Etiology of Severe Mosaic and Its Effect on Safflower

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ABSTRACT

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Severe mosaic, leaf distortion, and stunted growth were characteristic symptoms caused by turnip mosaic virus (TuMV) in safflower growing in the Sacramento Valley of California. Symptoms were reproduced in safflower plants inoculated with juice extracted from diseased plants. Rotting of seed ovules resulted in severe reduction of seed yield in TuMV-infected plants. Some cultivars developed similar necrotic local lesions and systemic necrosis reactions to both TuMV and lettuce mosaic virus. Spread of the virus from weed hosts to safflower by aphids in nature was indicated by transmission of TuMV from the commonly occurring weeds *Brassica geniculata* and *B. campestris* to safflower by *Myzus persicae*. The virus was identified on the basis of host range, physical and morphological properties, and serology.

Safflower (*Carthamus tinctorius* L.) is a natural host of cucumber mosaic (CMV), alfalfa mosaic (AMV), and lettuce mosaic (LMV) viruses (3,4). Infected plants develop systemic mosaic, but in some safflower cultivars, plants infected with LMV develop systemic necrosis (5). A severe viruslike disease first observed in safflower in 1979 in the Sacramento Valley of California was characterized by symptoms that were atypical of these viruses. Small dark green and pale green areas, pale green veinbanding, distortion, and bronzing were typical symptoms in leaves of the entire plant and bracts of the seed head. Affected plants were generally stunted and had reduced leaf and seed head size. Incidence of diseased plants ranged from 50% at field borders to 15–25% inward. Investigations were undertaken to identify the virus and to determine the reaction of safflower cultivars to the virus, including its effect on seed development and yield.

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MATERIALS AND METHODS

Transmission and safflower reactions. Safflower plants showing severe mosaic were collected in the field. Leaves were triturated in 0.01 M phosphate buffer, pH 7.0, with a mortar and pestle. Extracted juice was rubbed on the first two true leaves of Dart safflower plants in the greenhouse. Dart plants were maintained as a source of inoculum. Similar inoculations were made on safflower leaves with juice extracted from leaves of cruciferous weed hosts that showed mosaic and were growing near the safflower fields.

Nonviruliferous aphids, *Myzus persicae* (Sulzer), were reared on healthy Tendergreen mustard (*Brassica juncea* (L.) Coss) plants. Aphids were transferred to healthy safflower plants after 15-min acquisition periods on infected safflower, *B. juncea, B. geniculata* (Desf.) Ball (short-pod mustard), and *B. campestris* L. (wild yellow mustard) plants.

Nine safflower cultivars and 22 safflower plant introductions were inoculated and observed for symptoms. Plants of certain cultivars were inoculated with LMV and turnip mosaic virus (TuMV) (American Type Culture Collection T34) in parallel tests with the safflower virus for comparison of symptoms.

Host range. Six to 10 plants of 21 plant species were mechanically inoculated at the cotyledon or true leaf stage and kept in the greenhouse. Symptomless plants were assayed by back-inoculation to safflower.

Properties. Juice extracted from leaves of infected safflower was diluted 10-fold with buffer and assayed for infectivity on safflower. Equal amounts of undiluted juice were pipetted into glass tubes that were capped and stored at 20–22 C from 1 to 4 days. Frozen sap served as a control in assays on safflower. Extracts in hermetically sealed thin-walled glass pipettes were heated in a constant-temperature water bath for 10 min at 5 C increments from 30 to 70 C, then cooled and assayed on safflower. Leaf-dip preparations stained with neutral 2% potassium phosphotungstate were

examined in an electron microscope for particle morphology. Particle length was derived from measurements from electron micrographs.

Serology. Immunodiffusion tests were run in agar gels containing 0.2% sodium dodecyl sulfate (SDS) (2). Juice from infected turnip (*B. rapa* L.) and safflower plants was placed in tubes and kept in a water bath at 40 C for 4–6 min, cooled, and centrifuged for 1 hr at 10,000 rpm. Clarified sap from plants infected with the virus isolated from safflower, from plants infected with TuMV (T34), and from healthy plants was used in serological tests with TuMV antiserum.

Effect on plant growth and seed yield. Dart plants were grown in the greenhouse in 5-in. (12.7-cm-diameter) pots (one plant per pot) filled with autoclaved soil. Sixteen 2-wk-old plants were inoculated with the safflower virus by rubbing infected plant juice on the first two true leaves. A similar group of plants was inoculated with LMV, and a third group was not inoculated. Plants were randomized and received similar irrigation thereafter. Data were compiled on flowering, plant height and weight, and quantity and weight of seed.

Dart plants inoculated with the safflower virus and healthy plants were transplanted from pots to the field when they were 14 days old. Three replicates of seven plants were planted for each treatment. Data were taken on number of seed heads per plant and weight of seed composites from each replicate. Seeds collected from infected plants were planted in the greenhouse, and the resulting plants were observed for mosaic symptoms.

Safflower heads in the fresh or wilted flower stage that were collected from healthy and infected plants in the greenhouse and field were dissected and examined for damage to the receptacle and floral parts. Pollen grains were collected from florets and placed in tetrazolium bromide, which stains the viable grains (A. B. Hill, *unpublished*).

RESULTS

Transmission. The virus was readily transmitted by mechanical inoculation from leaves of safflower and the cruciferous weeds *B. campestris* and *B. geniculata*. Systemic mosaic symptoms developed in 4–6 days, and the plants became severely stunted. Mosaic developed in 16% of the plants inoculated by *M. persicae*. The virus was transmitted by the aphids from *B. campestris* and *B. geniculata* to safflower and from safflower to both weed hosts.

Safflower reactions. Mosaic developed systemically in plants of five cultivars and nine introductions, but reactions of inoculated leaves varied from mosaic and necrotic lesions to symptomless. Systemically infected leaves showed veinbanding; they were reduced in size and distorted. Necrotic lesions developed in 4 days on inoculated leaves of plants of four cultivars and 13 introductions. Lesion size on leaves of Pacific 7 and VFR-1 plants averaged 1 × 1.4 mm, compared with 1 × 2.5 mm for lesions on leaves inoculated with LMV. Plants with necrotic lesions had no mosaic, but 10–50% developed systemic necrosis that was lethal to about 21% of the affected plants. Systemic necrosis from LMV developed in 70–80% of the Pacific 7 and VFR-1 plants and was lethal to more than 50% of the plants. Plants inoculated with TuMV (T34) developed symptoms similar to those inoculated with the safflower isolate.

Host range. Systemic mosaic developed in *Brassica pekinesis* (Lour.) Rupr. (Chinese cabbage), *B. campestris, B. geniculata, Raphanus sativus* L. (wild radish), *B. juncea*, and *B. rapa*. Leaves of Tendergreen mustard and turnip plants showed moderate to severe blister-mottle. Systemic mottle and leaf rugosity developed in *Sonchus oleraceae* L. (sowthistle) and *Nicotiana clevelandii* Gray, respectively. Lesions that formed on inoculated leaves of *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. were chlorotic, and lesions on *Gomphrena globosa* L. were necrotic.

The virus was not recovered from symptomless plants of *Beta vulgaris* L. (sugar beet and red table beet), *B. oleracea* L. var. *capitata* L., *Capsella bursa-pastoris* L. Medik., *Cucumis sativus* L., *Lactuca sativa* L. (Paris Green Cos), *L. serriola* L. (prickly lettuce), *Lycopersicon esculentum* Mill., *Medicago sativa* L., *Phaseolus vulgaris* L., and *Helianthus annuus* L.

Properties. The safflower virus was inactivated between 55 and 60 C. The dilution end point was between 10^{-5} and 10^{-6}. The virus remained infective for 3 days in safflower sap extract stored at 24 C. Uniform-sized, slender, flexuous rods (760 nm long) were observed in safflower leaf-dip preparations (Fig. 1).

Serology. TuMV antiserum reacted in SDS-agar gel diffusion tests with antigens from turnip infected with the virus and from turnip infected with TuMV (T34) (Fig. 2). Precipitin lines of the antigens were fused. Reactions were less discrete with antigens from infected safflower and from unclarified antigens, regardless of the source plant.

Effect on plant growth and seed yield. Height and seed production were significantly reduced in safflower plants infected with the virus in the greenhouse (Table 1). Differences in plant dry weight and number of undeveloped seeds

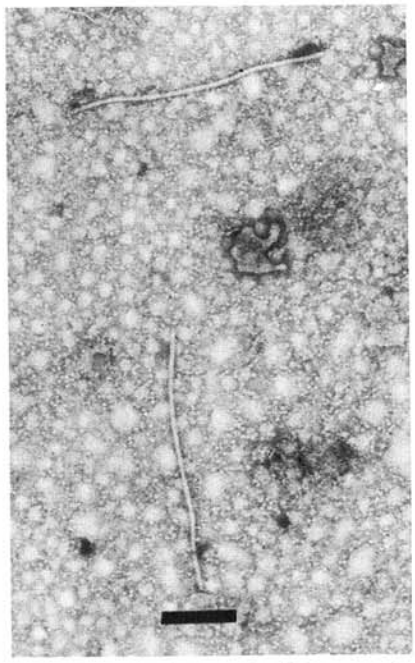

Fig. 1. Electron micrograph of flexuous rod-shaped particles in crude sap from turnip inoculated with a virus isolate from safflower. Scale bar = 200 nm.

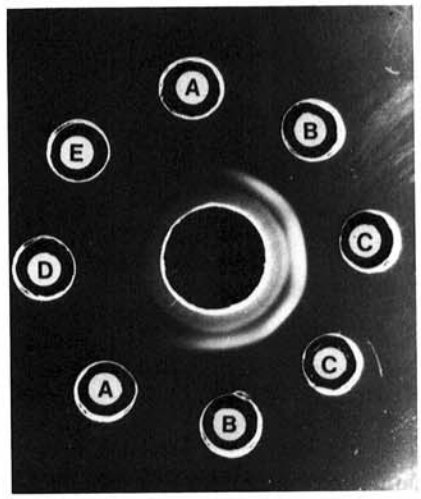

Fig. 2. Reaction of turnip mosaic virus (TuMV) antiserum (center well) with **(A)** clarified sap from healthy turnip, **(B)** clarified sap from turnip infected with TuMV isolated from safflower, **(C)** clarified sap from turnip infected with TuMV (T34), **(D)** saline, and **(E)** sodium dodecyl sulfate.

Table 1. Effect of turnip mosaic virus on growth and seed yield of safflower[y]

Treatment	Plant height (cm)	Plant dry weight (g)	No. undeveloped seeds/plant	No. fully developed seeds/plant	Weight (g) of fully developed seeds/plant
Uninoculated	53 a[z]	8.3 a	39 a	41 a	2.16 a
Lettuce mosaic	52 a	8.3 a	43 a	43 a	2.16 a
Turnip mosaic	47 b	7.2 a	57 a	15 b	0.92 b

[y]Tabular data represent means of 16 replicates.
[z]Values followed by a different letter are significantly different ($P = 0.01$).

between infected and healthy plants were significant ($P = 0.05$). The same number of seed heads developed on infected and healthy plants in the greenhouse. The weights (g) of seed composites from seven plants in each of three replicates in the field were 25, 32, and 25 for virus-infected plants and 127, 120, and 118 for healthy plants. The average numbers of seed heads per infected and healthy plants were nine and 19, respectively.

Heads of infected plants at flowering had only a few extended florets, usually located in the central part of the seed receptacle. Viable seeds were produced when florets were extended. Dissected seed heads showed rotting of some ovules and attached floret tubes and lack of seed development and floret extension from the periphery toward the center of the receptacle.

All plants (about 800) grown from seeds of infected plants were symptomless. Ninety-five and 98% of the pollen grains from florets of infected and healthy plants, respectively, stained positive for viability.

DISCUSSION

The virus isolated from safflower was identified as TuMV on the basis of physical properties, particle morphology, serology, and host range. The length of the virus particle was about 760 nm; a previously reported particle length of 788 nm was incorrect (6).

Disease patterns in safflower fields, particularly at the borders, indicated that *M. persicae* and perhaps other aphids transmitted TuMV from weed hosts growing near the fields. *B. geniculata* and *B. campestris* commonly growing along roadsides bordering safflower fields could serve as sources of TuMV.

Local necrotic lesions and systemic necrosis reactions induced by TuMV were difficult to distinguish from similar reactions to LMV in certain safflower cultivars (5), limiting the use of such cultivars in the diagnosis of either virus. Although reactions of cultivars VFR-1 and Pacific 7 to LMV and TuMV were distinctive in the greenhouse, it is unlikely that systemic necrosis in the field could be attributed to either virus with certainty without serological assays. Severe mosaic and stunting of mosaic-reacting cultivars in the field suggest that TuMV is the causal agent.

Among viruses known to cause mosaic in safflower cultivars, TuMV has the most adverse effect on growth and seed yield. Reduction of seed yield up to 65% was related to disruption of seed development. Development and extension of some florets were precluded by rotting of ovules. The effect of TuMV on seed development in safflower was similar to that of aspermy virus, which disrupts meiotic division of the megaspore mother cell in tomato, resulting in necrosis and rotting of tissue (1). In geraniums infected with tobacco and tomato ringspot viruses, there is a reduction in the number of florets that complete development (8). Lack of complete development of some florets might be a factor in the increase of undeveloped seed in TuMV-infected safflower seed heads. Some viruses decrease the vigor of pollen in infected hosts (7) and thereby reduce the number of viable pollen grains. TuMV transmission in safflower pollen was not ascertained, so it is not known whether the small reduction in the number of viable pollen grains observed from infected safflower is an effect of the virus.

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