Marble Leaf of Sugar Beet, Caused by a Juice Transmissible Virus

C. W. BENNETT[1]

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Introduction

In September 1959, sugar beet plants (*Beta vulgaris* L.) were received from a field in eastern Oregon for determination of the cause of yellowing of older leaves. The yellowing produced on leaves of these plants resembled that produced by beet yellows and beet western yellows in certain respects, but there was a type of mottling not characteristic of symptoms of either of these diseases. Tests showed that the plants were infected with a juice-transmissible virus, apparently not previously described on sugar beet. The disease and its causal virus were studied further and the results are presented herein. Because of the type of discoloration on mature leaves of affected plants the name beet marble leaf is suggested as a common name for the disease.

Symptoms and Host Range

Greenhouse symptoms of marble leaf have been studied on several species of plants inoculated with juice from diseased beet plants. The virus may produce both local lesions and systemic infection.

Local Symptoms

Local lesions are produced on juice-inoculated leaves of *Beta vulgaris* L. (sugar beet), *B. macrocarpa* Guss., *Chenopodium amaranticolor* Coste & Reyn., and *C. murale* L. (sowbane). Lesions are similar on all of these hosts. They begin to appear on sugar beet leaves as chlorotic spots about 1 mm in diameter about 9 days after inoculation. Lesions increase in size slowly and may attain a diameter of 2-3 mm (Figure 1A and B). A small necrotic spot may develop in the center of the lesion, sometimes surrounded by one or more rings ranging in color from yellow to green. If lesions are numerous, inoculated leaves of *C. amaranticolor* and *C. murale* often yellow and drop. In such leaves the lesions may be surrounded by a ring of green tissue.

Systemic Effects

The marble leaf disease has been found only on sugar beet. Its effects have been studied on sugar beet and other host plants under greenhouse conditions.

[1] Plant Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

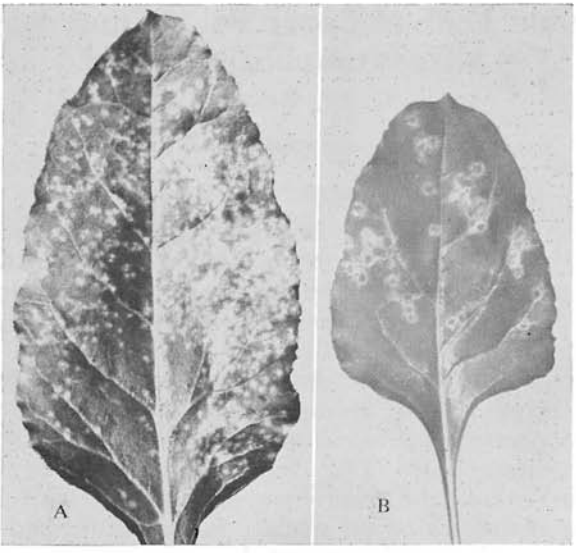

Figure 1.—Sugar beet leaves showing local lesions following juice inoculation. A, leaf with lesions in early stage of development; B, leaf with older lesions showing necrotic centers surrounded by chlorotic rings.

***Beta vulgaris* L. Sugar beet.** Systemic effects appear on sugar beet plants 10-30 days after inoculation. First systemic symptoms consist of vein chlorosis or mottling on young leaves and various patterns of chlorosis on half-grown leaves. Often the chlorotic areas are broader than the veins and they may be continuous or broken and accompanied by an indefinite mottle in some cases (Figure 2A). As the leaf matures, vein chlorosis becomes less conspicuous and the leaf becomes mottled. The chlorotic areas, however, are not conspicuous or well defined (Figure 2B). As the leaf ages the mottling becomes indefinite and the tissue between the main veins turns yellow prematurely (Figure 3). The leaves are not thickened as with beet yellows, but they appear dry and papery. Affected plants show no recovery. The range of symptoms described on leaves of different ages continues to be produced as long as affected plants remain reasonably vigorous. Except for the effects on young leaves, marble leaf could easily be mistaken for beet yellows or beet western yellows.

***Beta atriplicifolia* Rouy.** Symptoms are similar to those described on sugar beet.

***Beta macrocarpa* Guss.** About 20 days after inoculation, young leaves begin to curl downward at the tips and vein clearing is marked. New leaves are curled and stunted and growth of axillary buds is stimulated, often resulting in the production

of a type of rosette. Plants are stunted and the older leaves yellow prematurely. Seed yield is reduced drastically, and seeds from diseased plants are much smaller than those from healthy plants.

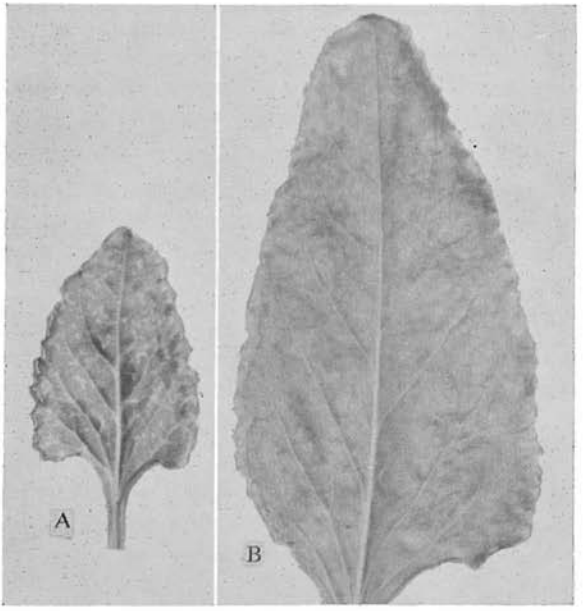

Figure 2.—A, young sugar beet leaf showing vein chlorosis and mottle; **B**, leaf approaching maturity, showing marbled type of mottling.

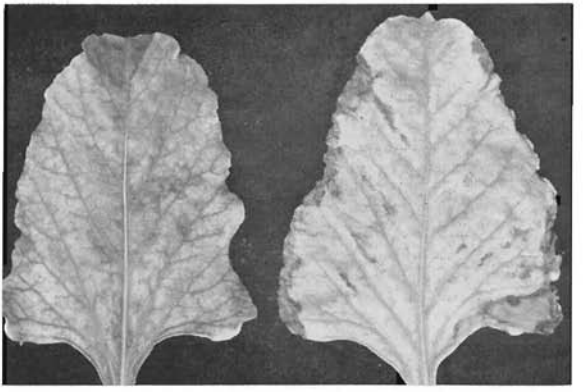

Figure 3.—Sugar beet leaves showing yellowing and necrosis produced on older leaves by the marble leaf disease.

Beta patellaris **Moq.** Leaves turn yellow or brownish, new growth yellows prematurely, and small dark spots appear on young leaves. No mottling is produced and there is little stunting of affected plants.

Beta patula **Ait.** Leaves begin to yellow as they approach maturity and may show a type of mottling or splotching. Leaves yellow and die prematurely.

Beta procumbens **Chr. Sm.** Yellowing, which is rather uniform without mottling, begins to appear when the leaves are about half-grown. Older leaves die prematurely and the plants are stunted.

Atriplex coronata **S. Wats. (Crown saltbush).** First symptoms begin to appear about 20 days after inoculation as broad chlorotic areas along the main veins. Leaves produced later show mottling and yellowing. Plants are not appreciably stunted.

Atriplex pacifica **Nels. (Dot scale).** Plants begin to produce leaves with reddish splotches on the blades and petioles and on the stems about 30 days after infection. Reddening is most intense at the leaf tips and around the edges of the leaves. Plants are not markedly dwarfed.

Chenopodium amaranticolor **Coste & Reyn.** Systemic infection begins to appear about 20 days after infection. First leaves that show symptoms may be only partially affected. Invaded portions show marked vein clearing of continuous or broken lines. Leaves produced later show distinct vein clearing and a type of mottle or splotching. Leaves are dwarfed and tend to bend downward and to roll slightly. Growth is reduced but infected plants produce seeds. The inflorescences of diseased plants have considerably more red pigment than healthy plants. Seeds of diseased plants are small and germination is reduced.

Chenopodium ambrosioides **L. (Mexican-tea).** Plants show marked mottling of a diffuse type. Tips of branches are distorted and leaves yellow and die. Seed production is much reduced.

Chenopodium murale **L. (Sowbane).** Systemic infection soon follows the production of local lesions on inoculated leaves. Young leaves are mottled and may show necrosis. Usually the blossoms become necrotic and the flowering parts die. Few seeds are produced on plants inoculated in the 5 to 10-leaf stage. In plants that are larger when inoculated, systemic infection usually does not become evident before the plants mature, or symptoms are produced on only the axillary shoots in the inflorescence. Plants are dwarfed and stems tend to bend and become distorted. Stems of parts of the plant showing leaf symptoms are brittle.

***Chenopodium urbicum* L. (City goosefoot).** Large chlorotic areas are produced on leaves that develop after infection. Plants are stunted and leaves become necrotic.

Plants on which no Infection was Observed

In host-range studies the following species in the indicated families, inoculated by means of rubbing juice from infected beet plants over leaves that had been lightly sprinkled with an abrasive, showed no evidence of infection.

AIZOACEAE.—*Tetragonia tetragonoides* (Pall.) Ktze. (New Zealand spinach).

AMARANTHACEAE.—*Amaranthus californicus* (Moq.) S. Wats., *A. caudatus* L. (Love-lies-bleeding), *A. cruentus* L. (Purple amaranth), *A. retroflexus* L. (Redroot amaranth).

CHENOPODIACEAE.—*Atriplex coulteri* (Moq.) D. Dietr., *A. expansa* (D. & H.) S. Wats. (Fogweed), *A. polycarpa* (Torr.) S. Wats. (Cattle saltbush), *Beta lomatogona* Fisch. & Mey., *B. maritima* L., *B. procumbens* Chr. Sm., *B. trigyna* Waldst. & Kit., *B. webbiana* Moq., *Chenopodium bonus-henricus* L. (Good King Henry), *C. capitatum* (L.) Aschers. (Strawberry-blite), and *Monolepis nuttaliana* (Schult.) Greene.

COMPOSITAE.—*Sonchus oleraceus* L. and *Zinnia elegans* Jacq. (Zinnia).

CRUCIFERAE.—*Capsella bursa-pastoris* L., (Shepherd's purse) and *Armoracia rusticana* Gaertn., B. Mey., & Scherb. (Horseradish).

CONVOLVULACEAE.—*Ipomoea purpurea* (L.) Roth (Morning glory).

GERANIACEAE.—*Erodium cicutarium* (L.) L'Her. (Filaree).

LEGUMINOSAE.—*Phaselous vulgaris* L. (Bean) and *Vigna sinensis* (Torner) Savi (Cowpea).

MALVACEAE.—*Malva parviflora* L. (Little mallow) and *M. sylvestris* L. (High mallow).

PRIMULACEAE.—*Samolus parviflorus* Raf. (Water pimpernel).

POLYGONACEAE.—*Rumex crispus* L. (Yellow dock).

SOLANACEAE.—*Capsicum frutescens* L. (Pepper), *Datura meteloides* DC. (Tolguacha), *D. stramonium* L. (Jimsonweed), *Lycopersicon esculentum* Mill. (Tomato), *Nicandra physalodes* (L.) Pers., (Apple-of-Peru), *Nicotiana bigelovii* S. Wats. (Indian tobacco), *N. glutinosa* L., and *Solanum dulcamara* L. (Bitter climbing nightshade).

URTICACEAE.—*Urtica dioica* L. (Stinging nettle).

Transmission of Causal Virus

Several methods of inoculation have been used with the mable leaf virus over a period of 3 years.

Juice Transmission. Marble leaf virus is readily transmissible by juice inoculation by the rubbing method of inoculation. Usually, high percentages of inoculated plants of susceptible species become infected.

Insect Transmission. The species of insects used in transmission tests were *Circulifer tenellus* (Baker), *Myzus persicae* (Sulz.), *Aphis fabae* Scop., *Hyalopterus atriplicis* L.,, *Pemphigus betae,* Doane, and *Macrosiphum euphorbiae* (Thomas).

Transmission was obtained with *M. persicae, A. fabae,* and *M. euphorbiae.* The transmission level was low with each of these species. In tests with *M. persicae* no infection was obtained with single aphids. With 2, 5, and 10 aphids per plant infection increased with number of aphids, but less than 20% infection was obtained with 10 aphids per plant. Fifty aphids per plant gave about 50% infection. Even lower percentages of infection were obtained with *A. fabae* and *M. euphorbiae.* No infection was obtained with the other species of insects even when used in very large numbers. It seems unlikely that any of the species shown to be vectors of the virus would be able to produce widespread transmission from beet to beet unless very large populations were present.

Tests for Seed Transmission. Plants of *Beta vulgaris* (an annual type), *B. macrocarpa, Chenopodium amaranticolor,* and *C. urbicum* were inoculated with marble leaf virus in early stages of development and retained until seeds were harvested. Seeds from these plants were planted in flats in the greenhouse and seedlings were watched to determine whether they showed symptoms of disease. Observations were made on 692 *B. vulgaris* seedlings, 439 *B. macrocarpa* seedlings, 742 *C. amaranticolor* seedlings and 740 *C. urbicum* seedlings. No evidence of infection was found on plants of any of these species. Since plants of each of these 4 species show marked symptoms when infected, it was concluded that the marble leaf virus probably is not seed-transmitted.

Tests for Transmission by Dodder. *Cuscuta californica* H. & A. was established on beet plants with marble leaf and stems were trained from diseased to healthy seedling sugar beet plants. In other tests, stem tips were broken from the dodder plants growing on diseased beets and placed on healthy seedling beet plants where they soon became established. Forty plants were inoculated by each of these methods, but no evidence of virus

transmission was obtained. Juice inoculations from dodder growing on diseased beet plants were made to *Beta macrocarpa*. No infection was obtained. It seems probable, therefore, that *C. californica* is not a host of marble leaf virus and that colonies growing on diseased beet plants contain little or no virus.

Properties of Causal Virus

In studies of properties of marble leaf virus, juice was pressed from diseased leaves of sugar beet plants and inoculated into leaves of healthy plants of *Beta macrocarpa* after the indicated treatment. Results were recorded on the basis of number of local lesions produced.

Thermal inactivation. In treatments of beet juice for 10 min. at 5° C. intervals, there was a progressive decrease in active virus from 50° through 60°. Thermal inactivation point of the virus appears to lie between 60 and 65°.

Tolerance of dilution. In tests in which beet juice was diluted with water, progressively fewer lesions were produced as dilution was increased, beginning with a 1-10 dilution. The dilution endpoint of the virus in beet juice appears to lie between 1-500 and 1-1000, indicating a low concentration of active virus in the beet plant.

Resistance to aging. Persistence of active virus in expressed juice of sugar beet varied widely in different tests at room temperature. In some tests no virus was recovered after 4 hours, whereas in others a small amount of infection was obtained after 24.

Damage Produced By Marble Leaf

Since the distribution and incidence of marble leaf are unknown, it is not possible to evaluate accurately the actual or potential economic importance of the disease in the field. It has been determined, however, that marble leaf is capable of producing appreciable stunting of plants in a greenhouse.

In 4 greenhouse tests, plants growing in 6-inch pots, 4 plants per pot, were inoculated in about the 4-leaf stage and harvested and weighed 60 to 78 days after inoculation. In a second type of test, plants growing singly in 3-gallon crocks and watered with Hoagland solution were inoculated in the 10 to 14-leaf stage. They were harvested and weighed in lots of 10 at monthly intervals 2 to 5 months after inoculation. In these tests (Table 1) marble leaf produced appreciable reductions in total plant weight, particularly in plants inoculated in the seedling stage. These losses are about the same as those produced by beet mosaic under similar conditions. Such losses indicate that marble leaf

Table 1.—Effect of marble leaf on weight of sugar beet plants (US 75) under greenhouse conditions.

Test number	Time from inoculation to harvest	Average weight of plants[1]	
		Healthy	Diseased
	Days	Grams	Grams
1	60	28	24
2	60	33	24
3	60	30	25
4	78	54	48
5	60	994	848
	90	1331	1230
	120	1690	1401
	150	1861	1595

[1] In tests 1 to 4, inclusive, 40 plants in 6-inch pots (4 plants per pot) were inoculated in the 4 to 6-leaf stage; in test 5, plants (singles) in 3-gallon crocks were inoculated in the 10 to 14-leaf stage and 10 plants were harvested after each indicated time interval. Inoculated plants were compared with equal numbers of check (non-inoculated) plants in each test.

would be capable of causing measurable yield reductions in the field if vectors were available to produce widespread dissemination of the causal virus in early stage of development of beet plants.

Tests were made also to determine the effects of marble leaf on seed production in *Beta macrocarpa*. Plants were inoculated in about the 6-leaf stage and retained for seed production along with appropriate healthy check plants. In 2 tests (Table 2) there were reductions in seed yield and weight of more than 50 percent. The effect of the disease on yield of seed of sugar beet has not been determined.

Table 2.—Effect of marble leaf on seed production and seed size in *Beta macrocarpa*.

Test number	Weight of seeds from 10 plants		Weight of 100 seeds	
	Healthy plants	Diseased plants	Healthy plants	Diseased plants
	Grams	Grams	Grams	Grams
1	68.5	31.2	3.0	1.3
2	82.4	36.2	3.5	1.2

Summary

A juice-transmissible virus which causes vein-yellowing and mottling of immature leaves and distinct yellowing of mature leaves of sugar beet plants was isolated from beets from Oregon. The virus produces local lesions on inoculated leaves of *Beta vulgaris, B. macrocarpa, Chenopodium amaranticolor,* and *C. murale.* Systemic infection was obtained in 6 species of *Beta,* 4 species of *Chenopodium* and 2 species of *Atriplex.* The virus appears to have a limited host range. *Myzus persicae, Aphis fabae,* and *Macrosiphum euphorbiae* are inefficient vectors of the virus. No evidence of seed transmission was found. The virus has a thermal inactivation point between 60 and 65 C, a dilution endpoint of about 1-1,000, and it remains active in extracted juice at room temperature for 24 hours, or less. Under greenhouse conditions the disease caused about a 10 percent reduction in plant growth. Yield and size of seeds of *Beta macrocarpa* were greatly reduced. The disease apparently has a very limited distribution and is not known to be causing measurable loss to the sugar beet crop in the areas where it was found.