A Severe Necrotic Disease of Sugar Beet Caused by a Strain of the Beet Mosaic Virus

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During the summer of 1963, Dr. F. J. Hills, Department of Agronomy, University of California, Davis, called our attention to sugar beet plants in a field near Davis that were showing a severe necrotic disease which hitherto had not been observed in this area. As a certain amount of distortion and vein clearing accompanied the symptoms of necrosis on affected plants, it was postulated that the causal agent might be a virus not previously found infecting sugar beets. Tests with the extracted sap of infected plants showed that a mechanically-transmissible virus was present. Further study of the disease and its causal virus showed that it was caused by an unusual strain of the beet mosaic virus. The results of this study and a description of the disease are presented herein.

Symptoms on Sugar Beet

The disease as originally observed on naturally infected beets consisted of a prominent necrosis of the leaf veins. This necrosis was associated only with the intermediate sized and smaller veins of partially developed leaves. These affected veins appeared as a dark necrotic network, while the rest of the leaf, with the exception of a small amount of inconspicuous mottling, appeared almost normal. Frequently the interveinal tissue of such leaves was puckered or blistered outward suggesting that there was no cessation of growth when the peripheral veinal tissue died. No necrosis, however, was observed on the larger veins, midribs, or petioles of affected plants. Vein-clearing and small necrotic flecks were present on the smaller immature leaves near the center of the crown on naturally infected plants.

Symptoms on mechanically inoculated beet seedlings in the greenhouse were somewhat different from those observed on naturally infected plants but the most conspicuous symptom was again necrosis. Generally the first symptoms developed about 3-5 days after inoculation and consisted of chlorotic or necrotic local lesions on the inoculated leaves (Figure 1, B & D). Initial systemic symptoms usually developed concurrently with the local symptoms or shortly thereafter and consisted of vein-clearing

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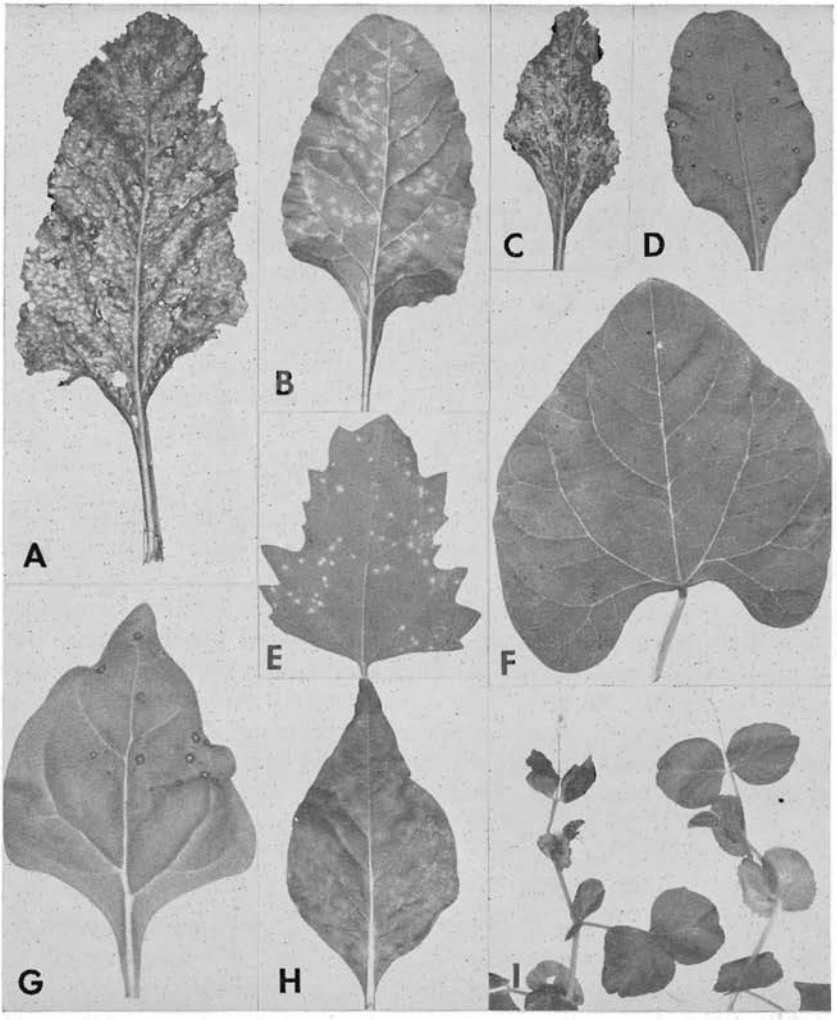

Figure 1.—Symptoms of the necrotic strain of beet mosaic virus on various hosts. Systemic symptoms on a well developed leaf of sugar beet (A) and a tip leaf from the same plant (C); local lesions on inoculated leaves of sugar beet (B & D), *Chenopodium quinoa* (E), Bountiful bean (F), and New Zealand spinach (G); Systemic mottle on *Nicotiana clevelandii* (H) and systemic terminal necrosis on Dwarf Telephone peas (left in I, on the right is a healthy pea plant for comparison).

and necrotic flecks on the youngest developing leaves in the crown. As these leaves continued to develop they exhibited prominent necrotic patches usually associated with the smaller veins and the neighboring tissue. Due to lack of growth as the leaves expanded, these necrotic areas caused tearing of the tissues giving the leaves a shot-holed appearance (Figure 1, A). Prominent chlorotic ringspots accompanied the necrosis and the leaves were generally somewhat distorted with an abnormal serration of the leaf margins (Figure 1, A). Generally, the plants were severely stunted.

Symptoms on Other Host Plants

The virus was inoculated to various other species of plants in the greenhouse in order to obtain some information on its host range. The plants were usually grown in 5-inch clay pots in a sterile composted greenhouse soil consisting of fine sand and peat supplemented with bone and blood meal. Generally, 4-10 plants of each species were mechanically inoculated by rubbing phosphate-buffered homogenates of infected leaves of *Nicotiana multivalvis* over corundum-dusted leaves with a clean forefinger. After allowing a period of 8-15 days for symptom development, an attempt was made to recover the virus from each plant by mechanical inoculation to beet seedlings. Symptoms on various plants were as follows:

Beta vulgaris var. *cicla* (L.) Moq. - Swiss Chard - Circular brown necrotic local lesions on inoculated leaves followed by a prominent systemic mosaic mottle with necrotic areas. *Chenopodium amaranticolor* Coste & Reyn. - Pale necrotic local lesions developing after 5-7 days on the inoculated leaves, followed shortly by stunting, yellowing, and downward curling of apical leaves, which together with the growing point, soon died.

C. capitatum (L.) Asch. - Reaction similar to *C. amaranticolor*.

C. quinoa Willd. - Local necrotic lesions (Figure 1, E) as on *C. amaranticolor* but accompanied by systemic development of a severe mottle with distortion and necrosis resulting in eventual death of the plants.

Cucurbita pepo L., var. Buttercup squash - No symptoms but virus recovered from small terminal leaves.

Gomphrena globosa L. - Chlorotic local lesions with pale necrotic centers after 7-10 days on inoculated leaves.

Hibiscus esculentus L. - A transient chlorotic line pattern on systemically invaded tissues; symptomless thereafter, but virus recovered.

Montia perfoliata (Domn) Howell. - Reddish-brown necrotic local lesions developing after about 10 days on inoculated leaves; no systemic symptoms.

Nicotiana clevelandii Gray. - Occasionally chlorotic local lesions on inoculated leaves; systemic chlorotic mottle.

N. multivalvis Lindl. - Reaction same as *N. clevelandii* (Figure 1).

Phaseolus vulgaris L. var. Bountiful, Morse Pole, Red Kidney and Sutter Pink - Very small reddish-brown necrotic local lesions (Figure 1, F); no systemic symptoms.

Pisum sativum L., var. Dwarf Telephone - Small local necrotic flecks on inoculated leaves; systemic terminal necrosis with eventual collapse and death of the plants (Figure 1, I).

Tetragonia expansa Thunb. - Dark necrotic local lesions on inoculated leaves (Figure 1, G); no systemic invasion by the virus.

Plants on Which No Infection was Obtained

No symptoms were observed or virus recovered from the following species after mechanical inoculation with the virus:

Althaea rosea L. Cav.; *Antirrhinum majus* L.; *Capsicum frutescens* L., var. California Wonder, Hungarian Yellow; *Cyomopsis tetragonoloba* (L.) Taub.; *Dahlia variabilis* (Willd.) Desf.; *Datura stramonium* L.; *Dolichos lablab* L.; *Helianthus annuus L.; Ipomoea purpurea* (L.) Lam.; *Matthiola incana* (L.) R. Br.; *Melilotus indica* (L.) All.; *Mirabilis jalapa* L.; *Nicotiana tabacum* L., var. Wisconsin Havana 425; *N. glutinosa* L.; *Phaseolus mungo* L.; *Physalis exocarpa* Brot.; *P. peruviana* L.; *Plantago lanceolata* L.; *Raphanus sativus* L.; *Rumex acetosa* L.; *Sesbania exaltata* (Raf.) Cory; *Trifolium incarnatum* L.; *T. pratense* L.; *Vicia faba* L.; *Vigna sinensis* (Torner) Savi; *Viola odorata* L.; *Zinnia elegans* Jacq.

The host range of the virus is similar to that of the beet mosaic virus, although somewhat different than that reported by others (3,4)[2].

Properties of the Virus *In Vitro*

The properties of the virus *in vitro* were determined by using the expressing sap from systemically infected sugar beet. After each treatment, the sap was rubbed over several young sugar beet seedlings to assay for infectivity. The following results were obtained: dilution, infection at 10^{-3}, none at 10^{-4}; longevity *in vitro* (ca. 20°C), infection after 2 days, none after 3 days; thermal inactivation (10 minute duration); infection after heating at 60°C, none after 65°C.

[2] Numbers in parentheses refer to literature cited.

These properties of the virus are similar to those reported by Pound (3) for the beet mosaic virus but also agree fairly well for those for the beet marble leaf (1), or ring mottle viruses (2).

Insect Transmission of the Virus

The virus was found to be readily transmissible by green peach aphids, *Myzus persicae* Sulz. Non-viruliferous insects were cultured on Chinese cabbage, *Brassica pekinensis* (Lour.) Rupr. When starved for 2 or more hours, followed by an acquisition feeding period of approximately 2 minutes on infected beets, slightly over 10 percent of the insects transmitted the virus to healthy sugar beet seedlings when single aphids were used. Thus, the green peach aphid is a fairly efficient vector of the virus.

Electron Microscopy of the Virus

Brandes' leaf dipping method (8) was used to prepare specimens for electron microscopy. Preparations from healthy and diseased *Nicotiana clevelandii* and sugar beet were shadowed with Uranium at an angle of 1 to 3 in a Kinney High Vacuum Evaporator and examined in an RCA EMU 3-G electron microscope. Flexuous rods which were presumed to be virus particles were seen in preparations from diseased material. Particles from *N. clevelandii* are shown in Figure 2.

Size determinations were made either by reference to polystyrene latex spheres 264 mμ in diameter which were included in the water droplets at the time of carrying out the leaf dips, or by reference to photographs of a diffraction grating, made at the same magnification as the virus. The two methods gave almost identical results. Though only a few particles were found, measurements were made of 25, the lengths of which varied between 641 mμ and 787 mμ. The mean particle length was determined to be 688 (± 8) mμ. About one-half of the total number of particles measured were approximately 650 mμ in length. This particle length is somewhat shorter than that reported for the beet mosaic virus in dip preparations (8).

Cross-Protection Tests

The various properties of the virus suggested it might be a strain of the beet mosaic virus although the symptoms it induced on beets were markedly different than the more commonly occurring strains of the virus. Several cross-protection tests were carried out to determine if infection with a conventional strain of the beet mosaic virus would immunize beets against infection by the necrotic virus. As the virus causes distinctive symptoms in beets, its presence in systemically infected plants would be readily discernible in plants previously infected with the beet mosaic virus.

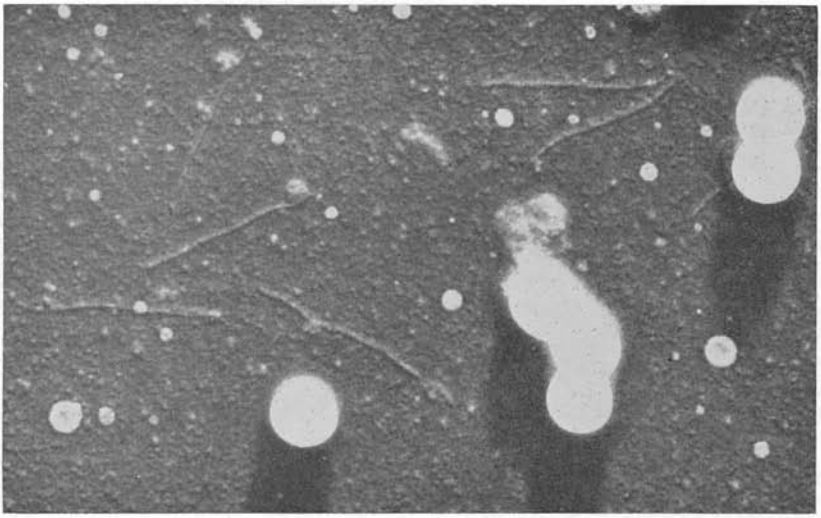

Figure 2.—Electron micrograph of the necrotic strain of the beet mosaic virus. The large white spheres are polystyrene latex particles 264 $m\mu$ in diameter. Magnification is approximately 34,800.

A batch of 17 young sugar beet plants were mechanically inoculated with a common strain of the beet mosaic virus; 8 days later these same plants and a batch of healthy seedlings of the same age, were inoculated with the necrotic virus. No symptoms characteristic of the necrotic virus developed on those plants previously infected with beet mosaic virus until several weeks had elapsed when two of the 17 plants developed a systemic necrosis. These results suggested that perhaps the beet mosaic virus protected against the necrotic virus but the results were not conclusive.

This experiment was repeated with another batch of 10 sugar beet seedlings. In this case a period of 3 weeks was allowed to elapse before superimposing the necrotic virus on beet mosaic virus infected plants. Moreover, only those leaves showing systemic symptoms of beet mosaic were inoculated with the necrotic virus. In this experiment, healthy plants of the same age, inoculated with the necrotic virus, developed numerous necrotic local lesions on the inoculated leaves but those already infected with beet mosaic developed no local lesions. Similarly, none of these plants developed systemic symptoms of the necrotic virus when previously infected with beet mosaic.

One additional experiment was done to confirm the previous results with the cross-protection tests. In this case, half-leaves of beet plants with several well-developed leaves were mechanically inoculated with a common strain of the beet mosaic virus. The opposite half-leaves were similarly rubbed with buffer alone. Eighteen days later, the entire surface of the same leaves was mechanically inoculated with the necrotic virus. Although in this test relatively few necrotic local lesions developed, and some developed on the half-leaves previously inoculated with the beet mosaic virus, the numbers of lesions on comparable half-leaves were markedly different in each case (Figure 3). The mean number of lesions of the necrotic virus on half-leaves previously inoculated with beet mosaic virus was 1.3, whereas an average of 14.2 was present on half-leaves previously rubbed with buffer

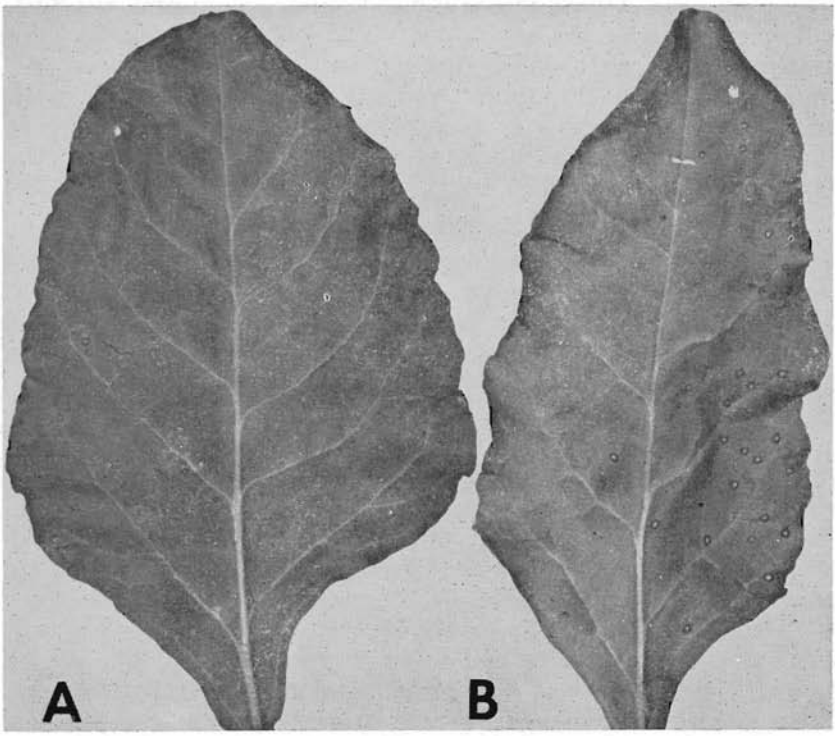

Figure 3.—Results of cross-protection tests with the necrotic strain of the beet mosaic virus. The right half of leaf A and the left half of leaf B were inoculated with a common strain of the beet mosaic virus which does not produce local lesions on sugar beet. Eighteen days later the entire surface of both leaves was inoculated with the necrotic strain of the beet mosaic virus. Note that the necrotic strain has not produced lesions on those half leaves previously inoculated with the common strain.

alone. Hence, this experiment confirmed previous results indicating that the beet mosaic virus can immunize plants against infection by the necrotic virus.

Effect of the Virus on the Growth of Sugar Beet Plants in the Greenhouse

After identifying the virus as a strain of the beet mosaic virus, a single test was done to obtain an estimate of the damage caused by the virus on sugar beet plants in the greenhouse. Uniform seedlings of sugar beet, variety US 75, were selected and transplanted into 6-inch clay pots. One batch of 20 plants was inoculated in a 2-4 leaf stage with the necrotic strain and a similar batch of 20 plants maintained as a control.

The plants were harvested 92 days after inoculation. The tops were removed at the soil line and weighed individually. The roots were removed from the soil, washed, and each weighed. The mean weight and standard deviation of tops and roots from infected plants were 62 $\pm$ 5 and 25 $\pm$ 4 ounces, respectively. The same from healthy plants was 246 $\pm$ 6 and 90 $\pm$ 5 ounces, respectively, for tops and roots. These results are indicative of the potentially serious effect of this strain of mosaic on the growth and yield of sugar beets.

Discussion

The host range, symptoms on hosts other than beet, properties *in vitro*, transmissibility, and morphology, indicate that the virus described herein is a strain of the beet mosaic virus. This tentative identification was confirmed by cross-protection tests with a conventional strain of the beet mosaic virus.

Several recently described virus diseases of beet (1,2,7), while similar to the virus described herein, and to other strains of mosaic, in their manner of aphid-transmissibility, have been distinguished on the basis of host range, symptomatology, and lack of cross-protection with known strains of beet mosaic. Although some of these viruses may be more distantly related strains of mosaic, this point can be resolved only by serology. The cucumber and alfalfa mosaic (6) viruses, also known to occur naturally on sugar beet and to be transmitted by aphids in a nonpersistent manner, differ from mosaic in host range, symptomatology, and morphology.

Experiments on the effect of this severe strain of mosaic on the growth of sugar beets in the greenhouse and on the yield in field experiments (5) show that the virus possesses the potential for causing very serious losses if it should become widespread in commercial plantings. At the present time, however, it is rarely encountered and thus, economically is of negligible importance.

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