Induction of Outgrowth Formation on Storage Roots of Sweet Potato due to Plant Pathogenic Bacteria

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ABSTRACT

Storage roots of sweet potato *(Ipomoea batatas)* formed outgrowths in cambium regions after inoculation with plant-pathogenic bacteria as well as some bacteria isolated from the plant rhizospheres. The outgrowths developed not only from the normal vascular cambium regions, but also from anomalous cambium regions. The size of outgrowths differed depending on the bacterial species inoculated, and also on the size of sweet potato slices. The most striking outgrowths were produced by bacteria in the *Pseudomonas syringae* group and several other plant pathogens. The corynebacteria, agrobacteria, and several saprophytic bacteria were either ineffective or produced very weak reactions. The outgrowths were not formed when the inoculated slices were either treated with streptomycin within 6 hours, washed with distilled water prior to inoculation, or left noninoculated for longer than 3 hours.

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There are several bacterial diseases of plants characterized by the development of galls. They include crown gall *(Agrobacterium tumefaciens)* (2), olive knot disease *(Pseudomonas savastanoi)* (9), bacterial gall of Japanese wisteria *(Erwinia milletiae)* (7) and fasciations of sweet pea *(Corynebacterium fascians)* (4). The hyperplastic tissue development in these diseases, except crown gall, is induced by the continued stimulation by the pathogen and considered to be due to an imbalance of the growth regulator substances (1, 2, 4, 5). Some of these bacteria have a restricted host range, and cause diseases only on a few plant species.

We found, however, that the storage root tissues of sweet potato showed a common reaction to the inoculation of many plant-pathogenic bacteria, resulting in the induction of outgrowths or galls. Great differences were noted in size or extent of outgrowths, depending on the bacteria, ranging from rapidly growing outgrowths due to *P. syringae* group at the one extreme, to a negative response due to the corynebacteria, agrobacteria, and saprophytic bacteria at the other.

This paper deals with the results of our work on the induction of outgrowth formation on sweet potato due to bacteria.

MATERIALS AND METHODS.—The isolates of *Corynebacterium michiganense, C. oortii,* and *Agrobacterium tumefaciens* were kindly provided by H. Mukoo, Tokyo University of Agriculture. The isolates of *Pseudomonas tabaci* were obtained from K. Ono, Morioka Tobacco Experiment Station. The isolates of *P. fragi* and *Serratia* sp. were obtained from Y. Yamada, Shizuoka University. The other bacteria all were obtained from the Collection of Phytopathogenic Bacteria at the Laboratory of Plant Pathology, Shizuoka University. The bacteria employed in the study included pseudomonads (15 species), xanthomonads (11 species), Erwiniae (three species), agrobacteria (one species), corynebacteria (three species), *Bacillus* sp., *Serratia* sp., and 18 unidentified bacteria isolated from rhizospheres of chinese cabbage.

Yeast extract-peptone medium was commonly used for growing the bacteria. It contained yeast extract, 5g; polypeptone, 10g; glucose, 10g; distilled water, 1,000 ml with or without addition of 1.5% agar, pH 6.8.

Sweet potato cultivars Kokei 14 and Norin 1, harvested in 1974, were used. The roots were stored in plastic bags at 15 C until used. Roots about 5 cm in diameter were cut into 10-mm-thick slices after thorough washing with water and subsequent surface sterilization with 0.5% sodium hypochlorite solution. The slices were put into petri-dishes, the inner surface of which were previously lined with pieces of sterilized wet filter papers. A loopful of the bacterial growth taken from agar slant cultures was diluted into 0.1 ml of distilled water deposited on the surface of slices and spread with a loop. Subsequently several wounds, about 2-mm deep and 5-mm long, were made with a sharp knife across normal cambium regions and on the central parenchyma regions. Inoculated slices were kept at 30 C. Filter papers in the dishes were moistened daily by supplying a few drops of sterilized distilled water so that the inside of the dishes maintained high humidity. Controls were prepared as above, except that sterilized distilled water was used instead of bacterial inoculum.

RESULTS.—*Ability of plant pathogenic bacteria to induce the outgrowths.*—The size, texture and external appearance of outgrowths were considerably different depending on the bacteria. As shown in Table 1, the most rapid and luxuriant growths were produced by the bacteria of *P. syringae* group such as *P. lachrymans, P. mori, P. phaseolicola,* and *P. syringae* (onion isolates). No differences were noted in the extent of outgrowths induced by different isolates of these bacteria. White, solid outgrowths with smooth surfaces were formed all over the slices 2 days after inoculation and continued to enlarge for several days. They reached a maximum size

(approximately 5-mm thick) in 5 to 6 days after inoculation, and then started to turn brownish in color followed by gradual deterioration. The outgrowths induced by the isolates of *P. tabaci* were suppressed at an earlier stage of development, resulting in the brown discoloration. In this bacterium, furthermore, considerable differences were noted in the extent of outgrowths depending on the isolates used. The outgrowths induced by *P. alboprecipitans* (teosinte and corn isolates), *X. geranii* and *X. pruni* (peach isolates) were similar to those produced by *P. syringae*. The outgrowths induced by these bacteria were collectively formed over the normal vascular cambium regions (3) of the slices 2 days after inoculation, and the large nodules scattering in parenchyma were also formed on some of the anomalous cambium regions (3). In these cases, the wounds given to the normal cambium regions had no particular effect on the induction of outgrowths because the outgrowths developed all over these regions. The isolates of *X. oryzae* and *X. phormicola* produced similar,

TABLE 1. Induction of outgrowth formation on sweet potato slices by bacteria

Bacterial species[a]	Number of isolates used	Outgrowths formed on: Normal cambium without wound	Normal cambium wound	Anomalous cambium without wound	Anomalous cambium wound
P. andropogonis	5	1+[b]	1+	1+	. . .
P. alboprecipitans	2	4+	4+	3+	4+
P. cissicola	4	1+	2+	. . .	1+
P. eriobotryae	4	2+	2+	. . .	. . .
P. lachrymans	2	4+	4+	3+	4+
P. maculicola	2	1+	2+	. . .	. . .
P. marginalis (bean)	1	1+	2+	. . .	. . .
P. marginalis (cucumber)	4	soft rot			
P. mori	3	4+	4+	3+	1+
P. phaseolicola	2	4+	4+	3+	4+
P. panici	1	. . .	3+	. . .	1+
P. solanacearum	5	wet brown rot, or sometimes white, powdery outgrowths			
P. syringae	10	4+	4+	3+	4+
P. tabaci	6	4+,v[c]	4+,v	4+,v	. . .
P. theae	8	. . .	2+	. . .	. . .
Soft-rotting pseudomonads	4	white and rough growths with loose and powdery texture of 1 to 2 mm in depth			
P. fluorescens	1	. . .	1+	. . .	. . .
P. fragi	1	. . .	. . .	. . .	. . .
X. begoniae	2	2+	3+	. . .	. . .
X. campestris	5	. . .	2+	. . .	. . .
X. citri	3	. . .	2+	. . .	. . .
X. geranii	1	3+	3+	1+	. . .
X. nigromaculans	3	. . .	2+	. . .	. . .
X. oryzae	4	1+	2+	2+	. . .
X. phaseoli	2	2+	2+	. . .	. . .
X. phormicola	3	2+	3+	3+	1+
X. pisi	2	soft rot			
X. pruni	2	3+	4+	3+	1+
X. tardicrescens	2	. . .	3+	. . .	2+
X. vitians	2	. . .	3+	. . .	. . .
A. tumefaciens	4	. . .	1+	. . .	. . .
C. michiganense	2	. . .	1+	. . .	. . .
C. oortii	2	. . .	1+	1+	. . .
C. sepedonicum	1	. . .	. . .	. . .	. . .
E. carotovora	3	soft rot			
E. herbicola	3	. . .	. . .	. . .	. . .
E. milletiae	3	. . .	3+	. . .	. . .
Bacillus sp.	1	. . .	. . .	. . .	. . .
Serratia sp.	1	. . .	1+	. . .	. . .
18 isolates of unidentified rhizosphere bacteria (chinese cabbage)	2	soft rot			
	2	. . .	1+,v	. . .	. . .
	4	3+,v	3+,v	2+,v	1+
	10	. . .	. . .	. . .	. . .
Control		. . .	. . .	. . .	. . .

[a]Abbreviated genus names: *P.* = *Pseudomonas*, *X.* = *Xanthomonas*, *A.* = *Agrobacterium*, *C.* = *Corynebacterium*, and *E.* = *Erwinia*.

[b]Arbitrary scale for size of outgrowths. 1+ - 1 mm in diameter, 4+ - 4-5 mm in diameter or in thickness.

[c]Response was variable depending on the isolates used. Figure shows the degree of the most active isolate.

but smaller, outgrowths. *Pseudomonas eriobotryae, P. maculicola, P. marginalis* (bean isolate), *X. phaseoli* and *X. begoniae* produced outgrowths only on the normal cambium regions, but not on the anomalous cambium regions (Fig. 1- A to D).

Isolates of *P. cissicola, P. panici* (rice isolate), *P. theae, X. campestris* (cabbage isolates), *X. citri, X. nigromaculans, X. tardicrescens, X. vitians, E. milletiae* and a pathogenic bacterium isolated from galls on *Melia azedarach* L. caused moderate outgrowths developing mainly at the site of wounds on the normal cambium regions, but scarcely on the other parts. The isolates of *P. solanacearum* (tobacco and tomato strains) scarcely produced any outgrowths on the cambium regions or other parts of the root slices. Although the parenchyma tissues of the slices usually developed moderate wet brown rot, white, powdery outgrowths were sometimes formed depending on either the isolates used or the conditions of incubation. Among the soft-rotting bacteria, *X. pisi, P. marginalis* (cucumber isolates) and *E. carotovora* caused typical soft rot with rapid collapse of the tissues. Some soft-rotting pseudomonads, however, produced outgrowths characterized by a white, powdery and dried appearance all over the surface of slices, from which raised masses of loose cells could be easily removed. Although no evidence has been obtained, it seemed that the quality and/or quantity of pectic enzymes produced by bacteria were responsible for the difference in the response of sweet potato tissues to these soft-rotting bacteria (Fig. 1-E,F).

Outgrowths were scarcely produced by some plant pathogenic bacteria such as *P. andropogonis, A. tumefaciens, C. oortii, C. michiganense* and *C. sepedonicum,* in which the reactions corresponded to those in the control. The same was true with nonpathogenic bacteria such as *P. fluorescens, P. fragi, Serratia* sp. and *Bacillus* sp. In contrast, some bacteria isolated from rhizosphere of chinese cabbage induced outgrowths to various extents. Some of them induced rather dense outgrowths corresponding to those produced by *X. phormicola* or *X. oryzae*. The surface of storage roots were inoculated by the plant pathogenic bacteria as well as some rhizosphere bacteria mentioned above with a needle through the epidermis and maintained on wet nonsterilized soil. The development of outgrowths was observed a few days after inoculation and they soon reached several millimeters in diameter. With prolonged incubation under nonsterile conditions, the nodules were invaded by secondary soil microorganisms and finally collapsed leaving dark-brown pit lesions on the root surface (Fig. 2).

Isolations were made from the tissues of outgrowths formed by inoculating plant pathogenic bacteria as well as the rhizosphere bacteria. The same bacteria used for inoculation were always recovered in large numbers within 24 hours after inoculation from the tissues 3 to 4 mm in depth from the surfaces which were sterilized with a sodium hypochlorite solution. After 3 days, the bacteria could be recovered from 5 to 6 mm deep regions. These results suggest that the bacteria stimulated the outgrowth induction in the interior regions of the tissues. Suspensions of the bacteria which produced the rapid outgrowths were held in a boiling water bath for 10 minutes and death of bacteria was confirmed by plating on nutrient agar prior to inoculation. No outgrowths developed on the slices inoculated with the heat-killed bacteria.

Relation between the number of bacterial cells and induction of outgrowths.—The isolates of *P. phaseolicola, P. syringae* and *X. pruni* were seeded to peptone broth and shaken at 28 C for 24 hours. The bacteria were then serially diluted into 10 ml of peptone broth and 0.1 ml of each dilution was spread on the surface of slices of sweet potato 5 cm in diameter. At the same time, the number of bacterial cells in the original culture was determined by plating on agar. The inoculated slices were given four wounds across normal vascular cambium regions with a knife and put in petri-dishes. They were incubated at 30 C and filter papers placed on the innersurface of glasswares were moistened everyday. Results taken 4 days after inoculation were shown in Table 2. The minimum inoculum density required for the outgrowth development was between 10^5 and 10^6 cells/slice for *P. phaseolicola* and *P. syringae*, but between 10^6 and 10^7 cells/slice for *X. pruni*. Minimum inoculum dose for *X. pruni* was about 10 times greater than for *P. phaseolicola*.

Induction period necessary for the bacteria to start the outgrowth formation.—Sweet potato slices inoculated with *P. phaseolicola* and *P. syringae* by the same procedures mentioned above were treated with streptomycin 0, 1, 2, 3, 6, 10, and 24 hours after inoculation. A 0.2 ml of 1,000 ppm solution of streptomycin sulphate were dropped on the surface of the slices and spread with a loop. Results were taken 4 days after inoculation. Treatment with streptomycin within 6 hours after inoculation, completely inhibited outgrowth formation. Development of outgrowths was also considerably suppressed on the slices treated with streptomycin 10 hours after inoculation. These results indicate that the bacteria multiply and invade the tissues between 6 and 10 hours after inoculation deeply enough to escape the chemotherapeutic effects of streptomycin.

Effect of delayed inoculation on outgrowth formation.—Slices placed in petri-dishes immediately after being cut were incubated at 30 C under humid conditions as usual. *Pseudomonas phaseolicola* and *P. syringae* were subsequently inoculated 0, 0.5, 1, 3, 6, 10, and 24 hours after preparation of the slices. Results were taken 4 days after inoculation. The outgrowths developed on the slices inoculated within 3 hours after they were cut. However, outgrowth formation was somewhat suppressed even on the slices which were left for 1 hour after being cut.

Effect of washing slices immediately after cutting on subsequent outgrowth formation.—Slices were repeatedly washed five times with distilled water immediately after cutting until the dispersion of white latex from the slices into water was not apparent. *Pseudomonas phaseolicola* and *P. syringae* were inoculated after excess water on the surface was removed with cheese cloths. Results were taken 5 days after inoculation. The small nodules developed only at the site of wounds given to the normal cambium regions 4 days after inoculation of *P. phaseolicola*. The growths were, however, stopped at an early stage when the nodules reached 1 to 2 mm in diameter, and did not continue to enlarge. When the slices were washed more thoroughly,

even the small nodules did not appear. No outgrowths were produced on washed slices inoculated with *P. syringae*.

Relation between the size of slices and the induction of outgrowth formation.—Roots were cut into different thicknesses and inoculated with *P. phaseolicola*. The results were taken 4 days after inoculation. No difference was observed between the outgrowths formed on the slices 5- or 10-mm thick. Luxuriant outgrowths measuring 4- to 5-mm thick were formed along the

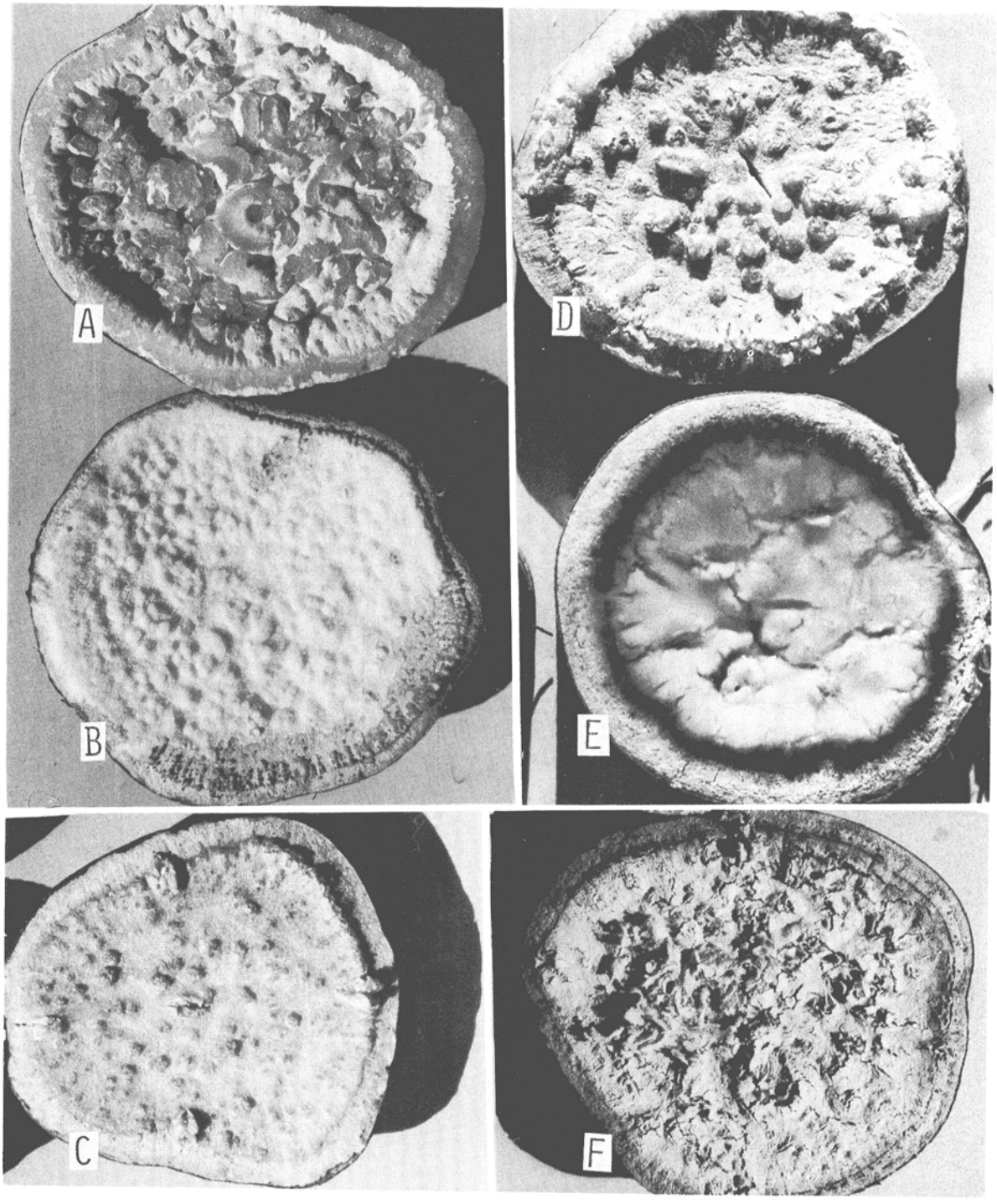

Fig. 1-(A to F). Outgrowths of cambial tissue on sweet potato slices induced by plant pathogenic bacteria. **A)** *Pseudomonas phaseolicola*, **B)** noninoculated control, **C)** *Xanthomonas begoniae*, **D)** *P. mori*, **E)** *X. pisi*, and **F)** a soft-rotting pseudomonad.

normal as well as on anomalous cambium regions after 4 days. On the slices 3 mm thick, however, the outgrowth formation was considerably depressed. Nodules 3 mm in diameter were formed in a line on the normal, but rarely on the anomalous cambium regions. These nodules exhibited rough surfaces in contrast to the white and smooth texture of outgrowths formed on the 5- to 10-mm-thick slices. Furthermore, the nodules turned brown and deteriorated soon after development. On the 1-mm-thick slices, only scattered small nodules were formed on normal cambium regions. These nodules, which measured 2 mm in diameter, also turned dark-brown and deteriorated soon after development. These facts indicated that outgrowths need, for their continued enlargement, a supply of nutrients from the underlying thick healthy tissues.

DISCUSSION.—Storage roots of sweet potato showed the intense reaction of the outgrowth formation with the inoculation of certain plant pathogenic bacteria. The most vigorous outgrowths were always observed on normal vascular cambium regions so that this tissue appeared to be very sensitive to the stimulation by the bacteria. The anomalous cambium regions also showed a positive reaction to some active bacteria such as *P. syringae,* but its response was generally less intensive than that of normal cambium regions. Although the outgrowths were resulted from marked hypertrophy of the cambium and neighboring storage parenchyma cells with a limited extent of hyperplasia (Goto and Makino, *unpublished*), the real mechanisms involved in the reaction are unknown. The outgrowths were not formed following either streptomycin treatment after inoculation, washing slices with water prior to inoculation, or simply leaving slices under humid conditions without inoculation for certain periods of time. These facts suggest that the establishment of the inoculated bacteria in the tissues and their subsequent growth are necessary for the induction of outgrowth formation. Washing slices with water may have depleted the nutrients required for bacterial growth, but it may be possible that the other factors are involved in the lack of response in washed slices because location of wound callus formation is different from that in unwashed slices (Goto and Makino, *unpublished*). The cambium regions no longer responded to the bacterial cells when inoculation was delayed. In this case, the preceding wound-healing reactions may prevent the response of the tissues to bacterial stimuli. It is not clear, however, whether the lack of response of the tissues is due to the competition between the reactions to wound stimuli and bacterial stimuli for growth regulation mechanism, or in some physiological changes of the tissues resulting in the inhibition of bacterial stimuli.

The induction of outgrowth formation due to bacteria is a conspicuous characteristic of sweet potato although it

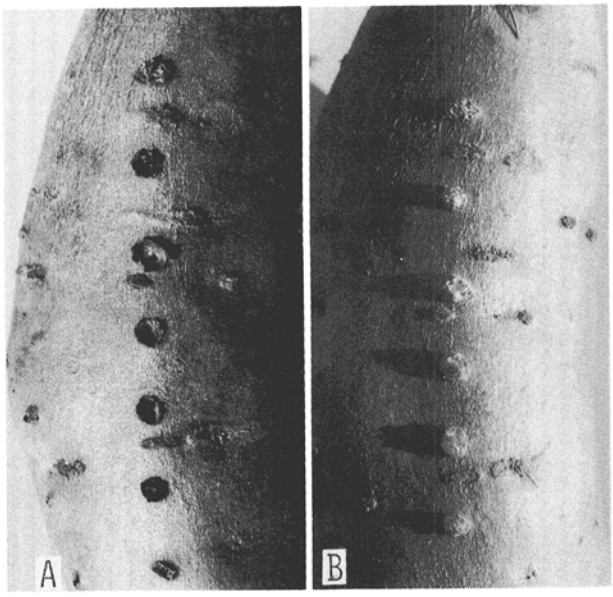

Fig. 2 (A, B). Outgrowth formation by *Pseudomonas phaseolicola* on the surface of sweet potato. **A)** brown pit lesions 4 weeks after inoculation. **B)** white nodules developed 5 days after inoculation.

TABLE 2. Relation between number of bacterial (two *Pseudomonas* spp. and one *Xanthomonas* sp.) cells in the inoculum and induction of cambial outgrowths in sweet potato slices

Bacteria	Experiment I		Experiment II	
	Cells per slice (no.)[a]	Response[b]	Cells per slice (no.)	Response
P. phaseolicola	5.2×10^7	3+	8.6×10^7	4+
	5.2×10^6	2+	8.6×10^6	4+
	5.2×10^5	. . .	8.6×10^5	. . .
P. syringae	5.8×10^7	3+	9.6×10^7	3+
	5.8×10^6	1+	9.6×10^6	2+
	5.8×10^5	. . .	9.6×10^5	. . .
X. pruni	1.9×10^8	4+	1.7×10^8	3+
	1.9×10^7	2+	1.7×10^7	2+
	1.9×10^6	. . .	1.7×10^6	. . .

[a]A 0.1 ml sample of 1/10 serial dilutions of bacterial suspensions was inoculated on slices. The actual number of cells per slice was determined from these dilutions by agar plating method.

[b]Arbitrary scale for size of outgrowths. 1+ - 1 mm in diameter, 4+ - 4-5 mm in diameter or thickness.

has also been observed with *Datura* (6, 8). Some bacteria could stimulate tissue outgrowths at the site of wounds on normal cambium regions, but not on the other parts. It seems that the wounds harbor the introduced bacteria, providing them the appropriate places for multiplication. Therefore, the differences in the outgrowth-inducing ability may be affected by how extensively the bacteria can multiply in the excised tissues. Thus, it seems likely that all active bacteria have something in common and differences observed between bacteria may be quantitative response to whatever is responsible.

Lovrekovich and Loebenstein (6) reported that *P. syringae, P. phaseolicola,* and *P. lachrymans* induced hypertrophy leading to gall formation on *Datura* leaf through inoculation with Carborundum. These bacteria also induced luxuriant outgrowths on sweet potato tissues in our work, but were different in that the heat-killed cells did induce gall formation on *Datura* leaf, but not on sweet potato tissues. Apparently stimuli of living bacteria are necessary for the induction of outgrowth formation in sweet potato, but not in *Datura.*

Some rhizosphere bacteria induced outgrowths. Some of them were pathogenic causing soft rot on vegetables to various extents, but the others were not pathogenic. It may be possible that these bacteria cause the same symptoms on sweet potato through the similar mechanisms of the outgrowth formation, and its subsequent collapse under natural conditions.

LITERATURE CITED

1. BRAUN, A. C. 1959. Growth is affected. Pages 189-241 *in* Plant Pathology. Academic Press, New York and London. 674 p.
2. BRAUN, A. C. 1972. Plant tumor research. Progress in experimental tumor research. Vol. 15. S. Karger, New York. 235 p.
3. ESAU, K. 1960. Pages 197-199 *in* Anatomy of seed plants. John Wiley & Sons, New York. 376 p.
4. GOODMAN, R. N. 1967. Pages 246-257 *in* The biochemistry and physiology of infectious plant diseases. D. Van Nostrand Co., Princeton, New Jersey. 354 p.
5. HILDEBRAND, D. C., J. P. THOMPSON, and M. N. SCHROTH. 1966. Bacterial enhancement of self-limiting outgrowth formation on Datura. Phytopathology 56:365-366.
6. LOVREKOVICH, L., and G. LOEBENSTEIN. 1966. Induction of non-hyperplastic galls by living and heat-killed phytopathogenic bacteria. Phytopathology 56:578-579.
7. OKAJIMA, T., M. GOTO, and N. OKABE. 1972. Histopathology of bacterial gall of Japanese wisteria (Wistaria floribunda). Bot. Mag., Tokyo 85:177-185.
8. SANDS, D. C. 1965. Hypertrophy induced in Datura and tobacco leaves by intercellular injections of bacteria. Phytopathology 55:1074 (Abstr.).
9. WILSON, E. E. 1965. Pathological histogenesis in oleander tumors induced by Pseudomonas savastanoi. Phytopathology 55:1244-1249.