

### A New Method of Mechanically Transmitting Curly Top Virus

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#### ABSTRACT

Curly top virus was mechanically transmitted to sugar beet by an injector instrument normally used in human mass immunization programs. Fifty per cent infection was

obtained when 48-day-old plants were inoculated with a single injection into the crown of each plant.

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*Additional key words:* virus injection, *Beta vulgaris*.

In 1924, Severin (3) reported the first successful mechanical transmission of curly top virus. Juice from diseased sugar beets (*Beta vulgaris* L.) was introduced into the crown of healthy beets by means of holes made by forcing a sterile needle 1-1.5 inches into the crown; 9 of 100 beets inoculated developed typical curly top symptoms in 12 to 39 days. Bennett (1) tried several methods of mechanically transmitting curly top virus. All were unsuccessful except one similar to that used by Severin. Needle punctures into the crowns of sugar beets through drops of inoculum resulted in 12 infected beets of 124 inoculated. More recently, Fulton (2) reported transmission of a Wisconsin isolate of curly top virus

by pricking an infective extract into axillary buds of tobacco plants.

Curly top virus exists in close association with the phloem tissue of its host plants, and multiplication may be limited to the phloem (1). Two factors which may have limited earlier attempts at mechanical transmission are failure of inoculum to reach phloem tissue and injury of phloem cells caused by needle punctures. To overcome these limitations, we tested a method of injecting a very fine stream of inoculum into or through the vascular tissue, under pressure and without a needle, in the hope that virus particles would enter and become established in the phloem.

An instrument which would produce such a jet

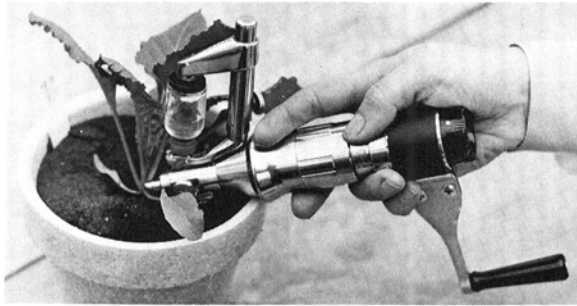


Fig. 1. Instrument used to inject curly top virus into the crown of sugar beet plants.

stream of inoculum under pressure was obtained from the Hypospray Division of R. P. Scherer Corp., Detroit, Mich., for use in the inoculations reported here. This instrument is normally used for mass immunization programs to inject medicaments through human skin by means of a jet stream instead of a hypodermic needle. Injections require only a few seconds. The injection consists of an initial stage in which the medicament penetrates the skin at ca. 12,000 psi. A second stage is of lower pressure and produces a dispersion pattern. Depth of penetration can be controlled by the size of the nozzle orifice. The pressure is created by winding up the injector with a handle which compresses a set of twelve springs and retracts a plunger. A preset dosage of inoculum is automatically supplied for each injection from a small serum bottle attached to the injector.

Inoculum was prepared from diseased sugar beet roots (cultivar US 33). Diseased tissue and 0.016 M phosphate buffer containing 0.01 M  $\text{Na}_2\text{SO}_3$  (1:1, w/v) was thoroughly homogenized in a blender operated at high speed. The juice, obtained by squeezing the macerated tissue in four layers of cheesecloth, was centrifuged at 35,000 g for 30 min, and the supernatant liquid frozen. When needed for inoculation, the frozen juice was thawed and centrifuged at 5,000 g for 10 min to remove freeze-denatured proteins. The resulting low-speed supernatant was concentrated 35:1 by centrifugation at 145,000 g for 40 min and resuspension of the pellets in 0.016 M phosphate buffer.

Preliminary tests indicated that different-sized injector nozzles caused varying amounts of injury. Therefore, three nozzles were tested on 36-day-old sugar beet seedlings of susceptible cultivar US 33. Inoculations were made at approximately a 45° angle into the crown of the beet at a point near the base of one of the outside petioles (Fig. 1). A dosage of 0.025 ml/injection was used for all inoculations.

The injector nozzle with the smallest orifice produced the highest percentage infection with very little plant injury. More infection was also obtained

TABLE 1. Effect of plant age in mechanical transmission of curly top virus to sugar beet

Age of plant at inoculation (days)	Percentage infection, days after inoculation <sup>a</sup>	
	18	28
27	19	29
34	30	30
41	25	42
48	38	50
56	25	38
63	21	33
70	17	25
77	13	21
Check	0	0

<sup>a</sup>Twenty-four plants (cultivar US 33) were inoculated for each treatment.

when the injector was operated at a reduced pressure of ca. 6,000 psi. This combination of nozzle and reduced injector pressure was used for a subsequent experiment for determination of the effect of plant age on susceptibility to mechanical inoculation.

Plants of susceptible cultivar US 33 ranging in age from 27 to 77 days were inoculated by a single injection into the crown of each plant as described above. Fifty per cent infection was obtained when 48-day-old plants were inoculated (Table 1). The percentage infection decreased when younger plants or older plants were inoculated.

This method of curly top virus transmission is much more rapid than previous attempts at mechanical transmission. It offers considerable advantage over insect transmission when it is necessary to inoculate individual plants. Maintaining colonies of insects and transferring them during virus transmission tests requires time, labor, and facilities. Maintaining insect-free areas for inoculation and symptom development requires constant attention. These difficulties would be eliminated if mechanical transmission of curly top virus could be used. In addition, this method may be applicable to other leafhopper-transmitted viruses, most of which have not been transmitted mechanically.

To be of maximum value in assay procedures or in screening for host resistance, the percentage infection obtained from any inoculation method must approach 100%. Work is now in progress to investigate various ways of increasing the percentage infection using the injector method.

#### LITERATURE CITED

1. BENNETT, C. W. 1934. Plant-tissue relations of the sugar-beet curly-top virus. *J. Agr. Res.* 48:665-701.
2. FULTON, R. W. 1955. Curly-top of tobacco in Wisconsin. *Plant Dis. Repr.* 39:799-800.
3. SEVERIN, H. H. P. 1924. Curly leaf transmission experiments. *Phytopathology* 14:80-93.