Effect of Previous Crop on Soil Populations of *Burkholderia solanacearum*, Bacterial Wilt, and Yield of Tomatoes in Taiwan

V. V. Michel, G. L. Hartman, and D. J. Midmore, Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan, 74199 Taiwan (Republic of China)

**ABSTRACT**


Three field experiments were conducted to evaluate populations of *Burkholderia solanacearum* and the occurrence of tomato bacterial wilt in soil left fallow, and following crops of cowpea, eggplant, and rice. Bacterial population declined after cowpea and rice, but not after eggplant. The population also declined after soil was left fallow, indicating that a suitable host plant is required to maintain the bacterial population. The area under disease progress curve (AUDPC), based on the incidence of wilted tomato plants, was significantly (*P < 0.05*) higher following eggplant compared with cowpea and rice for three experiments and in fallow for two of the three experiments. Yields of cowpea, eggplant, and rice were not affected by the presence of *B. solanacearum* in the soil; however, yields of tomato crops were significantly (*P < 0.05*) lower following eggplant than following rice. Under greenhouse conditions, bacterial populations decreased more when soil was cropped to rice under permanently flooded conditions.

Bacterial wilt caused by *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*) is a major soilborne disease of tomato (*Lycopersicon esculentum Mill.*) in the tropics and subtropics (8). At the Asian Vegetable Research and Development Center (AVRDC), located in southern Taiwan, breeding for resistance to bacterial wilt of tomato has had some success, as a moderately resistant hybrid was reported to have less bacterial wilt than other hybrids (6). Although host resistance has shown some promise for tomato bacterial wilt control in Taiwan, it may be rather site-specific, as a report indicated that the same tomato lines planted in Indonesia, the Philippines, and Taiwan had different levels of resistance to bacterial wilt (5).

Cultural controls have been used in an overall management scheme to control bacterial wilt. More specifically, crop rotation has in some cases been an effective control method, but it has been studied mainly in nontropical environments or uplands in the tropics with tobacco (*Nicotiana tabacum L.*) and potato (*Solanum tuberosum L.*) (10,14,17,26). Some crops, such as rice (*Oryza sativa L.*), were reported to reduce bacterial wilt of potato in Indonesia (31) and Sri Lanka (23). Other crops that are grown in the tropical lowlands before tomato, such as cowpea (*Vigna unguiculata L.*) and eggplant (*Solanum melongena L.*), were reported to decrease and increase bacterial wilt of tomato, respectively (26,30). Cowpea also has been reported as a host for *B. solanacearum* (20).

The objectives of this study were to monitor bacterial wilt of tomato and *B. solanacearum* population in the soil following a crop of either cowpea, eggplant, or flooded rice, and after soil was left fallow, and to determine the effect of irrigation and N fertilization of rice on *B. solanacearum* population under pot-cultured greenhouse conditions.

**MATERIALS AND METHODS**

**Field design and procedures.** Three field experiments were conducted at AVRDC. Experiment 1 started on 26 June 1992 and ended 1 December 1992. Experiments 2a and 2b started on 17 June and 13 July 1993 and ended 24 November and 3 December 1993, respectively. In all the experiments, four different crop sequences were investigated: cowpea-tomato, eggplant-tomato, rice-tomato, and fallow-tomato. In contrast to experiment 1, the factor infested/noninfested soil was added to experiments 2a and 2b. With the exception of the tomato cultivar, experiments 2a and 2b were identical. The soil was Fluventic Dystrochrept, a sandy loam, pH 7.5 (1:1.5 [wt/vol]; air-dry soil/0.01 M CaCl₂), total N = 0.059% and total C = 0.704%. After experiment 1, the field experimental site was deep-plowed and planted to wheat (*Triticum aestivum L.*) for the cool season before infesting the soil again for experiments 2a and 2b. Experiment 2a was located adjacent to experiment 2b at the same site where experiment 1 was conducted the previous year.

A bacterial wilt nursery was established to infest the soil with *B. solanacearum* before starting the experiments. A susceptible tomato line (AVRDC line L-390) was directly seeded in the field. Six weeks after sowing, a stock suspension of PSS4—AVRDC *B. solanacearum* strain 4, biovar 3 (7), race 1 (2)—was grown on 523 medium (11) for 1 day at 30°C and then mixed with distilled water. The stock suspension was diluted in the field with tap water to approximately OD₅₆₀ = 0.3, which corresponded to 6 × 10⁶ CFU/ml on triphenyl tetrazolium chloride (TTC) medium (12). Leaves were clipped with sickles prior to inoculation. Plants wilted 2 weeks later and were incorporated in the soil with a rotovator.

Experiment 1 was arranged in a randomized complete block design with four replications. Experiments 2a and 2b were arranged in a split-plot, randomized complete block design with three replications. The main treatment was infested/noninfested soil, as only half the plots were infested with *B. solanacearum*. In the noninfested plots, corn (*Zea mays L.*) was grown instead of tomato. The corn was cut at ground level when the tomato plants were inoculated in the bacterial wilt nursery. The aboveground corn parts were removed from the field. In all experiments, adjacent plots were separated by 1 m. Blocks were separated by 2 m. Furrow irrigation and drainage were designed so that surface water from one experimental plot could not contaminate another plot. In experiments 2a and 2b, all major field management practices such as soil preparation, forming beds, and transplanting were first completed in the noninfested plots to avoid contamination of the infested plots. Different sets of tools were used for the infested and noninfested plots.

Cowpea, eggplant, and rice were planted in the field 1 to 2 days after infesting the soil with *B. solanacearum*. Cowpea (land-
race Matou #1) was sown in two rows per bed, with a spacing of 0.7 m between and 0.1 m within rows. Before sowing, basal P-K fertilizer was broadcast and incorporated at a rate of 40 kg of P₂O₅ and 110 kg of K₂O per ha. No N fertilizer was applied. Eggplant seedlings (var. Pingtung Long, resistant to bacterial wilt) were raised in a greenhouse in 9-cm-diameter plastic pots filled with a steam-sterilized 1:3:1:1 mixture of sand/field soil/rice husk/sugarcane compost. Four- to 7-week-old seedlings were transplanted in two rows per bed with a spacing of 0.6 m between rows and 0.4 m within rows. Before transplanting, basal N-P-K fertilizer was broadcast and incorporated at a rate of 40 kg of N, 80 kg of P₂O₅, and 70 kg of K₂O per ha. The beds were covered with rice straw mulch. Two N-K side dressings at a rate of 40 kg of N and 40 kg of K₂O per ha per application were applied. In rice plots, beds were flattened and the soil was puddled after application of basal N-P-K fertilizer at a rate of 40 kg of N, 30 kg of P₂O₅, and 60 kg of K₂O per ha. Commercial rice seedlings (var. 90 Days Sticky Rice) were transplanted with a spacing of 24 × 24 cm between hills. Rice plots were flooded permanently until harvest and two N side dressings, each of 40 kg of N per ha, were applied.

All experimental plots were hand weeded. Areas between plots were kept weed-free with herbicide (glyphosphate). Insecticides and fungicides were used to minimize crop damage and yield losses as needed. Furrow irrigation was done regularly in accord with rainfall. Fallow plots were irrigated at the same rate as cowpea and eggplant plots.

At harvest, aboveground parts of rice and eggplant were removed from the field. Aboveground biomass of cowpea was removed in experiment 1 but not in experiments 2a and 2b. Basal N-P-K fertilizer, at a rate of 40 kg of N, 80 kg of P₂O₅, and 70 kg of K₂O per ha, was broadcast in all plots for the subsequent tomato crop. Plants were rotated and the beds were rebuilt with the same irrigation and drainage system before tomato seedlings were transplanted.

Tomato seedlings were raised the same way as eggplant seedlings. An indeterminate commercial tomato hybrid (var. Farmers 301, susceptible to bacterial wilt) was used in experiments 1 and 2b. For experiment 2a, a determinate AVRDC tomato line (CL-5915-93Dq-1-0-3-0, moderately resistant to bacterial wilt) was used. Seedlings were transplanted 4 to 5 weeks after sowing in one row per bed, with spacings of 0.5 m and 0.3 m within the row in experiments 1 and 2a/2b, respectively. The plot size was 10 × 1 m in experiment 1 and 6 × 1 m for either infested or noninfested soil in experiments 2a and 2b. The total number of tomato plants per plot was 20 in all experiments. Soil was covered with rice straw mulch and dark gray plastic mulch in experiments 1 and 2a/2b, respectively. In all experiments, an N-K side dressing, at a rate of 40 kg of N and 40 kg of K₂O per ha per application, was applied twice. Pest control was done as for the previous crops.

**Enumeration of B. solanacearum from soil.** Soil samples were taken at the following times: (i) after infestation of the soil; (ii) after establishment of the first crop; (iii) at harvest of the first crop before transplanting tomato; (iv) after establishment of tomato; and (v) at harvest of tomato. These times corresponded to 7, 35, 80, 105, and 158 days after infestation (DAI) of soil with B. solanacearum; 0, 35, 62, 89, 118, and 160 DAI; and 0, 35, 76, 112, and 143 DAI for experiments 1, 2a, and 2b, respectively. Soil was removed from each plot to a depth of 5 to 20 cm with a 3-cm-diameter soil auger. Ten and
four subsamples per plot were pooled in 1992 and 1993, respectively. Subsamples were collected between the tomato row and the bed edge alternately on both sides of each row to minimize root injury. The soil removed at each sampling was at least 20 cm away from the previous sampling site. Between plots, sampling tools were carefully cleaned, and for experiments 2a and 2b all the plots with noninfested soil were sampled before the plots with infested soil.

To avoid moving soil on footwear, no sampling was done after heavy rainfall.

Immediately after sampling, 10 g of soil per sample was added to 90 ml of sterile, distilled water in a 250-ml Erlenmeyer flask and shaken for 30 min before serial dilutions were made on modified SM-1 medium (32). The following components were added to the basic TTC medium: 5 μg of crystal violet per ml, 100 μg of polymyxin B sulfate per ml, 20 μg of tyrothricin per ml, 5 μg of chloromycin per ml, and 5 μg of cycloheximide per ml. The plates were incubated at 30°C. CFU counts were irregular, fluidal, white forms with white or pink centers, after 2 to 3 days incubation at 30°C. A 20-g sample of soil was oven dried to determine soil dry weight.

Wilt data. Eggplant and tomato wilt were recorded weekly in the late afternoon (except when heavy rainfalls prohibited access to the field) starting immediately after transplanting. A plant was counted as wilted if at least one leaf was partially wilted. Plants at the end of each row were not rated. The area under disease progress curve (AUDPC) was calculated based on wilt incidence (25).

Yield data. Yield was not measured in experiment 1. In experiments 2a and 2b, cowpea, eggplant, and tomato were harvested a number of times. Rice and cowpea grain was oven dried before weighing. At rice harvest, all remaining immature cowpea grains were also collected. Eggplant and tomato yields were measured as fresh weight. Each eggplant harvest was terminated at the time of rice harvest even though new eggplant fruits were still developing. At final tomato harvest, fully developed green fruits were included in yield. Total plot yields, including border plants, were assessed for cowpea and rice, while for eggplant and tomato, plants at the end of each row were excluded from yield assessment.

Data analysis. The number of CFU per g of dry soil was transformed (logarithmic [x + 1]) for statistical analysis (28). Wilt and yield data were subjected to analysis of variance and least significant difference (P < 0.05) was used for means separation (16).

Greenhouse experiments. Two greenhouse experiments were conducted from 22 March to 7 July 1993 and from 15 July to 6 October 1993. The two experiments consisted of the same treatments. Rice was grown in infested soil with two irrigation factors (flooded/nonflooded) and two N fertilizations (with/without N fertilizer) in all possible combinations. A control treatment without rice was included to provide information about survival of B. solanacearum in noncropped soil. Noninfested soil without rice plants was used as a second control to determine whether there was any natural occurrence of B. solanacearum in the soil. Soil collected adjacent to the field experimental site was sieved (1-cm mesh size) and air dried. Strain PSS4 was grown on 523 medium at 30°C for 1 day. A stock suspension was prepared with distilled water and adjusted to OD600 = 0.3. The stock suspension was diluted 1:4 with tap water prior to adding to the air-dried soil. The PSS4 suspension/soil ratio was 1:10 (vol/vol). The soil and suspension were mixed thoroughly, covered with black plastic sheets, and incubated for 6 days at room temperature. Infested soil was trans-

![Fig. 2. Bacterial wilt incidence in tomato following cowpea, eggplant, rice, or fallow. A tomato plant was considered wilted when at least one leaf was partially wilted. (A) Experiment 1 (1992). (B) Experiment 2a (1993). (C) Experiment 2b (1993). Ybar represents standard error of a mean.](image-url)
ferred to the experimental units (= boxes). For a control, soil was mixed with distilled water instead of PSS4 suspension.

Plastic boxes (50 × 30 × 30 cm) were equipped with a drainage system to control the water level. Two centimeters above the bottom, one side of the box was perforated and a 1-cm-diameter silicon tube was inserted. The tube inside the box was punctured at 5-cm intervals and was then embedded into gravel that was covered with a layer of synthetic insulation material and a double layer of nylon net (1-mm mesh size) to avoid the obstruction of the drainage system by soil particles. The boxes were insulated on all external surfaces with 2-cm-thick Styrofoam sheets to avoid excessive heating by solar radiation.

Before each box was filled with 35 liters of infested soil, equivalent rates of basal P fertilizer (30 kg of P₂O₅ per ha) and K fertilizer (60 kg of K₂O per ha) were added and mixed thoroughly with the soil. Urea was added to the treatments with N fertilization at a rate of 30 kg of N per ha as a basal application. The boxes were then arranged in a completely randomized design in a greenhouse with cooling and shading so that the controlled maximum air temperature was 30°C. Each treatment had three replications.

Table 1. Area under disease progress curve (AUDPC) based on wilt incidence in tomato following cowpea, eggplant, fallow, or rice in 1992 (experiment 1) and 1993 (experiments 2a and 2b)

<table>
<thead>
<tr>
<th>Previous crop</th>
<th>Experiment 1</th>
<th>Experiment 2a*</th>
<th>Experiment 2b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>345</td>
<td>71</td>
<td>49</td>
</tr>
<tr>
<td>Cowpea</td>
<td>925</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Eggplant</td>
<td>2,235</td>
<td>670</td>
<td>902</td>
</tr>
<tr>
<td>Fallow</td>
<td>494</td>
<td>180</td>
<td>28</td>
</tr>
<tr>
<td>Probability &gt; F</td>
<td>0.014</td>
<td>0.067</td>
<td>0.001</td>
</tr>
<tr>
<td>Least significant difference (5%)</td>
<td>1,103</td>
<td>506</td>
<td>277</td>
</tr>
</tbody>
</table>

* Only subplot treatments of the main-plot treatment “infested soil” are included.

Table 2. Yields of tomato following cowpea, eggplant, fallow, or rice in experiments 2a and 2b (1993), in which tomatoes were grown in infested (Burkholderia solanacearum) and noninfested soils*  

<table>
<thead>
<tr>
<th>Previous crop</th>
<th>Experiment 2a</th>
<th>Experiment 2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infested soil</td>
<td>Noninfested soil</td>
</tr>
<tr>
<td>Cowpea</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Eggplant</td>
<td>1.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Fallow</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Rice</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Mainplot error*</td>
<td>93.35</td>
<td>189.96</td>
</tr>
<tr>
<td>Subplot error*</td>
<td>384.64</td>
<td>81.32</td>
</tr>
<tr>
<td>Replications (no.)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Least significant difference (LSD) (5%) between subplot treatments for the same mainplot treatment</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>LSD (5%) between different mainplot treatments</td>
<td>1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Soil was infested by growing susceptible tomatoes in the field. Six weeks after seeding, tomatoes were injured and inoculated with B. solanacearum. Wilted plants were incorporated 2 weeks later. In noninfested plots, corn was grown instead of tomatoes.

RESULTS

Field experiments. The initial populations of B. solanacearum were similar in all three experiments and the populations decreased during the first crop and in fallow (Fig. 1A–C). In cowpea, fallow, and rice, B. solanacearum populations declined rapidly to nearly, or completely, undetectable levels, whereas the populations in soils planted to eggplant declined substantially in only one of the three experiments. When tomatoes were grown after eggplant, the bacterial population increased in all three experiments, reaching levels similar to those after initial soil infestation. The population also increased following fallow when the susceptible tomato cv. KY 301 was planted. The B. solanacearum population following cowpea only in experiment 1. B. solanacearum was not detected following rice in any of the experiments. In the noninfested plots in experiments 2a and 2b, B. solanacearum was not detected in soil on any sampling date.

Bacterial wilt of eggplant in the infested plots was 1, 3, and 4% in experiment 1, 2a, and 2b, respectively. Bacterial wilt was not observed in cowpea and rice, and not in eggplant grown in the noninfested plots. None of the treatments suppressed bacterial wilt of tomato completely. The highest wilt incidence occurred following eggplant in all three experiments (Fig. 2A–C). In contrast, very low wilt incidence was observed in rice treatments in all experiments. In experiments 2a and 2b, wilt after cowpea and fallow was as low as after rice. In experiment 1, wilt incidence was slightly higher after cowpea than after fallow, but wilt levels were intermediate compared with eggplant and rice. In the noninfested plots of experiments 2a and 2b, no bacterial wilt of tomatoes occurred.

In all experiments, AUDPC of bacterial wilt of tomato was significantly less after cowpea and rice than after eggplant (Table 1). The AUDPC after fallow was significantly less than after eggplant in two of the three experiments.

The yields of cowpea, eggplant, and rice were not affected by infestation of the soil with B. solanacearum. The average yield of experiments 2a and 2b for the infested/noninfested plots was 0.18/0.18 kg/m² for cowpea, 3.37/3.42 kg/m² for eggplant, and 0.40/0.37 kg/m² for rice. Tomato yields were relatively low, as they were affected by fruit worm (experiment 2a) and black leaf mold (experiment 2b) (Table 2).
both experiments there were no significant differences in yields between treatments in the noninfested plots. In the infested plots of experiment 2a, following cropping in eggplant caused a significantly lower tomato yield than following rice, cowpea, or fallow soil, for which yields were comparable. The tomato yield following eggplant was significantly lower in the infested plots than in the noninfested plots. In experiment 2b only the difference between the highest-yielding tomato after rice and the lowest-yielding tomato following eggplant was significant in the infested plots. Even though yield of tomato in the infested eggplant treatment was less than 60% of the yield in the noninfested treatment, the difference was not significant.

Greenhouse experiments. Initial \( B. \ solanacearum \) populations ranged from 3.4 to \( 8.9 \times 10^6 \) CFU per g of dry soil, but differences between treatments means within each experiment were not significant (\( P < 5\% \)) after transformation into log values. Over a 12-week period, \( B. \ solanacearum \) population of the control treatment declined in the first and second experiment by 1.9 and 1.1 log units, respectively (Fig. 3A,B). During the first 4 weeks after transplanting rice, population differences between treatments were small. Thereafter, \( B. \ solanacearum \) numbers declined considerably faster under flooded conditions than under nonflooded conditions. Decline was enhanced when urea was present, especially in the second experiment. In the first experiment, the effect of urea was obvious 8 weeks after transplanting rice. There was no effect of urea in the nonflooded treatments. In the noninfested control treatment, \( B. \ solanacearum \) was not detectable on any of the sampling dates.

DISCUSSION

Bacterial wilt of tomato is devastating under certain conditions in subtropical and tropical areas of the world. There are few reports about the effect of various crops on soil populations of \( B. \ solanacearum \). In our study of infested field soil, the population of \( B. \ solanacearum \) was comparable to populations found in naturally infested soil (18). A general decrease of the pathogen population in soil has been reported by several authors (3,21,22), indicating that \( B. \ solanacearum \) does not survive well in nonrhizosphere soil. In our study, the population of the bacterium declined once the tomatoes in the bacterial wilt nursery were dead and when soil was planted to nonhosts. Maintenance of a high population of \( B. \ solanacearum \) in soil cropped to eggplant agrees with a previous report that the pathogen multiplies in and is released from the roots of solanaceous crops (13). The pathogen may multiply in host plants without wilt symptoms. In a recent study, inoculated but symptomless tomato plants were all latently infected at the collar level regardless of the cultivar (4).

In the field, the decline in bacterial population in flooded soil planted to rice was not different from that in soil left fallow. The more detailed study in the greenhouse revealed that the decline under continuous flooding was more rapid than the decline in nonflooded soil. Nesmith and Jenkins (19) found that flooding reduced the survival of \( B. \ solanacearum \) in four different soils and that antagonistic microorganisms were important. Urea, as a readily available nitrogen source, might have enhanced the activity of these microorganisms; this would explain the more pronounced effect of the flooded treatments with N fertilizer. The characteristics of the microflora of flooded paddy soils are maintained after drainage (34). This might explain the long-term effect of flooded rice demonstrated in field experiment 1, in which \( B. \ solanacearum \) was still detectable after transplanting tomato but disappeared 2 months later (Fig. 1A). An increased decline of the pathogen population in nonrhizosphere soil planted to rice, compared with soil left fallow, was also reported in the Philippines (21).

Despite the suppressive action of flooded rice on the pathogen population, bacterial wilt of the subsequent tomato crop was not eliminated (Fig. 2). Survival of \( B. \ solanacearum \) in flooded rice fields has been reported in Sri Lanka (24), where low bacterial wilt incidences of potato occurred in fields cropped to paddy rice for several years. Two factors might influence the survival of \( B. \ solanacearum \) in flooded rice fields. First, rice transports atmospheric oxygen to the roots and releases it to the soil, thereby creating an aerobic rhi-

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**Fig. 3.** *Burkholderia solanacearum* populations in artificially infested soil planted to rice under flooded (+ water) and nonflooded (– water) conditions, with (+ N) and without (– N) N fertilizer. Nonflooded fallow soil without N fertilizer served as control. (A) First experiment. (B) Second experiment. Error bar represents standard error of a mean.
Scarcity of water in some areas restricts the extent of flooded-rice cultivation; economical reasons also militate against some farmers from including paddy rice in their crop rotation. In terms of weight, the yield of tomato after eggplant was only 44 to 57% of the yield of rice but, the average yield of eggplant was eight times greater than the rice yield. Depending on current production prices, a rotation of eggplant and tomato might be more profitable than one of rice and tomato. When farm size is limited and agriculture is the only income source, a highly labor-intensive crop such as eggplant might be more convenient than a more labor-extensive crop such as rice. If so, management methods other than crop rotation to control bacterial wilt need consideration.

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LITERATURE CITED


