Short Communication

Comparison of Disease Suppressiveness of Different Soils with or without Repeated Application of Organic Matters toward Bacterial Wilt of Tomato Caused by *Ralstonia solanacearum*

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Six different soils that have received different organic matter amendments at different rates for more than 7 years (OM-soils) were collected and their suppressiveness against bacterial wilt in tomato was compared with that of corresponding control soils, that is, those amended with only chemical fertilizers (CF-soils). Disease incidence was lower in a yellow soil (Toyohashi, Aichi, Japan) and a brown lowland soil (Ayabe, Kyoto, Japan) with repeated application of cow manure (OM-soils) than in the corresponding CF-soils. The dynamics of the pathogen in non-rhizosphere soils after inoculation showed a quite similar fate in both the OM-soils and CF-soils. However, *lux* activity tended to be lower in tomato roots grown in the OM-soils than CF-soils when a *lux*-marked pathogen was inoculated, suggesting that disease incidence is suppressed in the OM-soils because of the reduced growth of the pathogen in the rhizosphere. In contrast, there was no significant difference in disease incidence in four soils between the OM-soils and CF-soils. These results suggested that repeated application of organic matter does not always induce suppression of bacterial wilt, and that the kind of organic matter and application rate may affect the suppressiveness.

Key words: bacterial wilt, compost, cow manure, *lux* activity, rhizosphere, survival

Bacterial wilt caused by *Ralstonia solanacearum* ranks as one of the most important plant diseases occurring in tropical, sub-tropical and warm temperate regions of the world¹. Conventional control techniques have met with limited success because of better survival of the pathogen in soil, especially in deeper layers². Since the pathogen attacks the plant from the soil, it is difficult to control the disease using chemicals once infection has occurred. Therefore, alternative methods are needed to control bacterial wilt.

Sustainable agriculture requires a number of practices one of which is proper management of plant pathogens. Or-

ganic amendments play an important role in sustainable agriculture, but there are increasing environmental problems with the application of huge amounts of organic matter. Therefore, if effective ways of preventing soilborne diseases using organic wastes were to be developed, they would be an incentive for the recycling of organic wastes. Our earlier study³ demonstrated that a soil (FYM-soil) amended with 400 t ha⁻¹ yr⁻¹ of farmyard manure for 14 yr was highly suppressive of bacterial wilt, compared to a soil (CF-soil) amended with only chemical fertilizers, and that the growth of the pathogen in the non-rhizosphere soil and host roots was suppressed more in the FYM-soil. The FYM-soil was therefore considered pathogen-suppressive as well as disease-suppressive and this study indicated the possibility of suppressing disease through the repeated application

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of organic matter.

In this study, six different soils that have received different organic matter amendments at different rates for long periods were collected from different parts of Japan in order to estimate the relationship between repeated application of organic matter and suppressiveness toward bacterial wilt. The objective of this study was to compare disease incidence and the pathogen's dynamics between soils with or without repeated application of organic matter.

Soils having different histories of organic matter amendment were collected from the Aichi Prefecture Agricultural Research Center Higashi-Mikawa Agricultural Research Institute (Toyohashi, Aichi, Japan), the National Agricultural Research Center⁶⁾ (Tsukuba, Ibaraki, Japan), the National Agricultural Research Center for the Western Region (Ayabe, Kyoto, Japan) and Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan) (Table 1). Each soil was collected from a depth of 0 to 10 cm, sieved through a 2-mm screen and subjected to the experiments within two months of collection.

Two hundred grams of soil was taken into a vinyl pot 9 cm in diameter, inoculated with the pathogen, described below, at a density of 10^4 to 10^6 CFU g⁻¹ soil, and adjusted to 50% of maximum water-holding capacity (MWHC). Twenty grams of the inoculated soil was separately taken into a

50-ml flask to monitor the dynamics of the pathogen in the non-rhizosphere soil. The strain used for the inoculation was *R. solanacearum* YU1Rif43lux1 (the strain YU1Rif43⁸⁾ spontaneously resistant to rifampicin was marked with a *lux* gene using pSOL2⁷⁾). The bacterial inoculum was prepared by overnight culture at 28°C in 10⁻¹ strength of nutrient broth (Eiken Co., Tokyo, Japan).

Tomato (*Lycopersicon esculentum* Mill. Momotaro) seeds were pregerminated in a moist petri dish in the dark at 28°C for 2 days. Then, six tomato seedlings were transplanted into a pot and grown in a climatron, adjusted to 80% relative humidity and 30°C, with a light regime (day:night=12h:12h, light intensity=270 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Three pots were prepared per treatment. Hyponex fertilizer that contains nitrogen, phosphorous, potassium, etc., was added twice a week to each pot to support plant growth during the entire period of incubation. Watering was performed twice a day to maintain the water content at 50% MWHC. A disease index was recorded periodically for 30 days on the following basis: 0; no symptoms of wilting, 1; 0 to 25% of plants showing wilting, 2; 26 to 50%, 3; 51 to 75%, 4; 76 to 100%. Population density of the pathogen in non-rhizosphere soil was periodically determined by the dilution plate method using 10⁻¹ strength of nutrient agar (NA) containing 50 $\mu\text{g ml}^{-1}$ rifampicin, 50 $\mu\text{g ml}^{-1}$ polymyxin B, and 100 μg

Table 1. Characteristics of the soils used

Site (soil name)	Type of fertilization	Year of management	Application rate of OM (t ha ⁻¹ y ⁻¹)	MWHC (g g ⁻¹)	pH (H ₂ O)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N ratio
Fuchu (Cumulic andosol)	Chemical fertilizers (CF)	9	0	1.43	5.0	80.1	8.2	9.8
	Cow manure (CowM)	9	42	1.53	6.4	87.2	10.9	8.0
Tsukuba (Low-humic andosol)	CF	10	0	1.07	6.2	49.5	4.1	12.0
	Plant residue compost	10	20	1.06	6.2	52.5	4.6	11.4
Tsukuba (Yellow soil)	CF	10	0	0.56	5.3	9.0	1.1	8.5
	Plant residue compost	10	20	0.57	6.0	14.2	1.7	8.4
Tsukuba (Gray lowland soil)	CF	10	0	0.70	6.1	13.7	1.6	8.4
	Plant residue compost	10	20	0.64	5.9	18.9	2.3	8.3
Toyohashi (Yellow soil)	CF	7	0	0.58	5.1	10.6	1.2	8.6
	CF and cow manure (CF+CowM)	7	20	0.46	5.0	17.6	1.8	9.6
	Cow manure (CowM)	7	51	0.70	7.2	27.8	2.6	10.7
	Poultry manure (PoulM)	7	13	0.49	7.2	13.5	1.6	8.3
Ayabe (Brown lowland soil)	CF	11	0	0.48	5.3	6.9	1.0	7.3
	cow manure 3 times (3CowM)	11	113	0.65	6.2	25.6	2.5	10.2
	Cow manure (CowM)	11	38	0.51	5.9	13.3	1.4	9.3
	Pork manure (PorkM)	11	19	0.47	6.4	13.0	1.5	8.6

MWHC: maximum water-holding capacity, CF: chemical fertilizers, OM: organic matter.

Table 2. Disease index (DI) of bacterial wilt of tomato and *lux* activity of the roots grown in soils inoculated with *R. solanacearum* at different levels of initial density

Soil name	Type of fertilization	Initial density (cfu g ⁻¹)					
		5×10 ⁵		1×10 ⁶			
		DI	<i>Lux</i> activity	DI	<i>Lux</i> activity		
Low-humic andosol (Tsukuba)	CF-soil	1.1±0.2	4.1±1.0	1.6±0.8	3.9±0.3		
	OM-soil	1.1±0.3	3.9±0.2	1.8±0.6	4.0±0.5		
Yellow soil (Tsukuba)	CF-soil	2.7±0.6	4.9±0.6	2.8±0.8	5.4±0.8		
	OM-soil	2.8±0.5	4.1±0.3*	2.7±0.5	4.1±0.3*		
Gray lowland soil (Tsukuba)	CF-soil	3.6±0.6	4.0±0.3	2.4±0.2	4.1±0.3		
	OM-soil	3.3±0.9	3.8±0.3	3.6±0.3	4.0±0.3		
		1×10 ⁴		1×10 ⁵		1×10 ⁶	
		DI	<i>Lux</i> activity	DI	<i>Lux</i> activity	DI	<i>Lux</i> activity
Cumulic andosol (Fuchu)	CF-soil	1.9±0.1	3.8±0.6	2.9±1.3	4.2±0.2	3.5±0.2	5.0±1.2
	OM-soil	1.9±0.1	4.1±0.4	3.4±0.5	4.2±0.2	3.8±0.2	5.1±1.0
		5×10 ⁵ (Experiment 1)		1×10 ⁵ (Experiment 2)		5×10 ⁵ (Experiment 2)	
		DI	<i>Lux</i> activity	DI	<i>Lux</i> activity	DI	<i>Lux</i> activity
Yellow soil (Toyohashi)	CF-soil	3.4±0.6	ND	2.2±2.6	3.9±0.2	3.4±0.8	4.1±0.4
	CF+cowM-soil	1.8±0.8*	ND	1.2±1.2	3.8±0.2	1.8±2.1*	3.8±0.3
	CowM-soil	2.2±1.4	ND	1.3±0.4	3.6±0.3	2.8±0.1*	4.3±1.1
	PoulM-soil	3.3±0.5	ND	0.8±0.9*	3.8±0.2	2.8±0.7	3.7±0.4

Disease index after 30 days of cultivation is expressed as the mean±SD (2 or 3 replicates, each replicate contained 6 plants): 0; no symptoms of wilting, 1; 0 to 25% of plants showing symptoms, 2; 26 to 50%, 3; 51 to 75%, 4; 76 to 100%.

Lux activity after 14 days of cultivation is expressed as the mean (log photon counting (arbitrary unit) g⁻¹ root)±SD (n=6).

OM-soil: soil amended with organic matter, CF-soil: soil amended with chemical fertilizers.

* significantly (P<0.05) different between CF-and OM-soils in the same initial density. ND: not determined.

ml⁻¹ cycloheximide.

After two weeks of cultivation, six tomato plants were taken from the vinyl pot and their roots were washed in sterile distilled water. Bioluminescence of the washed roots was measured separately using an ARGUS-20 (Hamamatsu Photonics, Hamamatsu, Japan) with an exposure time of three minutes. Preliminary experiments showed that there was a significant positive correlation between *lux* activity and the number of pathogens in the tomato roots (data not shown).

Total C and N levels were always higher in the soils amended with organic matter (OM-soils) than the corresponding control soils, that is, those amended with chemical fertilizers only (CF-soils) (Table 1). Thus, the effect of an accumulation of carbon with repeated application of organic matter was confirmed. This was reflected in the maximum water-holding capacity and pH in some soils, but not in others.

In cumlic andosol (Fuchu), yellow soil (Tsukuba), low-humic andosol (Tsukuba) and gray lowland soil (Tsukuba), disease incidence was not significantly different between

the OM- and CF-soils (Table 2). In yellow soil (Toyohashi), the soils amended with CF and cow manure (CF+CowM-soil), cow manure (CowM-soil) and poultry manure (PoulM-soil) showed a significantly lower disease index in two, one and one, respectively, of 3 experiments (Table 2). When the disease index in the final days of the three experiments was analyzed using a two-way ANOVA, it was found to be significantly lower in the CF+CowM-soil than in the CF-soil. In brown lowland soil (Ayabe), the disease index was significantly lower in the soil (3 cow M-soil) amended with cow manure at three times the conventional dosage than in the CF-soil (Fig. 1).

Survival of the pathogen in the non-rhizosphere soil was monitored for 30 days. There was no remarkable difference in the fate of culturable populations of the pathogen between the CF- and OM-soils in the six soils (only data for brown lowland soil is shown as a typical example). *Lux* activity of the roots grown for 14 days showed that there was no difference in four out of six soils between the CF- and OM-soils. In the brown lowland soil amended three times with cow manure, in which disease incidence was signifi-

Fig. 1. Disease index of bacterial wilt in brown lowland soil (Ayabe) (a), dynamics of culturable population of the pathogen *R. solanacearum* YU1Rif43lux1 in the soils (b) and *lux* activity of roots grown in soils for 14 days (c). Each point shows the mean $\pm$ standard error ($n=3$ in (a) and (b)). Values with the same letters are not significantly ($P < 0.05$) different.

cantly lower, *lux* activity was also significantly lower than that in the CF-soil. In yellow soil (Tsukuba), *lux* activity was significantly lower in the OM-soil, although there was no difference in disease index.

This study demonstrated that the bacterial wilt of tomato was significantly suppressed in the yellow soil (Toyohashi) and brown lowland soil (Ayabe) amended with CF+cow manure and with cow manure 3 times, respectively, than in

the corresponding CF-soils. In addition, disease incidence tended to be lower in the yellow soil (Toyohashi) amended with cow manure or poultry manure than in the CF-soil. Therefore, it was likely that repeated application of organic matter resulted in a suppressive effect on bacterial wilt. Naturally occurring suppressive soils active against bacterial wilt of tobacco have been reported by Koga *et al.*⁵⁾ who found suppressiveness in a variety of soils, including volcanic soils and non-volcanic soils with different texture from sand to clay loam. This study indicates that organic matter management can create artificial suppressive soils.

There were, however, no significant differences in disease index in the other four soils between the CF- and OM-soils. These results suggest that repeated application of organic matter does not always bring about disease suppressive properties, and that the kind of organic matter and/or application rate might affect the suppressiveness. In the brown lowland soil (Ayabe), disease suppression was not observed in the soil amended with cow manure at the conventional rate, suggesting that a larger amount of organic matter was likely to be responsible for reducing the bacterial wilt. This result is supported by our earlier report⁴⁾, in which bacterial wilt was suppressed by a single application of farmyard manure and poultry manure at a rate of 40 g kg⁻¹ soil, but not at a rate of 20 g kg⁻¹. Complicated results, however, were obtained in the yellow soil (Toyohashi): The application rate and year were smaller and shorter respectively in the CF+CowM soil having disease suppressive properties, than in the brown lowland soil amended with cow manure having no such properties. More experiments are necessary to conclude that a relation exists between disease suppressiveness and repeated application of organic matter.

In the FYM-soil, highly suppressive of bacterial wilt³⁾, the survival of the pathogen was poorer than in the CF-soil and multiplication of the pathogen in the roots was greatly suppressed compared to that in the CF-soil. In six soils used, there were no remarkable differences in survival of the pathogen between the CF- and OM-soils, suggesting that survival of the pathogen may not be different between the CF- and OM-soils. *Lux* activity, however, was significantly lower in the 3CowM-soil (Ayabe), where a significantly lower disease index was observed, compared to that in the CF-soil, suggesting that suppression of growth of the pathogen in the host rhizosphere might be involved in the reduced disease index in the 3CowM-soil. In contrast, *lux* activity did not differ significantly between the CF+cowM-soil and CF-soil (Toyohashi), indicating that growth of the pathogen in roots was not suppressed in the CF+CowM soil, where

the disease index was significantly lower than in the CF-soil. The reason for this contradiction is not clear at present.

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