Use of Polyethylene Glycol and Glycerol as Carriers of Antibiotics for Reduction of *Xanthomonas campestris* pv. *phaseoli* in Navy Bean Seeds

L. Z. LIANG, J. M. HALLOIN, and A. W. SAETTLER (deceased), Department of Botany and Plant Pathology and Agricultural Research Service, USDA, Michigan State University, East Lansing 48824-1312

ABSTRACT

Liang, L. Z., Halloin, J. M., and Saettler, A. W. 1992. Use of polyethylene glycol and glycerol as carriers of antibiotics for reduction of *Xanthomonas campestris* pv. *phaseoli* in navy bean seeds. Plant Dis. 76:875-879.

Bean seeds (*Phaseolus vulgaris*), either noninfested or internally infested with *Xanthomonas campestris* pv. *phaseoli*, were immersed in solutions of polyethylene glycol (PEG) or glycerol as well as in solutions of these materials containing streptomycin, tetracycline, and chlorotetracycline. Immersion in either 25% PEG or 60% glycerol solutions did not diminish germination while seedling vigor was slightly reduced. PEG solutions were more effective than glycerol solutions for introduction of antibiotics into seeds. Concentrations of tetracycline and chlorotetracycline in PEG solutions that effectively reduced *X. c. phaseoli* were phytotoxic. PEG solutions with streptomycin reduced but did not eradicate internal populations of the bacterium from naturally contaminated seeds and caused few phytotoxic effects.

Common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye is one of the most serious seedborne diseases of dry edible beans (*Phaseolus vulgaris* L.) throughout the world (14,17,19). The major sources of primary inoculum for the disease are internally and externally contaminated seeds (16). Certification programs for the production and testing of pathogen-free seed are helpful, but blight outbreaks persist because many growers plant seed from sources other than those considered to be pathogen-free. Effective treatments to eradicate the bacterium from bean seed would contribute greatly to an integrated strategy for disease management.

External contamination with *X. c. phaseoli* on bean seeds is easily controlled with streptomycin sulfate (15) and sodium hypochlorite (18). However, there are no acceptable methods known for eradicating internal populations of the bacteria from seeds. Thus, improved seed treatments are needed.

Eradication of internal bacteria in seed with antibiotics can be accomplished only by introduction of those chemicals into the seeds. This can be done with aqueous solutions; however, such treatment allows rapid imbibition of seeds. Subsequent redrying of seeds for storage causes reductions in germination and seed vigor. Alternatively, antibiotics can be introduced into seeds with organic solvents, but these either fail to penetrate into embryo tissues, or they damage the embryos, probably through membrane disruption (3). Aqueous solutions of polyalcohols, especially polyethylene glycol (PEG), provide an osmotic environment that restricts seed imbibition while still allowing diffusion of solutions into embryos. Immersion of seeds in PEG solutions followed by drying and storage does not impair germination, can increase seedling resistance to adverse environments, and is called osmotic conditioning (1,5,10,13). Hepperly and Sinclair (4) used PEG solutions to introduce penicillin G and streptomycin into soybean seeds. Antibiotic activity was detected in all seed parts after 15 wk of storage.

Below we report an assessment of the potential of osmotic conditioning as an aid in eradicating or reducing internal populations of *X. c. phaseoli* from bean seeds. The objectives were 1) to assess the impact of concentration and duration of immersion in PEG and glycerol solutions on navy bean seed germination, 2) to test the efficacy of PEG and glycerol solutions for introducing antibiotics into seeds, 3) to test whether PEG- or glycerol-antibiotic combinations are phytotoxic, and 4) to determine if these treatments would effectively eliminate *X. c. phaseoli* from internally contaminated seed.

Accepted for publication 15 April 1992.

MATERIALS AND METHODS

Sources and infection of seeds. Seeds of commercial cultivar C-20, a white-seeded navy bean (*P. vulgaris*), were used throughout this study. A large sample was obtained from Michigan Foundation Seed Association and was stored dry, at room temperature, for 9 mo before the start of the study.

Seeds to be assayed for internal populations of *X. c. phaseoli* were shaken in a 1:1 commercial bleach–distilled water solution for 1 min as described by Weller (18), rinsed in sterile distilled water, and incubated individually in test tubes with 3 ml of liquid semiselective medium (LSSM; 1 g of yeast extract, 25 mg of cycloheximide, 2 mg of nitrofurantoin, 1 mg of nalidixic acid, and 0.5 mg of gentamicin in 1,000 ml of 0.01 M phosphate buffer, pH 7.2) and then placed on a shaker at 20 C for up to 10 days. Samples of turbid liquid were streaked on plates of yeast extract–calcium carbonate agar (YCA; 10 g of yeast extract, 15 g of agar, 2.5 g of $CaCO_3$, and 1 L of water. Pale yellow colonies were transferred to a semiselective medium (SSM; 1 g of yeast extract, 15 g of agar, 8 g of soluble potato starch, 6 μl of 1% methyl green, 3 μl of 1% methyl violet 2B, 25 mg of cycloheximide, 2 mg of nitrofurantoin, 1 mg of nalidixic acid, and 5 mg of gentamicin in 1,000 ml of 0.01 M phosphate buffer, pH 7.2) and streaked for purity. Presumptive colonies of *X. c. phaseoli* were diluted to 10^8 cfu/ml in sterile distilled water and infiltrated into leaves of 15-day-old C-20 bean seedlings. Cultures that caused development of bacterial lesions within 10 days after inoculation were considered pathogens.

Naturally infected seeds were produced in field plots at the Botany and Plant Pathology Farm, Michigan State University, East Lansing. Four different strains of *X. c. phaseoli* that had been isolated from infected bean seeds were used to inoculate a susceptible cultivar (C-20) of navy bean. A bacterial suspension (about 10^5 cfu/ml) was prepared from 24-hr cultures of *X. c. phaseoli* grown on plates of YCA. The bacteria were washed from the plates with sterile water, and the cell suspension was sprayed onto foliage, at 15, 30, and 45 days after the seeds had been planted. After harvest, seeds with visible symptoms of infection (discoloration) were selected as the naturally infected sample. These seeds exhibited 24% internal infection, as determined by growth of bacteria

from surface-disinfected seeds.

Large quantities of seed with internal populations of *X. c. phaseoli* were produced by vacuum infiltration as described by Goth (2), with the following modifications. A culture of the bacterium was grown on YCA plates for 48 hr and then transferred to buffered yeast extract (BYE) medium (1 g of yeast extract per liter of 0.01 M phosphate buffer, pH 7.2), which was shaken for 18 hr at 25 C. The resulting bacterial suspension was diluted with sterile water to an optical density of 0.2 at 620 nm. The cell suspension contained approximately 10^6 cfu/ml. Seeds were rinsed in a 1:1 dilution of commercial bleach (2.6% NaOCl) for 2 min to reduce surface microbes, then rinsed three times in sterile distilled water, blotted with sterile paper towels, and dried at room temperature in a laminar flow hood for 24 hr. The dried seeds were immersed in the bacterial suspension and exposed to a vacuum of approximately 500 mm of Hg for 2 min. The flask was shaken to enhance the escape of air from the seeds. The suction was released abruptly. The seeds remained immersed for 1 min and then were drained, blotted dry with sterile paper toweling, left in open trays in a laminar flow hood for 4 hr, and finally dried at 25 C for another 24 hr. Seeds with broken or cracked seed coats were removed. An average of 43% of the seeds treated in this manner contained internal propagules of the bacterium.

Germination tests and seed treatments. Germination tests were done by placing seeds between sheets of moistened paper toweling (9) at 25 C. Germination percentages and seedling fresh weights were determined after 7 days. Numbers of normal and abnormal seedlings also were recorded (8).

Glycerol and PEG 8000 powder were dissolved in distilled water at room temperature. Seeds were immersed in 100-ml solutions of these chemicals at 20 C for 1–5 days. The concentrations were 10, 15, 20, 25, and 30% PEG or 50, 60, and 70% glycerol; there were four replicate 100-seed samples for each solution. (Fermentation occurred when seeds were immersed for up to 4 days at 25 C.) After immersion, seeds were washed for 20 sec in tap water and dried in a laminar flow hood for 16 hr at 25 C.

To find whether antibiotics dissolved in PEG or glycerol solutions affected germination, we immersed four replicate samples of 100 seeds each in 100-ml solutions of 25% PEG or 60% glycerol mixed with 0.4 mg/ml or 0.8 mg/ml streptomycin sulfate, 0.4 mg/ml or 0.8 mg/ml chlortetracycline hydrochloride, or 0.4 mg/ml or 0.8 mg/ml tetracycline hydrochloride for 1, 3, or 5 days. The treated seeds were handled and tested for germination as described above. After soaking, the seeds were washed, dried, and germinated as previously described. Seeds soaked only in 25% PEG or 60% glycerol were used as controls.

Antibiotic activity in seeds. Antibiotic activity in seeds was measured on plates of buffered yeast extract (BYE) agar containing 10 g of yeast, 15 g of agar, and 1,000 ml of 0.01 M phosphate buffer, pH 7.2. The freshly autoclaved BYE agar was cooled to 40 C and mixed with *X. c. phaseoli* suspended in 0.01 M phosphate buffer to provide a final concentration of 10^8 bacterial cells per ml of 0.9-strength medium. Twenty milliliters of the seeded medium was pipetted into 90-mm-diameter petri plates, which then were stored at 4 C prior to use. Seeds were immersed for 1, 3, 5, 7, 24, or 72 hr in PEG or glycerol, mixed with either 0.4 mg/ml streptomycin sulfate, 0.4 mg/ml chlorotetracycline hydrochloride, or 0.4 mg/ml tetracycline hydrochloride. The treated seeds were rinsed three times in distilled water and halved longitudinally, and seed coats were removed. Cotyledons and embryos were separated, dried overnight in a laminar flow hood, and placed on bacteria-seeded BYE agar plates. Plates were placed in an incubator at 28 C for 24 hr, and zones of inhibition were measured from the edge of the assayed piece to the farthest point of the cleared zone. Cotyledons and embryos of seeds that were soaked only in PEG or glycerol served as controls. Forty-eight seeds were used for each treatment.

To determine if antibiotic residue in the seeds affected bacterial growth in semiselective liquid media, we immersed seeds in 25% PEG plus 0.8 mg/ml of streptomycin, chlorotetracycline, or tetracycline, respectively, for 3 days, rinsed the seeds in sterile distilled water, and soaked them individually in test tubes with 1, 2, 3, 4, or 5 ml of LSSM for 1 day at 5 C. Subsequently, 5 μl of a 10^2 cfu/ml bacterial suspension was delivered into each tube. These tubes were incubated for 7 days on a shaker at 25 C, and samples of liquid from each tube were streaked on YCA and SSM.

To evaluate the efficacy of eradication treatments, we immersed both artificially and naturally infested seeds for 1 or 3 days at 20 C in solutions containing 25% PEG or 60% glycerol mixed with 0.4, 0.8, or 1.6 mg/ml streptomycin, 0.4 or 0.8 mg/ml chlorotetracycline, or 0.4 or 0.8 mg/ml tetracycline separately. Additional treatments employing 3.2 mg/ml streptomycin and 1–5 days of immersion were included for naturally infested seeds. The treated seeds were rinsed in sterile distilled water for 15 sec and dried at room temperature in a laminar flow hood for 24 hr. The dried seeds were rinsed in a solution of commercial laundry bleach (5.25% NaOCl) and distilled water (1:10, v/v) for 1 min. The seeds then were added to test tubes containing 3 ml of LSSM, and the tubes were shaken for 10 days to test for the presence of *X. c. phaseoli*. This procedure allowed enrichment of pathogen populations. Turbid tubes were noted, and liquid samples were streaked on YCA and SSM plates. Four hundred seeds were tested in each treatment of artificially infested seeds, and 800 seeds were tested in each treatment of naturally infested seeds.

RESULTS

Seeds soaked in 25 and 30% PEG solutions or 60 or 70% glycerol solutions for 1–4 days had germination percentages equal to or greater than that of the control (Fig. 1). Seeds soaked longer than 1 day in solutions with 20% or lower PEG content or for 1 day or longer in 50% glycerol had lower germination percentages than the control.

Several of the above treatments were repeated with weighing of seeds before and after soaking to determine if the solutions were absorbed into the seeds. The 60% glycerol solution was not taken up by seeds, whereas the other solutions were (Table 1). This explains the absence of germination reduction in seeds treated with 60% glycerol. The remaining experiments were done both with 25% PEG and with 60% glycerol. However, because results were consistent with failure of 60% glycerol to enter most seeds, only results of experiments with 25% PEG are presented.

Seeds treated with PEG mixed with either chlorotetracycline or tetracycline produced lower ($P = 0.05$) seedling fresh weights (Table 2) but exhibited no reductions in seed germination compared with seeds soaked only in 25% PEG. No significant difference in germination percentage or seedling fresh weights was observed between seeds soaked in PEG with streptomycin and those soaked only in 25% PEG. Albinism was observed on seedlings from seeds treated with PEG plus 0.4 mg/ml tetracycline or chlorotetracycline, but not on those from seeds soaked in PEG and streptomycin (up to 1.6 mg/ml) under the same conditions.

Zones of inhibition around cotyledons gradually increased with period of immersion from 1 to 24 hr (Table 3). No further increases were observed with immersion periods longer than 24 hr. Antibiotic activity also was associated with embryos from these seeds; zones of inhibition averaged 1.3 mm around embryos from all antibiotic treatments that contained 0.4 mg/ml of antibiotic. Small zones of inhibition were observed around embryos and cotyledons soaked only in PEG.

Preliminary assays on the recovery of bacteria from seeds revealed that blight bacteria were detected in all tubes, regardless of liquid volume. However, those tubes that contained only 1 ml of liquid medium showed less than 70 bacterial colony-forming units per milliliter, whereas tubes that contained from

Table 1. Weight and germination of navy bean seeds immersed in aqueous solutions of glycerol or polyethylene glycol (PEG) for 3 days at 20 C

Treatment	Weight[a] (g/100 seeds)	Germination (%)
Dry seed[b]	16.0[c]	85
Glycerol 60%	16.8	87
Glycerol 50%	22.7	22
PEG 25%	24.9	91
PEG 20%	26.1	78
LSD (0.05)	2.1	6

[a]Of imbibed seeds.
[b]Control seeds, not immersed in solutions.
[c]Each value is the mean of four samples of 100 seeds.

Table 2. Germination percentages and seedling fresh weights of navy bean seeds previously immersed in solutions of 25% polyethylene glycol (PEG) plus streptomycin, chlorotetracycline, or tetracycline[a]

Solution	mg/ml	Germination (%) Days immersed 1	3	5	Mean seedling fresh weights (grams/seedling) Days immersed 1	3	5
PEG alone		94	86	79	0.96	0.85	0.73
Streptomycin	0.4	98	92	86	0.97	0.78	0.66
	0.8	97	95	87	0.86	0.76	0.69
Chlorotetracycline	0.4	95	84	76	0.60	0.45	0.45
	0.8	91	82	71	0.52	0.49	0.45
Tetracycline	0.4	98	95	72	0.64	0.54	0.48
	0.8	97	93	73	0.56	0.45	0.46
		LSD (0.05) = 5			LSD (0.05) = 0.10		

[a]Each value is the mean of four samples of 100 seeds.

2 to 5 ml of liquid medium all had more than 700 cfu/ml. Use of tubes with 3 ml of liquid medium was selected as a standard throughout evaluations of elimination of internally seedborne bacteria.

Blight bacteria were not detected in artificially infested seeds that had been immersed for 1 or 3 days in a 25% PEG solution containing 1.6 mg/ml streptomycin, 0.4 mg/ml tetracycline, or 0.4 mg/ml chlorotetracycline (Table 4). With all treatments, increases in either the antibiotic concentration or the immersion time reduced the population of *X. c. phaseoli* recovered.

With the naturally infested seed sample, large decreases in pathogen populations occurred in all treatments with mixtures of antibiotics in 25% PEG solutions, especially with 3 days of immersion. However, none of these treatments successfully eradicated the pathogen from the seeds. Seeds with impermeable coats accounted for 10.2, 8.5, 6.9, 5.5, and 4.5% of those immersed in 25% PEG plus 1.6 mg/ml streptomycin for 1, 2, 3, 4, and 5 days, respectively. With seeds immersed for 3 days, 67% of the cultures of *X. c. phaseoli* isolated were from seeds with impermeable coats, whereas the remainder grew on YCA containing 50 μg/ml of streptomycin and were considered resistant to the antibiotic. A slight reduction in seedling fresh weight was caused by the highest concentration of streptomycin.

DISCUSSION

Solutions of PEG can be used as antibiotic carriers for treatment of seeds without affecting germination percentage. A concentration of 25% appeared best for moving antibiotics into the seeds as measured by reductions in occurrences of *X. c. phaseoli* within seeds; seedling vigor as expressed by seedling fresh weight was slightly reduced by this treatment. Higher PEG concentrations had less effect on seedling vigor but led to less bactericidal activity, apparently because less of the solution entered the seeds.

Glycerol was not suitable as an antibiotic carrier. Solutions with concentra-

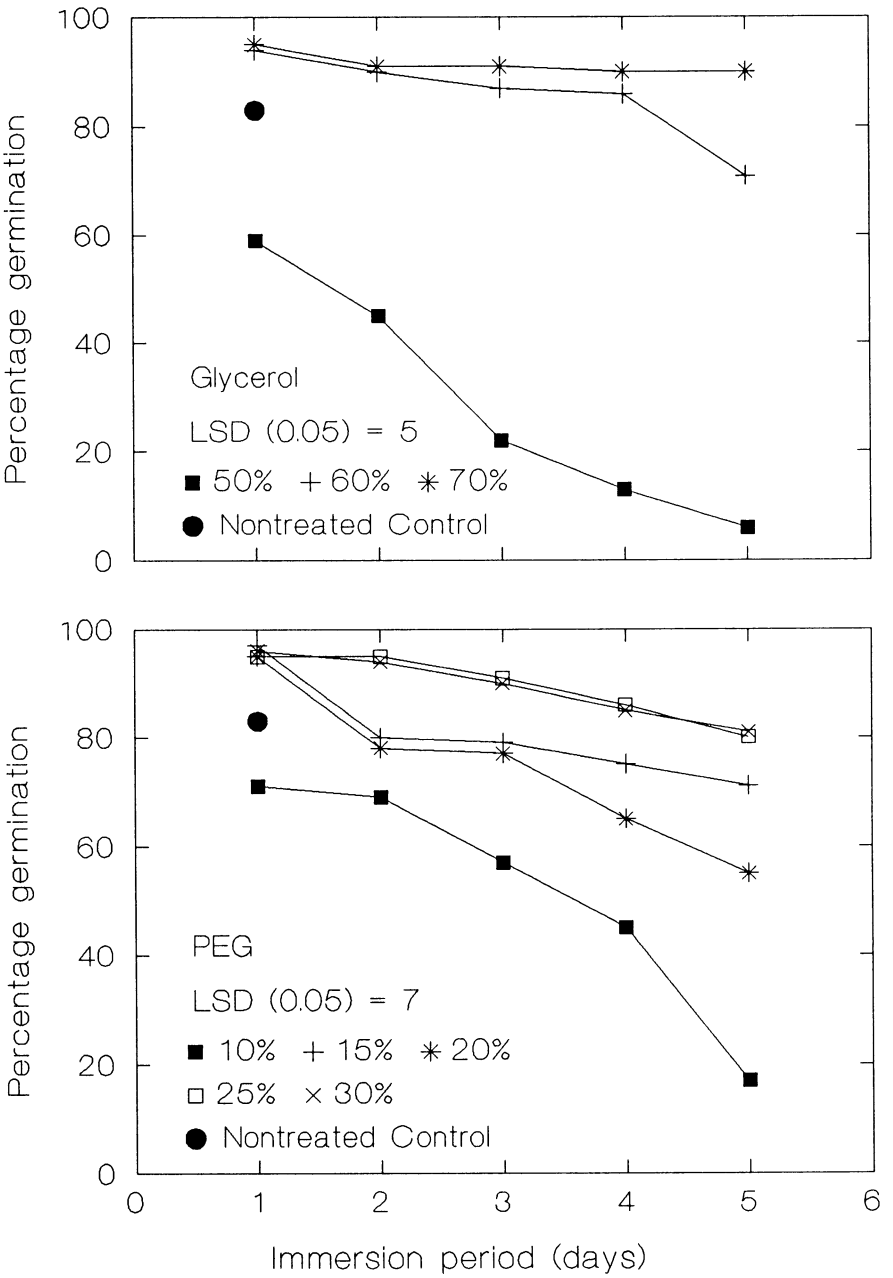

Fig. 1. Germination percentages of navy bean seeds immersed in different concentrations of glycerol or polyethylene glycol (PEG) for up to 5 days. Nonimmersed seeds were used as controls. Four hundred seeds were tested in each treatment.

Table 3. Effects of immersion of navy bean seeds in aqueous solutions of 25% polyethylene glycol (PEG) or PEG plus 0.4 mg/ml of streptomycin, chlorotetracycline, or tetracycline on subsequent growth of *Xanthomonas campestris* pv. *phaseoli*[a]

	Zone of inhibition (average width in mm)[b]						
	Immersion period (hours)						
Treatment	0	1	3	5	7	24	72
PEG alone	ND[c]	ND	ND	ND	ND	1.2	ND
PEG + streptomycin	0	1.8	2.7	3.1	4.3	5.2	5.3
PEG + chlorotetracycline	0	2.1	2.9	4.2	5.1	6.1	6.0
PEG + tetracycline	0	2.3	3.3	4.3	5.0	6.0	6.2
	LSD (0.05) = 0.4						

[a]Around cotyledons plated on buffered yeast extract agar containing the bacterium. Following immersion, seeds were rinsed three times in distilled water, halved longitudinally, and seed coats were removed. Cotyledons and embryos were separated and dried overnight in a laminar flow hood and placed on the agar medium.
[b]Each value is the mean of 48 cotyledons. Width represents the distance from the edge of the cotyledon to the farthest point of the cleared zone.
[c]ND = not determined.

Table 4. Effect of 1- or 3-day immersion of seed in antibiotics dissolved in 25% polyethylene glycol (PEG) on recovery of *Xanthomonas campestris* pv. *phaseoli* from navy bean seeds naturally infected or vacuum infiltrated with the bacteria

		Seeds with bacteria (%)			
		Naturally infected		Infiltrated	
		Immersion		Immersion	
Treatment	mg/ml	1 day	3 days	1 day	3 days
Untreated		23.0[a]	23.0	43.0	43.0
PEG only		19.4	18.6	20.0	19.0
PEG + streptomycin	0.4	ND[b]	ND	18.0	3.3
PEG + streptomycin	0.8	ND	ND	8.9	0.6
PEG + streptomycin	1.6	2.4	0.4	0	0
PEG + tetracycline	0.4	0.6	0.1	0	0
PEG + chlorotetracycline	0.4	0.8	0.2	0	0
	LSD (0.05)	1.8		2.1	

[a]Values are the means of eight and four samples of 100 seeds for naturally infected and infiltrated seeds, respectively.
[b]ND = not determined.

tions greater than 60% were not absorbed by the seeds, and lower concentrations reduced seed germination. This phenomenon explains why no phytotoxicity was observed when seeds were soaked in 60% glycerol solutions containing tetracycline and chlorotetracycline, whereas PEG solutions containing these antibiotics induced phytotoxicity. The glycerol solutions of antibiotics were much less effective than similar PEG solutions in reducing the occurrence of common blight bacteria within seeds.

Tetracycline and chlorotetracycline in PEG solutions proved effective in reducing internal populations of seedborne *X. c. phaseoli* from bean seeds; however, phytotoxicity rendered these treatments unacceptable. Streptomycin appears to be the most promising antibiotic for treating bean seeds. A concentration of 1.6 mg/ml in a 25% PEG solution reduced the incidence of naturally contaminated seed to 0.2–11% of control values. The inability of PEG plus streptomycin to completely eliminate *X. c. phaseoli* from naturally infected seeds was associated with "hard" seeds and antibiotic resistant strains of the pathogen. Although these findings are based upon a single seed sample, they seem likely to pertain more generally, since "hard" seeds are not rare among commercial bean seed lots, and streptomycin resistant strains of *X. c. phaseoli* were found in natural populations in Michigan in a previous study (18).

Antibiotics such as streptomycin (11) and chlorotetracycline (12) have been used to eradicate *X. c.* pv. *campestris* (Pammel) Dowson from *Brassica* seeds, but these treatments often resulted in reduced germination and seedling vigor (6). Humaydan et al (7) overcame this disadvantage by exposing antibiotic-treated seeds (tetracyclines and streptomycin) to a solution of 0.5% NaOCl. Seed lots that received the combined treatment were unaffected in either germination or seedling vigor. This method was not effective in our study (*unpublished*). The NaOCl treatment only partially overcame the phytotoxicity induced by tetracycline or chlorotetracycline, and seedlings from such treatments were severely etiolated. Success in eradicating *X. c. campestris* from *Brassica* seeds was probably due to the fact that this pathogen was borne externally and could be eliminated by conventional surface treatments. For example, a 1-hr soaking of seeds in aqueous solutions of antibiotics eliminated the bacteria (7). Such a short treatment period probably did not allow the antibiotics to penetrate into the embryo or cotyledon. Antibiotics on the seed surface and in the seed coat could be easily oxidized by NaOCl. In contrast, the failure of NaOCl to overcome completely the phytotoxicity associated with the tetracycline treatments in our tests was due to the localization of the antibiotics inside the seeds, where the oxidant failed to penetrate.

Populations of *X. c. phaseoli* in artificially infested seeds were more sensitive to antibiotic treatment than those in naturally infested seeds. Clearly, the vacuum inoculation technique did not completely simulate the natural situation. The vacuum inoculation technique would assure internal pathogen populations only with seeds with permeable seed coats. The antibiotic solutions used here should be quite efficient in eradicating those infestations, particularly when antibiotic-sensitive strains are involved.

LITERATURE CITED

1. Fu, J. R., Lu, X. H., Chen, R. Z., Zhang, B. Z., Liu, Z. S., Li, Z. S., and Cai, D. Y. 1988. Osmoconditioning of peanut (*Arachis hypogea* L.) seeds with PEG to improve vigor and some biological activities. Seed Sci. Technol. 16:197-212.
2. Goth, R. W. 1966. The use of partial vacuum to inoculate bean seeds with pathogenic bacteria. Plant Dis. Rep. 50:110-111.
3. Halloin, J. M., Minton, E. B., and Petersen, H. D. 1978. Fungicide application to cottonseed using methylene chloride carrier. Crop Sci. 18:909-910.
4. Hepperly, P. R., and Sinclair, J. B. 1977. Aqueous polyethylene glycol solutions for treating soybean seeds with antibiotics. Seed Sci. Technol. 5:727-733.
5. Heydecker, W. 1974. Germination of an idea: The priming of seeds. Part 3. Univ. Nottingham Sch. Agric. Rep.
6. Huber, G. A., and Gould, C. G. 1949. Cabbage seed treatment. Phytopathology 39:869-875.
7. Humaydan, H. S., Harman, G. E., Nedrow, B. L. and Dinitto, L. V. 1980. Eradication of *Xanthomonas campestris*, the causal agent of black rot, from *Brassica* seeds with antibiotics and sodium hypochlorite. Phytopathology 70:127-131.
8. International Seed Testing Association. 1979. Handbook for seedling evaluation. ISTA, Zurich.
9. International Seed Testing Association. 1985. International rules for seed testing. Seed Sci. Technol. 13:299-335.
10. Khan, A. A., Tao, K. L., Knypl, J. S., Borkowska, B., and Powell, L. E. 1978. Osmotic conditioning of seeds: Physiological and biochemical changes. Acta Hortic. 83:267-278.
11. Klisiewiecz, J. M., and Pound, G. S. 1961. Studies on control of black rot of crucifers by treating seeds with antibiotics. Phytopathology 51:495-500.
12. Lockhart, C. L., Gourley, C. O., and Chapman, E. W. 1976. Control of *Xanthomonas campestris* in brussels sprouts with hot water and aureomycin seed treatment. Can. Plant Dis. Surv. 56:63-66.
13. Muhyaddin, T., and Wiebe, H. J. 1989. Effects of seed treatments with polyethylene glycol (PEG) on emergence of vegetable crops. Seed Sci. Technol. 17:49-56.

14. Saettler, A. W. 1989. Common bacterial blight. Pages 261-283 in: Bean Production Problems in the Tropics. H. F. Schwartz and M. A. Pastor-Corrales, eds. Centro Internacional de Agricultura Tropical, Cali, Colombia.
15. Saettler, A. W,. and Anderson, A. L. 1978. Bean disease. Pages 172-179 in: Dry Bean Production. Mich. State Univ. Agric. Exp. Stn. Ext. Bull. E-1251.
16. Saettler, A. W., and Perry, S. K. 1972. Seed transmitted bacterial diseases in Michigan navy beans, *Phaseolus vulgaris*. Plant Dis. Rep. 56:378-381.
17. Sutton, M. D., and Wallen, V. R. 1970. Epidemical and ecological relations of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* on beans in south-western Ontario, 1961–1968. Can. J. Bot. 48:1320-2334.
18. Weller, D. M. 1978. Ecology of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in navy bean (pea) beans (*Phaseoli vulgaris* L.). Ph.D. thesis. Michigan State University, East Lansing. 137 pp.
19. Zaumeyer, W. J., and Thomas, H. R. 1957. A monographic study of bean diseases and methods for their control. U.S. Dep. Agric. Tech. Bull. 868. 255 pp.