The Relationship of *Xanthomonas campestris* pv. *translucens* to Frost and the Effect of Frost on Black Chaff Development in Wheat

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ABSTRACT

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The relationship of *Xanthomonas campestris* pv. *translucens* to frost injury and subsequent colonization and infection of wheat plants was investigated. Ice nucleation activity (INA) of nine strains of *X. c.* pv. *translucens* was determined according to the droplet test at −9 C. Frost damage and lesion development on plants were tested at −4 and −3 C. All nine strains of *X. c.* pv. *translucens* were INA-positive. Wheat, barley, bean, and corn plants sprayed to runoff with suspensions containing 10^8 colony-forming units (cfu) of *X. c.* pv. *translucens* per milliliter sustained greater frost damage than plants inoculated with water alone. The amount of frost damage to plants increased significantly when the time of exposure to frost or the time between inoculation and cold treatment increased. Lesions developed more rapidly in plants subjected to frost than in plants not exposed to frost. Lesions also increased in size and number as the incubation time increased between inoculation and freezing. Frost damage and disease severity in plants were directly related to the number of INA bacteria present on those plants during the frost period. As few as 30 cfu of *X. c.* pv. *translucens* per square centimeter of leaf area resulted in increased frost injury. *X. c.* pv. *translucens* is capable of epiphytic growth on wheat plants, and frost damage may be enhanced by epiphytic populations of this bacterium, which can, in turn, increase disease severity.

Additional key words: bacteria, ice nucleation activity.

Black chaff of wheat, caused by *Xanthomonas campestris* pv. *translucens* (34), is a serious disease of wheat worldwide (42). Seed is considered the most important source of primary inoculum (14,34) and little is known about secondary inoculum. Like many plant pathogenic bacteria, an injury, such as from windblowne sand or insects, is thought to provide an entrance for infection by *X. c.* pv. *translucens* (39). In many wheat-growing regions, black chaff occurs only sporadically. In eastern Idaho, black chaff is a serious disease in irrigated spring wheat (32), but its severity and distribution varies from year to year. The disease is most severe in areas of higher elevation. In an attempt to determine why the disease occurs sporadically, we investigated the possibility that 1) *X. c.* pv. *translucens* may increase frost injury to wheat plants and 2) black chaff development may be influenced by frost injury.

MATERIALS AND METHODS

Bacterial strains. Strains of *X. c.* pv. *translucens* and one strain of *Pseudomonas syringae* pv. *syringae* used in this study (Table 1) were maintained on slants of yeast extract-dextrose-calcium carbonate (YDC) (43) and King et al's medium B (KB) (15), respectively, at 4–5 C.

Ice nucleation activity (INA). All strains of *X. c.* pv. *translucens* and strain C-271 of *P. s.* pv. *syringae* were grown on YDC and KB agar plates, respectively, overnight at room temperature (23–25 C). Cells (approximately 10^9 cfu/ml) were suspended in 0.1 M phosphate buffer, pH 7.0, and tested for INA at −9 C using the droplet freezing procedure of Lindow et al (21). Aluminum foil weighing pans were sprayed with a solution of 1% paraffin in xylene. The pans were then placed in an oven set at 55 C to evaporate the xylene. The aluminum pans were floated in a 1-L beaker containing ethanol:water (1:1, v/v) and cooled to −9 C by Lauda-Brinkman K-2/R refrigerated circular water bath (Brinkman Instruments Inc., Westburg, NY). Ten 10-μl droplets of each bacterial suspension were placed on a pan. The time required for each droplet to freeze was recorded in seconds.

Ice nucleation frequencies of strain B-504 of *X. c.* pv. *translucens* and strain C-271 of *P. s.* pv. *syringae* were calculated at −5 and −9 C by the method of Vali (37) as modified by Lindow et al (22). Cell suspensions of strains B-504 and C-271 were prepared as above and serially diluted to 10^{-9}. Forty 10-μl droplets of each dilution were applied to paraffin-coated aluminum foil boats and INA was determined as described above. The number of droplets that froze within 3 min was recorded for each dilution. The number of ice nuclei per unit volume and the cell density (cfu/ml) for each dilution were calculated and the ice nucleation frequencies were then determined.

Effect of time of exposure at −4 C on frost damage and disease severity. Wheat (*Triticum aestivum* L. em Thell, cv. Borah), barley (*Hordeum vulgare* L., cv. Nk425), corn (*Zea mays* L., cv. ASG-5), and bean (*Phaseolus vulgaris* L., cv. Bush Blue Lake-47) were

TABLE 1. Strains of bacteria used in study of relationship of black chaff to frost

Organism	Laboratory strain no.	Source[a]	Host of origin	Location	Year isolated
Xanthomonas campestris pv. *translucens*	B-502	1	Wheat	Idaho	1982
	B-504	1	Wheat	Idaho	1982
	B-505	1	Barley	Idaho	1982
	B-506	1	Wheat	Idaho	1982
	B-520(4779)	2	Wheat	Brazil	1983
	B-523	1	Wheat	Idaho	1982
	B-524	1	Wheat	Idaho	1982
	B-528	1	Wheat	Idaho	1983
	B-607	1	Wheat	Idaho	1985
Pseudomonas syringae pv. *syringae*	C-271 (PSS-17)	3	Bean	Minnesota	1984

[a] Sources: 1 = N. W. Schaad, Moscow, ID; 2 = S. K. Mohan, Londrina, Brazil; 3 = J. P. Hubbard, Agrigenetics, Farmington, MN.

grown for 15 days (two- to three-leaf stage) in a greenhouse at 22 ± 2 C with a 12-hr photoperiod (under nonhardening conditions).

Inoculum was prepared as follows: Strains B-502, B-504, and B-607 of *X. c.* pv. *translucens* and strain C-271 of *P. s.* pv. *syringae* were grown on YDC and KB agar plates, respectively, for 24 hr at room temperature (23–25 C). Bacterial cells were suspended in sterile distilled water and the suspension adjusted to approximately 1×10^8 cfu/ml (38). The plants were gently sprayed to runoff with the inoculum using a chromatographic sprayer and placed in a Percival model E-54U-DL dew chamber (Percival Manufacturing Co., Boone, IA) at 25 C and 100% relative humidity (RH) for 24 hr. Control plants were sprayed with water. Each treatment consisted of three leaves on each of four plants and was replicated four times.

After incubation, plants were moved to a laboratory bench at 25 ± 2 C for 15 min to reduce the amount of free moisture on the plants. The plants were then placed in an upright freezer (Signature frostless freezer, Sears Roebuck and Co.) at −4 C for 0, 5, 10, 15, 20, or 30 min. After cold treatment, plants were returned to the dew chamber for 24 hr and checked for frost damage. Damage was expressed as percentage of leaf area injured by cold treatment. For disease severity, wheat and barley plants were inspected and rated for disease development 14 days after inoculation. A rating of 0–5 (0 for no symptoms and 5 for total leaf area with symptoms) was used.

Amount of frost damage and its effect on lesion development. Inocula of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* were prepared as described above. Fifteen-day-old wheat plants (grown in a greenhouse at 22 ± 2 C) of cultivars Borah or Waid were divided into three groups of 90 plants. Plants in groups 1, 2, and 3 were inoculated with sterile distilled water, *X. c.* pv. *translucens* strain B-504, or *P. s.* pv. *syringae* strain C-271, respectively. A 3-ml syringe with a 26-gauge needle was used to inoculate each plant in three places: 2 cm below each leaf tip, 2 cm above each leaf base, and at the growing point (leaf sheath puncture). Half of the plants from each group were kept on a laboratory bench at 25 ± 2 C and the other half were placed in the dew chamber at 25 C and 100% RH.

At time 0, 1, 2, and 3 days after inoculation, plants in each treatment were exposed to freezing temperature in a rate-controlled chest-type freezer (36). The freezer was equipped with an aluminum plate to serve as a heat sink. A fan circulated air around the plate, preventing a vertical thermal gradient. Heat was provided by a 168-W soil heating cable (1.2-m long) with current controlled by a Love model 51-8120 proportional controller linked with a Love model 105 cam-type programmer (Love Controls Corp., Wheeling, IL). Plants placed in the freezer at 0.5 C were cooled from 0.5 to −3 C at a rate of 0.2 C/min, held at −3 C for 10 min, and then returned to their previous locations. Twenty-four hours later, plants were checked for frost damage as described above. Fourteen days later, each leaf was detached and the length of each disease lesion or group of lesions was recorded to the nearest millimeter. Plants not subjected to frost treatment were used as controls. Three plants per pot were used in each treatment and each treatment was replicated three times. The experiment was also conducted in an upright freezer at −4 C. The exposure times for cultivars Borah and Waid in the upright freezer were 20 and 10 min, respectively.

Level of inoculum required to influence frost injury. Twenty-four-hour-old cultures of *X. c.* pv. *translucens* strain B-504 from YDC agar plates and *P. s.* pv. *syringae* strain C-271 from KB agar plates were suspended in sterile saline (0.85% sodium chloride) and their densities adjusted to 1.0 optical density (OD) at 540 nm. Tenfold serial dilutions of the suspensions were then atomized onto nonhardened, 2-wk-old wheat plants of cultivar Borah. The inoculated plants were placed in a dew chamber at 25 C for 5 hr (100% RH) and then transferred to a laboratory bench for 15 min. Three samples of one leaf each were collected from plants in each treatment. To determine the presence of *X. c.* pv. *translucens* and *P. s.* pv. *syringae*, each leaf sample was cut into approximately 2-cm-long pieces, placed in an 18 × 15-mm test tube containing 5 ml of sterile saline plus 0.01% Tween 20, and washed by being shaken on a reciprocal shaker for 5 min and spun for 1 min. Appropriate tenfold serial dilutions were made and 0.1 ml pipetted onto each of three plates of XTS agar (32) for *X. c.* pv. *translucens* and KBBC agar (26) for *P. s.* pv. *syringae*. Immediately after the leaf samples were collected, the plants from each treatment with 24 remaining leaves were placed in the rate-controlled chest freezer for 15 min at −3 C. The plants were returned to the dew chamber for 24 hr and then checked for frost damage as described above. Noninoculated plants and plants inoculated with saline or distilled water served as controls. Three plants were used per treatment and each treatment was replicated three times.

Epiphytic growth of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* on wheat. Two groups each of 18 nonhardened, 2-wk-old wheat plants, cultivar Borah, were inoculated with 3×10^8 cfu of *X. c.* pv. *translucens* strain B-504 and 1×10^8 cfu of *P. s.* pv. *syringae* strain C-271 per milliliter as described above. Each treatment consisted of three plants and was replicated three times. One group of plants was placed in a dew chamber at 25 C and the other was kept on a laboratory bench at 25 ± 2 C. After 0, 1, 3, 6, 10, 14, and 21 days, leaf samples were collected, washed, and tenfold serial dilutions of the wash water were assayed on XTS and KBBC, as above, to determine levels of each pathogen present.

RESULTS

Ice nucleation activity. All strains of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* C-271 were active as ice nuclei. The mean time for droplets of cell suspensions of *X. c.* pv. *translucens* strains to freeze ranged from 15 to 33 sec. In contrast, droplets of cell suspensions of *P. s.* pv. *syringae* strain C-271 required 52 sec to freeze. Droplets of phosphate buffer without bacteria required 286 sec to freeze. Ice nucleation frequency for strain B-504 of *X. c.* pv. *translucens* and strain C-271 of *P. s.* pv. *syringae* at −5 C were 4.6×10^{-2} and 7.2×10^{-3}, respectively. As the temperature decreased from −5 to −9 C, the nucleation frequency increased to 9.3×10^{-1} for strain B-504 and to 3.4×10^{-2} for strain C-271.

Time of exposure at −4 C on frost damage and disease severity. The temperature of −4 C inside the upright freezer increased to 3 ± 0.5 C as soon as the plants were placed inside the freezer. The temperature then gradually decreased and reached −4 C again after 20 min.

Results of inoculation with *P. s.* pv. *syringae* and *X. c.* pv. *translucens* varied with the host and time of exposure to −4 C (Table 2). Both bacteria caused significantly more frost damage (compared to water controls) to plants when exposed to −4 C for 20 min or longer (Table 2). Frost damage influenced by *X. c.* pv. *translucens* and *P. s.* pv. *syringae* did not differ significantly over all hosts and times of exposure (Table 2).

Both bacteria were pathogenic on wheat plants. Disease

TABLE 2. Relationship between the time of exposure to −4 C and the amount of damage in different plants influenced by *Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *translucens*

Plant	Treatment	Time of exposure (min)				
		5	10	15	20	30
Wheat	Water	0 a[a,b]	0 a	5 a	12 a	25 a
	P. s. pv. *syringae*	0 a	10 ab	30 b	60 b	95 b
	X. c. pv. *translucens*	0 a	18 b	25 a	67 b	100 b
Barley	Water	0 b	0 c	0 c	10 c	20 c
	P. s. pv. *syringae*	0 b	0 c	10 c	40 d	100 d
	X. c. pv. *translucens*	0 b	0 c	12 c	50 d	100 d
Corn	Water	0 c	10 d	15 d	40 e	55 e
	P. s. pv. *syringae*	0 c	18 de	55 e	100 f	100 f
	X. c. pv. *translucens*	2 c	25 e	64 e	100 f	100 f
Bean	Water	0 d	11 f	19 f	42 g	62 g
	P. s. pv. *syringae*	0 d	14 f	50 g	70 h	100 h
	X. c. pv. *translucens*	0 d	18 f	57 g	87 h	100 h

[a] Percentage of leaf area with frost damage. Figures are mean of four replicates of four plants, each with three leaves. Data for *X. c.* pv. *translucens* taken from mean of three strains, B-502, B-504, and B-607.

[b] Means in each column and within each host followed by the same letter are not significantly different (0.01 level) as determined by Duncan's new multiple range test.

symptoms started to appear 8 to 10 days after inoculation. Symptoms for both pathogens were the same as those described previously (42).

Plants inoculated with *X. c.* pv. *translucens* and *P. s.* pv. *syringae* and exposed to −4 C for 5 or 10 min did not show significantly greater disease severity than those not exposed to cold treatment (Table 3). However, the disease severity in plants exposed to −4 C for 15 min and longer was significantly higher than that in control plants with no frost exposure or in plants exposed to −4 C for only 5 or 10 min. The majority of plants exposed to −4 C for 30 min were killed (Table 2) and no measurement of disease severity was possible.

Amount of frost damage and its effect on lesion development. Plants inoculated and placed in the dew chamber for varying times between inoculation and freezing had significantly more frost damage over all times than plants kept on a laboratory bench (Table 4). As the time between inoculation and freezing increased, the amount of frost damage also increased, regardless of whether the plants were kept in a dew chamber or on a laboratory bench.

Significantly more and larger lesions developed in wheat plants subjected to frost than in plants not exposed to frost regardless of whether the plants were incubated in a dew chamber or kept on a laboratory bench before frost treatment (Table 5). Furthermore, lesions developed more rapidly in plants incubated in a dew chamber than in plants left dry prior to freezing. Lesions also increased in size and number as the time of incubation between application of bacteria and freezing increased.

TABLE 3. Effect of time of exposure of wheat and barley to −4 C on disease severity caused by *Pseudomonas syringae* pv. *syringae* and *Xanthomonas campestris* pv. *translucens*[a]

Time of exposure (min)	Wheat			Barley		
	P. s. pv. *syringae*	*X. c.* pv. *translucens*	Water	*P. s.* pv. *syringae*	*X. c.* pv. *translucens*	Water
0	1.2 a[b]	1.2 a	0	0.0 a	1.0 a	0
5	1.0 a	1.2 a	0	0.0 a	1.0 a	0
10	1.0 a	2.0 ac	0	0.0 a	1.2 a	0
15	1.5 a	2.7 bc	0	1.0 b	1.5 a	0
20	2.0 ab	3.5 b	0	2.0 c	2.7 b	0
30	2.6 b	ND[c]	0	ND[c]	ND[c]	0

[a] Treatments (plants) were the same as in Table 2.
[b] Numbers are disease ratings: 0 = no disease; 5 = total leaf area with symptoms. Ratings were done 14 days after inoculation. Means in each column followed by the same letter are not significantly different (0.05 level) as determined by Duncan's new multiple range test.
[c] ND = No data available because plants died, due to frost injury 48 hr after inoculation.

TABLE 4. Effect of incubation time before exposure to −3 C on frost damage influenced by *Xanthomonas campestris* pv. *translucens* and *Pseudomonas syringae* pv. *syringae*

Environment	Incubation time (days)	Inoculants[a]		
		Water	*X. c.* pv. *translucens*	*P. s.* pv. *syringae*
Laboratory bench	0	0 a[b]	15 a	0 a
	1	0 a	20 a	0 a
	2	0 a	35 b	10 b
	3	0 a	35 b	10 b
Dew chamber	0	0 a	14 a	0 a
	1	5 a	40 b	10 b
	2	5 a	48 c	35 c
	3	5 a	65 d	50 d

[a] Plants were inoculated with bacteria or water using a 26-gauge needle attached to a 3-ml syringe. Control plants inoculated with water or bacteria but not exposed to frost (−3 C) were negative.
[b] Percentage of leaf area with frost damage, mean of three replicates of four plants, each with three leaves. Means in each column followed by the same letter are not significantly different (0.01 level) as determined by Duncan's new multiple range test.

Results obtained with cultivar Waid were similar to those obtained for cultivar Borah except that cultivar Waid was even more sensitive to cold treatment.

Level of inoculum required to influence frost injury. Approximately 30 cfu of *X. c.* pv. *translucens* (log cfu = 1.48, Fig. 1) and 16 cfu of *P. s.* pv. *syringae* (log cfu = 1.2, Fig. 1) per square centimeter of leaves were necessary to influence frost injury in wheat plants. Populations of *X. c.* pv. *translucens* up to 10^4 cfu/cm^2 (log cfu = 4.76) on leaves had approximately the same influence on frost damage as 30 cfu/cm^2, whereas populations greater than 10^4 cfu/cm^2 had significant influence (Fig. 1). In contrast, populations of *P. s.* pv. *syringae* were not directly related to frost damage. For example, populations of 4.2×10^3 cfu/cm^2 (log cfu = 3.62) on leaves had a significantly greater influence on frost damage (26%) than did the populations of 6.7×10^4 (log cfu = 4.83) (20%) or 5.3×10^5 (log cfu = 5.72) (23%) cfu/cm^2 (Fig. 1). However, the relative relationship between population densities of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* and the amount of frost damage that result were similar. Noninoculated plants and plants inoculated with saline or distilled water sustained less than 4% frost damage (Fig. 1).

TABLE 5. Effect of incubation time before exposure to −3 C on lesion development caused by *Xanthomonas campestris* pv. *translucens* and *Pseudomonas syringae* pv. *syringae*

Environment[a]	Incubation time (days)	Inoculants		
		Water	*X. c.* pv. *translucens*	*P. s.* pv. *syringae*
Laboratory bench				
Freezing	0	0.0 a[b]	22.6 a	9.7 a
	1	0.0 a	28.3 a	22.1 b
	2	0.0 a	30.1 a	21.3 b
	3	0.0 a	27.4 a	27.6 c
No freezing	NA[c]	0.0 a	10.7 b	5.2 a
Dew chamber				
Freezing	0	0.0 a	9.8 b	21.8 b
	1	0.0 a	45.8 c	28.6 c
	2	0.0 a	52.3 c	36.7 d
	3	0.0 a	73.8 d	52.4 e
No freezing	NA	0.0 a	13.0 b	7.9 a

[a] Methods and plants the same as in Table 4.
[b] Figures are length of lesions (mm) measured 14 days after inoculation. Mean of three replicates of four plants, each with three leaves. Means in each column followed by the same letter are not significantly different (0.01 level) as determined by Duncan's new multiple range test.
[c] Not acceptable, because these plants were not exposed to frost.

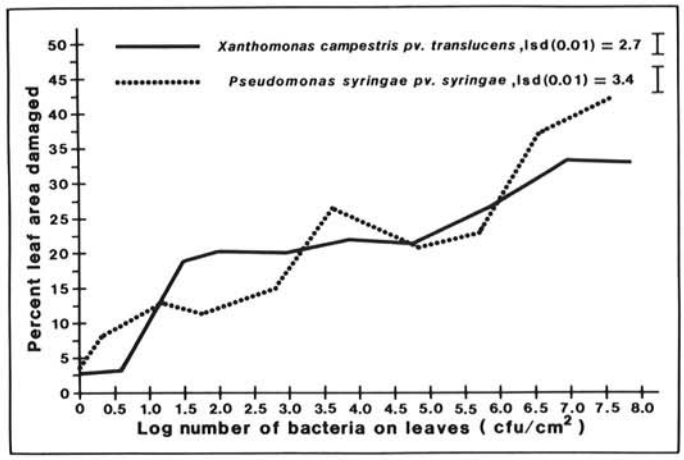

Fig. 1. Relationship between inoculum level of *Xanthomonas campestris* pv. *translucens* and *Pseudomonas syringae* pv. *syringae* and frost damage at −3 C for 15 min. Each point on X-axis is the average of three replicates of one leaf assayed 5 hr after inoculation. Each point on Y-axis is the average of three replicates of three plants, each with three leaves.

Multiplication of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* on wheat leaves. When plants were placed in a dew chamber, populations of *X. c.* pv. *translucens* increased very rapidly the first day and continued a slow increase until day 10, then decreased gradually for the next 11 days (Fig. 2). In contrast to *X. c.* pv. *translucens*, populations of *P. s.* pv. *syringae* increased more slowly and decreased much quicker (Fig. 2).

In contrast to plants kept in the dew chamber, plants kept under dry conditions on a laboratory bench did not support multiplication of *X. c.* pv. *translucens* and *P. s.* pv. *syringae*. Although cells of *P. s.* pv. *syringae* and *X. c.* pv. *translucens* could be isolated from plants 1 day after inoculation, neither organism could be isolated thereafter.

DISCUSSION

All strains of *X. c.* pv. *translucens* and the single strain of *P. s.* pv. *syringae* investigated herein were INA-positive. *X. c.* pv. *translucens* showed stronger INA and had higher ice nucleation frequencies than did *P. s.* pv. *syringae*; however, fewer cells of *P. s.* pv. *syringae* were required to influence frost damage to wheat plants. This might have been due to the fact that frost damage to wheat plants in the presence of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* was measured at −3 or −4 C (Fig. 1), but ice nucleation frequencies were determined at −5 and −9 C. The ice nucleation frequencies of a bacterial isolate at any given temperature can be very different (22). *P. s.* pv. *syringae* might have had a higher nucleation frequency at −3 or −4 C than *X. c.* pv. *translucens*, therefore requiring few cells to influence frost injury to plants.

Several INA bacteria, including *P. s.* pv. *syringae* (1,19,20,23), *P. fluorescens* biotype G (24) and biotype A (18), *P. s.* pv. *coronafaciens* (13), *P. s.* pv. *viridiflava* (8,30), *Erwinia ananas* (30), and *E. herbicola* (21), have been identified. This report identifies *X. c.* pv. *translucens* as another INA bacterium.

The presence of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* can accentuate the damage done to wheat, barley, corn, and bean plants at −4 C. These results agree with the observation that INA bacteria on plants prevents supercooling (9,11,21), thereby increasing plant sensitivity to cold temperatures. That wheat and barley are less damaged by frost than corn and beans is expected because the latter are warm season plants.

Growth of *X. c.* pv. *translucens* on wheat leaves increased more rapidly and continued longer than did growth of *P. s.* pv. *syringae*. Wheat apparently is a better host for the epiphytic growth for *X. c.* pv. *translucens* than for *P. s.* pv. *syringae* (Fig. 2). This agrees with O'Brien and Lindow's suggestion (27) that plants differ in relative receptivity to epiphytic bacteria and that some pathogenic strains show host preference for epiphytic colonization. The slow decline in populations of *X. c.* pv. *translucens* and a rapid drop in populations of *P. s.* pv. *syringae* may explain the difference in symptoms caused by the two pathogens. The hypersensitivelike lesions caused by *P. s.* pv. *syringae* (42) apparently do not support high levels of bacterial growth for long periods. In contrast, infections resulting in water-soaked lesions, like those caused by *X. c.* pv. *translucens* (42), do support high populations.

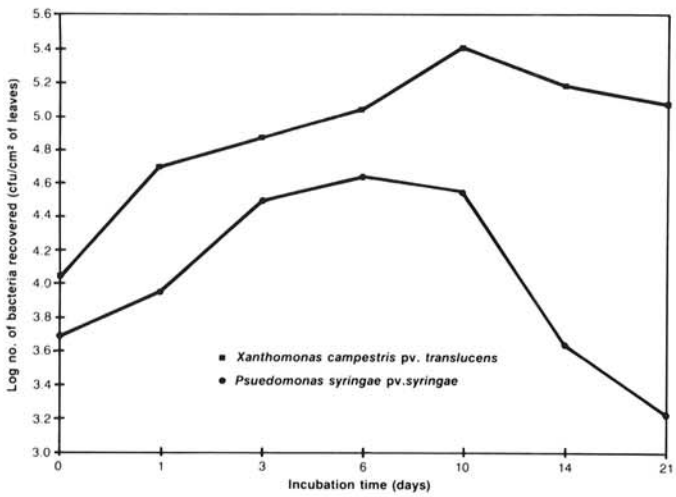

Fig. 2. Multiplication of *Xanthomonas campestris* pv. *translucens* strain B-504 and *Pseudomonas syringae* pv. *syringae* strain C-271 on wheat plants. Plants were sprayed with suspensions containing approximately 3×10^8 cells of *X. c.* pv. *translucens* or 1×10^8 cells of *P. s.* pv. *syringae* per milliliter and incubated in a dew chamber at 25 C. Samples were taken and assayed after 0, 1, 3, 6, 10, 14, and 21 days. Each point is the mean of the log population from three determinations.

Survival of *P. s.* pv. *syringae* as an epiphyte has been well documented (2,5,12,16,17,28). In this study we show that *X. c.* pv. *translucens* also is a good epiphyte on wheat.

Positive correlations were observed between increased populations of *X. c.* pv. *translucens* or *P. s.* pv. *syringae* and the amount of frost damage. This implies that the increase in the amount of frost damage observed as the result of increase in the incubation time between inoculation and freezing was indeed due to the increase in populations of these two bacteria in or on wheat plants. Positive correlations between frost damage and increased populations of bacteria reported here agree with earlier results with *E. herbicola* on corn plants (21). Similarly, the amount of frost damage and the number and size of lesions were directly correlated. Whether increase in lesion size and number is due to increase in frost damage, bacterial populations, or both has to be determined. However, our data indicate that, most likely, both increased frost damage and populations of bacteria have bearing on increased disease severity.

Previous investigators have suggested that frost injury predisposes pea plants (3), pear blossom (9,29,30), peach twigs (6,40), cherry leaves (44), and different tissues of plants from Oleaceae, Magnoliaceae, and Salicaceae families (2) to bacterial infection. Our results support these reports and suggest that the higher disease incidence in cold-injured wheat plants is directly related to the number of INA bacteria present (within limits) on those plants. High correlations between the amount of disease and populations of *P. s.* pv. *syringae* on wheat (33), *P. glycinea* on soybean (25), *P. syringae* pv. *tomato* on tomato (35), *X. c.* pv. *phaseoli* on bean (41), and *P. s.* pv. *garcae* on coffee (31) have been reported earlier.

X. c. pv. *translucens* and *P. s.* pv. *syringae* had approximately equal influence on frost damage to wheat plants with spray inoculations. Conversely, in needle inoculations, *X. c.* pv. *translucens* was more effective than *P. s.* pv. *syringae* in accentuating frost injury. Host specificity, the internal or external location of INA bacteria, and the type of the plant tissue may all play an important role in frost sensitivity. One explanation for *X. c.* pv. *translucens* being more influential following needle inoculation may be that the wounding provides avenues for ingress of the pathogen into the host tissue. Also, wheat is the natural host for *X. c.* pv. *translucens*, whereas bean is the natural host for the strain of *P. syringae* we used. This may explain why *X. c.* pv. *translucens* grows faster in wheat than *P. s.* pv. *syringae* (Fig. 2).

Populations of 10^2–10^3 cells of both *X. c.* pv. *translucens* and *P. s.* pv. *syringae*/cm^2 on leaves have been frequently recovered from field-grown wheat plants (*unpublished*). Therefore, sufficient ice nuclei perhaps are present on wheat leaves in the field to accentuate black chaff in higher elevations in southeastern Idaho.

In Idaho, strains of *P. s.* pv. *syringae* isolated from beans, wheat, or alfalfa will infect all three hosts (*unpublished*). Therefore, we support the view that *P. s.* pv. *syringae* is an assemblage of strains with a wide host range (7,10).

In the upright freezer, a significant change in the temperature was obtained as soon as plants were placed in or removed from the freezer. In contrast, the variations in temperature in the rate-controlled chest-type freezer were small (0.5–1.0 C). Therefore, we believe a rate-controlled freezer is more appropriate than a regular upright freezer in any type of study in which minimum variation in temperature is desired.

When grown under cool temperatures and especially under short daylight periods, wheat can tolerate ice formation at temperatures

to −10 C (4). This is due to the hardening of tissues by low temperatures. Substantial freezing injury, which was observed on wheat plants cooled to only −3 or −4 C, was due to the fact that we used nonhardened, greenhouse-grown spring wheat plants. Under Idaho's conditions, spring wheat normally does not get cold-hardened and therefore is prone to frost injury even at mild freezing temperatures (−2 to −4 C) (H. S. Fenwick, *personal communication*). In contrast, winter wheat is normally cold-hardened and is usually more resistant to frost injury than spring wheat. Black chaff is normally not a field problem in winter wheat in Idaho (*personal observations*). In addition, in Idaho, frost damage and disease development on spring wheat are observed throughout the growing season. Although wheat is more sensitive to frost at flowering stage than at seedling stage, we used seedlings in our study because frost injury and black chaff are observed first on seedlings and seedlings are easier to work with.

In conclusion, our results have established that both *X. c.* pv. *translucens* and *P. s.* pv. *syringae* are active ice nuclei bacteria and that the presence of *X. c.* pv. *translucens* and *P. s.* pv. *syringae* can increase frost damage to wheat plants grown in high altitude areas. Although frost is not essential to infection, the susceptibility of wheat plants to *X. c.* pv. *translucens* increases considerably following frost. Furthermore, the infection spreads more rapidly in frosted tissue. The extent of both frost injury and disease development are related to resident populations of *X. c.* pv. *translucens* or *P. s.* pv. *syringae* during frost periods.

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