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SPINACH YELLOW DWARF

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SPINACH YELLOW DWARF[1]

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INTRODUCTION

FIVE VIRUS DISEASES of spinach (*Spinacia oleracea* L.) have thus far been reported to occur under natural conditions in the United States—namely, spinach blight (McClintock and Smith, 1918)[4], which is identical with common cucumber mosaic (Hoggan, 1933); aster yellows (Kunkel, 1926; Severin, 1934; Severin and Frazier, 1945); sugar-beet curly top (Severin and Henderson, 1928; Scott, 1935; Adams, 1936); beet mosaic (Jones, 1931; Hoggan, 1933; Smith, 1934); and spotted wilt (Gardner, Tompkins, and Thomas, 1937).

This paper deals with still another naturally occurring virus disease of spinach—spinach yellow dwarf. The aspects covered include symptoms and host range of the disease, the properties and aphid vector of the virus, transmission of the virus by single aphids, a comparison of the transmission of the virus by one species of aphid with mechanical inoculation, and retention of the virus by aphids.

MATERIALS AND METHODS

Source of Virus. The source of the spinach-yellow-dwarf virus was naturally infected spinach plants obtained from the truck-crop fields near San Pablo. The virus was retained through repeated mechanical inoculations and aphid transmission to spinach.

Spinach Extract. Juice from diseased spinach plants was obtained by grinding the plants to a pulp in a sterilized food chopper or mortar. The pulp was then placed between two layers of cheesecloth and the sap expressed by hand into a sterile glass dish.

Mechanical Inoculation. The method of mechanical inoculation used is that described by Rawlins and Tompkins (1936). After inoculation, the inoculum and the carborundum were washed from the leaves with water. The virus extract from each preparation was inoculated into 5 healthy spinach plants.

Variety of Spinach. Long Standing Bloomsdale spinach was used in studies of properties and aphid transmission of the virus and as a source of virus in host-range studies. This variety grows rapidly and remains in good condition a long time without bolting to seed.

Noninfective Aphids. Noninfective green peach aphids were obtained from populations reared on healthy sugar beets maintained in the greenhouse.

Methods of Transferring Aphids. High populations of aphids were transferred by cutting off leaves which bore large numbers of aphids, then placing the leaves on the inner or youngest leaves of another plant. Small

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

numbers of aphids were transferred from plant to plant with a moistened camel's-hair brush.

SYMPTOMATOLOGY

The most conspicuous symptoms of the disease on naturally infected spinach are yellow blotches on the outer, or oldest, leaves, and dwarfed, puckered, curled, mottled inner, or youngest, leaves. Typical symptoms are illustrated in plate 1.

The first visible symptoms of the disease on spinach are a clearing of the veinlets (plate 2, *A*) and curvature of the midrib. These symptoms develop within from 12 to 14 days after inoculation in the greenhouse, and from 25 to 35 days out of doors during the winter.

The younger leaves next show yellow and green mottling, puckering, curling, and blisterlike elevations (plate 1). The older leaves show numerous small chlorotic areas (plate 2, *B*) which later coalesce and form conspicuous yellow blotches (plate 2, *C*). The yellow blotches become necrotic, the condition usually beginning at the basal margin of the leaf (plate 2, *D*) and progressing toward the tip. The heart of the infected plant is stunted, with shortened petioles on the younger leaves (plate 1).

As the disease progresses, the older leaves gradually become brown and dry. The young leaves usually remain mottled and distorted for a week or more after the older leaves are dead, but eventually they also become yellow and die.

HOST RANGE

Natural Infection. The natural host range of the yellow-dwarf virus so far known is limited to spinach. The virus was recovered from naturally infected spinach by inoculation of extracted sap into healthy spinach.

Experimental Infection. The following ten horticultural varieties of spinach have been experimentally infected with the virus by mechanical inoculation:

Chenopodiaceae:
Spinacia oleracea, varieties Broad Flanders, Giant Thick Leaved, Juliana, King of Denmark, Long Standing Bloomsdale, Prickly Seeded Dark Green, Savoy Leaved or Bloomsdale, Thick Leaved Nobel, Virginia Savoy, and Virofloy.

The variety Virginia Savoy spinach is resistant to spinach blight (Smith, 1920; Hoggan, 1933), but is susceptible to the yellow-dwarf virus.

Recovery of Virus. The virus was recovered from all the experimentally infected varieties of spinach by inoculation of extracted sap in healthy spinach.

Nonsusceptible Plants. The following economic and ornamental flowering plants, tested by mechanical inoculation, are nonsusceptible to the disease:

Aizoaceae:
Tetragonia expansa, New Zealand spinach
Campanulaceae:
Campanula medium, canterbury bells
Chenopodiaceae:
Beta vulgaris, varieties of garden beets: Crimson King, Crosby's Egyptian, Detroit Dark Red, Early Blood Turnip, Extra Early Flat Egyptian, Early Wonder, Ferry's Crosby, and Good for All

Beta vulgaris, varieties of mangel-wurzel or stock beets: Danish Red Giant Eckendorf, Danish Sludstrup, Danish Yellow Giant Eckendorf, Giant Half Sugar Green Top, Giant Half Sugar Rose Top, Giant Yellow Intermediate, Mammoth Long Red, Yellow Leviathan
Beta vulgaris var. *cicla*, varieties of Swiss chard: Large Ribbed Dark Green, Lucullus Dark Green
Compositae:
Callistephus chinensis, aster
Lactuca sativa, lettuce
Crucifereae:
Brassica oleracea var. *capitata*, cabbage
Brassica campestris, common yellow mustard
Mathiola incana var. *annua*, stock or gilly flower
Cucurbitaceae:
Cucurbita pepo, varieties of squash: Italian or Zucchini, White Bush Scallop
Citrullus vulgaris, varieties of watermelons: Kleckey's Sweet, Klondike
Cucumus sativus, White Spine cucumber
Geraniaceae:
Pelargonium zonale, geranium
Leguminosae:
Lupinus hartwegii
Malvaceae:
Gossypium hirsutum, cotton, Acala variety
Nyctaginaceae:
Mirabilis jalapa, four-o'clock
Primulaceae:
Primula veris, cowslip
Solanaceae:
Capsicum frutescens, California Wonder pepper
Lycopersicon esculentum, Marglobe tomato
Nicotiana glutinosa
Nicotiana alata var. *grandiflora*, tuberous-flowered tobacco
Nicotiana tabacum, Turkish tobacco
Petunia hybrida, petunia, Rosy Morn variety
Datura stramonium, stramonium
Solanum tuberosum, potato
Umbelliferae:
Foeniculum vulgare var. *dulce*, Florence fennel
Apium graveolens var. *dulce*, celery, Golden Self-Blanching variety

The host range of the virus so far discovered is thus limited to varieties of spinach. Plants in only thirteen families were tested, however, and it may be possible that economic or ornamental plants or weeds in other families are susceptible. A limited host range for this virus would not be suprising, for those of other mosaic viruses are limited. For example, Tompkins and Middleton (1941) found that the host range of a mosaic disease of *Primula obconica* is limited to two additional species within the same genus, namely, *P. malacoides* and *P. sinensis*.

PROPERTIES OF THE VIRUS

Thermal Inactivation. The thermal inactivation of the spinach-yellow-dwarf virus was determined in the extracted sap from leaves of experimentally infected spinach. Ten cubic centimeters of diseased sap was poured into thin glass test tubes, which were plugged with cotton and submerged in a water bath maintained at the desired temperature by an electric thermostat. An

agitator connected to an electric motor kept the water in circulation to maintain an evenly distributed temperature. The time of exposure in the water bath was 11 minutes, 1 minute being allowed for lag. After exposure at the desired temperature, the extract was cooled rapidly by partly submerging the test tubes in running tap water. Unheated controls were used in each test. Determinations were made at 5° C intervals. The results were as follows:

Temperature, ° C	Plants inoculated	Per cent infected
Unheated control	25	84
40	25	84
45	15	80
50	25	4
55	15	0
60	25	0
65	25	0
70	25	0

These data show that the virus remained active at 45° and that 1 infection was obtained from 25 inoculations at 50°; but no infections occurred at 55° to 70°.

Effect of Freezing Virus Extract. Two extractions of sap from leaves of experimentally infected spinach plants were placed in cold storage at –18° C immediately after extraction. Monthly inoculations of the virus extract in healthy spinach were made for a period of 6 months. The results were as follows:

Age of virus extract, months	Plants inoculated	Plants infected
Control	10	9
1	10	8
2	10	9
3	10	6
4	10	5
5	10	4
6	10	1

Thus freezing did not inactivate the virus at the end of 6 months, although only 1 infection was obtained with 10 plants inoculated at the end of this time.

Tolerance to Aging in Vitro. Tests were made to determine the inactivation of the virus in diseased spinach sap exposed to the air at room temperatures. Five cc of the expressed juice from experimentally infected spinach plants was poured into sterile test tubes which were plugged with cotton. Inoculations of the virus extract stored in test tubes were made at intervals of 24 hours for a period of 9 days. The following data were obtained, 25 plants being inoculated in each test:

Days exposed	Plants infected	Days exposed	Plants infected
0; Control	19	5	2
1	15	6	3
2	14	7	2
3	7	8	0
4	7	9	0

These data indicate that infectivity of the virus occurred after the extract was aged *in vitro* from 3 to 7 days and that no infections were obtained at the end of 8 and 9 days.

Tolerance to Dilution. In determining the tolerance of the virus to dilution, the juice expressed from experimentally infected spinach was diluted with sterile distilled water. The infections obtained were as follows:

Dilution	Plants inoculated	Per cent infected
Undiluted control	45	80
1:10	45	87
1:100	45	76
1:1,000	45	36
1:5,000	45	16
1:10,000	45	13
1:15,000	35	3
1:20,000	35	6
1:25,000	35	0
1:50,000	45	0

The tolerance to dilution was 1:20,000. There is considerable variation in the tolerance to dilution of the virus with different extractions; with three virus extracts, infections were obtained at dilutions of only 1:100.

APHID TRANSMISSION OF VIRUS

By Green Peach Aphid. Large populations of the green peach aphid, *Myzus persicae* (Sulzer) have been observed in the fields near San Pablo. This aphid transmitted the spinach-yellow-dwarf virus from experimentally infected to healthy spinach plants in the greenhouse.

By Single Aphids during Short Feeding Time. An experiment was conducted to determine whether single green peach aphids were able to transmit the virus during short feeding periods. Single previously noninfective wingless aphids were fed 5 minutes on a diseased plant and then 5 minutes on a healthy spinach plant. Two infections were obtained with 25 aphids tested.

Comparison of Transmission of Virus by Aphids with Mechanical Inoculation. Transmission of the virus by 20 green peach aphids was compared with that by mechanical inoculation. After a large population of previously noninfective green peach aphids was reared on experimentally infected spinach plants, lots of 20 aphids were transferred from diseased plants to each of 5 healthy spinach plants. The extract from each diseased plant on which the aphids fed was also inoculated into 5 healthy spinach plants. The results were as follows:

Test no.	Plants infected by aphid transmission	Plants infected by mechanical inoculation
1	4	5
2	4	3
3	2	4
4	0	4
5	0	3
Total	10	19

Infections obtained by aphids thus averaged 40 per cent and by mechanical inoculation 76 per cent.

Retention of the Virus. Two experiments were conducted to determine the length of time the green peach aphids would retain the virus. In the first experiment, 5 lots of 20 wingless green peach aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred daily to 3 successive healthy spinach plants. The aphids remained on the third plant for a period of 1 week. The results were as follows, the plus sign indicating production of the disease and the minus sign indicating that no disease resulted:

Test no.	1st day	2d day	3d day
1	+	−	−
2	+	−	−
3	+	−	−
4	+	−	−
5	+	−	−
Total +	5	0	0
Total −	0	5	5

Thus aphids infected only those plants fed upon the first day.

In the second experiment, 5 lots of 20 wingless aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred hourly to 10 successive healthy spinach plants. As appears in table 1, 5 lots of aphids infected a spinach plant during the first hour, and 2 lots during the second hour also, and none thereafter.

TABLE 1

RETENTION OF SPINACH-YELLOW-DWARF VIRUS BY LOTS OF 20 GREEN PEACH APHIDS, *Myzus persicae*, TRANSFERRED HOURLY TO 10 SUCCESSIVE HEALTHY SPINACH PLANTS

Test no.	Results* on successive plants, with hourly transfers									
	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
1	+	+	−	−	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−	−	−
3	+	−	−	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−	−	−
5	+	−	−	−	−	−	−	−	−	−
Total +	5	2	0	0	0	0	0	0	0	0
Total −	0	3	5	5	5	5	5	5	5	5

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

DESCRIPTION OF SPINACH-YELLOW-DWARF VIRUS

Name: Spinach yellow dwarf.

Host plant: Spinach (*Spinacia oleracea*).

Symptoms of disease: Vein clearing, yellow and green mottling, puckering, curling, blisterlike elevation, curvature of midribs and petioles, and stunting of young leaves. Small chlorotic areas, which fuse, forming yellow blotches, followed by necrosis and drying of outer leaves. Yellowing and death of younger leaves.

Incubation period of disease: 25 to 30 days out of doors during winter.

Properties of virus: Thermal inactivation 55° C in 10-minute exposures, resistance to aging *in vitro* 8 days, tolerance to dilution 1:20,000.

Modes of transmission: Natural inoculation by green peach aphid, *Myzus persicae;* mechanical inoculation with expressed juice.

SUMMARY

The symptoms of yellow dwarf on naturally and experimentally infected spinach are described.

The host range of this virus is limited to spinach. Twenty-six species of plants in 23 genera belonging to 13 genera, were nonsusceptible to the virus. Some of the properties of the spinach-yellow-dwarf virus are summarized as follows: thermal inactivation of the virus was 55° C in 10-minute exposures. Freezing sap extracted from diseased spinach kept in cold storage at –18° C did not inactivate the virus at the end of 6 months. An inactivation of the virus occurred after the virus extract was exposed to the air at room temperature for a period of 8 days. The tolerance to dilution of extracted diseased spinach juice was 1:20,000.

The green peach aphid, *Myzus persicae* (Sulzer), was demonstrated to be a vector of the virus. Two of 25 previously noninfective wingless aphids transmitted the virus after feeding 5 minutes on a diseased plant and 5 minutes on each healthy plant. Infections obtained by lots of 20 aphids averaged 40 per cent, and by mechanical inoculation of the virus extract from each plant on which the aphids had fed, 76 per cent. Lots of 20 wingless aphids transmitted the virus from diseased to healthy plants during the first day, but failed to infect plants during the second day and during the following week. Five lots of 20 infective aphids, when transferred hourly to successive sets of healthy spinach for a period of 10 hours, infected 5 plants during the first hour, 2 plants during the second hour, and none thereafter.

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PLATES

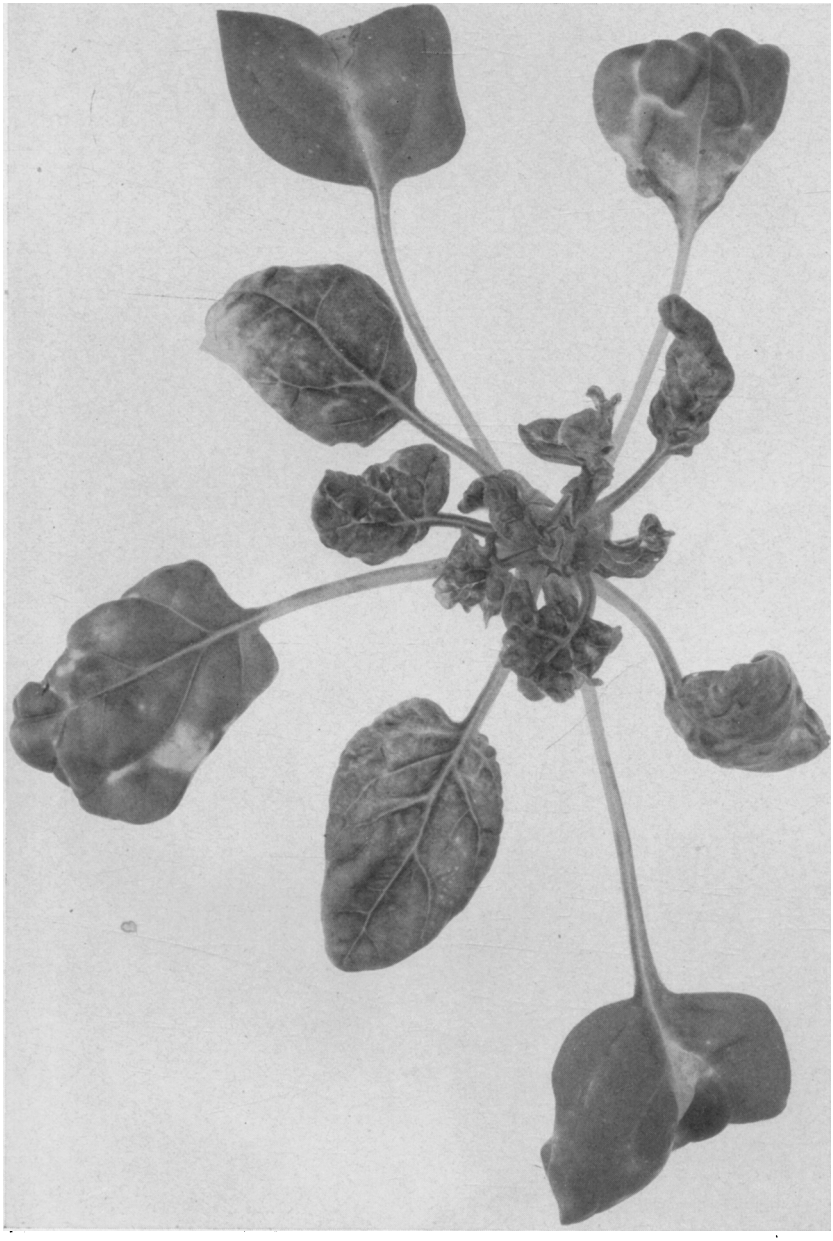

Plate 1.—King of Denmark spinach (*Spinacia oleracea*) experimentally infected with yellow-dwarf virus showing yellow blotches on the older, puckered, and curled leaves; and showing dwarfed, puckered, mottled, younger leaves, some displaying curved petioles.

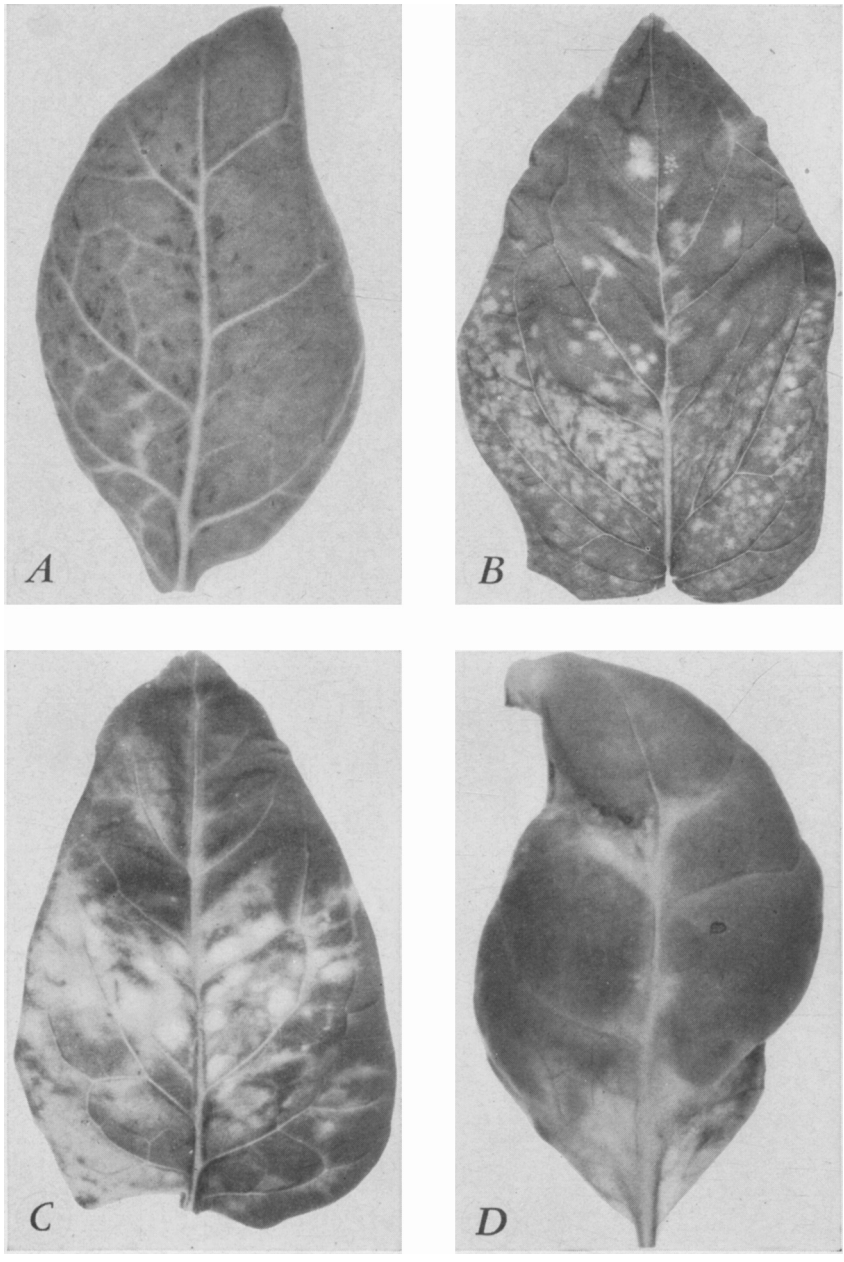

Plate 2.—King of Denmark spinach (*Spinacia oleracea*) infected with yellow-dwarf virus: *A*, cleared veinlets on youngest leaf; *B*, leaf showing numerous, small, circular, chlorotic areas, *C*, yellow blotches formed by fusion of the chlorotic areas; *D*, chlorosis and necrosis of leaf.