was collected from Field Wailua 5 in Dole Plantation, Haleiwa, Oahu, HI on 4 February 2010. The field had been fallow with minimal weed growth for 8 mo. after pineapple residues were deep plowed into the soil. Initial population density of *R. reniformis* in vermiform stages was 200/250 cm^3 soil. Soil was well mix prior to potting into 15-cm diam clay pots. Each pot was filled with approximately 1,000 cm^3 (= 986 g) field soil. A total of 40 pots were prepared. Pots were either 1) kept in a dry condition where no irrigation was provided (DRY), 2) irrigated daily (IRR), 3) planted with 8-10 seeds of 'Tropic Sun' sunn hemp (SH), or 4) planted with 'SCL 825' cowpea, *Vigna unguiculata* (L.) Walp. (Peaceful Valley Farm and Garden Supply, Grass Valley, CA) (CP) with 2 plants per pot. Ten pots were prepared for each conditioning. All pots except those in DRY treatment were irrigated with 100 ml water/pot every day and weeded periodically. This soil conditioning continued for 10 wk and was terminated on 30 April 2010. Cowpea and sunn hemp plants were cut at the soil level at the end of the experiment. At termination of the conditioning, 100 cm^3 soil was subsampled from each pot. The remaining soil was then transfered to 10-cm diam clay pots.

Five days after termination of the conditioning, soil in 5 pots from each conditioning were amended with dry sunn hemp foliage powder at 1% (w/w) (SH+), whereas the other 5 pots from the same conditioning treatment received no amendment (SH-). Dry sunn hemp foliage powder used for soil amendment was prepared from field-grown sunn hemp plants, oven-dried to a constant weight, and ground with a commercial blender (Winsted Conn, Waring Products Co., CT) to powder form. The experiment was a 4 × 2 (conditioning × sunn hemp amendment) factorial experiment with 5 replications arranged in randomized complete block design. At 1-wk after sunn hemp incorporation, three cowpea seeds were sown per pot as a bioassay for viability of *R. reniformis*. After germination, cowpea seedlings were thinned to 2 seedlings per pot. The trial was terminated 3-wk after cowpea was planted for bioassay.

Trial II

Soils used in Trial II were collected from the same field as Trial I except that in Trial II, soil had been fallow for 12 mo. *Rotylenchulus reniformis* infestation level was 10 vermiform life stages/250 cm^3 soil. Soils were conditioned as described in Trial I for 10 wk. Soil was then amended or not amended with dry sunn hemp foliage powder at 1% (w/w) the following day. Cowpea seeds were sown 1 wk later. At 3 wk after cowpea planting, cowpea roots and soil were processed for nematode extraction.

Soil nematode assay

Soil were extracted for nematodes at the beginning (250 cm^3 soil), termination of the conditioning (100 cm^3 soil), and termination of the cowpea bioassay (250 cm^3 soil) by elutriation (Byrd *et al.*, 1976) followed by centrifugal flotation (Jenkins, 1964). Extracted *R. reniformis* were counted at 100× magnification level using an inverted microscope (Fluovert, Leitz Wetzlar, Germany). Nematodes were categorized into active vermiform and non-active coiled states. Nematodes that moved or responded to a probe were categorized as active. Coiled nematodes were assumed to be in an anhydrobiotic state.

Root nematode assay: At termination of the bioassay, 0.3 g cowpea roots/pot were subsampled and stained with acid fuchsin (Daykin and Hussey, 1985). *Rotylenchulus reniformis* inside the roots were categorized into infective, slightly swollen, swollen, and mature female stages as described by Wang *et al.* (2001).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) for soil nematodes at conditioning, and 4 × 2 factorial ANOVA for soil and root nematodes at bioassay using the general linear model (GLM) procedure in Statistical Analysis System (SAS Institute, Cary, NC). Nematode numbers were log-transformed [log (x + 1)] prior to ANOVA to normalize data. Untransformed arithmetic means of data are presented. Means for specific sampling times were separated by Waller-Duncan k-ratio (k = 100) t-test wherever appropriate. When significant interaction occurred between conditioning and SH amendment, nematode populations were compared between amendment treatments for each conditioning.

Table 1. Number of reniform nematodes active or coiled in soil at termination of four conditioning treatments: remaining dry (DRY), irrigated (IRR), planted with sunn hemp (SH), and planted with cowpea (CP).

	Trial I		Trial II	
Conditioning	Active	Coiled	Active	Coiled
	Nematodes/250 cm^3 soil			
DRY	40[z] c	45 b	3 b	20 a
SH	65 b	10 c	8 b	0 b
IRR	72 bc	32 bc	3 b	0 b
CP	3875 a	362 a	78 a	45 a

[z]Means are average of 10 replications. Means in a column followed by same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test based on log (x + 1) transformed values.

Table 2. Effects of sunn hemp amendment on number of active and coiled reniform nematodes in soil with different conditioning.

	Active			Coiled		
Conditioning	SH+	SH-	means	SH+	SH-	means
	Nematodes/250 cm^3 soil					
			Trial I			
DRY[y]	16[z]	2*	9 c	2	0	1 b
IRR	4	12	8 c	0	0	0 b
SH	6	34*	20 b	2	2	2 b
CP	1056	2604*	1830 a	18	38	28 a
Means (amd)	270	663		5	10	
			Trial II			
DRY	2	6	4 b	0	0	0 b
IRR	0	2	1 b	0	0	0 b
SH	0	4	2 b	0	0	0 b
CP	10	102**	61 a	0	6	3 a
Means (amd)	2	28**		0	2	

[z]Data are means of 5 replications. Means in a column under Trial I and II followed by same letter do not differ according to the Waller-Duncan *k*-ratio (*k* = 100) *t*-test based on log (x+1) transformed values. *, and ** represent significant difference between log (x+1) transformed means of SH+ and SH- amendments at $P < 0.05$ and $P < 0.01$, respectively.
[y]Pre-plant soil conditionings, dry (DRY), irrigation (IRR), sunn hemp (SH), and cowpea (CP) were tested with (SH+) or without (SH-) sunn hemp amendment (amd).

RESULTS

Effects of conditioning

Conditioning affected active and coiled nematodes consistently in both trials. Conditioning by planting CP resulted in the highest number of active *R. reniformis* in both trials ($P < 0.05$, Table 1). The DRY conditioning resulted in a

higher ($P < 0.05$) number of coiled nematodes than SH in Trial I and II, and IRR in Trial II. Planting of SH consistently maintained lower coiled *R. reniformis* than the DRY conditioning ($P < 0.05$).

Effects of sunn hemp amendment

Based on the 4 × 2 factorial ANOVA at termination of cowpea bioassay, conditioning affected active and coiled nematodes in both trials, whereas the SH amendment only affected active nematodes in Trial II ($P < 0.05$). However an interaction between SH amendment and soil conditioning occurred for active nematodes in Trial I ($P < 0.05$), and active and coiled nematodes in Trial II ($P < 0.1$). Planting of CP resulted in the highest ($P < 0.05$) *R. reniformis* population density regardless of active or coiled form than the other conditioning treatments (Table 2). Amending soil with sunn hemp reduced ($P < 0.05$) active *R. reniformis* in SH and CP conditioning treatments in Trial I, but only reduced ($P < 0.01$) nematode numbers in CP in Trial II. In contrast, amending soil with sunn hemp increased ($P < 0.05$) active *R. reniformis* when soil was conditioning in DRY condition in Trial I. Over all, amending soil with SH did not affect numbers of coiled *R. reniformis*.

Conditioning affected all developmental stages of *R. reniformis* inside the cowpea roots ($P < 0.05$) in Trial I (Table 3). In Trial II, conditioning affected ($P < 0.05$) all stages except for the slightly swollen stage. Amendment of SH only affected vermiform stage in cowpea roots in Trial I ($P < 0.01$) but affected all stages in Trial II ($P < 0.01$). Interaction between conditioning and sunn hemp amendment occurred on vermiform and slightly swollen stages in Trial I ($P < 0.01$) but only on vermiform and swollen stages in Trial II ($P < 0.05$, Table 3).

CP conditioning resulted in the highest number of *R. reniformis* inside the roots among treatments (Table 3). Planting of SH did not reduce ($P > 0.05$) number of any stages of *R. reniformis* in the cowpea roots as compared to DRY and IRR. However, SH amendment suppressed ($P < 0.01$) number of vermiform stages in cowpea roots in Trial I and all stages in Trial II. In particular, suppression of *R. reniformis* infection by SH amendment was most effective in soil previously planted with CP. Lower number of all stages of *R. reniformis* was observed on CP planted and SH amended cowpea roots in both trials except for the mature stage in Trial I ($P < 0.10$) (Table 3). However, SH amendment did not reduce the number of any stages of *R. reniformis* in DRY. In Trial II, SH amendment also suppressed ($P < 0.05$) the vermiform and swollen stages in the SH planted soil, and the swollen stage in IRR conditioned soil ($P < 0.01$, Table 3).

Cowpea growth

In Trial I, conditioning by SH and IRR resulted in higher cowpea shoot biomass than DRY ($P < 0.05$, Table 4). However, amendment of SH did not affect cowpea shoot biomass in Trial I (Table 4). In Trial II, conditioning did not affect ($P > 0.05$) cowpea shoot biomass but SH amendment did ($P < 0.05$, Table 4). Amendment of sunn hemp only enhanced ($P < 0.05$) cowpea shoot biomass in CP conditioning soil ($P < 0.05$) but not in other conditioning (data not presented).

DISCUSSION

The current study demonstrated that planting of SH and irrigation created a favorable environment for *R. reniformis* and thus keep the nematode active. In contrast, the unirrigated dry soil conditioning kept the nematode in an anhydrobiotic state. This result was consistent with the findings of many researchers who found that *R. reniformis* was able to enter into anhydrobiosis in dry soil (Baujard and Martiny, 1995; Sehgal and Gaur, 1989; Tsai, 1978; Tsai and Apt, 1979). Under extreme moisture loss, soil dwelling nematodes such as *R. reniformis* will coil their body (Perry, 1999) and survive desiccation (Torres *et al.*, 2006). Sunn hemp is a poor host of *R. reniformis* but still allows a limited number of the nematode to penetrate the sunn hemp root (Caswell *et al.*, 1991; Marla *et al.*, 2008; Wang *et al.*, 2001). Thus, fewer *R. reniformis* may have penetrated into sunn hemp roots and remained active instead of adopting a survival state. Planting sunn hemp minimizes the anhydrobiotic state of *R. reniformis*. Thus, planting of sunn hemp would keep more *R. reniformis* active and vulnerable to subsequent control treatments than would exposure to environmental conditions like DRY where nematodes enter into an anhydrobiotic state.

The number of coiled *R. reniformis* from planting CP was high probably because the total number of *R. reniformis* in CP was high. Among all 4 conditioning treatments, only DRY had > 50% of *R. reniformis* coiled whereas, the other conditionings had < 10% coiled nematodes.

In the current experiment, sunn hemp amendment suppressed *R. reniformis* when the soils were conditioned with CP, a *R. reniformis* susceptible host. Soil conditioned with cowpea was dominated by active stage nematodes at the end of the conditioning. Management strategies are more effective when plant-parasitic nematodes are in their active and vulnerable stage (Deliopoulos *et al.*, 2010). LaMondia (2008) also suggested controlling tobacco cyst nematode, *Globodera tabacum*, by destroying crop roots immediately after crop harvest, i.e., when the nematodes were still in their active state. Similarly, Ornat *et al.* (1999) suppressed population densities of peanut root-knot nematode, *M. arenaria*, and lesion nematode, *Pratylenchus neglectus*, by destroying host plant roots when nematodes were active. Current greenhouse experiment also showed that sunn hemp would suppress *R. reniformis* more effectively when the nematodes were in their vulnerable states.

Although SH amendment suppressed *R. reniformis*

Table 3. Effect of sunn hemp amendment on different stages of reniform nematodes in cowpea roots under different soil conditioning.

	Vermiform			Slightly swollen			Swollen			Mature			Total		
Conditioning	SH+	SH-	Means	SH+	SH-	Means	SH+	SH-	Means	SH+	SH-	Means	SH+	SH-	Means
	Nematodes per g of cowpea root														
							Trial I								
DRY[y]	3[z]	6	4 b	5	2	3 b	5	3	4 b	6	2	3 c	18	11	14 b
IRR	3	2	2 b	2	2	1 b	7	9	8 b	7	17	12 b	18	29	23 b
SH	0	6	3 b	4	3	3 ab	5	2	3 b	6	7	6 bc	15	16	15 b
CP	2	27**	14 a	3	24**	13 a	29	105**	67 a	123	271	197 a	156	426[@]	290 a
Means (amendment)	2	10**		3	7		11	29		35	73		51	120	
							Trial II								
DRY	1	5[@]	3 b	2	4	3 a	3	3	3 b	1	3	2 b	6	14	10 ab
IRR	3	4	3 b	2	8	5 a	0	4**	2 b	0	1	0 b	5	16	11 ab
SH	2	6*	4 ab	1	4	2 a	0	4*	2 b	0	0	0 b	3	12[@]	7 b
CP	1	32*	18 a	1	26[@]	15 a	1	31**	17 a	1	13[@]	7 a	4	101*	57 a
Means (amendment)	1	11**		1	10**		1	10**		0	4**		4	36**	

[z]Data are means of 5 replications. Conditioning means in a column for each trial followed by same letter do not differ according to Waller-Duncan *k*-ratio ($k = 100$) t-test based on log (x+1) transformed values. [@], *, and ** represent significant difference between SH+ and SH- amendments at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively based on log (x+1) transformed values.

[y]Pre-plant soil conditionings are dry (DRY), irrigation (IRR), sunn hemp (SH), and cowpea (CP); amendment treatements are with (SH+) or without (SH-) sunn hemp amendment.

Table 4. Shoot wt (g) of bioassay cowpea affected by four conditionings and two amendments.

Factors	Trial I	Trial II
Conditionings		
DRY[y]	12.23[z] b	8.00 a
IRR	16.06 a	10.12 a
SH	16.14 a	8.12 a
CP	13.70 ab	9.06 a
Sunn hemp amendment		
SH+	14.73 a	10.21a
SH-	14.33 a	7.61b

[z]Means for each conditioning and amendment are average of 10 and 20 replications, respectively. Means in a column followed by the same letter(s) under Trial I and II do not differ according to the Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

[y]Four pre-plant soil conditionings are dry (DRY), irrigation (IRR), sunn hemp (SH), and cowpea (CP); two amendment treatments are with (SH+) or without (SH-) sunn hemp amendment.

effectively in both SH and CP planted soil, it is more effective to suppress *R. reniformis* in SH planted soil as indicated by the lower number of *R. reniformis* in SH compared to CP planted soil at the end of the cowpea bioassay. However, planting of SH followed by amendment of SH did not result in lower numbers of *R. reniformis* than DRY followed by no SH amendment at the end of the cowpea bioassay. Although fallow is a good method of nematode management (Barker and Koenning, 1998), it also comes with a price of poorer plant growth as indicated in this cowpea bioassay. In this experiment, SH amendment only improved bioassay cowpea shoot weight in Trial II but not Trial I. This was possibly due to a difference in the population densities of *R. reniformis* between the two trials. When *R. reniformis* was suppressed, it allowed the bioassay cowpea plants to exhibit more vigorous growth. In Trial I, population densities of *R. reniformis* in soil planted with CP was too high despite SH amendment (>1,000/250 cm^3 soil), thus SH amendment did not result in improvement of shoot weight of the cowpea bioassay plants. Whereas, in Trial II, the number of *R. reniformis* on SH+ was low (10/250 cm^3 soil)

In conclusion, sunn hemp amendment suppressed active states of *R. reniformis* better than the survival states. Growing sunn hemp in *R. reniformis* infested soil maintained the nematodes in an active and vulnerable state, whereas a dry fallow kept *R. reniformis* in its survival state, making the nematode difficult to control. The poor host effect of sunn hemp against *R. reniformis* could be combined with the soil incorporation of sunn hemp for greater suppression of *R. reniformis*.

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