Application of the Latex Flocculation Serological Assay to Curly Top Virus

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ABSTRACT

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Antiserum produced against curly top virus (CTV) was used to develop a latex flocculation (LF) assay for the virus. The LF assay was 100 times more sensitive than double diffusion in agar but was less sensitive than a plant infectivity assay. Results from the plant infectivity assay are obtained in 2 wk, whereas the LF assay can be completed in 1 hr with sensitized latex prepared in advance. By using the LF assay to measure virus concentration in extracts of diseased tissue, tobacco was found to contain much higher concentrations of CTV than sugar beet, tomato, or bean.

Additional key words: beet curly top virus, agglutination, antibody.

Curly top virus (CTV) has no known local lesion host. During CTV purification (7), assays to monitor virus concentration required feeding virus preparations to the leafhopper vector, caging the insects on susceptible plants, then waiting for symptoms to appear. This plant infectivity assay is laborious and requires about 2 wk to obtain results. The purification of CTV permitted the production of an antiserum suitable for developing a serological assay.

The relative concentration of antibody in antiserum produced against CTV may be measured by agar diffusion. However, a more sensitive serological assay was desirable for detecting and estimating concentrations of CTV. One such assay is the latex flocculation (LF) method first used by Singer and Plotz (8) in the diagnosis of rheumatoid arthritis. Since that report, latex particles coated with the gamma-globulin fraction of specific plant virus antisera have been used to detect and study serological relationships of several plant viruses (1, 2, 4, 5). Application of the LF method in CTV research is reported here. A preliminary report of this work has been made (6).

MATERIALS AND METHODS

Curly top virus was purified from *Nicotiana tabacum* L., using procedures previously described (7). Rabbits were immunized with four weekly intramuscular injections of 1 ml of antigen homogenized with an equal amount of Freund's complete adjuvant. Four wk after the final injection and for several months thereafter, serum was collected at biweekly intervals. Antisera from several collections were combined and frozen for use in this study. When tested by agar double diffusion, the dilution end point of the combined antiserum was 512.

With some modification as described in the Results section, the procedure outlined by Bercks et al. (3) was followed for coating latex particles (0.81 μm) with the separated gamma-globulin fraction of the CTV antiserum. The globulin fraction of the antiserum was obtained by precipitation with ammonium sulfate. Globulin dilutions from 1:500 to 1:1,200 were compared when adsorbed onto latex and a dilution of 1:800 gave maximum sensitivity. The resulting sensitized latex suspension was stored at 5 C until used.

Twofold serial dilutions of the antigen were made in 96-well, polystyrene agglutination plates, using the Microtiter system of Cooke Laboratory Products, Alexandria, VA 22314. The sensitized latex suspension was added to each dilution of antigen, and the plates were shaken for 50 min at 200 rpm on a rotary shaker (Model 0531; Fermentation Design, Inc., Allentown, PA 18103) at a temperature of 30-32 C. The wells of the agglutination plate were examined with a binocular microscope at ×30 to determine the highest dilution at which flocculation of the latex particles occurred. The following controls all gave negative results, or results easily distinguished from positive: (i) latex sensitized with globulin from normal serum vs. antigen; (ii) nonsensitized latex vs. antigen; and (iii) antibody-sensitized latex vs. the buffer used to prepare the antigen dilutions.

The LF assay was used to measure CTV concentration in infected tissue of each of four hosts. Seedlings of bean (*Phaseolus vulgaris* L. 'Tendercrop'), beet (*Beta vulgaris* L. 'US 33'), tomato (*Lycopersicon esculentum* Mill. 'Moscow'), and tobacco (*Nicotiana tabacum* L. 'Turkish') were inoculated with viruliferous leafhoppers when the plants were 7-, 28-, 28-, and 35 days old, respectively. Fifteen days after inoculation 5g of severely diseased tissues were harvested, using several plants of each host. An extraction from the tissue was clarified and concentrated as reported previously (7). Dilution end points, determined with the LF assay, were taken as a measure of relative virus concentration in each host.

RESULTS

The appearance of a positive flocculation reaction is compared to a negative reaction in Fig. 1. The transition from positive to negative reaction, indicating the dilution end point, usually was abrupt; however, an intermediate reaction showing a reduced amount of flocculation sometimes occurred. Unless the virus concentration was high, some difficulty was encountered in classifying the reaction when crude extracts were tested. At high virus concentrations, flocculation was reduced, or absent, in the first few wells of the dilution series, presumably because of antigen excess.

Effect of temperature, shaking time, and amount of sensitized latex used on sensitivity of latex flocculation assay.—The results reported in Tables 1, 2, 3, and 4 were based on experiments using virus preparations prepared at different times and having different concentrations. The virus preparations were extracted and clarified from diseased tobacco as previously reported (7). Positive reactions occurred at higher dilution end points at 30 and 32 C than at either higher or lower temperatures (Table 1). At temperatures below 28 C, it became increasingly difficult to identify positive reactions because of reduced flocculation.

Based on the results shown in Table 2, 50 min of shaking time was required for maximum sensitivity. The amount of sensitized latex used greatly influenced sensitivity of the LF assay (Table 3). The microtiter serial dilution system used in these studies restricted the quantity of each antigen dilution to 0.025 ml. Highest dilution end points were obtained when 0.004 ml of

TABLE 1. Effect of temperature on the dilution end point of a curly top virus preparation[a] assayed by the latex flocculation test[b]

Temp (C)	Flocculation of virus in preps at a reciprocal dilution of:				
	128	256	512	1,024	2,048
24	–[c]	–	–	–	–
28	+	+	+	–	–
30	+	+	+	+	–
32	+	+	+	+	–
34	+	+	+	–	–

[a]An extract from diseased tobacco tissue clarified with chloroform and butanol.
[b]Shaking time was 1 hr.
[c]Symbols: – = no flocculation; + = flocculation present.

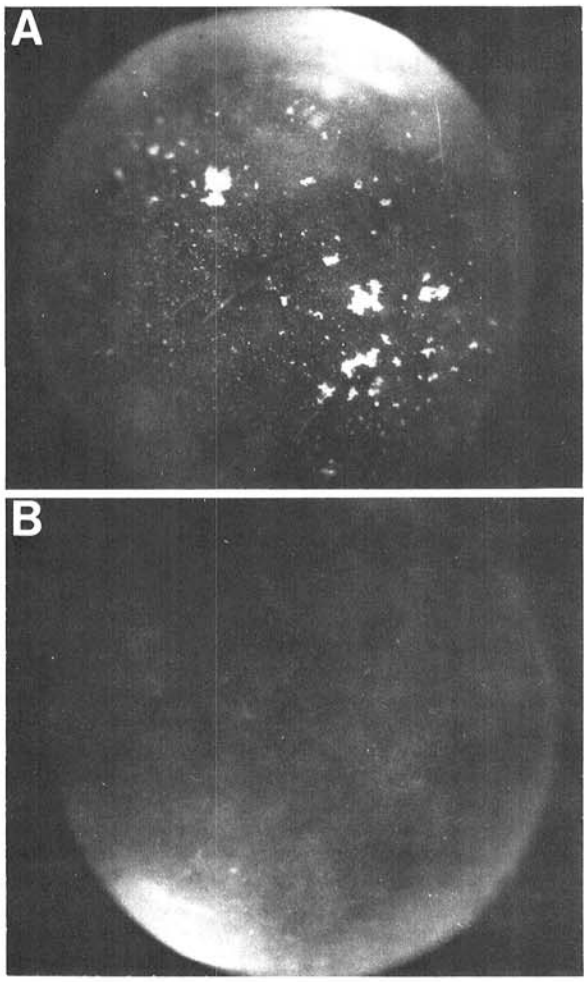

Fig. 1-(A, B). Latex flocculation reaction for curly top virus; **A)** positive flocculation in well of plastic plate (×30); **B)** negative reaction.

TABLE 2. Effect of shaking time on the dilution end point of a curly top virus preparation[a] assayed by the latex flocculation test[b]

Shaking time (min.)	Flocculation of virus in preps at a reciprocal dilution of:						
	2	4	8	16	32	64	128
20	–[c]	–	–	–	–	–	–
30	–	–	–	–	–	–	–
40	+	+	+	+	+	–	–
50	+	+	+	+	+	+	–
60	+	+	+	+	+	+	–
70	+	+	+	+	+	+	–
80	+	+	+	+	+	+	–
90	+	+	+	+	+	+	–

[a]An extract from diseased tobacco tissue clarified with chloroform and butanol.
[b]Temperature was 30-32 C.
[c]Symbols: – = no flocculation; + = flocculation present.

sensitized latex was mixed with each 0.025-ml antigen dilution.

Comparison of the sensitivity of latex flocculation, agar diffusion, and plant infectivity assays.—The LF assay was over 100 times more sensitive than an agar double diffusion assay, and a plant infectivity assay was four times more sensitive than the LF assay in detecting CTV (Table 4). The agar and plant infectivity assays required 24-48 hr and 10-14 days, respectively, to obtain results. In contrast the LF assay could be completed in 1 hr, using sensitized latex prepared in advance.

Use of latex flocculation assay to compare CTV concentration in four hosts.—Based on assays of clarified extracts from diseased tissue, the concentration of CTV was four times higher in tobacco than in bean and eight times higher than in sugar beet or tomato (Table 5). Preliminary tests of cultivars of sugar beet differing in resistance to CTV indicated that resistant cultivars had lower concentrations of virus than susceptible ones.

DISCUSSION

The LF assay of CTV was not as sensitive as a plant infectivity assay which, until now, has been the only reliable method of assay for this virus. But the LF assay offers two major advantages over plant infectivity assays. First, results can be obtained with the LF assay in 1 hr, whereas the plant infectivity assay requires 2 wk. Secondly, much of the variability encountered in the plant infectivity assay, due to differences in seedling development and growing conditions during and after inoculation with leafhoppers, is eliminated using the LF assay.

My studies indicated that, to obtain maximum sensitivity with the LF assay the optimum dilution of globulin for adsorption onto latex should be determined for each new batch of sensitized latex prepared. Also the optimum amount of sensitized latex added to each antigen dilution may differ slightly with different batches

TABLE 3. Effect of amount of sensitized latex added to each virus dilution on the dilution end point of a curly top virus preparation[a] assayed by the latex flocculation test[b]

Amount of sensitized latex (ml)	Flocculation of virus in preps at a reciprocal dilution of:						
	2	4	8	16	32	64	128
.001	–[c]	–	–	–	–	–	–
.002	–	+	–	–	–	–	–
.003	+	+	+	+	+	–	–
.004	+	+	+	+	+	+	–
.005	+	+	+	+	+	–	–
.010	+	+	+	+	–	–	–
.015	+	+	+	+	–	–	–
.200	+	+	+	–	–	–	–

[a]An extract from diseased tobacco tissue clarified with chloroform and butanol.
[b]Shaking time was 50 min and temperature was 30-32 C.
[c]Symbols: – = no flocculation; + = flocculation present.

TABLE 4. Comparison of sensitivity of latex flocculation, agar diffusion, and plant infectivity assays in detecting curly top virus

Type assay	Flocculation of virus in preps at a reciprocal dilution of:										
	2	4	8	16	32	64	128	256	512	1,024	2,048
Agar diffusion	+	–	–	–	–	–	–	–	–	–	–
LF	+	+	+	+	+	+	+	+	–	–	–
Infectivity	+	+	+	+	+	+	+	+	+	+	–

TABLE 5. Use of the latex flocculation assay to compare curly top virus concentration in four hosts

Host	Flocculation of virus in preps at a reciprocal dilution of:[a]							
	2	4	8	16	32	64	128	256
Tomato	+[b]	+	+	+	–	–	–	–
Sugar beet	+	+	+	+	–	–	–	–
Bean	+	+	+	+	+	–	–	–
Tobacco	+	+	+	+	+	+	+	–

[a]Extracts from 5 g of diseased tissue clarified with chloroform and butanol. The test was repeated five times with similar results.
[b]Symbols: + = flocculation present; – = no flocculation.

of sensitized latex. In my studies, as in others, the sensitized latex was stable when kept at 5 C for many months.

The results of comparing CTV concentration in different hosts are also in agreement with preliminary tests which led to the selection of tabacco as the best host for producing quantities of this virus for purification (7).

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