Differences in Aggressiveness of *Erwinia carotovora* subsp. *betavasculorum* Strains and Their Reactions to Sugar Beet Cultivars

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ABSTRACT

Whitney, E. D., and Mackey, B. E. 1989. Differences in aggressiveness of *Erwinia carotovora* subsp. *betavasculorum* strains and their reactions to sugar beet cultivars. Plant Disease 73:220-222.

In tests conducted in the greenhouse, strains of *Erwinia carotovora* subsp. *betavasculorum* caused significantly different amounts of disease in sugar beet. The apparent aggressiveness of the strains varied because of a cultivar resistance × strain interaction. A large difference in cultivar resistance to individual strains was also found. In tests conducted in the field, very aggressive strains infected a higher percentage of beets than less aggressive strains (19 vs. 3.7%), increased the percentage of rot per diseased beet from 8.7 to 44.3%, and raised the disease index from 0.2 to 3.5%. Because of cultivar × strain interactions, we suggest that several of the most aggressive strains of the bacterium be used when selecting for resistance, to allow reliable identification of the most resistant plants.

Additional keyword: selection

After the identification of *Erwinia carotovora* (Jones) Bergey et al subsp. *betavasculorum* Thomson et al as a pathogen of sugar beet (*Beta vulgaris* L.) (3,4), techniques were developed for field and greenhouse identification of resistant plants (6–8). Subsequently, both a major gene and minor genes for resistance were suggested on the basis of inheritance studies in field-grown sugar beet (2). One of the priorities in the selection of resistant cultivars is that the most aggressive strains available be used because they identify a larger percentage of susceptible beets. Therefore, we studied the aggressiveness of different strains of *E. c.* subsp. *betavasculorum* and the possible interaction of aggressiveness with cultivar resistance. A preliminary report of this study has been published (5).

Accepted for publication 17 October 1988 (submitted for electronic processing).

MATERIALS AND METHODS

Strains of *E. c.* subsp. *betavasculorum* isolated from several beet-growing areas in the United States (Table 1) were stored in sterile tap water at room temperature and increased on King's medium B (1). An L-shaped rod was used to suspend 24-hr cultures in sterile tap water. Suspensions of each strain were diluted to 10^7 cfu/ml based on a colorimetic analysis of the concentrated stock suspension (6,8). Cultivars used in each test are listed in Table 2.

Greenhouse tests. In tests conducted in the greenhouse during 1973–1974, 1980, and 1981, four petioles per 6-wk-old plant were each pierced with a dissecting needle 1 cm above the crown. About 0.25 ml of the inoculum was atomized onto each injured plant. Plants were rated for disease 2 mo later on a scale of 0 = no disease, 1 = some rot, and 2 = dead (6). Potted plants in tests in the greenhouse were arranged in completely randomized designs, with three or four plants per 15-cm pot (plot) during 1973–1974, 1980, and 1981. The number of replications per cultivar per test was 10 in 1973–1974 and 1980 and five in 1981, amounting to 30, 40, and 20 plants, respectively, per test. Two of the less aggressive strains used in 1980 were replaced by two more aggressive strains in 1981. Also, cultivars were varied to determine if a more significant interaction would occur. The mean rating of plants in each plot was analyzed in a 4 × 6 factorial in 1973–1974 and a 6 × 9 factorial in 1980 and 1981. The results could not be summed over experiments in 1980 and 1981 because of a change in cultivars, strains, and heteroscedasticity. The inoculation dates were 2 April 1973,

Table 1. Identity, source, aggressiveness, and use of strains of *Erwinia carotovora* subsp. *betavasculorum*

Strain	Source	Aggressiveness[a]	Test[b]
UR-7	S. Thomson, CA	Variable	1,2,3,4
SB-4	S. Thomson, CA	5	1,4
SB-6	S. Thomson, CA	1	1
SB-13	S. Thomson, CA	3	1,4
163	S. Thomson, CA	2	2,3
WE-1	E. Ruppel, WA	5	2,3,4
CB-2	M. Stanghellini, AZ	2	2
KSB-9	M. Stanghellini, AZ	2	2,3
SP-5	E. Whitney, CA	5	2,3,4
MR-1	E. Whitney, CA	5	2,3,4

[a] Rated on a scale of 0–5, where 0 = not aggressive and 5 = very aggressive.
[b] Test 1 = 1973–1974, test 2 = 1978–1979, test 3 = 1980, test 4 = 1981.

2 May 1974, 4 February 1980, and 11 September 1981.

Field tests. In two separate tests on 11 July 1978 and 6 July 1979 (data combined), about 10 ml of inoculum was sprayed on 2-mo-old uninjured plants per 30 cm of row with a pressurized sprayer (7). The plants were harvested, individually cut, and scored on a scale of 0, 7, 25, 50, 75, 93, and 100% rot 3 mo later (2). A split-split plot design with three replications was used. Strains were used in randomized complete blocks in the main plots, with cultivars as subplots and years as sub-subplots. Each cultivar plot (single row) was 7.0 m long × 0.7 m wide, with plants thinned to a 20-cm spacing. One noninoculated main plot per block was included in each test and each strain, separated by two buffer rows. Because the variance of the two tests was homogeneous, the statistical analysis was an 8 × 4 × 2 factorial analysis over years.

RESULTS

In all but the test conducted in the greenhouse during 1973–1974, significant differences in aggressiveness of the strains were observed (P = 0.06 in 1973–1974 to P = 0.001 in 1981) (Table 3). A significant cultivar × strain interaction occurred only in the greenhouse in 1980 and 1981, when nearly equal numbers of U.S. and European cultivars were included in each test with six strains of the bacterium (Table 4). The use of more aggressive strains and some different cultivars led to a greater significance of the interaction (1980 vs. 1981).

Greenhouse tests. Aggressiveness of the strains differed considerably on a scale of 0–2, from 0.1 to 1.8 in 1980 (Fig. 1A) and from 0.1 to 1.75 in 1981 (Fig. 1B). A significant cultivar, strain, and cultivar × strain interaction occurred in 1980 and 1981 (Table 3). Ten of the 12 cultivar × strain combinations between the three U.S. cultivars common to the 1980 and 1981 tests—E840, 546, and C17—reacted similarly to strains SP-5, WE-1, UR-7, and MR-1. The exception was UR-7, which was significantly less aggressive (LSD 0.05) on cultivars C17 and 546 in 1981.

In 1980, strains SP-5, WE-1, UR-7,

Table 2. Identity, source, resistance rating, and use of cultivars

Cultivar	Source	Resistance rating[a]	Test[b]
C17	USA	S	1,2,3,4
C36	USA	R	2
546	USA	MR	2,3,4
Y526	USA	MR	2,3
US 75	USA	MR	1
Y03	Europe	MR	1,4
Y04	USA	MS	1,4
554H1	USA	MR	1,4
Sp7035	USA	MR	1
E840	USA	VS	3,4
D2	USA	MR	3
Mono 81	Europe	MS	3
Hh Mono	Europe	MS	3,4
Beta 1443	Europe	MS	3,4
Hh 545	Europe	MS	4
Monatuno	Europe	MS	3

[a] S = susceptible, MS = moderately susceptible, VS = very susceptible, R = resistant, MR = moderately resistant.

[b] Test 1 = 1973–1974, test 2 = 1978–1979, test 3 = 1980, test 4 = 1981.

Table 3. R^2 values for tests and probabilities associated with F ratios for strains, cultivars, or strain × cultivar disease effects for each test based on a disease index

Test	R^2	Strain	Cultivar	Strain × cultivar
Greenhouse, 1973–1974	1.00	0.06 NS[a]	0.001***	0.199 NS
Greenhouse, 1980	0.94	0.001***	0.001***	0.014*
Greenhouse, 1981	0.74	0.001***	0.001***	0.001***
Field, 1978–1979	0.61	0.04*	0.001***	0.097 NS

[a] NS = not significant, * P = 0.05, *** P = 0.001.

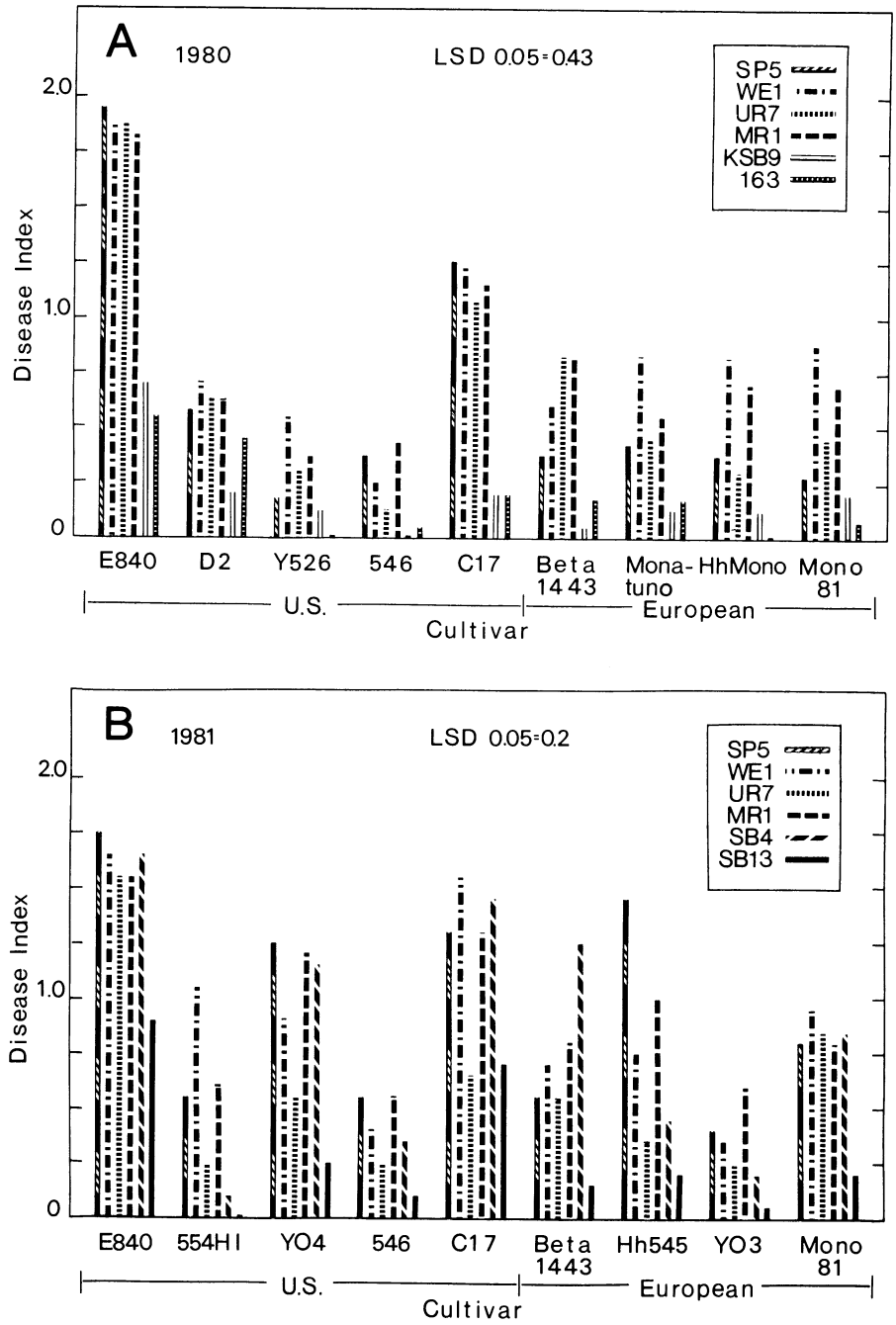

Fig. 1. The interaction of several strains of *Erwinia carotovora* subsp. *betavasculorum* on sugar beet cultivars differing in resistance in greenhouse tests during **(A)** 1980 and **(B)** 1981.

and MR-1 produced similar amounts of disease in cultivars E84O, 546, and C17, although significant differences in resistance resulted in less disease in these cultivars when strain KSB-9 or 163 was used. SP-5, however, caused less disease in the European cultivars than in the U.S. cultivars. It was less aggressive than UR-7 and MR-1 in Beta 1443, WE-1 in Monatuno and Hh Mono, and WE-1 and MR-1 in Mono 81. These variations in aggressiveness are responsible for significant cultivar × strain interactions. This same scenario was associated with other interactions, e.g., UR-7 and MR-1 caused similar amounts of disease in Beta 1443 but differed in aggressiveness to Hh Mono (Fig 1A).

Many interactions occurred in the 1981 test (Fig. 1B) and can be observed by comparing two strains on two cultivars as above, e.g., SP-5 and WE-1 on 554H1 and Hh 545 (a significant difference in the direction of the response) or SP-5 and WE-1 on Y04 and 546 (a significant difference in the magnitude of the response).

Field tests. Some disease was observed in the noninoculated treatment. Large differences in cultivars and strains were noted for each variable measured: disease index (DI), percentage of diseased beets, and percentage of rot per diseased beet (Tables 4 and 5). The correlations between all possible combinations of the dependent variables were highly significant. Each measurement differed as to the amount of damage, however, as the aggressiveness of the strain increased or the cultivar resistance increased (Duncan's multiple range test).

Table 4. Means of all strains for disease index, percentage of diseased beets, and percentage of rot per diseased beet for cultivars in the 1978–1979 field test

Cultivar	DI[x,y]	Percent diseased beets[y]	Percent rot/diseased beet[y]
C36	0.20 a[z]	3.0 a	8.1 a
546	0.26 a	3.8 a	11.1 a
Y526	1.34 b	9.5 b	26.2 b
C17	15.67 c	32.2 c	59.6 c

[x]Disease index = (percent rot per beet)/(number of beets per plot).
[y]Correlations between DI and percent diseased beets, DI and percent rot/diseased beet, and percent diseased beets and percent rot/diseased beet were 0.99, 0.96, and 0.99, respectively ($P = 0.01$).
[z]Duncan's multiple range test, $P = 0.05$ level of probability within columns.

Table 5. Means of all cultivars for disease index, percentage of diseased beets, and percentage of rot per diseased beet for strains in the 1978–1979 field test

Strain	DI[x,y]	Percent diseased beets[y]	Percent rot/diseased beet[y]
CB-2	0.23 a[z]	3.7 a	8.7 a
UR-7	0.25 a	3.8 a	11.6 a
163	0.60 ab	8.6 b	14.3 a
KSB-9	0.84 ab	8.9 b	24.4 b
SP-5	1.72 b	12.3 bc	33.5 c
WE-1	3.05 b	17.7 c	36.4 c
MR-1	3.55 b	19.1 c	44.3 d

[x]Disease index = (percent rot per beet)/(number of beets per plot).
[y]Correlations between DI and percent diseased beets, DI and percent rot/diseased beet, and percent rot/diseased beet were 0.98, 0.95, and 0.96, respectively ($P = 0.01$).
[z]Duncan's multiple range test, $P = 0.05$ level of probability within columns.

DISCUSSION

The discovery of large differences in two of three aggressiveness tests conducted in the greenhouse and an interaction between cultivars and strains should alert researchers to the possibility of a loss of durability if bacterial rot occurs in resistant cultivars. A single dominant gene in selected cultivars such as E536 and C36 (2,9) confers high resistance, and no evidence for its loss in durability has been noted. If highly aggressive biotypes became predominant in the population in the field, however, the effectiveness of this gene could be reduced. Minor genes have also been suggested as sources of resistance in the same cultivar and could help to reduce the probability of epiphytotics (2). The more aggressive strains caused a higher incidence as well as a greater severity of disease. Thus, a strain's aggressiveness is not limited to its ability to cause disease but also includes its ability to macerate tissue. Therefore, several highly aggressive strains should be used for selecting for durable resistance to *E. c.* subsp. *betavasculorum*. DI, percentage of diseased beets, or percentage of rot per diseased beet could be used as the criterion for making field selections or evaluating cultivars, but choice would be influenced by whether selections were for qualitative genes (percentage of infection) or quantitative genes (percentage of rot per diseased beet).

Whether the increase in the significance (0.01 vs. 0.001) of the cultivar × strain interaction between the 1980 and 1981 tests was due to the use of more aggressive strains, to the use of different cultivars, or to environmental factors is not known. Biotypes that can cause substantial disease in resistant cultivars were found in greenhouse tests. However, how well these strains would compete in the general population of *E. c.* subsp. *betavasculorum* would require field tests over several years.

Why differences in strain reaction occurred between the tests in 1980 and the tests in 1981 is not clear. However, because the inoculations were at different times of the year, February vs. September, environmental conditions such as day length and greenhouse temperatures would be considerably different. Strain UR-7 was one of the least aggressive in the field studies but one of the most aggressive in the greenhouse tests, suggesting that the environment could affect the apparent aggressiveness of certain strains.

This is the first report on the aggressiveness of strains and of a cultivar × strain interaction between sugar beet and *E. c.* subsp. *betavasculorum*.

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