Location of Curly Top Virus Antigen in Bean, Sugarbeet, Tobacco, and Tomato by Fluorescent Antibody Staining

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ABSTRACT

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The fluorescent antibody staining method was used to locate curly top virus antigen in bean, sugarbeet, tobacco, and tomato. Antigen was found only in phloem tissue. The occurrence of specific staining corresponded to the appearance of foliar symptoms. Susceptible tomato plants had more than eight times as many specifically stained sites as did resistant plants. No localized staining was observed in viruliferous leafhoppers although a generalized fluorescence was detected.

Additional key words: serology, fluorescein, fluorescence microscopy, conjugates, resistance.

Much has been learned about the distribution of curly top virus (CTV) in host plants from vector transmission techniques and histological studies. The virus is restricted to the phloem (1). Application of the fluorescent antibody (FA) method to CTV would provide direct evidence to support this conclusion and also would permit valuable comparisions of virus distribution and movement in resistant and susceptible hosts. Use of the FA method to identify viruliferous sugarbeet leafhoppers would be of great value in evaluating the threat to the sugarbeet, tomato, and bean crops from natural populations of the vector.

An antiserum suitable for serological tests has been produced against CTV (4, 5). This antiserum was used to locate CTV antigen by the FA method (8, 9, 10) in diseased plant tissue and in the leafhopper vector, *Circulifer tenellus* (Baker). A preliminary report on this work has been made (6).

MATERIALS AND METHODS

Seed of bean (*Phaseolus vulgaris* L.), susceptible cultivar Tendercrop and resistant cultivar Apollo, supplied by Matt Silbernagel, Prosser, Washington; sugarbeet (*Beta vulgaris* L.) cultivar US33; tobacco (*Nicotiana tabacum* L.) cultivar Turkish; and tomato (*Lycopersicon esculentum* Mill.) susceptible cultivar Moscow and resistant cultivar C5, supplied by Mark Martin, Prosser, Washington, were used. Seedlings of each cultivar were inoculated with CTV when 7, 28, 35, and 28 days old, respectively. Inoculation was accomplished by caging viruliferous leafhoppers on the seedlings for 3 days.

Freshly harvested stem or petiole tissue from inoculated and noninoculated plants was embedded in a glycerol-gelatin mixture and then quick-frozen in acetone and dry ice (2). Twenty percent gelatin was dissolved in a 5% glycerol-in-water solution and the temperature was adjusted to slightly above the melting point. Tissue was embedded as the glycerol-gelatin mixture was cooled in small, plastic containers. Quick freezing was accomplished by pressing the plastic, embedding container into a thick slurry of dry ice and acetone. Sections 10-20 μm thick were cut within 48 hr in a cryostat at −15 C and mounted directly on glass slides. Sections were air-dried, then stored at 5 C until used.

Similar sections were prepared from nonviruliferous leafhoppers and from leafhoppers that had fed overnight through a membrane on concentrated preparations of purified CTV. In addition, whole leafhoppers, salivary glands, or intestines were smeared on glass slides for examination.

The globulin fraction of CTV antiserum was separated by ammonium sulfate precipitation (9). The optimal labeling method (9) was used to conjugate the globulin with fluorescein isothiocyanate isomer I (FITC) (BBL, Div. Becton Dickinson Co., Cockeysville, MD). The conjugate was passed through Sephadex to remove the free FITC, and then aliquots were stored at −20 C until used for staining. A similar conjugate was prepared with globulin from normal rabbit serum to be used as a control.

Tissue sections were stained by covering them with a few drops of the FITC-antiserum conjugate and incubating the preparation at 35 C for 2 hr in a moist chamber. The stained sections were rinsed with phosphate-buffered saline (PBS), pH 7 and mounted in a glycerol-phosphate mounting medium. The mounting medium was prepared by adding 10 ml of PBS to 90 g of

TABLE 1. Comparison of the occurrence of foliar symptoms and fluorescent antibody (FA) staining in sections from resistant and susceptible bean and tomato plants inoculated with curly top virus

Host	No. inoculated	No. with foliar symptoms	No. with positive FA staining	(Average no.) positive sites per tissue section[a]
Bean				
Susceptible	20	20	20	59
Resistant	20	0	0	0
Tomato				
Susceptible	20	18	18	35
Resistant	20	9	7	4

[a]Number of positive sites per tissue section was based on observations of 10 sections for each of the 20 plants inoculated. Sections from resistant and susceptible plants were approximately the same diameter.

glycerol. Observations were made with a Zeiss Universal fluorescence microscope employing vertical illumination and barrier filters 50/44 and exciter filter BG12. Photographs were made with Ektachrome-X film.

Initially, sections were counterstained before mounting with a PBS solution containing 0.03 mg/ml of tetramethyl-rhodamine isothiocyanate isomer R. This was done in an attempt to quench the autofluorescence of cell walls that was particularly troublesome in sugarbeet sections. It was determined later that changing from a commercial mounting medium to the mounting medium described here reduced autofluorescence and counterstaining was not considered helpful.

RESULTS

Specific FA staining was observed only in diseased phloem tissue of all four hosts examined (Fig. 1). Results with bean and tomato were similar to those illustrated for sugarbeet and tobacco. No specific staining was observed in healthy tissue stained with FITC-antiserum conjugate or in diseased tissue stained with FITC-normal serum conjugate. Specific fluorescence was a characteristic blue-green color which could be distinguished readily from a yellow-green autofluorescence. Cell walls of xylem vessels exhibited a bright-yellow autofluorescence.

The shortest length of time from inoculation to observation of specific staining was 5, 5, 6, and 8 days for bean, sugarbeet, tobacco, and tomato, respectively. This is also the sequence of occurrence of first foliar symptoms in the four hosts.

When resistant cultivars of bean and tomato were compared with susceptible ones (Table 1), FA staining only occurred in tissue from plants showing foliar symptoms. Also, in resistant tomato where approximately one-half of the inoculated plants developed foliar symptoms, the number of sites with FA staining was much lower than in comparable susceptible plants.

No localized specific staining was observed in sections prepared from viruliferous leafhoppers. Smears of intestines from leafhoppers fed on highly concentrated preparations of CTV had a general fluorescent glow when stained and examined with the fluorescent microscope. This fluorescent glow was distinguishable from smears prepared from nonviruliferous leafhoppers.

No specific staining occurred in plant or leafhopper tissue when healthy tissue was stained with conjugated antiserum or when diseased tissue was stained with conjugated normal serum.

DISCUSSION

A relatively short time is required for locating CTV antigen in diseased tissue by the method described here. With conjugated antibody and embedding medium prepared in advance, the procedure from diseased plant to microscopic examination can be completed in less than 3 hr.

These results, obtained with direct rather than indirect techniques, confirmed earlier histological and vector-transmission studies (1) which concluded that CTV was primarily restricted to phloem tissue. This method should also enable us to determine to what extent CTV may occur in other tissues, such as meristem and seeds.

Esau and Hoefert (3) observed CTV only in the nuclei of phloem parenchyma cells. Sections produced by our method were not thin enough to determine the location of the virus antigen within the cell. However, application of paraffin procedures (8) to obtain thinner sections or examination of dissociated cells (7) should provide information on the extent to which CTV antigen is restricted to the nucleus.

Much of the evidence collected thus far suggests that CTV does not multiply in the leafhopper vector (1). Our failure to observe any localized sites of CTV antigen in the leafhopper suggests an absence of multiplication in the vector. Although we could identify leafhoppers that had fed on highly concentrated CTV preparations, it is doubtful that leafhoppers from field collections would carry enough virus to be identified by this method.

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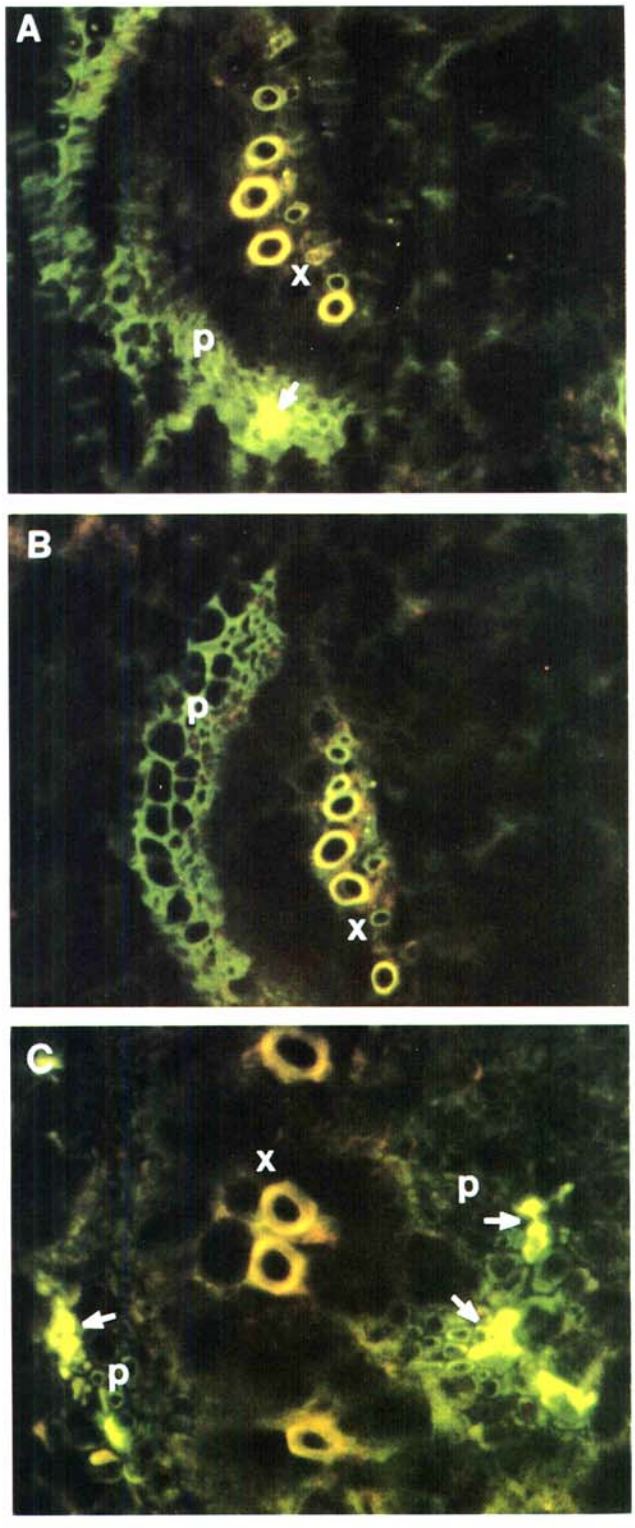

Fig. 1-(A to C). Fluorescent antibody staining of curly top virus antigen in transverse sections through vascular bundles of infected sugarbeet and tobacco (×320). X = xylem (thick-walled vessels have natural yellow fluorescence), and P = phloem. **A)** Sugarbeet section showing a single site of specific staining (arrow) in the phloem. **B)** Absence of specific staining in infected sugarbeet control section stained with fluorescein conjugated with globulin from normal serum. **C)** Tobacco section showing several specifically stained sites (arrows) found only in the phloem.

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