Differences in Vectoring Ability and Aggressiveness of Isolates of *Polymyxa betae*

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ABSTRACT

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Six nonviruliferous isolates of *Polymyxa betae* from California, Colorado, Nebraska, and Alberta, Canada, were tested for their ability to acquire and transmit beet necrotic yellow vein virus (BNYVV) and cause damage to sugar beet plants. All of the isolates tested transmitted BNYVV from systemically infected *Beta macrocarpa* to sugar beet. Infection by viruliferous isolates of *P. betae* resulted in decreased root weight and increased root branching and root tip mortality when compared with noninfected controls. Nonviruliferous isolates of the fungus decreased the top weight and root weight of sugar beet when compared to controls. Differences in the amount of damage caused by BNYVV were observed when the virus was transmitted by individual isolates. An isolate from the Sacramento Valley was found to be more aggressive than other isolates.

Additional keywords: *Beta vulgaris*, rhizomania.

Polymyxa betae Keskin is an obligate parasite of sugar beet and other plant roots and the vector of beet necrotic yellow vein virus (BNYVV) (7,10,17), the causal agent of rhizomania (18). The fungus was first recognized in the western hemisphere in California in 1977 (6) and since then has been reported in additional areas of North America (2,4,13).

Although the fungus appears to infest most soils with a history of sugar beet culture, BNYVV is not present in all fungal populations. In a selective survey of California sugar beet fields, the fungus was found in 82% of the soils, but BNYVV was detected in association with only 45% of the fungal isolates (Gerik and Duffus, *unpublished*). Some isolates of *P. betae*, from hosts other than sugar beet, are unable to transmit BNYVV (1). It is not known whether the entire population of *P. betae* from sugar beet is able to acquire and transmit BNYVV.

P. betae has been reported to cause necrosis of small roots, leaf chlorosis, and stunting in infected plants (11,12). These observations were made before it was discovered that BNYVV is frequently associated with the fungus. A question remains as to the type of damage caused from infection by *P. betae* with and without BNYVV.

This paper reports on the ability and efficiency of isolates of *P. betae*, from different geographical areas, to acquire and transmit BNYVV. The paper also reports differences in pathogenicity among the isolates. A preliminary report has been given (8).

MATERIALS AND METHODS

Fungal isolation and inoculum production. The isolates of *Polymyxa betae* used in this study are listed in Table 1. Isolations were made from infected sugar beet roots obtained from infested fields, and from greenhouse plants growing in soil from infested fields. Unifungal cultures of *P. betae* were established by the methods of Barr (2) and Fujisawa and Sugimoto (7). Fungi were grown on roots of sugar beet plants grown in a pasteurized greenhouse soil mixture and maintained at 28 C in a growth chamber. Inoculum was obtained from the air-dried washed roots of these plants, stored at room temperature in glass vials.

Establishment of viruliferous fungal cultures and transmission tests. Except where indicated, all plants were grown in a

pasteurized greenhouse soil mixture (six volumes Chular loam, one volume Super Soil potting mix, Rod McLellan Co. So. San Francisco, CA), pH 7.0, contained in pots (10 cm square). A two factorial (fungal isolate, viruliferous and nonviruliferous) experiment was established in a completely randomized design with three replications per test. Each test was on a separate greenhouse table in a room maintained between 28 and 20 C. A method was developed to establish viruliferous fungal cultures. Three weeks after they were transplanted into pasteurized soil, seedlings of *Beta macrocarpa* L., a systemic host of BNYVV (J. E. Duffus, *unpublished*), were mechanically inoculated with an isolate of BNYVV obtained from field-grown sugar beet near Paso Robles, CA; a second set of seedlings remained uninoculated. Two weeks after viral inoculations each pot was infested with inoculum of one of six nonviruliferous isolates of *P. betae*. Other pots containing plants with and without virus were not infested with the fungus. Plants were watered cautiously to minimize the possibility of pot-to-pot contamination by splashing. Plants of *B. macrocarpa* were cut at the soil surface 4 wk after fungal infestation and seeds of sugar beet USH11 were planted in each pot in the same soil in which the *B. macrocarpa* had grown. Plants were thinned to five per pot. After 2 mo, soil cores (9 × 2 cm) were collected from each pot with a no. 15 cork borer and dried, all plant tissue above the soil was cut and weighed, and the root tissue was washed from the soil, dried, and weighed. Two 0.5-g (fresh weight) samples of feeder root tissue from each pot were assayed for virus infection by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (3).

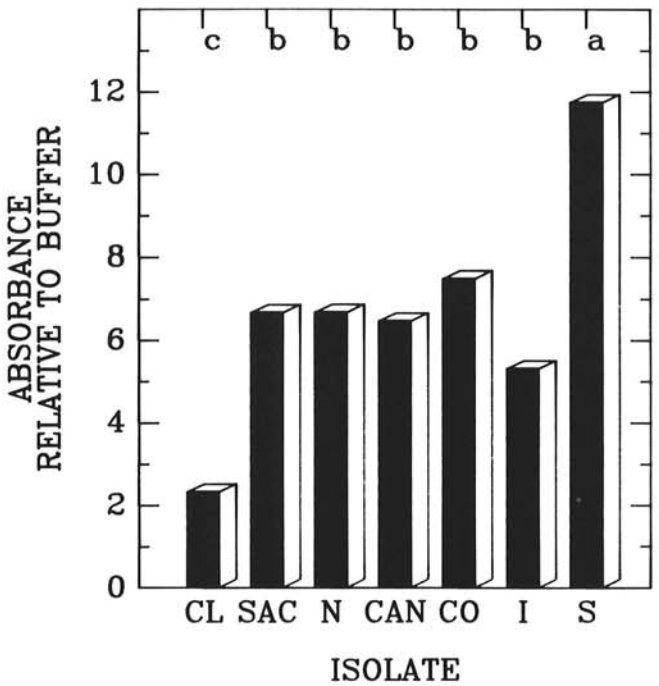

Fig. 1. Relative enzyme-linked immunosorbent assay absorbance values indicating the concentration of beet necrotic yellow vein virus in sugar beet root samples. The virus was transmitted by one of six isolates of *Polymyxa betae*. The isolates of *P. betae* are represented by: SAC (Sacramento Valley), N (Nebraska), CAN (Canada), CO (Colorado), I (Imperial Valley) and S (Salinas Valley); CL represents healthy tissue (control). Each value is the mean of 18 samples (0.5 g fresh weight of feeder root tissue). The standard error was 1.26. Actual absorbance values for the buffer controls ranged from 0.033 to 0.049. The same letters above the bars represent no significant differences at $P = 0.05$ according to Fisher's protected LSD.

Root assays. The root tissue was washed from 10 g of the cork borer samples. The total root length of normal and necrotic roots was determined by the line intersect method of Newman (14) as modified by Tennant (19). The number of branch points and root tips for each sample was counted. The infection index (0–5) of *P. betae* was determined for each sample by a modification of the plus-minus slide method for quantifying mycorrhizal infection in roots as described by Giovannetti and Mosse (9). Five 2-mm-long feeder root segments from each sample were randomly selected and mounted on a microscope slide. The entire length of each segment was observed with a compound microscope (100× magnification) and each segment was scored plus or minus for infection by *P. betae*. The infection index equaled the mean number of positive segments of each sample from a treatment.

RESULTS

Transmission of BNYVV. All six isolates of *P. betae* were able to acquire BNYVV from systemically infected *B. macrocarpa* and transmit it to sugar beet (Fig. 1). The virus content in roots infected by the Salinas Valley isolate was significantly greater than that in roots infected by the other isolates.

Vector infection. The six isolates of *P. betae* differed significantly in their ability to infect sugar beet (Table 2). The highest incidence of infection was caused by the Sacramento isolate and the lowest by the Canada isolate (Table 3). The infection rate was significantly greater for viruliferous isolates (Table 2). A significant interaction was observed between isolate and viruliferous state (Table 2).

Effect of vector and virus on growth of sugar beet. Infection by all nonviruliferous isolates of *P. betae* significantly reduced the mass of the top portion of sugar beet (Table 3). The reduction was greatest for the Sacramento isolate. Infection by the viruliferous isolates resulted in no significant reduction of top mass, when compared with the control. Plants infected with the viruliferous Sacramento isolate had less top mass than plants infected with the viruliferous Nebraska and Salinas isolates.

TABLE 1. List of isolates of *Polymyxa betae* used in this study and their source

Isolate	Source	Collector
Sacramento Valley	Roots from Dixon, CA	E. D. Whitney
Nebraska	Roots from Lincoln, NE	W. G. Langenberg
Canada	Soil from Taber, Alberta	P. Bergen
Colorado	Roots from Longmont, CO	E. G. Ruppel
Imperial Valley	Soil from Brawley, CA	J. E. Duffus
Salinas Valley	Soil from Gonzales, CA	M. H. Yu

TABLE 2. *F* values[a] and error mean square values for six parameters 2 mo after planting sugar beet in soil infested with beet necrotic yellow vein virus (BNYVV) viruliferous or nonviruliferous isolates of *Polymyxa betae*

Source of variation	df	Infection index	Top weight	Root weight	Root necrosis	Branches per cm	Tips per branch
Greenhouse table	2	1.28	4.01*	15.36**	11.88**	4.91**	5.37**
Treatments							
BNYVV	1	7.72**	28.93**	1.64	1.39	15.69**	12.21**
P. betae isolate	6	24.34**	2.56*	3.29**	0.69	2.75*	1.42
BNYVV × Isolate	6	2.51*	2.08	1.58	0.62	1.36	0.99
Error mean square	110	1.123	29.831	0.197	0.022	0.198	0.034
Total	125						

[a] Coefficients followed by two asterisks are significant at $P < 0.01$; those with one asterisk at $P < 0.05$.

TABLE 3. Mean values,[a] least significant differences, and coefficients of variation for six parameters 2 mo after planting sugar beet in soil infested with beet necrotic yellow vein virus (BNYVV) viruliferous or nonviruliferous isolates of *Polymyxa betae*

Isolate	BNYVV[b]	Infection index[c]	Top weight (g)	Root weight (g)	Root necrosis (%)	Branches per cm	Tips per branch
Control[d]	+	0.0	34.7	1.4	28.6	0.35	0.36
	−	0.1[e]	36.8	1.5	25.3	0.38	0.56
Sacramento	+	4.6	32.2	1.0	29.3	1.16	0.34
	−	2.8	27.3	0.9	32.8	0.59	0.31
Nebraska	+	3.1	38.7	0.9	24.3	1.11	0.26
	−	2.0	29.4	1.2	28.3	0.42	0.49
Canada	+	1.2	34.8	1.0	24.6	0.80	0.34
	−	1.9	31.0	1.1	32.1	0.37	0.47
Colorado	+	2.6	36.0	1.3	30.4	0.68	0.29
	−	2.1	27.7	0.9	39.5	0.46	0.38
Imperial	+	3.8	34.9	1.1	33.1	0.70	0.29
	−	3.3	28.9	0.9	27.5	0.40	0.41
Salinas	+	2.4	38.3	1.6	27.0	0.46	0.39
	−	1.8	31.5	1.2	33.8	0.39	0.47
LSD ($P \leq 0.05$)		1.0	5.1	0.4	n.s.	0.42	0.17
C.V. (%)		47	17	39	50	76	48

[a] Each value is based on three replicates in each of three blocks.
[b] + = BNYVV viruliferous, − = BNYVV nonviruliferous *P. betae*.
[c] The number, out of five, of 2-mm-long root segments infected with *P. betae*.
[d] Neither control was infested with *P. betae*. The controls differ in that the + treatments were grown in soil in which BNYVV infected *Beta macrocarpa* had previously grown. The − treatments were in soil which had previously grown noninfected *P. macrocarpa*.
[e] This low reading was due to a low amount of contamination by *P. betae* in the controls.

Infection by several isolates of *P. betae* reduced total dry root mass of sugar beet (Table 3). The reduction in root mass resulted from infection by both viruliferous and nonviruliferous isolates (Table 2). Neither infection by viruliferous nor nonviruliferous isolates of *P. betae* resulted in an increase of feeder root necrosis.

The number of root branches in the samples was counted. BNYVV infection resulted in a significantly greater number of branches relative to total root length (Table 2). The incidence of root branching differed among the plants infected with the viruliferous isolates of *P. betae*. The greatest incidence was observed with the Sacramento isolate. The least amount of branching was observed with the Salinas isolate (Table 3). Nonviruliferous isolates did not increase root branching.

The number of root tips in the samples was observed. BNYVV infection significantly reduced root tip numbers in infected plants. The differences in the number of root tips among plants infected with the different isolates of *P. betae* were not significant.

DISCUSSION

The six isolates of *P. betae* in this study were collected from a wide geographical area, and all were able to transmit BNYVV. This observation indicates the potential of nonviruliferous populations of *P. betae* to acquire and transmit BNYVV. In the United States, rhizomania is now limited to California (5) and Texas (4). In addition to these two states, nonviruliferous *P. betae* is known to infest sugar beet soils in Nebraska (13), Colorado, Minnesota, Idaho, Oregon, Utah, and Wyoming (J. S. Gerik and J. E. Duffus, *unpublished*). From our data, one could assume that rhizomania has the potential to become established in these other areas.

The greater levels of infection by *P. betae* observed with the viruliferous isolates when compared with the nonviruliferous isolates is an interesting phenomenon. This observation indicates that an introduced viruliferous population of *P. betae* into a sugar beet field might outcompete an endogenous nonviruliferous population of *P. betae*. It is not known why this difference exists. It could be due either to the virus affecting the host or vector. *P. betae* only infects primary root tissue. BNYVV causes a proliferation of root branching, which results in more susceptible tissue for *P. betae* to infect. The increase in susceptible tissue would not in itself result in increased infection per unit root length, but would maintain adequate inoculum for subsequent infection by *P. betae*. Stress induced by viral infection has been shown to increase root exudation (16). Increases in root exudation have been shown to increase root colonization by soil fungi (15). This is one possible explanation for this phenomenon.

Of the parameters measured, much of the direct damage to roots appears to be root tip death, which would result in a loss of apical dominance, an increased amount of root branching, and lower accumulation of root mass. This damage is caused by BNYVV and not *P. betae*. Infection by *P. betae* alone significantly decreased top plant weight so the fungus must have a deleterious effect on the host. It is not known if infection by *P. betae* alone will reduce sugar yields.

Differences are evident among the six isolates of *P. betae* in this study. The Sacramento Valley and Imperial Valley isolates appear to be the more aggressive isolates. Plants infected with these isolates exhibited a high infection index and reduced root weight, whether or not the isolates were viruliferous. The treatments infected with the Salinas Valley, Canada, and Nebraska isolates had the lowest infection indices, and generally had less damage. These and other differences between the isolates may represent inherent differences in their aggressiveness.

Although virus concentrations were significantly greater for the plants infected with the viruliferous Salinas Valley isolate, relatively less damage to the plants was observed with this viruliferous isolate. The data analysis indicates that most of the damage caused by this fungus-virus complex is due to the virus, but it appears that the aggressiveness of the vector must be an important aspect of the disease. This apparent contradiction may be due to the nonsystemic nature of BNYVV. BNYVV moves systemically in plants only rarely (James E. Duffus, *unpublished*) and depends on the vector for most cell-to-cell movement. A more aggressive isolate would move the virus to more cells, which might result in tissue death. A less aggressive isolate might move the virus to fewer cells, not killing the tissue, and thus allowing for greater viral replication in the cells that are infected.

In conclusion, it appears that several populations of *P. betae* from North America can be effective vectors of BNYVV. Isolates of *P. betae* from different areas do differ biologically. Field experiments will be needed to determine if nonviruliferous *P. betae* will cause yield loss in sugar beet.

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