# Extraction of the Curly Top Agent from Segmented Petioles of Beet

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ABSTRACT

A convenient and effective technique for extracting the curly top causal agent consists of immersing 4- to 6-mm segments of recently infected beet petiole and leaf midrib for 24 to 48 hr in 0.01 M sodium citrate containing 0.1 to 0.4% 2-mercaptoethanol. Beet leafhoppers fed upon such extracts transmitted at rates comparable to those reported for leafhoppers fed on conventional phloem exudates from beets.

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Previous failure to isolate the curly top virus, assuming the agent is a virus, may be attributed to the laborious assay required to detect the agent since it cannot be transmitted by rubbing and to it being associated, perhaps exclusively, with host phloem tissues (1, 3). For the latter reason, it may occur in tissue homogenates in very low concentrations. However, a rich source of causal agent, which is relatively free of extraneous cellular material, is the phloem exudate which appears on the surface of diseased leaves and stems. This material has been a useful source of the causal agent but it is very difficult to obtain in quantity. The present report describes experiments aimed at the development of more convenient and reliable methods for extraction of the curly top agent.

The source of curly top agent used herein was an isolate from sugar beets (*Beta vulgaris* L.) collected near Five Points, California. An unnamed cultivar of curly top-susceptible beets (designated 72103-0) supplied by D. D. Dickenson, Holly Sugar Corp., Colorado Springs, Colorado, was used to propagate the virus and for bioassay. The infectivity of extracts was assessed by feeding portions to beet leafhoppers (*Circulifer tenellus* [Baker]) which were subsequently allowed to feed on healthy sugar beet seedlings. The percentage of plants which became infected was used as a rough indication of the quantity of curly top agent in an extract. For assay, a few drops of extract were placed on the surface of a Parafilm membrane (6) stretched over short, cylindrical glass jars; to prevent evaporation, another piece of the same material was stretched over the first to trap the extract between the membranes. Extracts were usually dialyzed to remove detrimental low molecular weight substances and then sucrose was added to 4 to 5%. After acquisition feeding, leafhoppers were placed singly on sugar beet seedlings beneath cages which covered the entire seedling. About 30 to 40 seedlings were inoculated in each treatment.

It was hypothesized that phloem contents including the causal agent might diffuse out of short stem and petiole segments if these were immersed in liquid for an extended period of time. Some success was experienced in several initial trials in which petioles from plants showing severe symptoms, including phloem necrosis and exudation, were sliced and immersed for various periods in a variety of media. When petioles and leaf midribs of infected plants were cut into segments 4- to 6-mm in length and immersed 24 to 48 hr in an equal volume of 0.01 M sodium citrate containing 0.1 to 0.4% 2-mercaptoethanol at about 25 C and the resulting extracts fed to leafhoppers, 10 to 80% of the latter transmitted the virus. These rates of transmission are similar to those experienced with phloem exudates by Bennett (2).

The most important consideration for starting material was found to be the time from infection until extraction of tissue. Consistently higher rates of transmission were obtained with recently infected plants which exhibited phloem necrosis. As reported by others (5), plants which had been infected 10 to 12 weeks were found to be a poor source of causal agent.

Extracts obtained by petiole immersion were compared

with those prepared in a conventional fashion by homogenization in a Waring Blendor. A batch of petioles and midribs from infected plants was divided at random into two groups. One group was cut into short segments, immersed in an equal volume of extraction liquid for 24 hr and the other group was homogenized in the same quantity of medium. Upon assay the undiluted homogenate gave 22% infection whereas the phloem extract gave 33% infection. Although the results may have been influenced somewhat by leafhopper behavior, it is obvious that the phloem extract was a preferred source of starting material for purification in view of its relatively slight content of extraneous cellular matter compared with the tissue homogenates.

Infected petioles were sectioned into segments of different sizes and tested to determine the effect of stem length on release of causal agent during immersion. When uniformly selected batches of petioles from infected plants were sliced into segments either 1-mm, 4- to 6-mm, 1.0-cm, or 3- to 4-cm in length, the rate of transmission by leafhoppers fed on the extracts was 31, 45, 40, and 23%, respectively. Thereafter, petioles were sectioned into pieces about 5-mm thick for extraction.

To evaluate the effectiveness of extraction of causal agent by the immersion procedure, a batch of sliced petioles was covered with an equal volume of extraction medium for 34 hr and the medium then replaced by a second volume of medium for a 12-hr period. When fed to leafhoppers, the first and second extracts gave 39.5 and 3.5% infection in inoculated plants. In a second experiment, the first extract was removed and the tissues were subsequently homogenized with a mortar and pestle in an equal volume of extraction medium. When the filtered homogenate was fed to leafhoppers, they infected 16.5% of the assay plants. A second batch of leafhoppers fed on the first extract at the same time gave 33% infection. From these results it appears that the immersion procedure is only partially effective in extracting the causal agent.

When one of two randomly selected batches of petioles with phloem exudates was washed thoroughly with tap water to remove all phloem exudate on the surface before both batches were extracted by immersion in the usual manner, the washed and unwashed material gave extracts with 34.5 and 44% infection, respectively. From this test it appears that most of the causal agent in these extracts originated from the phloem rather than from the exudates on the surface of the tissue.

It was theorized that the osmotic concentration of the medium may influence the diffusion or transport of materials out of the phloem. However, in several comparisons in which 4-6 mm segments were extracted in concentrations of sucrose varying from 0 to 55% in 0.01 M citrate, 0.2% 2-mercaptoethanol, followed by dialysis of each extract against 4% sucrose before feeding to leafhoppers, no appreciable differences were found among the various extracts.

Precipitation by ethanol as described by Bennett (2), was found to be a useful method for further concentration of curly top agent from petiole extracts and gave higher rates of leafhopper transmission. It was observed that addition of a small amount (1/50th volume) of 3 M sodium acetate, pH 5.0, before adding ethanol, accelerated the formation of a visible precipitate and gave an increased rate of transmission. In our trials with various concentrations of citrate for resuspending ethanol precipitates, no appreciable differences were found between 0 to 0.05 M citrate, in contrast to published reports (4). Higher concentrations of citrate were detrimental.

TABLE 1. Leafhopper transmission of curly top virus after resuspension of ethanol precipitates of petiole extracts[a]

| Relative concentration of extract | Leafhopper transmission | |
|---|---|---|
| | No. transmitting/ total no. used | Percent transmission |
| Experiment 1 | | |
| 1[b] | 6/15 | 40 |
| 5[c] | 11/17 | 64 |
| Experiment 2 | | |
| 1 | 4/12 | 33 |
| 9 | 10/18 | 56 |
| Experiment 3 | | |
| 1 | 2/18 | 11 |
| 10 | 9/15 | 60 |

[a] The initial extracts were prepared by the petiole immersion method. The ethanol precipitate was prepared by adding 1.5 vol of 95% ethanol. After 1-3 hr at −20 C and low speed centrifugation, the precipitate was resuspended in 0.01 M citrate for 12-18 hr.

[b] Initial extract before ethanol precipitation.

[c] Ethanol precipitate resuspended in citrate equal to one-fifth the initial volume of extract.

In several trials with ethanol precipitated material layered onto sucrose density gradients followed by centrifugation, neither visible nor ultraviolet absorbing components unique to infected material were detected.

The method described herein appears to afford a method for extracting the curly top entity from the phloem which averts the great bulk of host material obtained by grinding whole tissue and the concomitant risk of virus loss by entrainment with discarded extraneous material.

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