Using Enzyme-Linked Immunosorbent Assay to Identify Beet Leafhopper Populations Carrying Beet Curly Top Virus

D. L. MUMFORD, Plant Pathologist, U.S. Department of Agriculture, Agricultural Research Service, Utah State University, Logan 84322

ABSTRACT

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Beet leafhoppers carrying beet curly top virus acquired from feeding on infected plants or on extracts from infected plants were readily identified by enzyme-linked immunosorbent assay. Results correlated very well with a bioassay consisting of caging leafhoppers individually on sugar beet seedlings and determining percentage of infection. This method was used to compare virus levels in field populations of beet leafhoppers.

Additional key words: *Circulifer tenellus*

A major control measure for curly top disease in sugar beets, tomatoes, beans, and cucurbits in California consists of locating large populations of the beet leafhopper vector (*Circulifer tenellus* Baker) and reducing their number with insecticide sprays. The effectiveness of this control measure could be increased if populations of leafhoppers that carry the highest level of beet curly top virus (BCTV) could be quickly identified. Attempts to identify leafhoppers carrying BCTV using fluorescent antibody staining were not successful (6), probably because the virus does not multiply in the insect and is present in very low concentrations (1). Enzyme-linked immunosorbent assay (ELISA) has been used to detect cucumber mosaic virus in aphid vectors (3). Because of the sensitivity of this method, it was investigated for identifying beet leafhoppers carrying BCTV. This paper reports the use of ELISA in detecting BCTV in beet leafhoppers and its application to the curly top control program in central California.

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MATERIALS AND METHODS

Antiserum against BCTV was obtained from rabbits as described previously (5). The antiserum had a dilution end point of 512 when tested by agar double diffusion. The ELISA method of Clark and Adams (2) was used with only slight modification. The globulin fraction of the antiserum was precipitated with ammonium sulfate and conjugated to alkaline phosphatase. The protein concentration of the globulin was adjusted to 1.6 mg/ml before use in conjugation or for coating plates. Polystyrene plates (Dynatech Laboratories, Inc., Alexandria, VA 22314) containing 96 wells were coated with globulin diluted 1:20,000 in sodium carbonate buffer by incubation at 37 C for 4 hr. After coating, plates were stored at 5 C until used. Sensitivity was increased and occurrence of occasional erratic readings was reduced if phosphate-buffered saline containing 0.05% Tween 20, 20% polyvinylpyrrolidone (mol wt 40,000), and 1% bovine serum albumin (PTPB) was incubated in the plate wells for 1 hr at 37 C before and after incubation of the test sample.

Extracts for testing were prepared by grinding leafhoppers in 0.6 ml of PTPB in 2-ml glass tissue grinders. Extracts were introduced directly into coated wells and incubated overnight at 37 C. Conjugate diluted 1:1,000 in PTPB was incubated in plate wells for 4 hr at 37 C after incubation of the extracts. The substrate *p*-nitrophenyl phosphate was next incubated in each well for 1 hr at room temperature. The resulting dephosphorylation reaction was stopped by the addition of 3 M sodium hydroxide, and absorbance at 405 nm (A_{405}) was determined for the contents of each well with a spectrophotometer.

Viruliferous leafhoppers were obtained

Table 1. Enzyme-linked immunosorbent assay values of extracts from beet leafhoppers after acquisition access on a sugar beet plant infected with beet curly top virus

No. of leafhoppers extracted/sample	Values after acquisition time (hr)[a] 0	1	4	8	24
1	0.007	0.007	0.007	0.008	0.017[b]
5	0.02	0.02	0.09*	0.11*	
10	0.07	0.09	0.11	0.15*	
20	0.11	0.13	0.17	0.72*	

[a] Values (A_{405}) are averages of 20 readings where leafhoppers were assayed singly and 10 readings where leafhoppers were assayed in groups of 5, 10 or 20.
[b] * = Significantly different ($P = 0.05$) from control (0 hr).

Table 2. Comparison of enzyme-linked immunosorbent assay (ELISA) and infectivity assay for detection of beet curly top virus in beet leafhoppers

Dilution fed to leafhoppers[a]	Leafhoppers giving ELISA values >0.05 (%)[b]	Infection in seedlings (%)[c]
Control[d]	0	0
1:324	0	0
1:162	3	5
1:54	17	16
1:18	43	47
1:6	77	83

[a] Seventy leafhoppers were fed on each dilution of clarified extract for 24 hr.
[b] Thirty leafhoppers assayed individually.
[c] Forty leafhoppers caged individually on sugar beet seedlings.
[d] Control consisted of leafhoppers fed on extraction medium without beet curly top virus.

either by allowing them to feed on infected sugar beet plants or feeding them clarified extracts from infected tobacco plants through a Parafilm membrane (4). Infected sugar beets were obtained by inoculating 5-wk-old sugar beet plants and allowing 3 wk for symptom development. Leafhoppers caged for different periods of time on the infected sugar beets were assayed either individually or in groups of 5, 10, or 20 and compared with similar leafhoppers fed on healthy plants.

Viruliferous leafhoppers for comparing ELISA and a plant assay were obtained by feeding them for 24 hr on different dilutions of a clarified extract from infected tobacco. The plant assay consisted of caging the leafhoppers singly on 3-wk-old susceptible sugar beet seedlings for 5 days and determining percentage of infection 3 wk later.

To evaluate field populations of beet leafhopper, 30 leafhoppers were collected from each reproduction site in California on each of 10 dates and sent frozen in dry ice to my laboratory. The leafhoppers were assayed in groups of 10 and compared with similar assays of leafhoppers that had been feeding on healthy plants.

RESULTS AND DISCUSSION

BCTV was detected in groups of 20, 10, or 5 beet leafhoppers after an acquisition period of 8 hr on a BCTV-infected sugar beet and in single leafhoppers after an acquisition period of 24 hr (Table 1). These results suggested that field populations of leafhoppers could be monitored for BCTV.

Additional tests were conducted to compare the detection of BCTV in single leafhoppers by ELISA and by a plant infectivity assay. Previously, the only way to detect viruliferous leafhoppers was to cage them on seedlings, which required 2–3 wk. Leafhoppers were fed on dilutions of a virus extract because this was a more precise method of obtaining leafhoppers carrying different amounts of virus. The percentage of these leafhoppers that gave ELISA values greater than an arbitrarily selected value of 0.05 were compared with the percentage infection that similar leafhoppers produced when caged individually on susceptible sugar beet seedlings. The 0.05 value was selected because the percentage of leafhoppers exceeding this value ranged from 77% when fed a 1:6 dilution to zero when fed a 1:324 dilution of virus extract.

ELISA results correlated well with infectivity data (Table 2) and support the earlier finding that BCTV can be detected in single leafhoppers by ELISA.

BCTV in field populations of leafhoppers from 21 and 38 sites in California was monitored by ELISA during 1979 and 1980, respectively. Sites selected were those annually treated with insecticides to reduce leafhopper populations. Three groups of 10 leafhoppers were tested from each site on 10 different dates (January to April) each year; controls were nonviruliferous leafhoppers raised in the greenhouse. Leafhoppers from two sites in 1979 consistently gave ELISA values that were more than double those of nonviruliferous controls. These two sites received additional treatment during the insecticide spray period. All other groups of leafhoppers assayed in 1979 and 1980 gave ELISA values less than double those of controls and were not considered a threat to crops.

Curly top disease in sugar beet, tomato, and other susceptible crops in central California was not considered to be of economic importance in 1979 or 1980. ELISA determinations of low levels of BCTV in field populations of beet leafhoppers corresponded to low levels of disease during subsequent crop seasons.

Curly top disease is noted for its unpredictable, sporadic, severe outbreaks. This is because unpredictable environmental conditions influence leafhopper reproduction, time of migration, and extent of virus acquisition. An abundance of viruliferous leafhoppers is essential for a severe disease outbreak. If high levels of BCTV were detected in field populations of leafhoppers, it would be advisable to utilize all available protective measures. If, on the other hand, very low levels of virus were detected in leafhopper populations before planting, costly protective measures may be avoided during those years. Data for additional years, similar to those obtained in 1979 and 1980, are needed to develop confidence that monitoring virus content of leafhoppers can be used to predict high or low potential for a curly top disease outbreak.

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