Epiphytic Movement and Survival of Pseudomonas syringae on Spring Wheat

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ABSTRACT

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Field, greenhouse, and growth chamber experiments demonstrated that *Pseudomonas syringae*, the incitant of bacterial leaf necrosis of wheat, moved from inoculated wheat seed to the seedling and survived as an epiphyte on the leaves. In greenhouse studies, 80-98% relative humidity favored movement of *P. syringae* to aerial parts of the seedlings. Movement was not different on wheat cultivars susceptible or resistant to bacterial leaf necrosis. Under 70-98% relative humidity in a growth chamber, *P. syringae* moved to a significantly higher ($P = 0.01$) percentage of seedlings at 10 C than at 16 or 22 C. In the field, with a serotype VI isolate as a marker, seedborne *P. syringae* was recovered from Bounty 208 wheat first true leaves, but not from upper leaves up to 43 days after emergence. From date of seedling emergence to day 41, total precipitation received was only 1.09 cm. After a rainfall of 3.48 cm on day 42, other *P. syringae* serotypes (III, IV, and V) were isolated from upper leaves. This suggests that other sources of inoculum for bacterial leaf necrosis also were present.

A leaf necrosis incited by the bacterium, *Pseudomonas syringae* van Hall, has been widespread on certain spring and winter wheat (*Triticum aestivum*) cultivars in South Dakota from 1968 to 1974 (13, 14). Outbreaks of this disease were reported in Minnesota (21) and Montana (19) in 1975. Bacterial leaf necrosis probably is more prevalent than has been reported since *P. syringae* has been isolated from winter wheat seed from Nebraska, North Dakota, South Dakota, and the Canadian provinces of Alberta and Saskatchewan (13, 15).

The etiology of bacterial leaf necrosis was unknown until 1972 when Otta (13, 14) determined that *P. syringae* was the incitant. In Minnesota, Sellam and Wilcoxson (21) also identified the bacterium isolated from blighted wheat leaves as *P. syringae*.

Little is known about the epidemiology of bacterial leaf necrosis. Widespread outbreaks have appeared suddenly while the wheat is in the boot stage. Severity of the disease in South Dakota seems to be linked with the amount of wind-driven rain received in the last week of May and the first 3 wk of June (14).

In recent years, resident epiphytic populations of *P. syringae* have been detected on many host and nonhost plants (1, 2, 4, 5, 18, 20). Under field conditions pseudomonads identified as being either *P. syringae* or a closely related species have been isolated from apparently healthy plants such as peach (3, 4), cherry (1, 2), pear (18), apricot, almond, olive, rose, juniper (4), soybean (12), tomato (20), and certain weeds (4, 5, 20). Ercolani et al. (5) established a correlation between large epiphytic populations of *P. syringae* on hairy vetch and subsequent outbreaks of bacterial brown spot of bean in adjacent fields. Others also have suggested that epiphytic populations of *P. syringae* can be a source of disease-inciting inoculum (1, 2, 4, 20). The ability of phytopathogenic bacteria to move from inoculated seed to the aerial parts of the seedling and become part of the epiphytic microflora also has been demonstrated (9).

The objective of this investigation was to determine if *P. syringae* from artificially contaminated seed could become established as an epiphyte on the aerial parts of wheat seedlings and, if so, to determine what influence relative humidity (RH), temperature, and cultivar susceptibility to bacterial leaf necrosis might have on bacterial movement to the seedling.

MATERIALS AND METHODS

Wheat seed inoculation.—A *P. syringae* serotype VI isolate (15) from diseased wheat leaves (culture number 488–J. D. Otta, Plant Science Dept., South Dakota State University, Brookings, SD 57007) was grown on King's medium B (MB) (7) for 24 hr and then suspended in sterile deionized water at a concentration of about 1×10^7 cells/ml. Wheat seed was placed in the suspension under vacuum (produced by a water aspirator) for 5 min, and then air dried. The inoculated seed was used in greenhouse, growth chamber, and field studies to determine movement of seedborne *P. syringae* to the seedling.

Isolations from wheat seedlings.—Two methods were used to isolate *P. syringae* from wheat seedlings and leaves. One method involved triturating a leaf or the excised aerial part of a seedling in three drops of sterile

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deionized water. The resulting suspension was pipetted onto a plate of MB, spread with a sterilized glass L-shaped rod, and incubated at 22-24 C for 72 hr. In the other isolation method, individual excised seedlings or leaves were placed aseptically into test tubes containing 8 ml of D-4 broth (6). The tubes were incubated as stationary cultures without aeriation for 20 hr at 22-24 C. Tubes then were thoroughly agitated and two drops of a culture suspension (delivered from a Pasteur pipette) were placed onto a plate of MB. The drops of suspension were spread on the agar surface and incubated as previously described.

Bacterial colonies were identified tentatively as *P. syringae* on the basis of colony appearance, fluorescent pigment production, and negative oxidase reaction (8). Colonies obtained in the field study which appeared to be *P. syringae* were randomly selected from the plates and subcultured. After 24-36 hr of incubation these isolates were tested for the characteristics previously listed. Most of the isolates obtained in the field study also were tested for serotype with the somatic ('O') antigen and Ouchterlony (17) double-gel diffusion plates as reported by Otta and English (16). Isolates were tested for homologous reactions with antisera of the six major *P. syringae* serotypes (16).

Isolation of epiphytic Pseudomonas syringae populations from wheat seedlings in greenhouse studies.—The effect of humidity and cultivar susceptibility (to leaf necrosis) on movement of *P. syringae* from the seed to the seedling was studied in the greenhouse. The experiment consisted of growing inoculated and noninoculated seed of three spring wheat cultivars, Bounty 208, World Seeds 1809, and Chris under high (80-98%) and low (30-65%) RH. There were two replications of each treatment and two planting dates (A and B). Pots were placed in a completely randomized design. Different, recently-inoculated seed lots were used on each date. The wheat cultivars Bounty 208, World Seeds 1809, and Chris are very susceptible, moderately susceptible, and resistant to bacterial leaf necrosis (14), respectively. Seeds were planted (10 seeds per 10-cm diameter pot) in a nonsterilized peat, soil, and sand (4:3:5, v/v) mixture. A greenhouse bench served as the low-RH area and a plastic-enclosed chamber with a wet sand bottom was the high-RH area. Relative humidity and temperature in both areas were recorded with hygrothermographs. Seedlings were excised aseptically approximately 2 mm above the soil line 4 days after emergence. Both the D-4 broth and the trituration isolation method were used to detect *P. syringae* on the seedlings. The epiphytic nature of *P. syringae* on wheat seedlings had been determined previously by leaf prints on MB (J. D. Otta, *unpublished*). Owing to temperature fluctuations in the greenhouse, further experimentation was modified and performed in a controlled-environment chamber.

Isolation of epiphytic Pseudomonas syringae populations from wheat seedlings in growth chamber studies.—Experiments involving temperature effects on movement of seedborne *P. syringae* to seedlings were conducted in a growth chamber. Pots were planted with *P. syringae*-inoculated Bounty 208 seed as in the greenhouse experiment and were kept moist with sterile deionized water. Pots were placed in an ISCO E-2 growth chamber with a 14-hr light period, and the chamber was operated at 10, 16, or 22 C with 10 pots per temperature. Relative humidity was maintained between 70-98% with fluctuations from maximum to minimum consistently occurring four-to-five times per hour. Seedlings were excised 4 days after emergence, and the D-4 broth isolation method was used. Treatments were repeated at a later date with a different recently-inoculated seed lot and two sets of 10 pots per temperature.

Isolation of epiphytic Pseudomonas syringae populations from field collected wheat leaves.—A field plot was planted with *P. syringae*-inoculated Bounty 208 seed and an adjacent area was planted with noninoculated Bounty 208. Attempts to isolate *P. syringae* from wheat leaves from both areas were made periodically with the D-4 broth method. Randomly selected leaves were removed aseptically and individually placed in test tubes, capped, and transported to the laboratory. Leaf position was recorded for each leaf collected. The first, second, and third true foliage leaves were sampled. As plants matured, main tillers were selected and all leaves on a tiller were sampled. The number of first true foliage leaves sampled was 500 and 1,050 for the noninoculated and inoculated areas, respectively. The number of other foliage leaves sampled was 1,500 and 1,750 for the noninoculated and inoculated areas, respectively. Precipitation data were obtained from the Plant Science Farm weather station located 0.8 km from the plot.

RESULTS

Epiphytic Pseudomonas syringae on seedlings in the greenhouse.—*Pseudomonas syringae* was recovered from the aerial parts of seedlings grown from inoculated seed but not from seedlings grown from noninoculated seed. The D-4 broth and trituration methods detected *P. syringae* on 43 and 49% of the seedlings, respectively. These percentages were not significantly ($P = 0.05$) different; therefore, the data were combined. Under high-RH conditions a significantly ($P = 0.01$) higher percentage of the seedlings had detectable *P. syringae*, but there was no significant difference in percentages due to cultivar (Table 1).

On planting date A, 57% of the seedlings yielded *P. syringae*. This percentage was significantly ($P = 0.01$)

TABLE 1. Effect of wheat cultivar and relative humidity on movement of *Pseudomonas syringae* from inoculated wheat seeds to aerial parts of seedlings grown in the greenhouse

	Seedlings with *P.* syringae[x]			
Relative Humidity	Bounty 208 (%)	World Seeds 1809 (%)	Chris (%)	Means[z] (%)
High (80-98%)	72	57	52	60 a
Low (30-65%)	24	41	27	31 b
Means[y]	48 a	49 a	39 a	

[x]Values represent the mean of eight pots.

[y]Means in the same row followed by the same letter are not significantly different ($P = 0.01$).

[z]Means in the same column followed by the same letter are not significantly different ($P = 0.01$).

higher than the 37% obtained on planting date B. However, there was no treatment × date interaction. All other interactions also were nonsignificant.

Epiphytic Pseudomonas syringae on seedlings in a growth chamber.—*Pseudomonas syringae* was isolated from 65, 35, and 35% of the seedlings grown at 10, 16, and 22 C, respectively. The percentage of seedlings with *P. syringae* was significantly ($P=0.01$) higher at 10 C than at 16 or 22 C (Table 2). Time from planting to seedling emergence varied greatly depending on temperature. Emergence time at 10, 16, and 22 C was 9-10, 4, and 3-4 days, respectively.

Epiphytic Pseudomonas syringae on wheat leaves in the field.—Leaf sampling in the inoculated area began one day after seedling emergence. *Pseudomonas syringae* was found on a small percentage of apparently healthy first true leaves up to 11 June 43 days after emergence (Fig. 1) at which time sampling of these leaves was discontinued. Only one *P. syringae* isolate (serotype IV) was recovered from first true leaves from the noninoculated area.

Isolates from the first true leaves sampled from the inoculated area were of the same serotype (VI) as the *P. syringae* isolate used to inoculate the seed. Twenty-five isolates from first true leaves were randomly selected and tested for serotype. All were found to be serotype VI, the same serotype as the *P. syringae* isolate used to inoculate the seed. Thus, it was assumed that all 45 isolates obtained (Fig. 1) were serotype VI.

Pseudomonas syringae was not isolated from any upper leaves in either inoculated or noninoculated plants from 30 April through 9 June 1976. From day of emergence to 9 June, total precipitation amounted to 1.09 cm with no single rainfall exceeding 0.5 cm. After a rainfall of 3.48 cm on 10 June, *P. syringae* serotypes III, IV, and V were isolated from upper leaves of tillers in both inoculated and noninoculated plants. A few *P. syringae* serotype VI isolates were obtained from upper leaves of plants in the noninoculated area but no isolates of this serotype were recovered from upper leaves of plants in the inoculated area.

DISCUSSION

Pseudomonas syringae moved from the wheat seed to the seedling and survived on healthy leaves under greenhouse, growth chamber, and field conditions. These results indicate that *P. syringae* can survive as an epiphyte on wheat and that seedborne *P. syringae* is a possible source of inoculum for bacterial leaf necrosis.

In the greenhouse, high RH had a favorable effect on the movement or survival of *P. syringae* on the seedling. This agrees with data reported by Leben (10) who found that migration of epiphytic bacteria from cucumber seed to aerial parts of the seedlings was greater under high RH.

The temperature fluctuations in the greenhouse probably were responsible for the drop in the percentage of seedlings that yielded *P. syringae* on date B. The temperature ranged between 21-27 C during the initial experimental period. During the second experimental period, temperatures also were in this range except for a fluctuation that occurred 1 day after seedling emergence when the temperature reached a maximum of 43 C in the high humidity area and remained above 32 C for 5 hr. A corresponding temperature increase was observed in the low-RH area where the temperature reached 32 C and exceeded 27 C for 6 hr. In work with resident populations of *P. tomato* (*P. syringae*) Schneider and Grogan (20) reported that the population dropped to a very low level when exposed to 32 C.

Greenhouse results also indicated that movement of *P. syringae* to the seedling was not different on cultivars susceptible or resistant to bacterial leaf necrosis. This suggests that, for the cultivars tested, resistance is not related to the inhibition of movement of seedborne inoculum to the seedlings.

Movement of *P. syringae* was influenced by temperature in the growth chamber experiment. The higher percentage of seedlings yielding *P. syringae* at 10 C than at 16 or 22 C may reflect the slower germination and emergence rate at the lower temperature. Slower germination may give the bacteria more time to become established on the seedling. The mechanism of movement from the seed to the seedling is unknown, but it has been suggested that the bacteria "swim" up the seedling or are carried up by the growing point (11).

Under extremely dry field conditions *P. syringae* not only moved from the seed to the seedling, but was able to persist on the leaves. The bacteria apparently were unable, however, to move beyond the first true leaves.

TABLE 2. Effect of temperature on movement of *Pseudomonas syringae* from inoculated wheat seeds to aerial parts of seedlings grown in a growth chamber

Temperature (C)	Seedlings with *P. syringae* (%)
10	65 a
16	35 b
22	35 b

[z]Values represent the mean of 30 pots. Values followed by the same letter are not significantly different ($P = 0.01$).

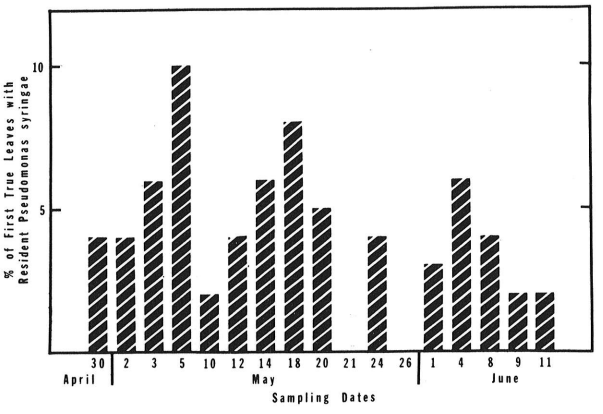

Fig. 1. Occurence of resident *Pseudomonas syringae* serotype VI on the first true leaves collected from plants grown in the field from Bounty 208 wheat seed preplant inoculated with that bacterium.

The apparent inability of *P. syringae* to move to the upper leaves may have been due to the unusually dry conditions. The appearance of other *P. syringae* serotypes on upper leaves conincided with a 3.48 cm (10 June) rainfall. These serotypes probably were from natural seedborne inoculum or from epiphytic populations from nearby weeds or crop plants.

We believe that seedborne *P. syringae* can be spread to upper leaves once it is present on the first true leaf. Absence of spread of serotype VI *P. syringae* to upper leaves probably resulted from lack of significant amounts of rainfall until late in the growing season when the first leaves were senescent. The presence of other sterotypes after the first substantial rain indicates that *P. syringae* can be spread and established as an epiphyte on wheat leaves. Additional studies on the movement of epiphytic *P. syringae* on wheat under more normal rainfall conditions are needed

An epiphytic phase of *P. syringae* on wheat has important implications in the epidemiology of bacterial leaf necrosis. It was demonstrated that seedborne *P. syringae* could move to the wheat seedlings and persist on the leaves. Considering the common occurrence of *P. syringae* in wheat seed lots (15), infested seed could be an important source of inoculum in the field. Natural inoculum, whether from seed or other sources, may result in the buildup of epiphytic populations of *P. syringae* which could be spread with each successive rainfall. Under the right predisposing conditions these populations may become pathogenic, resulting in the observed sudden, widespread outbreaks of bacterial leaf necrosis (14).

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