The Spread of Beet Necrotic Yellow Vein Virus from Point Source Inoculations as Influenced by Irrigation and Tillage

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ABSTRACT

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A 3-year study was initiated in 1992 to map the spread of beet necrotic yellow vein virus (BNYVV) from known point sources of inoculum by irrigation and tillage practices. The experiment each year consisted of four plots, each containing 12 30-m rows, on 76-cm centers. Sugar beet seeds (cv. HH39) coated with BNYVV-infested *Polymyxa betae* were planted in the first 3 m of the two outside rows of each plot and constituted the point sources of inoculum. The remaining plot area was planted with uninfested seeds. Plots were furrow irrigated every 2 weeks. Plant and soil samples were collected from the point source areas and other predetermined locations in each plot before tillage operations. Plant roots were assayed by indirect double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for BNYVV incidence. Soil samples were planted with sugar beet seeds in the greenhouse, and after 10 to 12 weeks, roots of bait plants were assayed for BNYVV. To further evaluate movement, soil samples were collected from the previous locations after tillage events and after a second sugar beet crop before being bioassayed. All plant samples taken from point sources exhibited typical symptoms of rhizomania, and both plant and soil samples tested positive for BNYVV in DAS-ELISA tests. Out of 336 plants collected in the field from non–point-source areas, only 1 tested positive. Before tillage events, 2% of the soil samples were positive for BNYVV for the first experiment and 0.9% tested positive the second, suggesting negligible movement of viruliferous *P. betae* by irrigation. After tillage and harvest operations, 9 and 6% of the soil samples were infested for the first and second experiments, respectively. After a successive sugar beet crop, 15% of the soil samples were positive for BNYVV in the first experiment and 12% in the second. Our results show that physical movement of soil during tillage and harvest operations exert a much greater influence on spread of BNYVV than furrow irrigation, which contradicts the generally accepted concept that viruliferous *P. betae* is rapidly disseminated by irrigation.

Rhizomania is a serious disease of sugar beets worldwide. It is caused by beet necrotic yellow vein virus (BNYVV), which is transmitted by the obligate fungal parasite *Polymyxa betae* (Keskin). The host range of *P. betae* is primarily confined to species of the Chenopodiaceae, Amaranthaceae, and Portulacaceae (3,11). Symptoms of rhizomania include severe stunting and constriction of taproots and massive proliferation of secondary rootlets, known as root bearding. The virus seldom becomes systemic; as a result, BNYVV usually is confined to these secondary roots. Because of the tremendous damage inflicted by rhizomania, it is one of the more important diseases of sugar beets.

Since its discovery in Italy in 1959, rhizomania has spread to most sugar beet production areas of the world (10). California was the first state in the United States to identify BNYVV from sugar beet in 1983 (7), followed by Texas in 1987 (6). Recently, it has also been reported in Idaho, Wyoming, Nebraska, and Colorado (9,13,28). In some of these states, by the time rhizomania was positively identified, both the vector and the virus were already widespread, which limited the opportunity to determine the initial dispersal of the pathogen. For example, by 1989, 6 years after its discovery in California, the pathogen contaminated over 35,000 ha (10). The general consensus was that the extremely rapid spread of the disease was primarily due to California growers irrigating from canals. In their system, water runoff from fields drains back into canals and is used to irrigate other fields. Such a system potentially could contaminate thousands of hectares in a very short period of time. Because of the rapid contamination of farms in California, researchers focused on methods of disease control and management (2,22,34), and fewer studies were devoted to the mode of dispersal (12).

In Texas, BNYVV also is more widespread than initially was believed and has been reported in 8 of 10 sugar beet-growing counties (20). However, because numerous farms are still free of the pathogen, factors that impact movement of the pathogen and its rate of spread when introduced into a field are of great interest. Therefore, a 3-year study was initiated in 1992 with the objective of determining how viruliferous *P. betae* is dispersed throughout plots from infested point sources as influenced by irrigation and tillage operations. Preliminary reports have been published (16,18).

MATERIALS AND METHODS

The study was conducted from 1992 to 1994 at the Texas Agricultural Experiment Station, Bushland, on crop land never before used for sugar beet production. Before beginning the study, eight 9 × 30-m plots were disced, leveled, and prepared for planting. Four to five soil samples, each consisting of approximately 0.5 liters of soil taken from the upper 10 cm of the soil profile, were collected from each plot and planted with sugar beet seeds in the greenhouse. After 10 to 12 weeks, roots of plants grown in these soil samples were checked microscopically for cystosori of *P.*

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betae and assayed by indirect double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for the presence of BNYVV (14,15).

Field design and agronomic practices. Land preparation followed conventional agronomic procedures for sugar beet production in the Texas Panhandle that have been previously published (17,19). This involved a preplant injection of anhydrous ammonia, followed by a phorate (Thimet) and ethofumesate (Nortron) application for insect and weed control, respectively. At 6 to 8 weeks after emergence, trifuralin (Treflan) was sprayed and incorporated as a lay-by treatment for additional weed control.

The first experiment (1992 to 1993) consisted of four plot areas, each containing 12 30-m rows on 76-cm centers, completely enclosed on all sides by a border dike. During the first year of this study, two irrigation regimes were included in the test: half of each plot was furrow irrigated every 14 days, and the other half was irrigated approximately every 30 days. A furrow separated the two irrigation treatments in each plot and was not watered, so movement of viruliferous zoospores of *P. betae* between the two treatments was unlikely.

Sugar beet seeds of cv. HH39 (Holly Hybrids, Colorado Springs, CO) were coated with ground sugar beet roots infested with viruliferous *P. betae*, with 2% methyl cellulose as the carrier in a 1:10:10 ratio (wt/vol/wt; inoculum/methyl cellulose/seeds) (14). The first 3 m of the 2 outside rows in each 12-row plot were planted with inoculated seeds by hand during mid-May and constituted the point source regions. The seeds were placed at the edges of the plots to simulate accidental infestation of a field by a farm implement. The remainder of the plot was planted with uninfested seed at a depth of 2 cm and a rate of 15 to 20 seeds per m.

Plots were irrigated immediately after planting for emergence. After the first year, the differential irrigation treatments were dropped, and each plot was irrigated every 14 days by quickly flooding the furrows until the water was just below the top of the beds. Water was allowed to soak into the beds for 20 to 30 min and then the enclosed plots were flooded again. This technique applied 6 to 8 cm of water per irrigation. Each bed and furrow was blocked at the opposite end from the water source so the water filling the furrow would remain contained within that furrow. This prohibited water passing from one furrow to another (14).

At the end of the season, beets were topped with a four-row topper and mechanically harvested with a two-row beet digger. After harvesting in 1992, these same four plots were rebedded and, after application of pesticides, planted with uninfested seed during May 1993. The second planting was designed to evaluate the impact of a successive sugar beet crop on pathogen dispersal from the original point sources.

Sampling. To monitor spread of BNYVV, each plot was marked with flags at distances of 0.0, 4.5, 13.5, 22.5, and 30 m down the row from the beginning of the point sources (Fig. 1). The down-row sampling location closest to the point source (4.5 m), actually only 1.5 m from the end of the point source in rows 1 and 12, was selected because we wanted to have one down-row sampling location close to the inoculum source where plants would likely become infected. The remaining three sampling locations were equidistantly separated at 9 m, but a farm road was put in, and we lost 1.5 m from the end of the plots—therefore, there was 7.5 m at the distal end of the plot rather than 9 m. Plant samples were collected twice (at midseason and shortly before harvest) during 1992 from these five distances in the three outside rows from each side of each plot. Each plant sample consisted of one to two beet roots removed from the flagged sampling locations. This sampling scheme was undertaken to save time and expense. Had plant samples from the two rows adjacent to the point sources tested positive, the remainder of the plot would have been sampled. Samples also were taken from each point source area.

The most accurate method of detecting BNYVV in a field is to bioassay soil samples (C. M. Rush, *personal observations*). Therefore, 60 soil samples, 5 from each of the 12 rows, were collected, as described previously, before harvest from each plot. These samples were taken from the same general locations as the plant samples (five points in each row), except all 12 rows in the four plots were sampled. Plots were harvested mechanically during September 1992. Detection of BNYVV in any of the plant or soil samples collected from outside a point source area before harvest was attributed to movement of viruliferous *P. betae* in irrigation water. The moisture, or irrigation effect, included rainfall and was meant to include all variables affecting movement of the virus prior to the first tillage event. For simplicity, we will refer to this as the irrigation effect.

Before replanting the successive sugar beet crop in 1993 (to conclude experiment 1), plots were rebedded and prepared using the same methods as the year before. To determine spread of BNYVV due to tillage (1992 harvesting and 1993 land preparation), 60 soil samples were collected from each plot from the same general locations as the year before. After harvesting the 1993 plots, 60 soil samples again were collected from the same locations to evaluate additional spread of the virus after a successive sugar beet crop. No plant samples were taken from the successive crop.

The study was repeated in four new plots (experiment 2), beginning in 1993, concluding with another sugar beet crop in 1994. All plant and soil sampling procedures were the same as in experiment 1, except plant samples were collected and assayed only once before the 1993 harvest. Even though differential irrigation treatments were dropped after the first year of the study, two point sources were included in the repeated study to maintain continuity of initial inoculum locations between tests.

BNYVV assays. Roots of plant samples collected from the field were assayed by DAS-ELISA for the presence of BNYVV with commercially available antisera and enzyme conjugates from BioReba Ag (Chapel Hill, NC). Root tissue selected for assaying consisted of small, secondary feeder roots in the axial groove of the taproot—the portion of the beet where BNYVV most likely would be found (21). Sugar beet controls, positive and negative for BNVYY, which were maintained in the greenhouse, were included in all DAS-ELISA tests. Absorbance values ≥3× the negative controls were considered positive for BNYVV (14). For purposes of analysis and mapping of ELISA results, a positive ELISA value was designated as 1 and a negative value as 0.

Soil samples were brought into the greenhouse, potted in Ray Leach Cone-Tainers (Stuewe and Sons, Inc., Corvallis, OR), and seeds of susceptible cultivars were planted. After 10 to 12 weeks,

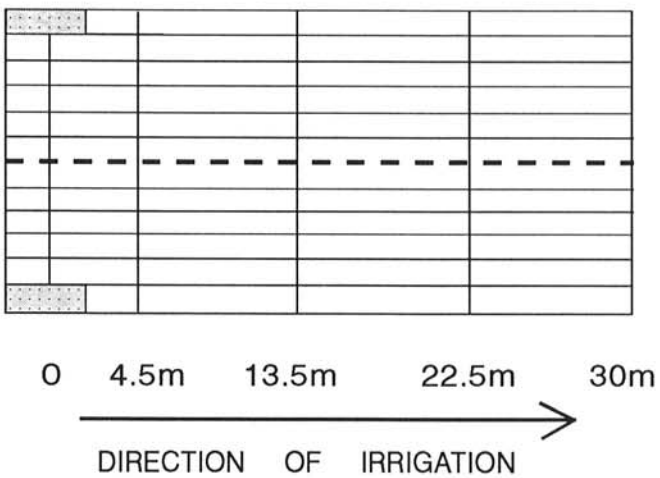

Fig. 1. Field map of one plot of sugar beet containing 12 30-m rows. Shaded boxes in the two outside rows represent the beet necrotic yellow vein virus-inoculated point source areas. Numbers and vertical lines depict locations (in meters) where plant and soil samples were collected for assay to monitor movement of viruliferous *Polymyxa betae*.

bait plants grown in the soil samples were harvested. Roots were washed free of soil and assayed by DAS-ELISA for the presence of BNYVV as described previously (14,15).

Analysis. Results from ELISA tests were analyzed by analysis of variance (ANOVA) to determine whether replications were different and whether interactions between replications and sampling positions existed. Positive ELISA results from the bioassays were labeled on plot maps to visualize spread of BNYVV from the infested point sources before tillage, after tillage, and after a successive crop (Fig. 2). In an attempt to quantitatively describe the observations, several models were tested.

Results from the ELISAs were given a spatial designation from the point sources. The point sources were placed on the edge of rows 1 and 12, so data from these rows were combined, as were rows 2 and 11, 3 and 10, 4 and 9, 5 and 8, and 6 and 7. These six positions were given distance measurements of 0.00, 0.76, 1.52, 2.28, 3.04, and 3.8 m across rows from the point sources, respectively. Distances down rows from point sources of BNYVV were 0.0, 4.5, 13.5, 22.5, and 30 m. The rate of spread of the virus was tested across rows, down rows, and the interaction across and down rows by the model ELISA = intercept + a(ACROSS + b(DOWN) + c(ACROSS × DOWN), where a, b, and c are parameters to be estimated. Modifications of this model also included square root and natural log transformations of the factors (ACROSS and DOWN) and quadratic terms of these factors. Models also were tested by natural log-transformed ELISA results (values of 0 were set to 0.1). Models with $P \leq 0.05$ were accepted if all individual factors were significant with a t test ($P \leq 0.05$). The model with the highest R^2 was selected. If the rate of spread across rows was not significantly different than the rate of spread down rows and there was no interaction between rates of spread across and down rows, then the tested models were simplified to the following gradient models (5): ELISA = a(DIST)^b and ELISA = $a$exp[$-b$(DIST)], where DIST is the distance from the point source and a and b are parameters to be estimated. These models were linearized and fitted with linear regression (SAS [29]). Models were selected based on the criteria listed above. The data from each test and sampling period were modeled separately.

RESULTS

BNYVV was not detected in any soil samples collected from plots prior to beginning the study (data not shown). Therefore, we were confident that BNYVV detected during the study came from inoculum we applied in the field.

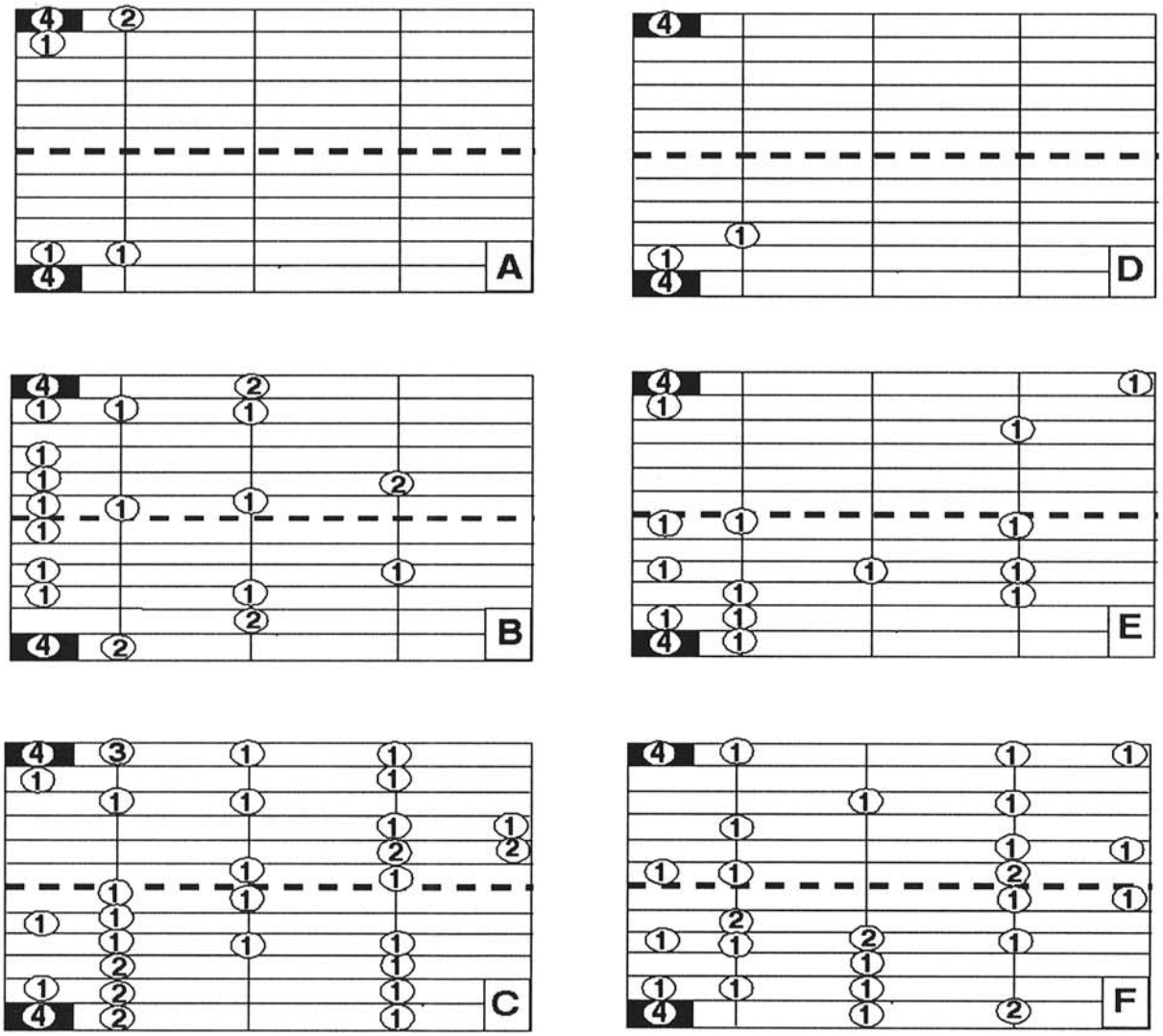

Fig. 2. Spread of beet necrotic yellow vein virus (BNYVV) in continuous sugar beet plots in Bushland, TX, during 1992 to 1994. Each field (A through F) is a composite of four replications. Fields A, B, and C represent the first experiment (1992 to 1993). Fields D, E, and F represent the repeat experiment (1993 to 1994). Shaded boxes in the two outside rows depict point sources inoculated with BNYVV. Ovals represent the location and number of soil samples that tested positive for BNYVV after one season's irrigation (A and D); after tillage operations (B and E), including harvest; and after a successive sugar beet crop (C and F). All soil samples were assayed in the greenhouse by planting susceptible sugar beet seeds and testing roots for BNYVV by indirect double-antibody sandwich enzyme-linked immunosorbent assay after 10 to 12 weeks.

All studies were planted late for sugar beet production in Texas (mid-May) to maximize the chances for infection. Soil temperatures at this time varied between 18 to 20°C. Twenty-four hours after irrigating with ground water that was approximately 13 to 15°C, soil temperatures were 16 to 18°C. This is within the range necessary for *P. betae* to infect but is below the 25°C optimum (4,11).

Assays after irrigation. During both years of the study, very little movement of viruliferous *P. betae* occurred after irrigation. During 1992, plants were collected at midseason and harvest. Only 1 of 224 plants outside the point sources was infected with BNYVV and occurred at harvest. Because the differential irrigation treatments had no effect on virus movement from point sources, the treatments were dropped. In the second experiment, initiated in 1993, only one plant sampling was conducted, and no non–point-source samples tested positive for the pathogen (Table 1).

ANOVA indicated no significant differences between replications or interactions between replications and sampling locations with regard to ELISA results from the soil bioassays (Table 2). Therefore, the results from the four replicates were combined and are presented in Figure 2. Figure 2A and D show the effects of irrigation on virus movement as determined by DAS-ELISA results from soil bioassays for the first and second years, respectively. Each figure represents a composite of all four replications for each year, and the ovals represent the number and locations of soil samples that were positive for BNYVV after bioassaying in the greenhouse. For 1992, 5 of 232 non–point-source samples (2%) were positive, whereas only 2 of 232 samples were positive for 1993 (0.9%) (Table 1). The virus was detected in both plant and soil samples collected from point sources in every assay. The plants collected from these areas exhibited typical symptoms of rhizomania.

Assays after tillage. Soil samples collected after harvest in 1992 and before replanting in 1993 also showed few positive reactions in DAS-ELISA tests (Table 1). Only 9% of the non–point-source samples (21 of 232) were positive for BNYVV. In the study initiated in 1993, the virus was detected in only 14 of 232 soil samples (Table 1), indicating that soil movement due to tillage dispersed the pathogen to a greater extent than did irrigation. At least three samples with a down-row distance of 22.5 m from a point source tested positive in both tests (Fig. 2B and E).

Assays after the successive crop. A second crop of beets, along with its associated agronomic practices, resulted in an even greater number of BNYVV-positive soil samples and spread the vector even further away from point sources within the plots (Table 1; Fig. 2C and F). In both experiments, there were three BNYVV-infested soil samples identified from the 30-m location, the maximum distance sampled from the sources of inoculum.

Analysis. The models selected among those tested had a linear function for spread down rows, a linear or quadratic function for spread across rows, and an interaction between spread down and across rows (Table 3). These models, although significant ($P = 0.05$) and with R^2 values ranging from approximately 0.68 after irrigation events to approximately 0.17 after the second year's planting, poorly described the dynamics of viral spread. However, the models did demonstrate that spread was affected by direction (across and down rows), so models using only distance from the point source were less adequate than the models selected. Sporadic spread of the virus by soil movement in tillage, harvesting, and planting operations was not well described by any of the tested models. For example, the number of soil samples testing positive for BNYVV declined in some locations over time (Fig. 2B versus C [at the 0- and 13.5-m distances]), and the number of positive soil samples did not necessarily decrease as distance from the point source increased (Fig. 2).

TABLE 1. Non–point-source sugar beet plant and soil samples collected and assayed for beet necrotic yellow vein virus (BNYVV) during 1992 to 1994 [a]

Samples	Experiment 1 (1992 to 1993)			Experiment 2 (1993 to 1994)		
	Total collected	No. positive	% positive	Total collected	No. positive	% positive
Plant	224	1	0.9	112	0	0
Soil (after irrigation)	232	5	2.1	232	2	0.9
Soil (after tillage)	232	21	9.0	232	14	6.0
Soil (after second crop)	232	35	15.1	232	28	12.1

[a] Roots of plant samples were tested for BNYVV by indirect double-antibody sandwich enzyme-linked immunosorbent assay. Soil samples were planted in the greenhouse with susceptible sugar beet seeds, and roots of bait plants were assayed for BNYVV after 10 to 12 weeks.

TABLE 2. Analysis of variance for combined analysis over the entire study (1992 to 1994) concerning beet necrotic yellow vein virus (BNYVV) incidence in soil samples assayed by indirect double-antibody sandwich enzyme-linked immunosorbent assay

	df	Mean square	$Pr > F$
Test[a]	1	0.18774	0.1291
Sampling date[b]	2	1.65578	0.0001
Replication[c]	3	0.01687	0.8914
Row[d]	11	0.91594	0.0001
Location[e]	4	1.83149	0.0001

[a] Study 1 (1992 to 1993) or 2 (1993 to 1994).
[b] Sampling date: soil samples were collected after irrigation; soil samples were collected after tillage; and soil samples were collected after second successive crop.
[c] Each 9 × 30-m plot was considered one replication.
[d] Each plot contained 12 rows, and all were sampled and assayed for BNYVV incidence.
[e] Each row contained five sampling locations, and all were assayed for BNYVV incidence.

TABLE 3. Response surface curves describing the spread of beet necrotic yellow vein virus in sugar beet plots from infested point sources after irrigation, harvest, and replanting

Model[a] terms	Parameter (SE) after irrigation		Parameter (SE) after harvest		Parameter (SE) after planting in year 2	
	Exp. 1 (1992)	Exp. 2 (1993)	Exp. 1 (1992)	Exp. 2 (1993)	Exp. 1 (1993)	Exp. 2 (1994)
I	–1.95 (0.03)	–2.06 (0.03)	–1.92 (0.08)	–1.87 (0.05)	–1.71 (0.07)	–1.97 (0.10)
D	–0.12 (0.01)	–0.11 (0.01)	–0.13 (0.02)	–0.11 (0.02)	–0.07 (0.03)	–0.07 (0.02)
A	–0.36 (0.02)	–0.38 (0.02)	–0.40 (0.06)	–0.32 (0.04)	–0.39 (0.06)	–0.43 (0.07)
$D \times A$	0.13 (0.01)	0.12 (0.01)	0.09 (0.02)	0.11 (0.01)	0.13 (0.02)	0.10 (0.02)
A^{2b}		0.01 (0.01)	0.03 (0.01)			0.04 (0.02)
R^{2c}	0.59	0.68	0.26	0.31	0.19	0.17

[a] The dependent terms of the model were log(enzyme-linked immunosorbent assay + 0.01) and intercept (I); the independent terms were D = log(distance down the row), and A = log(distance across the rows). The distances at the point sources were given a measurement of 0.1 so the natural log could be taken.
[b] A^2 is the quadratic term for distance across rows. There were no acceptable models with a quadratic term for distance down rows.
[c] Coefficient of determination.

DISCUSSION

The current study was designed to monitor the spread of viruliferous *P. betae* in virgin sugar beet ground from point sources of inoculum. This was intended to represent a small, accidental introduction of BNYVV and its subsequent spread within a field through conventional agronomic practices for the Texas Panhandle. Based on prior studies involving zoosporic fungi and the tremendously rapid spread of BNYVV throughout California sugar beet regions, the lack of movement of the virus due to irrigation was extremely surprising. It could be argued that viruliferous *P. betae* had moved throughout the plots, but possibly, virus levels in plants were too low to be detected by ELISA in the plants. To correct this potential problem, soil samples were collected from the rhizosphere and bioassayed in the greenhouse for 10 to 12 weeks under optimum conditions for disease development. Using this technique, even very low densities of viruliferous *P. betae* could be detected, confirming the extent of spread of the virus even if it went undetected in the plant assays. Therefore, we believe the reported results are accurate.

The results concerning effects of tillage and soil movement also were somewhat unexpected. There was not a large number of samples that tested positive for BNYVV compared to the total collected and assayed, but the ones detected were well distributed within the plots (Fig. 2). This illustrates that mechanical harvesting and subsequent tillage practices the next year (bed formation and insecticide and herbicide applications and incorporations) resulted in movement of the pathogen at least 23 m down the row and 5 to 6 m across rows from the point sources. After a second crop, the pathogen had been moved the entire length of the plots. No symptoms were ever seen on plants outside of the inoculated regions, but the presence of the virus was confirmed by assaying roots of bait plants grown in the soil samples collected from the field.

Because the virus was always present in point source regions, both in plant and soil samples, we know the pathogen was successful in becoming established in test plots. Therefore, environmental conditions were suitable for zoospore release and infection to occur, and the method of infesting plots also was effective. These results support a previous study (14) that evaluated this method of infesting seeds with viruliferous *P. betae* for use in rhizomania field studies.

Numerous other studies have been conducted to examine the epidemiology and ecology of zoosporic pathogens under field conditions. Several of these have revealed that disease severity and incidence were independent of initial inoculum densities (23,26, 27). Irrigation and rainfall had larger affects on time of onset and final disease incidence than did inoculum density of *Phytophthora capsici* (26).

Disease severity and symptomatology of *Phytophthora parasitica* on tomatoes under furrow irrigation, however, decreased as distance from inoculated areas increased (24). In the furrow-irrigation-*Phytophthora parasitica* system, Neher and Duniway (24) concluded that levels of root disease within one growing season were more related to inoculum levels in soil than to inoculum dispersal. Other studies involving zoosporic pathogens indicate similar results (25).

The *P. betae*-BNYVV complex appears to operate more in a density-dependent manner than in an inoculum density-independent system. Tuitert and Hofmeester (33) found that initial inoculum level and primary infection by *P. betae* cystosori were major factors in disease incidence, severity, and yield loss, regardless of irrigation. They also determined that under drip irrigation, a 10,000-fold increase of *P. betae* occurred during the first year a sugar beet crop was grown (32). This shows the potential for pathogen increase to occur wherever BNYVV inoculum is found in a field. However, drip irrigation by itself did not spread the virus to a great extent. Their results indicated that horizontal movement of BNYVV by means of zoospores was less than 5 cm. Dispersal of viruliferous *P. betae* through growth of roots away from infested sites, likewise, was limited (20 to 25 cm). This could help explain why in our study soil samples from certain locations tested positive for BNYVV in one sampling but not the next. If the sample was taken from as little as 30 cm away from the original sample, results could vary. This could have occurred by sampling on opposite sides of a bed. Tuitert (31) also showed that soil movement by tillage practices moved viruliferous *P. betae* at least 8 m after three successive crops of sugar beets. This was the furthest distance from inoculum sources that was tested. The experimental design, methods, and objectives of our study were different than those of Tuitert (31), yet our results correspond closely to and support the data collected by Tuitert (31).

Based on the results of this study, furrow irrigation plays much less of a role in the epidemiology of BNYVV in Texas than was believed previously. The soil type in this study was a silty clay loam and possibly could have contributed to reducing dispersal of the vector through irrigation water. Duniway (8) has shown that clay loam soils restrict dispersal of zoospores to a greater extent than do coarser soils, which indicates that pore spaces of most finer textured soils are too small to permit significant zoospore movement. The obligate nature of *P. betae* also could explain the lack of BNYVV detection after irrigation. Even if conditions were adequate for zoospore release and passive dispersal by furrow irrigation water, the zoospores would die unless they came into contact with a live host. *Phytophthora* spp. and other facultative saprophytic zoosporic pathogens would be much easier to detect in this manner because they colonize leaves, stems, and other residues (24). Also, Gerik and Duffus (12) demonstrated that six isolates of *P. betae* capable of acquiring and transmitting BNYVV differed in ability to infect sugar beet. Infection by some isolates resulted in significantly greater virus concentration in and damage to plants than others. However, it is unknown whether every viruliferous zoospore that infects a plant transmits the virus.

Plant samples from point source regions exhibited the typical symptoms of severe rhizomania infection. However, we observed no foliar or severe root symptoms of rhizomania on beets outside the point source regions, even in the successive crop in inoculated field plots, but viruliferous *P. betae* was confirmed only after bioassaying soil samples for the presence of BNYVV. This supports the hypotheses that development of symptoms and severe yield losses may be the result of BNYVV introduction as many as 10 to 15 years earlier (1,30). It has been suggested that two to three sugar beet crops must be grown before populations of *P. betae* and BNYVV are increased to the levels necessary to generate symptoms, unless initial contamination has been extensive (1). This could explain observations in Texas and Colorado, where sugar beet fields were infested with BNYVV, but severe rhizomania never developed, and yields were unaffected (28).

In conclusion, under the conditions of this study in the Texas Panhandle, BNYVV did not spread as rapidly as expected. One season's tillage practices distributed the pathogen to a greater extent around the field than did irrigation. Because spread appears to occur primarily through mechanical means and not by water, it is difficult to predict where new infections will occur with respect to known infested sites. Therefore, BNYVV's potential for destroying sugar beet crops is still a threat. This is evidenced by both the severe field losses experienced in California (10) and by growth-chamber experiments conducted under ideal environmental conditions (15). Every possible effort to reduce the introduction of BNYVV to new growing areas should be made to help insure the longevity of the sugar beet industry in the United States.

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