A Semiselective Agar Medium for Isolating *Xanthomonas campestris* pv. *translucens* from Wheat Seeds

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ABSTRACT

Schaad, N. W., and Forster, R. L. 1985. A semiselective agar medium for isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds. Phytopathology 75: 260-263.

A semiselective agar medium (XTS agar) was developed and tested for the isolation of *Xanthomonas campestris* pv. *translucens* (hereafter referred to as *X. translucens*) from seeds of wheat (*Triticum aestivum*). XTS agar contained Difco nutrient agar, glucose, cycloheximide, gentamycin, and cephalexin. More than 91% of the seed-associated saprophytic bacteria washed from seeds of six wheat lots and one barley (*Hordeum vulgare*) lot were inhibited on XTS agar. *X. translucens* was isolated on XTS agar from 15 of 19 randomly obtained commercial wheat seed lots in Idaho but from only three of the 19 lots assayed on nutrient glucose agar. Comparison of results of assaying washings from samples of seed lots with different levels of natural contamination by *X. translucens* onto XTS agar and sowing samples of the same seed lot in the field showed the former to be more sensitive. Our frequent isolation of *X. translucens* from wheat seed lots suggests that seed contamination may be a factor in recent epiphytotics of black chaff in sprinkler-irrigated wheat fields in southern Idaho. We suggest that wheat seeds be assayed for *X. translucens* and that contaminated seed lots not be grown under sprinkler irrigation.

Additional key words: bacteria, seedborne pathogens.

Black chaff of wheat, caused by *Xanthomonas campestris* pv. *translucens* (hereafter referred to in this paper as *X. translucens*), continues to reduce wheat yields world-wide (1,8,10,14,21). Losses of 40% have occurred in sprinkler-irrigated fields in southern Idaho and control efforts have been unsuccessful (7). Black chaff of wheat was first described by Smith in 1917 although he was aware of the disease since 1902 (20). *X. translucens* was first described in 1916 as the causal agent of bacterial blight of barley (*Hordeum vulgare* L.) (9). Jones et al (10) established that the pathogen was carried in barley seed produced in Montana. The bacterium was shown to be transmitted in wheat seed in 1919 and the causal organism was named *X. translucens* var. *undulosum* (21). *X. translucens* infects many different cereals and grasses (2,4,6,14). Most efforts at controlling black chaff have been directed toward resistance and seed treatments. Seeds have been treated with mercury (6,10) and hot water (10); however, no method of assaying the seed to determine the effectiveness of such treatments has been available. Selective media have been described for xanthomonads (11), *Xanthomonas campestris* pv. *campestris* (3,16,19), *X. campestris* pv. *pruni* (5), and *X. campestris* pv. *juglandis* (15), but none works well for *X. translucens*. Kim developed a selective medium (KM-1) for isolating *X. translucens* from plant tissue and soils in Montana (12), however, we found that many strains of *X. translucens* from Idaho grew poorly on KM-1 agar.

The purpose of this study was to develop a simple, semiselective, agar medium for isolating *X. translucens* from seeds.

MATERIALS AND METHODS

Cultures and cultivars. Most strains of *X. translucens* were freshly isolated from wheat and barley in southeastern Idaho (Table 1). Wheat cultivars used in this investigation included: Bliss (PI 486350), Borah (CI 17267), Daws (CI 17419), Dirkwin (CI 17745), Fielder (CI 17268), Fieldwin (CI 17425), Hill-81 (CI 17954), Lewjain (CI 17909), Owens (CI 17904), Stephens (CI 17596), and WAID (CI 17806). Barley cultivars included: Karla (CI 15860) and Klages (CI 15478).

Development of semiselective agar medium. Preliminary tests comparing growth of 16 strains by dilution plating (22) on yeast-extract, dextrose, $CaCO_3$ (YDC)(23); medium 523 (11); basal KM-1 medium (12); Difco nutrient agar; and nutrient agar plus 0.5% glucose (NG agar) showed that highest recoveries of all strains were on KM-1 basal medium and NG agar. Whereas colonies of all strains were 2.5 mm or greater in diameter after 5 days on NG agar, colonies of eight strains failed to grow beyond 1.0 mm in diameter after 7 days on KM-1 basal medium (colony diameters of four strains were 0.5 mm or less).

To make NG agar selective for *X. translucens* we screened compounds for specific inhibition of wheat seed flora. Eighteen antibiotics and nalidixic acid were screened for selectivity by agar paper disk and liquid growth tests as described by Randhawa and Schaad (16). Compounds that failed to inhibit growth of *X. translucens* in the paper disk test were tested further in NG broth. Finally, those that did not inhibit *X. translucens* in liquid growth tests were added to NG agar and the recovery of *X. translucens* and reduction of wheat seed flora were determined (16).

To measure inhibition of wheat seed flora, 2 g of seed from each of six seed lots were added separately to 10 ml of 0.85% NaCl (saline) and soaked at 3–5 C. After 30 min, 1 ml from each seed sample was pooled and 0.1 ml of the mixture was pipetted onto each of three plates of test media. Sixteen strains of *X. translucens* were tested for growth and plating efficiency (average recovery) on the final selective medium. Bacteria were grown in liquid medium 523 in a New Brunswick environmental incubator shaker at 100 rpm for 18 hr at 30 C. Cell concentrations of the cultures were standardized with a colorimeter, diluted to 10^{-5} and 10^{-6} with sterile saline, and 0.1 ml was plated onto four plates each of NG agar and test medium as described (22). Plates were incubated at 30 C and colonies were counted after 4 days. Percent plating efficiencies were calculated as follows: number of colony-forming units (CFU) recovered on test medium $\times 10^2$ / number of CFU on NG agar.

Detection of *X. translucens* in naturally contaminated seeds. Seed samples were obtained at random from commercial grain elevators in southern Idaho, a wheat breeder at Moscow, ID, and known infected wheat fields in southern Idaho.

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Seed samples (125 g, approximately 3,000 seeds) were added to 125 ml of cold (2–5 C) sterile saline containing two drops (Pasteur pipette) of Tween-20 in a 500-ml Erlenmeyer flask and shaken at room temperature. After 3 min, 1 ml was removed and diluted to 10^{-2}. One-tenth milliliter of undiluted and 10^{-1} and 10^{-2} dilutions was pipetted onto four plates of each agar medium and spread with a *L*-shaped glass rod. The plates were incubated at 30 C for 5 days. Colonies typical of *X. translucens* were cloned and presumptively identified by streaking onto YDC agar. Representative colonies were tested for pathogenicity by injecting wheat seedlings (at the two- to three-leaf stage) with an inoculum of approximately 10^5 CFU/ml (22) by using a 0.46-mm-diameter (26-guage) needle attached to a 1-ml syringe.

Correlation of laboratory assay results to development of black chaff in the field. Different levels of contaminated seed (naturally infected and/or infested) were made by mixing contaminated seed of wheat cultivar WAID (lot 1084) and Bliss (lot 1106) seed with WAID foundation (lot 1202) seed as follows: 3,000 WAID foundation, 0 contaminated; 2,970 WAID foundation, 30 contaminated; 2,700 WAID foundation, 300 contaminated; and 0 WAID foundation, 3,000 contaminated. Based on the seed wash assay, lots 1084, 1106, and 1202 had a high level, low level, and trace level of contamination, respectively (Table 2). Lot 1084 seed was also assayed for seed infection by plating four samples of 1,000 surface-disinfested (4.2% calcium hypoclorite for 15 min) seeds onto XTS agar as described (17).

Eight replications of each mixture of WAID lot 1084 and Bliss were made. Four replications were assayed in the laboratory for *X. translucens* and four replications were sown in replicated plots on 7 May 1983 at Kimberly, ID. Plots were 1.5 × 5.5 m and spaced a minimum of 4.6 m from an adjacent plot. Horse-beans (*Vicia faba* L.) were planted between the plots to provide a height barrier, thereby reducing the possibility of pathogen spread between plots. Plots were sprinkler irrigated for 6–8 hr at weekly intervals to provide favorable conditions for disease development. Disease ratings were made 19 July when the wheat was in the flowering stage (Feeckes growth stage 10.5)(13). Presence of disease was confirmed by isolating the pathogen from leaves of one or more plants with black chaff symptoms from each plot.

RESULTS

Development of semiselective medium. Nalidixic acid and most antibiotics were inhibitory to *X. translucens*. However, gentamycin and cephalexin at 8 and 10 μg/ml, respectively, suppressed the growth of most seed-associated saprophytic bacteria without inhibiting the growth of *X. translucens*. In fact, most bacteria antagonistic to *X. translucens* on agar media were inhibited by these two antibiotics. The final selective medium designated XTS agar contained the following (per liter of distilled water): 23 g of nutrient agar (Difco), 5 g of glucose, 200 mg of cycloheximide (20 ml of a 100 mg/ml stock solution in 75% ethanol), 8 mg of gentamycin (0.8 ml of a 10 mg/ml stock solution in 75% ethanol), and 10 mg of cephalexin (1.0 ml of a 10 mg/ml stock solution in 75% ethanol). The antibiotics were added after the medium was autoclaved. Plates of XTS agar could be stored for 1–2 mo at 4–5 C without reduction in plating efficiency. Colonies were visible after 3 days. Colonies were light yellow, shiny, round, smooth, convex, and 2.0–2.5 mm in diameter after 5 days (Fig. 1) and 3–4 mm in diameter after 7 days, depending upon the strain. Similarly, colonies on YDC agar were typically 1–1.5 mm after 3 days and 2–2.5 after 5 days. Plating efficiencies on XTS agar ranged from 59.3 to 105.9 with a mean efficiency of 86.4 (Table 1). Colonies of *X. translucens* on XTS agar were similar to those on NG agar except for a slight reduction in size.

Reduction in seed-associated bacteria. Ninety-one percent or more of the seed-associated saprophytic bacteria growing on NG agar failed to grow on XTS agar (Table 3). CFU ranged from 76 to 320 per plate at 10^{-2} on NG agar and from 14 per plate at 10^{-2} to five per plate at 10^{-1} on XTS agar (Table 3).

Recovery of *X. translucens* from naturally contaminated seed. *X. translucens* was isolated from 15 of 19 seed lots assayed on XTS agar but from only 3 of 19 on NG agar (Table 2). Bacteria antagonistic to *X. translucens* were observed on XTS agar in three WAID seed lots, 1084, 1101, and 1103. No colonies of *X. translucens* were observed on XTS agar in the undiluted sample of

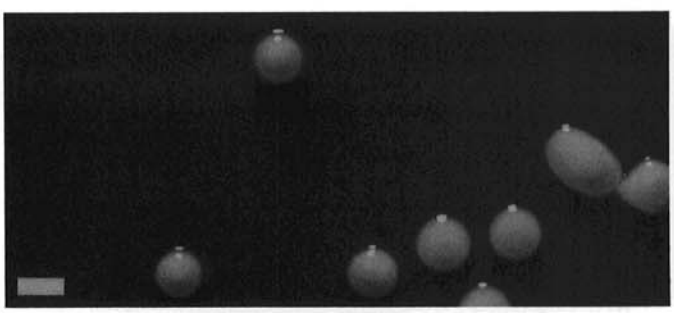

Fig. 1. Colonies of *Xanthomonas campestris* pv. *translucens* growing on XTS selective agar after 5 days at 30 C. Bar represents 2.0 mm.

TABLE 1. Source and growth of strains of *Xanthomonas campestris* pv. *translucens* on semiselective XTS agar medium compared to growth on nutrient glucose agar

Strain	Year collected	Source		Mean recovery (%)[a]
		Host	Location	
B-519(4734)[b]	1982	Wheat	Lodrina, Brazil	116.6
B-501	1981	Barley	Logan, UT	105.9
B-500	1981	Barley	Malheur County, OR	102.9
B-508	1982	Barley	Power County, ID	102.5
B-524	1982	Wheat	Minidoka County, ID	100.0
B-520(4779)[b]	1982	Wheat	Lodrina, Brazil	100.0
B-503	1982	Barley	Power County, ID	96.5
B-499	1981	Wheat, seed	Bingham County, ID	93.8
B-498	1981	Wheat, seed	Cassia County, ID	93.2
B-504	1982	Wheat	Power County, ID	87.5
B-505	1982	Barley	Bingham County, ID	86.6
B-507	1982	Barley	Power County, ID	86.1
B-506	1982	Wheat	Power County, ID	83.3
B-502	1982	Wheat	Power County, ID	81.4
B-523	1982	Wheat	Power County, ID	79.3
B-518(4733)[b]	1982	Wheat	Lodrina, Brazil	59.3

[a] Recovery from sterile 0.85% NaCl. Mean recovery = (no. colonies recovered on XTS × 10^2)/(no. colonies on Difco nutrient glucose agar). Figures are calculated from the mean number of colonies per plate, four plates per strain.

[b] Strains received from S. K. Mohan, Fundação, Instituto Agronomico Do Paraná, Londrina, Paraná, Brazil.

these seed lots and considerable inhibition of colonies of *X. translucens* was observed at a dilution of 10^{-1} (Fig. 2A). In contrast, colonies of *X. translucens* were easily counted on XTS agar at a dilution of 10^{-2} with no evidence of antagonism (Fig. 2B).

Correlation between laboratory assays and black chaff development in the field. *X. translucens* was isolated from all contamination levels including the control foundation seed, but black chaff infections in field plots developed only from seed lots from which seed assays resulted in 1,000 or more CFU of *X. translucens* per milliliter (Table 4). Seeds of WAID lot 1084 surface disinfested and plated onto XTS agar resulted in a mean of 14 infected seeds per 1000.

DISCUSSION

X. translucens was easily isolated from naturally contaminated wheat and barley seed on XTS agar. The nutrient agar based XTS agar allowed for development of the yellow pigment and good growth of all strains of *X. translucens* that were tested. At the same time, XTS prevented growth of 91% or more, depending upon the seed lot, of the seed-associated saprophytic bacteria.

Antagonistic bacteria were generally not a problem on XTS agar. However, growth of *X. translucens* was inhibited in three of 19 seed lots tested. Two of these seed lots were from wheat fields near Paul, ID, that had black chaff. *X. translucens* was detected in these three seed lots only because the pathogen occurred in very high numbers. The presence of such antagonists may have resulted in the failure to detect *X. translucens* in our negative seed lots from Moscow. If antagonists do occur in certain seed lots, one can easily check for their presence by overspraying an assay plate without colonies of *X. translucens* with a suspension of *X. translucens*. The antagonist could be isolated and a specific antibiotic could be

TABLE 2. Isolation of *Xanthomonas campestris* pv *translucens* from seeds of barley cultivars Karla and Klages and 11 wheat cultivars on nutrient glucose (NG) agar and semiselective agar medium (XTS agar)[a]

Seed			CFU[b]/ml × 10^2	
Cultivar	Lot no.	Source	NG agar	XTS agar
Stephens	1086-A	Breeder, Moscow, ID	0	0.0
Hill-81	1086-B	Breeder, Moscow, ID	0	0.0
Lewjain	1086-C	Breeder, Moscow, ID	0	0.0
Daws	1086-D	Breeder, Moscow, ID	0	0.0
WAID	1202	Foundation, Pullman, WA	0	0.4
Owens	1119	Elevator, Lewiston, ID	0	0.7
Owens	1070	Elevator, Blackfoot, ID	0	3.0
Fieldwin	1080	Elevator, Idaho Falls, ID	0	10.0
Bliss	1106	Breeder, Aberdeen, ID	0	11.0
Owens	1082	Elevator, Rupert, ID	46	12.0
Dirkwin	1072	Elevator, Blackfoot, ID	0	33.0
Klages	1071	Elevator, Blackfoot, ID	0	95.0
Fielder	1073	Elevator, Blackfoot, ID	0	320.0
Borah	1107	Infected field, Rockford, ID	0	580.0
Karla	1081	Elevator, Rupert, ID	0	680.0
WAID	1103	Certified, Logan, UT	ND[c]	2,060.0
WAID	1101	Infected field, Paul, ID	ND	2,920.0
WAID	1084	Infected field, Paul, ID	21	5,700.0
WAID	1083	Infected field, Rockford, ID	23	17,000.0

[a] Figures are means of three samples of 125 g of seed from each seed lot. All seeds were obtained after normal cleaning operations.
[b] CFU = colony-forming units.
[c] ND = not determined.

TABLE 3. Reduction in population of saprophytic bacteria isolated from seeds of barley cultivar Karla and six wheat cultivars on semiselective agar medium (XTS agar)

			CFU of saprophytic bacteria/ml		
Cultivar	Lot no.	Source[b]	Nutrient glucose agar	XTS agar	Reduction of seed bacteria (%)
Borah	1107	Infected field	2.1×10^5	7.3×10^2	99.7
Fieldwin	1080	Elevator	7.6×10^4	5.0×10^2	99.3
WAID	1084	Infected field	3.2×10^5	3.9×10^3	98.8
Karla	1081	Elevator	2.2×10^5	4.0×10^3	98.2
WAID	1083	Infected field	2.9×10^5	6.6×10^3	97.7
Bliss	1106	Breeder	3.2×10^5	1.4×10^4	95.6
Owens	1082	Elevator	1.1×10^5	9.3×10^3	91.6

[a] Figures are means of three samples of 125 g of seed from each seed lot. Reduction in seed bacteria were determined as follows: 100 − (no. bacteria recovered on XTS agar per milliliter of seed washings × 10^2)/total no. seed-associated saprophytic bacteria recovered on nutrient glucose agar per milliliter of seed washings.
[b] Origin of seed is given in Table 2.

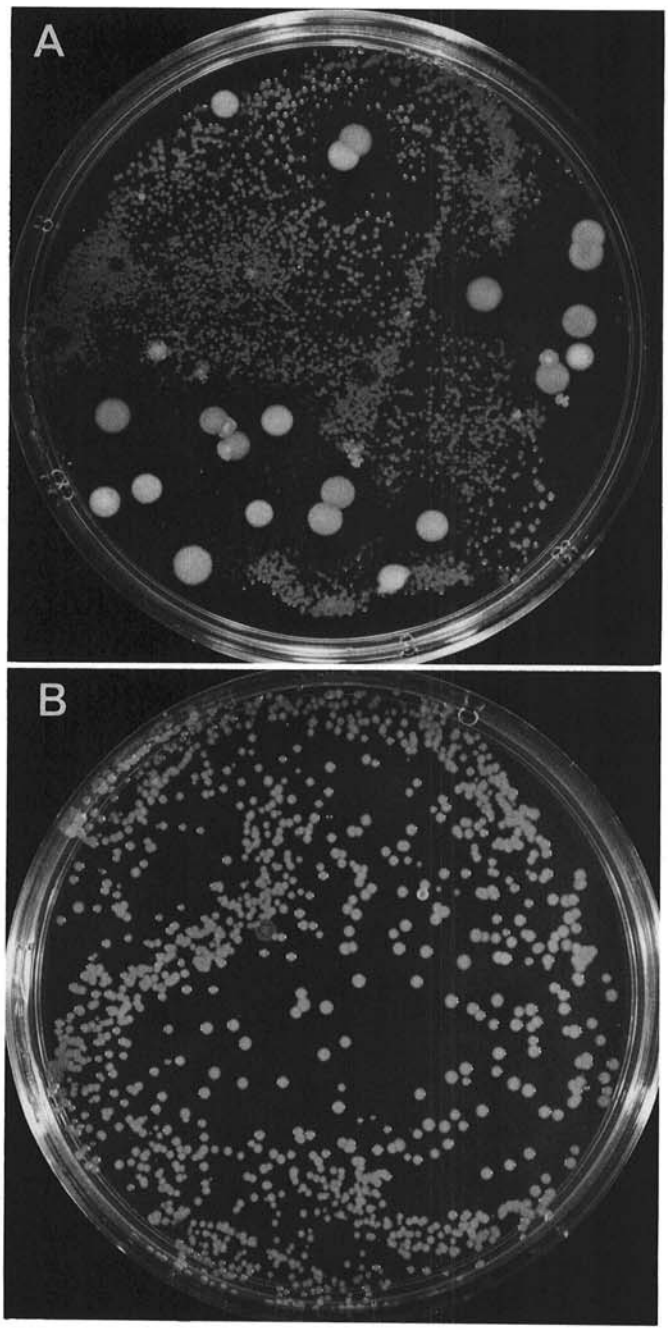

Fig. 2. Effect of antagonistic bacteria on the recovery of *Xanthomonas campestris* pv. *translucens* on XTS selective agar: **A,** plated seed washings of lot 1084 diluted 10^{-1}, note the inhibition of *X. translucens* near the larger antagonistic colonies; *B,* same plated seed washings diluted 10^{-2}. Note numerous colonies of *X. translucens* and no antagonistic bacteria.

TABLE 4. Correlation of laboratory assay results to black chaff development in the field[a]

Infected cultivar and seed lot	No. of seeds from: Foundation lot	No. of seeds from: Infected lot	Laboratory assay (mean CFU[b] *X. translucens*/ml)	Field disease: Plots with black chaff (no.)	Field disease: Disease rating[c]
Bliss-1106	3,000	0	7	0	0.00
	2,970	30	79	0	0.00
	2,700	300	266	0	0.00
	0	3,000	1,205	1	0.25
WAID-1084	3,000	0	0	0	0.00
	2,970	30	1,170	1	0.25
	2,700	300	42,400	4	2.50
	0	3,000	363,083	4	4.00

[a] Eight replications were made of each of four mixtures of naturallv contaminated wheat seeds of Bliss lot 1106 and WAID lot 1084 with seeds of foundation WAID lot 1202. Four replications of 125 g of seed were assayed as described in Materials and Methods and four replications were sown in the field 7 days later on 7 May 1983 at Kimberly, ID. Each plot was at least 4.6 m (=15 ft) from an adjacent plot.

[b] CFU = colony-forming units.

[c] Disease rating: 0 = no black chaff, 1, 2, 3, and 4 = one site with one to two infected plants, two sites with one to two infected plants, three to four sites with three to four infected plants, and numerous sites with three to four infected plants, respectively. Plants were read on 19 July. Isolations from each plot were made at random from leaves of plants with black chaff symptoms. Figures are the means of four plots.

found that could be added to XTS agar to prevent growth of the antagonist.

Kim (12) developed KM-1 agar, a lactose-based antibiotic agar, for isolating *X. translucens* from barley leaf debris and soil in Montana. We found that basal KM-1 agar (without antibiotics) resulted in high recoveries of most strains as reported (12), however, most of the strains of *X. translucens* we tested grew so slowly that seed flora quickly overgrew them, making identification very difficult. Lack of development of the yellow pigment makes differentiation very difficult. Whereas Kim (12) reported the average diameter of colonies of his Montana strains to be 2.5 mm or greater and 1.5–2.0 mm for seven and four strains, respectively, after 7 days at 28 C, we found that colonies of eight of the 16 strains we tested to be 1.0 mm or less on KM-1 after 7 days. The remaining strains ranged in size from 1.5 to 2.5 mm after 7 days.

Results of laboratory seed assays (CFU of *X. translucens* on XTS agar) agreed favorably with development of black chaff in the field. *X. translucens* was detected by laboratory assays in all six levels of contamination tested, including one of the control foundation seed samples used for diluting the contaminated seeds. However, black chaff developed in the field from seeds with only the highest and two highest levels of contamination for the Bliss and WAID seed, respectively. Under the conditions of our field tests, contamination levels of approximately 1,000 CFU of *X. translucens* per milliliter of seed washings are needed for black chaff to develop. For the WAID seed, this level of contamination was equivalent to a level of infection of 0.014%. Apparently, an infection level somewhat higher than this is required for development of black chaff. This is in close agreement to a seed infection of (0.03%) required for development of epiphytotics of black rot of crucifers (18). Our results suggest that seed washing assays with XTS agar can be used for black chaff certification and/or quarantine programs.

LITERATURE CITED

1. Bamberg, R. H. 1936. Black chaff of wheat. J. Agric. Res. 52:397-417.
2. Boosalis, M. G. 1952. The epidemiology of *Xanthomonas translucens* (Dowson) on cereals and grasses. Phytopathology 42:387-395.
3. Chun, W. W. C., and Alvarez, A. M. 1983. A starch-methionine medium for isolation of *Xanthomonas campestris* pv. *campestris* from plant debris in soil. Plant Dis. 67:632-635.
4. Cunfer, B. M., and Scolari, B. L. 1982. *Xanthomonas campestris* pv. *translucens* on triticale and other small grains. Phytopathology 72:683-686.
5. Civerolo, E. L., Sasser, M., Helkie, C., and Burbage, D. 1982. Selective medium for *Xanthomonas campestris* pv. *pruni*. Plant Dis. 66:39-43.
6. Dickson, J. G. 1956. Diseases of Field Crops. Pages 27-30. 2nd ed. McGraw-Hill Book Co., New York. 517 pp.
7. Forster, R. L. 1982. The status of black chaff disease in Idaho. Pages 1-4 in: Idaho Wheat (December issue). Idaho State Wheat Growers Association. Owyhee Plaza Hotel, Boise. 20 pp.
8. Gorlenko, M. V. 1961. Bacterial diseases of plants (study of bacterioses). Pages 113-115. Vysshaya Shkola, Moskva. 174 pp.
9. Jones, L. R., Johnson, A. G., and Reddy, C. S. 1916. Bacterial blights of barley and certain other cereals. Science 44:432-433.
10. Jones, L. R., Johnson, A. G., and Reddy, C. S.. 1917. Bacterial blight of barley. J. Agric. Res. 11:625-643.
11. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Phytopathology 60:969-976.
12. Kim, H. K. 1982. Epidemiological, genetical, and physiological studies of the bacterial leaf streak pathogen, *Xanthomonas campestris* pv. *translucens* (J.J.R.) Dowson. PhD thesis. Montana State University, Bozeman. 94 pp.
13. Large, E. C. 1965. Growth Stages in Cereals. Illustrations of the Feekes Scale. Plant Pathology 3:128-129.
14. Moffett, M. L. 1982. Bacterial plant pathogens recorded in Australia. Page 333 in: Plant Bacterial Diseases (A Diagnostic Guide). P. C. Fahy and G. J. Persley, eds. Academic Press, New York. 393 pp.
15. Mulrean, E. N., and Schroth, M. N. 1981. A semiselective medium for the isolation of *Xanthomonas campestris* pv. *juglandis* from walnut buds and catkins. Phytopathology 71:326-339.
16. Randhawa, P., and Schaad, N. W. 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. Phytopathology 74:268-272.
17. Schaad, N. W., and Kendrick, R. 1975. A qualitative method of detecting *Xanthomonas campestris* in crucifer seed. Phytopathology 65:1034-1036.
18. Schaad, N. W., Sitterly, W. R., and Humaydan, H. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers in the field. Plant Dis. 64:91-92.
19. Schaad, N. W., and White, W. C. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.
20. Smith, E. F. 1917. A new disease of wheat. J. Agric. Res. 10:51-53.
21. Smith, E. F., Jones, L. R., and Reddy, C. S. 1919. The black chaff of wheat. Science 50:48.
22. Vidaver, A. K. 1980. Gram-positive bacteria. Pages 12-16 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
23. Wilson, E. E., Zeitoun, F. M., and Fredickson, D. L. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618-621.