Interactions of Antagonist and Pathogen in Biological Control of Onion White Rot

R. S. Utkhede and J. E. Rahe

Research scientist, Agriculture Canada Research Station, Summerland, British Columbia, Canada V0H 1Z0, and associate professor, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

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ABSTRACT

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This study was undertaken to identify stable antagonists to isolates of *Sclerotium cepivorum* for biological control of onion white rot. Antagonism was measured by inhibition zone size in vitro and percent white rot infection in plants of cultivar Autumn Spice in pot trials. Regression analysis and deviation from regression were used to determine the stability of the antagonists for biological control of onion white rot. Significant differences in antagonism were detected among the antagonists. The order of antagonists, ranked according to antagonism and ability to control onion white rot, depended on the isolate of *S. cepivorum* being tested. However, the statistical model quantitatively identified useful characteristics in the B2 isolate of *Bacillus subtilis*. This antagonist showed a high degree of stability for inhibition zone in vitro and percent infection to white rot in pot trial for the 13 *S. cepivorum* isolates that were tested. This indicates that the bacterial antagonist B2 has a potential for biological control of onion white rot over a range of isolates of *S. cepivorum* from five countries. It is also suggested that relationships of values obtained by the statistical analysis will be useful in selecting stable biological agents for control of plant diseases.

Sclerotium cepivorum Berk., the causal agent of white rot, is pathogenic to *Allium* spp. under field conditions and is a longstanding pathogen with widespread distribution in many parts of the world (8). No field treatment has yet been devised that will eradicate the fungus from soil. Various chemical treatments provide partial control (3,5). Four isolates of *Bacillus subtilis* provided significant season-long protection for the partially resistant cultivar Festival, and the best of these also provided significant protection for the susceptible cultivar Autumn Spice in muck soil of the Fraser Valley of British Columbia (6).

Sclerotia of seven of the 12 exotic isolates of *S. cepivorum* resulted in isolation of bacterial isolates, tentatively identified as *B. subtilis* on the basis of colony morphology on potato-dextrose agar. These bacterial isolates were antagonistic to *S. cepivorum* when tested in dual culture plate tests.

This paper describes a comparison of the antagonistic activity of seven exotic and four domestic antagonistic isolates of *B. subtilis* as measured in dual culture tests and also of the effectiveness of the 11 antagonist isolates for control of white rot on the cultivar Autumn Spice growing in soil in pots.

MATERIALS AND METHODS

Four isolates (S201A, S201B, S197A, and S197B) were obtained from I. D. Geard, Department of Agriculture, New Town Research Laboratories, New Town 7008, Tasmania, Australia; three isolates (J191, J192, and J202) were obtained from J. R. Coley-Smith, University of Hull, U.K.; one isolate (VDM) was obtained from P. van der Meer, Institute of Horticultural Research, Wageningen, The Netherlands; two isolates (NZ32 and NZ37) were obtained from B. Hawthorne, Lincoln Research Center, DSIR, Private Bag, Christchurch, NZ; isolate BBY was isolated from naturally infested muck soil of the Fraser Valley of British Columbia, Canada. All the five countries from which we received *S. cepivorum* isolates have a serious white rot problem in field-grown onions.

During the plating of sclerotia of *S. cepivorum* on PDA (potato-dextrose agar) petri plates, a number of contaminating bacteria were isolated and cultured on PDA. Thus, bacterial antagonist HB2 was isolated from sclerotia of *S. cepivorum* obtained from P. van der Meer; NZB1 was from sclerotia obtained from B. Hawthorne; AB1, AB6, and AB9 were from sclerotia obtained from I. D. Geard; EBW2 and EBW6 were from sclerotia obtained from J. R. Coley-Smith; B1, B2, B4, and B8 were from sclerotia recovered from naturally infested muck soils of the Fraser Valley of British Columbia, Canada. The bacterial isolates were then individually tested for antagonism of a local isolate (BBY) of *S. cepivorum* by point transfer of bacteria to one side of a 100 × 15-mm petri plate containing PDA; 5-mm-diameter disk cut from an actively growing culture of the BBY on PDA was placed 55 mm from the point of inoculation with the bacterial isolate. All pathogen-bacterial dual culture tests were repeated three times, and the plates were stored at 22–25 C. Bacterial isolates showing antagonism were transferred to PDA petri plates and after 10 transfers were tested again for antagonism against the local isolate of BBY.

Eleven bacterial isolates that showed consistent antagonism against the growth of the BBY isolate were tested further for their antagonism to 13 isolates of *S. cepivorum* obtained from five countries. The tests for antagonism were made on 100 × 15-mm petri plates containing 10 ml aliquots of PDA. A 5-mm-diameter disk cut from an actively growing culture of *S. cepivorum* isolate on PDA was placed in the center and four bacterial antagonists were placed near the periphery equidistant from each other and from the center. All pathogen-bacterial pairings were repeated three times, and the plates were stored at 22–25 C. The width (in centimeters) of the inhibition zone was measured from the edge of the *S. cepivorum* colony to the edge of the bacterial colony after 2 wk of incubation.

The same 11 bacterial isolates that showed antagonism against the growth of 13 *S. cepivorum* isolates on PDA were further tested in a greenhouse pot trial for their ability to control white rot infection on plants of susceptible cultivar Autumn Spice onions growing in nonsterile muck soil in plastic pots. Cultures of the bacterial antagonist were grown in 250-ml flasks containing 100 ml of potato-dextrose broth incubated in a continuously illuminated reciprocating shaker (75 rev/min) at 25 C for 6 days. Seeds of the cultivar Autumn Spice were immersed in broth culture for 5 min and then sown (30 seeds per pot) in muck soil (pH = 5.2) infested with *S. cepivorum* isolates (1,000 sclerotia in 500 cc of soil per pot). The seeded pots were kept in a greenhouse at 20 ± 2 C. The trial was

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seeded on 21 March and harvested on 25 August 1980. Percent infection was calculated from the differences between numbers of emerged plants and harvested plants apparently free of white rot as a percentage of the number of emerged plants. Microscopic examination of dead plants when plated on PDA indicated that all plants that died before harvest were infected with *S. cepivorum*. The pot trial experiment was replicated three times. Analysis of variance, Duncan's multiple range test, and correlation between inhibition zone width and percent infection were conducted as described by Cochran and Cox (1).

The statistical analysis for stability of antagonism was carried out according to the method given by Eberhart and Russell (2). This technique recently was used to test stability of cultivar resistance to onion white rot (7) and for durability of general resistance evaluating cultivar × isolate interactions (4). We used this technique to measure the stability of antagonist *B. subtilis* to isolates of *S. cepivorum* and also on percent infection by isolates of *S. cepivorum* in the cultivar Autumn Spice. Our approach was similar to that used by Eberhart and Russell (2) to measure stability parameters of maize genotypes exposed to various environments. We considered each bacterial isolate to be a cultivar and each isolate of *S. cepivorum* to be an environment and measured the interaction between them. The stability parameters (the mean, the regression coefficient of each bacterial isolate on the isolate index of *S. cepivorum*, and the squared deviations from this regression) measure the consistency of performance of bacterial isolates against various isolates of *S. cepivorum*. These parameters are defined as follows: $Y_{ij} = \mu_i + b_i I_j + X_{ij}$, in which Y_{ij} is the antagonistic zone mean of the ith bacterial isolate at jth isolate of *S. cepivorum*, μ_i is the mean of the ith bacterial isolate over all isolates of *S. cepivorum*, b_i is the regression coefficient that measures the response of the ith bacterial isolate to various isolates of *S. cepivorum*, X_{ij} is the deviation from regression of the ith bacterial isolate at the jth isolate of *S. cepivorum*, and I_j is the isolate index of *S. cepivorum* obtained as the means of all bacterial isolates at the jth isolate of *S. cepivorum* minus the grand mean. Environments considered here are various isolates of *S. cepivorum* that were obtained from five countries.

RESULTS

The antagonistic isolates of *B. subtilis* and isolates of the onion pathogen, *S. cepivorum*, differed significantly ($P = 0.01$) on the basis of their mean antagonistic zone and white rot infection percentages. Mean inhibition zone between bacterial antagonists and isolates of *S. cepivorum*, and the stability parameters for antagonistic zone are presented in Table 1. Bacterial isolate B2 produced significantly larger inhibition zones with eight isolates of *S. cepivorum* than with the remaining three isolates tested on PDA plates. Generally, most of the bacterial isolates showed larger zones of inhibition with isolate J202 of *S. cepivorum* from the U.K. (ie, J202 was most sensitive to all antagonists). Table 2 shows the mean percentage infection with *S. cepivorum* isolates and the stability parameters for percent infection with 11 bacterial antagonists. Differences among isolates of *B. subtilis* were observed when they were ranked for inhibition zone and percent white rot infection (Tables 1 and 2). This indicated the influence of isolates of *S. cepivorum* on the order of *B. subtilis* for inhibition zone and percent white rot infection. The infection with all isolates of *S. cepivorum* was lowest when bacterial isolate B2 was present in soil.

Correlation between width of inhibition zone in vitro and percent infection in the cultivar Autumn Spice in the pot trials of 11 bacterial isolates was significant at $P = 0.01$ ($r = 0.96$). This indicates a nearly perfect correlation between width of inhibition zone on PDA plates by antagonists and protection from infection by isolates of *S. cepivorum* in the pot trial. Figures 1 and 2 give a tabular summary that is useful in selecting the stable antagonist.

DISCUSSION

Antagonist × pathogen interactions are of importance to plant pathologists seeking to identify a stable antagonist for biological control of plant diseases. Comparison of antagonists against a series of plant pathogenic isolates reveals differences in the relative rankings. This would cause a difficulty in demonstrating the significant superiority of any one antagonist over the others. If stability of antagonism (ie, the ability to show a minimum of interaction with changes in pathogen isolates) is a genetic characteristic, the identification of stable antagonists is of obvious value to plant pathologists.

Regression coefficients measure the sensitivity of bacterial antagonists to differences in the isolates of *S. cepivorum*. Squared deviation from regression provided a measure of the predictability of the relationship between variation in pathogenic isolates of *S. cepivorum* and the bacterial antagonist.

According to the model of Eberhart and Russell (2), the stable antagonist would show a large inhibition zone, a regression coefficient near one, and nonsignificant deviation from regression

TABLE 1. Width of inhibition zone (cm) between isolates of *Sclerotium cepivorum* and isolates of *Bacillus subtilis*

Isolates of *S. cepivorum*	Isolates of *B. subtilis*											
	HB-2	NZB1	AB-1	EBW6	EBW2	AB-6	B-8	B-4	AB-9	B-2	B-1	Mean
S201A	0.25	0.42	0.52	0.37	0.32	0.32	0.42	0.50	0.75	0.65	0.72	0.47 a[x]
S197B	0.15	0.38	0.35	0.45	0.33	0.35	0.48	0.65	0.47	0.83	0.80	0.48 a
S197A	0.05	0.32	0.38	0.37	0.32	0.37	0.55	0.85	0.42	0.90	1.00	0.50 ab
S187B	0.17	0.25	0.42	0.50	0.37	0.35	0.75	0.42	0.70	0.90	0.82	0.51 ab
J192	0.35	0.30	0.37	0.35	0.47	0.42	0.50	0.72	0.85	0.98	0.53	0.53 b
S187A	0.37	0.42	0.37	0.42	0.45	0.47	0.50	0.47	0.85	0.98	0.77	0.55 b
VDM	0.30	0.42	0.37	0.35	0.45	0.50	0.50	0.83	1.07	0.95	1.03	0.62 c
J191	0.42	0.55	0.45	0.57	0.55	0.65	0.67	0.50	0.98	0.85	0.62	0.62 c
BBY	0.37	0.50	0.30	0.45	0.38	0.45	0.65	1.33	0.57	0.70	1.10	0.62 c
NZ32	0.28	0.47	0.40	0.32	0.45	0.45	0.60	0.55	1.13	1.05	1.12	0.62 c
NZ37	0.15	0.52	0.50	0.47	0.42	0.50	0.65	0.62	1.03	0.95	1.05	0.62 c
S201B	0.20	0.42	0.55	0.35	0.40	0.52	0.62	0.57	1.05	1.12	1.37	0.65 c
J202	1.42	0.70	0.83	1.10	1.22	1.25	1.07	1.15	0.83	1.60	1.73	1.17 d
Mean	0.34 a[y]	0.44 b	0.45 b	0.46 b	0.48 bc	0.51 c	0.61 d	0.70 e	0.82 f	0.96 g	0.97 g	
b	1.78 *[z]	0.53 *	0.63 *	1.04 *	1.21 *	1.31 *	0.80 *	0.79 *	0.33	1.11 *	0.45 *	
S^2d	1.18 *[z]	0.07	0.08	0.08	0.06	0.03	0.08	2.02 *	0.61 *	0.16	0.45 *	

[x]Within this column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[y]Within this row, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[z]In the last two rows, the asterisks indicate statistical significance, $P = 0.05$.

TABLE 2. Percent white rot in relation to isolate of *Sclerotium cepivorum* and isolates of *Bacillus subtilis* applied as seed treatment

Isolates of *S. ceptivorum*	Isolates of *Bacillus subtilis*												
	B-2	B-1	AB-9	B-4	B-8	EBW6	EBW2	AB-6	AB-1	NZB1	HB-2	Control	Mean
J202	16.6[x]	16.8	16.5	18.1	9.9	10.9	13.0	12.1	16.5	30.4	15.0	25.6	16.8 a[x]
NZ37	6.2	12.8	10.5	38.9	34.8	51.0	45.4	55.7	49.9	51.2	85.5	90.1	44.3 b
J191	11.6	37.0	29.3	49.9	30.1	36.8	41.8	36.5	55.3	45.8	62.4	95.8	44.4 b
VDM	8.3	10.1	12.2	19.0	49.9	58.5	51.6	51.6	68.0	57.8	67.9	83.3	44.9 b
BBY	12.4	11.9	33.2	14.5	34.5	49.3	57.0	55.4	77.5	49.9	62.5	91.8	45.8 b
NZ32	10.1	11.9	10.9	43.9	40.7	62.6	51.6	57.1	60.4	54.2	72.1	83.4	46.6 bc
S201B	11.5	17.3	12.6	44.9	37.3	57.4	56.7	46.0	43.8	61.4	80.7	96.2	47.1 bcd
S187A	10.0	24.1	13.7	40.6	49.0	50.4	51.1	50.6	63.6	59.4	66.5	91.3	47.5 bcd
S187B	10.1	18.7	29.6	39.3	25.2	45.8	60.2	65.1	57.2	76.0	79.2	93.7	50.0 cde
J192	12.2	45.6	14.3	28.3	47.8	58.4	51.1	54.5	62.9	69.3	65.0	95.0	50.6 def
S201A	34.4	25.1	25.7	41.1	58.8	55.9	63.8	64.9	46.2	60.2	74.3	93.4	53.7 ef
S197B	9.9	16.5	53.2	33.8	51.9	58.9	52.7	64.1	66.5	63.2	84.7	89.9	53.8 ef
S197A	12.4	11.0	57.8	37.2	43.3	56.0	63.1	60.7	60.4	68.2	91.6	91.7	54.4 f
Mean	12.8 a[y]	19.9 b	24.6 c	34.6 d	39.5 e	49.7 f	51.5 g	51.7 g	56.0 h	57.5 h	69.8 h	86.3 i	
b	0.03 [z]	0.11	0.65 *	0.49 *	1.01 *	1.39 *	1.32 *	1.40 *	1.09 *	0.99 *	1.74 *	1.76 *	
S^2d	68.11[z]	126.86*[z]	235.61*	117.10*	74.33	53.86	26.51	30.10	96.46*	45.57	77.57*	56.23	

[x]Within this column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[y]Within this row, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[z]In the last two rows, the asterisks indicate statistical significance, $P = 0.05$.

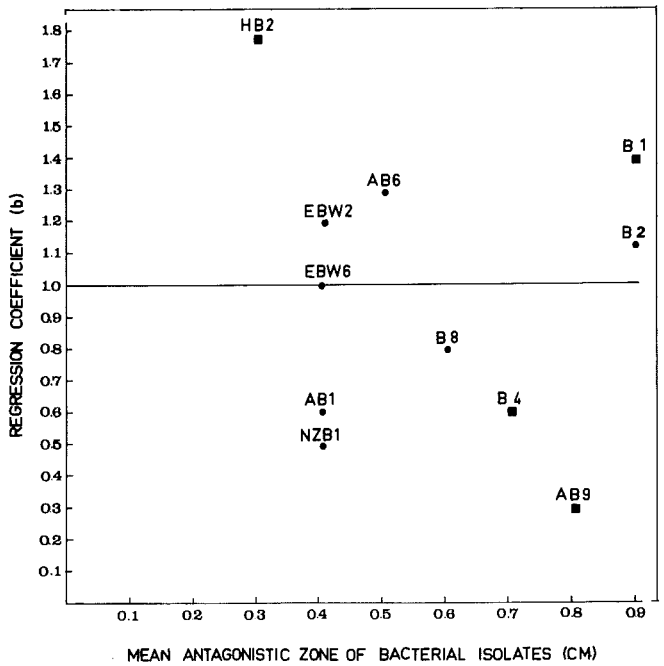

Fig. 1. Stability of the in vitro antagonism of isolates of *Bacillus subtilis* to *Sclerotium cepivorum* isolates. Estimates of S^2d are significant ($P = 0.05$) only for bacterial isolates indicated by ■.

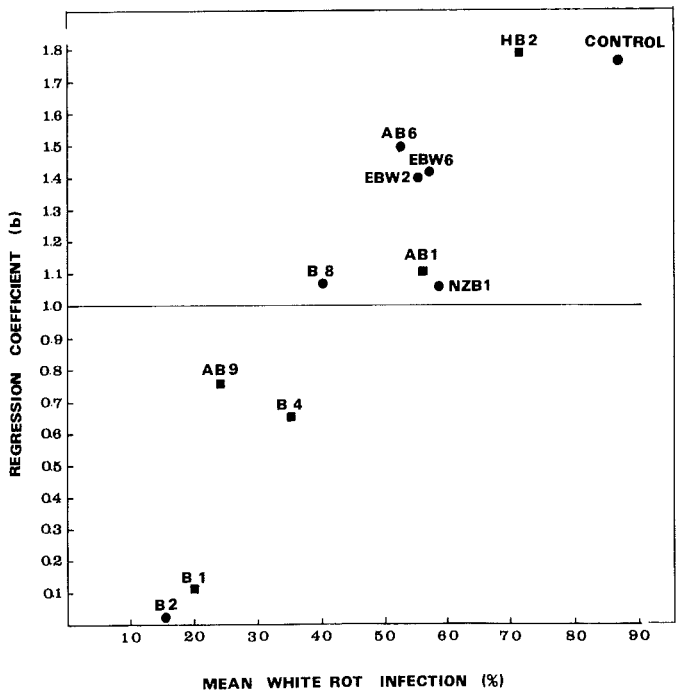

Fig. 2. Stability of the in vivo antagonism of 11 selected isolates of *Bacillus subtilis* to *Sclerotium cepivorum* infection in plants of onion cultivar Autumn Spice in a pot trial. Estimates of S^2d are significant ($P = 0.05$) only for bacterial isolates indicated by ■.

for antagonistic zone values. The stable antagonist able to provide protection from infection by *S. cepivorum* would allow a low percentage of host plant infection by isolates of *S. cepivorum*, and would have a regression coefficient near zero and a nonsignificant deviation from regression. On both of these bases, bacterial antagonist B2 is the most stable antagonist that has shown a high degree of in vitro stability when tested with different isolates of *S. cepivorum* and in soil under greenhouse conditions (Tables 1 and 2). B1, B4, AB9, and HB2 showed significant deviations from regressions for antagonistic zone and also for percent white rot infection, which are undesirable characteristics of these antagonists.

This analysis is useful in testing the stability of an antagonist because it provides a better measurement of the antagonist over a wide range of isolates of *S. cepivorum*, and thus broadens our understanding of using the stable antagonists for biological control of white rot over a wide range of isolates of *S. cepivorum* from five countries.

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