In vitro soil temperature tolerance and field overwintering of soybean bacterial blight pathogen, *Pseudomonas syringae* pv. *glycinea*

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A pure culture of *Pseudomonas syringae* pv. *glycinea* adhering to nylon threads buried in sterile and non-sterile soil in petri plates survived and retained its pathogenicity for 11 months at temperatures ranging from -5° to 35°C. The pathogen survived the winter in field plots at Ottawa where infested soybean (*Glycine mad* debris was either left on the soil surface or plowed under at depths of 15-22 cm and 30-37 cm the previous fall and infected soybean plants the following spring.

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Une culture pure de *Pseudomonas syringae* pv. *glycinea,* adhérant a des fils de nylon enterrés dans du sol sterile et non-sterile dans des plats de petri, a survecu et a conserve sa pathogénicité durant 11 mois à des temperatures allant de -5" a 35°C. Le pathogene a survécu a l'hiver dans des parcelles au champ à Ottawa sur des debris de soja (*Glycine mad* infestes, laissés a la surface ou enterres à une profondeur de 15-22 cm et 30-37 cm l'automne precedent, et a infecté les plants de soja le printemps suivant.

introduction

Bacterial blight of soybean (*Glycine max* (L.) Merr.) caused by *Pseudomonas syringae* pv. *glycinea* Young, Dye & Wilkie (4,8) is a common disease in most soybean production areas (1,10.14), particularly during cool, wet seasons with frequent rain-storms; hot, dry weather on the other hand may arrest disease development (6,21). The pathogen may survive in infected seed (11), infested host debris (7,13,20) or volunteer soybeans (9). In Minnesota (13) and Nebraska (20) the pathogen survived better when infested soybean debris was kept on the soil surface than when burried. In Brazil (10), however, it was concluded that leaf debris was not a good between-season survival site for the pathogen; the lack of survival was attributed to moderate to high temperatures leading to rapid decay of host tissues and increased activity of antagonistic microorganisms (16). Under laboratory conditions, the pathogen can survive in dried infected leaves for several years (3) and also withstand repeated periods of freezing and thawing (12). Although the thermal death point of fresh cultures of the pathogen can be as high as 49°C, it usually failed to grow at 35°C in liquid or on solid media (4). Pure cultures, used to infest sterilized or non-sterilized soil, lost their viability within a week, whereas the pathogen in leaf tissues buried in similar soil remained viable for 6 weeks, particularly when the soil was relatively dry (20). The effect of temperature on survival of the pathogen in soil is not clearly known. From the evidence available, it is conceivable that if the pathogen does not survive in soil, then crop rotation and/or deep plowing of infested soybean debris should provide some control of the disease if healthy seed is used.

The main objectives of the present work were to determine 1) the influence of a wide range of temperatures on the survival of the pathogen in soil in the absence of host debris in the laboratory and 2) if deep plowing of infested soybean debris in field plots in the fall would reduce disease incidence the following spring.

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Materials and methods

The pathogen. An isolate of *Pseudomonas syringae* pv. *glycinea* Young, Dye & Wilkie (hereafter called pv. *glycined,* used in this work, was obtained from naturally infected soybean leaves at Ottawa in 1977 and stock cultures were maintained on yeast-dextrose-carbonate agar (19) slants at 4°C. Initially, several routine bacteriological tests (2, 19) were carried out and eventually four differential culture media plus a pathogenicity test (5) were employed to distinguish pv. *glycinea* from other soil bacteria. Color (17) and growth characteristics of the pathogen on these media were as follows: 1) on King's B medium (19), it grew copiously, was buff in color and produced a green fluorescence under ultraviolet radiation; 2) on Kado's D4 medium (19) colonies were glistening bluish-gray (pearly); 3) on Leben's M-71 medium they were reddish to ox-blood with a narrow translucent border (19); and 4) on nutrient agar (19) they were whitish to creamy in color but their growth was poor when compared to other three media.

Survival under laboratory conditions. The survival of the pathogen in soil was tested by a thread method (18) and virulence by a method used by Kennedy (13) and Schuster (20). Pieces of 5 cm long nylon thread were soaked in a water-suspension of a 48-h-old culture (on King's B medium) of the pathogen (10^8 cells/ml) for 15-20 minutes and then buried in sterilized and non-sterilized soil (3:1:1 mixture of loam, sand and peat by volume) held in 9 cm petri plates with two pieces per plate. The initial soil moisture content was 20% ± 2. Plates were individually wrapped in plastic bags to prevent moisture loss and incubated at 9 temperatures ranging from -5" to 35°C. At each temperature there were 5 sets of 3 plates. After certain periods of incubation (4,41,210,330 and 365 days) threads from one set (3 plates) at each temperature were removed, shaken gently to dislodge loosely adhering soil particles and plated on Leben's M-71 medium. In 3-4 days, the development of reddish to ox-blood bacterial colonies

along or near the pieces of thread indicated recovery of the pathogen. Using the host as a selective medium (13,20) the virulence of representative isolates of the pathogen was tested as follows: for each test, a slightly turbid bacterial suspension was prepared by rinsing 4 pieces of thread in 50 ml water and sprayed on the lower (abaxial) surface of 4 unifoliate soybean leaves with a Paasch air brush until water-soaked (5). Two kinds of controls were included to compare results: a) leaves sprayed with water only and b) leaves sprayed with a suspension (10^6 cells/ml) of pv. ***glycinea*** from stock cultures. Bacterial blight lesions developed on leaves in 5-8 days and the symptoms were rated visually as mild (+), moderate (++) and severe (+++).

Survival under field conditions. In a 42 × 66 m field plot at Ottawa, bacterial blight infected soybean plants (previously inoculated with pv. ***glycinea)*** were plowed under at normal (15-22 cm) and deep (30-37 cm) plowing depths in long strips (4.6 x 66 m, replicated twice). Also included were two non-plowed strips where plant debris was left on the soil surface during the winter of 1979-80. Each of the six strips was separated by a 2.1 m pathway to allow for equipment and vehicle movement. In the spring of 1980, each strip was divided into two equal parts to obtain half-size duplicates, and cultivation (before planting soybeans) was done only in one direction to avoid cross-contamination of soil between the strips. Three soybean cultivars, Maple Presto, Evans and PI 153.293 (Plant Introduction, U.S. Regional Soybean Laboratory, Urbana, Illinois) were planted in the strips in a split plot design (3 plowing types, 3 cvs, 2 replications and 2 duplicates (within replicates). A plot contained three 5.4 m long rows, 15 cm apart. The number of infected plants in the middle row of each plot was counted periodically from the time of emergence until 8-9 leaves were fully expanded. It was assumed that early infections from soil inoculum would begin from the lower leaves. Seed samples (100 per cv.) were tested for seed-borne infection by plating them on Leben's M-71 medium and conducting a pathogenicity test of suspected colonies.

Results and discussion

Survival at different temperatures in soil plates. The pathogen remained viable in soil for 12 months (mo) through a temperature range of -5°C to 35°C but its pathogenicity was affected by prolonged incubation at higher temperatures particularly in non-sterile soil (Table 1). At low temperatures (-5° to 0°C) in both sterile and non-sterile soil it remained virulent (+++) throughout the test period (12 mo). Slight reduction of virulence (++) was noticed after 41 days at or above 30°C. After 7 mo of incubation at 5°-35°C, the pathogen produced moderate symptoms (++), and after 11 mo at 10"-35°C only mild symptoms (+) developed. After 12 mo, the pathogen from sterile soil at 20"-35°C caused mild symptoms as those at 11 mo but suspensions from non-sterile soil produced no symptoms. These results clearly show that pure cultures of the pathogen can survive in soil for a much longer period than previously reported (20). The retention of virulence was greatest at low temperatures indicating that the pathogen should be able to overwinter in temperate regions. The reduction in symptom development was probably related to a loss in the number of virulent cells of the pathogen after prolonged in-

Table 1. Pathogenicity of ***Pseudomonas syringae*** **pv. *glycinea*** from sterile (ST) and non-sterile (NS) soil incubated at temperatures from -5° to 35°C during a 12-month period.

Soil	Temp °C	4 days	41 days	7 mo	11 mo	12 mo
ST	-5	+++*	+++	+++	+++	+++
NS	-5	+++	+++	+++	+++	+++
ST	0	+++	+++	+++	+++	+++
NS	0	+++	+++	+++	+++	+++
ST	5	+++	+++	++	++	++
NS	5	+++	+++	++	++	++
ST	10	+++	+++	++	+	+
NS	10	+++	+++	++	+	+
ST	15	+++	+++	++	+	+
NS	15	+++	+++	++	+	+
ST	20	+++	+++	++	+	+
NS	20	+++	+++	++	+	-
ST	25	+++	+++	++	+	+
NS	25	+++	+++	++	+	-
ST	30	+++	++	++	+	+
NS	30	+++	++	++	+	-
ST	35	+++	++	++	+	+
NS	35	+++	++	++	+	+
Control Pathogen		+++	+++	+++	+++	+++
Water spray		-	-	-	-	-

*+++ = severe, ++ = moderate, + = mild, and - = no symptoms.

cubation (7 mo or more) at relatively higher temperatures (5°-35°C). However, the pathogen did survive 11 mo at all temperatures tested, indicating its temperature tolerance. The adverse effect of non-sterile soil due to microbial antagonism (10,16) only occurred after 12 mo of incubation at higher temperatures (20°-35°C).

Overwintering in field strips plowed at different depths. Seed used for planting gave no evidence of seed-borne infection; therefore, it would be assumed that the primary inoculum for bacterial blight development in the spring of 1980 was from soil. The disease first appeared (June 17) on the lower leaves of three plants in a plot within a deep-plowed strip; 10 days later it was detected in a non-plowed strip, and in the following week, it was found in 13-15 plots within each plowing type. Ten days later (July 14), the number and percentage of infected plants in all 108 plots were recorded (Table 2) and alalysed (AOV). The largest number of infected plants was found in the deeply plowed strips when compared with other treatments but differences were not significant. However, cultivars differed significantly ($P = 0.01$) as PI 153.293 was more susceptible than Maple Presto or Evans. Thus depth of plowing had little influence on overwintering of the pathogen in this test at Ottawa.

Table 2. Number and percent of infected plants of three soybean cultivars in deep, normal and non-plowed strips on July 14, 1980.

	Deep		Normal		Non-plowed	
	No.	%	No.	%	No.	%
Maple Presto	76	10.7	18	2.4	15	2.2
Evans	70	11.6	18	2.9	15	2.3
PI 153.293	339	42.8	176	21.5	265	32.3
Mean	162	23.0	71	9.6	98	13.6

Conclusions

The soybean bacterial blight pathogen, P. ***syringae*** pv. ***glycinea*** withstood a wide range of soil temperatures (-5° to 35°C) for 12 mo, although its virulence was affected by prolonged incubation at higher temperatures, especially in non-sterile soil. Under field conditions at Ottawa, it overwintered in soil plowed at depths of zero to 37 cm. ***In vitro*** results indicate that the pathogen has the ability to oversummer at higher temperatures. At the present time not enough information is available to propose a rotation procedure to reduce the inoculum potential in soil.

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