South Dakota State University

Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

Electronic Theses and Dissertations

1977

Epiphytic Movement and Survival of *Pseudomonas Syringae* on Spring Wheat

Sandra J. Fryda

Follow this and additional works at: <https://openprairie.sdstate.edu/etd>

Recommended Citation

Fryda, Sandra J., "Epiphytic Movement and Survival of *Pseudomonas Syringae* on Spring Wheat" (1977). *Electronic Theses and Dissertations*. 5066.
<https://openprairie.sdstate.edu/etd/5066>

This Thesis - Open Access is brought to you for free and open access by Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact michael.biondo@sdstate.edu.

EPIPHYTIC MOVEMENT AND SURVIVAL OF
PSEUDOMONAS SYRINGAE ON SPRING WHEAT

BY

SANDRA J. FRYDA

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Plant Pathology, South Dakota
State University
1977

SOUTH DAKOTA STATE UNIVERSITY LIBRARY

EPIPHYTIC MOVEMENT AND SURVIVAL OF
PSEUDOMONAS SYRINGAE ON SPRING WHEAT

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of the thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Date / /

Head, Plant Science
Department

Date ' /

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major advisor, Dr. Jack D. Otta for all his assistance during my research and the preparation of this thesis.

Special thanks are extended to Mr. Paul D. Evenson and Dr. W. Lee Tucker for their advice on statistical design and analysis and to Dr. Wayne S. Gardner for his help and words of encouragement.

Finally, I want to thank my parents, Joe and Eleanor Fryda, for their loving support.

SJF

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	6
Isolations from wheat seeds and wheat leaves	6
Wheat seed surface sterilization	7
Wheat seed inoculation	7
Isolation of resident <u>P. syringae</u> populations from wheat seedlings in the greenhouse	8
Isolation of resident <u>P. syringae</u> populations from wheat seedlings in a growth chamber	9
Isolation of resident <u>P. syringae</u> populations from field collected wheat leaves	10
Isolation of toxin	11
RESULTS	15
Resident <u>P. syringae</u> on seedlings in the greenhouse	15
Resident <u>P. syringae</u> on seedlings in a growth chamber	17
<u>P. syringae</u> resident leaf populations - 1975	17
<u>P. syringae</u> resident leaf populations - 1976	20
Toxin production	28
Long term survival of seedborne <u>P. syringae</u>	28
DISCUSSION	35
LITERATURE CITED	39

LIST OF TABLES

Table	Page
1. Effect of wheat cultivar and relative humidity on movement of <u>P. syringae</u> from inoculated seeds to aerial parts of seedlings grown in the greenhouse	16
2. Effect of temperature on movement of <u>P. syringae</u> from inoculated wheat seeds to aerial parts of seedlings grown in a growth chamber	18
3. Occurrence of resident <u>P. syringae</u> populations on 'Bounty 208' wheat leaves collected from the 1975 field plot	19
4. Occurrence and serotype of resident <u>P. syringae</u> populations on 'Bounty 208' wheat leaves collected from the 1976 field plot	21
5. Occurrence of resident <u>P. syringae</u> populations on 'Bounty 208' wheat spikes collected from the 1976 field plot	29
6. Determination of toxin production by <u>P. syringae</u> and <u>P. atrofaciens</u>	32
7. Long term survival of <u>P. syringae</u> on inoculated 'Winoka' wheat seed	33

LIST OF FIGURES

Figure	Page
1. 1976 field plot design for detection of spread of <u>P. syringae</u> resident leaf populations	13
2. Occurrence of resident <u>P. syringae</u> populations on 'Bounty 208' first true leaves collected from inoculated strip in the 1976 field plot	25
3. Occurrence and serotype of resident <u>P. syringae</u> on 'Bounty 208' leaves collected from the 1976 field plot	27
4. <u>P. syringae</u> serotype distribution of 1976 field isolates	31

EPIPHYTIC MOVEMENT AND SURVIVAL OF
PSEUDOMONAS SYRINGAE ON SPRING WHEAT

Abstract

SANDRA J. FRYDA

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 experiments 80-98% relative humidity favored movement of P. syringae to the coleoptile and first true leaf 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 10C than at 16 or 22C. In a field experiment, using serotype VI as a marker, P. syringae was recovered from the first true leaf, but not from upper leaves up to 41 days after emergence. From date of seedling emergence to day 41, total precipitation received was only 1.09 cm. However, after a substantial rainfall (3.48 cm) on day 42, P. syringae serotypes III, IV, and V, in addition to VI, were isolated from upper leaves in both inoculated and control areas. This suggests that other sources of inoculum for bacterial leaf necrosis were also present.

INTRODUCTION

A leaf necrosis incited by the bacterium, Pseudomonas syringae Van Hall, has been widespread on certain spring and winter wheat cultivars in South Dakota from 1968 to 1974 (24, 25). Outbreaks of this disease were reported in Minnesota (32) and Montana (30) in 1975. Bacterial leaf necrosis is probably more prevalent than 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 (24, 26).

Symptoms of this disease usually appear on the leaves during the boot stage. The initial symptoms are small water soaked areas which quickly take on a grey-green desiccated appearance. These areas soon become necrotic and light tan in color. As much as 75-100% of the leaf blade may become necrotic (25) with the flag leaf and the next two leaves below it being the most seriously affected. Many of the winter wheat cultivars grown in South Dakota are susceptible to leaf necrosis. The cultivars Winoka, Hume, and Lancer are particularly susceptible (24, 25). Most of the spring wheat cultivars show some resistance to leaf necrosis (25, 32) but Bounty 208, Bounty 309, Bonanza, and Lark have been reported to be very susceptible (25, 32).

Little is known about the epidemiology of this bacterial leaf necrosis. Outbreaks have appeared suddenly throughout entire fields of susceptible cultivars with severity in South Dakota apparently linked with the amount of wind driven rain received in the last week of May and the first 3 weeks in June (25). Estimates of yield loss due to this

disease alone have not been reported. However, an estimate of a 12% overall yield loss in susceptible winter wheat cultivars due to leaf spotting diseases was made in 1975 in Montana (30). This loss included leaf necrosis caused by P. syringae. It was observed that the bacteria, P. syringae and Xanthomonas translucens, were responsible for most of the damage on the flag leaves whereas leaf spotting fungi were more prevalent on lower leaves.

The etiology of this leaf necrosis was unknown until 1972 when Otta (24, 25) determined that P. syringae was the incitant. Symptoms of this disease are similar to those described for basal glume rot (23, 33) caused by P. atrofaciens. Using pathological, serological, physiological and biochemical tests, Otta (24, 25) found no differences between known P. syringae cultures and Pseudomonas isolates from diseased wheat. Sellam and Wilcoxson (32) also identified the bacteria isolated from blighted wheat leaves as P. syringae. This agrees with a report by Koishibaev (13) who inoculated wheat plants with P. syringae and discovered that this bacterium was pathogenic on wheat. Otta (25) points out that there may not be enough consistent differences between P. syringae and P. atrofaciens to warrant separating them into two species. P. atrofaciens and a number of other nomenspecies are included in P. syringae in the 8th edition of Bergey's Manual of Determinative Bacteriology (4), but this is done with a conditional statement that some of these nomenspecies may deserve independent specific rank. Thus, the validity of some Pseudomonas nomenspecies, including P. atrofaciens, is still uncertain.

In recent years epiphytic populations of P. syringae have been found on a number of host and nonhost plants. By the definition of Leben (17), the term microbial epiphyte refers to any microorganism that can be cultured from a plant surface. Furthermore, there are two types of microbial epiphytes, casuals and residents. Casuals do not have the ability to multiply on a plant surface and are present on a plant accidentally. However, they may grow saprophytically on foreign debris on the plant. In contrast, residents do have the ability to multiply on the surface of healthy, living parts of the shoot system without noticeably affecting the plant. The term epiphyte as used in this paper refers to a resident microbial epiphyte.

In the greenhouse Leben et al. (22) recovered P. syringae from healthy buds, stipules, and newly unfurled leaves 9-22 days after inoculating terminal buds of Phaseolus vulgaris L. 'Red Kidney' with the bacterium. These plants had been growing in a high humidity chamber, but even after placing them in the drier environment of the greenhouse, P. syringae could still be isolated from some of the leaves for 7-16 additional days. Under field conditions pseudomonads resembling P. syringae were isolated by Leben and Miller (21) from buds, pollinated flowers, and young pods of healthy soybean plants. Schneider and Grogan (31) found resident populations of P. tomato (P. syringae) on apparently symptomless tomato leaves in the field. They also isolated this bacterium from the roots and foliage of other crop plants and various weeds.

Large epiphytic populations of P. syringae were found on hairy vetch, Vicia villosa, in Wisconsin (7, 9) where bacterial brown spot of bean

has been a serious problem. P. syringae was the main component of the epiphytic microflora on this weed during most of the year. A correlation was made between high epiphytic populations on hairy vetch and later outbreaks of brown spot in adjacent bean fields.

Epiphytic populations of P. morsprunorum, which is either closely related or identical to P. syringae, were discovered by Crosse (1, 2) on healthy cherry leaves. This bacterium was abundant on leaf surfaces throughout the growing season with cell numbers/cm² ranging from an average of 1018.4 on a susceptible variety to 119.6 on a resistant variety (1). Occasionally the number of P. morsprunorum per leaf exceeded 4 million (2). Because of the high numbers of P. morsprunorum on the leaves, it was suggested that these epiphytic populations are the main source of inoculum for bacterial canker of stone fruits in the fall (1).

English and Davis washed P. syringae from the surface of healthy peach and almond leaves, fruits, and twigs in California orchards (6). The bacterium was also isolated from apparently healthy woody plants such as olive, apricot, rose, dwarf juniper, firethorn, Chinese wingnut, Penstemon antirrhinoides, Viburnum tinus, and Albizzia julibrissen (6). Occasionally P. syringae was isolated from weeds in the orchard.

Dowler and Weaver (5) found P. syringae, or a closely related pseudomonad, prevalent on healthy peach trees in Georgia and South Carolina. They suggested that these pseudomonads represent a potential disease threat but that bacterial canker is less likely to occur without predisposing factors.

The effect of frost on a potentially pathogenic epiphytic population of P. syringae was studied by Panagopoulos and Crosse (29). They stated that this pathogen seemed to be universally distributed on pear trees as the dominant component of the surface microflora. In their study they showed that, under the predisposing condition of frost, epiphytic populations of P. syringae became pathogenic and caused blighting of pear blossoms.

Thus, several studies have shown that epiphytic populations of P. syringae are a common occurrence and that these bacteria can serve as a source of disease inciting inoculum under predisposing conditions. It has also been reported that some seedborne bacteria can migrate to aerial parts of the seedling and become established as part of the microflora under greenhouse conditions (15, 17, 18, 19, 20). Several Russian studies have demonstrated similar movement under field conditions (as cited by 17). These studies have involved nonpathogenic bacteria but Leben (16) demonstrated that seedborne plant pathogenic bacteria may also possess this ability. He inoculated tomato seed with the tomato pathogen Xanthomonas vesicatoria and later recovered the bacteria from apparently healthy tomato seedlings.

One of the objectives of this investigation was to determine if P. syringae could live as an epiphyte on wheat, and if so, what implications this might have on the epidemiology of bacterial leaf necrosis of wheat. Another objective was to determine whether seedborne P. syringae could serve as an important source of overwintering inoculum.

MATERIALS AND METHODS

Isolations from wheat seeds and wheat leaves. -- P. syringae was isolated from wheat seeds by placing individual seeds aseptically into test tubes containing 4 ml of D-4 broth (10), a selective medium for Pseudomonas species. The tubes were incubated as stationary cultures without aeration for 24 hours at 22-24C. The tubes were then thoroughly agitated and two Pasteur pipette drops of each culture were placed onto a separate plate of King's medium B (MB) (11). Plates were spread with a sterilized glass L-rod and incubated at 22-24C for 72 hours.

Two methods were used for isolating Pseudomonas syringae from wheat leaves. One method involved triturating an entire leaf or aerial part of a seedling in 3 drops of sterile deionized water. The resulting liquid was pipetted onto a plate of MB and incubated at 22-24C for 72 hours. A variation of this method in which only a 5 cm midsection of a leaf was triturated was used in some isolations in the 1975 field study. In the second isolation method individual leaves were placed into 8 ml of D-4 broth in capped test tubes. The rest of the procedure was the same as that used for seed isolations except the incubation time was shortened to 18-20 hours. In 1976 field studies the second method was also used to isolate from wheat spikes by placing the entire spike into D-4 broth.

In light of past experience, isolated colonies were tentatively identified as P. syringae on the basis of colony appearance, fluorescent pigment production and negative oxidase reaction (14). Most of the 1976 field isolates were also tested for serotype using the somatic

('O') antigen and Ouchterlony (28) double-gel diffusion plates as reported by Otta (26). Isolates were tested for homologous reactions with antisera of the six major P. syringae serotypes; I, II, III, IV, V, and VI (27). Serological testing was carried out after all field sampling was completed.

Wheat seed surface sterilization. -- Surface sterilization procedures were used to remove possible natural P. syringae contamination from Bounty 208 spring wheat seed used for field studies. Seed for the 1975 field plot was soaked in a 0.52% sodium hypochlorite solution for 2 minutes, rinsed with nonsterile tap water, and allowed to air dry. This process was then repeated. After this procedure seed isolation results revealed that P. syringae was still present on 2 out of 100 seeds sampled. Due to the failure of this method to eradicate P. syringae from the seed, a different technique was used on the seed for the 1976 field study. Seed was placed in a 1.31% sodium hypochlorite, 0.1% Triton X-100 (Eastman) solution and placed under vacuum for 5 minutes. The seed was then rinsed with nonsterile tap water and allowed to air dry. Prior to this procedure P. syringae had been detected on one seed in a 200 seed sample. After the sterilization process, no P. syringae was detected in 200 seeds tested. However, it is difficult to assess the effectiveness of this method with such a small sample because the percentage of seeds with P. syringae was so low before the sterilization process.

Wheat seed inoculation. -- A P. syringae isolate from diseased wheat leaves (culture number 488- J. D. Otta, Plant Science Department, South Dakota State University, Brookings, SD) was used to inoculate seed for

all studies. This isolate was grown on MB for 24 hours and suspended in sterile deionized water. For the 1975 field plots, surface sterilized seed was placed in a suspension of about 1×10^8 P. syringae cells/ml, vacuum infiltrated for 20 minutes, and then air dried. A sample of Winoka seed inoculated in the same manner on September 9, 1974 was stored at room temperature. Samples of this seed were checked periodically for about 2.3 years for the presence of P. syringae. Seed for the greenhouse, growth chamber, and 1976 field plot studies was vacuum infiltrated for 5 minutes in a suspension containing about 1×10^7 P. syringae cells/ml.

Isolation of resident P. syringae populations from wheat seedlings in the greenhouse. -- The effect of humidity and varietal susceptibility to leaf necrosis on the movement of seedborne P. syringae to the seedling was studied in the greenhouse. Two different leaf isolation methods were also tested to determine which was more sensitive in detecting P. syringae. Each experiment consisted of the following: two treatments, inoculated and noninoculated seed; three spring wheat cultivars, Bounty 208, World Seeds 1809, and Chris; two isolation methods, trituration and D-4 broth; two humidities, high and low; and two replications. The wheat cultivars Bounty 208, World Seeds 1809, and Chris are respectively very susceptible, moderately susceptible, and resistant to bacterial leaf necrosis (25). Ten seeds were planted per 10 cm pot in an unsterilized peat, soil, and sand (4:3:5) mixture. The same soil mixture was used in all greenhouse and growth chamber studies. Pots were placed in a completely randomized design. A

greenhouse bench served as the low humidity area and a plastic enclosed chamber with a wet sand bottom produced a high humidity area. Relative humidity and temperature in both areas were recorded with hygrothermographs. Seedlings were excised aseptically approximately 2 mm above the soil line four days after emergence. The D-4 broth isolation method or the trituration method described previously was then used. The experiment was repeated twice and for each experiment a different recently inoculated seed lot was used. In the second and third experiments pots were watered carefully using a beaker instead of a hose to avoid possible spread of P. syringae by splashing water.

Isolation of resident P. syringae populations from wheat seedlings in a growth chamber. -- Inconsistent results obtained in the greenhouse experiments (described in results) may have been produced by large fluctuations in temperature. Thus, studies involving temperature effects on movement of seedborne P. syringae to seedlings were carried out in a temperature controlled growth chamber. Pots planted with P. syringae inoculated Bounty 208 seed were placed into an ISCO E-2 growth chamber at 10, 16, or 22C with 10 pots per temperature and 10 seeds per pot. Temperature sequence in each experiment was randomly chosen. Pots were kept moist with sterile deionized water. Seedlings were removed four days after emergence and the D-4 broth isolation method was used as previously described. This experiment was repeated twice with a different recently inoculated seed lot used each time. In the first experiment there was no precise control over relative humidity and it

fluctuated daily between 80-90% during the light cycle and between 90-98% during the dark cycle. In experiments 2 and 3 relative humidity was kept consistently between 70-98% with fluctuations from maximum to minimum occurring 4-5 times per hour. The third experiment differed from the previous two in that there were two sets of 10 pots per temperature instead of one.

Isolation of resident *P. syringae* populations from field collected wheat leaves. -- In 1975 and 1976 plots were set up at the Plant Science farm, Brookings, South Dakota, to check for the presence of resident populations of *P. syringae* on wheat grown from *P. syringae* inoculated seed. In 1975 a randomized complete block design with eight replications and two treatments (inoculated and noninoculated Bounty 208 seed) was used to determine possible yield loss due to bacterial leaf necrosis. Drill strips were planted with either *P. syringae* inoculated seed or with surface sterilized noninoculated seed that had been vacuum infiltrated with sterile water. All drill strips were separated by 1.8 m alleys. Attempts to isolate *P. syringae* from wheat leaves were made periodically. Randomly selected leaves from inoculated and control strips were removed aseptically with a scalpel. Leaf position was recorded for each sample collected. The first, second, and third true foliage leaves to develop were designated as first, second, and third leaves, respectively. As plants matured main tillers were selected and leaves on the tiller were sampled. Leaf positions on a tiller were designated as fourth, fifth, sixth, and seventh with the flag leaf representing the seventh leaf. Leaves were placed individually in

plastic bags and transported to the lab where leaf isolation procedures were carried out.

Due to the spread of P. syringae into the control strips in the 1975 field plot, the 1976 field plot (Fig. 1) was designed to determine the spread of P. syringae from a drill strip planted with inoculated Bounty 208 seed to a control area planted with surface sterilized seed. Precipitation data for the growing season was obtained from the Plant Science farm weather station located 0.8 km east of the plots. Wind data was obtained from the weather station on top of the Agricultural Engineering building located 0.4 km south of the plots. Randomly selected leaves were collected periodically as previously described. Leaves were placed individually in capped test tubes and taken to the lab where the D-4 broth method of isolation was used. Isolates appearing to be P. syringae were tested for serological reaction with antiserum produced against known P. syringae cultures. Isolates not homologous to known P. syringae serotypes were tested for Geotrichum candidum inhibition (3), hypersensitive reaction (12) and pathogenicity on sorghum. For the pathogenicity test, two week old sorghum plants, SD 104, were preconditioned before inoculation by covering with plastic bags for 24 hours. Plants were then atomized with sterile deionized water suspensions (about 1×10^8 cells/ml) of 24 hour old cultures of the field isolates to be tested. After inoculation plants were placed in a mist chamber for 24 hours and then moved to a greenhouse bench.

Isolation of toxin. -- Two different methods were used to test for toxin production by Pseudomonas isolates from wheat. One method

1976 FIELD PLOT

involved using the syringomycin isolation procedure of Gross and DeVay (8) and checking the resulting extract for G. candidum inhibition (3). The other method was the direct bioassay of G. candidum inhibition (3) by bacterial colonies growing on potato dextrose agar plus 0.2% casamino acids (PDA+). P. syringae isolates from peach, cherry, wheat seed, and diseased wheat leaves and two P. atrofaciens isolates were tested.

RESULTS

Resident *P. syringae* on seedlings in the greenhouse. -- *P. syringae* was recovered from the aerial parts of seedlings grown from inoculated seed but not from seedlings grown from noninoculated seed. Table 1 shows the percentage of seedlings with *P. syringae* as affected by experiment, wheat cultivar, relative humidity, and isolation method. *P. syringae* was recovered from a significantly ($P=0.01$) higher percentage of the seedlings grown under high humidity than under low humidity, but there was no significant difference in percentages due to cultivar or isolation method (Table 1). All interactions were also nonsignificant.

The percentage of seedlings which yielded *P. syringae* in experiment 1 was significantly ($P=0.01$) higher than in experiment 2 (Table 1). *P. syringae* was detected on only 1.5% of the seedlings in experiment 3 and, due to this extremely low percentage of detection, the experiment was excluded from the statistical analysis. Much of the variation between experiments may have been caused by erratic fluctuations in temperature and humidity in the greenhouse. The temperature generally ranged between 21-27C in all three experiments. Relative humidity usually ranged between 80-98% in the high humidity area and between 30-65% in the low humidity area. No major deviations from these values were seen in experiment 1 but in experiment 2, during one day only, the temperature reached a high of 43C in the high humidity area and remained above 32C for five hours. This was accompanied by a drop in relative humidity to 65-75%. A corresponding temperature increase was observed

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

		% of seedlings with <i>P. syringae</i> ^a									
		Isolation method	Cultivar			Means					
Relative humidity	Bounty 208		World 1809	Seeds Chris	Broth	Trit.	High	Low	Expt.		
										1	2
Expt. 1	High ^b	Broth	82	81	67	77					
		Trit. ^e	79	85	70						
	Low ^c	Broth	25	62	33	40					
		Trit.	22	37	46						
Expt. 2	High ^b	Broth	50	16	32	33					
		Trit.	77	47	41						
	Low ^c	Broth	22	31	10	21					
		Trit.	29	33	20						
Means ^d											
Cultivars			48a	49a	40a						
Isolation methods						43a	40a				
Humidities								60a	30b		
Experiments										57a	34b

^aValues represent the mean of 2 pots.

^bRanged between 64-99% RH.

^cRanged between 25-68% RH.

^dMeans in the same row followed by the same letter are not significantly different (P=0.01).

^eTrituration method.

in the low humidity area. However, the temperature only reached a maximum of 32C and exceeded 27C for 6 hours.

In experiment 3 there was again a dramatic fluctuation during one day. The temperature in the low humidity area was 32-38C for two hours and remained above 27C for 5 hours. It can only be assumed that there was a corresponding increase in temperature in the high humidity area since the hygrothermograph in that location malfunctioned during this period. However, the temperature may have exceeded 49C since the maximum temperature in the high humidity chamber was 11 degrees higher than in the low humidity area during the fluctuation in experiment 2.

Resident *P. syringae* on seedlings in a growth chamber. -- A significantly lower percentage of seedlings yielded *P. syringae* in experiment 1 than in experiments 2 and 3 (Table 2). Differences in humidity conditions as described previously may explain this variation. *P. syringae* was found on a significantly ($P=0.01$) higher percentage of seedlings grown at 10C than at 16 or 22C (Table 2). It was noted that the time from planting to seedling emergence varied greatly depending on temperature. Emergence time at 10, 16, and 22C was 9-10, 4, and 3-4 days respectively.

P. syringae resident leaf populations - 1975. -- Leaf sampling for the detection of *P. syringae* in the field began two days after seedling emergence and continued until necrotic lesions appeared on the leaves during the boot stage. Results of these leaf isolations appear in Table 3. Early isolations from the first true leaves from inoculated drill strips yielded many plates containing almost exclusively *P.*

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

Temperature	% of seedlings with <i>P. syringae</i> ^a				Means ^d
	Expt. 1 ^b	Humidity controlled ^c			
		Expt. 2	Expt. 3		
			Set 1	Set 2	
10 C	34	70	64	60	57a
16 C	20	34	39	34	32b
22 C	14	37	36	30	29b
Means ^e	23a	47b	44b		

^aValues represent the mean of 10 pots.

^bRelative humidity was not controlled and it fluctuated daily between 80-90% during the 14 hr light cycle and between 90-98% during the 10 hr dark cycle.

^cRelative humidity constantly fluctuated between 70-98% (maximum to minimum 4-5 times/hr) for all three temperatures.

^dMeans in the column followed by the same letter are not significantly different ($P=0.01$).

^eMeans in the row followed by the same letter are not significantly different ($P=0.01$).

TABLE 3. Occurrence of resident *P. syringae* populations on 'Bounty 208' wheat leaves collected from the 1975 field plot.

Location	Date sampled	Leaf position	No. sampled	% with <i>P. syringae</i>
Inoculated	May	12 1st	80	12.5 ^a
		14 1st	100	33.0 ^a
		20 3rd	100	0.0 ^a
		21 3rd	50	0.0 ^a
		23 3rd	96	0.0 ^a
		27 1st & 2nd ^d	84	23.8 ^a
	June	3 1st & 2nd ^d	24	20.8 ^b
		3rd	12	0.0 ^b
		4th	12	0.0 ^b
		5th	11	0.0 ^b
		6th	3	0.0 ^b
		9 3rd	23	43.5 ^c
		4th	23	51.5 ^c
		5th	23	39.1 ^c
		6th	11	9.0 ^c
		7th	1	0.0 ^c
		12 4th	33	30.3 ^c
		5th	33	30.3 ^c
		6th	32	34.4 ^c
		7th	10	40.0 ^c
Control	May	15 1st	98	0.0 ^a
	June	12 4th	45	20.0 ^c
		5th	45	26.7 ^c
		6th	45	20.0 ^c
		7th	24	20.8 ^c

^aTrituration isolation method.

^bLeaf midsection variation of trituration method.

^cCD-4 broth isolation method.

^dDue to poor condition of the bottom two leaves it could not be determined with any certainty which was the first or second leaf. Therefore, they were not listed separately.

syringae colonies. Other bacterial colonies, particularly yellow mucoid types and other pseudomonads, became more prevalent in isolations made later in the growing season. P. syringae was not detected on leaves above the first true leaf until June 9 when the D-4 broth isolation method was used. Distribution of P. syringae on plants appeared to be random since bacteria were often detected on one or two upper leaves of a tiller but not on the other leaves of that same tiller. No P. syringae was found in a May 15 sampling of first true leaves from control strips even though P. syringae was present on 33% of the first true leaves from inoculated strips at this time. However, after finding P. syringae on upper leaves in the inoculated strips, isolations from upper leaves in the control areas were made and about 22% of these leaves yielded P. syringae.

Frequent rainshowers occurred from June 9 to 20. Symptoms similar to those of bacterial leaf necrosis appeared about June 16 when plants were in the early boot stage. P. syringae was isolated from these early leaf lesions. Leaf necrosis was observed in both inoculated and control strips, and only slight differences in yield were observed.

P. syringae resident leaf populations - 1976. -- Leaf sampling in the inoculated area began one day after emergence. P. syringae was found on a small percentage of the first true leaves up to June 11, 43 days after emergence (Table 4). By this time all of the first true leaves were quite necrotic due to senescence, and sampling of these leaves was discontinued. There appeared to be a general decline in the percentage of first true leaves with resident P. syringae as the season

TABLE 4. Occurrence and serotype of resident *P. syringae* populations on 'Bounty 208' wheat leaves collected from the 1976 field plot.

Location	Date sampled	Leaf position	No. of leaves sampled	% of leaves with serotype VI	% of leaves with other serotypes
Inoculated	April 30	1st	50	4	0
	May 2	1st	50	4	0
	3	1st	50	6	0
	5	1st	50	10	0
	10	1st	50	2	0
	12	1st	50	4	0
		2nd	50	0	0
	14	1st	100	6	0
	18	1st	50	8	0
		2nd	50	0	0
	20	1st	100	5	0
		2nd	100	0	0
	21	1st	50	0	0
		2nd	50	0	0
	24	1st	50	4	0
		2nd	50	0	0
	26	1st	50	0	0
		2nd	50	0	0
	June 1	1st	100	3	0
		2nd	100	0	0
		3rd	100	0	0
		4th	100	0	0
	4	1st	100	6	0
		2nd	50	0	0
		3rd	50	0	0
		4th	50	0	0
	8	1st	50	4	0
		2nd	50	0	0
		6th	50	0	0
		7th	50	0	0
	9	1st	50	2	0
		5th	50	0	0
		6th	50	0	0
		7th	50	0	0
	11	1st	50	2	0
		5th	50	0	6
		6th	50	0	2
		7th	50	0	4
	22	6th	50	0	10
		7th	50	0	0

TABLE 4. continued

	July	1	6th	50	0	6
			7th	50	0	4
		8	6th	50	0	2
			7th	50	0	6
		19	6th	50	0	0
			7th	50	0	0
		27	6th	50	0	0
			7th	50	0	0
Adjacent control	May	10	1st	100	0	0
		17	1st	100	0	0
		26	1st	100	0	1
	June	16	6th	100	1	10
			7th	100	1	5
		23	6th	100	1	13
			7th	100	1	9
	July	8	6th	50	2	8
			7th	50	0	4
North control	May	12	1st	100	0	0
		20	1st	100	0	0
	June	16	6th	100	0	8
			7th	100	0	4
		23	6th	100	0	6
			7th	100	0	5
		29	6th	100	0	2
			7th	100	0	0
	July	7	6th	100	0	3
			7th	100	0	2
		20	6th	100	0	0
			7th	100	0	0

progressed (Fig. 2). However, the slope of the regression line is not significantly different ($P=0.01$) from zero which implies that the percentage of leaves with resident P. syringae may not be linearly dependent on time.

Isolates from the first true leaves were found to be the same serotype (serotype VI) as the seedborne inoculum (Fig. 3). Of the 43 isolates collected between April 30 and June 8, 23 were randomly selected and tested for serotype and all were found to be serotype VI. Thus, an assumption has been made on the tables and figures that all 43 of these isolates were serotype VI. All isolates collected after June 8 were checked for serotype. P. syringae was isolated only from the first true leaves through June 9. In control areas it had not been found through June 9 on either the first true leaves or the upper leaves, except for one first leaf from the adjacent control area collected May 26. This isolate was found to be serotype IV (Fig. 3).

The weather was extremely dry from day of emergence to June 9 with total precipitation during the period amounting to 1.09 cm. On June 10 3.48 cm of precipitation was received along with a south-southwest wind. Isolations from leaves sampled after the rain yielded P. syringae from upper leaves in both the inoculated and control areas. These post rainfall isolations yielded isolates of serotypes III, IV, and V in addition to serotype VI (Fig. 3). Due to time limitations only the top 2 leaves were sampled after June 11. There was no observed increase in percentage of leaves with P. syringae after a second rainfall of 3.05 cm on June 26. However, by this time leaves were beginning to dry. All leaves

FIGURE 3. Occurrence and serotype of resident P. syringae on 'Bounty 208' leaves collected from the 1976 field plot. No P. syringae was isolated from any upper leaves collected before June 11. Therefore, only the results from first true leaf isolations are represented on left side of the figure.

appeared completely brown and dried by July 19 and no P. syringae was detected from July 19, 20, and 27 leaf samplings. Three hundred wheat spikes were also sampled and five out of 150 taken from the inoculated area yielded P. syringae (Table 5). Four of these isolates were serotype III and one was serotype V. No bacterial leaf necrosis was observed in the plots.

Of the 173 P. syringae-type field isolates collected, 150 were homologous to known P. syringae serotypes (Fig. 4). Of the remaining 23 isolates, 20 produced a hypersensitive reaction in Nicotiana glutinosa indicating that they were potentially plant pathogenic pseudomonads. Thirteen of these 20 isolates inhibited G. candidum. Production of a toxin that inhibits this fungus is one characteristic of P. syringae although not all P. syringae isolates are toxin producers. Two of the isolates which inhibited G. candidum were pathogenic on sorghum. This indicates that at least two of the isolates were probably P. syringae and that they may represent a previously unknown serotype of P. syringae.

Toxin production. -- A syringomycin type toxin was produced by 5 of the 6 wheat P. syringae isolates tested and by one of the P. atrofaciens isolates (Table 6). Whenever toxin production was detected by the syringomycin extraction process it was also detected by the direct bioassay method.

Long term survival of seedborne P. syringae. -- Periodic isolations from the same lot of inoculated Winoka wheat seed detected some viable P. syringae on the seed 855 days after the seed was inoculated with the bacteria (Table 7). In general, however, there was a continual decline

TABLE 5. Occurrence of resident *P. syringae* populations on 'Bounty 208' wheat spikes collected from the 1976 field plot.

Date sampled	Location	No. sampled	% with <i>P. syringae</i>
6/29	North control	100	0
7/1	Inoculated	50	6
7/8	Inoculated	50	0
7/8	Adjacent control	50	0
7/19	Inoculated	50	4

TABLE 6. Determination of toxin production by P. syringae and P. atrofaciens.

Isolate No.	Species	Source	Geotrichum candidum inhibition	
			Living bacteria	culture extract
B5	<u>P. syringae</u>	peach	+	+
HE885a	<u>P. syringae</u>	cherry	+	+
218	<u>P. syringae</u>	wheat seed	+	+
276	<u>P. syringae</u>	wheat seed	+	+
295	<u>P. syringae</u>	wheat seed	-	-
399	<u>P. syringae</u>	wheat seed	+	+
376	<u>P. syringae</u>	wheat leaves	+	+
488	<u>P. syringae</u>	wheat leaves	+	+
P-8	<u>P. atrofaciens</u>	wheat	+	+
P-51	<u>P. atrofaciens</u>	wheat	-	-

Control^a

^aSterile potato dextrose broth plus 0.2% casamino acids (PDB+).

Sources of isolates:

P. syringae

- J. E. DeVay, Department of Plant Pathology, University of California, Davis, California. Isolate B-5.
- H. English, Department of Plant Pathology, University of California, Davis, California. Isolate HE885a.
- J. D. Otta, Department of Plant Science, South Dakota State University, Brookings, South Dakota. Isolates 218, 276, 295, 376, 399, 488.

P. atrofaciens

- J. Cumming, Research Branch, Chemistry and Biology Research Institute, Canada Department of Agriculture. Isolates P-8, P-51.

TABLE 7. Long term survival of P. syringae on inoculated 'Winoka' wheat seed.

Date	Days after inoculation	% of seeds with <u>P. syringae</u> ^a
9/23/74	7	100
1/2/75	108	98
8/29/75	347	54
3/11/76	542	31
7/21/76	644	14
1/18/77	855	1 ^b

^aValues based on sample of 100 seeds.

^bValue based on sample of 200 seeds.

in the percentage of seeds with detectable P. syringae (Table 7).

DISCUSSION

P. syringae moved from the seed to the seedling and survived on the 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 one possible source of inoculum for bacterial leaf necrosis.

In the greenhouse, high humidity had a favorable effect on the movement or survival of P. syringae on the seedling. This might have been expected since Leben (18) found that humidity had considerable influence on the migration of epiphytic bacteria from cucumber seed to seedlings. Leben (18) reported that at 30-40% relative humidity the bacteria did not move to aerial plant parts; at 70-80% relative humidity the bacteria were present on certain aerial parts; and at 90% they were widely distributed.

The temperature fluctuations which occurred in greenhouse experiments 2 and 3 may have been responsible for the drop in the percentage of seedlings yielding P. syringae. In working with resident populations of P. tomato (P. syringae) Schneider and Grogan (31) discovered that the population dropped to a very low level when exposed to 32C even under free moisture conditions. Thus, exposure to temperatures above 32C for several hours may have reduced the population of P. syringae on the wheat seedlings in greenhouse experiments 2 and 3 to a point where bacteria were not detected. It should also be noted that plating efficiency of both isolation methods used is probably low. A number of bacteria are no doubt left in the plant tissue in the trituration method,

and D-4 broth has a reported plating efficiency of only 6.5% for P. syringae (10). Thus, P. syringae may have been present on more of the seedlings than was detected. According to the greenhouse results there was no significant difference between isolation methods in detection of P. syringae on the seedlings.

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

Movement of P. syringae, however, was influenced by temperature. The fact that a higher percentage of seedlings yielded P. syringae at 10C may be due to the slower germination and emergence rate at that 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 either "swim" their way up the seedling or are carried up with the growing point (20).

Under even extremely dry field conditions, P. syringae was not only able to move from the seed to the seedling, but it was able to persist for long periods on the leaves. The bacteria seemed unable, however, to move systematically up the plant. Spread seemed dependent upon rain since P. syringae could only be found on leaves other than the first true leaf after substantial rainfall had occurred in the Brookings 1976 plot.

Serotype distribution of field isolates indicates that seedborne P.

syringae was the initial source of P. syringae detected on the first true leaves. P. syringae was detected only on the first true leaves through June 9 and since all the isolates from the inoculated strip were of the same serotype as the inoculum on the seed, it seems reasonable to assume that these isolates originated from the seed. Considering the common occurrence of P. syringae in wheat seed lots (26) and its long term survival on the seed, infected seed could be an important source of inoculum in a field.

The one isolate obtained from a first leaf in the adjacent control strip was probably from natural seedborne inoculum or a weed growing in the area since it was not a serotype VI isolate. Some P. syringae may have survived even though the seed underwent surface sterilization procedures. Leben and Daft report that some bacterial residents still remained on cucumber seeds after using drastic surface sterilization procedures which killed many seeds (20).

The P. syringae on the first true leaves may have spread to other leaves during the rain. However, the serotype VI isolates obtained from leaves other than the first true leaf may also have come from other sources. Resident populations of P. syringae have been isolated from many weeds (6, 7, 9, 31) and from crop plants (1, 2, 5, 6, 21, 29, 31). The recovery of numerous serotype III, IV, and V isolates indicate that P. syringae may have come from crop plants or weeds growing in the area.

Since seed was inoculated with a serotype VI isolate, it was expected that isolates of this serotype would be the most prevalent.

Early isolations supported this expectation, but after the rain serotype VI isolates were a very small percentage of the total isolates obtained. It is possible that serotypes III and IV were better adapted to surviving on wheat leaves. The absence of serotypes I and II in the isolates obtained in this study is in agreement with findings that P. syringae serotypes I and II are least prevalent on wheat, corn, sorghum, and foxtail and occur most frequently on stone fruits (26).

An epiphytic phase of P. syringae on wheat has important implications in the epidemiology of bacterial leaf necrosis. It was demonstrated that P. syringae could spread and become established on leaves with just one substantial rainfall. Even with small amounts of initial inoculum, whether from seed or other sources, epiphytic populations of P. syringae could become more widespread with each successive rainfall. Under the right predisposing conditions these populations could become pathogenic resulting in the observed sudden widespread outbreaks of bacterial leaf necrosis (25).

LITERATURE CITED

1. CROSSE, J. E. 1959. Bacterial canker of stone fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. *Ann. Appl. Biol.* 47:306-317.
2. CROSSE, J. E. 1963. Bacterial canker of stone fruits. V. A comparison of leaf-surface populations of *Pseudomonas mors-prunorum* in autumn on two cherry varieties. *Ann. Appl. Biol.* 52:97-104.
3. DEVAY, J. E., F. L. LUKEZIC, S. L. SINDEN, H. ENGLISH, and D. L. COPLIN. 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. *Phytopathology* 58:95-101.
4. DOUDOROFF, M., and N. J. PALLERONI. 1974. Genus I. *Pseudomonas* Migula 1894, 237 Nom. Cons. Opin. 5. *Jud. Comm.* 1952, 121, pages 217-243 in R. E. Buchanan and N. E. Gibbons (ed.). *Bergey's Manual of Determinative Bacteriology*, 8th ed. Williams and Wilkins, Baltimore, Maryland. 1268 p.
5. DOWLER, W. M., and D. J. WEAVER. 1975. Isolation and characterization of fluorescent pseudomonads from apparently healthy peach trees. *Phytopathology* 65:233-236.
6. ENGLISH, H., and J. R. DAVIS. 1960. The source of inoculum for bacterial canker and blast of stone fruit trees. *Phytopathology* 50:634 (Abstr.).
7. ERCOLANI, G. L., D. J. HAGEDORN, A. KELMAN, and R. E. RAND. 1974. Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. *Phytopathology* 64:1330-1339.
8. GROSS, D. C., and J. E. DEVAY. 1974. In vitro production of syringomycin by isolates of *Pseudomonas syringae* in relation to pathogenicity (holcus spot) on maize. *Proc. Am. Phytopath. Soc.* 1:75-76.
9. HAGEDORN, D. J., R. E. RAND, and G. L. ERCOLANI. 1972. Survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean. *Phytopathology* 62:762 (Abstr.).
10. KADO, C. I., and M. G. HESKETT. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology* 60:969-975.
11. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.

12. KLEMENT, Z., G. L. FARKAS, and L. LOVREKOVICH. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology* 54:474-477.
13. KOISHIBAEV, M. 1971. [Bacteriosis of millet in the Aktyubinsk district.] *Vest. Sel'-Khoz. Nauki, Alma Ata* 14:42-46. (Rev. *Plant Pathol.* 50:669. 1971).
14. KOVACS, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (London)* 178:703.
15. LEBEN, C. 1961. Microorganisms on cucumber seedlings. *Phytopathology* 51:553-557.
16. LEBEN, C. 1963. Multiplication of *Xanthomonas vesicatoria* on tomato seedlings. *Phytopathology* 53:778-781.
17. LEBEN, C. 1965. Epiphytic microorganisms in relation to plant disease. *Ann. Rev. of Phytopath.* 3:209-230.
18. LEBEN, C. 1965. Influence of humidity on the migration of bacteria on cucumber seedlings. *Can. J. of Microbiol.* 11:671-676.
19. LEBEN, C. 1968. Colonization of soybean buds by bacteria: observations with the scanning electron microscope. *Can. J. of Microbiol.* 15:319-320.
20. LEBEN, C., and G. DAFT. 1966. Migration of bacteria on seedling plants. *Can. J. of Microbiol.* 12:1119-1123.
21. LEBEN, C. and T. D. MILLER. 1973. A pathogenic pseudomonad from healthy field-grown soybean plants. *Phytopathology* 63:1464-1467.
22. LEBEN, C., M. N. SCHROTH, and D. C. HILDEBRAND. 1970. Colonization and movement of *Pseudomonas syringae* on healthy bean seedlings. *Phytopathology* 60:677-680.
23. MC CULLOCH, L. 1920. Basal glume rot of wheat. *J. Agr. Res.* 18: 543-552.
24. OTTA, J. D. 1972. Wheat leaf necrosis incited by *Pseudomonas syringae*. *Phytopathology* 62:1110 (Abstr.).
25. OTTA, J. D. 1974. *Pseudomonas syringae* incites a leaf necrosis on spring and winter wheats in South Dakota. *Plant Dis. Rep.* 58: 1061-1064.
26. OTTA, J. D. 1977. Occurrence and characteristics of isolates of *Pseudomonas syringae* on winter wheat. *Phytopathology* 67:22-26.

27. OTTA, J. D., and H. ENGLISH. 1971. Serology and pathology of *Pseudomonas syringae*. *Phytopathology* 61:443-542.
28. OUCHTERLONY, O. 1958. Diffusion-in-gel methods for immunological analysis. *Prog. Allergy* 5:1-78.
29. PANAGOPOULOS, C. G., and J. E. CROSSE. 1964. Frost injury as a predisposing factor in blossom blight of pear caused by *Pseudomonas syringae* Van Hall. *Nature* 202:1352.
30. SCHAREN, A. L., J. W. BERGMAN, and E. E. BURNS. 1976. Leaf diseases of winter wheat in Montana and losses from them in 1975. *Plant Dis. Rep.* 60:686-690.
31. SCHNEIDER, R. W., and R. G. GROGAN. 1977. Bacterial speck of tomato: sources of inoculum and establishment of a resident population. *Phytopathology* 67:388-394.
32. SELLAM, M. A., and R. D. WILCOXSON. 1976. Bacterial leaf blight of wheat in Minnesota. *Plant Dis. Rep.* 60:242-245.
33. WILKIE, J. P. 1973. Basal glume rot of wheat in New Zealand. *N. Z. J. Agric. Res.* 16:155-160.