Nyolate Seed Treatment of *Brassica* spp. to Eradicate or Reduce Black Rot Caused by *Xanthomonas campestris* pv. *campestris*

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ABSTRACT

Harman, G. E., Norton, J. M., Stasz, T. E., and Humaydan, H. S. 1987. Nyolate seed treatment of *Brassica* spp. to eradicate or reduce black rot caused by *Xanthomonas campestris* pv. *campestris*. Plant Disease 71:27-30.

Nyolate, a material manufactured as a general disinfectant by the Alcide Corporation, has potential as a seed treatment for eradicating seedborne bacteria. Nyolate is provided as two components that are mixed and diluted with water immediately before use. The first component (Nyolate solution A) contains $NaClO_2$; the second (Nyolate solution B) contains lactic acid, which acts as an activator. Initial experiments were performed with a formulation that contained 1.3% $NaClO_2$ in solution A and 7.5% lactic acid in solution B. Subsequently, the manufacturer changed to a second formulation containing 2.73% $NaClO_2$ in solution A and 15.1% lactic acid in solution B. Trials were conducted with seed lots of *Brassica* species naturally infected with *Xanthomonas campestris* pv. *campestris*. In initial experiments with the first formulation, soaking seeds for 30 min with a 1:1:8 (Nyolate A/Nyolate B/water) mixture eradicated the pathogen from the seed lots tested and caused only a low level of phytoxicity. In later experiments with the second formulation, treatment with a mixture containing 10:3:90 (A/B/water) completely eradicated *X. c.* pv. *campestris* from most of the seed lots tested and greatly reduced the detectable level of the pathogen in the remaining lots. Treatment with Nyolate at the 10:3:90 concentration did not reduce stands of kale, collards, turnip, cabbage, or Chinese cabbage but did reduce stands of cauliflower and broccoli. Treating seeds at a 20:6:75 concentration reduced emergence of most of the seed lots tested. Nyolate seems to have promise as a alternative to hot-water seed treatment for *Brassica* spp.

A variety of crops are plagued by seedborne bacterial pathogens. Among the most severely affected are cruciferous crops, including cabbage and other *Brassica* spp. The most prevalent bacterial pathogen in these crops is *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot.

The standard treatment for eradicating these pathogens from seeds is a hot-water soak (50 C for 20–30 min) (1). However, this treatment is ineffective with some seed lots (4) and frequently results in reduced seed germination and seedling vigor (3). Other treatments have been reported to reduce or eliminate this pathogen from seeds but were very strongly phytotoxic (5,7). In addition, we (4) reported a promising streptomycin/NaOCl seed treatment that effectively eradicated *X. c.* pv. *campestris* and had a low level of phytotoxicity under greenhouse conditions. However, under some field conditions, a phytotoxic response was noted that precluded use of this treatment (G. E. Harman, *unpublished*).

Thus, an effective, reliable, and nonphytotoxic antibacterial seed treatment is still badly needed. We have obtained promising results with a material manufactured by the Alcide Corp., Norwalk, CT, that was labeled for use as a hospital disinfectant. This paper reports data indicating that this material may be useful in reducing infection or eradicating *X. c.* pv. *campestris* from *Brassica* seeds.

MATERIALS AND METHODS

Nyolate is provided as two components, an activator and a base, that are combined with water immediately before use. In the formulation used in early experiments (formulation I), component A contained 1.3% sodium chlorite ($NaClO_2$) and component B contained 7.5% lactic acid. The manufacturer then changed the product, and in the formulation used in later experiments (formulation 2), component A contained 2.73% $NaClO_2$ and component B contained 15.1% lactic acid. Both formulations also contained surfactants, and these surfactants differed between the two.

Seed treatment consisted of soaking seeds in Nyolate solutions for 30 or 60 min, rinsing the seeds in water, and air-drying them. Seeds were planted within a month of treatment.

Seed lots were naturally infected, except for one obtained from systemically infected plants that were inoculated when young. Seeds from systemically infected

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plants can be expected to contain bacteria both on the surface and inside the seed coat (2). Because seed lots were of limited size and rapidly depleted, we used samples taken from a number of lots.

Procedures for detecting *X. c.* pv. *campestris* were similar to those described earlier (4), except Randhawa and Schaad's nutrient starch cycloheximide antibiotic agar (NSCAA) (6) was substituted for SX-1 as a semiselective medium. Treated or untreated seeds were soaked for 6 hr at 30 C in 100 ml of sterile saline on a rotary shaker. The resulting supernatant was serially diluted, and 0.1-ml aliquots were plated on NSCAA. Plates were incubated for 5–7 days at 30 C, and the number of starch-hydrolyzing, *X. c.* pv. *campestris*-like colonies and the total number of bacterial colonies were determined (4). Presumptive *X. c.* pv. *campestris* colonies were transferred to yeast-dextrose-$CaCO_3$ agar (YDC) (9) for subsequent testing. Alternatively, dilution plates with heavy growth of bacteria presumed to be mostly *X. c.* pv. *campestris* were washed with sterile saline and the resulting suspension tested immediately. Presumptive *X. c.* pv. *campestris* isolates were tested by inoculating Market Prize cabbage plants by immersing leaves in the bacterial suspension and piercing them with a paper punch. The inoculated plants were kept in a moist chamber in the greenhouse at 27–30 C for 5–7 days, and the presence or absence of characteristic black rot symptoms was noted (4).

A field trial was conducted at the Harris Seed Co. in Rochester, NY, to determine efficacy of treatments. An infected lot of collard seeds was soaked for 30 min in a solution containing 1:1:18 (Nyolate component A/Nyolate component B/distilled water), then rinsed and dried. Checks included untreated seeds from the same seed lot and untreated seeds from a noninfested lot of Market Prize cabbage. Four thousand seeds from each treatment were sown in a raised bed containing three rows 30 cm apart and 15 m long. Beds containing each treatment were separated by 1.8–2.4 m from adjacent treatments. Observations for black rot symptoms began when the cotyledons were fully expanded and continued until plants reached the eight- to 10-leaf stage 7 wk after planting. Any plants with suspected black rot symptoms were harvested, and the presence of *X. c.* pv. *campestris* was confirmed by plating tissue on a semiselective medium, transferring suspect colonies to YDC, and finally by inoculating susceptible plants as described earlier (4). Data on stand counts were also taken during these trials.

Phytotoxicity trials were conducted by treating noninfested seeds from seed lots of various cultivars with Nyolate at concentrations previously determined effective in eradicating *X. c.* pv. *campestris*. Untreated seeds served as a control. For each treatment × seed lot combination, 50 treated and 50 untreated seeds were planted in bands 5 cm wide 40 cm long in flats containing a peat/vermiculite mix. Treatments contained four replicates. Seven centimeters separated each band within flats. A completely randomized design was used for the placement of bands within the flats.

RESULTS AND DISCUSSION

In preliminary experiments, four naturally infected seed lots were treated by soaking for 30 min in a 1:1:18 (A/B/water) dilution of Nyolate formulation I. Assay of 10,000 treated seeds from each of these four lots detected no *X. c.* pv. *campestris*, whereas the pathogen was readily detected in untreated samples.

Because this treatment appeared effective, the field trial described in Materials and Methods was conducted. Plants arising from the untreated collard seeds included 57 infected plants by the end of the experiment. Plants from the noninfected Market Prize lot included two infected plants, whereas one infected plant was found in the plants from Nyolate-treated seeds. Thus, this 1:1:18 treatment appeared effective in both laboratory and field trials.

Subsequently, a very heavily infested lot was obtained, and the 1:1:18 treatment for 30 min was found to reduce levels of infection from about 16,000 *X. c.*

Table 1. Effects of a 30-min seed treatment with a 10:3:90[a] solution of Nyolate formulation II on total numbers of bacteria and of *Xanthomonas campestris* pv. *campestris* from various lots of crucifer seeds

Seed lot	Treatment	No. seeds tested per replicate	No. replicates	Total bacteria recovered (cfu/ml)	*X.c.* pv. *campestris*-like colonies[b] (cfu/ml)
Broccoli lot A	Untreated	2,000	1	>625,000	156,000
	Nyolate	10,000	2	20	0
	Nyolate	5,000	1	200	1
Broccoli lot B	Untreated	2,000	1	125,000	19,000
	Nyolate	5,000	2	0	0
Broccoli lot C	Untreated	4,000	1	575	225
	Nyolate	4,000	1	2	0
Broccoli lot D	Untreated	2,000	1	93,700	NR[c]
	Nyolate	5,000	2	0	0
Broccoli lot E	Untreated	10,000	1	25,000	250
	Nyolate	10,000	2	10	0
Collards lot A	Untreated	5,000	1	>626,000	NR
	Nyolate	10,000	2	0	0
Cabbage lot A	Untreated	10,000	1	437,000	4,375
	Nyolate	10,000	2	35	0
Brussels sprouts lot A	Untreated	2,000	1	12,000	8,100
	Nyolate	5,000	3	0	0
Brussels sprouts lot B	Untreated	10,000	1	325	70
	Nyolate	10,000	2	10	0
Cauliflower lot A	Untreated	10,000	1	1,875	750
	Nyolate	10,000	1	5	2
	Nyolate	10,000	1	20	0

[a] Solution A/solution B/water.
[b] The identity of *X. c.* pv. *campestris* from all untreated seed samples and from the two treated samples from which *X. c.* pv. *campestris*-like colonies were obtained was confirmed by inoculation of Market Prize cabbage in the greenhouse.
[c] Numbers of *X. c.* pv. *campestris*-like colonies not recorded.

pv. *campestris* colony-forming units (cfu) to 5 cfu/ml of assay medium from 10,000 seeds. Because as few as one infected seed in 10,000 may give rise to significant levels of disease (8), we sought more effective concentrations. A seed soak of 1:1:8 (A/B/water) for 30 min was found to provide the maximum dosage (time × concentration) that did not cause excessive phytotoxicity.

An experiment was then conducted to determine whether residual Nyolate might be active during the assay procedure rather than during the treatment itself. During the soaking procedure, residual Nyolate might be leached from seeds and kill bacteria during the assay procedure rather than during the actual seed treatment. To determine whether or not assays might be affected by this problem, a heavily infected broccoli seed lot was treated with Nyolate at 1:1:8 for 30 min. After drying, 400 seeds of the treated sample, a mixture of 200 treated and 200 untreated seeds, and 400 untreated seeds were assayed separately. About half as many total bacteria or *X. c.* pv. *campestris*-like colonies were found on NSCAA in the mixture of treated and untreated seeds as were found in totally untreated seeds, as would be expected from the fewer numbers of infected seeds. Conversely, no *X. c.* pv. *campestris*-like colonies were found in the treated seeds. Thus, the assay seems little affected by any residual effects of the Nyolate treatment and should provide a reliable indication of the efficacy of these seed treatments.

Subsequently, the manufacturer changed the formulation of Nyolate. Formulation II is twice as concentrated as formulation I, so we expected to be able to simply prepare a 1:1:16 dilution of this second formulation. However, when we did this, tests proved this formulation to be unacceptably phytotoxic with some seed lots. Therefore, we tested other concentrations and found that a 1:1:30 dilution was required before phytotoxicity was completely eliminated. This concentration was, however, incapable of eradicating *X. c.* pv. *campestris* from some seed lots.

The apparent difference in phytotoxicity between formulations I and II is not understood. It may be due to the different wetting agents of formulation II permitting more rapid penetration of the seed coat, or it may simply reflect the fact that because earlier seed samples were exhausted, different seed lots were used for phytotoxicity tests for the two formulations.

Given this, we tried a number of ratios of A/B/water in an effort to find a suitable compromise between phytotoxicity and efficacy for the new formulation. We found that phytotoxicity could be alleviated by reducing the concentration of solution B (lactic acid).

A ratio of 10:3:90 (A/B/water) gave a reasonable compromise between efficacy and phytotoxicity. To test efficacy, we treated a total of 164,000 seeds from nine seed lots in this Nyolate concentration (Table 1). In two samples from two lots, very low levels of *X. c.* pv. *campestris* were detected. Thus, Nyolate provides a high level of control of the black rot bacterium, although occasional bacteria escape the treatment.

We also tested the phytotoxicity of the 10:3:90 mixture and a 2× level (20:6:75) on 21 noninfected seed lots (Table 2). Of the 21 seed lots, most were damaged by the 2× treatment, but only eight lots showed some reduction in germination after treatment with the 10:3:90 mixture. Most of these (seven) were lots of either broccoli or cauliflower. Apparently, seeds of these crops are more easily damaged by this treatment. The remaining 13 seed lots were not damaged by the 10:3:90 mixture.

Treatment with Nyolate provides a method for eradication of *X. c.* pv. *campestris* in some seed lots and great reduction in levels of the pathogen in others. Because as few as one infected seed in 10,000 may give rise to significant levels of disease (8), treatment of infected lots should be followed with an assay for the black rot bacterium to determine that the pathogen has been completely eliminated from the seed lot in question. Similarly, Nyolate is phytotoxic to some seed lots, and seed lots should be tested for sensitivity to this material before being treated. However, Nyolate may provide an alternative to hot-water treatment because hot water frequently damages seeds and sometimes is not effective. Nyolate has been granted an exemption from tolerance by the Environmental Protection Agency because all ingredients are on the generally recognized as safe (GRAS) list, and efforts to register this seed treatment are under way. Gustafson, Inc. will market this material. Nyolate seems to be an attractive possibility for the control of seedborne bacterial plant pathogens, and we will investigate the utility of Nyolate to eradicate bacterial plant pathogens from seeds of other crop species.

Table 2. Effects of 30-min seed treatment with Nyolate on various seed lots at 1× (10:3:90) and 2× (20:6:75) concentrations[y] relative to untreated seeds or seeds soaked for 30 min in water alone

	Greenhouse emergence (from 50 seeds)				Top weight (g) after 3 wk of growth			
Seed lot	Untreated	Water-treated	1×	2×	Untreated	Water-treated	1×	2×
Vates kale	50 a[z]	49 a	48 a	41 b	52 a	50 a	46 a	28 b
Vates collards	48 a	46 a	46 a	45 a	60 a	60 a	54 a	53 a
Champion collards	46 a	46 a	45 ab	42 b	48 a	48 a	46 ab	42 b
Just Right turnip	49 a	48 a	44 b	30 c	100 a	83 ab	76 b	73 b
Waltham broccoli	48 a	43 b	42 bc	40 c	49 a	44 ab	41 b	25 c
Premium Crop broccoli	45 a	37 b	28 c	18 d	44 a	38 b	32 c	10 d
Citation broccoli	44 a	44 a	36 b	23 c	48 a	45 a	36 b	23 b
Green Comet broccoli	46 a	46 a	41 b	33 c	55 a	52 a	49 a	37 b
Savoy King cabbage	43 a	40 ab	36 ab	31 c	46 a	45 a	42 a	38 a
Red Ruby Ball cabbage	36 a	33 ab	32 ab	26 b	32 a	31 a	31 a	23 b
Excel cabbage	45 a	44 a	40 a	33 b	45 a	41 ab	39 ab	26 c
Market Prize cabbage	48 a	47 a	47 a	45 a	62 a	60 a	57 a	55 a
Rio Verde cabbage	47 a	47 a	47 a	44 a	35 a	41 a	38 a	35 a
Snowball cauliflower	44 a	40 ab	39 b	37 b	47 a	45 a	41 a	39 b
Andes cauliflower	46 a	45 a	37 b	35 b	36 a	36 a	30 a	22 b
Snow Crown cauliflower	42 a	42 a	32 b	30 b	44 a	44 a	33 b	31 b
Michili chinese cabbage	49 a	46 a	46 a	46 a	54 a	59 a	50 a	59 a
Jade Pagoda chinese cabbage	37 a	38 a	32 ab	30 b	49 b	57 a	48 bc	42 c
Early Hybrid chinese cabbage	49 a	49 a	48 a	47 a	88 a	38 a	83 a	73 a
Early White Vienna kohlrabi	48 a	48 a	47 a	47 a	46 a	45 a	44 a	43 a
American Purple Top rutabaga	49 a	49 a	48 a	47 a	72 a	69 a	78 a	79 a

[y] Solution A/solution B/water.

[z] Dissimilar letters within rows (not columns), and for emergence or top weight, indicate significant differences among treatments according to Waller and Duncan's test ($K = 100$).

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