Chemical and Biological Control of Onion White Rot in Muck and Mineral Soils

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ABSTRACT

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Four isolates of *Bacillus subtilis* applied as seed treatments singly or in combination with broadcast chemicals significantly reduced white rot of onions in unsterile muck soils in a controlled-environment chamber. Field trials conducted on muck soil that contained high levels of natural inoculum of *Sclerotium cepivorum* in the 1978 and 1979 growing seasons had established that vinclozolin and iprodione provided significant control of onion white rot when applied as preplant broadcast treatments. Trials in the same seasons had shown that seed inoculation with the BACT-2 strain of *B. subtilis* also provided significant control of white rot. White rot of onion was completely controlled by vinclozolin and iprodione, and bacterial seed treatment significantly reduced infection on the pickling onion cultivar Silverqueen in mineral soil. Although vinclozolin continued to provide effective control of white rot, iprodione and BACT-2 were generally ineffective in 1980 in muck soil. In a growth-chamber pot trial, bacterial colony-forming units were significantly higher in the onion root zone in BACT-2 seed-treated soils. A significant ($P = 0.05$) correlation ($r = 0.64$) occurred between viable sclerotia present in the soil after harvest and the percent infection.

White rot of onion (*Allium cepa* L.) and other *Allium* species is caused by the soilborne fungus *Sclerotium cepivorum* Berk (12). No field treatment has been devised that will eradicate the fungus from soil. Chemical (9) and biological control of *S. cepivorum* with antagonist *Penicillium nigricans* (5,6) and *Coniothyrium minitans* (2) under laboratory and greenhouse conditions and with *Bacillus subtilis* (Ehrenb.) Cohn isolates under laboratory, greenhouse, and field conditions have been reported (11).

This paper describes the evaluation of bacterial antagonists applied to onion seeds singly and in combination with broadcast chemical treatments under greenhouse and field conditions for control of onion white rot on muck and mineral soils of British Columbia.

MATERIALS AND METHODS

All field trials, except the trial in mineral soil, were carried out in Burnaby, BC, in muck soil in which white rot had occurred previously and where white rot trials with or without added inoculum had been evaluated for the last five seasons. The inoculum level in the field-trial area was 0.5–1.5 sclerotia per gram of air-dry soil. Soil pH in the trial field was 5.1 (determined on a thick suspension of soil in 0.01 M $CaCl_2$). Temperature and rainfall during the 3 yr of trials were recorded in the centers of the fields.

1979 Pot trial in muck soil. Four isolates of *B. subtilis* as seed treatments and two chemicals as broadcast treatments were evaluated separately and in combination in a randomized complete block design. There were three replicates using the cultivar Autumn Spice. Bacterial cultures were grown in 250-ml flasks containing 100 ml of potato-dextrose broth (PDB) incubated in a continuously illuminated reciprocating shaker (75 strokes per minute) at 25 C for 6 days. The cultures were frozen and kept at −4 C for 10 days. Seeds of Autumn Spice were immersed in freshly thawed bacterial broth culture for 5 min and sown in muck soil. The chemicals were incorporated into the soil before seeding. Seeds of Autumn Spice were sown (18 per pot) in muck soil after inoculation with bacterial isolates. The soil was infested with *S. cepivorum* (800 sclerotia per 400 cm^3 of soil per pot, three pots per treatment). The sclerotia were distributed uniformly throughout the soil. The seeded pots were held in a growth chamber at 23 C and supplied with a 12-hr photoperiod (15 klux at plant height). Infected plants were counted 20 wk after sowing.

Disease incidence was assessed as the percentage of bulbs infected by *S. cepivorum*. Isolates BACT-1, BACT-2, BACT-4, and BACT-8 were from sclerotia of *S. cepivorum* recovered from naturally infested muck soils of the Fraser Valley of British Columbia. The dilution plate technique (7) was used for isolating bacterial colonies from healthy onion roots. Three randomly selected bulbs per treatment were removed from the soil and a 1-cm-long root from each bulb was cut. Weights of these roots were recorded. Root pieces were shaken well with 1 L of distilled water. From this, 0.06 ml of water was taken and mixed with 14.94 ml of molten potato-dextrose agar (PDA) before pouring into petri plates. Bacterial colony-forming units were counted after 48 hr of incubation at 23 C. All quantitative estimates of bacterial populations were replicated three times.

Sclerotia from soils of each treatment were recovered after harvest by a wet-sieving flotation technique and plated on PDA to determine their viability (10).

1978 Chemical and biological field trial on muck soil. Isolates of *B. subtilis* as seed treatments and two chemicals as broadcast treatments were tested on two cultivars, Autumn Spice and Festival, in a replicated trial as described earlier (11). Bacterial cultures were prepared and applied as seed treatments as described earlier. Chemical broadcast treatments were incorporated to a depth of approximately 5 cm and were applied immediately before seeding. The two chemicals used in this trial were vinclozolin (BASF [Canada] Ltd.) and iprodione (May and Baker, Canada). Plant and plot spacing, fertilization, and weed and insect controls were as described previously (8).

1979 Chemical and biological field trial on muck soil. Three chemicals, vinclozolin, iprodione, and benomyl (as broadcast treatments), and bacteria (as seed treatments) were evaluated singly and in combination in a 4 × 4 simple lattice design using Autumn Spice and Ailsa Craig. Chemical broadcast treatments were incorporated to a depth of about 5 cm immediately before seeding. Bacterial cultures were prepared and applied as seed treatments as described. Plant and plot spacing, fertilization, and weed and insect controls were also as described earlier (8). The trial was seeded on 24 April and harvested on 13 September 1979.

1980 Chemical and biological field trial on muck soil. Two chemicals as broadcast and the bacterial seed treatment were evaluated singly and in combination, using a randomized block design with three replicates. The cultivar planted was Autumn Spice. The bacterial seed treatment and chemical broadcast treatments were applied as described for the 1979 chemical and biological field trial on muck soil. Bacterial cultures were prepared and applied as the seed treatment as described. Plant and plot

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spacing, method of planting, fertilization, and weed and insect controls were also as described earlier (8). The trial was seeded on 5 May and harvested on 11 September 1980.

1979 Chemical and biological field trial on mineral soil. This trial was done at a commercial vegetable farm in Grand Forks, BC, in mineral soil that was moderately infested with *S. cepivorum.* Three chemicals (vinclozolin, iprodione, thiophanate-methyl) and a bacterial seed treatment were evaluated in a split-plot design with three replicates on Silverqueen. Chemical broadcast treatments were incorporated by disking to a depth of about 15 cm 4 days before seeding. Individual treatment plots consisted of 12 rows, alternately 25 and 71 cm apart and 19 m long. A mixture of BACT-2 + BACT-4 was tried because the mixed isolates produced a larger zone of inhibition in vitro against *S. cepivorum* on PDA than did either isolate alone (*unpublished*). Bacterial seed treatments were applied immediately before seeding. The trial was seeded at the rate of 45 kg/ha on 24 April with a tractor-mounted Planet Jr. Seeder and harvested on 13 July 1979. Data were collected from 1-ft random segments (about 500 plants) of the middle eight rows of each plot. Percent infection at harvest was calculated from the difference between number of total harvested and apparently healthy bulbs.

All results were analyzed for statistical significance (3). Arc sine transformation was followed where necessary before analyzing the data. Duncan's new multiple range test at the 5% level of significance was used to compare treatments.

Table 1. Results of a growth-chamber pot trial on chemical and biological treatments for control of onion white rot on the cultivar Autumn Spice 1979

Treatment		Chemical rate				
Chemical	Biological	(a.i.)	Mode	Infection (%)[v]	BACT colonies[w]	Viable sclerotia[x]
Vinclozolin	BACT-2	0.02 g/pot	Broadcast + seed[y]	0.0 a[z]	249.8 a[z]	0.27 cd[z]
Vinclozolin	BACT-4	0.02 g/pot	Broadcast + seed	0.0 a	28.2 b	0.13 abc
Vinclozolin	BACT-8	0.02 g/pot	Broadcast + seed	0.0 a	33.1 b	0.05 a
Vinclozolin	BACT-1	0.02 g/pot	Broadcast + seed	1.8 ab	79.5 b	0.25 bcd
Iprodione	BACT-2	0.02 g/pot	Broadcast + seed	18.0 e	85.0 b	0.10 ab
Iprodione	BACT-4	0.02 g/pot	Broadcast + seed	17.2 e	47.3 b	0.08 a
Iprodione	BACT-8	0.02 g/pot	Broadcast + seed	9.9 c	54.1 b	0.13 abc
Iprodione	BACT-1	0.02 g/pot	Broadcast + seed	22.2 e	54.5 b	0.15 abc
···	BACT-2	···	Seed	7.9 bc	170.6 ab	0.23 bcd
···	BACT-4	···	Seed	5.1 abc	39.2 b	0.33 d
···	BACT-8	···	Seed	10.8 cd	36.6 b	0.15 abc
···	BACT-1	···	Seed	16.7 de	36.0 b	0.28 cd
Vinclozolin	···	0.02 g/pot	Broadcast	1.7 ab	48.8 b	0.20 abcd
Iprodione	···	0.02 g/pot	Broadcast	5.7 abc	30.6 b	0.25 bcd
Control				44.2 f	36.3 b	0.85 e
SE				2.2	47.6	0.05

[v] Measured 20 wk after planting; infection was assessed as the percentage of bulbs affected by white rot.
[w] Adjusted number of bacterial colonies per gram of onion root counted after 48-hr incubation.
[x] Viable sclerotia of *S. cepivorum* per gram of soil immediately after harvesting.
[y] Chemical and biological treatments were applied as broadcast and seed treatments, respectively.
[z] Values within a column followed by the same letter do not differ significantly ($P=0.05$) according to Duncan's new multiple range test.

Table 2. Results of a field trial on chemical and biological treatments for control of onion white rot on bulb onions in muck soil in 1978–1980

Treatment		Chemical rate		Infection (%)[x]		
Chemical	Biological	(kg/ha)	Mode	1978	1979	1980
			Autumn Spice			
Vinclozolin	···	20 kg/ha	Broadcast	12.4 ab[y]	8.5 abc[y]	29.6 a[y]
Vinclozolin	BACT-2	20 kg/ha	Broadcast + seed[z]	···	6.2 ab	32.9 ab
Iprodione	···	20 kg/ha	Broadcast	20.7 abc	15.9 bcd	56.8 bc
Iprodione	BACT-2	20 kg/ha	Broadcast + seed	···	13.9 bcd	49.3 b
Benomyl	···	31 kg/ha	Broadcast	···	52.7 i	···
Benomyl	BACT-2	31 kg/ha	Broadcast + seed	···	48.4 hi	···
	BACT-2	···	Seed	37.0 c	31.6 fg	73.7 c
Control				74.6 e	54.1 i	78.7 c
			Festival			
Vinclozolin	···	20 kg/ha	Broadcast	7.7 a	···	···
Iprodione	···	20 kg/ha	Broadcast	29.3 abc	···	···
	BACT-2	···	Seed	11.9 ab	···	···
Control				58.7 d	···	···
			Ailsa Craig			
Vinclozolin	···	20 kg/ha	Broadcast	···	16.9 cde	···
Vinclozolin	BACT-2	20 kg/ha	Broadcast + seed	···	2.0 a	···
Iprodione	···	20 kg/ha	Broadcast	···	23.5 def	···
Iprodione	BACT-2	20 kg/ha	Broadcast + seed	···	23.5 def	···
Benomyl	···	31 kg/ha	Broadcast	···	40.6 gh	···
Benomyl	BACT-2	31 kg/ha	Broadcast + seed	···	44.2 hi	···
	BACT-2	···	Seed	···	26.1 ef	···
Control				···	54.5 i	
SE				6.6	3.2	7.4

[x] Infection was assessed as the percentage of bulbs affected by white rot.
[y] Values within a column followed by the same letter do not differ significantly according to Duncan's new multiple range test ($P = 0.05$).
[z] Chemical and biological treatments were applied as broadcast and seed treatments, respectively.

RESULTS

White rot infection on Autumn Spice growing in unsterile muck soil in pots was significantly less than that of the untreated control for all four bacterial isolates used as seed treatment applied singly or in combination with chemical broadcast treatments (Table 1). Bacterial populations were significantly higher in the BACT-2 seed treatment root zone. All bacterial isolates were identified as *B. subtilis.* Populations of viable sclerotia of *S. cepivorum* in the soil were significantly low in all treatments compared with the check soil.

Biological seed treatment with BACT-2 and vinclozolin and iprodione broadcast treatments used singly provided significant season-long protection on both cultivars in 1978 (Table 2). On Festival, the bacterial seed treatment BACT-2 gave protection comparable with that provided by the vinclozolin and iprodione broadcast treatments.

Vinclozolin, iprodione, and BACT-2 also provided continued control on two cultivars in 1979 (Table 2). Where chemicals and BACT-2 were tested in combination, only benomyl appeared to interfere with the protective activity of BACT-2. BACT-2 plus vinclozolin and iprodione provided slightly higher levels of control than did the corresponding vinclozolin and iprodione tested alone, but the control was significant only in the case of vinclozolin plus BACT-2 on Ailsa Craig.

Broadcast treatments of vinclozolin, but not iprodione, controlled onion white rot in 1980 (Table 2). BACT-2, an effective biological control agent in the 1978 and 1979 trials, did not provide significant season-long protection in 1980.

White rot of onion was completely controlled by vinclozolin and iprodione in mineral soil at Grand Forks on Silverqueen (Table 3). Thiophanate-

Table 3. Results of a field trial on chemical and biological treatments using commercial equipment for control of onion white rot on pickling onions in 1979

Treatment		Chemical rate		Infection
Chemical	Biological	(kg/ha)	Mode	(%)[x]
Vinclozolin	···	20 kg/ha	Broadcast	0.00 a[y]
Vinclozolin	BACT-2 + BACT-4	20 kg/ha	Broadcast + seed[z]	0.00 a
Iprodione	···	20 kg/ha	Broadcast	0.00 a
Iprodione	BACT-2 + BACT-4	20 kg/ha	Broadcast + seed	0.33 a
Thiophanate-methyl	···	16 kg/ha	Broadcast	26.42 b
Thiophanate-methyl	BACT-2 + BACT-4	16 kg/ha	Broadcast + seed	9.00 a
	BACT-2 + BACT-4	···	Seed	10.75 a
Control				35.83 b
SE				4.10

[x] Percent infection at harvest was calculated from the difference between number of total harvested and apparently healthy bulbs.
[y] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[z] Chemical and biological treatments were applied as broadcast and seed treatments, respectively.

methyl was not significantly effective for control of white rot. None of the chemicals affected the number of emerged plants. Bacterial seed treatment (BACT-2 plus BACT-4 mixed) significantly reduced infection in mineral soil on Silverqueen. The protective effect of bacterial seed treatment was apparent when tested alone or in combination with thiophanate-methyl.

DISCUSSION

The complete control afforded by vinclozolin and iprodione obscured the possible effect of bacterial seed treatment when tested in combination with these chemicals on mineral soil.

Trials in the 1978 and 1979 seasons had established that vinclozolin and iprodione as broadcast treatments and BACT-2 strain of *B. subtilis* as seed treatment provided significant control of white rot in muck and mineral soils of British Columbia. Trials in 1980, however, provided considerable new information about chemical and biological control of white rot. While vinclozolin continued to provide effective control of white rot, iprodione and BACT-2 were generally ineffective in 1980.

Almost continuous cool and wet weather prevailed for the 1980 growing season. Whether this condition was a factor in the failure of iprodione and BACT-2 in 1980 is unknown. Iprodione is more water-soluble than vinclozolin, and it is possible that differential leaching could have contributed to the failure of the former chemical in 1980. Another possible explanation is that the cool, wet weather was more favorable for infection of onions. It is also possible that *S. cepivorum* may have developed resistance to iprodione. Such possibilities are being checked in the laboratory.

Significant ($P = 0.05$) correlation ($r = 0.64$) was observed between viable sclerotia present in soil after harvest and percent infection in the greenhouse trial. Increased population of sclerotia resulting in increased incidence of white rot disease in onion and garlic was also observed by Crowe et al (4) and Adams (1).

The significantly high number of bacterial colony-forming units of BACT-2 in soils from BACT-2 seed treatments and significant control of onion white rot by this isolate for 2 yr in muck and mineral soils indicate the role of survival and multiplication of bacteria as onion rhizosphere colonists in controlling the white rot. Whatever the mechanism(s) of control of onion white rot may be, the level of protection provided by these bacterial isolates and chemicals indicates a potential practical field control of onion white rot in mineral and muck soils of British Columbia.

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