Control of Black Chaff of Wheat with Seed Treatment and a Foundation Seed Health Program

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ABSTRACT

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The eradication of *Xanthomonas campestris* pv. *translucens* from wheat seeds (*Triticum aestivum*) together with a foundation seed health program was evaluated for control of black chaff of wheat. The following chemical treatments were evaluated for eradicating *X. c.* pv. *translucens*: methoxyethylmercury acetate, ethylmercury-toluene sulphonanilide, phenylmercury acetate, cupric hydroxide, calcium hypochlorite, sodium hypochlorite, calcium propionate, and acidified cupric acetate (ACA). Naturally contaminated seeds were treated and assayed for *X. c.* pv. *translucens* by 1) plating seeds and seed washings onto XTS semi-selective agar and 2) sowing treated and untreated seeds in field plots in Kimberly, ID. The pathogen was detected by laboratory assays of seed from all treatments, except those treated with ACA. Similar results were obtained in field tests. The ACA treatment adversely affected germination and stand, but was the only one that controlled black chaff. To produce *X. c.* pv. *translucens*-free seed for the foundation seed health program, seed of three naturally contaminated seedlots was treated with ACA, assayed, and sown for increase. This procedure (using untreated seed) was repeated the 2 subsequent years. No disease was observed in the 3 years of the study. *Xanthomonas campestris* pv. *translucens* was not detected in seed assays following the ACA treatment or in first and third generation seed. However, a trace level of the pathogen was detected in the second generation seed. A foundation seed health program can be an effective control for black chaff in the absence of other sources of inoculum.

Additional keywords: bacteria, bacterial streak, bacterial stripe, seedborne pathogens, *Triticum durum*, *Xanthomonas translucens*

Black chaff (also called bacterial stripe and bacterial streak) of wheat (*Triticum aestivum* L.), caused by *Xanthomonas campestris* pv. *translucens*, is a disease of worldwide importance (10) and has recently become severe in sprinkler-irrigated fields in south central and eastern Idaho. Disease losses are estimated to be 30–40% in the most severely diseased fields in Idaho. In these cases, 5–10% of the wheat heads are sterile due to the infection and all the plants exhibit extensive leaf necrosis (2). The pathogen was first reported to be seedborne in 1919 (8), and seed is generally considered to be an important primary source of inoculum (1,8,9).

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The pathogen may both infest (6) and infect seed (9). Once plants become infected in the field, control becomes very difficult (*personal observations*). Pathogen-free seed, whether produced naturally or by chemical eradicants, has been the preferable means of black chaff control (1,10).

There has been speculation that the recent epidemics of black chaff in the United States were related to the withdrawal of registrations for mercury seed treatments in 1978 (5). A newly developed laboratory method for detecting *X. c.* pv. *translucens* in wheat seeds (6) was used to evaluate several seed treatments, including the ethyl and methyl mercuries, for efficacy in eradication of *X. c.* pv. *translucens* from wheat seeds. Naturally contaminated seeds were treated, assayed, and sown in field plots for evaluation. A preliminary report indicated the pathogen was eradicated by a hot, acidified cupric acetate (ACA) treatment originally described for crucifer seed treatment (7) but not by various mercury treatments (3). The purpose of this paper is to report details of the seed treatment study and indicate the potential of a foundation seed health program for control of black chaff.

MATERIALS AND METHODS

Seed treatment studies. In 1983, naturally contaminated durum wheat seed (*Triticum durum* Desf. 'WAID') was used and in 1984 bread wheat seed (*T. aestivum* 'Borah' and 'WestBred 906R') were used. Seeds were assayed in 1983 for *X. c.* pv. *translucens* by 1) plating seed washings onto XTS agar (6) and 2) direct plating of seeds onto XTS agar. For direct plating, 10 mg of Benlate 50W (E. I. DuPont de Nemours & Co., Wilmington, DE 19898) was mixed with 2,000 seeds to prevent growth of fungi on the XTS agar plates. After mixing and drying under a laminar flow hood, 250 seeds were plated onto each of eight plates (15 cm diameter) of XTS agar. Representative colonies were cloned and tested for pathogenicity using wheat seedlings (3–4 leaf stage of growth) as described (6). Foundation grade cultivar WAID seed free of the pathogen was included both years as a "healthy" check. In 1983, samples contained 23,000 seeds and were divided as follows after treatment: 1) three replications of 3,000 seeds for dilution plating assay, 2) three replications of 2,000 seeds for direct plating, and 3) four replications of 2,000 seeds for sowing in the field. In 1984, four replications of 2,000 seeds each were sown in the field, but no laboratory assays were performed.

The treatments and method of application in 1983 were as follows: cupric hydroxide (Kocide SD) (Kocide Chemical Corp., Houston, TX 77045), 260.8 ml/100 kg (4 fl oz/cwt) and phenylmercury acetate (Gallotox) (Guard Chemical Co., Ossining, NY), 54.1 ml/100 kg (0.83 fl oz/cwt) were applied as slurries; and ethylmercury-toluene sulphonanilide (Ceresan M-DB) (E. I. DuPont de Nemours & Co., Wilmington, DE 19898), 51.9 g/100 kg (0.83 oz/cwt) was applied as a dust. Seed for five other treatments were soaked in the following liquids: 0.5% ACA for 20 min at 45 C and then washed twice with 500 ml of sterile distilled water; 4.2% calcium hypochlorite for 15 min at 20–25 C, then washed in running tap water for 15 min; 1.6% calcium propionate for 20 min at 45 C;

0.5% sodium hypochlorite for 15 min at 20–25 C; and water for 20 min at 45 C. In the soak treatments, seeds in the flasks were swirled every 1–2 min and spread out on a laboratory bench to dry immediately after treatment.

Two treatments were added in 1984: 1) methoxyethyl mercury acetate (Panogen) (KenoGard AB, Stockholm, Sweden), 54.1 ml/100 kg applied as a slurry; and 2) calcium hypochlorite fumigation. A slurry of 18 g of calcium hypochlorite in 30 ml of distilled water was added to 900 g of seed moistened with distilled water in a flask, stoppered and shaken gently to distribute the chemical evenly, allowed to stand 12 hr at 20–25 C, rinsed 2 min in 2 L of sterile distilled water, and spread out to dry. A variation of the ACA treatment was also added in which the treatment was conducted at 25 C.

In 1984 two seedlots, Borah and WestBred 906-R, that had moderate and high levels of *X. c.* pv. *translucens* contamination, respectively, were both treated with Gallotox to determine whether this mercury seed treatment might be effective at the lower contamination level but not at the higher one. Untreated contaminated checks were included both years.

Seeds of each treatment were sown in the field on 7 May, 1983 and 12 May, 1984 in a randomized complete block design. Plots were fertilized with nitrogen (112 kg/ha) before seeding, and plants were sprayed with bromoxynil plus MCPA esters (Brominal Plus, 2.34 L/ha) for weed control at Feeke's growth stage 5–6 (4). Individual plots were 1.5 × 4.2 m and spaced a minimum of 4.6 m from an adjacent plot. Faba beans (*Vicia faba*) were sown between individual plots to provide a ground cover and height barrier to minimize interplot spread of the pathogen. Plots were irrigated for 6–8 hr at weekly intervals with solid set impact sprinklers to provide 1–2 acre inches of water and favorable conditions for disease development. Counts of emerged plants (growth stage 1, one to two leaves) and plants with symptoms of black chaff (growth stage 3), respectively, were made 26 May and 17 June, 1983 and 5 and 28 June, 1984.

Production of *X. c.* pv. *translucens*-free seed. Five kg each of three seedlots of the wheat cultivars Fielder, WestBred 906-R, and WAID naturally contaminated with *X. c.* pv. *translucens* were treated with ACA in 1984 and reassayed to verify eradication efficacy. Seeds of these lots were sown in an isolated, rain-fed tract on the Plant Science farm at Moscow, ID. This nonirrigated region was chosen because of a history of freedom from black chaff and infrequent precipitation during the growing season.

The seed increase plots were inspected during the growing season for symptoms of black chaff. The seed was harvested and three replications of 3,000 seeds were assayed by seed washing as described above for the presence of the pathogen. Five kg of this seed was planted for increase in 1985 in an isolated, rill-irrigated field at the Kimberly Research and Extension Center in south central Idaho. The seed was not treated again with ACA. Increase plots were inspected and the seed was assayed by washing as in 1984. This procedure was repeated in 1986 at Kimberly.

RESULTS

Seed treatment studies. In 1983, the pathogen was detected by the seed wash assay after all seed treatments, except the ACA, calcium hypochlorite, and untreated "clean" check (Table 1). However, *X. c.* pv. *translucens* was detected in the calcium hypochlorite-treated seeds by the direct plating assay, while the ACA treated seeds were still free of *X. c.* pv. *translucens* and all other bacteria. All presumptive colonies of *X. c.* pv. *translucens* tested for pathogenicity were positive.

Stand counts made 19 and 24 days after planting in 1983 and 1984, respectively, revealed that the ACA soak at 45 C caused a significant stand reduction of about 15 percentage points (Tables 1,2). Percentage values in the tables represent the percentage of germinated seed out of the total seeded. Young plants were also less vigorous and growth was retarded in this treatment compared with the untreated checks. These phytotoxic effects were not observed in the ACA soak treatment at 25 C. Stands and plant vigor in the other treatments appeared normal.

In 1983, the untreated "clean" check and ACA treatment had significantly fewer diseased plants than the other treatments (Table 1). Little or no disease was detected in plots seeded with ACA-treated seed. There was no significant difference in numbers of infected plants early in the season between the ACA and Kocide SD treatments. At the soft dough stage (growth stage 11.2), the ACA and "clean" check plots still had little or no disease. Only two diseased plants were observed in the four plots sown with ACA-treated seed (unchanged from the 17 June reading), whereas the Kocide SD and other treatments had numerous diseased plants scattered throughout the plots. Neither of the mercury treatments was efficacious. In 1984, no significant differences were detected in the numbers of infected plants among the treatments (Table 2).

Production of *X. c.* pv. *translucens*-free seed. No symptoms of black chaff were found in the visual inspections of seed plots in 1984–1986. The pathogen was not detected in the 1984 or 1986 harvested seed, but a trace level (8 cfu of

Table 1. Evaluation of 1983 seed treatments for eradication of *Xanthomonas campestris* pv. *translucens* and control of black chaff

		Seed assay method[x]				
		Seed wash	Seed plating			
Treatment	Method	Cfu of *X. c.* pv. *translucens*/ml (× 10^3)	No. seeds with *X. c.* pv. *translucens*	No. seeds with Bacteria	Stand count 26 May (%)[y]	No. of diseased plants in 4 replications (17 June)[y]
Check, untreated "clean"	None	0	0	2,000	71 a	0 a
Cupric acetate 0.5%, 45 C	Soak	0	0	0	57 b	2 a
Kocide SD 260.8 ml/100 kg	Slurry	260.0	1	260	70 a	33 ab
Calcium hypochlorite 4.2%	Soak	0	29	117	76 a	51 bc
Gallotox 54.1 ml/100 kg	Slurry	7.5	21	813	77 a	57 bc
Calcium propionate 1.6%	Soak	48.0	9	615	75 a	57 bc
Ceresan M DB 51.9 g/100 kg	Dust	400.0	3	1,453	76 a	66 bc
Sodium hypochlorite 0.5%	Soak	43.0	4	1,641	76 a	83 c
H_2O, 45 C	Soak	190.0	ND[z]	2,000	76 a	127 d
Check, untreated contaminated	None	1,600.0	ND	2,000	72 a	74 bc

[x] Seed source is naturally contaminated WAID durum, except the first entry is "clean" foundation WAID.
[y] Per 2,000 seeds sown. Numbers in a column with a letter in common are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[z] ND = none detected.

X. c. pv. *translucens*/ml of seed washing) was detected in the 1985 harvested seed.

DISCUSSION

X. c. pv. *translucens* can be eradicated from seed by the ACA seed treatment. Black chaff would be controlled unless subsequent inoculation occurred during the growing season. In another test (*unpublished*), plants grown from "clean" seed under sprinkler irrigation had no detectable level of the pathogen on leaf surfaces at growth stage 5, whereas plants from a contaminated seedlot had high levels. At growth stage 10, plants from both seedlots had high epiphytic populations, thus demonstrating the possibility of reinfestation during the growing season. The ethyl and methyl mercury and other seed treatments in the current study were ineffective. This is in contrast to the recommended use of mercury seed treatments for control of black chaff (1).

The ACA soak treatment at 45 C is somewhat phytotoxic as a seed treatment for wheat, but much less so than with crucifers (7). However, application of ACA at 25 C did not injure WAID wheat seed. Efficacy of ACA treatment at the two temperatures should be compared for other cultivars before initiating an eradication program for those cultivars. The slight reduction in stand counts with the 45 C ACA treatment is of little or no consequence because the ACA treatment is used only in the first year of foundation seed production. The phytotoxic effect did not persist when harvested seed from the 1983 ACA plots was replanted in 1984. Yields from these plots (2,603 kg/ha, 2,322 lb/a) and from plots planted with seed harvested from untreated "clean" check plots the previous year (2,608 kg/ha, 2,327 lb/a) were within 0.2% of each other when grown together in 1984. Since wheat seed is inexpensive, seeding rates can be increased to compensate for any potential reduced germination and/or vigor. The cost of the ACA treatment is mainly in the labor to treat and dry the seed, because the cost of the chemicals is small.

Comparison of results in 1983 of the two laboratory seed assay methods reveals the importance of using more than one assay technique when evaluating the effectiveness of seed treatments. Although no colonies of *X. c.* pv. *translucens* were detected on agar media plates from the calcium hypochlorite treatment in the seed washing technique, direct plating revealed the presence of the pathogen. This apparent contradiction may be explained by the visible presence of small clumps of calcium hypochlorite (≤3 mm diameter) remaining with the seed after washing. This residual calcium hypochlorite could have interfered with the recovery of the pathogen in the seed wash assay. Another possibility is that bacteria were still present in the seed coat or funiculus in the calcium hypochlorite-treated seed.

Similarly, the prolific growth of saprophytic bacteria on the untreated contaminated check seed in the direct plating assay prevented identification of any *X. c.* pv. *translucens* colonies that might have been present. Although XTS agar does suppress growth of many microorganisms associated with wheat seeds (6), the nutrient-rich substrate from germinating seed permitted many saprophytes to grow. This problem is not observed in the seed wash technique.

A disadvantage of the seed wash technique is that only numbers of viable cells of the pathogen washed from the seed can be detected. The number of contaminated seeds cannot be determined. One heavily contaminated seed, viable or nonviable, among 2,999 "clean" seeds may produce the same number of colonies of *X. c.* pv. *translucens* per ml in the seed wash assay as many lightly contaminated seeds.

In the seed wash assay there was a ten-fold lower level of contamination by *X. c.* pv. *translucens* among seeds treated with water at 45 C as compared with those in the untreated contaminated check. However, the mean number of diseased plants was significantly higher in the 45 C water treatment. The reason for the significantly higher number of infected plants in this treatment is not known. Since we know that this WAID seedlot contained bacterial antagonists (6), it is possible that treatment eliminated these antagonists. Therefore, the pathogen might have had a more favorable environment in which to multiply. Another more likely explanation is that all cells of *X. c.* pv. *translucens* were not killed by the 45 C treatment and were spread to other seeds during the treatment.

In both 1983 and 1984, the untreated "clean" check and the ACA treatments had the lowest numbers of diseased plants, but statistically significant differences were only detected in 1983. The lack of significance in 1984 was due to a higher background level of disease and greater variability in numbers of infected plants between replications. Poor growth of the faba beans between the individual plots in 1984 may have permitted a higher level of interplot disease spread, hence the higher level of diseased plants in the untreated "clean" check in 1984 than in 1983. No diseased plants were observed in the untreated "clean" check in 1983, whereas in 1984 a total of 11 plants were found. The fact that the same seedlot was used for the untreated "clean" check both years further suggests that infection came from outside the plot.

Based on the studies reported here, we conclude that *X. c.* pv. *translucens* can be eradicated from wheat seed by the ACA treatment. The ACA treatment at either 25 or 45 C soak temperature would be suitable in a foundation seed health program. After treatment, however, it would be advisable to produce the seed crop in isolation in an arid environment in order to maintain the seedlot free of the black chaff pathogen. Avoidance of sprinkler irrigation and areas with a history of black chaff are prerequisites for a successful program. Following this procedure, we have been able to increase seed for 3 years without a recurrence of black chaff.

Table 2. Evaluation of 1984 seed treatments for eradication of *Xanthomonas campestris* pv. *translucens* and control of black chaff

Treatment	Method	Stand count 5 June (%)[w]	No. diseased plants in 4 replications (12 July)[w]
Check, untreated "clean"[x]	None	67 a	11 a
Heavily contaminated seed[y]			
Cupric acetate 0.5%, 45 C	Soak	56 b	15 a
Cupric acetate 0.5%, 25 C	Soak	71 a	17 a
Kocide SD 260.8 ml/100 kg	Slurry	72 a	45 a
Check, untreated contaminated	None	73 a	54 a
Gallotox 54.1 ml/100 kg	Slurry	75 a	56 a
Panogen 54.1 ml/100 kg	Slurry	76 a	57 a
Moderately contaminated seed[z]			
Check, untreated contaminated	None	76 a	41 a
Calcium hypochlorite	Fumigation	75 a	55 a
Gallotox 54.1 ml/100 kg	Slurry	74 a	60 a

[w] Per 2,000 seeds sown. Numbers in a column with a letter in common are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[x] Cultivar WAID.
[y] Cultivar Borah.
[z] Cultivar WestBred 906-R.

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