Population Dynamics and Pathogenesis of Pseudomonas syringae in Maize and Cowpea in Relation to the In Vitro Production of Syringomycin

D. C. Gross and J. E. DeVay

Department of Plant Pathology, University of California, Davis, CA 95616. Present address of senior author: Department of Plant Pathology, University of Nebraska, Lincoln, NB 68503.
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ABSTRACT

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Fifteen species of *Pseudomonas* were compared for pathogenicity in maize and cowpea and for synthesis of syringomycin (SR) in agar and broth media. Fifty-five out of 75 isolates of *P. syringae* from various hosts produced SR; some isolates produced up to 3,300 units SR/ml of potato-dextrose broth (PDB). Twenty-eight representative strains of other *Pseudomonas* spp. (primarily plant pathogens) did not produce SR. Partial vacuum inoculation (10^8 cells/ml) of all *Pseudomonas* isolates into maize delineated a marked specificity of grass isolates of *P. syringae* for maize. Maize leaves were infiltrated with either of two isolates of *P. syringae*, pathogenic on maize, with inocula concentrations to give initial populations of 2×10^2 and 2×10^5 bacteria/0.32 cm^2 leaf disk; each isolate grew exponentially for 24-48 hr and attained final populations of 5×10^6 and 3×10^7 cells/leaf disk corresponding to the low and high inoculum concentrations, respectively. The end of the exponential growth phase of the bacteria in leaf tissue marked a cessation of lesion development. Growth of nonpathogenic isolates of *P. syringae* in maize tissue, including both producers and nonproducers of SR, was arrested with the development of a hypersensitive reaction (HR). All isolates that caused holcus spot on maize produced significant amounts of SR in PDB. Maize was at least twice as sensitive to SR than was the standard bioassay fungus, *Geotrichum candidum*. There was a strong relationship between systemic necrosis (SN) in inoculated cowpea hypocotyls and SR production by *P. syringae*. Systemic necrosis was caused by most SR-producing (in vitro) isolates, but not by any of the SR-negative isolates. However, population studies with SN-positive and SN-negative isolates of *Pseudomonas* spp. did not show large differences in multiplication in host tissues.

Pseudomonas syringae [species concept according to Dye et al. (10) in contrast to Doudoroff and Palleroni (9)] causes holcus spot of maize, sorghum, millet, and many other grasses (19); it is also a pathogen on at least 40 different plant genera (35). Few investigators have identified ecological or host-specific types of *P. syringae*. Kendrick (19) described the inoculation of maize with isolates of *Pseudomonas* sp. from maize, sorghum, foxtail, and other grass hosts; however, his results did not indicate whether or not the isolates were a distinctive ecotype. Saad and Hagedorn (29) found specificity for bean for isolates of *P. syringae;* only isolates from bean and lima bean produced typical symptoms of bacterial brown spot at both low and high concentrations of inocula. Pear strains of *P. syringae* were described by Crosse and Garrett (6) and Garrett et al. (13) as an ecotype specific for pear and distinct from citrus isolates based on phage and bacteriocin sensitivity. Ercolani (11) proved the epiphytic success of certain strains of *P. syringae* on pear trees. These results suggested host specificity among the ecotypes of *P. syringae*. In contrast, Otta and English (25) described general susceptibility of peach seedlings to isolates of *P. syringae* from other hosts. Lai and Hass (22) inoculated cowpea leaves with over 45 isolates of *P. syringae* from various hosts; they found that peach and cowpea were suitable hosts for screening pathogenic isolates of *P. syringae* regardless of their host of origin. Arsenijevic (1) reported similar results from inoculations of herbaceous and woody plants with *P. syringae*. However, these observations on peach, cowpea, and other plants (1, 4, 16, 27, 28, 31) did not distinguish disease from the hypersensitive reaction (HR) that is induced at high inoculum concentrations.

Pathogenic isolates of *P. syringae* characteristically produce syringomycin (SR) (2, 32, 33). DeVay et al. (7) ascribed a major role for this toxin in the bacterial canker disease of peach and the systemic necrosis (SN) disease on cotton (31) caused by *P. syringae*. Hoitink et al. (17) found that all isolates of *P. syringae* from bean that caused bacterial brown spot also produced SR. However, Otta and English (25), Baigent et al. (4), and Perlasca (26) found isolates of *P. syringae* that were pathogenic on peach seedlings, but were not inhibitory to *G. candidum* on potato-dextrose agar (PDA). Also, Rudolph et al. (28) found no positive correlation between SR production by various bacterial isolates on PDA and pathogenicity or virulence on bushbean.

In the present study, the production of SR by plant pathogenic and saprophytic species of *Pseudomonas* was compared. Their production of SR in semisolid and liquid media was quantified and compared with their ability to cause holcus spot on maize or a systemic necrosis (SN) disease on cowpea. Of particular interest was whether or not the nonhost-specific phytotoxin, SR, contributes to pathogenesis in more than one disease (33) within the wide host range of *P. syringae*. The population dynamics and pathogenesis of *P. syringae* in maize and cowpea were determined (i) to differentiate disease symptoms from the hypersensitive reaction (HR), and (ii)

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to determine the relationship between SR production and symptom development.

MATERIALS AND METHODS

Bacterial isolates.—All bacterial isolates (Table 1) were either single-cell cultured by the method of DeVay and Schnathorst (8) or restreaked for colony uniformity on King's MB (20). The isolates were stored on MB and in water suspensions (10^6 cells/ml) at 4 C. All isolates were checked for oxidase reaction (34), ultraviolet (UV) fluorescence (20), arginine dihydrolase (36), and tobacco hypersensitivity (21). The *P. syringae* isolates used in the population studies were Ps 17 from maize (*Zea mays*) in

TABLE 1. Comparison of strains of *Pseudomonas* species for syringomycin (SR) production on potato-dextrose agar (PDA) or potato dextrose broth (PDB) and virulence on maize and cowpea[a]

Pseudomonas spp.	Host of *P. syringae* isolates	Isolates[b] (no.)	Production		Holcus spot or HR on maize[e]	Cowpea disease or HR[f]
			PDA-SR[c]	PDB-SR[d]		
P. syringae	grasses	12	+	+	+	+
		2	+	+	HR	HR
		1	+	+	−	−
		5	−	+	HR	HR
		9	−	−	HR	HR
	bean	1	+	+	+	+
		1	+	+	HR	+
		1	−	+	HR	+
		1	−	+	HR	HR
	sugar beet	2	+	+	HR	+
		1	+	+	+	+
	tomato	2	+	+	HR	+
	pepper	1	−	−	HR	HR
	stone fruits	13	+	+	HR	+
		1	+	+	HR	HR
		1	−	+	HR	+
		1	+	+	+	+
		9	−	−	HR	HR
	pome fruits	4	+	+	HR	+
		1	+	+	HR	HR
	lilac	2	+	+	HR	+
		1	−	−	HR	HR
	walnut	1	+	+	HR	+
	rose	1	+	+	HR	+
	lemon	1	+	+	HR	+
P. pisi, et al.[g]		22	−	−	HR	HR
P. fluorescens		3	−	−	−	−
P. cepacia		1	−	−	ND	ND
P. avenae[h]		2	−	−	−	ND

[a]Table 1 presents only a portion of the data. Other data are available in complete form upon request.

[b]Strains were received from A. K. Vidaver, University of Nebraska, Lincoln; J. Otta, South Dakota State University; M. P. Starr, International Collection of Phytopathogenic Bacteria, Dept. of Plant Pathology, University of California, Davis.

[c]Production (+) or absence (−) of a zone of inhibition to *Geotrichum candidum* surrounding the margins of bacterial colonies. Average of four tests per plate.

[d]Production (+) or absence (−) of significant quantities of SR per 250 ml of PDB culture. Average of two cultures per strain.

[e]Maize selection, ACCO WF9-C, was used. + = virulent; HR = hypersensitive reaction; − = no reaction; ND = not determined. Each reaction value was based on an average of two pots, four plants per pot.

[f]Cowpea systemic necrosis disease. Cowpea cultivar Wisconsin Blackeye was used. Disease evaluation was done as described in footnote c. Each reaction value was based on an average of at least four plants.

[g]*Pseudomonas pisi*, *P. tabaci*, *P. coronafaciens*, *P. mori*, *P. phaseolicola*, *P. glycinea*, *P. tomato*, *P. lachrymans*, *P. mors-prunorum*, *P. savastanoi*, and *P. viridiflava* (10).

[h]Pathogenic on maize but caused symptoms unlike those of holcus spot (30).

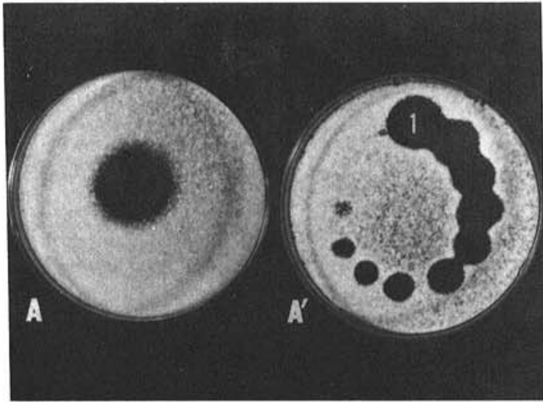

Fig. 1. *Geotrichum candidum* bioassay method for syringomycin (SR) production by *Pseudomonas syringae*, isolate B-301D. Plate A = SR production on 10 ml PDA after 6 days. Inhibition zone was approximately 8 mm from the edge of the bacterial colony. Plate A′ = SR extract from a 6-day-old PDB still culture. Sample was diluted 12-fold in a 1:1 dilution series from a 4-ml aqueous concentrate (spot #1) of the original *n*-butanol SR extract. Recovery of SR from the 250-ml broth culture was 20.48×10^4 units.

Nebraska; 5D430 from millet (*Panicum milaceum*) in Australia; 475 from foxtail (*Setaria lutescens*) in South Dakota; B-301D from pear (*Pyrus communis*) in England; and Ps 251 from plum (*Prunus domestica*) in New Zealand.

Syringomycin production in culture media.—Each isolate of *Pseudomonas* sp. was grown on PDA or in potato-dextrose broth (PDB) (14, 33) for in vitro production of SR. The PDA plates received a 10-μliter droplet of a 24-hr nutrient broth (NB) culture of each bacterial isolate in the center of each of four plates and then incubated 6 days at 23 C. The plates then were sprayed with a suspension of *G. candidum* (33) and inhibition zones were measured from the margins of bacterial colonies 24 hr later (Fig. 1). Also, for each isolate 0.5 ml of the NB inoculum was added to each of two PDB flasks and incubated in still culture for 6 days at 23 C. The PDB cultures then were killed with an equal volume of acetone, acidified, and extracted with *n*-butanol to recover SR (14). The extract was concentrated and adjusted to 4 ml, serially diluted (1:2, v/v) tenfold with 0.01 N HCl, and bioassayed with *G. candidum* (33) (Fig. 1).

Host plants.—Two selections of maize were used: ACCO WF9-C (ACCO Seed Co., Belmont, Iowa), and NK PX89 (Northrup King Co., Modesto, California). Plants were grown in UC potting mix (3) and thinned to

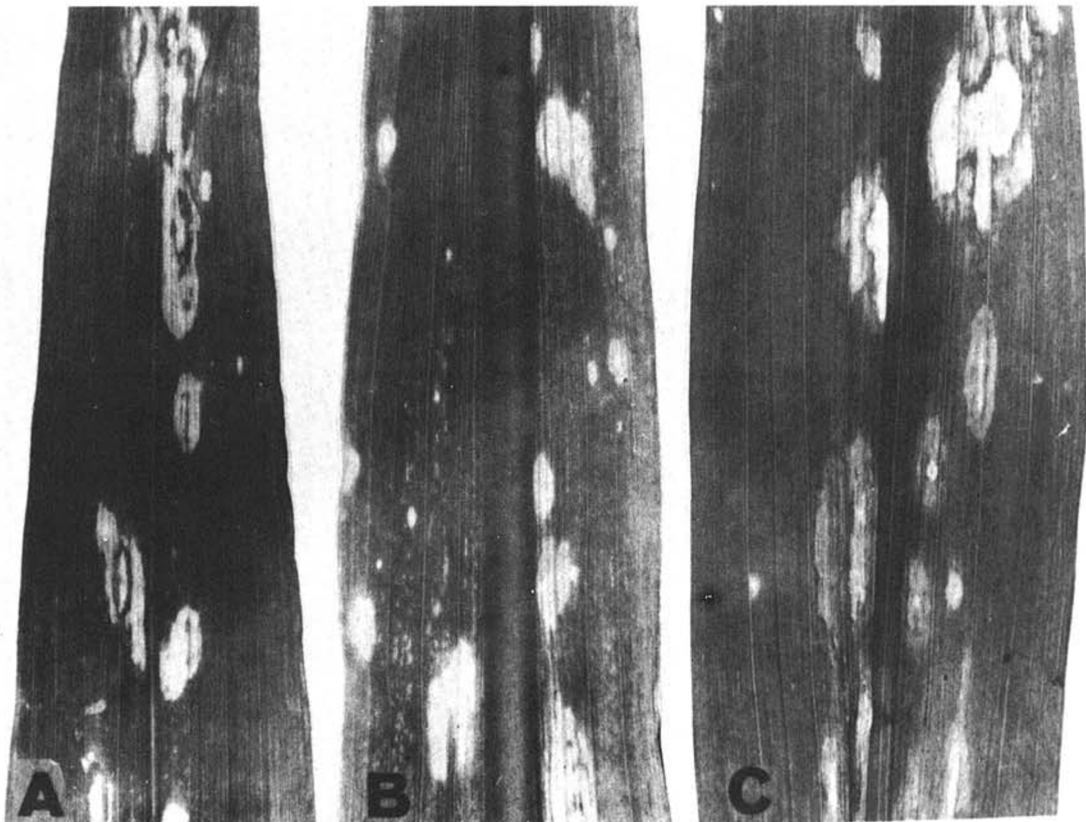

Fig. 2-(A to C). Holcus spot symptoms on maize 5 days after inoculation with **A)** maize, **B)** sorghum, and **C)** foxtail strains of *Pseudomonas syringae*.

five plants per 10-cm diameter pot. The cowpea (*Vigna sinensis*) cultivar, Wisconsin Blackeye, was planted in 20-cm pots (10 seeds/pot) containing UC potting mix. Plants were grown in the greenhouse at 20 to 25 C with daylength ranging from 12 to 14 hr.

Inoculum preparations for plant inoculations.—Bacterial inocula were grown in NB for 20 hr at 23 C in shake culture. The cultures were centrifuged at 11,800 *g* for 20 min; the pellet was resuspended in distilled water to give concentrations of approximately 10^8 and 10^5 cells/ml. Triton X-100 (0.1%) was added to the bacterial suspensions.

Maize inoculation techniques.—*Whorl method.*—Pathogenicity tests of all the bacterial isolates were made on 2- to 3-wk-old maize seedlings (ACCO WF9-C). Plants were sprayed with bacterial suspensions (10^8 cells/ml) until run-off, placed in a vacuum chamber (15), and subjected to a partial vacuum of 15 cm Hg, which was released suddenly; the procedure was repeated once. The plants were placed in a mist chamber for 2 days at 20-25 C and then moved back to the greenhouse bench at 20-25 C. Disease ratings were made on the 5th day after inoculation.

Partial-vacuum leaf immersion method.—Two-wk-old

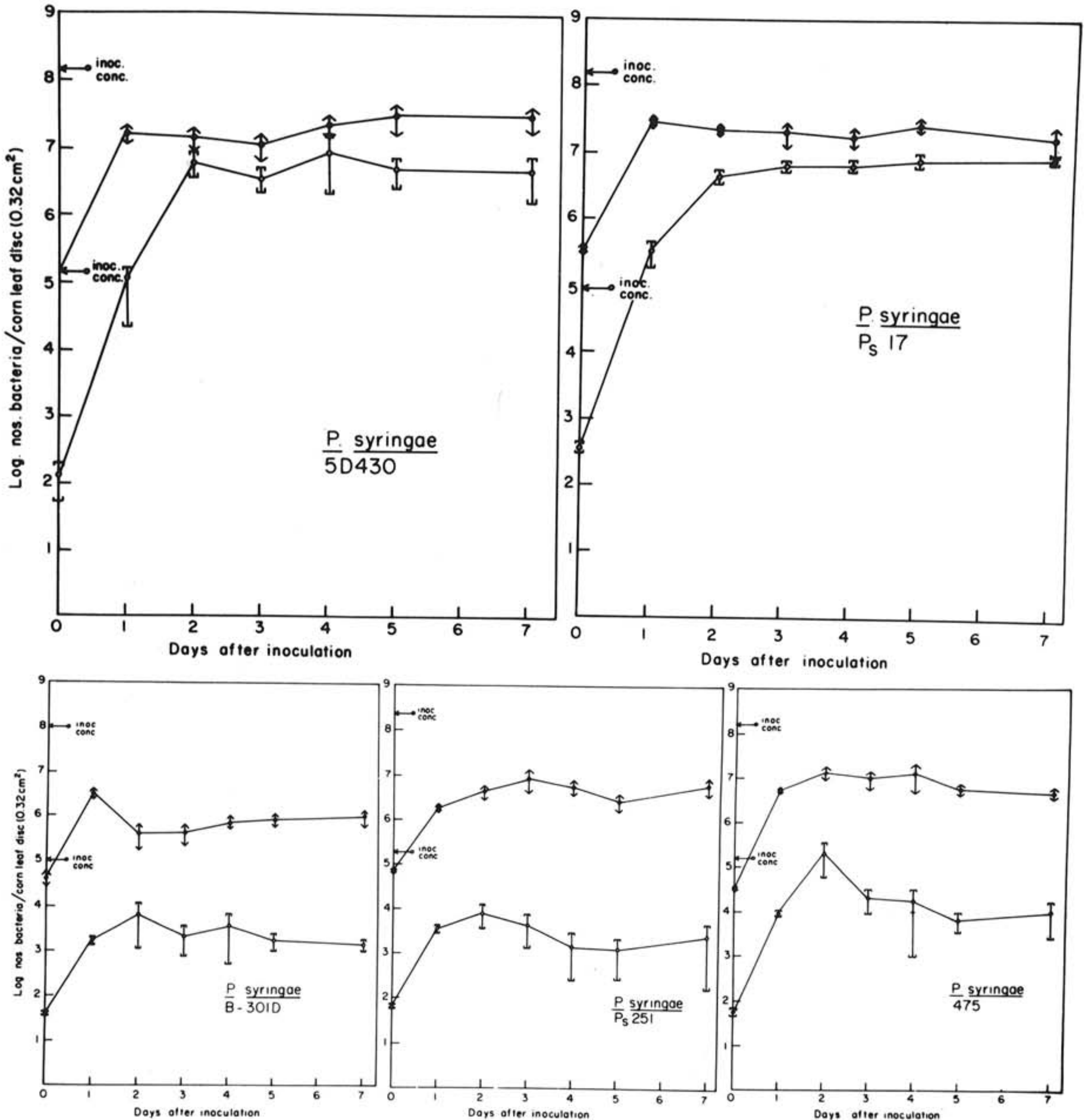

Fig. 3. Growth curves of *Pseudomonas syringae* strains 5D430, Ps 17, B-301D, Ps 251, and 475 in maize (corn) leaves. Concentrations of inocula were 10^8 bacteria/ml (•) and 10^5 bacteria/ml (o). Both inocula concentrations for all five strains are denoted by the arrows at day 0. Standard deviations were calculated for 10^8 (↕) and 10^5 (I) at the various intervals.

maize seedlings (NK PX89) were inoculated by a partial vacuum infiltration technique for studies on changes in bacterial populations in the leaves. The soil surface surrounding the plants was covered with moist paper towels; each pot was inverted and the aerial portions of plants were immersed in the proper dilutions of inocula contained in 1-liter crocks placed in the vacuum chamber and vacuum-infiltrated as described above. The plants then were rinsed under running water and placed in the greenhouse without incubation under mist.

Leaf-sampling method for bacterial populations.—Maize leaves (NK PX89) inoculated by the immersion method were sampled on days 0, 1, 2, 3, 4, 5, and 7. Leaf disks (0.32 cm^2) were cut with a paper punch. Four plants were sampled daily starting from the leaf tip; 10 disks were cut from one side of each of the second and third leaves. The disks from the four plants were placed in a sterile petri plate and mixed. The disks were subdivided into four 20-disk samples (volume, 0.1 ml/sample). Each sample was ground to a slurry in a sterile mortar containing 0.5 ml of sterile Triton-PM buffer, pH 7.25 [7 g K_2HPO_4, 2 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, and 0.1% Triton X-100 in 1 liter of double distilled water (DDW)]. The slurry was diluted with 9.5 ml of sterile Triton-PM buffer followed by standard $\log_{10}$ serial dilutions in sterile PM buffer without Triton X-100. At the appropriate dilutions, three 1-ml samples were mixed with Crosse's crystal violet sucrose medium (6) and incubated for 3-5 days at 23 C. The numbers of bacteria per leaf disk were calculated from the colony counts.

TABLE 2. Doubling times (d.t.) of pathogenic (Ps 17, 5D430) and nonpathogenic (B-301D, 475, Ps 251) strains of *Pseudomonas syringae* in maize leaves (line ACCO WF9-C)

Pseudomonas syringae isolate	Doubling times (hr) following inoculation[a] with:	
	10^8 cells/ml	10^5 cells/ml
Pathogenic:		
Ps 17	3.75	3.50
5D430	3.41	3.04
Nonpathogenic:		
B-301D	3.84	4.41
475	3.27	4.02
Ps 251	5.09	4.16

[a]Leaves were inoculated by the partial-vacuum leaf immersion method.

Cowpea inoculation technique and bacterial population measurement.—For the cowpea inoculations, inocula of approximately 10^8 cells/ml were prepared for each *Pseudomonas* sp. isolate as before, but without Triton X-100. Plant stems of 2- to 3-day-old plants were inoculated with 20 to 30 μliters of bacterial suspension injected with a sterile hypodermic syringe 1 cm below the cotyledon. The plants were rinsed under running water and placed in the greenhouse. Symptoms were read 6 days after inoculation. For the bacterial population study, plants were inoculated with concentrations of 10^8 and 10^5 cells/ml (isolates B301D, Ps 251, 475) as described above and then sampled on days 0, 1, 2, 3, 4, and 6. At time of harvest, 1-cm hypocotyl sections centered at the point of inoculation were excised and rinsed. Sixteen 1-cm sections were collected from 16 plants for each concentration of bacterial inoculum, divided into samples of four sections each, and ground in a mortar with sterile Triton-PM buffer. Standard $\log_{10}$ dilutions were made with sterile PM buffer without Triton X-100; at the appropriate dilutions, three 0.1-ml samples were spread with a glass rod on MB. Bacterial colonies were counted after 3 days at 23 C to determine the number of bacteria per 1 cm of hypocotyl.

Effects of syringomycin on maize tissue.—One mg of SR (from isolate 5D430), purified to a specific activity of

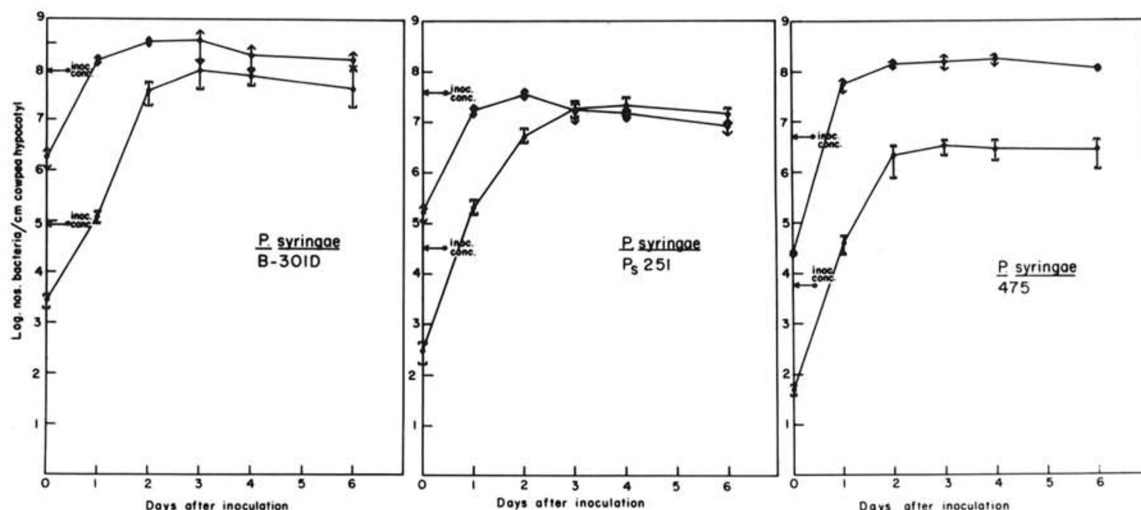

Fig. 4. Growth curves of *Pseudomonas syringae*, strains B-301D, Ps 251, and 475, in cowpea hypocotyls. Inocula concentrations were 10^8 bacteria/ml (•) and 10^5 bacteria/ml (o). Both inoculum concentrations for all three strains are denoted by arrows at day 0. Standard deviations were calculated for 10^8 (‡) and 10^5 (Ī) at the various intervals.

48 units/ μg (15), was lyophilized and then dissolved in 1 ml DDW and diluted serially 1:2 tenfold in DDW in duplicate. Samples from each dilution were bioassayed with *G. candidum* (33). Maize leaves (NK PX89) 15 cm in length from plants 3 wk old were excised and placed in tubes with 1 ml of each dilution of SR. Twenty leaves were tested for the toxicity of each dilution under continuous fluorescent light. Symptoms were read after 24 hr.

RESULTS

Syringomycin production.—Inhibition zones caused by SR in PDA varied from a trace to 20 mm, and SR produced in PDB ranged from zero to 819,200 units/250 ml of medium. Most pathogenic isolates of *P. syringae*, regardless of host origin, produced SR in PDA and PDB. All isolates which produced SR on PDA also produced SR in PDB; however, several isolates (Table 1) produced detectable amounts of SR only in PDB culture.

Apparently SR production was not a characteristic limited to fluorescent forms of *P. syringae*, since two nonfluorescent strains also produced SR. Production of SR was characteristic of strains of *P. syringae* and was not produced by the other pseudomonad nomenspecies included in this study (Table 1).

Pathogenicity tests.—Greenhouse inoculations of maize seedlings (ACCO WF9-C) by the whorl technique with pathogenic (holcus spot) isolates of *P. syringae* produced water-soaked lesions within 48 hrs. After 4 days, the scattered, round, elliptical, or irregular-shaped leafspots reached maximum lesion size, which ranged from 2 to 10 mm in diameter. The leafspots were water-soaked and appeared to be dark-green at first and then became sunken, desiccated, and tan in color. An intense disease reaction was produced within the plant whorl area. The whorl technique was effective in producing a full range of holcus spot symptoms which was useful for comparing bacterial isolates for pathogenicity.

Only 15 of the 75 isolates of *P. syringae* that were tested produced the holcus spot disease (Table 1). Twelve of the 15 isolates that produced holcus spot were isolated from grass hosts; these isolates were highly virulent and able to cause the full range of holcus spot symptoms (Fig. 2). The bean, stone fruit, and sugar beet isolates were weakly virulent on maize. They produced fewer and smaller lesions than the more virulent holcus spot isolates. All of the remaining *P. syringae* isolates, except one, produced a hypersensitive reaction (HR) within the area of the whorl. The HR was characterized by the rapid development of desiccated lesions from less than 1 mm in diameter to larger bleached areas of irregular shape. These HR lesions were nonspreading and were particularly visible 2-3 days after inoculation. The 15 isolates of *P. syringae* that caused holcus spot on maize produced significant amounts of SR in both PDA and PDB. Three isolates of *P. syringae* from holcus spot lesions on grass hosts produced high concentrations of SR in vitro, but were nonpathogenic on maize. This suggested that an ability to grow in maize, usually characteristic of holcus spot pathogens, had been reduced. In addition, there were numerous isolates of *P. syringae* from other hosts which produced SR, but did not produce holcus spot lesions (15). None of the various species of *Pseudomonas*, other than *P. syringae*, caused holcus spot. The plant pathogenic strains (excluding *P. avenae*) produced an HR within the whorl area similar to that produced by the nonpathogenic isolates of *P. syringae*.

Population changes of bacterial isolates in inoculated

TABLE 3. Doubling time (d.t.) of pathogenic (B)301D) and nonpathogenic (475, Ps 251) strains of *Pseudomonas syringae* in cowpea (cultivar, Wisconsin Blackeye) hypocotyls

Pseudomonas syringae isolate	Doubling times (hr) following inoculation [a] with:	
	10^8 cells/ml	10^5 cells/ml
Pathogenic:		
B-301D	3.75	3.48
Nonpathogenic:		
475	2.16	3.11
Ps 251	3.56	3.44

[a]Hypocotyls were inoculated by injection of 20 to 30 μliters of bacterial suspension.

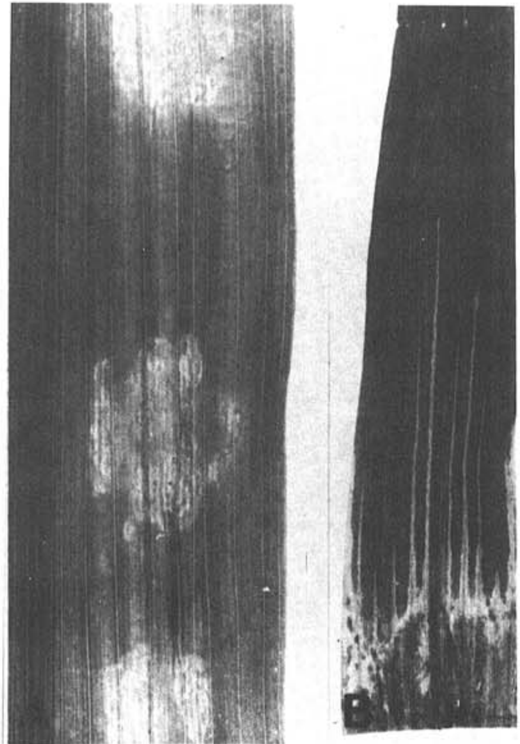

Fig. 5-(A,B). Maize leaves 24 hr after treatment with SR (specific activity 48 units/ μg) isolated from PDB cultures of the holcus spot and SR-positive strain 5D430. Leaf **A**) exhibits stomatal lesions within the area of application of a 10-μliter droplet of SR (1,600 units/ml). Leaf **B**) shows water-soaking, chlorosis, necrosis, and vascular streaking after uptake of SR in the transpiration stream or through stomata. Cut end of leaf directly immersed in a solution containing 1,600 units of SR/ml is water-soaked and chlorotic.

maize seedlings.—Growth curves for the two holcus spot isolates, Ps 17 and 5D430, at 10^5 and 10^8 cells/ml inocula concentrations were similar (Fig. 3) despite more than a 16-fold difference in SR production in vitro. From an initial population of 2×10^5 bacteria/leaf disk, exponential growth occurred during the first day reaching $3\text{-}5 \times 10^7$ bacteria/leaf disk. When the initial population was approximately 2×10^2 bacteria/leaf disk, bacterial growth and symptom development continued until the 2nd day. The end of the exponential phase of bacterial growth for both inocula concentrations was marked by a cessation of symptom development. However, the growth of isolates B-301D and Ps 251, which did not cause holcus spot, was curtailed at approximately 5×10^6 and 5×10^3 bacteria/leaf after growth of initial populations of approximately 5×10^4 and 50 bacteria/leaf disk, respectively. With both isolates, visible HR appeared after 1 day, but only at the higher inoculum concentrations. Isolate 475 exhibited intermediate growth in host tissue when compared with the holcus spot isolates (Ps 17 and 5D430) and the other isolates (B-301D and Ps 251). For isolate 475, at an initial inoculum concentration of 10^5 cells/ml, growth occurred until the second day, and then declined; however, visible HR symptoms developed only at 10^8 cells/ml.

Doubling times were calculated from values interpolated from the growth curves near the beginning and the end of the logarithmic growth phase (Table 2). The doubling times of two holcus spot isolates (Ps 17 and 5D430) at low inoculum concentration were 0.5 to 1.0 hr shorter than those of the nonpathogenic HR-producing isolates.

Population changes of bacterial isolates in inoculated cowpea seedlings.—After inoculation of all bacterial isolates into cowpea, 44 out of 75 isolates of *P. syringae* caused the SN disease reaction on cowpea (Table 1). These isolates were of medium-to-high virulence on cowpea. The remaining isolates of *P. syringae* except one and various other *Pseudomonas* species caused only the HR. No reactions were apparent from inoculations with *P. fluorescens*. The bacteria that caused SN disease in cowpea had no apparent natural host preference for cowpea, as indicated by their original source (Table 1). Many holcus spot pathogens were as virulent on cowpea as several of the pear and stone fruit pathogens. All of the isolates that caused holcus spot on maize also caused the SN disease in cowpea and there was a striking correlation between SN disease and SR production. Forty-four of the 55 SR-producing isolates of *P. syringae* caused the SN disease in cowpea, but none of the SR-negative isolates was pathogenic to cowpea.

Inoculation of cowpea hypocotyls with B-301D at 10^8 cells/ml, which caused SN disease, resulted in exponential growth during the first day until a population of 6×10^7 bacterial per centimeter of hypocotyl was reached (Fig. 4). Typical SN disease symptoms in hypocotyls occurred on day 3 at 10^5 cells/ml for B-301D. At 10^8 cells/ml, strains 475 and Ps 251 exhibited exponential growth during the first day and eventually reached levels of 2×10^8 and 3×10^7 bacteria/cm of hypocotyl tissue, respectively; HR symptoms caused by both strains appeared after the second day. For strains 475 and Ps 251 at inoculum concentration of 10^5 cells/ml, exponential growth continued to relatively high populations until the 2nd day; reddish-brown discoloration (HR) within the infiltrated tissue was visible after the third day. The doubling times (Table 3) reflected a similarity in growth rate between the SN disease-positive and HR-positive strains. However, the final population for B-301D in hypocotyls ranged from two-to thirtyfold greater than that for the HR-positive strains (475 and Ps 251).

Phytotoxicity of syringomycin on maize leaves.—Syringomycin purified to homogeneity (15) caused water-soaking, chlorosis, and necrosis of maize leaf tissue (Fig. 5). These symptoms first were noticeable after 12 hr and continued to develop for another 10 to 16 hr. Symptoms often were most intense around stomata and leaf wounds. At high concentrations of SR, water-soaking and streaking occurred along the vascular tissue to the leaf tip. Streaks initially were water-soaked then became chlorotic and necrotic. At low SR concentrations, only the portion of the leaf submerged in the SR solution became water-soaked and chlorotic. On the basis of symptom development, maize leaves were about twice as sensitive to SR as the standard bioassay fungus, *G. candidum*, since leaf symptoms developed at the low SR concentration of 50 units/ml (1 μg SR/ml).

DISCUSSION

All isolates of *P. syringae* that caused holcus spot of maize produced significant quantities of SR in vitro, but the amount produced in vitro did not correlate directly with virulence in maize as reported for cotton and peach (7, 31). Contradictory results (4, 25, 27, 28) on SR production on PDA and the pathogenicity of *P. syringae* in peach or bean may have resulted from the use of the variable, and comparatively insensitive, PDA test for SR (33). The wide differences in quantities of SR produced in PDA and PDB by various isolates of *P. syringae* attest to the dependence of SR production on nutritional and environmental conditions, as well as on genetic differences (15).

At present, still culture of *P. syringae* in PDB is the best method for estimating SR production. All SR-positive isolates on PDA produced SR in PDB; moreover, of 75 isolates of *P. syringae* approximately two-thirds produced significant quantities of SR ($\ngtr$ 6 units/ml) in PDB.

It appears that most, if not all, SR-negative isolates were cultural variants from SR-producing isolates of *P. syringae* (7, 24, 33). Loss of exotoxin production also occurs with other pathogenic nomenspecies of *Pseudomonas* (18). In this study, 9 of 29 isolates of *P. syringae* from grass hosts did not produce SR either in liquid or semi-solid PD culture. None of these SR-negative bacteria caused holcus spot or the SN disease of cowpea and cotton tissue (15).

Tests for SR production in PDA and PDB cultures of 12 pathogenic and two saprophytic *Pseudomonas* nomenspecies (excluding *P. syringae*) were negative. It appears that SR production is characteristic of the species, *P. syringae*.

The host specificity of strains of *P. syringae* from grass hosts for maize was apparent, although in this study three isolates of *P. syringae* from bean, cherry, and sugar beet caused weak holcus spot symptoms (Table 1).

Quantitative measurements of pathogenic and nonpathogenic bacterial populations in inoculated maize leaf tissue corresponded to growth patterns described for bacteria in certain other diseases (5, 12, 23). The populations of the holcus spot pathogens, 5D430 and Ps 17, were about 10^5 times greater by the 3rd day after inoculation than were those of the other strains. The relatively low population levels in inoculated maize seedlings for SR-producing strains, exemplified by B-301D, which were pathogenic on certain hosts other than grasses, indicated that some unknown factor in addition to SR was necessary for disease development. In maize leaves, the growth of incompatible strains (e.g., Ps 251), which did not produce SR in vitro was similar to the populations of incompatible strains (e.g., B-301D), which did produce SR in vitro. However, the ability to grow in maize leaves without the capability of SR production or pathogenesis resulted in an earlier cessation of growth compared with that of pathogens like Ps 17. An exception appeared to be the grass strain, 475, which did not possess the ability to produce SR and was nonpathogenic but still grew 100 times better by the 3rd day than strain B-301D or Ps 251 in maize tissue. In the case of strain B-301D, its inability to attain the critical growth level in host tissue necessary for production of toxic levels of SR may explain its nonpathogenicity in grasses. These examples indicated the interdependence of growth and toxin production by holcus spot pathogens in disease development.

Differences between growth rate of holcus spot-positive strains and holcus spot-negative isolates were especially striking at an inoculum concentration of 10^5 cells/ml where the holcus spot-positive strains had a shorter doubling time. Doubling times of 5.28 and 5.25 hr were described for an isolate of *P. syringae* from pear, heterologous in both cherry and bean leaves, respectively (12), for inoculum at 10^7 cells/ml. These doubling times were considerably longer than the doubling times obtained for both pathogenic and nonpathogenic strains of *P. syringae* in maize leaves (Table 20.

Quantitative measurements of bacterial populations from cowpea hypocotyls indicated that pathogenic strains such as B-301D reached slightly higher population levels than the HR strains, 475 and Ps 251, from the same initial inocula concentrations. However, the final hypocotyl populations and doubling times of these strains did not reflect the distinct differences between the SN and HR reactions of cowpea. SR appeared to be a primary cause of SN in cowpea. Indeed, the cowpea disease reaction was perhaps more a reflection of a strain's ability to produce SR (Table 1) than its potential to cause the cowpea disease under natural field conditions.

Syringomycin produced by the holcus spot strain 5D430 was highly toxic to maize, causing water-soaking, chlorosis, and necrosis of tissue within 24 hr, even at low SR concentrations (approximately 1 μg/ml) (Fig. 5). The lesions produced around stomata by SR mimicked holcus leaf spots produced by pathogenic bacteria. At high concentrations, SR caused systemic lesions or streaks, but at low concentrations symptoms were confined to the entry site.

Maize leaf tissue was more sensitive to SR than *G. candidum*, which agrees with the finding by Sinden et al. (32, 33) that peach shoots were more sensitive to SR than to *G. candidum*. Visible symptoms were induced by SR within 12 hr, a rapid enough response to enhance the establishment of the bacterial pathogen and to increase disease development even at concentrations of SR less than 2 μg/ml.

In conclusion, it appears that pathogenicity of *P. syringae* in maize and cowpea is dependent on both bacterial growth and SR production. The results also support the hypothesis that SR acts as a phytotoxin in more than one disease caused by ecotypes of *P. syringae*.

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