Influences of a radish and sweet potato continuous ridge use system on root-knot nematode damage to sweet potato

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The influences of continuous ridge use (CRU) on root-knot nematode (RKN) damage to sweet potato was investigated in a double cropping system that uses radish in winter and sweet potato in summer. The CRU system tended to show reduced RKN damage to sweet potato relative to the conventional system (TR system), in which the field was tilled and ridged before sweet potato cultivation. RKN population density after the radish harvest was low in the upper area of the ridge in both the CRU and the TR systems. In the TR system, density in the upper area of the ridge was slightly increased after tillage and ridging. Low density in the upper area of the ridge is suggested as one of the reasons for the decrease in damage to sweet potato in the CRU system. Adoption of the CRU system would be one method of reducing RKN damage to sweet potato. *Nematol. Res.* 44 (1), 1-8 (2014)

Key word: cultivation system, *Meloidogyne* spp., ridging, soil temperature, tillage

INTRODUCTION

In warm upland farming areas in Japan, the southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White), causes severe damage to many crops, particularly sweet potato, *Ipomoea batatas* L. The damage includes decreases in yield, formation of galls on fibrous roots, and degradation of the external appearance of tuberous roots (Lawrence *et al.*, 1986; Cervantes-Flores *et al.*, 2002; Tateishi *et al.*, 2007; Min *et al.*, 2011).

Reducing labor costs, conserving agricultural materials, and using soil nutrients efficiently are important considerations in farming. To decrease expenses for labor and mulch material, Kajimoto and Eto (1977) attempted the field cultivation of multiple crops in a time series with continuous ridge use (CRU). Recently, Niimi *et al.* (2011) showed the advantage of a double cropping system, using radish, *Raphanus sativus* L., in winter and sweet potato in summer with CRU. In the CRU system, competing farming practices on multiple crops in the spring are effectively avoided, and

crops can use soil nutrients efficiently.

In our preliminary experiment, root-knot nematode (RKN) damage to sweet potato decreased in the CRU system plots compared to single cropping plots of sweet potato. Suzuki *et al.* (2013) reported that radish cropping during the fall and winter cropping seasons had little influence on the nematode density before the succeeding sweet potato cropping and on nematode damage to sweet potato. Therefore, adoption of a CRU system, rather than radish cropping, should be more effective in reducing damage to sweet potato. In the radish and sweet potato double cropping system, the ridge used for radish cultivation is usually destroyed after the harvest to prepare for subsequent sweet potato cultivation, and the soil is disturbed by rotary tillage and ridging. In the CRU system, the soil in the ridge is not disturbed before sweet potato cultivation. This difference could affect RKN damage to sweet potato.

The objectives of this study were to confirm the influences of the CRU system on RKN damage to sweet potato in a set of field experiments. In addition, cultivation systems similar to the CRU system were also investigated to examine influences of CRU.

MATERIALS AND METHODS

Experimental site:

The fields for the experiments were located at the Miyakonojo Research Station, NARO Kyushu Okinawa Agricultural Research Center, Miyakonojo, Miyazaki,

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Japan. The soil type in the fields is Andosol, and the fields were naturally infested with RKN. Although RKN species were not identified in this study, in the central and southern region of Kyushu, including the experiment site, the predominant species in the sweet potato fields are *M. incognita* and *M. arenaria* (Neal) (Iwahori *et al.*, 2000).

CRU system, radish cultivation and sweet potato cultivation:

An illustration of the CRU system used in this study is presented in Fig. 1. The ridge was semicylindrical with an angular top and was mulched with polyethylene film. Height at the top of the ridge was 25 cm, and height at the shoulder was 20 cm; width between the shoulders was 65 cm, and width at the bottom was 80 cm.

Two experiments (experiments 1 and 2) were conducted in this study. Radish 'Syunpûta' was sown at 25 cm (experiment 1) or 20 cm (experiment 2) intervals in 2 rows on the shoulder of the ridge. The radish plants were appropriately covered with non-woven fabric with or without support for the winter. Weights of and RKN damage to storage roots and degree of galls on fine roots of radish were evaluated at harvesting; however,

Fig. 1. Illustration of the continuous ridge use system. Radish (left) and sweet potato (right) were cultivated using the same ridge continuously.

the harvest was favorable, and no galls were observed on fine roots in either of the 2 experiments (data not shown). After the harvest, the holes in the film left by the standing radish crop were closed, and vine cuttings of susceptible sweet potato cultivar 'Koganesengan' were planted at 30 cm intervals in a single row at the top of the ridge.

Basal fertilizer for radish was applied at rates of 15 g of N, 15 g of P_2O_5 , and 15 g of K_2O per square meter. Basal fertilizer for sweet potato was applied at rates of 5 g of N, 7 g of P_2O_5 , and 12 g of K_2O per square meter using the application methods described later for each experiment. Conventional weed and insect pest control methods were applied for radish and sweet potato. Rotary tillage and ridging were conducted using 22- to 25-kW tractors.

Nematode damage to sweet potato:

The fibrous roots of each sweet potato plant harvested were rated for galls using a root gall severity rank ranging from 0 to 4 (Suzuki *et al.*, 2012), and the values were converted to a root gall index (RGI) ranging from 0 to 100. Fresh weights of tuberous roots that weighed 50 g or more were recorded. Degradation of the external appearance of tuberous roots, *e.g.*, cracks, holes, and constrictions, were graded from 0 to 2, defined as follows: 0, good shape corresponding to marketable; 1, slightly irregular shape corresponding to marketable; and 2, obviously irregular shape corresponding to non-marketable (Fig. 2). The grades did not include color degradation or damage caused by other insects.

RKN population density evaluation:

One or 2 soil sample cores were collected at 4 points in the investigation area, described later for each

Fig. 2. Grades of tuberous roots classified on the basis of the external appearances in sweet potato. Grade 0, good shape corresponding to marketable; Grade 1, slightly irregular shape corresponding to marketable; and Grade 2, obviously irregular shape corresponding to non-marketable. Pictures were obtained from another study.

experiment, using a cylindrical soil sampler (5.5 cm in diameter) and mixed well. RKN population density was evaluated by the number of second-stage juveniles (J2) and/or the degree of parasitism to rose balsam, *Impatiens balsamina* L. For the J2 count, nematodes were extracted from two or three 20-g subsamples of fresh soil by the Baermann funnel method for 3 days at room temperature and counted under a microscope. The values were converted per 10 ml of soil. For the rose balsam plant assay, a pot (9 cm in diameter) was filled with 150 g of fresh soil; 2–3 replicate pots were used for a soil sample. Rose balsam seedlings were raised in plug trays (3 cm square, 4.5 cm deep) until a rootball formed, and then 1 seedling was transplanted into each pot. The pots were maintained in a phytotron under natural sunlight, with temperature set at 25°C. After 3–4 weeks, the roots were rated using a root gall severity rank (the same index as that used for sweet potato) to assess the galls. The values were converted to an RGI ranging from 0 to 100.

Experiment 1:

Four cultivation systems were compared in 2008–2009: TR (the field was tilled twice and ridged conventionally after the radish harvest), R (the field was not tilled and was only ridged after the radish harvest), CRU, and FTR (a soil fumigant (97% 1,3-dichloropropene) was injected into the soil conventionally (15–20 ml/m²) before radish sowing, and then the field was tilled twice and ridged conventionally after the radish harvest, like in the TR system) (Table 1). Basal fertilizer for sweet potato was applied over the entire plots before the first tillage in the TR and FTR systems or in the furrows in the R and CRU systems.

The inter-ridge distance was 120 cm. The size of the plot for each system was 7.0 m × 3.6 m (3 ridges), and the size of the investigation area, in the middle of each plot, was 5.0 m × 1.2 m (1 ridge). The plots were arranged in a randomized block design with 2 replicates per system.

Radish was cultivated in all systems from December 1, 2008, to March 23, 2009. After the harvest, soil samples were collected from both shoulders of the ridge at depths of 0–20 and 20–35 cm on April 8. Samples were also collected from the furrows of both sides of the investigation ridge at a depth of 0–15 cm. RKN population density was only evaluated by the rose balsam plant assay. Then, the fields in the TR and FTR systems were tilled, and the fields in the TR, R and FTR systems were ridged. Sweet potato was cultivated from April 24 to October 8. All sweet potato plants in the center of the investigation area (4.8 m × 1.2 m, 16 plants including those that died) were harvested and the damage was evaluated.

Experiment 2:

Three cultivation systems were compared in 2010–2011: TR, CRU, and CRU-D (a mulched ridge was constructed at the same time as CRU, and the fallow condition was maintained for the radish cultivation period). We also designed a fourth system, Fallow (a ridge was not constructed, the fallow condition was maintained for the radish cultivation period, and tillage and ridging before sweet potato cultivation were the same as those used in the TR system), for reference (Table 1). Basal fertilizer for sweet potato was applied over the entire plots before the first rotary tillage in the TR and Fallow systems and in the furrows in the CRU and CRU-D systems. The size of the plot for each

Table 1. Details of the cultivation systems in experiments 1 and 2.

Experiment (year)	Cultivation system	Before radish cultivation	Radish cultivation (D)	Before sweet potato cultivation	
		Soil fumigation (F)		Tillage (T)	Ridging (R)
Experiment 1 (2008–2009) <i>n</i> = 2	TR	-	+	+	+
	R	-	+	-	+
	CRU	-	+	-	-
	FTR	+	+	+	+
Experiment 2 (2010–2011) <i>n</i> = 4	TR	-	+	+	+
	CRU	-	+	-	-
	CRU-D	-	- ¹	-	-
	Fallow	-	- ²	+	+

¹ A mulched ridge was constructed at the same time as the CRU system without radish cultivation, and the fallow condition was maintained in winter and early spring.

² A ridge was not constructed, and the fallow condition was maintained in winter and early spring.

system was 4.2 m × 1.2 m (1 ridge), and the size of the investigation area, in the middle of each plot, was 3.0 m × 1.2 m. The plots were arranged in a randomized block design with 4 replicates per system.

Soil samples were collected on December 16 at depths of 10–15 and 30–35 cm from ground level before radish cultivation, and then the field was tilled in all systems. Radish was cultivated in the CRU and TR systems from December 17, 2010, to April 11, 2011. After the harvest, soil samples were collected from the eastern shoulder of the ridge at depths of 0–10, 10–20, and 20–30 cm in the TR, CRU, and CRU-D systems and at depths of 0–10, 10–20, and 20–30 cm from ground level in the Fallow system on April 14 or April 18. Then, the fields in the TR and Fallow systems were tilled and ridged, and soil samples were collected again from the ridge at depths of 0–10, 10–20, and 20–30 cm on April 26. Sweet potato was cultivated from April 27 to September 27. All sweet potato plants in the investigation area (10 plants including those that died) were harvested and the damage was evaluated.

Soil temperature was measured at 1-hr intervals using a button-type data logger (SEC-CD16TB or SEC-CD18TB, SANYO Electric Co., Ltd., Japan). Loggers were installed in 1 of the 4 plots in each system; 2 loggers were set in the investigation area at a depth of 10 cm from the top of the ridge for the CRU and CRU-D systems, and 2 loggers were set in the investigation area at a depth of 10 cm from ground level for the Fallow system. The total effective temperatures of RKN from December 19 (2 days after radish sowing) to April 18 (after radish harvest) were calculated using the mean of the values of the loggers (values from a broken logger were excluded), according to the lower threshold temperature of *M. incognita* in the central region of Kyushu (12°C) (Gotoh *et al.*, 1973).

Other investigation methods and growth environments were the same as those used in experiment 1.

Data analysis:

RKN J2 numbers and RGIs by the rose balsam plant assay were analyzed by a paired *t*-test for comparison of values after the radish harvest (before tillage and ridging) and after tillage and ridging in the TR system. RGIs, percentages of grade 0 and grade 0+1 (marketable) tuberous roots, and fresh weights of grade 0, grade 0+1, and total tuberous roots in sweet potato were analyzed by Dunnett's test for multiple comparisons of means, with the TR system as the control. The computations were carried out using SAS

9.2 (SAS Institute Inc., USA) at the Agriculture, Forestry and Fisheries Research Information Technology Center for Agriculture, Forestry and Fisheries Research, MAFF, Japan. RGI and percentage data were statistically analyzed after arcsine transformation. In experiment 2, RGI data were obtained from 3 of 4 blocks because 1 block had a missing value, and the data for that block were deleted from the analysis.

RESULTS

Experiment 1:

RGIs by the rose balsam plant assay after the radish harvest were approximately the same in the TR, R, and CRU systems and was low in the FTR system (Table 2). RGIs in sweet potato were significantly lower in the FTR system than in the TR system ($P < 0.05$) (Table 3) and slightly but insignificantly lower in the CRU system than in the TR system. The percentage of grade 0 and 0+1 tuberous roots in the CRU system was more than double that in the TR system; fresh weight of grade 0 and 0+1 tuberous roots in the CRU system was also more than double that in the TR system, but the differences were not significant. The percentage of grade 0 and 0+1 tuberous roots and fresh weight of grade 0 and 0+1 tuberous roots in the R system were between those in the TR and CRU systems. The percentage of grades 0 and 0+1 tuberous roots, and fresh weight of grade 0 tuberous roots were significantly higher in the FTR system than in the TR system ($P < 0.05$).

Experiment 2:

Before radish cultivation, the numbers of RKN J2 were approximately the same among systems and depths (Table 4). After the radish harvest, the numbers in the ridge were approximately the same among the TR, CRU, and CRU-D systems. The numbers were lowest at 0–10 cm, second lowest at 10–20 cm, and highest at

Table 2. Root gall index by rose balsam plant assay after radish harvest on April 8 in experiment 1.

Cultivation system ¹	Sampling area and depth (cm)		
	Shoulder of ridge		Furrow
	0–20	20–35	0–15
TR	25	54	54
R	21	54	38
CRU	17	46	54
FTR	4	4	4

¹ See Table 1.
Root gall index ranges from 0 to 100. Data are the means of 2 replicates.

Table 3. Influence of cultivation systems in experiments 1 and 2 on root-knot nematode damage to sweet potato.

Experiment (year)	Cultivation system ¹	Root gall index ² (0–100)	Percentage of each grade of tuberous roots ²		Fresh weight of each grade of tuberous roots (g/m ²)		
			Grade 0	Grade 0+1	Grade 0	Grade 0+1	Total
Experiment 1 (2008–2009) <i>n</i> = 2	TR	86	3	44	61	1360	2878
	R	86 NS	5 NS	74 NS	105 NS	2619 NS	3327 NS
	CRU	73 NS	26 NS	94 NS	572 NS	3131 NS	3424 NS
	FTR	30 *	65 *	100 *	1919 *	3699 NS	3699 NS
Experiment 2 (2010–2011) <i>n</i> = 4 ³	TR	48	14	82	290	3215	4079
	CRU	39 *	29 NS	99 *	650 NS	3419 NS	3448 *
	CRU-D	33 *	35 *	96 *	967 *	3726 NS	3854 NS
	Fallow ⁴	45	13	69	273	2425	3569

¹ See Table 1.² Data were statistically analyzed after arcsine transformation.³ Data are the means of 3 replicates only in the root gall index.⁴ Data were not statistically analyzed.* Significant difference from the TR system in each experiment (Dunnett's test, $P < 0.05$). NS: not significant.

Table 4. Influence of cultivation systems on fluctuation of root-knot nematode (RKN) density at each depth in experiment 2.

Before radish cultivation on December 16 in 2010

Depth (cm)	Density evaluation methods	TR ^{1,2}	CRU ^{1,2}	CRU-D ^{1,2}	Fallow ^{1,2}
10–15	No. of J2 ³	100 ± 44	104 ± 27	93 ± 28	122 ± 22
30–35	No. of J2 ³	123 ± 41	118 ± 52	119 ± 49	90 ± 29

Tillage in all systems and ridging in TR, CRU, and CRU-D systems
Radish cultivation in TR and CRU systems

After radish harvest on April 14 or 18 in 2011

Depth (cm)	Density evaluation methods	TR ^{1,4}	CRU ^{1,4}	CRU-D ^{1,4}	Fallow ^{1,2}
0–10	No. of J2 ³	9 ± 4	7 ± 4	5 ± 2	13 ± 5
	RGI ⁵	53	56	31	63
10–20	No. of J2 ³	32 ± 11	37 ± 13	32 ± 10	74 ± 24
	RGI ⁵	75	88	59	84
20–30	No. of J2 ³	107 ± 28	125 ± 32	119 ± 51	76 ± 18
	RGI ⁵	94	91	78	88

Tillage and ridging in TR and Fallow systems

After tillage and ridging on April 26 in 2011

Depth (cm)	Density evaluation methods	TR ^{1,4}	Fallow ^{1,4}
0–10	No. of J2 ^{3,6}	19 ± 4 NS	28 ± 9
	RGI ^{5,7}	78 *	91
10–20	No. of J2 ^{3,6}	37 ± 8 NS	43 ± 13
	RGI ^{5,7}	81 NS	91
20–30	No. of J2 ^{3,6}	52 ± 5 NS	96 ± 42
	RGI ^{5,7}	75 NS	88

¹ See Table 1 for abbreviations of the cultivation systems.² The density at each depth from ground level.³ The number of RKN second-stage juveniles (J2) per 10 ml of soil. The values are the mean ± standard error ($n = 4$).⁴ The density at each depth from the eastern shoulder of the ridge.⁵ The root gall index (RGI, 0–100) by rose balsam plant assay. The values are the mean ($n = 4$).⁶ Data were statistically analyzed after log ($X + 0.5$) transformation.⁷ Data were statistically analyzed after arcsine transformation.* Significant difference between "After radish harvest" and "After tillage and ridging" (paired t -test, $P < 0.05$). NS: not significant.

20–30 cm. The numbers in the Fallow system were lower at 0–10 cm than in the deeper areas. RGIs by the rose balsam plant assay showed the same trend in numbers of RKN J2 after the radish harvest in all systems and among all depths, although RGIs in the CRU-D system were slightly lower than those in the TR and CRU systems after the radish harvest. After tillage and ridging, the number of RKN J2 in the TR system increased to more than double at 0–10 cm and decreased to less than half at 20–30 cm in comparison with before tillage and ridging; however, these differences were not significant. RGIs increased significantly at 0–10 cm ($P < 0.05$) and decreased slightly but insignificantly at 20–30 cm.

RGIs in sweet potato were significantly lower in the CRU and CRU-D systems than in the TR system ($P < 0.05$) (Table 3). Percentage of grade 0+1 tuberous roots was significantly higher in the CRU system than in the TR system ($P < 0.05$). Percentage of grade 0 and 0+1 tuberous roots and fresh weight of grade 0 tuberous roots were significantly higher in the CRU-D system than in the TR system ($P < 0.05$).

Total effective temperatures from December 19 (2 days after radish sowing) to April 18 (after radish harvesting) were 163, 361, and 95 degree-days for the CRU, CRU-D, and Fallow systems, respectively.

DISCUSSION

In experiment 2, the RKN population density decreased for the radish cultivation period, and was low in the upper area of the ridge after the radish harvest in the TR and CRU systems (Table 4). Radish is a well-known host for *M. incognita*, which damages storage roots during warmer seasons (Araki and Iizuka, 1991; Suzuki *et al.*, 2013). The total effective temperature for *M. incognita* from infection to the beginning of egg deposition was tentatively determined to be 265 degree-days (Gotoh *et al.*, 1973). On the basis of the low total effective temperature in the CRU system, it was not expected that RKN would propagate in radish during the cropping season. Although the number of RKN in the roots was not investigated in this study, the infection might accelerate the density decrease in the soil during radish cultivation. As observed in the Fallow system in this study, normally, the RKN population density decreases during overwintering in the field (Starr and Jeger, 1985; Windham and Barker, 1988; Sano, 1990). The proportion of motile *M. incognita* J2 decreases linearly with degree-days ($>10^{\circ}\text{C}$) under conditions approximately equal to field capacity conditions

(Goodell and Ferris, 1989). In this study, mulching and a non-woven fabric cover warmed the soil in the ridge. Although the data were obtained from the condition without a non-woven fabric cover, daily mean and maximum soil temperatures in the upper area of the ridge were higher than those in the lower area in the 5 cm to 20 cm range (Niimi, unpubl. data). The temperature differences among the areas are likely useful in explaining the low RKN population density in the upper area, although soil moisture and aeration could affect the decrease intricately.

In the TR system, the RKN population density increased slightly at a depth of 0–10 cm of the ridge after tillage and ridging (Table 4). The increase was probably caused by the processes of tillage and ridging, which mix the low-density soil in the upper area of the ridge with the high-density soil in the deeper area of the ridge. A similar density shift was also observed in the Fallow system after tillage and ridging (Table 4). Sweet potato vine cuttings are planted above the ridge, and most of the underground portions of these cuttings lie in the upper area of the ridge. In sweet potato, adventitious roots arise from these portions, and some of these develop into tuberous roots (Firon *et al.*, 2009). Damage to sweet potato tended to be less in the CRU system relative to that in the TR system (Table 3). Hence, a lower density in the upper area of the ridge is considered as one of the reasons for the decrease in damage to sweet potato in the CRU system.

Damage in the R system was moderate relative to that in the TR and CRU systems (Table 3). It is assumed that the soil in the upper area of the ridge was not sufficiently mixed with that in the deeper area because of the lack of tillage. This finding reveals that preparing the soil by the process of tillage and ridging prior to sweet potato cropping is an important factor that influences RKN population dynamics and damage to the crop.

In experiment 2, in the CRU-D system, in which radish was not cultivated, the RKN population densities at each depth decreased to levels slightly lower than those in the CRU system (Table 4). The total effective temperature was higher in the CRU-D system than in the CRU system because the radish plant cover did not provide shielding and no holes left in the film from the standing radish crop. Hence, eggs would be expected to hatch earlier in the CRU-D system than in the CRU system, and the mortality rate would probably be increased because of the higher temperature.

In experiment 1, the damage to sweet potato clearly

decreased in the FTR system compared to that in the TR system (Table 3). This finding indicates that the reduction in damage in the CRU system was somewhat less than that in the soil fumigant treatment, the FTR system. Therefore, further improvements to the CRU system, regarding crop, season, agricultural materials, and application of organic matter, are future tasks. In particular, based on the results in the CRU-D system, delaying sweet potato planting in spring will reduce RKN density and damage if high soil temperatures in the upper area of the ridge are maintained during this period.

Adoption of the CRU system would be one method for reducing RKN damage to sweet potato. The CRU system could currently be used for RKN damage control as part of an integrative pest management program. In the future, an improved CRU system will reduce the need for soil fumigants, which are commonly used in upland farming.

ACKNOWLEDGEMENT

We deeply appreciate the useful suggestions for improving the manuscript provided by anonymous reviewers.

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Received: May 24, 2012