

Distribution of Beet Necrotic Yellow Vein Virus, Beet Distortion Mosaic Virus, and an Unnamed Soilborne Sugar Beet Virus in Texas and New Mexico

G. B. HEIDEL and C. M. RUSH, Department of Plant Pathology, The Texas A&M University System, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland 79012

ABSTRACT

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The Texas sugar beet-growing area was surveyed to determine the incidence of beet necrotic yellow vein virus (BNYVV), beet distortion mosaic virus (BDMV), and an unnamed soilborne sugar beet virus designated as Texas 7 (Tx7). In late 1990, 302 soil samples were collected from seven Texas counties and one New Mexico county from fields scheduled for 1991 production. Sugar beet seed was planted in the soil samples, and root tissue was later harvested and tested by ELISA. Of 174 soil samples screened for BNYVV, 19 were positive. Of 128 samples tested for BNYVV and Tx7, 12 were positive for Tx7, three were positive for BNYVV, and 23 were positive for both BNYVV and Tx7. In 1991, 159 soil samples were collected from around symptomatic beets. Root tissue from sugar beets grown in the soil samples were tested for BNYVV, Tx7, and BDMV. Twenty samples were positive for Tx7, 27 were positive for BNYVV, and 37 were positive for both Tx7 and BNYVV. Twelve of 72 sugar beets pulled at the time soil samples were collected were positive for BDMV. Sugar beets grown in soil samples collected from eight of the 10 Texas sugar beet-growing counties were positive for BNYVV. Tx7 and BDMV were identified in the three major Texas sugar beet-growing counties. BDMV was identified in one New Mexico county; this is the first report of BDMV in New Mexico. No soil samples, including those collected from around beets positive for BDMV, were positive for BDMV.

Beet necrotic yellow vein virus (BNYVV), a furovirus transmitted by *Polymyxa betae* Keskin, causes rhizomania, a severe disease of sugar beets (*Beta vulgaris* L.). Rhizomania is characterized by heavy lateral root proliferation, decreased taproot weight, and stunted and slightly yellowed top growth. Systemic BNYVV, seen rarely in infected beets, causes diagnostic vein yellowing, followed by veinal necrosis (6,11,29,30). BNYVV occurs in several European countries (20) and Japan (30). In the United States, BNYVV has been reported in California (9), Idaho (12), and Nebraska and Wyoming (15). BNYVV was reported in Texas in 1987 (8).

Since BNYVV was identified in Texas, two other sugar beet viruses in the state have been reported. Beet distortion mosaic virus (BDMV) (23), a flexuous rod with reported lengths ranging from 650 nm in leaf dips to 2,000 nm in purified preparations (10), causes distortion and mottling in infected leaves. The second virus, as yet to be named but referred to as Texas 7 (Tx7), is a member of a complex of viruses associated with BNYVV in sugar beets. Tx7 is morphologically similar to, but serologically different from, BNYVV (22). Like BNYVV, Tx7 is transmitted by *P. betae* (22; C. M. Rush, unpublished). Foliar

symptoms, when present, can include pale yellow vein-banding that sometimes progresses to bright yellow broad vein-banding in older leaves. Leaves can be mottled or slightly distorted. Taproots can appear healthy, unlike those of beets infected with BNYVV. It is not known if BDMV or Tx7 affects sugar beet yield or quality.

In the Texas Panhandle, sugar beets are a relatively small but economically important crop. Approximately 17,100 ha of sugar beets are grown in 12 counties in Texas and eastern New Mexico, and approximately 85% of the production is concentrated in three adjacent Texas counties: Castro, Deaf Smith, and Parmer. In 1987, when BNYVV was reported in Texas, it had been identified on one farm. This was assumed to be an isolated incident because there was no widespread occurrence of symptomatic beets in the area. However, because of the potential for severe economic damage to the sugar beet crop from infection by BNYVV, a survey was initiated in 1990 to determine the distribution of BNYVV in Texas and New Mexico. Midway through the survey, foliar symptoms were observed on sugar beets that had been transplanted in the greenhouse. Results of subsequent ELISA tests of symptomatic leaves indicated that Tx7 and BDMV occurred more frequently than had been suspected. To determine the distribution of these viruses, and whether they occurred with BNYVV in the field or coinfecting field sugar beets,

the survey was expanded to include BDMV and Tx7.

MATERIALS AND METHODS

The survey for BNYVV, Tx7, and BDMV in the Texas sugar beet-growing area was divided into three studies. The first two were soil surveys initiated in 1990 in cooperation with the Imperial Holly Sugar Corporation in Hereford, Texas. Soil samples were assayed for BNYVV in the first study and for BNYVV and Tx7 in the second study. The third study was a sugar beet and soil survey conducted in 1991. Beet and soil samples were screened for BNYVV, Tx7, and BDMV.

Soil survey for BNYVV, 1990. Soil samples were collected in late fall and early winter 1990 from fields scheduled for 1991 sugar beet production. The samples were collected by agronomists with the Imperial Holly Sugar Company. Soil was sampled from fields previously planted to sugar beets and suspected of being infested with BNYVV. Three to five subsamples from the upper 15 cm of soil were randomly collected in each field and bulked. Soil from each bulked sample was placed in 9-cm² square pots and planted with seed of sugar beet cv. HH39. Two replications of 174 bulked samples from seven Texas counties (Castro, Deaf Smith, Hale, Oldham, Parmer, Randall, and Swisher) and one New Mexico county (Roosevelt) were planted. The sugar beets were grown 9–12 wk in the greenhouse, adequate time to allow colonization by *P. betae* and infection by BNYVV if viruliferous *P. betae* was present in the soil (13). Roots were harvested, washed, and assayed serologically for BNYVV using anti-serum from several sources (Bioreba Inc., Chapel Hill, NC; Boehringer Mannheim Biochemicals, Indianapolis, IN; Agdia Inc., Elkhart, IN). Positive and negative controls were included in each test.

The procedure used was a modification of the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (7,26). Polyvinyl chloride (PVC) microtiter plates were coated with BNYVV immunoglobulin G (IgG) diluted per the manufacturer's recommendation in 0.1 M phosphate-buffered saline (PBS), pH 7.1. Except during the substrate reaction, plates were incubated at 37 C for 1 hr. Plates were washed 10 times between steps with wash buffer (WB) (0.02 M sodium phosphate buffer,

pH 7.6, with 0.015 M NaCl, 0.05% [v/v] Tween 20, and 0.00125% [w/v] thimerosal or 0.02% [w/v] sodium azide) and were incubated in a humidified box. After coating, wells were blocked with WB containing 1% bovine serum albumin (BWB). Except for the blocking step, in which the wells were filled, reagent and sample volumes were 50 μ l. Sample extracts were prepared by grinding tissue in BWB (1:10, w/v). Alkaline phosphatase-conjugated BNYVV IgG was diluted according to the manufacturer's recommendation in BWB. Substrate (*p*-nitrophenyl phosphate, 4.0 mg/ml in 10% diethanolamine, pH 9.8) was added, and plates were incubated in the dark at 25 C for 1–18 hr, until the positive controls reacted. Absorbance values at 410 nm were read with a Dynatech MR 300 microplate reader. Absorbance values equal to or greater than three times the average of the healthy control absorbance values were considered positive.

Soil survey for BNYVV and Tx7, 1990. A total of 128 unreplicated soil samples from Castro, Deaf Smith, and Parmer counties were tested for BNYVV and Tx7. Sugar beet bait plants were grown, processed, and tested for BNYVV as described above. Tx7 antiserum was provided by J. E. Duffus and H.-Y. Liu, USDA-ARS, Salinas, California. Roots were tested by ELISA for Tx7. Briefly, IgG to Tx7 was affinity purified using a protein-A sepharose column (P3391, Sigma, St. Louis, MO) (25,26). The secondary antibody was biotinylated (3). Plates were processed and the ELISA

procedure was carried out under conditions described above. The biotinylated secondary antibody was probed with avidin-conjugated alkaline phosphatase (ExtrAvidin, Sigma) diluted to 0.16 μ g/ml in BWB, and the substrate was added after the final wash. Tx7 IgG and biotinylated IgG were used at 9.0 or 5.0 μ g/ml.

Soil and sugar beet survey for BNYVV, Tx7, and BDMV, 1991. In late summer and early fall of 1991, rhizosphere soil was collected from around sugar beets that exhibited viruslike symptoms. Viruslike symptoms could have included foliar symptoms usually associated with BNYVV (including stunting, faint overall yellowing, or wilting), Tx7 (broad vein-banding, some mottling), or BDMV (distortion, mottling). Soil samples were also collected from around sugar beets exhibiting typical root symptoms caused by infection with BNYVV. Some sugar beets exhibited symptoms of vein yellowing and distortion unlike those caused by BNYVV, Tx7, or BDMV. If no symptomatic sugar beets were detected in a field, soil samples were collected randomly within the field. Symptomatic sugar beets were pulled and transplanted to pots in the greenhouse. Leaf tissue from 72 beets and root tissue from sugar beets grown in soil samples were later assayed by ELISA for BNYVV, Tx7, and BDMV. Foliar ELISA results were compared directly with ELISA results of plants grown in the soil sample collected from around them.

Three replications of 159 soil samples (inclusive of the 72 soil samples mentioned above) from nine Texas and two New Mexico counties were placed in cylindrical containers 4 cm in diameter and 17 cm long (Super Cell Cone-Tainer, Stuewe and Sons, Inc., Corvallis, OR). Sugar beets were grown in the soil samples, processed, and tested by ELISA for BNYVV and Tx7 as described above. Root samples were also tested for BDMV. BDMV antiserum was provided by J. E. Duffus and H.-Y. Liu. BDMV IgG and biotinylated IgG were prepared and used in ELISA as described for Tx7. Leaves of sugar beets collected in the field were tested for BDMV, BNYVV, and Tx7 by ELISA as described for root samples.

RESULTS

Soil survey for BNYVV, 1990. BNYVV was detected in 19 of 174 fields screened and was identified in sugar beets grown in soil samples collected from the three primary Texas sugar beet-growing counties (Table 1). ELISA results of bait plants indicated that BNYVV occurred in four of 29 fields sampled in Castro County, six of 84 fields sampled in Deaf Smith County, and four of 19 fields sampled in Parmer County.

Soil survey for BNYVV and Tx7, 1990. BNYVV was identified in bait plants grown in soil samples collected from Castro, Deaf Smith, and Parmer counties, and Tx7 was identified in Castro and Deaf Smith counties (Table 2). Sugar beets grown in only three of 128 soil samples screened were positive for BNYVV and negative for Tx7. Sugar beets grown in the remaining 23 soil samples that were positive for BNYVV were also positive for Tx7. Bait plants from 12 soil samples were positive for Tx7 and negative for BNYVV.

Soil and sugar beet survey for BNYVV, Tx7, and BDMV, 1991. As in the previous two studies, BNYVV was identified in Castro, Deaf Smith, and Parmer counties (Table 3). BNYVV was also identified, alone or in combination with Tx7, in Floyd, Hale, Oldham, Randall, Roosevelt (New Mexico), and Swisher counties. Tx7 was identified in Castro, Deaf Smith, Floyd, Parmer, and Randall counties. Among 159 samples tested, BNYVV and Tx7 were detected together in bait plants grown in soil samples more often than either virus alone. Beets grown in over one-half of the soil samples collected, either from around symptomatic sugar beets or randomly within a field, were positive for BNYVV or Tx7 or both. None of the sugar beet roots grown in soil samples was positive for BDMV.

When ELISA results from leaves of field-grown beets and bait plants grown in rhizosphere soil of the same beets were compared, BDMV was identified in leaf tissue from 12 field-grown plants but not in any bait plants. Sugar beets grown in six of these samples were negative for all three viruses, two were positive for Tx7, two were positive for BNYVV, and two were positive for both Tx7 and BNYVV (Table 4). BDMV was identified

Table 1. Soil samples collected in 1990 from fields scheduled for 1991 sugar beet production in Texas and New Mexico and screened for BNYVV^a

County ^b	No. tested/ no. positive ^c
Unknown	21/5
Castro (6,259; 36)	29/4
Deaf Smith (6,586; 38)	84/6
Hale (490; 3)	1/0
Oldham (157; 0.9)	6/0
Parmer (1,935; 11)	19/4
Randall (227; 1)	6/0
Roosevelt (NM) (162; 0.9)	5/0
Swisher (1,102; 6)	3/0
Total	174/19

^aThree to five soil samples were collected from each field and bulked.

^bNumbers in parentheses indicate total number of hectares in sugar beets in the county and percentage of total sugar beet production, based on hectareage, respectively. Information was provided by Imperial Holly Sugar Corporation, Hereford, Texas. Other sugar beet-growing counties in the area and their hectareage in sugar beets and percentage of total sugar beet production, respectively, are Curry, NM (137; 0.8), Lamb (106; 0.6), Floyd (18; 0.1), and Potter (4; 0.01).

^cHH39 sugar beet seed was planted in two replications of each sample, and root tissue was harvested 9–12 wk later and tested by ELISA.

Table 2. Soil samples collected in 1990 from fields scheduled for 1991 sugar beet production in Texas and screened for BNYVV and Tx7^a

County	No. tested ^b	No. positive ^c		
		BNYVV	Tx7	BNYVV + Tx7
Unknown	15	1	0	3
Castro	39	0	7	8
Deaf Smith	62	0	5	12
Parmer	12	2	0	0
Total	128	3	12	23

^aThree to five soil samples were collected from each field and bulked.

^bHH39 sugar beet seed was planted in each soil sample, and root tissue was harvested 9–12 wk later and tested by ELISA.

^cValues in the BNYVV and Tx7 columns do not include samples positive for BNYVV + Tx7.

in four Texas counties (Castro, Deaf Smith, Parmer, and Floyd) and one New Mexico county (Roosevelt). Foliar BDMV did not occur with foliar Tx7 or foliar BNYVV in this survey.

Foliar Tx7 was detected in two of 72 sugar beets tested but in none of the corresponding soil samples (Table 4). Foliar BNYVV was detected in one sugar beet. The leaf tissue of that sugar beet was also positive for Tx7, and sugar beets grown in the soil sample collected from around that beet were positive for BNYVV and Tx7. Viruses were often detected in bait plants grown in rhizosphere soil from plants testing negative for foliar infection. Sugar beets grown in 25 of the 72 soil samples tested positive for Tx7 or BNYVV or both, even though foliar tests were negative.

Positive results of soil samples tested in 1990 and 1991 were combined, and among 461 samples, sugar beets grown in 11% of soil samples were positive for BNYVV, 7% were positive for Tx7, and 13% were positive for both BNYVV and Tx7 (Table 5).

DISCUSSION

BNYVV was identified in eight and Tx7 was identified in five of the 10 sugar beet-growing counties in the Texas Panhandle. Approximately 85% of the sugar beets in the area are grown in Castro, Deaf Smith, and Parmer counties, and BNYVV and Tx7, alone or in combination, were identified in each county. Both viruses are more widespread in the area than was thought when the survey was initiated. When a field is initially infested with BNYVV, inoculum levels may be so low that effects on the crop may go unnoticed. By the time inoculum levels increase to the point that yield is reduced or symptoms are evident, BNYVV has probably been spread throughout the field (21). In Texas, sugar beets are typically grown in 4- to 5-yr rotations. It is likely BNYVV was in Texas well before 1987, when it was first reported.

BNYVV was reported in 1992 in Idaho (12) and in 1989 in Britain (4). In Idaho, a survey of 354 fields in nine counties indicated that BNYVV was present in only 27 fields, all located in an area of about 7 mi in diameter. By 1990 in Britain, rhizomania had been identified five times since 1984 (5). Steps taken in Idaho to slow the spread of BNYVV include wearing protective footwear, cleaning equipment leaving the field, and posting no-trespassing signs. Sugar beets grown in known infested fields are not purchased by the local sugar processing plant, and sugar beets grown in fields within a 1-mi radius of infested fields are not purchased unless a soil survey indicates no BNYVV is present (12). In Britain, no sugar beets are grown on farms where BNYVV is identified, equipment is cleaned between fields, and movement of farm animals is restricted.

The crop is destroyed if BNYVV is identified, and the grower is compensated for its estimated value (27).

In California, BNYVV was reported in 1984 and was identified on over 30,000 ha by 1989. Crop losses have been reduced primarily by avoidance, planting cultivars tolerant to BNYVV, and fumigating with 1,3-dichloropropene (Telone II) until its use was suspended in 1990 (2,16,19,24).

Because of the widespread distribution of BNYVV in Texas, as in California, quarantines are pointless. Still, measures

are warranted to slow further spread of the pathogen. Tare soils should not be returned to fields and equipment should be cleaned before going from one farm to the next.

Because cystosori of *P. betae* can remain viable and viruliferous in soil for years (1), crop rotations are of limited value in controlling BNYVV. In fields suspected of being infested with BNYVV, BNYVV-tolerant sugar beet cultivars should be grown. However, the success of these cultivars will depend on their tolerance to the numerous soilborne

Table 3. Soil samples collected in 1991 from sugar beet fields in Texas and New Mexico and screened for BNYVV, Tx7, and BDMV^a

County	No. tested ^b	No. positive ^c			
		BNYVV	Tx7	BNYVV + Tx7	BDMV
Unknown	2	0	2	0	0
Castro	33	3	4	18	0
Curry (NM)	10	0	0	0	0
Deaf Smith	14	6	1	3	0
Floyd	5	0	1	1	0
Hale	8	2	0	0	0
Lamb	2	0	0	0	0
Oldham	7	1	0	0	0
Parmer	52	12	10	14	0
Randall	10	0	2	1	0
Roosevelt (NM)	7	1	0	0	0
Swisher	9	2	0	0	0
Total	159	27	20	37	0

^aSoil samples were collected from the rhizosphere of beets that appeared symptomatic for BNYVV, Tx7, or BDMV. If no symptomatic beets were found in a field, soil samples were collected randomly.

^bHH39 sugar beet seed was planted in three replications of each soil sample, and root tissue was harvested 9–12 wk later and tested by ELISA.

^cValues in the BNYVV and Tx7 columns do not include samples positive for BNYVV + Tx7.

Table 4. Number of samples of leaves of field-grown sugar beets and of roots of bait plants grown in rhizosphere soil of the same field-grown beets with positive ELISA results^a

Viruses detected in leaves of field beets	Viruses detected in roots of bait plants				
	Tx7	BNYVV	BNYVV + Tx7	BDMV	None
Tx7	0	0	0	0	2
BNYVV	0	0	0	0	0
BNYVV + Tx7	0	0	1	0	0
BDMV	2	2	2	0	6
None	9	8	8	0	0

^aRhizosphere soils were collected from around 72 symptomatic (foliar or root) plants, and the plants were transplanted in the greenhouse. Leaf tissue was tested by ELISA, and the results were compared with ELISA results of roots of beets grown in rhizosphere soil collected from around the symptomatic field plants. Samples positive for Tx7 and BNYVV do not include those positive for BNYVV + Tx7.

Table 5. Soil samples collected in 1990 and 1991 and screened for BNYVV and Tx7

County	No. tested ^a	No. positive ^b		
		BNYVV	Tx7	BNYVV + Tx7
Castro	101	7	11	26
Deaf Smith	160	12	6	15
Parmer	83	18	10	14
Others combined	117	12	5	5
Total	461	49	32	60
Percent positive samples		11	7	13

^aSoil samples collected in 1990 were from fields scheduled for 1991 sugar beet production. Rhizosphere soil samples collected in 1991 were from around sugar beets that exhibited virus-like symptoms in roots or leaves. HH39 sugar beet seed was planted in the soil samples, and root tissue was harvested 9–12 wk later and tested by ELISA.

^bValues in the BNYVV and Tx7 columns do not include samples positive for BNYVV + Tx7.

fungal pathogens that are prevalent in Texas (17,28). Protecting sugar beet seedlings from infection by *P. betae* and BNYVV during the first few weeks of plant growth may also help improve yields. This can be accomplished by planting early when soil temperatures are too low for fungal activity or by transplanting sugar beets into the field (5,16). Transplanting beets is an economically viable option used in Japan (5), but no economic studies of the practice have been conducted in the United States.

The effects of BNYVV on sugar beets and its potential for damage to a beet crop have been well documented (6,11,18, 24,29,30), but the effect of Tx7 on beets is unknown. The significance of the distribution of Tx7 and its occurrence with BNYVV will not be realized until its effects on sugar beet yield and quality have been determined. Sugar beets can be infected by both BNYVV and Tx7, but it is not known if *P. betae* cotransmits BNYVV and Tx7 or if mixed populations of viruliferous *P. betae* occur. Beets positive for BDMV were identified in four Texas counties and one New Mexico county. This is the first report of BDMV in New Mexico. BDMV was not detected in sugar beets grown in any soil samples, including rhizosphere soils collected from around beets that were positive for BDMV. Attempts made since this study was completed to transmit BDMV by *P. betae* and to bait BDMV out of field soil samples have not been successful. BDMV is not vectored by *P. betae*. Some bait plants grown in rhizosphere soils collected from around beets positive for BDMV were positive for Tx7 or BNYVV or both. These three viruses occurred together in fields, but it was not determined if BDMV coinfects field beets with Tx7 or BNYVV. Foliar Tx7 or BNYVV was not identified in leaf tissue infected with BDMV in this study.

BNYVV rarely moves systemically in field sugar beets (14). In this survey, any BNYVV detected in leaf tissue occurred with Tx7. Although ELISA results of the foliar assay presented here do not indicate it, Tx7 systemically infects field sugar beets more frequently than BNYVV (*personal observation*). In an earlier foliar assay of 58 symptomatic sugar beets maintained in the greenhouse, 58% were positive for Tx7, 5% were positive for BNYVV, and 26% were positive for both Tx7 and BNYVV (*unpublished*). BNYVV, when it was systemic, occurred five times more often with Tx7 than it did alone in leaf tissue. This may indicate that Tx7 somehow facilitates the move-

ment of BNYVV from root to leaf tissue.

In this study, foliar Tx7 or foliar BNYVV and Tx7 combined were detected in only three of 72 sugar beets tested (4%). Unlike sugar beets infected with BDMV, in which leaves remain consistently symptomatic, foliar symptoms associated with BNYVV and Tx7 have been observed to fade and reappear in sugar beets maintained in the greenhouse. Day length, temperature, or maintenance in relatively small containers for long periods of time may contribute to the appearance of symptomatic leaf tissue.

Although foliar symptoms caused by Tx7 are observed more frequently in the field than those caused by BNYVV, Tx7 apparently does not move systemically as a regular or sustained part of its infection process. A better test to determine coinfection by Tx7 and BNYVV in field beets would be useful.

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