

## Eradication of *Xanthomonas campestris* pv. *translucens* from Barley Seed with Dry Heat Treatments

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### ABSTRACT

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Dry heat eliminated *Xanthomonas campestris* pv. *translucens*, the seed-transmitted causal agent of bacterial black chaff and leaf streak of cereal grains, from barley seed without an appreciable reduction in germination. The pathogen was not detected in heavily infested seed exposed to temperatures of 71, 75, or 84 C for 11 days. In contrast, the pathogen was apparently eliminated from moderately infested seed after exposure to 72 C for 4 days. The heat treatment decreased bacterial numbers much more rapidly the first day than on subsequent days. Reduction in germination was practically negligible for seed treated at 71 or 72 C for 7 days or less. Germination was reduced an average of only 8% for 25 cultivars of barley treated for 11 days at 71 C. Heat treatment may prove to be a convenient, nonchemical way to produce viable bacteria-free seed.

Additional keywords: bacterial diseases, seed treatment

*Xanthomonas campestris* pv. *translucens*, a plant pathogen that causes leaf streak and black chaff, is a worldwide problem affecting cereal grain crops (4,9). The pathogen survives between growing seasons on plant residue and seed (3). Inoculum is spread to new locations primarily by infested seed, resulting in crop losses. In an analogous situation, with *Pseudomonas syringae* pv. *phaseolicola* in beans, as few as five diseased seedlings per 10,000 could lead to substantial crop losses (10).

Efforts to eradicate *X. c.* pv. *translucens* have been directed toward

producing bacteria-free seed. Infested seeds have been treated with pH extremes, hot water, and chemicals (1,5-9,11,14). The cited eradication techniques have failed, have proved phytotoxic, or are cumbersome. The use of dry heat to eradicate *X. c.* pv. *translucens* on barley (*Hordeum vulgare* L.) seed is presented herein.

### MATERIALS AND METHODS

**Heat treatment.** Two seed lots of barley cultivar Moravian III, naturally infested with different levels of *X. c.* pv. *translucens*, were treated with dry heat. Seed lots from several infested fields were assayed initially for *X. c.* pv. *translucens*, stored at room temperature, and placed in paper packets before treatment in one of three convection ovens maintained at

71, 75, and 84 C. The metal oven shelves were lined with paper to avoid uneven heat distribution. Packets containing seed from the most heavily infested lot were placed in the ovens sequentially in such a way that all packets were removed from the ovens at the same time after treatment for 15, 11, 7, 5, 3, 1, or 0 days. This test was repeated twice, for a total of three replicates. The heat-treated seed was evaluated by a single bacterial assay and germination test.

Moderately infested seeds were exposed to 72 C for 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 days. This test was repeated, for a total of two replicates. The treated seed was tested for bacteria eight times and for germination four times.

The germination of seeds from 25 barley cultivars exposed to 71 C for 11 days was compared with that of 25 control samples. About two-thirds of the selected cultivars represented a mixture of "well-adapted" two-row and six-row malting and feed barleys grown in the western United States. The others were a sample of waxy, heavy-fiber barleys from the Montana Intrastate Nursery in Bozeman.

**Response measurements.** For bacterial assays, 10 g of seed was added to 100 ml of sterile phosphate-buffered saline (PBS; 6.8 g of  $\text{KH}_2\text{PO}_4$ , 1.16 g of NaOH, 8.5 g of NaCl, and 1,000 ml of distilled water). The mixture was added to a sterile plastic blender container. To sterilize the blender container and blade components, we soaked them in 70%

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ethanol and then rinsed them in sterile distilled water. Several drops of Tween 20 (Sigma) were added. The mixture was comminuted for 20 sec at medium-high speed. The suspension was shaken by hand for 3 min and then serially diluted to  $10^{-6}$  with PBS. The dilutions, in 0.1-ml aliquots, were spread on an agar medium (XTS-X) that is semiselective for *X. c. pv. translucens*. The medium contained, per liter of distilled water, 23 g of nutrient agar, 8 g of sucrose, 200 mg of cycloheximide, 8 mg of gentamicin sulfate, 10 mg of cephalixin, and 20 mg of 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactopyranoside (X-gal). The medium was modified from Schaad's semi-selective (XTS) agar (13) by the addition of 20 mg of X-gal dissolved in 1 ml of *N,N*-dimethylformamide and 3 g more of sucrose. The X-gal reacts with  $\beta$ -galactosidase, turning the normally yellow colonies of *X. c. pv. translucens* a bluish green (12). Plates were incubated in continuous darkness at 28 C for 7 days.

To test germination, 100 seeds were placed on moistened blotters in polycarbonate boxes with lids and kept in continuous darkness for 7 days at 20 C (2). Seeds with a radical at least 1 cm long were given a positive germination rating.

**Statistical analysis.** Regression analysis was used to model the effect of heat treatment on bacterial populations and seed germination. The model used for bacterial populations was  $\log_{10}$  (population count) =  $A - B \times \text{day}^C$ , with intercept  $A$ , coefficient  $B$ , and exponent  $C$ . The model used for seed germination was  $\arcsin$  (proportion germinated) =  $A + B \times \text{day} + C \times \text{day}^2$ , with intercept  $A$  and coefficients  $B$  and  $C$ .

Lack-of-fit tests used results from analysis of variance procedures. Response variables were the logarithms of bacterial populations and the arcsine of the square root of the percentage of seed that germinated. The differences in germination rates for the treated and control seeds for the 25 barley cultivars were analyzed by a paired-*t* procedure for which the treatment-by-cultivar interaction becomes the statistical error.

## RESULTS

*X. c. pv. translucens* was not isolated from heavily infested seed treated for 11 days or from moderately infested seed treated for 4 days. Initial bacterial counts for untreated seed (day 0) were  $5.37 \times 10^6$  and  $1.02 \times 10^3$  per gram of the heavily and moderately infested seed, respectively. The bacterial count was reduced much more during the first day than on subsequent days of treatment.

For heavily infested seed, the fitted values for  $A$  and  $C$  in the equation  $\log_{10}$  (count) =  $A - B \times \text{day}^C$  were 6.76 and 0.47, respectively, for all temperatures, and the values for  $B$  were 1.88, 2.24, and 2.61 for the three treatment temperatures

of 71, 75, and 84 C, respectively. The fitted values for moderately infested seed treated at 72 C were 3.15, 2.10, and 0.46 for  $A$ ,  $B$ , and  $C$ , respectively.

All coefficients for both infestation levels differed significantly from zero ( $P < 0.001$ ). The three values of  $B$  for the three treatment temperatures applied to the heavily infested seed differed significantly from each other ( $P < 0.001$ ). The  $R^2$  values for the regression of population over time at the specific temperature were 0.89 and 0.92 for heavily and moderately infested seed, respectively.

Although the close fit of the model to the treatment means is apparent visually in Fig. 1, the fitted model is nevertheless an approximation of the true change in bacterial count over time. Model adequacy is supported by the

large proportion of the variation in the treatment means that is explained by the model, as measured by  $R^2$  for regressions of treatment means over time. The values for  $R^2$  were 0.96 and 0.98 for heavily and moderately infested seed, respectively. The lack-of-fit was not significant for heavily infested seed and significant ( $P < 0.01$ ) for moderately infested seed. The latter model included observations from more periods and greater replication. We were unable to provide a more suitable model.

The slopes (first derivatives) of the curves in Fig. 1, after transformation from logarithms, are estimates of the daily bacterial survival rate (or persistence) at selected times. The first derivative was  $-B \times C \times \text{day}^{(C-1)}$ ; the estimated survival rate after 0.5 days of treatment at 84 C is antilog  $[-2.61 \times$

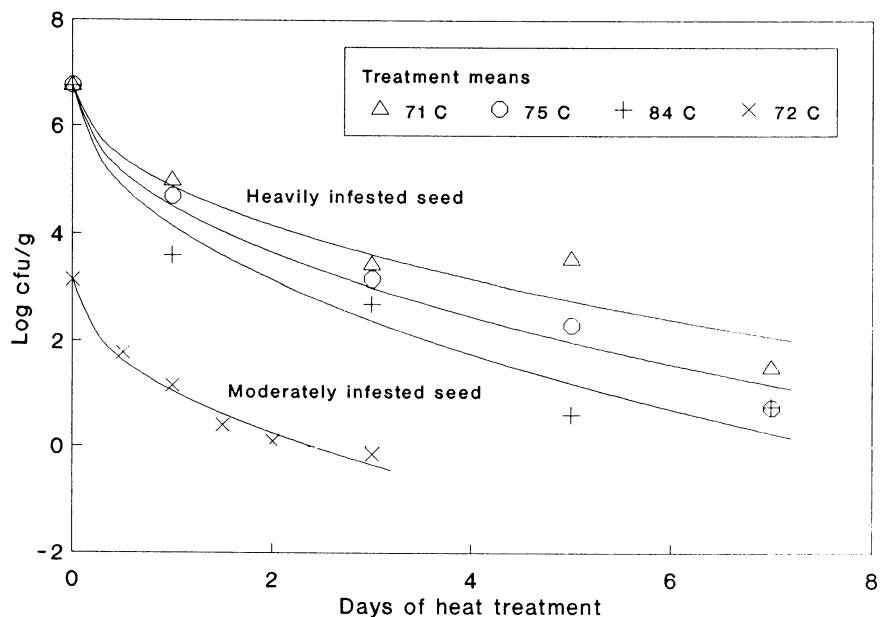


Fig. 1. Reduction in bacterial count of *Xanthomonas campestris* pv. *translucens* as a result of heat treatment of two seed lots of barley cultivar Moravian III with varying levels of infestation.

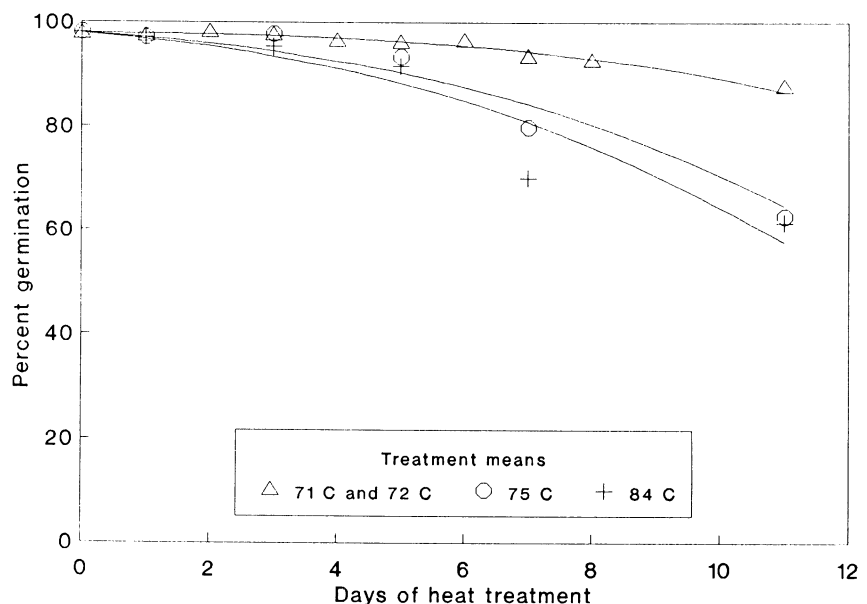


Fig. 2. Reduction in barley seed germination after dry heat treatments.

$0.47 \times 0.5^{(0.47-1)}] = 0.017$ , or 1.7%. The daily survival rates after 0.5 days of treatment varied from 1.7 to 5.3% across the four temperatures. Rates increased to 26–38% after 4 days of treatment. The rates for 72 C were between those for 71 and 75 C. Thus temperature, but not level of infestation, affected survival.

The most obvious features of the pattern of treatment means are a low initial bacterial survival rate that was affected by temperature followed by a much higher survival rate that was largely independent of temperature. A two-phase linear model was fit to the data to examine these obvious features. It produced moderately larger  $R^2$  values than the initial, more biologically common model. Specifically, one slope was used for the time period up to 1 day, and a second slope was used for 1.5 days and beyond. For the heavily infested seed, slopes (or survival rates) varied with temperature for the first period ( $P < 0.001$ ) but did not differ statistically among the three temperatures for the subsequent period. The estimated nominal daily survival rate was  $38 \pm 4\%$  with 95% confidence for seed treated 1.5 or more days.

For the germination data, the values obtained for the coefficients  $A$  and  $C$  for all temperatures were 1.42 and  $-0.0018$ , respectively, and the values for  $B$  for the temperatures 71 and 72 C combined, 75 C, and 84 C were 0.0,  $-0.024$ , and  $-0.031$ , respectively. The data for the two infestation levels treated at 71 and 72 C were combined into one regression line because no differences in germination due to treatment could be detected statistically. All coefficients differed significantly from zero ( $P < 0.001$ ), and the three slopes in the model for 71 and 72, 75, and 84 C differed significantly from each other ( $P < 0.001$ ). Adequacy of the shape of the fitted model is indicated by  $R^2 = 0.94$  (when using treatment means as data) and nonsignificance of a lack-of-fit test. The curve for 71 and 72 C combined, with its much higher rate of replication, fit the data much better than those for 75 and 84 C.

Germination of the untreated seed for the 25 cultivars averaged 95%. Germination

decreased to 87% for seeds treated at 71 C for 11 days. The decrease of  $8 \pm 4\%$  with 95% confidence for the 25 cultivars conforms closely to that observed for the seed of cultivar Moravian III treated for 11 days at 72 C (Fig. 2).

## DISCUSSION

Surface-acting antimicrobial substances are not often effective in controlling the transmission of bacteria on systemically infested seed. Treatments must focus on penetrating the seed coat because some *X. c. pv. translucens* can be found in vessels of the seed coat or within the embryo tissue (10). Dry heat treatment accomplishes this goal, while leaving the seed viable.

The pathogen was eliminated from heavily infested seed by 11-day heat treatments at 71, 75, or 84 C and from moderately infested seed by a 4-day treatment at 72 C, with relatively little effect on the seed germination rate. After accounting for the lower initial daily survival rate associated with higher temperatures, bacterial survival rates for moderately and heavily infested seed were similar. Note, however, that because survival rates were similar, the duration of treatment needed to eradicate *X. c. pv. translucens* or to reduce populations of the bacterium to acceptably low levels did depend on the level of infestation. The high infestation rates reported here ( $5 \times 10^6$  per gram) were unusual and were found in only two of the seed lots examined. More commonly, populations of  $10^3$ – $10^4$  per gram are found. These levels could be eliminated by treatment for 5–7 days at 72 C.

Barley seed infested with *X. c. pv. translucens* can be heat-treated to reduce populations of the pathogen by as many as six orders of magnitude. The dry heat treatments described here can be easily applied to the large numbers of seed envelopes used in international breeding and selection programs. Adoption of our heat treatment could lead to reduced dissemination of this pathogen around the world.

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