Occurrence and Identification of Lucerne Transient Streak Virus in Alberta, Canada

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ABSTRACT

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A previously unrecognized virus isolated from severely diseased alfalfa (*Medicago sativa*) plants at the University of Alberta farm was identified as a strain of lucerne transient streak virus on the basis of host range, particle morphology, serology, and nucleic acid and coat protein analysis.

During a survey of viruses affecting alfalfa (*Medicago sativa* L.) crops, a previously unidentified virus was isolated from the University of Alberta farm. Diseased alfalfa plants showed yellow streaks or flecks along the lateral veins of trifoliolate leaves. We identified the causal agent as a strain of lucerne transient streak virus (LTSV) that we will refer to as LTSV-Alt. LTSV is known to occur in Australia (1), New Zealand (3), and eastern Canada (7). This is the first report on the occurrence of LTSV from western North America.

MATERIALS AND METHODS

Leaf tissue from the alfalfa plants showing transient streak symptoms under field conditions was ground in 10 mM phosphate buffer, pH 7.0, and inoculated mechanically to *Chenopodium quinoa* Willd. A biologically pure virus culture was obtained by single-lesion transfers in peas (*Pisum sativum* L.), then maintained and propagated on *C. quinoa*. The virus was purified from inoculated leaves of *C. quinoa* as described by Randles et al (10) and centrifuged in 5–25% sucrose density gradients prepared in 20 mM phosphate buffer, pH 7.5. Purified virus samples were examined with a Phillips-300 transmission electron microscope after staining with 2% uranyl acetate, pH 7.0, and samples for immune electron microscopy (IEM) were prepared as described previously (2). An antiserum to LTSV-Alt was prepared in rabbits, and serological tests were done by double diffusion in agar (11). Ribonucleic acid (RNA) was isolated from purified virus preparations by phenol-sodium dodecyl sulfate (SDS) extraction and analyzed by electrophoresis in 3% polyacrylamide under nondenaturing and denaturing gels containing 7 M urea (5). Virus coat protein was analyzed by discontinuous electrophoresis in 9% polyacrylamide gels containing SDS (6).

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RESULTS AND DISCUSSION

Symptoms of LTSV-Alt on alfalfa. LTSV-Alt was isolated from naturally infected alfalfa plants. Under field conditions, most plants showed transient streaks along the veinal areas (Fig. 1A). In addition, some plants also showed yellow flecks and leaf distortion was apparent on severely infected plants. Under greenhouse conditions, alfalfa plants mechanically inoculated with LTSV-Alt also produced transient streaks and yellow flecks on the trifoliolate leaves (Fig. 1B). Pronounced leaf distortion was also evident on severely infected plants.

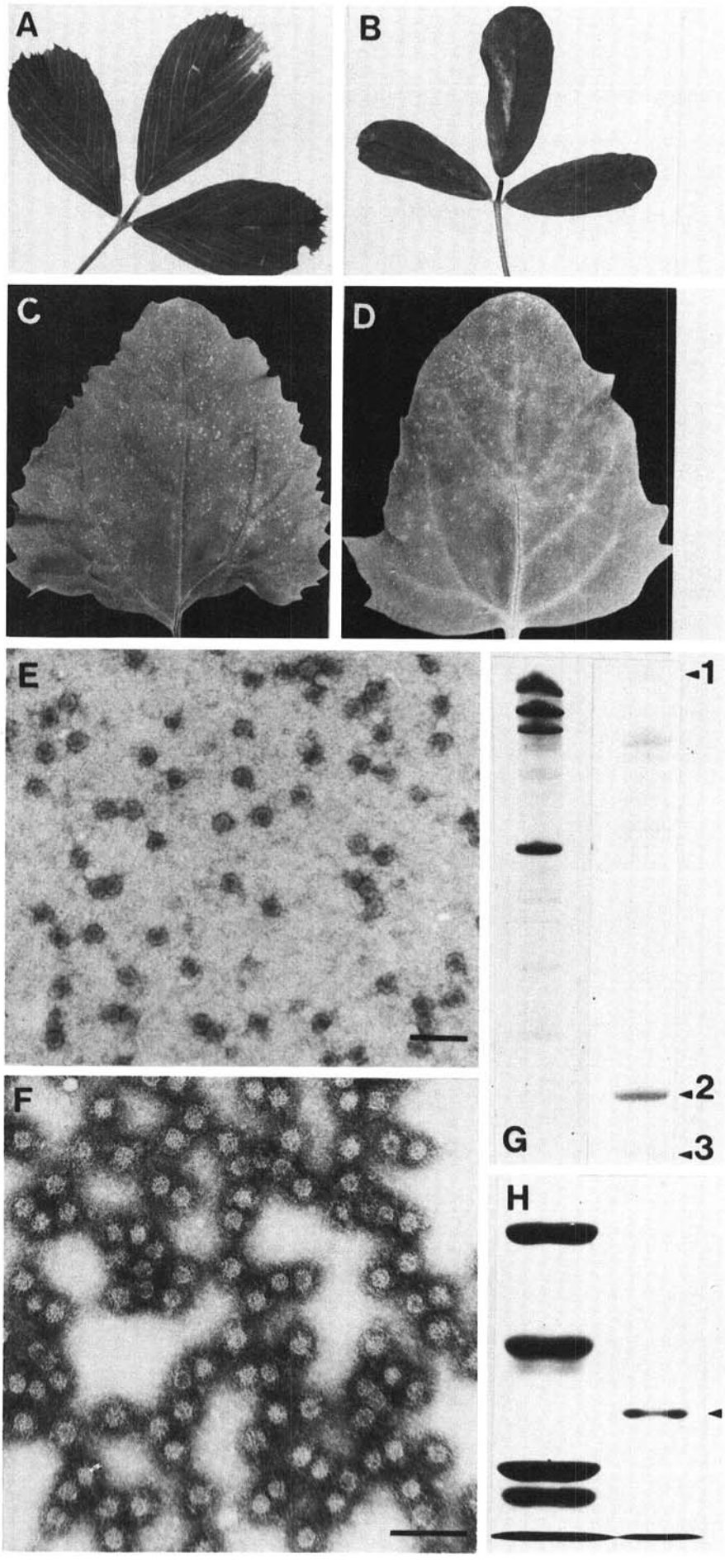
Host range. LTSV-Alt was transmitted to several plant species that have been shown susceptible to LTSV strains (1,3,7), producing characteristic symptoms of the virus on *C. amaranticolor, C. quinoa*, and *P. sativum*. LTSV-Alt has several hosts in common with Australian (LTSV-Aus, one), New Zealand (LTSV-NZ, three), and Canadian (LTSV-C, seven) isolates of the virus. Like LTSV-Aus and LTSV-C, it did not infect *Nicotiana clevelandii*, which was reported susceptible to LTSV-NZ (3). However, LTSV-Alt, like LTSV-NZ and LTSV-C, was distinguished from LTSV-Aus by its ability to produce local necrotic lesions on *P. sativum*. LTSV-Alt also produced necrotic local lesions on *C. amaranticolor* Coste & Reyn. (Fig. 1C), whereas in *C. quinoa*, it produced chlorotic local lesions (Fig. 1D) followed by systemic infection. The virus did not infect any of the following hosts: *Cucumis sativus* L., *Gomphrena globosa* L., *N. tabacum* 'White Burley,' *Tetragonia expansa*, *Vicia faba* L., and *Vigna unguiculata* 'Blackeye.'

Electron microscopy. Negatively stained purified preparations of LTSV-Alt contained homogeneous populations of polyhedral particles of about 30 nm in diameter (Fig. 1E), similar to those reported for other isolates of LTSV (3,9).

Serology. Serum samples taken from rabbits immunized with purified preparations of LTSV-Alt had homologous titers up to 1/128 when examined by immunodiffusion tests. Purified preparation of LTSV-Alt reacted strongly with antiserum to LTSV-Aus (homologous titer 1/128) and LTSV-C (unknown titer) at dilutions of 1/64 and 1/128, respectively, but failed to give positive reactions with antiserum to southern bean mosaic virus-B (SBMV-B) and SBMV-Cp. The relationship between LTSV-Alt and two other LTSV isolates was also examined by IEM. When purified preparations of LTSV-Alt were allowed to react with anti-LTSV-C, heavy decoration of the particles with heterologous antibodies (Fig. 1F) confirmed the antigenic relationship between the two isolates. Similar results were obtained when anti-LTSV-Aus was used to decorate the particles of LTSV-Alt.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis under denaturing conditions of RNA from LTSV-Alt preparations revealed the presence of three fractions annotated as 1, 2, and 3 in order of increasing mobility, and the bands corresponding to RNAs 1 and 3 were less intensive than RNA 2 (Fig. 1G). However, when RNA preparations of LTSV-Alt were subjected to electrophoresis under nondenaturing conditions, the electrophoretic pattern was different in that RNAs 2 and 3 migrated as a single band (data not shown). As observed for other isolates of the virus (8,9), from their electrophoretic behavior we assume that RNAs 2 and 3 of LTSV-Alt are also circular and linear molecules, respectively. Protein dissociated from purified preparations of LTSV-Alt migrated as a single band with a molecular weight of about 34×10^3 when subjected to electrophoresis under denaturing conditions (Fig. 1H), similar to that reported for Australasian isolates (4). In addition, the two Australasian isolates and LTSV-C also contained an additional band corresponding to a molecular weight of about 29×10^3, which was not observed in our preparation. Recently, it has been shown that 29×10^3 protein band is the product of limited proteolysis of the viral protein resulting from long storage in vitro (8).

From the results presented in this paper, it is clear that LTSV also occurs in western North America. In spite of its widespread occurrence in alfalfa (A. L. N. Rao and C. Hiruki, *unpublished*), we do



not know why the virus has not been detected by previous workers in Alberta. Because mixed infections are common in alfalfa (7), it is possible that the mild symptoms produced by LTSV would have been masked by the severe symptoms produced by other viruses such as alfalfa mosaic virus. Hence it is reasonable to assume that LTSV could be present wherever alfalfa is grown.

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Fig. 1. Symptoms of lucerne transient streak virus (LTSV) strain from Alberta (LTSV-Alt) infection of **(A)** alfalfa under field conditions, **(B)** alfalfa under greenhouse conditions, **(C)** *Chenopodium amaranticolor*, and **(D)** *C. quinoa*. **(E)** Electron micrograph of purified preparation of LTSV-Alt negatively stained with uranyl acetate. Scale bar = 100 nm. **(F)** Immune electron micrograph showing the virus particles of LTSV-Alt decorated with anti-LTSV-C. Scale bar = 100 nm. **(G)** Polyacrylamide gel electrophoretic analysis under denaturing conditions of RNAs from (right) LTSV-Alt and (left) alfalfa mosaic virus. **(H)** Polyacrylamide gel electrophoretic analysis of (right) coat protein from LTSV-Alt (arrow); the band at the bottom of the electrophoretogram is β-lactoglobulin, which was added as an internal marker. (Left) Series of standard molecular weight markers (from top): bovine serum albumin (66,000 daltons), ovalbumin (45,000 daltons), trypsinogen (24,000 daltons), and β-lactoglobulin (18,400 daltons).

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