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Relation between Resistance to Bacterial Wilt and Calcium Nutrition in Tomato Seedlings

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Abstract

To contribute to the development of new integrated practices for the control of soilborne diseases, the relation between the development of bacterial wilt, a serious soilborne disease induced by *Ralstonia solanacearum*, and Ca nutrition in tomato (*Lycopersicon esculentum* Mill.) seedlings was investigated. Increased Ca concentrations in the nutrient solution reduced the disease severity in the seedlings of resistant cultivars, and decreased the populations of the pathogen in stems. This Ca-dependent resistance was also observed in susceptible tomato seedlings grafted onto rootstocks of a highly resistant cultivar. The resistance was affected by the Ca concentration after infection with the pathogen, but not before infection, suggesting that the Ca concentration in the cell walls before infection might not be directly involved in the Ca-dependent resistance. When varietal differences in the resistance and nutrient uptake by the seedlings were examined, highly resistant cultivars were characterized by a high Ca uptake. However, the differences in Ca uptake might not be related to the expression of the resistance, based on the results of experiments using mutually grafted seedlings of cultivars differing in resistance. Application of composts with various Ca concentrations reduced the disease severity, and the degree of reduction was correlated with the increase of the Ca uptake in shoots. These results indicate that the resistance of tomato to bacterial wilt is markedly affected by Ca nutrition of the host.

Discipline: Plant disease / Soils, fertilizers and plant nutrition
Additional key words: Ca, cultivar, disease, *Lycopersicon esculentum*, *Ralstonia solanacearum*

Introduction

In vegetable cultivation in Japan, intensive cultivation with continuous cropping is common, and injury by continuous cropping, mainly due to soilborne diseases, has been a major constraint on vegetable production[16]. Growers avoid the soilborne diseases mainly by soil fumigation with chemical fumigants such as methyl bromide and/or by cultivation of resistant cultivars or grafted plants using resistant rootstocks. However, chemical fumigants are hazardous to human health and the global environment, and it has been decided that the use of methyl bromide as a soil fumigant will be prohibited in future. Moreover, the resistance of cultivars and rootstocks is known to be unstable, especially under unfavorable growth conditions[20]. Thus the development of new integrated practices for the control of the diseases is urgently required.

On the other hand, successive and excessive application of fertilizers and soil amendments, which is commonly carried out in vegetable fields in Japan, results in the accumulation and imbalance of nutrients in soil[18]. Such unfavorable nutrient conditions disturb the plant nutritional status, and promote outbreaks of plant diseases[14]. The elucidation of these phenomena may contribute to the development of new integrated practices for the control of the diseases.

In this context, calcium (Ca) is an important nutrient element. It has been observed that the application of Ca (e.g. lime or gypsum) exerts a suppressive effect on a wide range of diseases[5,10]. Especially, many investigators have reported that increased Ca concentration in plant tissues suppresses some diseases[1,3,4,12,19]. Various mechanisms have been proposed as follows: inhibition by Ca of the activity or gene expression of pectolytic enzymes, especially polygalacturonase, produced by the pathogens as a virulent factor[4,6], increase of the resistance of cell walls to these enzymes by Ca[2,13,17], inhibition by Ca of ethylene production[15,19], or a combination of all these mechanisms.

Therefore, studies were carried out on the relation

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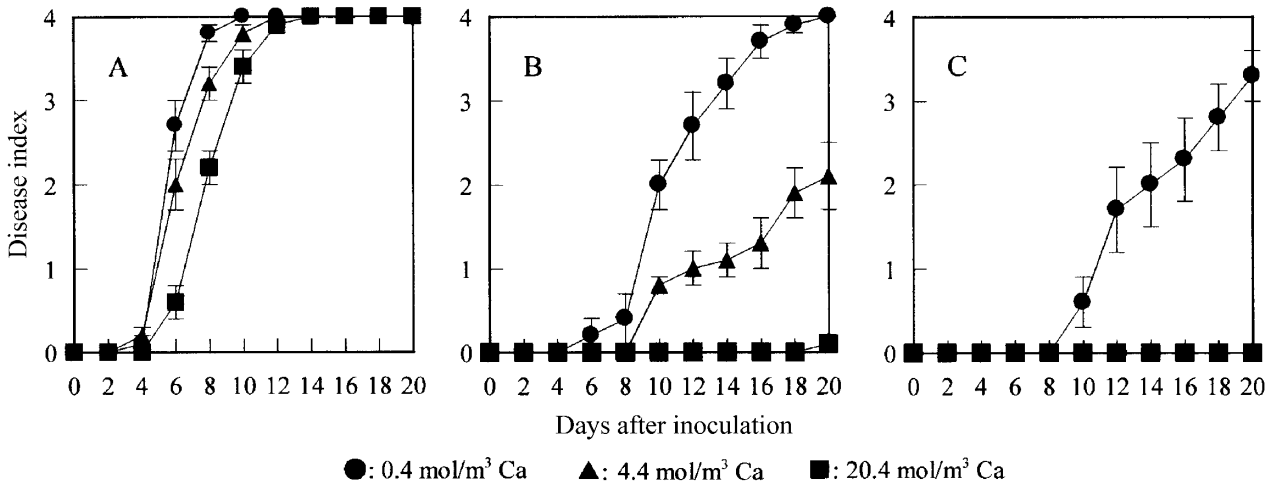

Fig. 1. Effect of Ca concentration in nutrient solutions on the development of bacterial wilt on 3 tomato cultivars
A: Ponderosa (susceptible), B: Zuiei (moderately resistant), C: Hawaii 7998 (highly resistant).
Mean disease indices were determined using 12 seedlings, with scores from 0 = healthy to 4 = dead.
Standard errors are represented as vertical bars.

between Ca nutrition in plants and the development of diseases, especially bacterial wilt induced by *Ralstonia solanacearum* on tomato (*Lycopersicon esculentum* Mill.), which is a serious soilborne disease of tomato plants cultivated in tropical, subtropical, and warm temperate regions of the world[8]. The objectives of this study were to reveal the role of Ca nutrition in the resistance of tomato to the disease, and to elucidate the mechanisms underlying the Ca-dependent changes in the resistance. These may contribute to the development of new integrated control practices of bacterial wilt in tomato. In this report, recent experimental results about the relation between the resistance to bacterial wilt and Ca nutrition in tomato seedlings are described.

Effect of calcium concentration in nutrient solution on the resistance to bacterial wilt

The relation between the Ca concentration in the nutrient solution and the resistance to bacterial wilt was studied using seedlings of 3 tomato cultivars with various degrees of resistance to the disease[21]. Seedlings were transferred to nutrient solutions (pH 5.8) with Ca concentrations of 0.4, 4.4, or 20.4 mol/m^3, representing low, medium, and high concentrations, respectively, and hydroponically grown in a phytotron (12 h daylength, 25/18ºC day/night before inoculation, 28/20ºC after inoculation). One week after the initiation of the Ca treatment, the seedlings were inoculated by wounding the stem with scissors dipped in a suspension (10^8 cfu / mL) of the pathogen. Disease indices were visually recorded on a scale of 0 to 4 (0 = healthy and 4 = dead) for 20 days after inoculation. In a separate experiment, stem segments (5 cm long) were collected from the inoculation sites of the Ca-treated seedlings that were grown and inoculated as described above, 5 days after inoculation, and populations of the pathogen in the segments were counted by plating on a selective medium[7].

Disease development was rapid in a susceptible cultivar 'Ponderosa', and all the seedlings died 2 weeks after

Table 1. Effect of Ca concentration in nutrient solution on *Ralstonia solanacearum* populations in stem segments[a)]

Ca concentration in nutrient solution	Population (log cfu mL^{-1})		
	cultivar[b)]		
(mol/m^3)	Ponderosa (S)	Zuiei (MR)	Hawaii 7998 (HR)
0.4	10.1	9.0	7.1
4.4	9.4	9.5	6.1
20.4	9.1	8.5	5.1

a): Stem segments were collected 5 days after inoculation.
b): S, susceptible; MR, moderately resistant; HR, highly resistant.

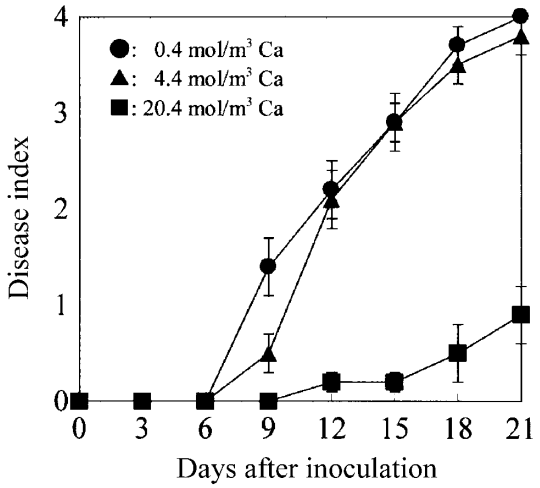

Fig. 2. Effect of Ca concentration in nutrient solution on the development of bacterial wilt on grafted tomato seedlings [scion: 'Momotaro' (susceptible), rootstock: Hawaii 7998 (highly resistant)]

Mean disease indices were determined using 10 seedlings, with scores from 0 = healthy to 4 = dead. Standard errors are represented as vertical bars.

inoculation at all the Ca concentrations (Fig. 1A). However, the Ca concentration in the solution markedly affected the disease severity in a moderately resistant cultivar 'Zuiei'. Disease development on seedlings using a solution with a low Ca concentration was apparently stimulated, although no disease symptoms on plants were present until 18 days after inoculation when a solution with a high Ca concentration was used (Fig. 1B). For a highly resistant cultivar 'Hawaii 7998', disease symptoms were lacking on seedlings treated with Ca at medium and high concentrations during the experiment, while the seedlings treated with a low Ca concentration severely wilted 20 days after inoculation (Fig. 1C). Populations of the pathogen in the stem segments were negatively correlated with Ca concentrations, and were significantly different among cultivars depending on the degree of varietal resistance (Table 1). These results indicate that the resistance of tomato seedlings to bacterial wilt is markedly affected by the Ca nutrition of the host, and that the reduction in the population of the pathogen with increasing concentration of Ca contributes to the Ca-dependent resistance of tomato seedlings.

Effect of calcium concentration on the resistance of grafted tomato seedlings

Similar results were obtained in another experiment using tomato seedlings of a susceptible cultivar 'Momotaro' grafted onto rootstocks of the highly resistant cultivar Hawaii 7998[26]. The grafted seedlings were grown and treated with Ca under almost the same conditions as those described above, and inoculated with a bacterial suspension by stem puncture at the base of the stem of the rootstock. In a separate experiment, xylem exudates were collected from decapitated scions of the Ca-treated seedlings 5 days after inoculation, and Ca concentration and populations of the pathogen in the exudates were determined.

The grafted seedlings treated with low and medium concentrations of Ca in the solution began to wilt 9 days after inoculation, and the disease progressed rapidly (Fig. 2). In contrast, the development of the disease in the seedlings treated with a high Ca concentration was significantly suppressed. Calcium concentration in the xylem exudates collected from scions increased, while populations of the pathogen in the exudates significantly decreased with increasing concentration of Ca in the solution (Table 2). These results indicate that Ca-dependent resistance to bacterial wilt is also observed in grafted tomato seedlings, and that the reduction in the pathogen population in the xylem of the scion probably contributes to the suppression of the disease at high concentrations of Ca.

Table 2. Effect of Ca concentration in nutrient solution on Ca concentration and populations of *Ralstonia solanacearum* in xylem exudates collected from scions of grafted tomato seedlings[a)]

Ca concentration in nutrient solution (mol/m^3)	Ca concentration in xylem exudates (mol/m^3)	Pathogen populations in xylem exudates (log cfu mL^{-1})
0.4	2.01c[b)]	11.4a
4.4	8.39b	10.2b
20.4	28.9a	9.2c

a): Xylem exudates were collected 5 days after inoculation.
b): Means in the same column with different letters were significantly different at $P < 0.05$ according to Tukey's method of multiple comparisons.

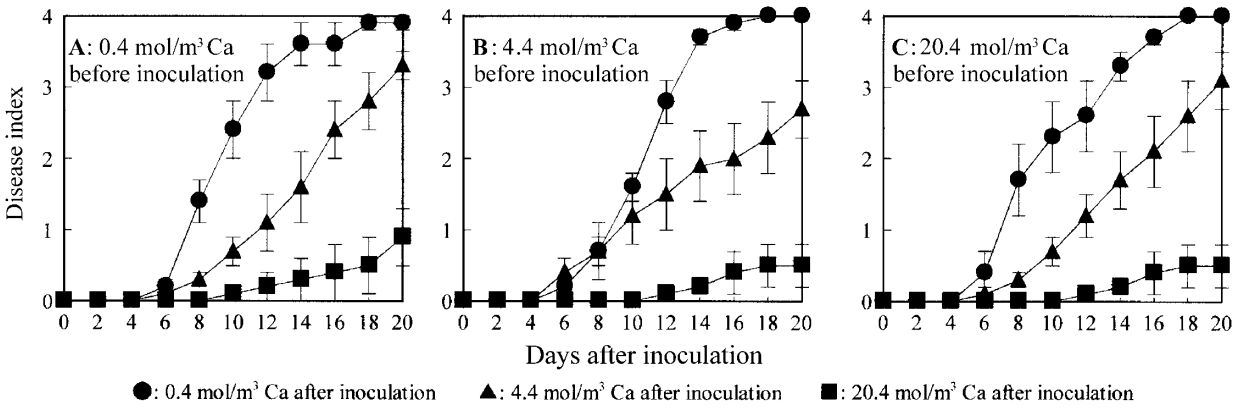

Fig. 3. Effect of Ca concentration in nutrient solution before [(in mol/m^3) A, 0.4; B, 4.4; C, 20.4] and after [(in mol/m^3) ● , 0.4; ▲ , 4.4; ■ , 20.4] inoculation with *Ralstonia solanacearum* on the development of bacterial wilt on moderately resistant tomato seedlings (Zuiei)

Mean disease indices were determined using 10 seedlings, with scores from 0 = healthy to 4 = dead. Standard errors are represented as vertical bars.

Effect of calcium concentration in nutrient solution before and after infection on the resistance

One possible mechanism of the Ca-dependent resistance of tomato to bacterial wilt is the increased resistance of plant cell walls associated with Ca to the pectolytic enzymes produced by the pathogen[2,13,17]. To reveal the role of cell wall Ca in the resistance, the author examined the effect of the Ca concentration in the nutrient solution before and after inoculation with the pathogen on the resistance of tomato seedlings to bacterial wilt[24].

One week before inoculation, tomato seedlings of a moderately resistant cultivar Zuiei were transferred to solutions containing 3 concentrations of Ca (0.4, 4.4, or 20.4 mol/m^3), and grown in a phytotron. Soon after inoculation with a bacterial suspension, the seedlings that were treated with each concentration of Ca before inoculation were transferred to solutions containing the same 3 concentrations of Ca.

Disease development of the seedlings was not affected by the concentration of Ca in the solution before inoculation (Fig. 3). However, a higher concentration of Ca after inoculation reduced the disease severity, indicating that the resistance to bacterial wilt is affected by the concentration of Ca in the host tissues after infection, but not before infection. In this study, the concentration of Ca in the cell walls had probably increased with increasing Ca concentrations in the tissues, based on the increase of Ca supply before inoculation, because a high proportion of Ca in plant tissues is often located in the cell walls[11]. Thus, it is suggested that the concentration of Ca in the cell walls before infection may not be directly involved in the Ca-dependent resistance of tomato seedlings to bacterial wilt.

Varietal differences in calcium uptake and resistance

To reveal the relation between the resistance and nutrient uptake, especially Ca uptake by tomato, varietal differences in the resistance to bacterial wilt and nutrient uptake by tomato seedlings were examined in a pot experiment[22].

Twenty-three cultivars with a wide range of bacterial wilt resistance were selected. Seedlings were transplanted into pots filled with commercial soil, and grown in a glasshouse. Fifteen days after transplanting, seedlings were inoculated by wounding the stem with scissors dipped in a bacterial suspension, and the disease severity was visually recorded for 20 days after inoculation. Twenty days after transplanting, non-inoculated seedlings of each cultivar were sampled and subjected to plant

Table 3. Calcium accumulation in seedlings of tomato cultivars differing in their reaction to bacterial wilt

Reaction to bacterial wilt[a)]	No. of cultivars	Ca accumulation (mg plant^{-1})
Susceptible	12	45.0b [b)]
Moderately resistant	6	49.3b
Highly resistant	5	58.6a

a): Reaction of tomato cultivars to bacterial wilt was based on mean disease indices of each cultivar 20 days after inoculation. Susceptible: 3.0–4.0, Moderately resistant: 0.1–2.9, Highly resistant: 0.

b): Means in the same column with different letters were significantly different at $P < 0.05$ according to Kruskal-Wallis test.

Table 4. Calcium concentration and accumulation in shoots of mutually grafted tomato seedlings

Cultivar[a)]		Ca concentration	Ca accumulation
Scion	Rootstock	(mg g^{-1})	(mg $shoot^{-1}$)
Ponderosa (S)	Ponderosa (S)	15.1	48.9
Ponderosa (S)	Zuiei (MR)	15.3	49.0
Ponderosa (S)	Hawaii 7998 (HR)	20.1	60.7
Zuiei (MR)	Ponderosa (S)	15.0	46.6
Zuiei (MR)	Zuiei (MR)	16.4	48.3
Zuiei (MR)	Hawaii 7998 (HR)	19.1	66.4

a): S, susceptible; MR, moderately resistant; HR, highly resistant.

analysis.

According to the degree of resistance to the disease, 23 tomato cultivars were classified into 3 groups (susceptible, moderately resistant, and highly resistant). Although differences in nutrient uptake among cultivars were observed for all the elements tested, the relation between the resistance and nutrient uptake was observed only for the Ca uptake. Highly resistant cultivars absorbed more Ca than susceptible and moderately resistant ones, and thus, were characterized by a high Ca uptake (Table 3). These results suggest that there is a close relation between the Ca-dependent resistance to the disease and the high Ca uptake in resistant cultivars.

Calcium uptake and resistance of mutually grafted seedlings

To determine the role of varietal differences in Ca uptake on the resistance, the relationship between the Ca uptake and the resistance was examined in a pot experiment using mutually grafted seedlings of tomato cultivars differing in their resistance[25)].

A susceptible (Ponderosa) or moderately resistant (Zuiei) cultivar was grafted onto the rootstock of a susceptible, moderately resistant, or highly resistant cultivar (Hawaii 7998). After acclimation, mutually grafted seedlings were transplanted into pots, each filled with commercial soil, and grown in a glasshouse. One week after transplanting, the grafted seedlings were inoculated with a bacterial suspension by the root-injuring or petiole-cutting method. After inoculation, the seedlings were grown in a phytotron (28/22ºC day/night), and the disease severity was visually recorded as described above. Non-inoculated seedlings in each combination were sampled 11 days after transplanting, and subjected to plant analysis.

The concentration of Ca of the scion was signifi-

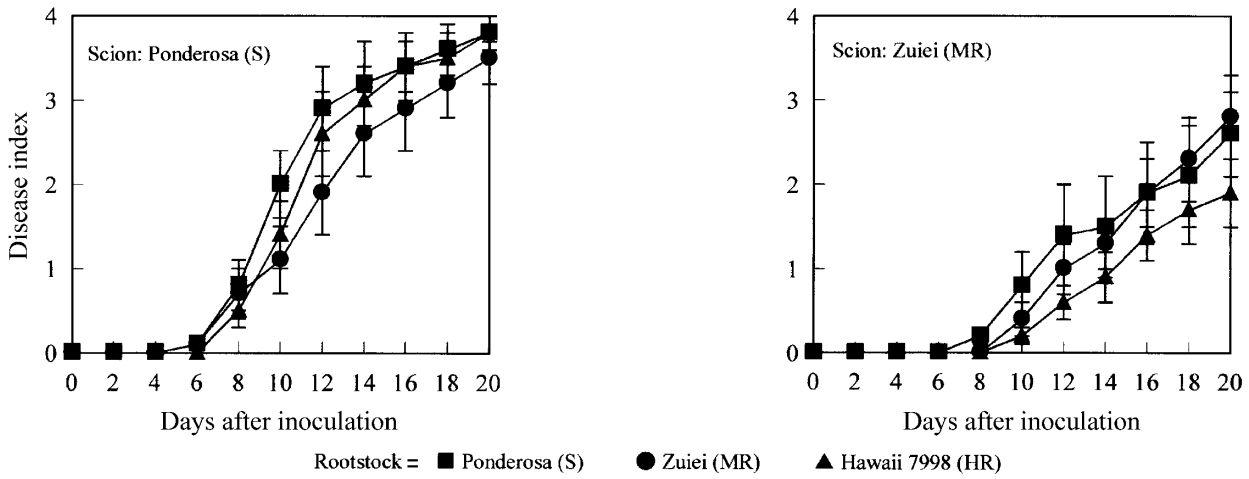

Fig. 4. Development of bacterial wilt on mutually grafted tomato seedlings by petiole inoculation with *Ralstonia solanacearum*
Scions from susceptible (left) and moderately resistant (right) cultivars were grafted onto the rootstocks from susceptible (■), moderately resistant (●), and highly resistant (▲) cultivars. Mean disease indices were determined using 10 seedlings, with scores from 0 = healthy to 4 = dead. Standard errors are represented as vertical bars.

Table 5. Effect of compost application on Ca concentration and accumulation in tomato seedlings

Treatment	Ca concentration in compost (mg g^{-1})	Ca concentration in shoot (mg g^{-1})	Ca accumulation in shoot (mg $shoot^{-1}$)
No compost	–	15.1c[a)]	44.5b[a)]
Bark compost	36.3	22.4b	79.9a
Cattle manure-bark compost	59.8	30.9a	89.6a

a): Means in the same column with different letters were significantly different at $P < 0.05$ according to Tukey's method of multiple comparisons.

cantly high when the scion was grafted onto the rootstock of a highly resistant cultivar, resulting in a significant increase in the amount of accumulated Ca per shoot (Table 4). The symptoms of bacterial wilt in the grafted seedlings inoculated at either the level of roots or petioles depended on the resistance of the cultivar to which the inoculated part of the graft belonged. Among the root-inoculated seedlings, the disease was mild in those grafted onto the rootstock of the resistant cultivars and was severe in those grafted onto the susceptible cultivar (data not shown). Among the seedlings inoculated at the level of the petioles, the disease was severe in the scions of the susceptible cultivar, and the resistance of the cultivar used as the rootstock did not affect the disease severity (Fig. 4). These results suggest that the differences in Ca uptake in the shoots of grafted seedlings may not be related to the expression of the resistance to bacterial wilt.

Effect of compost application on calcium uptake and development of bacterial wilt

It is generally recognized that the application of composts made of organic materials affects the development of a variety of soilborne diseases[9)]. However, the mechanisms underlying the changes in disease severity due to compost application have not been fully elucidated. Based on the results described above, the application of composts containing Ca might have enhanced the Ca uptake by plants, and affected the development of diseases. To confirm this assumption, the effect of compost application on Ca uptake and bacterial wilt development of tomato seedlings was analyzed in a pot experiment[23)].

Two kinds of composts differing in Ca concentration were used in this experiment (Table 5). Tomato seedlings (Ponderosa) were transplanted to pots, each filled with 1 kg of commercial soil, and were grown in a phytotron. Composts were well mixed with the soil at the rate of 20 g / kg. Eleven days after transplanting, the seedlings were inoculated by the soil-drenching, root-injuring, or petiole-cutting method with a bacterial suspension (10^7 cfu / mL for soil-drenching, 10^8 cfu / mL for other methods), and disease indices were visually recorded for 20 days. Non-inoculated seedlings from each treatment were sampled 17 days after transplanting, and Ca concentration in shoots was determined.

Calcium concentration and accumulation in the shoots of the seedlings increased by compost application, especially when cattle manure-bark compost, which had a higher concentration of Ca than bark compost, was

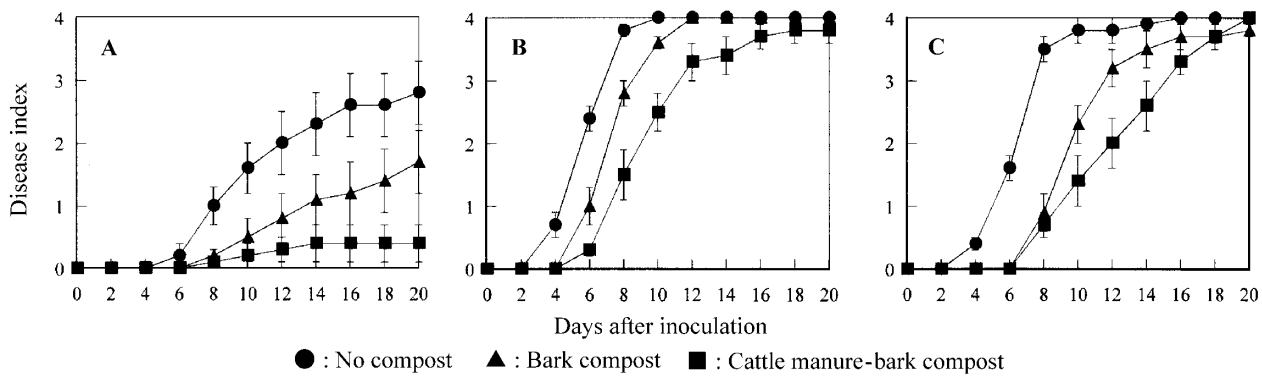

Fig. 5. Effect of compost application on the development of bacterial wilt on tomato seedlings (Ponderosa)
Seedlings were inoculated with *Ralstonia solanacearum* by soil-drenching (A), root-injuring (B), or petiole-cutting (C) method. Mean disease indices were determined using 12 seedlings, with scores from 0 = healthy to 4 = dead. Standard errors are represented as vertical bars.

applied (Table 5). Compost application delayed or suppressed the disease development in all the inoculation methods (Fig. 5). The degree of delay in or suppression of the disease development was high when cattle manure -bark compost was applied, i.e. the degree of reduction in the disease severity was correlated with the increase of the Ca uptake in shoots. These results suggest that increased Ca uptake by tomato seedlings, which is probably supplied from the composts, may contribute to the reduction in the disease severity.

Conclusion

As previously described, the resistance of tomato to bacterial wilt is markedly affected by Ca nutrition of the host. These results may contribute to the development of new methods for integrated control of bacterial wilt in tomato. However, the mechanisms of Ca-dependent resistance to the disease remain unknown, and further studies should be carried out.

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