Effect of Introducing Nematode-Resistant Sweet Potato Cultivars on Crop Productivity and Nematode Density in Sweet Potato-Radish Double-Cropping Systems

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Abstract: The root-knot nematode (RKN) is a significant pest in upland farming. We studied the effects of introducing nematode-resistant sweet potato cultivars on crop yield, crop quality, and RKN population dynamics in sweet potato-radish double-cropping systems. Three cropping systems with and without nematicide treatment (6 systems in total) were arranged for a 4-yr field experiment from 2003 to 2006. In two nematode-suppressive cropping systems, highly nematode-resistant J-red (J) or Sunny red (S) and moderately nematode-resistant Kyushu No. 139 (K139) or Murasakimasari (M) sweet potato cultivars were cropped in alternate years beginning with the former and the latter, and in the non-nematode-suppressive cropping system, nematode-susceptible Kokei No. 14 (K14) and M were cropped in alternate years beginning with the former, from 2003 to 2005. In all cropping systems, K14 was cropped in 2006 to estimate the nematode-suppressive effect of the preceding 3-yr cropping. Introduction of J and S to the cropping system decreased the number of RKNs. In 2006, the extent of injury of K14 was decreased in nematode-suppressive cropping systems. The RKN population density, however, recovered during the cropping of K14 even after cropping of J or S or after nematicide treatment. This suggests that the effects of these measures last for only 1 yr. Nematode injury in radish decreased after nematicide treatment and after cropping of highly nematode-resistant J or S. These results indicate that the introduction of nematode-resistant sweet potato cultivars in cropping systems is effective for reduction of agrochemical use for sustainable agriculture.

Key words: Double-cropping system, Nematode-resistant cultivar, Radish, Root-knot nematode, Sweet potato.
The host plant range of *M. incognita* is extensive and this makes it difficult to establish rational cropping systems. However, in sweet potato, resistance to RKN has been evaluated, and nematode-resistant cultivars have been bred in Japan (Shibuya, 1952; Tarumoto, 1992) and in the U.S. (Cervantes-Flores et al., 2002). These tuberous roots are not heavily injured by RKN, and the cultivation of highly nematode-resistant cultivars has been shown to decrease the nematode population density (Inagaki and Momota, 1983; Tabuchi and Sakamoto, 1983; Ueda et al., 1986; Fukunaga and Iwahori, 2002; Suzuki et al., 2005).

In the southern region of Kyushu, many types of vegetables can be cultivated after sweet potato cropping in summer, and these sweet potato-vegetable double-cropping systems are more profitable for farmers than single cropping systems. However, the effects of introducing nematode-resistant sweet potato cultivars into these cropping systems are not well understood. The objective of this study was to determine the effects of introducing nematode-resistant sweet potato on crop quality, crop yield, and RKN population dynamics in sweet potato-radish double-cropping systems in a 4-yr field experiment.

### Materials and Methods

1. **Experimental design and crop management**

   Field experiments were conducted from 2003 to 2006. The field was located at the Miyakonojo Research Station, National Agricultural Research Center for Kyushu Okinawa Region (Miyakonojo, Miyazaki, Japan, 31º45’ N 131º01’ E). The soil was andosol and its texture was loam. The field was naturally infested with RKNs, and the sweet potato cultivar “Kokei No. 14,” which has been identified as a nematode-susceptible sweet potato cultivar (Sano et al., 2002), was cultivated in 2002. The cropping sequence involved continuous annual double cropping of sweet potato in the summer and radish in the fall. In this experiment, we examined the effects of the cropping systems with different sweet potato cultivars and nematicide treatment on the yield and nematode injury (Table 1).

### Table 1. Scheme of sweet potato-radish double cropping systems with different potato cultivars, with and without nematicide treatment.

| Cropping system | Nematicide treatment | Year and sweet potato cultivar | Replication |
|-----------------|----------------------|-------------------------------|-------------|
| CR1             | Control              | 2003: J, 2004: M, 2005: J, 2006: K14 | 6–3*        |
| CR2             | K139                 | 2003: S, 2004: M, 2005: K14   | 6–3*        |
| CS              | K14                  | 2003: M, 2004: K14, 2005: K14 | 4           |
| NR1             | Nematicide           | 2003: J, 2004: M, 2005: K14   | 3           |
| NR2             | K139                 | 2003: S, 2004: M, 2005: K14   | 3           |
| NS              | K14                  | 2003: M, 2004: K14, 2005: K14 | 2           |

| Abbreviation | Name of sweet potato cultivar | Expected resistance** | Resistance from the result of this experiment |
|-------------|-------------------------------|-----------------------|---------------------------------------------|
| J           | J-red                         | Highly resistant      | Highly resistant                            |
| S           | Sunny red                     | Highly resistant      | Highly resistant                            |
| K139        | Kyushu No. 139                | Highly resistant      | Moderately resistant                        |
| M           | Murasakimasari                | Susceptible           | Moderately resistant                        |
| K14         | Kokei No. 14                  | Susceptible           | Susceptible                                 |

*Three of the 6 plots of CR1 and CR2 were removed before radish cultivation in 2003 for another experiment which is not discussed in this paper.

**We expected resistance of these cultivars with reference to the report of Sano et al. (2002). Radish was excluded in this scheme, but was the same cultivar (Taibyousoubutori) in all systems and years. For nematicide treatment, a soil fumigant (97%, 1,3-dichloropropene) was injected before planting of sweet potato cuttings, and a non-fumigant nematicide (1.5%, fosthiazate) was applied before sowing radish.
systems to evaluate the nematode suppressive effect of the preceding 3 yr of cropping.

In addition to CR1, CR2 and CS cropping systems, the same cropping system but with nematicide (nematode-suppressive) treatment, NR1, NR2 and NS cropping systems, respectively, were arranged. For nematicide treatment, a soil fumigant (97% 1,3-dichloropropene) was injected into soil before planting sweet potato at a rate of 15–20 mL m\(^{-2}\), and a non-fumigant nematicide (1.5% fumziazate) at a rate of 25 g m\(^{-2}\) before sowing radish. Each system was replicated 2–6 times. The size of the plot for each system was 4.0 × 6.75 m and that of the investigation area, which was set in the middle of each plot, was 1.8 × 2.25 m.

Ridges for planting of sweet potato were mulched with black polyethylene film. Ridge distance was 75 cm and hill distance was 30 cm. Cuttings of sweet potato were planted in single rows during April or May and harvested in August or September (111–122 d after planting). Radish (Raphanus sativus L. “Taibousoubutori”) was sown in September and harvested in December (63–77 d after sowing). The row distance was 50 cm and the hill distance was 30 cm (25 cm in 2004). Basal fertilizer was applied at rates of 5 g of N m\(^{-2}\), 7 g of P\(_{2}\)O\(_{5}\) m\(^{-2}\), and 12 g of K\(_{2}\)O m\(^{-2}\) for sweet potato, and 15 g of N m\(^{-2}\), 20 g of P\(_{2}\)O\(_{5}\) m\(^{-2}\), and 13 g of K\(_{2}\)O m\(^{-2}\) for radish each year. Conventional weed and insect pest control methods were applied.

2. Measurements

Soil samples for the assessment of nematode density were collected from the investigation area at a depth of 10–15 cm by using a garden trowel before planting of sweet potato cuttings or at the hillside at mid-height of the ridge at the time of harvesting of sweet potato. Nearly equal amounts of samples were collected at 4 points and mixed well. Second-stage RKN juveniles were extracted from 20 g of fresh soil by using the modified Baermann funnel method (Sano, 2004); each soil sample was placed on a Japanese-paper filter supported by a wire gauze in a glass funnel (approximately 9 cm in diameter) filled with tap water for 3 d at room temperature (approximately 25°C). Finally, the number of RKNs was counted under a microscope. Extraction and counting of RKNs in each soil sample was replicated 2–3 times.

All sweet potato plants in the investigation area (approximately 18 plants) were harvested. Tuberous roots were removed, and the remaining fibrous roots were also removed as far as possible. Fibrous roots of individual plants were rated for the degree of RKN parasitism by galls, using an index ranging from 0 to 4 as follows: 0, no galls; 1, light galling; 2, moderate galling; 3, heavy galling; and 4, severe multiple galling and deterioration of the roots. The index values were converted to a range of 0–100 (the root-gall index [RGI]; Fig. 1). The fresh weight of individual tuberous roots which was 50 g or more, was recorded. Tuberous roots with heavy nematode injuries (Fig. 2), e.g. cracks, holes, and constrictions, were identified as injured tuberous roots.

Twenty radish plants from each experimental plot were sampled randomly. Fibrous roots of individual plants were used for the measurement of the RGI, by the same method used for sweet potato. The fresh weight of each individual storage root and injury (constrictions) on the tip of the storage root were measured (Fig. 3). Injury was rated on 5 or 3-score indices. In 2005, the index was set as 0, no injury; 1, light injury; 2, moderate injury; 3, heavy injury; and 4, very heavy injury. In 2004 and 2005, the index was revised as 0, no injury; 1, moderate injury; and 2, very heavy injury. These index values were converted to a range of 0–100.

3. Statistical Analysis

In this experiment, the initial nematode density and nutrient conditions were similar in the plots. Therefore, data obtained from this study were analyzed by analysis of variance with SAS 9.2 using the GLM model. Type II sum of squares was adopted for the procedure.

Results

1. Fluctuations in RKN population density

The RKN population density before planting and that at the time of harvesting of sweet potato are shown in Fig. 4. Before planting of the sweet potato in 2003, the density ranged from 101 to 149 nematodes per 20 g of soil in each system. In 2003, the density at the time of harvesting differed with the sweet potato cultivar. The cultivation of J in the CR1 cropping system resulted in a low density. In contrast, the cultivation of K14 in the CS cropping system heavily increased the density. The cultivation of K14 in the CR2 cropping system resulted in an intermediate density between those of the CR1 and the CS cropping system. The nematicide treatment had little influence on the density. In 2004, the cultivation of S decreased the density in the CR cropping system. After the cropping of M in the CR1, the density was low, whereas the cultivation of M in the CS resulted in a high density. In 2005, the density was lower in the CR1 and CR2 cropping systems than in the CS cropping system at the time of harvesting of sweet potato. In 2006, before planting of sweet potato, the density was relatively high in the CS cropping system. In every cropping system, the density increased after cultivation of K14.

The RGI of sweet potato in different cropping systems is shown in Table 2. RGI was lower in the nematicide group than in the control group during 2003–2006, indicating that RGI was affected by the cropping system. RKN caused severely galled roots in K14 and moderately galled roots in K139 and M. There were almost no galls on roots of J and S. Table 3 shows the RGI of radish in different cropping
systems. RGI was lower in the nematicide group than in the control group, and the indicating that the RGI of radish was affected by the cropping system of sweet potato cultivars. RGI was the highest in the CS system for 3 yr.

2. Crop Productivity

(1) Sweet potato
The tuberous roots of K14 were consistently injured. In 2003, 72% and 54% of tuberous roots in the CS and NS systems were injured, respectively. In 2005, 37% and 2% of

![Image](Fig. 1. Example of the root-gall index (RGI). Fibrous roots of individual plants were rated for the degree of RKN parasitism by galls, using an index ranging from 0 (no galls) to 4 (severe multiple galling and deterioration of the roots). The index of the roots at the bottom picture on the left is 0, and that on the right (severe multiple galling with egg masses) is 4. The index values were converted to a range of 0–100.]

![Image](Fig. 2. Nematode injuries on the tuberous root of sweet potato. a: crack, b: hole, c: constriction.]

![Image](Fig. 3. Fibrous roots and the tip of a storage root of radish. a: fibrous roots (from which the RGI was evaluated), b: injury (constrictions) at the tip of the storage root. Injury was rated using an index (0 [no injury], 1 [moderate injury], and 2 [very heavy injury] in 2004). The indices on the left, middle, and right are 0, 1, and 2, respectively. The index values were converted to a range of 0–100.)
Table 2. Influence of different cropping systems on root-gall indices (RGIs) of sweet potato.

| Cropping system | Year | 2003 | 2004 | 2005 | 2006 |
|----------------|------|------|------|------|------|
| CR1            |      | 0\(^\text{ii}\) | 19\(^\text{iv}\) | 0\(^\text{v}\) | 48\(^\text{v}\) |
| CR2            |      | 48\(^\text{v}\) | 1\(^\text{i}\) | 12\(^\text{iv}\) | 51\(^\text{v}\) |
| CS             |      | 90\(^\text{vi}\) | 26\(^\text{v}\) | 49\(^\text{vi}\) | 79\(^\text{v}\) |
| NR1            |      | 1\(^\text{v}\) | 10\(^\text{iv}\) | 0\(^\text{v}\) | 26\(^\text{v}\) |
| NR2            |      | 23\(^\text{v}\) | 0\(^\text{v}\) | 5\(^\text{v}\) | 27\(^\text{v}\) |
| NS             |      | 85\(^\text{v}\) | 13\(^\text{v}\) | 20\(^\text{v}\) | 40\(^\text{v}\) |

2-Way ANOVA

Nematicide: *** * ** ***

System: *** ** *** ***

Nematicide x System: *** NS ** NS

Fibrous roots of individual plants were rated for galls by using an index ranging from 0 (no galls) to 4 (severe multiple galling and deterioration of the roots), and the index values were converted to RGI, ranging from 0 to 100.

Cultivars: 1) J red, 2) Sunny red, 3) Kyushu No. 139, 4) Murasaki san, 5) Kokei No. 14.

*, **, and *** significant at P < 0.05, 0.01, and 0.001, respectively. NS, not significant.

For abbreviations of the cropping systems, refer to Table 1.

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Table 3. Influence of different cropping systems on root-gall indices (RGIs) of radish.

| Cropping system | Year | 2003 | 2004 | 2005 |
|----------------|------|------|------|------|
| CR1            |      | 16   | 32   | 14   |
| CR2            |      | 36   | 16   | 24   |
| CS             |      | 63   | 63   | 52   |
| NR1            |      | 4    | 1    | 0    |
| NR2            |      | 16   | 1    | 3    |
| NS             |      | 17   | 0    | 18   |

2-Way ANOVA

Nematicide: *** *** ***

System: *** *** ***

Nematicide x System: ** *** NS

Fibrous roots of individual plants were rated for galls by using an index ranging from 0 (no galls) to 4 (severe multiple galling and deterioration of the roots), and the index values were converted to RGI, ranging from 0 to 100.

*, **, and *** significant at P < 0.05, 0.01, and 0.001, respectively. NS, not significant.

For abbreviations of the cropping systems, refer to Table 1.

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Fig. 4. Fluctuations in root-knot nematode (RKN) population densities (mean ± S.E.) in each cropping system during 4 years. B: before planting, and H: at the time of harvesting of sweet potato.

For abbreviations of the cropping systems, refer to Table 1.
the tuberous roots in CS and NS were injured, respectively. The shapes of the tuberous roots of S were irregular, and the injuries could not be evaluated. In the remaining cultivars, namely, J, K139 and M injuries were rare for all treatments (data not shown).

The yield of tuberous roots and the number of tuberous roots were lower in the nematicide-treated systems in 2003. The nematicide treatment did not influence the yield or the number of tuberous roots in 2004 and 2005 (Table 4). Analysis of variance showed that the nematicide treatment and the cropping system had a significant influence on the fresh yield (fresh weight of tuberous roots and that of non-injured tuberous roots). Both of these measures were very low in CS cropping system (Table 5).

(2) Radish

Table 6 shows the effects of the nematicide treatment and the cropping system on the fresh weight and nematode injury of radish storage root determined by analysis of variance. The nematicide treatment affected the nematode injury level. The cropping system affected the nematode injury level of radish in 2003 and 2005, and the fresh weight in 2004, although the fresh weights varied with the yr. Nematode injury levels in CS were the highest throughout the entire 3-yr period.

Discussion

In the sweet potato-radish double cropping system, the difference in the sweet potato cultivars was found to influence the RKN population density. Cropping of K14 which is a nematode-susceptible cultivar increased the density in 2003 and 2005 in the CS cropping system. In contrast, the density did not increase after cropping of J and S, which are nematode-resistant cultivars, and RKN was seldom found to parasitize the roots of J in the CR1 cropping system and S in the CR2 cropping system. It is notable that in 2003, cultivation of J decreased the density in spite of the very high density before cropping. These results highlight the effectiveness of introducing nematode-resistant cultivars to the sweet potato cropping system. Meanwhile, the RKN density increased in 2006 after 1 yr of cropping of a nematode-susceptible sweet potato cultivar K14, not only in the CS cropping system but also in the CR1 or CR2 cropping systems. These results

| Crop | Crop 1 (g) | Crop 2 (g) | Crop 3 (g) | Crop 4 (g) |
|------|------------|------------|------------|------------|
| CR1  | 2037       | 2866       | 2486       | 3677       |
| CR2  | 2412       | 2716       | 2548       | 3626       |
| CS   | 1331       | 15.93      | 16.89      | 15.68      |

2-Way ANOVA

Nematicide *** Nematicide x System NS
System *** NS

Table 5. Effects of different cropping systems on the yield and injuries of Kokei No.14 in 2006.

| Crop | Fresh weight (g m^-2) | Non-injured tuberous (g m^-2) | Ratio |
|------|-----------------------|-------------------------------|-------|
| CR1  | 1617                  | 1464                          | 90.1  |
| CR2  | 1464                  | 1464                          | 90.1  |
| CS   | 761                   | 761                           | 100.0 |

2-Way ANOVA

Nematicide *** System ***
Nematicide x System NS NS

* *, **, and *** significant at P<0.05, 0.01, and 0.001, respectively. NS, not significant. For abbreviations of the cropping systems, refer to Table 1.
show that the effects of the introduction of a highly nematode-resistant cultivar last only for 1 yr if a nematode-susceptible sweet potato cultivar is cultivated thereafter. Sano et al. (2002) also demonstrated that J and S have high nematode resistance in 4 different M. incognita populations collected from different areas. The populations included the Miyakonojo population from the Miyakonojo Research Station. On the other hand, they showed that nematode resistance of K139 and M depends on the M. incognita populations. In that study, K139 was highly resistant to the Miyakonojo population, and M was susceptible. We expected the same results in our study; however, the nematode resistance of K139 and M differed from our expectation, and these are regarded as moderately nematode-resistant cultivars (Table 1). The RGI in 2003 indicated that RKN parasitized the roots of K139 and the RKN population density in the cropping systems with cultivation of K139 were at an intermediate level with respect to those of the cropping systems with K14 and the cropping systems with J. These results indicate that K139 was not completely resistant to RKNs. The difference may have been caused by differences in the fields investigated and properties of the RKN populations inhabiting the fields. Our results obtained in 2004 in the CR2 cropping system indicate that M also has moderate resistance because the cultivation did not increase the nematode density to a great extent, and nematode injury of tuberous roots was rare. Lawrence et al. (1986) also reported that more eggs and juveniles of M. incognita were recovered from a nematode-susceptible cultivar than from a moderately nematode-resistant cultivar when the initial density was high.

On the other hand, RKN decrease the yield and the number of tuberous roots not only in K14 but also in the nematode-resistant cultivars J and K139 under high RKN density conditions in 2003. Interestingly, although Minagawa (1976) previously reported that M. incognita affects the number of tuberous roots in a nematode-susceptible cultivar in Japan, RKNs also seem to affect the number of tuberous roots in highly nematode-resistant cultivars in the roots in which RKNs cannot propagate. In this regard, M. incognita appears to penetrate the root system of both nematode-resistant and susceptible cultivars (Komiyama et al., 2006). RKNs tend to damage roots at an early stage of development, thus inhibiting tuberous root formation, regardless of the resistance of the cultivar. Makumbi-Kidza et al. (2000) reported that M. incognita reduces the number of storage-roots in cassava. This indicates that such a phenomenon might be common among tuber crops. However, the yield of J was not greatly decreased in 2003 because of an increase in the number of heavy tuberous roots.

Nematicide treatments have been shown to decrease RKN population density and the extent of associated injuries in sweet potato (Nielsen and Sasser, 1959; Johnson and Cairns, 1971; Hall et al., 1988). We observed injury in K14 in 2003, 2005 and 2006. Although nematicide treatment did not sufficiently prevent injury because of the very high initial density in 2003, it prevented injuries because of the lower initial density in 2005 and 2006. However, the density recovered during cropping of K14 even though nematicide treatment was introduced. This is similar to the result obtained upon introduction of highly nematode-resistant cultivars and suggests that the effects of

Table 6. Influences of different cropping systems on fresh weight and nematode injury of radish storage root in 2003–2005.

| Cropping system | Year | 2003 | 2004 | 2005 |
|-----------------|------|------|------|------|
|                 | Crop | weight | Nematode injury | Fresh weight | Nematode injury | Fresh weight | Nematode injury |
| CR1             | 555  | 16   | 708  | 41   | 1335 | 27   |
| CR2             | 505  | 27   | 725  | 19   | 1233 | 40   |
| CS              | 550  | 47   | 657  | 67   | 1324 | 72   |
| NR1             | 565  | 12   | 845  | 14   | 1313 | 7    |
| NR2             | 610  | 20   | 815  | 14   | 1336 | 23   |
| NS              | 571  | 19   | 624  | 3    | 1192 | 34   |

2-Way ANOVA

| Nematicide | System | NS | NS | NS | NS | NS |
|------------|--------|----|----|----|----|----|

NS, not significant.

Injury was rated using an index ranging from 0 (no injury) to 4 (very heavy injury) in 2003 or 0 (no injury) to 2 (very heavy injury) in 2004 and 2005, and these index values were converted to a range of 0–100.

For abbreviations of the cropping systems, refer to Table 1.
a nematicide and cropping of resistant cultivars last only 1 yr. Continuous cropping of nematicide-susceptible sweet potato cultivars is difficult.

Nematode injury of radish was also observed in our study. Araki and Iizuka (1991) reported radish injury caused by *M. incognita* during cultivation in Kumamoto Prefecture in the fall. In this study, nematode injury was influenced by both the sweet potato cultivar in the cropping system and the nematicide treatment. Furthermore, these treatments also affected the parasitism by RKNs. The levels of parasitism and nematode injury were high in the CS cropping system and low after the cropping of highly nematode-resistant sweet potato cultivars in CR1 and CR2 cropping systems. These results show that cropping of highly nematode-resistant sweet potato cultivars is helpful for the management of nematode injury in radishes.

In our study, highly nematode-resistant sweet potato cultivars were introduced and rotated with moderately nematode-resistant cultivars from 2003 to 2005 in the CR1 cropping system and from 2004 to 2005 in the CR2 cropping system. These cropping systems kept the RKN density low until 2005. In 2006, injury of a nematode-susceptible sweet potato cultivar decreased after nematode-suppressive cropping systems without nematicide treatment. Rotation of highly and moderately nematode-resistant sweet potato cultivars is more useful than continuous cropping of highly nematode-resistant cultivars, because many types of cultivars can be introduced. In Japan, sweet potato cultivars are cultivated and developed for many applications such as the production of alcohol, starch, and vegetables (Kumagai, 2000). Farmers can cultivate multiple marketable cultivars, including moderately nematode-resistant cultivars. In addition, this cropping system is useful for avoiding the emergence of new nematode strains. Continuous cropping of highly nematode-resistant cultivars often results in the development of new nematode strains (Okamoto and Mitsui, 1974). Nishizawa (1974) described a new *M. incognita* pathotype, which has been shown to break the resistance of nematode-resistant cultivars. Furthermore, continuous cropping of the nematode-resistant sweet potato cultivar “Shiroyutaka” increases nematode density in the third and fourth years (Fukunaga and Iwahori, 2002).

In Japan, highly nematode-resistant sweet potato cultivars are not in high commercial demand at this time. However, our study showed that introduction of nematode-resistant sweet potato cultivars in cropping systems is effective for reducing the use of agrochemicals in sustainable agriculture. Therefore, the present study suggests that if highly nematode-resistant cultivars could be marketed in the near future, the use of these cultivars will be highly beneficial in practical cropping systems.

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