Survival of *Xanthomonas campestris* pv. *translucens* Between Successive Wheat Crops in Arkansas

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ABSTRACT

Reducing the level of initial inoculum may be a feasible means of controlling bacterial streak and black chaff of wheat caused by *Xanthomonas campestris* pv. *translucens*. The objective of this research was to determine the principal source of inoculum under Arkansas conditions. Using artificially infested seeds, a rifampicin-resistant mutant of the pathogen (strain 88-14Rd) was established in field plots of wheat cultivars Florida 302 (susceptible) and Tarral 101 (moderately resistant) at two locations and in a plot of Florida 302 at a third location. Bacterial streak caused by strain 88-14Rd developed in all plots. Strain 88-14Rd was not detected in crop debris, soil, or possible alternative host plants in the field 3 mo after harvest at any location. No bacterial streak symptoms were observed at any location on Florida 302 planted with disinfested seed 4 mo after harvest. However, strain 88-14Rd was isolated from one of 480 Florida 302 leaves assayed from one location at one sampling time. The percentage of harvested seed infested with strain 88-14Rd 2 mo after harvest ranged from 9.8 to 37.7% for Florida 302 and from 0.3 to 5.3% for Tarral 101. Under growth chamber conditions, transmission of seedborne strain 88-14Rd to seedlings ranged from 4.0 to 24.5% for Florida 302 and from 0.0 to 0.2% for Tarral 101. Under field conditions, however, strain 88-14Rd was isolated only from one seedling of one Florida 302 seed lot. Based on the poor survival of the pathogen in the field and the relatively high percentage of infested seed and transmission to seedlings under growth chamber conditions for the susceptible cultivar, infested seed is suspected to be the principal source of inoculum.

Bacterial streak (black chaff) of wheat (*Triticum aestivum* L. em. Thell), caused by *Xanthomonas campestris* pv. *translucens* (Jones et al) Dye, was recently identified as an important disease in Arkansas. Bacterial streak was found only occasionally in Arkansas from 1984 to 1986 and was found statewide in 1987, predominantly on cultivars Hunter and Florida 302 (T. L. Kirkpatrick, personal communication). There was circumstantial evidence that these early occurrences of bacterial streak may have been associated with infested seeds. Since 1987, bacterial streak increased in incidence and severity on several soft red winter wheat cultivars in Arkansas (10).

Growing resistant cultivars appears to be a feasible means of control (13), and reducing the level of initial inoculum also may contribute to control if the principal inoculum sources are known. Seed, crop debris, alternative hosts, and soil are the most likely sources of bacterial inoculum for annual crops such as wheat (7,15).

*X. c. translucens* is seedborne and has been reported to overwinter in Minnesota on weedy grasses and crop debris of spring wheat, and in soil (1,2). Infested seeds were the principal source of bacterial streak inoculum for spring wheat in Idaho (3) and spring barley and wheat in Brazil (9). Forster and Schaad (3) were able to nearly eliminate bacterial streak by planting pathogen-free seed.

There have been no studies on the ability of *X. c. translucens* to overwinter in the field between successive winter wheat crops. The objective of this study was to determine the importance of seed, crop debris, alternative hosts, and soil as sources of initial inoculum between successive crops of soft red winter wheat. A preliminary report has been published (12).

MATERIALS AND METHODS
Strain 88-14Rd of *X. c. translucens*, which is resistant to rifampicin at 100 μg/ml and can be readily isolated and distinguished from wild type strains (11), was used in this study. This research involved three steps: 1) establishing strain 88-14Rd in the field to obtain naturally infested seed, crop debris, soil, and potential alternative hosts; 2) determining how well strain 88-14Rd survived on naturally infested seed and was transmitted to the next crop; and 3)
determining how well strain 88-14 \textsuperscript{Rd} survived in the field and was transmitted to the next crop.

Seed of soft red winter wheat cultivars Florida 302 (susceptible) and Terral 101 (moderately resistant) was treated with dry heat at 60 C for 10 days to disinfect the seed of any naturally occurring "X. c. translucens" (4). Disinfested seeds were assayed for the pathogen by comminuting four 30-g samples in cold (4 C) sterile saline containing 0.05% Tween 20 and dilution plating on XTS medium (14) without gentamycin. Disinfested seeds were infested with strain 88-14 \textsuperscript{Rd} by vacuum infiltration of inoculum (5 \times 10^6 cfu/ml) as described previously (11). The average population of strain 88-14 \textsuperscript{Rd} associated with the seed was determined at planting by comminuting four 30-g samples of each seed lot as described above and dilution plating on NDA-RC medium (11). Data were analyzed in log_{10} units, and the mean was transformed to the original scale.

One plot of each cultivar was planted at the Strawberry Substation, Bald Knob, Arkansas, and at the Vegetable Substation, Kibler, Arkansas, in October 1990. Cultivars were separated by 8 m of oats. One plot of Florida 302 was planted at the Southwest Research and Extension Center, Hope, Arkansas, in November 1991. Plots were approximately 30 m square. One large plot of each cultivar rather than several smaller plots was used to limit spread of the pathogen between plots.

At Feakes growth stage (GS) 11.2 (soft dough) (6), plots were evaluated for bacterial streak severity, and a random sample of 20 lesions per cultivar per location was assayed to determine if symptoms were caused by strain 88-14 \textsuperscript{Rd}. At harvest in June, all crop debris and seed except for a 25-kg seed sample were left in the field to maximize the amount of potential inoculum in the field. Plots at Bald Knob and Kibler were disked, and soybean was planted in a fourth of the plot areas to determine if soybean, commonly double-cropped with wheat in Arkansas, was an alternate host. The plot at Hope was not tilled or planted to soybean.

Samples of harvested seed were cleaned twice using an Almaco air blast seed cleaner and stored at room temperature (approximately 22 C). The average population of strain 88-14 \textsuperscript{Rd} associated with the harvested seed was determined 1 mo after harvest as described above. Percent infestation of strain 88-14 \textsuperscript{Rd} in each seed lot was determined at 2 and 13 mo after harvest by a tube assay in NB-RCMP medium (11) on six subsamples of 60 individual seeds. Briefly, the tube assay involves incubating individual seeds or leaves in nutrient broth amended with certain antibiotics and detecting strain 88-14 \textsuperscript{Rd} by characteristic turbidity in the medium after several days. Incidence of transmission to seedlings in a growth chamber at 25 C was determined 2-3 mo after harvest by the tube assay on two subsamples of 300 symptomless primary leaves from seedlings of each seed lot.

Samples of the harvested seed were planted in the field to determine the incidence of transmission to plants under field conditions. Seed lots from Bald Knob and Kibler were tested at Fayetteville in 1992 and at Fayetteville and Bald Knob in 1993. The seed lot from Hope was tested at Fayetteville and Bald Knob in 1993. For each location-year, each seed lot was planted in one plot approximately 8 m. Incidence of transmission was determined by a tube assay at GS 2 (seedling stage) on 240 individual seedlings and at GS 10 (boot stage) on flag-1 and flag-2 leaves from 120 tillers.

Possible alternative hosts, crop debris, and soil at Bald Knob, Kibler, and Hope were sampled in September from the plot area where Florida 302 was harvested the previous June. Samples were taken only from areas where the susceptible cultivar was grown to increase the probability of finding the marked strain. The predominant plant species at each location were sampled and included volunteer wheat, crabgrass (Digitaria sanguinalis (L. Scop.), purple nutsedge (Cyperus rotundus L.), broadleaf signalgrass (Brachiaria platyphylla (Griseb.) Nash), sudangrass (Sorghum sudanense (Piper) Stapf), tall fescue (Festuca arundinacea Schreb.), goosegrass (Eleusine indica (L.) Gaertn.), wood sorrel (Oxalis corniculata L.), soybean (Glycine max (L.) Merril), Palmer amaranth (Amaranthus palmeri F. Wats.), spotted spurge (Euphorbia maculata L.), and johnsongrass (Sorghum halepense (L.) Pers.).

Four 10-g subsamples of crop debris, soil, and each plant species were comminuted and dilution plated on NDA-RC. One hundred twenty individual leaves each of volunteer wheat, crabgrass, fescue, nutseed, broadleaf signalgrass, sudangrass, and johnsongrass also were tested for strain 88-14 \textsuperscript{Rd} using the tube assay.

The plot area was disked, and disinfested Florida 302 seed was planted in the plot area in October as another measure to determine if strain 88-14 \textsuperscript{Rd} could survive in the field between successive wheat crops. At GS 10 and 11.2 the following spring, wheat plants were examined for disease symptoms, and 120 flag and flag-1 leaves were tested using the tube assay.

RESULTS AND DISCUSSION

No naturally occurring "X. c. translucens" was detected in seeds disinfested using dry heat at 60 C for 10 days. Although initial seedborne populations of strain 88-14 \textsuperscript{Rd} ranged from 3,800 to 9,300 cfu per seed at planting, all populations were sufficient to cause disease and resulted in naturally infected seed of both cultivars at all locations (Table 1). Strain 88-14 \textsuperscript{Rd} was recovered from all lesions assayed.

Strain 88-14 \textsuperscript{Rd} was detected in all seed lots 2 and 13 mo after harvest (Table 2).

\begin{table}
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\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Cultivar lot} & \textbf{Location}\textsuperscript{a} & \textbf{Population (cfu/seed)} & \multicolumn{2}{c|}{\textbf{Disease severity\textsuperscript{b}}} \\
 & & & \textbf{Planting} & \textbf{Harvest} \\
\hline
Florida 302 & Bald Knob & 7,200 & 2,260 & 35 \\
 & Kibler & 7,200 & 87 & 12 \\
 & Hope & 9,300 & 180 & 15 \\
Terral 101 & Bald Knob & 3,800 & 87 & 2 \\
 & Kibler & 3,800 & 5 & 1 \\
\hline
\end{tabular}
\caption{Population of \textit{Xanthomonas campestris} pv. \textit{translucens} strain 88-14 \textsuperscript{Rd} associated with wheat seed lots at planting (after vacuum infiltration of inoculum) and seed harvested from the plants, and disease severity on flag leaves at Feakes growth stage 11.2}
\end{table}

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\begin{tabular}{|l|l|l|l|}
\hline
\textbf{Cultivar lot} & \textbf{Location}\textsuperscript{b} & \textbf{Seed infested (\%)} & \multicolumn{1}{c|}{\textbf{Seedlings infested (\%)}} \\
 & & \textbf{2 mo} & \textbf{13 mo} \\
\hline
Florida 302 & Bald Knob & 37.7 (3.7)\textsuperscript{c} & 23.3 (1.9)\textsuperscript{d} & 24.5 (2.8)\textsuperscript{e} \\
 & Kibler & 9.8 (1.2) & 9.9 (1.5) & 7.3 (1.7) \\
 & Hope & 27.3 (1.9) & 1.5 (0.6) & 4.0 (1.7) \\
Terral 101 & Bald Knob & 5.3 (1.1) & 3.4 (0.4) & 0.2 (0.2) \\
 & Kibler & 0.3 (0.3) & 0.3 (0.3) & 0.0 (0.0) \\
\hline
\end{tabular}
\caption{Incidence of \textit{Xanthomonas campestris} pv. \textit{translucens} strain 88-14 \textsuperscript{Rd} on wheat seeds harvested from plants grown from artificially infested seed 2 and 13 mo after harvest, and incidence of infested seedlings grown from this seed in a growth chamber 2 mo after harvest\textsuperscript{a}}
\end{table}
Where direct comparisons could be made, percentage of infested seed was higher for the susceptible cultivar (Florida 302) than for the moderately resistant cultivar (Terral 101). Under growth chamber conditions, percentage of infested seedlings ranged from 4.0 to 24.5% for Florida 302 and from 0.0 to 0.2% for Terral 101. Average seedborne populations associated with harvested seed were lower for Terral 101 than for Florida 302 (Table 1). Thus, lower levels of seedborne inoculum and lower transmission rate appear to be components of resistance.

Strain 88-14Rd was not isolated at any of the three locations from crop debris, soil, or the plant species sampled from the plots approximately 3 mo after the wheat was harvested. No bacterial streak symptoms were observed at any of the locations on Florida 302 that was planted approximately 4 mo after harvest. Strain 88-14Rd was isolated from one of the 480 Florida 302 leaves at Kibler but was never isolated from Florida 302 leaves at Bald Knob or Hope.

Under conditions typical for soft red winter wheat production in Arkansas, X. c. translucens did not survive well over summer in the field between harvest in June and planting the next crop the following October. Therefore, crop debris, plants commonly found in Arkansas wheat fields during the summer, and soil are not important sources of initial inoculum, and crop rotation or weed control are not necessary to reduce inoculum.

High air and soil temperatures in Arkansas from June through September may be responsible for the inability of X. c. translucens to survive in the field. McCarter et al (8), working with Pseudomonas syringae pv. tomato in Georgia, and Jones et al (5), working with Xanthomonas campestris pv. vesicatoria in Florida, also concluded that high summer temperatures were unfavorable for bacterial survival.

Based on survival of inoculum associated with seed and transmission to plants under growth chamber conditions, infested seed is the most likely source of initial inoculum. However, in experiments to determine the transmission of seedborne inoculum under field conditions (involving four seed lots in 1992, five seed lots in 1993, and a total of 6,720 individual seedlings or leaves), strain 88-14Rd was isolated from only one seedling of the Florida 302–Bald Knob seed lot tested at Fayetteville in 1993. The low rate of transmission in this study is similar to transmission rates for other seedborne bacterial plant pathogens (15).

Incidence of infested seed or seedlings under controlled conditions was not associated with incidence of infested or diseased plants under field conditions. Schaad and Forster (14) determined that seed lots with fewer than 1,000 cfu/ml in seed washing (approximately 42 cfu per seed) of X. c. translucens were unlikely to cause disease under irrigated growing conditions in Idaho. Compared to washing seeds as recommended by Schaad and Forster (14), comminuting seeds as done in this study approximately doubles the number of cfu recovered in seed assays (E. A. Milus, unpublished). Also, the tube assay used in this study can detect as few as one viable cell of strain 88-14Rd per leaf or seed (11). Therefore, it is likely that many of the infested seeds had too few cells of the pathogen for successful transmission and establishment under field conditions in Arkansas. More information is needed on thresholds and the effects of environmental conditions for transmission of seedborne inoculum.

ACKNOWLEDGMENTS

We thank Charlie Parsons and Terry Kirkpatrick for assistance with field plots and Carol Becton for weed identification.

LITERATURE CITED