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Lettuce chlorosis virus – A new whitefly-transmitted closterovirus

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Abstract

A previously undescribed virus disease of lettuce, sugarbeets, other crop and weed hosts was found in the southwest desert regions of U.S.A. Affected lettuce and sugarbeet hosts exhibited interveinal yellowing, stunting, rolling, and brittleness of affected leaves. Since 1990, yellowing symptoms on desert lettuce and sugarbeet were shown to be induced by a mixture of lettuce infectious yellows virus (LIYV) and this previously undescribed virus termed lettuce chlorosis virus (LCV). LCV is a closterovirus with flexuous, filamentous particles 800–850 nm long. The virus is transmitted efficiently by both *Bemisia* biotypes. LCV differs significantly from LIYV and other previously described viruses in host range (LCV does not infect the Cucurbitaceae), insect transmission, and serology.

Introduction

Lettuce infectious yellows virus (LIYV) has been a limiting factor in the production of crops in the desert regions of southwestern U.S.A. since 1981. The virus, which is vectored by the sweetpotato whitefly [*Bemisia tabaci* (Gennadius)] attacks at least 10 major agricultural crops including lettuce, cantaloupe, cucumber, melons, sugarbeet, and carrots (Duffus et al., 1986). Following the introduction of biotype 'B' into the southwest desert whitefly population in 1990 and the establishment of its gene pool into the resident 'A' population, a number of important changes in LIYV epidemiology took place (Cohen et al., 1992; Liu et al., 1992; Duffus and Liu, 1994). The new populations were much poorer vectors of LIYV (almost 100 fold less efficient), developed more rapidly, and were so destructive to melons that virtually no fall melons have been planted since 1992. Thus, in spite of record whitefly populations, the absence of the major source of LIYV (fall melons) has resulted in very low incidence of LIYV in the period 1991–1994.

Since 1991, a close monitoring of yellowing symptoms on weeds, lettuce, and sugarbeet crops uncovered a number of yellowing virus isolates that did not react to antiserum against LIYV.

A whitefly-transmitted virus termed lettuce chlorosis virus (LCV) was recognized as being distinct from previously reported viruses that cause yellowing diseases. A preliminary report of this research has been given (Duffus et al., 1994).

The purpose of the study on the LCV was to verify its viral nature, to measure some of its biological properties, and to investigate its relationship with its whitefly vector.

Materials and methods

Virus source and whitefly maintenance. The virus isolates used were obtained from several commercial lettuce plants collected in the Imperial Valley, California during 1991. These virus isolates did not react in ELISA tests with antiserum against LIYV. The virus was maintained in *Chenopodium murale* L., *C. capitatum* (L.), Asch., or *Lactuca sativa* L. and transferred from plant-to-plant via inoculation with the whitefly vector.

The sweetpotato whitefly, *Bemisia tabaci* Gennadius (biotypes A and B by isozyme analysis) (Liu et al., 1992), was reared on sweetpotato (*Ipomoea batatas* (L.) Lom.) in muslin-covered cages, as above. *B. tabaci*, biotype A, was originally collected from

cotton, *Gossypium hirsutum* L., in 1981, and biotype B (*B. argentifolii*) (Bellows and Perring, 1994) from melon (*Cucumis melo* L.) in 1990. Virus sources in the various experiments were lettuce plants inoculated about 30 days earlier. *Malva parviflora* L. were used as test plants. Following inoculation with LCV via whiteflies, the plants were sprayed with resmethrin (to eliminate the vectors) and placed in greenhouses. All plants were grown in screened greenhouses fumigated at weekly intervals with dichlorvos and resmethrin.

Host range. The host range was determined by allowing whiteflies 24 h acquisition feedings on diseased tissue and then transferring 30–50 insects to each of 10 seedlings of each species tested and feeding them for 48 h. The presence of virus in each plant species tested for susceptibility was determined by whitefly transfer (30–50/plant) to *N. clevelandii* and lettuce seedlings 30–45 days after inoculation. The virus was recovered from field plants by feeding nonviruliferous *Bemisia* whiteflies on the collected plants for 24 h, transferring the insects to healthy indicator seedlings, and feeding them for 48 h.

Transmission. Attempts were made to transmit LCV mechanically using 0.1 M sodium phosphate buffer, pH 7.0, containing 0.02 M sodium sulfite. The virus sources included lettuce, *C. murale*, and *N. clevelandii*. The plants inoculated included these and a number of other species found to be susceptible when inoculated via the whitefly vector.

Transmission attempts with *B. tabaci* (biotypes A and B) were made by confining the insects to diseased plants and transferring them to healthy indicator species as described herein.

Virus-vector relationships. Transmission tests for different biotypes of whiteflies were made by the leaf cage method described previously (Cohen et al., 1983). Five tests (ten plants per replication) were performed in each of the experiments for determination of transmission by different insect biotypes, vector efficiency, acquisition, inoculation, and transmission threshold periods. Unless otherwise stated, 30 insects per cage were used on each plant in the different tests. The ability of viruliferous whiteflies to retain LCV was determined by allowing the insects acquisition periods of 24 h on diseased plants and then transferring them in groups of 30 in daily serial transfers on healthy indicator seedlings.

Virus purification. Virions were purified using modifications of methods for lettuce infectious yellows virus (LIYV) (Duffus et al., 1986; Klaassen et al., 1994). LCV-infected *N. clevelandii* plants were harvested 2–3 wk after inoculation. Plants were homogenized in a meat grinder with cold extraction buffer (0.1 M Tris-HCl, pH 7.4) plus 0.5% (w/v) Na_2SO_3 and 0.5% (v/v) 2-mercaptoethanol (Klaassen et al., 1994). The plant material was further homogenized with a ball mill for 2 h at 4 °C. The crude extract was expressed through cotton muslin. Triton X-100 was added to a final concentration of 2% (v/v) and the extract was stirred overnight at 4 °C. The mixture was centrifuged in a Sorvall GSA rotor at 10,000 g for 10 min. The supernatant was ultracentrifuged for 1 h at 118,000 g in a Beckman 70 Ti rotor over a 5-ml cushion of 20% sucrose in extraction buffer plus 2% Triton X-100. The pellets were resuspended overnight at 4 °C in TE (10 mM Tris-HCl, pH 7.4, 1mM EDTA). Triton X-100 was added to the suspension at a final concentration of 2% (v/v) and again stirred for 1 h at 4 °C, followed by centrifugation in a Sorvall SS-34 rotor at 8,000 g for 10 min. The supernatant was layered over 4 ml of 20% (w/v) sucrose in TE and centrifuged in a Beckman 70 Ti rotor for 2 h at 90,000 g. The pellets were resuspended overnight in TE, then followed by centrifugation in a Sorvall SS-34 rotor at 8,000 g for 10 min. The supernatant was layered on sucrose-cesium sulphite gradients prepared as described by Gumpf et al. (1981). Gradients were centrifuged in a Beckman SW 28 rotor for 7 h at 83,000 g at 10 °C. Fractions containing LCV virions were dialyzed at 4 °C against three changes of TE.

Antiserum production and serology. Purified LCV antigen, in aliquots of 0.5 ml, were administered, one injection subcutaneous (0.4 A_{260} OD), and 3 intramuscular (0.4, 0.8 and 1.6 A_{260} OD) into a rabbit. Each aliquot was mixed with an equal volume of Freund's complete adjuvant. Bleedings were initiated at the sixth week after the first injection. Indirect ELISA was conducted by first coating plates with antigens prepared from infected and control leaf tissues in 10 volumes of coating buffer (0.05 M sodium carbonate, pH 9.6). Antisera of LCV and LIYV (Duffus et al., 1986) were cross-absorbed with healthy sap of *Nicotiana clevelandii* leaf (1:10, w/v in PBS) (Deng, 1995) and purified as described by Clark and Adams (1977). After reaction of immunoglobulins, goat anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate (Sigma) was added to the plates and incubated. A_{405}

values were measured after addition of substrate, usually 2–4 h at room temperature.

Results

Host range and symptoms. LCV induced severe yellowing and/or reddening symptoms, stunting, rolling, and brittleness of affected leaves in a wide range of weed and crop species. Species that naturally show red pigment tended to show intensification of red color in interveinal areas when infected. Symptoms on many of the common hosts were very similar to those induced by beet yellows or beet western yellows viruses, which are aphid-transmitted, or to lettuce infectious yellows and beet pseudo yellows viruses, which are whitefly-transmitted.

Plants susceptible to LCV infection:

Amaranthaceae – *Gomphrena globosa* L.
Chenopodiaceae – *Beta macrocarpa* Guss., *B. vulgaris* L., *Chenopodium amaranticolor* Coste & Reyn., *C. capitatum* (L.) Asch., *C. murale* L., *Spinacia oleracea* L.
Compositae – *Lactuca sativa* L., *Zinnia elegans* Jacq.
Convolvulaceae – *Ipomoea nil* (L.) Roth.
Cruciferae – *Capsella bursa-pastoris* (L.) Medic., *Thlaspi arvense* L.
Geraniaceae – *Geranium dissectum* L.
Leguminosae – *Trifolium subterraneum* L.
Linaceae – *Linum lewisii* Pursh., *L. grandiflorum* Desf.
Malvaceae – *Abutilon theophrastii* Medic., *Anoda abutiloides* A. Gray, *Malva parviflora* L.
Polygonaceae – *Rumex crispus* L.
Solanaceae – *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. glutinosa* L., *N. tabacum* L., *Physalis wrightii* Gray.
Umbelliferae – *Conium maculatum* L., *Daucus carota* L.

Plants not susceptible to LCV infection:

Apocynaceae – *Vinca minor* L.
Caryophyllaceae – *Dianthus chinensis* L., *Spergula arvensis* L.
Compositae – *Cynara cardunculus* L., *C. scolymus* L., *Helianthus annuus* L., *Picris echioides* L., *Sonchus oleraceus* L., *Tagetes patula* L., *Taraxacum officinale* Webber.
Convolvulaceae – *Dichondra repens* Forst., *Ipomoea* tricolor Cav.
Cruciferae – *Brassica oleraceae* L. var. *botrytis*, *Raphanus sativus* L.
Cucurbitaceae – *Citrullus vulgaris* Schrad, *Cucumis sativus* L., *C. melo* L., *Cucurbita metuliferus* Naud., *C. pepo* L., *C. palmata* S. Wats.
Euphorbiaceae – *Ricinus communis* L.
Leguminosae – *Medicago sativa* L., *Phaseolus vulgaris* L., *Pisum sativum* L.
Malvaceae – *Anoda cristata* (L.) Schlecht., *Gossypium hirsutum* L.
Solanaceae – *Capsicum annuum* L., *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Gaertn., *Nicotiana glauca* Graham, *Petunia hybrida* Vilm., *Physalis alkekengi* L., *P. floridana* Rybd., *P. ixocarpa* Brot., *Solanum elaeagnifolium* Cav., *S. melongena* L., *S. nigrum*.
Umbelliferae – *Apium graveolens* L. var. dulce.

Transmission tests

Mechanical. No transmission was detected by mechanical inoculation in any test.

Insect. Insect collections taken during the initial separation of LCV from the LIYV complex were mixtures of *B. tabaci* biotypes A and B. LCV was transmitted by both biotypes reared in the greenhouse. Because the A biotype was easier to control under greenhouse conditions, this insect was used for the virus-vector relationship studies unless otherwise indicated.

Virus-vector relationships

Transmission efficiency. Single *Bemisia* (biotype A) allowed a 24 h acquisition feeding on diseased plants are capable of transmitting LCV. Transmission rates established by 1, 5, 10, 20, and 40 A biotype whiteflies per plant were: 2.9, 25.7, 31.4, 50.0, and 74.3%, respectively; for B biotype whiteflies the rates were: 0.0, 5.0, 12.5, 35.0, and 57.5%, respectively.

Minimum acquisition access period. LCV was acquired by whiteflies in a feeding period of 1 h, but was transmitted more efficiently after longer feeding periods – 4.0, 4.0, 32.0, 84.0, and 96.0% after 1, 3, 6, 24, and 48-h acquisition feedings.

Minimum inoculation access period. Whiteflies given 24-h acquisition feeding on diseased plants transmitted LCV to 34.0, 56.0, 68.0, 80.0, and 76.0% of plants after

Table 1. Malva seedlings infected (+) and noninfected (–) in daily serial transfers using groups of 30 viruliferous *B. tabaci*

Whitefly colony no.	Days after acquisition feeding[a]										
	0	1	2	3	4	5	6	7	8	9	10
1	+	–	–	–	–	–	–	–	–	–	–
2	+	–	–	–	–	–	–	–	–	–	–
3	+	–	–	–	–	–	–	–	–	–	–
4	+	+	–	–	–	–	–	–	–	–	–
5	+	+	+	–	–	–	–	–	–	–	–
6	+	+	+	+	+	–	–	–	–	–	–
7	–	+	–	–	–	–	–	–	–	–	–
8	+	–	+	–	–	–	–	–	–	–	–
9	+	+	+	–	–	–	–	–	–	–	–
10	+	+	+	–	–	–	–	–	–	–	–
11	–	–	–	+	–	–	–	–	–	–	–
12	+	+	+	+	+	–	–	–	–	–	
13	+	+	+	–	–	–	–	–	–	–	–
14	+	–	–	–	–	–	–	–	–	–	–
15	+	–	+	–	+	–	–	–	–	–	–
16	+	+	+	+	–	–	–	–	–	–	–
17	+	+	+	+	–	–	–	–	–	–	–
18	+	+	–	–	–	–	–	–	–	–	–
19	+	–	+	–	–	–	–	–	–	–	–
20	+	+	+	–	–	–	–	–	–	–	–

[a] No seedlings were infected by the whitefly colonies in daily serial transfers from the 10th to the 15th day after acquisition feeding.

inoculation feeding periods of 1, 3, 6, 24, and 48-h, respectively.

Persistence. The ability of viruliferous *Bemisia* to retain LCV was determined by daily serial transfers to healthy plants. The insects retained the virus for 4 days and were fairly efficient vectors through 3 days. The insects did not transmit virus during days 5 through 10 (termination of test) (Table 1).

Virus purification. One band was located approximately 28–30 mm from the bottom of the sucrose-Cs_2SO_4 gradients of partially purified preparations. The band, after dialysis, had an absorption spectrum typical of viral nucleoprotein, and a $A_{260/280}$ value of 1.20. This band was absent in gradients of healthy material subjected to the same purification technique.

Electron microscopy. Partially purified preparations from sucrose-Cs_2SO_4 gradients showed numerous flexuous filamentous particles of variable lengths (Figure 1). Measurements from over 250 particles indicated the modal length in the 750–800 nm range.

Table 2. Reciprocal tests of lettuce chlorosis virus (LCV) and lettuce infectious yellows virus (LIYV) with indirect ELISA

Antigen	Absorbance at 405 nm[1]	
	LCV IgG	LIYV IgG
LCV	1.222	0.122
Healthy control	0.113	0.139
LIYV	0.102	0.449
Healthy control	0.127	0.150

[1] Absorbances were taken 4 h after addition of substrate and represent the averages of four wells.

The diameter of the particles was approximately 12 nm. Sap from LCV-infected leaves of *N. clevelandii* contained long, flexuous, rod-shaped particles with a modal length of 800–850 nm (Figure 2).

Serology. After cross-absorption with healthy tobacco leaf sap, the titer of LCV immunoglobulin was 1/8000 as determined in indirect ELISA tests by reaction against 1/10 dilution of LCV-infected *C. murale* leaf sap.

Repeated tests using nonabsorbed polyclonal antisera against LCV and LIYV in indirect ELISA resulted in high background readings with the healthy control. However, after cross-absorption, immunoglobulins against both LCV and LIYV were suitable for serological detection in indirect ELISA (Table 2).

LCV IgG reacted with its homologous antigen but not with LIYV antigen. In several different tests, the average ratio of A_{405} values of the LCV-infected to healthy samples was $10 \times (n > 100)$. Similarly, LIYV IgG reacted only with its homologous antigen but not with LCV antigen. The average ratio of A_{405} values of the LIYV-infected to healthy samples was $3 \times (n \geq 25)$. In other ELISA tests, LCV IgG did not react with other whitefly-transmitted viruses, i.e., beet pseudo yellows (BPYV), cucurbit yellow stunting disorder (CYSDV) and tomato infectious chlorosis virus (TICV).

Discussion

Whitefly-transmitted clostero-like viruses are emerging as one of the most numerous subgroups of the closteroviruses. The viruses induce interveinal yellowing, thickening, and brittleness of affected leaves, and/or vein yellowing symptoms (Duffus, 1987). They are transmitted by whitefly vectors in a semipersistent manner, are associated with the phloem, and most

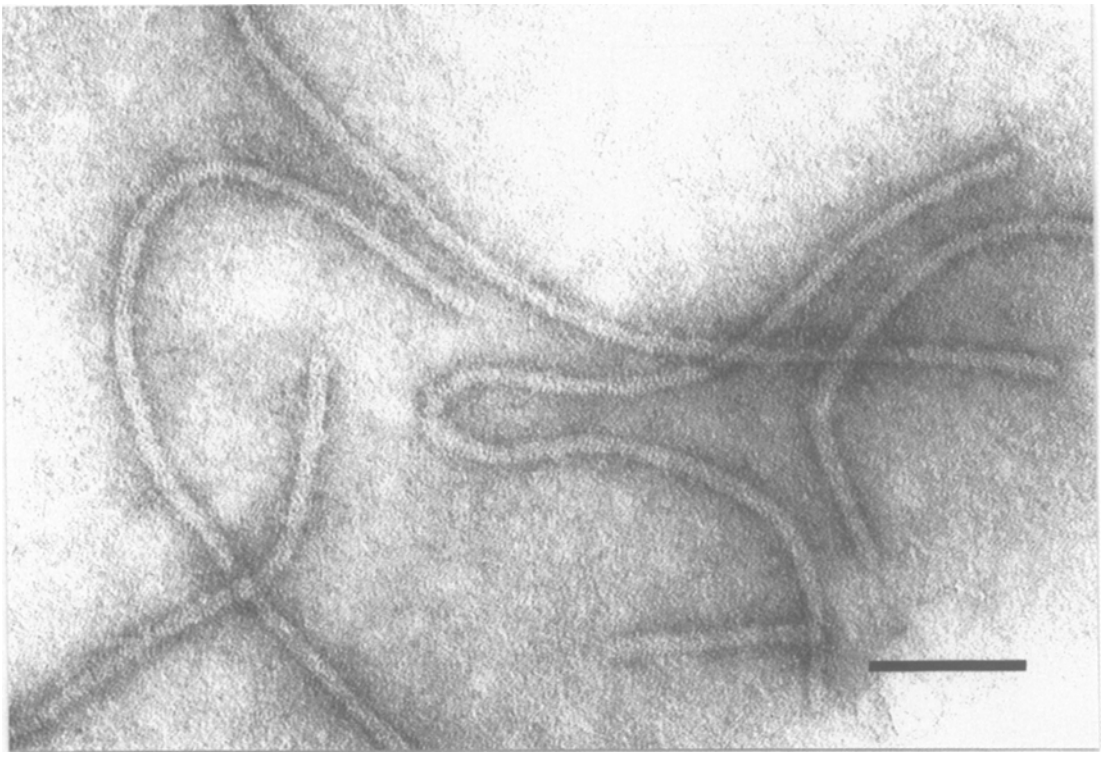

Figure 1. Electron micrograph of purified lettuce chlorosis virus particles stained in 2% uranyl acetate. Bar represents 100 nm.

appear to produce cytoplasmic vesicles. The virions of the group including LCV, LIYV, CYSDV, TICV and sweetpotato sunken vein virus (SPSVV) are filamentous and flexuous, with modal lengths of 700–900 nm, only BPYV appears to be longer (Cohen et al., 1992; Duffus et al., 1995; Wisler et al., 1996; and H. Y. Liu, unpublished results).

Several whitefly-transmitted closteroviruses differ from the aphid-transmitted viruses of the group by their significantly shorter length, (700–900 vs. 1200–2200 nm) and the fact that at least some members have a divided genome (Klaassen et al., 1995; Wisler et al., 1996).

Biologically the viruses of these two closterovirus subgroups are very similar. Both have strong affinities to the phloem, are not transmitted mechanically or with great difficulty, have moderate to restricted host ranges, and induce almost identical symptoms. LCV is shown to be distinct from LIYV and other whitefly-transmitted viruses in a number of significant ways. The virus is serologically distinct from LIYV, BPYV, CYSDV, and TICV in ELISA serological reactions.

LCV also differs from these viruses in insect-vector relationships supporting virus protein differences indicated by serology. Both biotypes of *Bemisia* transmit LCV with approximately equal efficiency. LIYV is transmitted efficiently by the A biotype, but inefficiently by the B type, whereas CYSDV is transmitted efficiently by the B biotype, but inefficiently by the A biotype. BPYV and TICV are transmitted by *Trialeurodes vaporariorum* but the other viruses by the *Bemisia* biotypes. LCV is retained by *Bemisia* significantly longer than LIYV but significantly shorter than CYSDV (Duffus, 1995).

An important difference in host range between LCV and LIYV is the fact that LCV does not infect members of the Cucurbitaceae. Fall melons have played a major role in the epidemiology of LIYV in the southwest desert region. The crop was the major source of late season whiteflies and LIYV. The absence of the major source of the LIYV (fall melons) resulted in very low incidence of LIYV in the 1991–1994 fall crops (less than 0.1%) (Duffus and Liu, 1994).

Since the cucurbits are not a host of LCV the incidence of LCV has not been affected by this major cropping change. It appears at this point that weed hosts are the major source of LCV, since none of the late summer, early fall crops grown in the region are

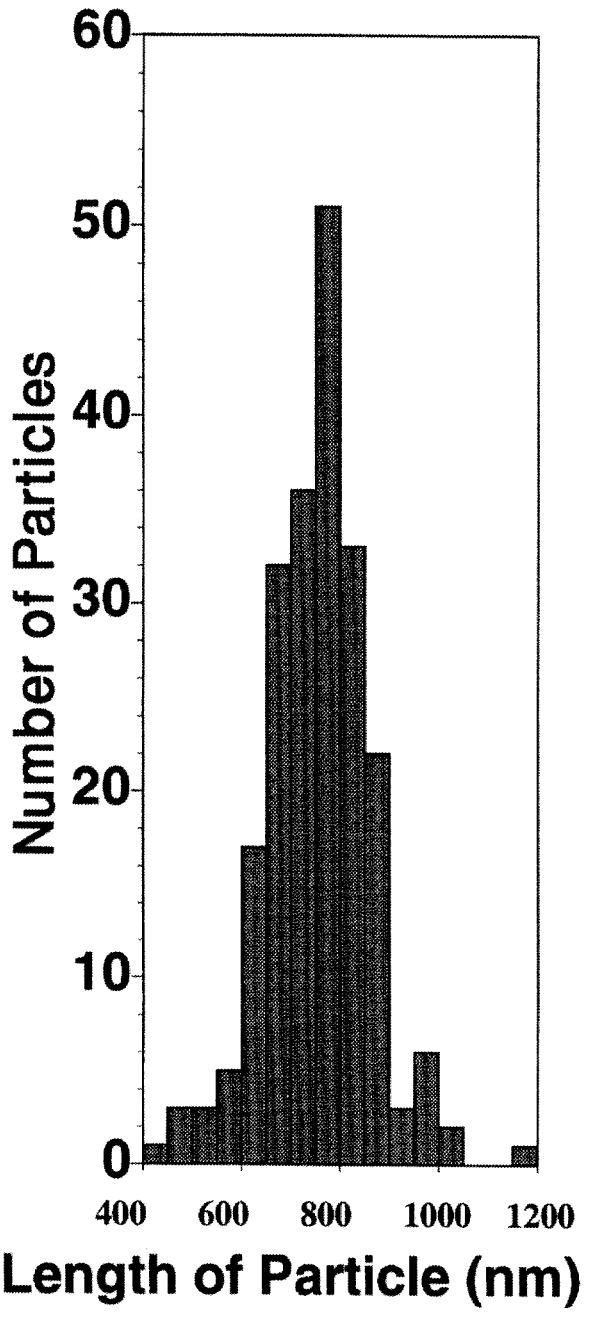

Figure 2. The length distributions of lettuce chlorosis virus particles in leaf dip preparations from LCV infected *Nicotiana clevelandii* and negatively stained with 2% uranyl acetate.

susceptible to the virus. Unless other susceptible crops are planted during the late summer planting period, the incidence of LCV should remain moderate.

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