Eradication of *Xanthomonas campestris*, the Causal Agent of Black Rot, from *Brassica* Seeds with Antibiotics and Sodium Hypochlorite

H. S. Humaydan, G. E. Harman, B. L. Nedrow, and L. V. DiNitto

A joint contribution of the Harris Seed Co., Inc., Rochester, NY 14624 (first and fourth authors) and of the New York State Agricultural Experiment Station, Geneva, NY 14456 (second and third authors).

We thank E. P. Carney and J. Millette for assistance in field plots.
Approved by the Director of the New York State Agricultural Experiment Station as Journal Series Paper No. 3224.
Accepted for publication 30 July 1979.

ABSTRACT

HUMAYDAN, H. S., G. E. HARMAN, B. L. NEDROW, and L. V. DiNITTO. 1980. Eradication of *Xanthomonas campestris*, the causal agent of black rot, from *Brassica* seeds with antibiotics and sodium hypochlorite. Phytopathology 70:127-131.

A method is needed for eradicating *Xanthomonas campestris* from *Brassica* seeds without causing damage to seeds or phytotoxicity to seedlings. The pathogen was eradicated from most lots of *Brassica* seeds by a 1 hr soak in a 500 μg/ml solution of either aureomycin, terramycin, or streptomycin. These treatments were phytotoxic, but if the antibiotic soak was followed with a water rinse and a soak for 30 min in 0.5% (w/v) NaOCl, the phytotoxicity was eliminated. Laboratory, greenhouse, and field tests indicated that a soak for 2 hr in a solution containing any one of these antibiotics followed by the water rinse and a NaOCl soak eradicated *X. campestris* from all seed lots tested and was not phytotoxic. When preceded with a reduced-pressure 0.2% (w/v) thiram soak for 24 hr, both *Phoma lingam* and *X. campestris* were eradicated from cabbage seeds with no loss of seed quality. By comparison, the standard hot water soak did not eradicate *X. campestris* from three of 11 lots tested, and it frequently damaged seed quality. A soak in 500 μg/ml of vancomycin HCl was not phytotoxic, but in field tests seeds treated with this antibiotic produced infected plants. Efforts are under way to register a seed soak in 500 μg/ml streptomycin for 2 hr followed by a water rinse and a 30-min soak in 0.5% NaOCl for eradication of seedborne *X. campestris* in the United States.

Cruciferous crops, including cabbage and other members of the *Brassica oleracea* L. group, have suffered epiphytotics of black leg caused by *Phoma lingam* (Tode ex. Fr.) and black rot caused by *Xanthomonas campestris* (Pam.) Dowson. Both pathogens are seedborne and epiphytotics frequently arise from diseased seeds.

The standard treatment for eradicating these pathogens from seeds has been a hot water soak (50 C for 20–30 min) (3). This treatment frequently results in reduced seed germination and reduced seedling vigor (7).

Thus, improved seed treatments are needed for eradicating these pathogens. Either a benomyl-Arasan slurry (4) or a reduced pressure thiram soak (6) will eradicate *P. lingam* without seed injury. Therefore, the most critical need is for a seed treatment capable of eradicating *X. campestris*. A number of antibiotics, including aureomycin, streptomycin, and terramycin, eradicate or greatly reduce *X. campestris* in infested *Brassica* seeds (8,10). Unfortunately, these substances are highly phytotoxic (8), and, although a number of substances reduce antibiotic-induced injury, none completely prevents phytotoxicity (2,5,9,10,14).

In this paper we report: an antibiotic/sodium hypochlorite (NaOCl) treatment that does not result in phytotoxicity or a reduction of seed vigor or viability of the sort induced by hot water; data indicating that this treatment will eradicate *X. campestris* from seeds as determined in laboratory, greenhouse, and field tests; and data indicating that this treatment, combined with the reduced pressure thiram soak, will eradicate both *X. campestris* and *P. lingam* without damage to seeds.

MATERIALS AND METHODS

Antibiotics. The following antibiotics reported to be effective against *X. campestris* (8,10,13) were evaluated for effects on seed germination, vigor, phytotoxicity, and seedborne *X. campestris:* streptomycin (AgriStrep, 21.2% streptomycin sulfate, Merck & Co., Inc., Rahway, NJ 07065), aureomycin (14% chlortetracycline HCl, American Cyanamid Co., Princeton, NJ 07470), terramycin (Tree injection formulation, 20% oxytetracycline HCl; Pfizer, Inc., New York, NY 10007), and vancomycin HCl (Eli Lilly and Co., Indianapolis, IN 46206).

Seed soak in antibiotic solutions. Unless otherwise mentioned, *Brassica* seeds, naturally infected with *X. campestris,* were soaked for 1 hr at 24 C in a 500 μg a.i./ml solution of the various antibiotics. Seeds to be treated were placed in a 400-ml beaker, to which the antibiotic solutions were added. The volume of the solution was twice that of the seeds. After soaking, seeds were rinsed for 2 min in running tap water and dried for 24 hr at 34 C. Seeds soaked in water and dried similarly served as controls.

Prevention of phytotoxicity. Seeds that had been soaked in solutions of the various antibiotics were rinsed in running tap water before being dried and immediately resoaked in a freshly prepared solution of 0.5% sodium hypochlorite (NaOCl) for 30 min. NaOCl was prepared by dilution from a 15% (w/v) commercial formulation (Bison Laboratories, Buffalo, NY 14211). After the final soak, seeds were rinsed for 2 min in running tap water and dried as described above.

Seed germination, seedling vigor, and phytotoxicity. Germination tests were done according to the rules of the Association of Seed Analysts of North America (1). Eleven days after seed sowing, counts were made of seedling emergence and also the number of seedlings showing antibiotic-induced injuries, such as chlorosis, stunting, and deformation of the cotyledons. Seedling vigor was rated subjectively on a scale of one to four, in which one and four represented the lowest and highest vigor, respectively. The height and general health of the seedlings were the major criteria for estimating vigor. Each treatment consisted of three 100-seed replicates. Each test was repeated three times.

Laboratory assay for the detection of *X. campestris*. Seeds (10,000 per lot) from each *Brassica* seed lot to be assayed were placed in a 250-ml Erlenmeyer flask containing 100 ml of sterile deionized water. The flasks were placed on a shaker for 6 hr at 30 C. From the resultant supernatant, four successive 1:5 serial dilutions were made. Aliquots (0.1 ml) of liquid from the undiluted

0031-979X/80/02012705/$03.00/0

supernatant and from each dilution were separately streaked onto one plate of SX agar (12). The plates were then incubated in the dark for 5 days at 30 C. After the incubation period, the plates were examined for the presence of *X. campestris*-like colonies (12). Suspected colonies were streaked onto yeast extract-dextrose-$CaCO_3$ agar (15) and the pathogenicity of yellow, mucoid colonies was verified by inoculating them onto radish and cabbage plants. This procedure has been tested by the senior author, in cooperation with N. Schaad (University of Georgia, Griffin 30223) and found to be as sensitive as the test developed by Schaad et al (11) which will detect samples with 0.01% infected seeds. Assayed seed lots were classsified into four categories as follows:

–Clean, if no *X. campestris* colonies were detected on SX agar.
–Lightly infested if 1–3 colonies of *X. campestris* were detected.
–Moderately infested if 4–9 colonies of *X. campestris* were detected.
–Heavily infested if 10 or more colonies of *X. campestris* were detected on SX agar.

All infected seed lots used in this study, except DB75, were of Japanese origin, and determined to be heavily infested with *X. campestris*. DB75 is a lot of broccoli seeds the parents of which were artificially inoculated with a virulent culture of *X. campestris* (Isolate PHW42 in P. H. William's collection, Dept. of Plant Pathology, University of Wisconsin, Madison 53706) during the summer of 1975. By plating 2,000 individual seeds on SX agar (12), we determined that 10% of the seeds in DB75 were infected with *X. campestris*. Lots containing 0.01% infected seeds may give rise to significant levels of disease in field plantings (11).

Greenhouse assays. Ten thousand treated seeds of each lot to be tested were planted 5-mm deep in a flame-pasteurized clay loam:sand:peat (l:l:l, v/v) mix in flats. Each flat contained 500 seeds. As a check, untreated seeds were planted in similar soil in flats. In 1977, untreated check seeds were all from the DB75 broccoli seed lot described above; 1,000 seeds were planted per test. In 1978, untreated seeds were of the same seed lot as the treated seeds; 10,000 seeds were planted per test. Greenhouse temperatures both years varied between 13 C at night to 27 C during the day. Plants were watered as needed.

Seedlings from untreated seeds were observed for the development of black rot symptoms. Approximately 1 wk after definite symptoms were observed, all seedlings, regardless of treatment, were individually pulled and examined. If symptoms suggestive of black rot were present, suspect seedlings were labelled, and placed in a capped 10 ml polypropylene vial. These plants were stored at 5 C (never more than seven days) until tested further.

To confirm the presence of *X. campestris,* individual plants with symptoms were briefly rinsed (1 min) in 0.87% (w/v) NaOCl (a 1:6 dilution of commercial Clorox), rinsed in 95% ethanol, rinsed in distilled water, and ground in 5 ml of sterile water in a mortar and pestle that had been steamed previously at 100 C for at least 30 min. Samples (0.1 ml) were placed onto each of two SX agar (12) plates, spread with a sterile glass rod, and then incubated on a laboratory bench. Seven to 10 days later, starch-hydrolyzing colonies were transferred to duplicate Difco Bacto-nutrient agar plates, which also were incubated on the laboratory bench. Five to 7 days later, the bacteria were washed from the plates in 1–2 ml of sterile water, and the bacterial suspension was diluted to 15 ml. Leaves of young (second-true-leaf stage) Market Prize cabbage plants were immersed in the suspension and perforated with a sterile paper punch. Two leaves of each plant were inoculated with each culture and plants were placed in a chamber maintained at 85–100% relative humidity at 20–25 C. Light at 21,600 lux (2,970 watts cool white fluorescent and 360 watts incandescent) was alternated with 12 hr of darkness. Each inoculation experiment included two plants inoculated with a known strain (PHW 42) of *X. campestris* and two inoculated with water. Seven to 10 days later, plants were observed and if characterisitic yellow lesions with black veins developed around the site of inoculation, the test was considered positive.

In 1977, four lots of Jade Cross Brussels sprouts treated with aureomycin/NaOCl were tested for black rot, and in 1978 Ruby Ball cabbage seeds treated with terramycin/NaOCl or with streptomycin/NaOCl were tested. Also in 1978, a mixture of 5,000 *P. lingam*-infested Market Prize cabbage and 5,000 *X. campestris*-infested Ruby Ball cabbage seeds were treated first with the reduced-pressure thiram treatment (6), and then with a 2-hr terramycin/NaOCl soak. This last test was conducted to determine whether the reduced pressure thiram and the terramycin/NaOCl treatments could be combined without seed damage and to determine whether both *P. lingam* and *X. Campestris* could be eradicated by the combined treatment.

Field assays. All field studies were conducted near Geneva, New York, in fields with no history of *Brassica* culture within the past 10 yr. They were separated from other *Brassica* plantings by at least 0.5 km. Treated and untreated seeds were planted in separate fields at least 2 km apart. Each treated or untreated seed sample contained 10,000 seeds and was separated from adjacent plantings by a thick planting of corn (1977) or oats (1978) 3 m wide. Each block of 10,000 seeds was planted in 16 rows 20 m long and 0.7 m apart. Each row received 625 seeds.

In 1977, seeds of the very heavily infested DB75 broccoli seed lot and a composite of samples of the Jade Cross commercial seed lots were treated with vancomycin or with aureomycin/NaOCl.

In 1978, only commercial infested seed lots were treated. Separate samples of a seed lot of Ruby Ball cabbage were treated either with streptomycin/NaOCl or terramycin/NaOCl. A sample of a lot of Nagaoka # 2 Chinese cabbage was treated with streptomycin/NaOCl. Finally a composite sample containing

TABLE 1. The effects of various treatments on seed germination, seedling vigor, and injury in a seed lot of Savoy King cabbage[a]

Treatment	Germination (%)	Injured seedlings (%)[b]	Seedling vigor[c]	Visible *X. campestris* infection
None	81	0	4.0	+
Hot water	76	0	3.0	–
NaOCl[d]	83	0	4.0	+
Vancomycin[d]	83	0	4.0	–
Aureomycin[d]	71	61	2.5	–
Streptomycin[d]	73	74	2.5	–
Terramycin[d]	73	28	3.0	–
Aureomycin/NaOCl[d]	85	0	4.0	–
Streptomycin/NaOCl[d]	82	0	4.0	–
Terramycin/NaOCl[d]	82	0	4.0	–
LSD (0.05)	7.0	3.0	0.2	

[a]Representative data from one seed lot; similar results were obtained with other *Brassica* crops and seed lots (see text).
[b]Injured seedlings were chlorotic, stunted, and/or possessed deformed cotyledons.
[c]A subjective rating of 1 to 4, in which 4 represents highly vigorous seedlings, and 1 represents seedlings of low vigor.
[d]All antibiotic treatments were soaks in 500 μg a.i./ml solution of the antibiotic for 1 hr at 24 C. The NaOCl treatment was a soak in a 0.5% (v/v) solution for 30 min at 24 C. Antibiotic/NaOCl treatments were soaks in 500 μg/ml antibiotic solutions, followed by a water rinse, and a final soak in 0.5% NaOCl for 30 min.

TABLE 2. Presence (+) or absence (−) of *Xanthomonas campestris* as determined by laboratory, greenhouse, and field assay of seed lots of various *Brassica* spp. cultivars after various seed treatments. Lots were known to be infected prior to treatment

Crop and cultivar	Treatment[a]	Assays[b]		
		Laboratory	Greenhouse	Field
Broccoli				
Green Comet	None	+	N.D.	N.D.
	Aureo/NaOCl	−	N.D.	N.D.
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Hot water	−	N.D.	N.D.
DB75	None	+		
	Aureo/NaOCl	+[c]	−	
	Strep/NaOCl	+[c]	N.D.	N.D.
	Terra/NaOCl	+[c]	N.D.	N.D.
	Vancomycin	+		
	Hot water	+	N.D.	N.D.
Brussels Sprouts				
Jade Cross lot 1	None	+	N.D.	
	Aureo/NaOCl	−	−	−
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Vancomycin	−	−	
	Hot water	−	N.D.	N.D.
Jade Cross lot 2	None	+	N.D.	
	Aureo/NaOCl	−	−	−
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Vancomycin	−	−	
	Hot water	−	N.D.	N.D.
Jade Cross E lot 1	None	+	N.D.	
	Aureo/NaOCl	−	−	−
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Vancomycin	−	−	
	Hot water	−	N.D.	N.D.
Jade Cross E lot 2	None	+	N.D.	
	Aureo/NaOCl	−	−	−
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Vancomycin	−	−	
	Hot water	−	N.D.	N.D.
Jade Cross E lot 3	None	+	N.D.	N.D.
	Strep/NaOCl	+[c]	N.D.	N.D.
	Terra/NaOCl	+[c]	N.D.	N.D.
	Hot water	−	N.D.	N.D.
Cabbage				
Ruby Ball	None	+	+	+
	Aureo/NaOCl	−	N.D.	N.D.
	Strep/NaOCl	−	−	−
	Terra/NaOCl	−	−	−
	Hot water	+	N.D.	N.D.
Cauliflower				
Snow Crown	None	+	N.D.	N.D.
	Aureo/NaOCl	−	N.D.	N.D.
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D.	N.D.
	Hot water	−	N.D.	N.D.
Chinese cabbage				
Nagoaka #2	None	+	N.D.	N.D.
	Aureo/NaOCl	−	N.D.	N.D.
	Strep/NaOCl	−	N.D.	-
	Terra/NaOCl	−	N.D.	N.D.
	Hot water	+	N.D.	N.D.
Turnip				
Just Right	None	+	N.D.	N.D.
	Aureo/NaOCl	−	N.D.	N.D.
	Strep/NaOCl	−	N.D.	N.D.
	Terra/NaOCl	−	N.D	N.D.
	Hot water	−	N.D.	N.D.

[a]Antibiotics/NaOCl treatments consisted of soaking seeds for 1 hr in 500 μg/ml (a.i.) of the antibiotic followed by 30 min soak in a 0.5% solution of sodium hypochlorite. Hot water treatment consisted of soaking seeds for 25 min at 50 C.

[b]In tests, 10,000 were assayed; N.D. indicates test not done. For laboratory assays 10,000 seeds were placed in 100 ml sterile deionized water and incubated for 6 hr at 30 C. Portions of the liquid or dilution thereof were streaked on SX agar and incubated at 30 C. Colonies suspected of being *X. campestris* were streaked on yeast extract-dextrose-$CaCO_3$ agar plates, and the pathogenicity of yellow, mucoid colonies was verified by inoculation onto radish and cabbage plants.

[c]*X. campestris* was not detectable in these lots when the cultivar soak was 2 hr long.

5,000 Market Prize and 5,000 Ruby Ball seeds was treated with the reduced pressure thiram treatment (6) followed by a 2 hr terramycin/NaOCl treatment.

Plants were observed at weekly to bi-weekly intervals throughout the next 2 mo and any plant or plant portion showing symptoms suggestive of black rot was tested for presence of the pathogen by the same procedures used for greenhouse-grown plants.

RESULTS

Phytotoxicity of seed treatments. Seeds treated for 1 hr with 500 μg/ml aureomycin, streptomycin, or terramycin alone showed signs of phytotoxicity, both reduced germination and seedling vigor. Hot water treatment also reduced vigor and germination (Table 1). Antibiotic-induced toxicity was characterized by chlorosis of cotyledons and subsequent death of some seedlings. Streptomycin was the most phytotoxic, followed by terramycin and aureomycin in order of decreasing phytotoxicity. Vancomycin or NaOCl alone was not toxic (Table 1).

Phytotoxicity caused by streptomycin, terramycin, or aureomycin soaks could be overcome by rinsing the seeds with water, then soaking them 30 min in 0.5% NaOCl (Table 1). Seed lots that received this combined treatment were unaffected in either germination or seedling vigor (Table 1). These tests were repeated using the streptomycin/NaOCl treatment with many other seed lots of other *Brassica* spp. crops, including Green Comet and Waltham 29 broccoli; Jade Cross and Jade Cross E Brussels sprouts; Danish Ballhead, Excell, Red Head, Savoy Ace, Harris Resistant Danish, Ruby Ball, and Market Prize cabbage; Imperial 10-6, Idol, Self-Blanche, and Snow Crown cauliflower; Nagoaka Pride, Early Hybrid G, and Nagoaka # 2 Chinese cabbage; and Just Right turnips.

Efficacy of seed treatments. The standard hot water treatment failed to eradicate *X. campestris* from three of 11 infested lots (Table 2). A second treatment eradicated *X. campestris*, but germination was severely reduced; only 25% germinated in one seed lot.

Antibiotic soaks for 1 hr followed by NaOCl treatments failed to eradicate *X. campestris* from the heavily infested DB75 or Jade Cross E lot 3. However, *X. campestris* was eradicated from these lots by a 2 hr antibiotic treatment (Table 2). Therefore, all seed lots were tested with a 2 hr antibiotic treatment, and *X. campestris* was eradicated from all lots with little or no reduction in germination or seedling vigor.

NaOCl alone did not eradicate *X. campestris* from infested seeds (Table 1, and other authors' data [*unpublished*]). Vancomycin soaks for 1 hr appeared to eradicate *X. campestris* from Jade Cross Brussels sprouts lots 1 and 2, and Jade Cross E lots 1, 2, and 3 in laboratory assays, but a composite sample of treated seeds gave rise to field infection. Vancomycin failed to eradicate *X. campestris* from broccoli lot DB75 either in laboratory or field tests (authors' *unpublished*).

The mixture of 5,000 *P. lingam*-infected Market Prize and 5,000 *X. campestris*-infected Ruby Ball cabbage seeds treated first with the reduced pressure thiram soak followed by a 2 hr terramycin/NaOCl soak gave rise to neither *P. lingam*- nor *X. campestris*-infected plants. Both pathogens were present in plants grown from untreated seeds in both greenhouse and field assays. This combined treatment was not injurious to seeds; in greenhouse tests seedlings emerged from 84% and 88% of seeds planted from untreated and treated samples, respectively. In field plantings, the same treatments resulted in 273 and 211 plants per row of the 625 seeds planted, respectively. Low emergence of both samples was probably due to hot (soil temperatures in excess of 30 C), dry conditions prevailing when the seeds were planted.

DISCUSSION

The effectiveness of NaOCl in preventing antibiotic-induced phytotoxicity is probably due to its being a strong oxidizing agent; thus inactivating residual antibiotics on seeds and under seed coats. Some divalent cations (eg, Ca^{++}, Mg^{++}, and Mn^{++}) reduce antibiotic-induced phytotoxicity either by competing with antibiotics for intracellular binding sites (16) or by reducing uptake of antibiotics by plant tissues (14). This difference in mode of action may explain why NaOCl is more effective than cations in preventing antibiotic-induced injury.

The 2-hr antibiotic/NaOCl treatment more effectively eradicates *X. campestris* than does hot water seed treatment and it causes no seed injury. Thus, it is an attractive alternative to hot water seed treatment. Efforts are underway to obtain FIFRA registration for this seed treatment, using the 2 hr antibiotic soak, since a 1 hr antibiotic soak is not effective with some seed lots.

Antibiotic/NaOCl treatment will not eradicate *P. lingam*. However, seed treatments have been developed that will eradicate this fungus (4,6), and we suggest that either one of the following combined treatments will eradicate both pathogens:

1. Treat seeds with antibiotic/NaOCl, dry them, and follow with a benomyl-thiram slurry (4) treatment.
2. Treat seeds using the reduced-pressure thiram soak (6), and without drying, follow immediately with the antibiotic/NaOCl treatment.

Both treatments should be effective and noninjurious to seeds. The benomyl-thiram treatment will no doubt be used if registration is obtained because it is very convenient.

Care must be taken with the antibiotic/NaOCl treatment to avoid the selection of antibiotic-resistant *X. campestris* strains. All treated seeds, whether they are commercial or stock seed lots, should be assayed for *X. campestris*, either by the laboratory procedure described in this work, by the technique developed by Schaad et al (11), or by other tests capable of detecting as few as one infested seed in 10,000. If the pathogen is detected after treatment, the seed should be subjected to hot water treatment, even though this may lower seed quality. No seed lots should be used for either commercial production or stock seed if *X. campestris* could not be eliminated by either or both of the above seed treatments. Also, it is less likely that any strain will simultaneously develop resistance to two antibiotics; therefore we plan work to register a seed soak consisting of 250 μg/ml streptomycin and 250 μg/ml terramycin. Preliminary results indicate that this combined treatment is as effective as 500 μg/ml of either antibiotic singly. These procedures combined may avoid loss of this treatment due to the development of resistant *X. campestris* strains.

LITERATURE CITED

1. ANONYMOUS. 1965. Rules for testing seeds. Proc. Assoc. Off. Seed Anal. N. Am. 54:1-112.
2. ARK, P. A., and J. P. THOMPSON. 1958. Prevention of antibiotic injury with Na- K- chlorophyllin. Plant Dis. Rep. 42:1203-1205.
3. CLAYTON, E. E. 1924. Investigations of cauliflower diseases on Long Island. N.Y.S. Agric. Exp. Stn. Bull. 506. 15 pp.
4. GABRIELSON, R. L., M. W. MULANAX, K. MATSUOKA, P. H. WILLIAMS, G. P. WHITEAKER, and J. D. MAGUIRE. 1977. Fungicidal eradication of seedborne *Phoma lingam* of crucifers. Plant Dis. Rep. 61:118-121.
5. GRAY, R. A. 1955. Inhibition of root growth by streptomycin and reversal of the inhibition by manganese. Am. J. Bot. 42:327-331.
6. HARMAN, G. E., and G. NASH. 1978. Soaking Brassica seeds in fungicide solutions to eradicate seedborne fungi: A comparison of aqueous and organic solvent infusion techniques. Plant Dis. Rep. 62:408-412.
7. HUBER, G. A., and C. J. GOULD. 1949. Cabbage seed treatment. Phytopathology 39:869-875.
8. KLISIEWICZ, J. M., and G. S. POUND. 1961. Studies on control of black rot of crucifers by treating seeds with antibiotics. Phytopathology 51:495-500.
9. McMEEKIN, D. 1973. Streptomycin inhibition of *Peronospora parasitica* and its host reversed by manganese and calcium. Phytopathology 63:34-36.
10. SUTTON, M. D., and W. BELL. 1954. The use of aureomycin as a treatment of swede seed for the control of black rot (*Xanthomonas campestris*). Plant Dis. Rep. 38:547-552.
11. SCHAAD, N. W., W. R. SITTERLY, and H. HUMAYDAN. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to

black rot of crucifers. Plant Dis. 64:91-92.
12. SCHAAD, N. W., and W. C. WHITE. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.
13. THIRUMALACHAR, M. J., M. K. PATEL, N. B. KULKARNI, and G. W. DHANDE. 1956. Effects in vitro of some antibiotics on thirty-two *Xanthomonas* species occurring in India. Phytopathology 46:486-488.
14. VENIS, M. A. 1969. Streptomycin inhibition of protein synthesis in peas reversed by divalent cations. Nature 221:1147-1148.
15. WILSON, E. E., F. M. ZEITOUN, and D. L. FREDRICKSON. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618-621.
16. ZAHN, G. 1962. Streptomycin and metal ions. I. The influence of some heavy metal ions, micronutrients and macronutrients on the phytotoxicity of streptomycin. Phytopathol. Z. 45:345-363.