Xanthomonas campestris pv. *translucens* on Triticale and Other Small Grains

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ABSTRACT

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Bacterial blight symptoms on triticale are elongated translucent, water-soaked lesions similar to those observed on other small grains. Physiological characters of *Xanthomonas campestris* pv. *translucens* (syn. *X. translucens*) from triticale are identical to those of strains from other hosts. Strains from triticale were equally virulent to triticale, wheat, and rye; much less virulent to barley; and nonpathogenic to oats, timothy, and *Bromus* spp. Strains from barley were restricted in host range, being pathogenic primarily to barley. Strains Xt-202 and Xt-211, isolated from triticale at two locations in Mexico, produced a host response nearly identical to that caused by two strains from Georgia and Alabama on 35 triticale lines. The results indicated that indigenous strains of *X. campestris* pv. *translucens* from wheat and rye can infect triticale when it is introduced into a new region. The greater severity of bacterial blight observed among triticales in the field compared to that on wheat and rye is most likely due to high susceptibility of triticale rather than changes in virulence in *X. campestris* pv. *translucens*. Both hypodermic injection and spray inoculation of seedlings of different small grain lines caused a similar host response. The chlorotic-fleck resistant reaction was easily detected by the spray-inoculation method. Triticale lines UP 7th ITSN #20, UPT 72142, S-69 (M2A-BgC), and Siskiyou were resistant to four or more of six *X. campestris* pv. *translucens* strains from the various geographic areas.

Bacterial blight caused by *Xanthomonas campestris* pv. *translucens* (J. J. and R.) Dye (syn. *X. translucens*) was first identified by Jones et al (13,14) on barley (*Hordeum vulgare* L.) in 1917. Earlier, Arthur (as cited in 22) and Heald (11) discovered a disease of wheat (*Triticum aestivum* L. em. Thell.) that was probably caused by this bacterium, but they did not identify the causal agent. The pathogen was identified on wheat (13,23) in 1919 and on rye (*Secale cereale* L.) in 1924 (20). Bamberg (3) reported the disease on wheat at two locations in Mexico during the early 1930s. The bacterium caused a severe leaf blight on triticale (*Triticosecale* Wittmack) in 1968 in the International Maize and Wheat Improvement Center (CIMMYT) breeding nurseries in Mexico (26). Since then, the disease has occurred in varying degrees of severity at CIMMYT test locations around the world (12,21). *X. campestris* pv. *translucens* was isolated from severely blighted triticale leaves in a nursery at Tifton, GA, in 1975 (B. M. Cunfer, *unpublished*). Because of its widespread occurrence and severity, bacterial blight is a threat to the development of triticale as a successful grain crop. There have been no detailed studies of the disease on triticale.

Recently, many nomenspecies of *Xanthomonas* included in the seventh edition of Bergey's manual (5) (including *X. translucens*) have been placed in *X. campestris* (6,7). Formae speciales epithets (eg, *hordei* and *secalis*) are now given pathovar status as well as the former species epithet *translucens* (7). Pathovars *hordei*, *secalis*, etc., retain their definitions based on host range, but the definition of pv. *translucens* is unclear. The neopathotype strains of both pv. *hordei* and pv. *translucens* are from barley. Strains from triticale have not been evaluated in either the old or new systems. As will be discussed, most strains that we studied exhibited a variable host range and the reaction on individual host cultivars was also quite variable. Therefore, the bacterium is referred to as *X. campestris* pv. *translucens* in that it is associated with small grains.

The objectives of this study were: to compare strains of *X. campestris* pv. *translucens* from triticale in the southeastern U.S. with strains from other geographic areas; to determine whether strains of *X. campestris* pv. *translucens* from triticale are specific to that host; and to identify potential sources of resistance among triticale lines.

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MATERIALS AND METHODS

Thirteen strains of *X. campestris* pv. *translucens* from seven hosts were obtained from N. W. Schaad (Table 1). These cultures were originally obtained from the National Collection of Plant Pathogenic Bacteria (Harpenden, UK) and the Culture Collection of Plant Diseases Division (Auckland, NZ). Additional strains of *X. campestris* pv. *translucens* were collected by the authors from triticale and other small grains in the southeastern USA and at various CIMMYT test locations in Mexico (Table 2). Strain Xt-226, isolated from triticale in Ethiopia, was obtained from the Commonwealth Mycological Institute.

Lesions with water-soaked tissues, and, if possible, bacterial exudate were triturated in sterile saline and allowed to stand for at least 15 min. Then a portion of the suspension was streaked on Bacto nutrient agar or yeast dextrose calcium carbonate agar (YDC) (25) and incubated for several days at 30 C. Isolated colonies suspected to be *X. campestris* pv. *translucens* were restreaked on YDC. Cells from a single colony were inoculated onto the host from which it was isolated. Following a positive test for pathogenicity, cells from the original colony were subcultured and lyophilized for long-term storage.

Physiological tests. A series of 13 physiological tests was performed with strains Xt-2, Xt-5, Xt-6, Xt-9, Xt-10, Xt-103, triticale strains Xt-110, Xt-114, Xt-202, Xt-211, Xt-216, and Xt-226, which were collected at widely separated geographic locations. Reference strains of *X. campestris* pv. *campestris* B-24 from cabbage and B-87A from *Brassica nigra* (obtained from N. W. Schaad) were also tested. Casein hydrolysis, nitrate reduction, and starch hydrolysis were determined as described by Király et al (17). The procedure of Stanier et al (24) was used to test for oxidase. Gelatin hydrolysis and action on milk agar were determined according to the methods specified by Goodman (9); citrate utilization was determined in Koser's citrate medium (1); catalase,

TABLE 1. *Xanthomonas campestris* strains obtained from national culture collections

Received as[a]	Designation	Type collection	Host	Location
X. translucens	Xt-1	(NCPPB[b] 973)	*Hordeum vulgare* (barley)	USA
X. translucens cerealis	Xt-2	(NCPPB 1836)	*Secale cereale* (rye)	USA
X. translucens cerealis	Xt-3	(NCPPB 1943)	*Hordeum vulgare*	USA
X. translucens hordei	Xt-4	(NCPPB 2181)	*Dactylis glomerata* (orchard grass)	Japan
X. translucens phleipratensis	Xt-5	(NCPPB 1839)	*Phleum pratense* (timothy)	USA
X. translucens undulosa	Xt-6	(NCPPB 1945)	*Triticum aestivum* (wheat)	Canada
X. translucens	Xt-7	(PDDCC[c] 5752)	*Hordeum vulgare*	USA
X. translucens cerealis	Xt-8	(PDDCC 1409)	*Bromus inermis* (smooth bromegrass)	USA
X. translucens hordei	Xt-9	(PDDCC 5735)	*Hordeum vulgare*	India
X. translucens oryzicola	Xt-10	(PDDCC 5743)	*Oryza sativa* (rice)	Malaysia
X. translucens phleipratensis	Xt-11	(PDDCC 5744)	*Phleum pratense*	USA
X. translucens secalis	Xt-12	(PDDCC 5749)	*Secale cereale*	Canada
X. translucens undulosa	Xt-13	(PDDCC 5755)	*Triticum aestivum*	Canada

[a] All *X. translucens* strains are incorporated into *X. campestris* (Bergey's Manual, 8th ed.).
[b] National Collection of Plant Pathogenic Bacteria, Harpenden, Hertfordshire, England.
[c] Culture Collection of Plant Diseases Division, Auckland, New Zealand.

TABLE 2. *Xanthomonas campestris* pv. *translucens* strains collected in the United States, Mexico, and Ethiopia

Strains	Host	Location
Xt-102, Xt-103	Rye	Tifton, GA
Xt-105, Xt-108, Xt-109, Xt-112	Rye	Griffin, GA
Xt-104	Rye	Ft. Valley, GA
Xt-116	Wheat	South Dakota
Xt-110, Xt-111	Triticale	Tifton, GA
Xt-114	Triticale	Huntsville, AL
Xt-203, Xt-209	Rye	Ciudad Obregon, Mexico
Xt-201	Wheat	Hermosillo, Mexico
Xt-214	Wheat	El Rufugio, Mexico
Xt-202, Xt-206, Xt-207	Triticale	Ciudad Obregon, Mexico
Xt-210	Triticale	Mexico CMI B5562
Xt-211	Triticale	Celaya, Mexico
Xt-215	Triticale	El Batan, Mexico
Xt-216, Xt-217	Triticale	Toluca, Mexico
Xt-226	Triticale	Ethiopia CMI B6106

and growth on sucrose agar (levan test) were determined according to Mitruka (19). Both Bacto triple-sugar iron agar and Bacto peptone iron agar were used to test for H_2S production (1). Hydrolysis of Tween-80 was determined in neutral-red agar with 2% Tween-80 (2). For salt-tolerance tests, nutrient broth was adjusted to the desired concentration with NaCl. All strains were grown through two transfers in liquid medium 523 (15) prior to the initiation of each test. All tests were performed at least twice.

Inoculation tests. Bacterial cultures were grown overnight in liquid medium 523 on a reciprocal shaker at 30 C. Cells were suspended in physiological saline and adjusted to 10^5 colony-forming units (cfu) per milliliter with a colorimeter. Seedlings at the three- to five-leaf stage were inoculated by hypodermic injection of the suspension into the leaves. Inoculations were made in such a way that only a few microliters of inoculum were infiltrated into the leaves. Plants were incubated in the greenhouse for 10–14 days at 20–27 C.

Special precautions were employed for handling strains from outside the USA. Plants were inoculated and incubated within a Plexiglas enclosed growth room within a greenhouse. Plants to be inoculated were set inside deep plastic containers so that the leaves did not extend beyond the container. The plants were watered from below so that inoculated leaves were not wetted. After inoculating and examining the plants, workers washed their hands in sodium hypochlorite. All inoculation materials as well as plants, soil, and containers were autoclaved at the conclusion of each experiment.

Plants were also inoculated in some tests by atomizing a bacterial suspension onto seedlings that had been held overnight in a mist chamber to induce water-soaking. After inoculation, the plants were kept in the mist chamber for 30 hr, then returned to the growth room.

Thirty-four strains of *X. campestris* pv. *translucens* from eight hosts were inoculated onto 6TB-059 triticale, Holley wheat, Athens Abruzzi rye, Volbar barley, and Coker 66-22 oats (*Avena sativa* L.). Strains Xt-110 and Xt-114 from the southeastern USA, Xt-202, Xt-211, and Xt-216 from Mexico, and Xt-226 from Ethiopia, all isolated from triticale, were inoculated on 75 cultivars and breeding lines of five small grain species to determine any similarities or differences in pathogenicity. Five plants of each cultivar were inoculated in each test and all tests were conducted at least twice.

RESULTS

Symptoms. The susceptible reaction on triticale was a narrow translucent, water-soaked lesion similar to that observed on other hosts. The bacterium caused irregularly elongated stripes sometimes extending along the entire length of the leaf. Bacterial exudate was present on most lesions and the amount increased at higher humidity.

In some susceptible reactions, only slight or mottled water-soaking was observed until the lesions became older. In other cases, chlorosis was associated with water-soaking from the beginning of symptom expression. At other times, necrosis occurred within a few days after water-soaking and chlorosis developed. Necrosis was never observed prior to or at the same time that the translucent response was seen.

Resistant reactions varied from no reaction, as sometimes seen on oats and some triticales, to mild chlorosis, or to necrosis. The chlorotic and necrotic reaction developed 2–4 days after inoculation. The chlorotic area sometimes enlarged to as much as 1 cm in diameter around the inoculation site. Necrotic lesions were rarely >5 mm in diameter. Numerous chlorotic flecks 1–2 mm in diameter developed on some resistant lines; this response was most easily observed when plants were inoculated by the spray method.

Physiological tests. The reaction of all *X. campestris* pv. *translucens* strains from triticale and other Gramineae was generally quite similar. All strains gave positive reactions for casein hydrolysis, catalase, citrate utilization, gelatin hydrolysis, mucoid growth on sucrose agar (levan test), and hydrolysis of Tween-80. Negative results were observed for oxidase, nitrate reduction, and H_2S production. Action on milk was alkaline. Most strains grew at 2.5% NaCl and some grew very slowly at 3%. Only two strains, Xt-6 and Xt-9, grew poorly above 1% NaCl. Strains of pv. *campestris* hydrolyzed starch whereas all strains from Gramineae did not. Otherwise, no differences between pv. *campestris* and pv. *translucens* strains were noted among the tests conducted. Therefore, these tests did not serve as determinative characters to differentiate strains from the various graminaceous hosts.

Host range. Strains of *X. campestris* pv. *translucens* from wheat, rye, and barley were usually most virulent on the host from which they were isolated when only one cultivar of each host species was inoculated (Table 3). Strains from triticale were about equally virulent on all species except oats. The reaction on the most susceptible host was exhibited 1–2 days before moderately

susceptible hosts developed symptoms. None of the 34 strains were pathogenic to Coker 66-22 oats. Oats exhibited either no reaction or very slight chlorosis or necrosis. Individual strains from rye and wheat were also pathogenic to all four species of small grains, although there was more variation among them than among the strains from triticale. Strains from barley were the most restricted in host range, being highly virulent to barley, but only occasionally pathogenic to other hosts (Table 3). Likewise the strains from wheat, rye, and triticale were least virulent to barley (Tables 3 and 4).

Strains from rice and several forage grasses were avirulent to the small grains (Table 3). In additional inoculations, selected strains from triticale were avirulent to timothy and several *Bromus* spp.

Comparison of *X. campestris* pv. *translucens* strains from diverse geographic locations. *X. campestris* pv. *translucens* from triticale at two locations in the southeastern USA, three widely separated locations in Mexico, and a strain from Ethiopia were selected for inoculation on 35 triticales, 14 wheats, 14 ryes, eight barleys, and four oats. A great diversity in reaction was noted. However, disease severity on triticale, wheat, and rye lines was nearly identical but much less on barley (Table 4). As in the previous experiment none of the strains infected oats. The similarity in pathogenicity among strains from triticale, wheat, and rye was also noted.

Based on a resistant or susceptible reaction triticale showed the greatest diversity of reaction among the small grains. Fourteen lines (39%) were susceptible to all six strains (Table 4). Siskiyou and

TABLE 3. Host range reactions of small grains to strains of *Xanthomonas campestris*[a]

Original host and strain	Triticale (6TB-059)	Wheat (Holley)	Rye (Athens Abruzzi)	Barley (Volbar)	Oats (Coker 66-22)
Triticale					
Xt-110	+++[b]	++	++	+++	C
Xt-111	+(V)	+(C)	+++	+(C)	0
Xt-114	+++	+++	+++	++	C
Xt-202	+++	++	++	++	C
Xt-206	+	++	++	++	C
Xt-207	++	++	++	++	C
Xt-210	+++	++	++	++	C
Xt-211	++	++	++	++	C
Xt-215	++	++	++	++	C
Xt-216	+	+++	+++	++	C
Xt-217	++	++	++	++	0
Xt-226	++	+	++	+++	N
Wheat					
Xt-6	+	+++	+	C	C
Xt-13	+	++	C	C	N
Xt-116	+	+++	++	+	N
Xt-201	+++	++	C	C	N
Xt-214	+(C)	++	++	+	N
Rye					
Xt-12	+	C	++	+	0
Xt-102	++	0	++	+	C
Xt-103	+	++	+++	+++	N
Xt-104	+(B)	+	+++	+++	N
Xt-105	C	++	++	+	N
Xt-108	++	0	++	+	0
Xt-109	C	C	++	+	0
Xt-203	++	+++	++	+	C
Xt-209	++(C)	+	+	+	0
Barley					
Xt-1	C(B)	+	C	++	C
Xt-7	+	C	0	+++	C
Xt-9	C	C	C	+++	0
Other					
Xt-4	C	0	0	+	N
Xt-5	0	N	C	N	0
Xt-8	C	+	N	+	0
Xt-10	0	0	0	0	0
Xt-11	0	0	0	0	0

[a] Symptom readings are averaged from five plants per treatment replicated three times.

[b] + = Mild water-soaking, ++ = moderate water-soaking, +++ = severe water-soaking, 0 = no symptoms, B = bleached lesion, C = chlorosis, N = necrosis, and V = variable reaction.

TABLE 4. Virulence of six *Xanthomonas campestris* pv. *translucens* strains from triticale inoculated onto breeding lines and cultivars of five small grains and frequency distribution of resistance among small grains to the bacterial strains

Host	No. of lines inoculated	No. of lines × strain combinations	Average disease severity[a]	No. of lines resistant to 0–6 strains of *X. campestris* pv. *translucens*						
				0	1	2	3	4	5	6
Triticale	35	210	2.03	14	6	8	3	1	1	2
Wheat	14	84	1.99	2	6	3	2	1	0	0
Rye	14	84	1.99	4	3	3	3	0	1	0
Barley	8	48	1.27	0	0	1	2	3	2	0
Oats	4	24	0	0	0	0	0	0	0	4

[a] Disease severity scale: 0 = no disease, 1 = lesion <5 mm (resistant), 2 = water-soaked lesions 5–15 mm in diameter (susceptible), and 3 = water-soaked lesion >15 mm in diameter.

UPT 72142 were resistant to four and five strains, respectively. Lines S-69 (M2A-BgC) and UP 7th ITSN #20 were resistant to all six strains.

Strains Xt-110 and Xt-114 caused identical reactions on all 35 triticales inoculated. Therefore, comparisons were made to see how many lines of the other host species also reacted the same to these two strains. Next, each time Xt-110 and Xt-114 produced the same response on individual lines of each species, comparisons were made to see if strains from outside the USA also caused the same response. Lines susceptible to all six strains were excluded from the set. Four of the foreign strains caused a similar host response as the two U.S. strains on triticale. Only Xt-216 caused a different host reaction greater than 66% of the time. Strains Xt-110, Xt-114, Xt-202, and Xt-216 caused identical responses to nine lines of wheat. When the two wheat lines susceptible to all strains are included, these four strains caused identical responses on 11 of 14 wheats. The highest rate of identical responses on rye cultivars was obtained with Xt-226 and the least with Xt-202. None of the barleys was susceptible to all strains and only one, cultivar Keowee, had the same response to Xt-110 and Xt-114.

DISCUSSION

Physiological tests have not proved to be determinative characters in the identification of *X. campestris* pv. *translucens* from graminaceous hosts. The reaction of *X. campestris* pv. *translucens* from triticale to 13 tests was essentially the same as that reported for *X. campestris* pv. *translucens* from other hosts (4,8). One major difference was the inability of the strains to produce H_2S. The source of sulfur in the current tests was sodium thiosulfate rather than cysteine as in previous tests (8).

Bamberg (3) found that a strain of *X. campestris* pv. *translucens* from barley and three strains from wheat were more virulent on the host from which they were isolated when only one barley and wheat cultivar were inoculated. When 20 wheat cultivars were inoculated with these four strains, the mean disease rating was similar for each strain. Our results were similar to Bamberg's for six strains from triticale inoculated on triticale, wheat, and rye, but the reaction among eight barleys was markedly different. We had available only three strains from barley. These reacted as pv. *hordei* by being restricted primarily to barley. A similar response has been found in previous studies (4,10). Strains from barley appear to exhibit two pathogenicity patterns. Some are restricted to barley, pv. *hordei*-type (4,8,10), and others are similar to strains from wheat, pv. *undulosa*-type (3). Most studies have utilized small numbers of strains and small numbers of host genotypes. Similarly, some studies have found evidence for strains restricted largely to rye, pv. *secalis* (4). Other strains from rye (3), including those of this study, fit the pv. *undulosa* pattern. According to the taxonomy of Hagborg (10), recognized in Bergey's eighth edition (6), strains from triticale fit the description of both pv. *undulosa* and pv. *secalis*.

The present work was not intended to be an in-depth taxonomic study. However, our results and the studies cited above show that a collection of strains from individual small grain species exhibits a variable host range and the pathovars are not as clear cut as designated. The data from strains from barley and the avirulence of strains from rice and forage grasses to small grains indicate some specialization within *X. campestris* on Gramineae.

Bamberg (3) also noted that among 50 wheat cultivars inoculated with four strains of *X. campestris* pv. *translucens* no pathogenicity patterns emerged to suggest physiological races. Our results with triticale concur with those of Bamberg. *X. campestris* pv. *translucens* appears to encompass genotypes with a wide variability in host range and cultivar response within host species. Katznelson and Sutton (16) found such wide variability among *X. campestris* pv. *translucens* strains that phage typing was not possible.

The greater incidence and severity of bacterial blight on triticale in the field in comparison to its relatively minor importance on rye and wheat is not the result of a change in virulence in *X. campestris* pv. *translucens* but the result of a more susceptible population of host genotypes. The appearance of bacterial blight when triticale is introduced into a new area probably results from the dissemination of the *X. campestris* pv. *translucens* population indigenous on wheat and rye and possibly other hosts to susceptible triticales.

The discovery among the 35 lines tested of four triticales with resistance to strains of *X. campestris* pv. *translucens* from diverse geographic locations is encouraging. The results are from seedling inoculations only, however. We are presently comparing results on adult plants in field tests. UPT 72142, UP 7th ITSN #20, and Siskiyou have performed well in yield trials in Georgia (18). Highly resistant lines have been found during natural epidemics at CIMMYT test locations (12). The continued selection of parents with bacterial blight resistance for use in development of new triticales appears to be an attainable goal.

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