Control of Xanthomonas campestris in Brussels sprouts with hot water and Aureomycin seed treatment'

C. L. Lockhart, C. O. Gourley, and E. W. Chipman

Eight chemicals employed at various temperatures, concentrations, and time periods were screened for the control of ***Xanthomonas campesrris*** on inoculated seed of cabbage ***Brassica oleracea*** var. ***capitata.*** Excellent control was obtained when seed was soaked in 500 ppm Aureomycin (22.5% chlortetracycline hydrochloride) at 50°C for 25 minutes; seed exposed to 16% acetaldehyde was also free of *X. campestris.* In field tests Brussels sprouts, *B. oleracea* var. ***gemmifera,*** were free from black rot when grown under good cultural conditions and sanitary practices from naturally infected seed that had been treated with aureomycin at 50°C. Aureomycin residues were not detected in Brussels sprouts at harvest.

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On a compare l'efficacité de huit antiparasitaires à diverses temperatures, concentrations et durées de traitement dans la lutte contre ***Xanfhomonas campestris*** sur des semences inoculées de choux (***Brassica oleracea*** var. ***capitata***). D'excellents résultats ont été obtenus lorsque les semences ont été tremphes dans une solution contenant 500 ppm d'auréomycine, maintenue à 50°C pendant 25 minutes. Les semences traitées à l'aide d'une solution à 16% d'acétaldéhyde étaient également indemnes. Dans les essais de plein champ, les choux de Bruxelles (*B. oleracea* var. ***gemmifera***) issus de semences naturellement infectées et traitees à l'aureomycine à 50°C etaient exempts de la nervation noire lorsque les pratiques culturales et sanitaires étaient satisfaisantes. Aucun résidu d'auréomycine n'a été décelé dans les choux de Bruxelles au moment de la récolte.

Black rot of crucifers caused by the bacterium ***Xanthomonas campestris*** (Pamm.) Dowson, is a seed- and soil-borne disease that is found throughout the world (2,6). Recent evidence indicates that the bacterium may survive for as long as 615 days in the soil and that one infected seed may produce enough inoculum to cause an epidemic (4,5).

In 1974 a serious outbreak of black rot occurred in Brussels sprouts in the province of New Brunswick. A survey of 26 ha showed an average loss of 24%, with losses in individual fields ranging from 0 to 50%. Although the seed used to plant these fields had been hot-water treated, it was found to be infected with *X. campestris.* Subsequent tests revealed that the recommended treatment of soaking seed in water at 50°C for 25 minutes was ineffective.

In rutabaga, ***Brassica napobrassica,*** *X. campestris* was controlled by soaking infected seed in Aureomycin for 30 minutes (6). However Aureomycin has not replaced the hot-water treatment of crucifer seed, apparently because the Aureomycin treatment discolors young seedlings, and because it does not control seed-borne alternaria and phoma diseases, which are controlled by hot water. This paper reports the successful use of Aureomycin in hot water and the screening of seven other chemicals for the control of *X. campestris* in seed of Brussels sprouts and cabbage.

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Materials and methods

Inoculated cabbage, ***B. oleracea*** var. ***capitata,*** seed was used in screening tests for treatments to control *X. campestris.* Seeds were inoculated by dipping them in a strong suspension of a pathogenic isolate of the bacterium followed by drying for 3 days at 23°C. For each of the liquid treatments (Table 1) 100 inoculated seeds loosely bagged in cheesecloth were soaked for the required interval. In the hot water treatments the time required to raise the temperature of the seed to 50°C is not included in the treatment times recorded. Following treatment the seeds were spread out on the cheesecloth to dry for 2-3 hours at room temperature. The volatile, acetaldehyde, was injected into 3.6-liter sealed jars each containing 100 inoculated seeds and left for 24 hours. Treated seeds were plated on potato dextrose agar (PDA) and after 7 days were examined for the presence of bacteria, seed germination, and evidence of phytotoxicity.

To test the effect of Aureomycin (22.5% chlortetracycline hydrochloride, Cyanamid of Canada Ltd.) and hot water seed treatment on Brussels sprouts, ***B. oleracea*** var. ***gemmifera,*** inoculated seed was treated (Table 2) and sown in a mixture of soil, peat, and sand (1:2:1) containing added nutrients in 10-cm clay pots. These were placed in a mist bed until seedling emergence and then moved to the greenhouse bench, temperature ca. 18°C. Samples of the seeds were also tested for *X. campestris* on PDA.

Table 1. The effect of various treatments on the recovery of ***Xanthomonas campestris*** from inoculated cabbage seed

Treatment	Treatment time	*X. campestris*	Germination %	Discoloration of seedlings
Penicillin G–potassium 1000 ppm at 50°C	30 min	+*		–
Cycloheximide 1000 ppm at 50°C	30 min	+		–
Streptomycin sulfate 1000 ppm at 50°C	30 min	+		–
Candicidin 1000 ppm at 50°C	30 min	+		–
Acetic acid 0.5 M	10 min	+	66	–
Acetic acid 0.25 M	20 min	+	50	–
Ethanol 95%	5 min	+	80	–
Acetaldehyde 2%	24 h	+	82	–
Acetaldehyde 4%	24 h	+	72	–
Acetaldehyde 8%	24 h	+	76	–
Acetaldehyde 16%	24 h	–	78	–
Water at 50°C	25 min	+	46	–
Water at 50°C	30 min	+	36	–
Water at 50°C	35 min	+	46	–
Aureomycin 2000 ppm at 50°C	25 min	–	45	+
Aureomycin 1500 ppm at 50°C	25 min	–	46	+
Aureomycin 1000 ppm at 50°C	25 min	–	61	+
Aureomycin 750 ppm at 50°C	25 min	–	75	+
Aureomycin 500 ppm at 50°C	25 min	–	78	+
Untreated seed		+	78	–

*+ denotes presence, – denotes absence of **X. *campestris,*** and blank space denotes no observation recorded.

Table 2. The effect of aureomycin seed treatments on control of ***X. campestris,*** on germination, and on the health of seedlings of Brussels sprouts

Treatment	Agar plate tests		Seedling discoloration in greenhouse after		Appearance
	X. campestris	Germination %	7 days	10 days	
Aureomycin 1000 ppm at 50°C for 25 rnin	–	57	+	–	Fair
Aureomycin 750 ppm at 50°C for 25 min	–	73	+	–	Fair
Aureomycin 500 ppm at 50°C for 25 rnin	–	77	+	–	Good
Water at 50°C for 25 min	+	53	–	–	Good
Untreated	+	64	–	–	Good

In 1975 field trials on the use of Aureomycin for black rot control were done at Kentville, Nova Scotia, and at Grand Falls and Rogersville, New Brunswick. At Kentville they were incorporated in a trial of 24 cultivars of Brussels sprouts from various seed companies. The incidence of naturally occurring ***X. campestris*** on the seed was determined by plating 100 seeds of each cultivar on D5 medium (3). The remaining seed from each cultivar was then divided into two equal parts; one part was treated with 500 ppm Aureomycin at 50°C for 25 minutes and the other was left untreated. The treated seed was planted in nursery rows in an area considered to be free of ***X. campestris*** and the untreated seed in the regular garden area at the Research Station. Seedlings

Table 3. Decay in Brussels sprouts at harvest (average of 24 cvs)

Seed treatment	% of decay losses (based on weight) caused by				
	X. carnpestris	*Alternaria*	*Botrytis*	Bacteria	Tipburn
Aureomycin *500* ppm at 50°C for 25 min	0	0.5	1.8	1.4	*0.8*
Untreated	12.7	0.2	1.4	2.2	2.2

from both areas were later transplanted into each of four randomized blocks located in the same areas in which they had been growing. At transplant time the seedlings from each garden were examined internally and externally; in addition cross sections of the stems at the soil line were taken from nine cultivars that had been found to have infected seed in the plating test and were plated on D5 medium. The plants were examined periodically. At harvest in October the weight of healthy and diseased sprouts was recorded. The diseased sprouts were examined visually for diseases and the quantity of each was recorded.

In New Brunswick at each location a comparison was made on single 0.8 ha adjoining plots between Brussels sprouts seed, cultivar Jade regular, treated with 500 ppm Aureomycin at 50°C for 25 minutes and seed soaked in water at 50°C for 25 minutes. The land at each location was considered to be free of *X. **campestris.*** An agar test prior to treatment showed that 10% of the seed carried *X. **campestris.*** After treatment, sample lots of 100 seeds **of** each treatment were plated on D5 medium to check for the presence of the pathogen. The plots were examined for black rot on September 22 and 23; in each plot 100 plants were examined in each of four sections selected at random.

For residue analysis 1000-g samples of Brussels sprouts were collected from each treatment at each location and stored at -20°C until required for bioassay. The samples were assayed against ***Bacillus cereus,*** ATCC, No. 11778, using the methods for detecting Aureomycin described **by** the Association **of** Official Analytical Chemists (1).

Results and discussion

Two of the chemical treatments tested were effective in freeing inoculated seed of Brussels sprouts and cabbage from *X. **campestris.*** Cabbage seed soaked in Aureomycin at 50°C for 25 minutes or exposed to 16% acetaldehyde for 24 hours was free of *X. **campestris*** (Table 1). Treatment with Aureomycin at 50°C for 25 minutes was selected for further study because, in addition to its effectiveness in controlling black rot, it, like hot water alone, also controlled alternaria and phoma diseases.

Brussels sprouts seedlings grown in the greenhouse from Aureomycin-treated seeds had a reddish discoloration which under good growing conditions disappeared in 7 – 10 days (Table 2). Other experiments not described here showed that this discoloration continued almost indefinitely under poor growing conditions of low light intensity or short days. Treatment with Aureomycin at 1000 ppm or 750 ppm at 50°c for 25 minutes caused stunting of Brussels sprouts seedlings; however at 500 ppm seedling growth was good and *X. **campestris*** was controlled.

Seed of 15 of the 24 commercial cultivars tested at Kentville yielded *X. **campestris*** on agar medium. Seed infection ranged from 1% to 20%. However *X. **campestris*** was not isolated from any of the seed treated with Aureomycin at 50°C for 25 minutes, and all cultivars grown in the field from treated seed remained free of black rot. Seedlings from untreated seed appeared healthy, but the bacterium was isolated from stems at transplanting. Black rot symptoms were first observed in plants from untreated seed on September 9, and by mid October plants of all 24 cultivars grown from untreated seed exhibited symptoms of black rot. Although plants grown from infected seed were the first to exhibit black rot symptoms, the disease spread so rapidly throughout the plots that at harvest time there was no correlation between the incidence of black rot in the field and that of infected seed. Black rot infections in decayed sprout samples ranged from 14% to 100% at harvest, but the average loss by weight of the harvested crop of the 24 cultivars was 12.7% (Table 3). All plants grown in the black-rot-free area from seed treated with 500 ppm Aureomycin at 50°C for 25 minutes remained free from the disease. There were some losses from other diseases in cultivars grown from treated seed (Table 3).

In the New Brunswick trial the agar test showed that 10% of the Brussels sprouts seed lots selected for the commercial test plots were infected with the black rot organism. After treatment with 500 ppm Aureomycin at 50°C for 25 minutes all samples were free of *X. **campestris;*** however 2% of those soaked in water at 50°C for 25 minutes yielded *X. **campestris.***

In the plots at Grand Falls black rot was not observed in Brussels sprouts grown from Aureomycin treated seed but 1% of those grown from hot water treated seed were

infected. At Rogersville 1% of the plants from both Aureomycin-treated and hot-water-treated seed became infected with *X. campestris.* Before cultivating or working the plots at Grand Falls the equipment was cleaned by washing with hot water and steam, whereas this precaution was not taken at Rogersville. The plots in both areas were harvested before the disease spread further.

Losses from black rot in commercial Brussels sprouts fields in New Brunswick were less in 1975 than in 1974. In 1975, a survey of 18 ha showed an average loss of 3% with individual losses ranging from 0 to 10% compared with a loss of 24% in 1974. The lower incidence in 1975 is attributed to the better use of crop rotations and, the planting of later maturing crops. Observations in 1974 suggested that black rot was more severe in crops maturing in early September than in those maturing later in September or in early October and in fields where Brussels sprouts had been grown the previous year.

Samples of sprouts grown from Aureomycin-treated seed were collected at harvest time and bioassayed against *Bacillus cereus;* none had any detectable residues of Aureomycin.

The methods described here may be applied to other crucifer crops as tests in the greenhouse have shown that 500 ppm Aureomycin at 50°C for 25 minutes also Freed cabbage seed from natural infections of *X. campestris*.

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