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TRANSMISSION OF CELERY-YELLOW-SPOT VIRUS BY THE HONEYSUCKLE APHID, RHOPALOSIPHUM CONII (DVD.)[1]

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CELERY YELLOW SPOT was first observed during the summer of 1934 in the Santa Clara Valley near Milpitas and has since been found in the celery fields near San Jose, Hollister, Salinas, and Sacramento. Symptoms have been briefly described (Severin and Freitag, 1938),[4] and results of insect-transmission experiments (Freitag and Severin, 1939) presented, in previous papers. The disease causes no appreciable loss to celery growers, although 40 per cent of the plants were infected in some fields. Because the plants are only slightly stunted and because the spotted outer leaves are normally discarded in the harvesting of celery for the market, the disease is of small economic importance.

In 1935 an investigation was undertaken to study the symptoms and host range of celery-yellow-spot virus. Attempts were made to transmit the virus by means of different aphid species and by mechanical inoculations. Virus transmission by single specimens of winged and mature wingless aphids and retention by the aphid vector were the subject of experiment.

SYMPTOMS

Usually the first symptom noticed in the greenhouse, about 14 days after inoculation of celery plants by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.) [*R. melliferum* (Hottes)], was the irregular pale green areas or spots and stripes, which rapidly became yellow (plate 1, *B*). This yellow spotting is the most characteristic sign of the disease. The spots and stripes are mostly along the veins (plate 1, *C;* plate 2, *A*), but are also scattered irregularly over the leaflets (plate 1, *E;* plate 2, *E*).

The yellow areas are irregular in shape and variable in size (plate 1, *D;* plate 2, *B, C*). The spots along the veins are often elongate (plate 1, *C;* plate 2, *E*) and sometimes occur at the basal portion of veinlets, where the latter join the main and lateral veins (plate 2, *A*). Some of the yellow spots are round and form small circular chlorotic areas (plate 2, *D*). The chlorotic spots may be numerous (plate 2, *D*) and may result in a general yellowing of the leaflet (plate 1, *E*). In advanced stages of the disease the small chlorotic spots may coalesce, forming enlarged spots (plate 1, *D;* plate 2, *B, D*) and mottled areas (plate 1, *F*). Their yellow color gradually fades and may become white as the leaf matures.

The petioles of naturally infected celery plants develop circular white spotting (fig. 1). When the epidermis is removed from these areas, brown specks may be seen along the veins of the celery stalk (plate 2, *F*).

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

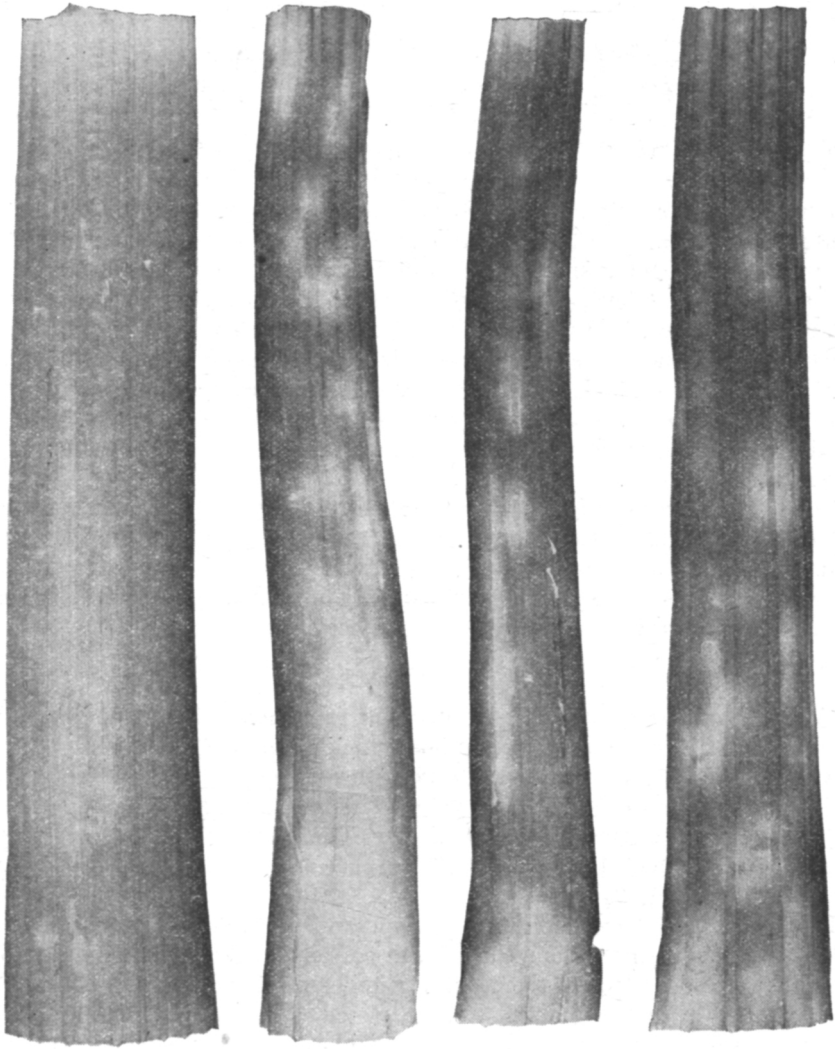

Fig. 1.—Petioles from celery plants: left, petiole from a healthy plant; right, three petioles from celery naturally infected with yellow spot, showing white spotting. (Milpitas, October 17, 1935.)

HOST RANGE

Natural Infection.—Celery (*Apium graveolens* L. var. *dulce* DC.), poison hemlock (*Conium maculatum* L.), and parsnip (*Pastinaca sativa* L.) are the only plants that were found to be naturally infected with celery yellow spot. Virus was recovered, by the honeysuckle aphid, from naturally infected parsnip and poison hemlock. Although the virus has not yet been recovered from celery either by aphids or by mechanical inoculation (see p. 380), the symptoms on naturally infected celery are identical with those on celery experimentally infected by the honeysuckle aphid from infected poison hemlock and parsnip.

Naturally infected parsnip plants, found at Stockton, showed a mild mottling of the leaves, whereas poison hemlock proved to be a symptomless carrier.

Experimental Infection of Celery.—Experiments were conducted to infect celery with yellow spot. Flower clusters from poison-hemlock plants heavily infested with honeysuckle aphids were collected from several localities. None of the plants from which the aphids were taken manifested yellow-spot symptoms. Poison hemlock that showed mottling, spotting, chlorotic rings, and line patterns was always demonstrated to be infected with the poison-hemlock-ringspot virus (Freitag and Severin, 1945). Plants, however, rarely suffered from a virus complex of ringspot and yellow spot. The poison-hemlock flower clusters infested with aphids were cut so that each piece had 50 to 100 aphids on it. From 5 to 10 pieces were cut from a single infested plant; then each piece was placed in a cage with a healthy celery plant. As the flower clusters wilted and dried, the aphids would move to the healthy celery leaves. There they fed for 2 to 5 days, after which they were fumigated with Nico-fume tobacco paper. Samples were taken near Alvarado, Sacramento, San Pablo, and San Jose from 25 poison-hemlock plants which proved to be naturally infected. The results obtained were as follows:

Locality and poison-hemlock plant no.	Celery plants inoculated	Celery plants infected
San Pablo		
No. 1	10	10
No. 2	10	6
No. 3	10	1
San Jose		
No. 4	10	2
Sacramento		
No. 5	5	5
No. 6	5	3
No. 7	5	3
No. 8	5	2
No. 9	5	1
Alvarado		
No. 10	10	10
No. 11	10	10
No. 12	10	10
No. 13	10	9
No. 14	10	7
No. 15	10	7
No. 16	10	6
No. 17	10	6
No. 18	10	6
No. 19	10	5
No. 20	10	4
No. 21	10	2
No. 22	5	3
No. 23	5	3
No. 24	5	3
No. 25	5	2
Totals	205	126

These data show that the aphids infected 126 of 205 celery plants, or 61.5 per cent. Transmission by aphids was erratic; the number of plants infected ranged from 10 to 100 per cent. This variation may have resulted from differences in virus concentration in the infected plants; or perhaps the aphids varied greatly in their ability to acquire and transmit the virus.

Experimental Infection of Poison Hemlock and Celery.—Since poison hemlock is a symptomless carrier of the yellow-spot virus, celery plants were inoculated in these experiments to serve as indicators of the presence of the virus. The honeysuckle aphids used for this purpose came from the same source as those used to infect the poison hemlock. They were collected on

TABLE 1

EXPERIMENTAL INFECTION OF CELERY AND POISON HEMLOCK WITH CELERY-YELLOW-SPOT VIRUS BY HONEYSUCKLE APHIDS FROM NATURALLY INFECTED POISON HEMLOCK

Naturally infected poison hemlock no.	Celery plants		Poison-hemlock plants	
	Inoculated	Infected	Inoculated	Infected*
1	10	7	5	3
2	10	5	5	1
3	10	4	5	2
4	10	2	10	0
5	10	1	2	0
6	5	3	5	3
7	5	3	5	0
8	5	3	5	0
9	5	3	5	5
10	5	3	2	0
11	5	2	2	0
12	5	1	3	0
Total	85	37	54	14
Percentage	..	*43.5*	..	*25.9*

* As determined by recovery of the virus by aphids and transfer to healthy celery plants.

naturally infected poison hemlock, and those from each plant were transferred to from 2 to 10 healthy poison hemlock and 5 to 10 healthy celery plants. About a month after the celery had developed symptoms, noninfective honeysuckle aphids were taken to the inoculated poison hemlock, where they were allowed to feed for about a week. Then they were moved to 5 healthy celery plants. Infection of one or more celery plants was considered proof that the poison hemlock was infected, although the latter displayed no symptoms.

As table 1 shows, the aphids were apparently able to infect celery more readily than poison hemlock. In each of 12 trials conducted, they infected 1 to 7 celery plants, whereas other aphids from the same sources infected poison hemlock in only 6 of 12 trials. A total of 37 of 85, or 43.5 per cent, of the celery plants and 14 of 54, or 25.9 per cent, of the poison-hemlock plants became infected.

RECOVERY OF VIRUS FROM POISON HEMLOCK AFTER INOCULATION

Experiments were conducted to test poison hemlock as a source of virus. Inoculations were made in the usual way by transferring honeysuckle aphids

Poison-hemlock plant no. and date of recovery test	Celery plants infected, of 5 inoculated
No. 1 (inoculated June 18); recovery of virus on:	
July 18	2
August 16	0
No. 2 (inoculated June 18); recovery of virus on:	
July 15	1
August 17	3
October 10	2
November 25	0
December 9	0
December 21	0
January 10	0
No. 3 (inoculated June 18); recovery of virus on:	
July 18	1
August 17	0
No. 4 (inoculated June 18); recovery of virus on:	
July 15	5
August 9	0
October 14	0
December 9	0
December 21	0
No. 5 (inoculated June 18); recovery of virus on:	
July 15	3
August 17	0
No. 6 (inoculated June 18); recovery of virus on:	
July 15	2
August 17	0
No. 7 (inoculated June 18); recovery of virus on:	
July 15	1
August 17	0
No. 8 (inoculated September 24); recovery of virus on:	
January 4	2
February 12	1
March 22	0
April 13	2
No. 9 (inoculated August 18); recovery of virus on:	
September 11	0
September 22	1
October 16	1
No. 10 (inoculated August 18); recovery of virus on:	
September 11	0
September 21	5[5]
October 16	0
Total	32
Percentage	19.2

[5] Out of 10 celery plants inoculated.

from naturally infected plants to healthy plants grown from seeds. These plants, as in previous experiments, failed to develop symptoms. The first recovery tests were conducted 24 days after the inoculation. Additional recovery tests were made at irregular intervals. The results were as shown on the preceding page.

The data indicate that experimentally infected poison hemlock is a poor source of the virus. In 6 of 10 tests the aphids recovered the virus from poison hemlock the first month after inoculation, but failed to recover it thereafter. The aphids infected only 32 of 165 celery plants, or 19.4 per cent. The low percentage of transmission obtained by using experimentally infected poison hemlock as a source of virus made it difficult to conduct many experiments. Why the aphids are unable to recover the virus readily from experimentally infected poison hemlock, whereas they were far more successful in acquiring it from naturally infected plants, is not understood.

FAILURE TO RECOVER VIRUS FROM CELERY

Nine species of aphids (listed in table 2) were reared on celery that showed symptoms of yellow spot. The aphids from each infected plant were transferred in lots of 25 to each of 5 healthy celery plants. After this transfer, the juice of the infected plants was extracted by crushing the leaves in a mortar with a pestle and pressing the juice through cheesecloth by hand. Five healthy celery plants were then inoculated by dusting the leaves with powdered carborundum and lightly rubbing the leaves with absorbent cotton that had been dipped in the extracted juice according to the method described by Rawlins and Tompkins (1936).

The transmission results with aphids and with mechanical inoculations are summarized in table 2. As this experiment conclusively demonstrates, the aphids were not able to recover virus from 66 infected celery plants and consequently did not infect any of the 330 celery plants to which they were transferred. Mechanical inoculation of 285 celery plants with the juice from plants used as a source of virus for the aphid tests resulted in no infections.

TRANSMISSION OF VIRUS BY SINGLE APHIDS

A comparison was made of the transmission of yellow-spot virus by single specimens of winged and mature wingless honeysuckle aphids reared on naturally infected poison hemlock. The aphids were collected at Alvarado on poison-hemlock plants of this kind. Each was transferred singly, by means of a camel's-hair brush, to healthy celery and fed for 1 day.

The winged honeysuckle aphids proved to be more efficient vectors than the mature wingless ones. The former infected 20 out of 50 celery plants; the latter only 6 out of 50.

RETENTION OF VIRUS BY APHIDS

Some plant viruses transmitted by aphids are retained, whereas others are lost soon after the aphids leave the infected plants. Experiments were conducted to determine how long the honeysuckle aphids retained the yellow-spot virus. The aphids were collected on naturally infected poison hemlock and transferred with a camel's-hair brush to successive healthy celery plants

daily for 6 to 23 days. Five experiments were performed; and in each, from 10 to 25 lots of 25 aphids were transferred daily to healthy celery, a total of 85 lots being used. Every day, when the aphids were thus transferred, several would die, so that after 10 days only a few remained alive.

TABLE 2

ATTEMPTS TO TRANSMIT CELERY-YELLOW-SPOT VIRUS FROM CELERY TO CELERY BY APHIDS AND BY MECHANICAL INOCULATION

Aphid	Number of infected celery plants tested	Aphid transmission		Mechanical inoculation	
		Plants inoculated	Plants infected	Plants inoculated	Plants infected
Celery aphid, *Aphis apigraveolens* Essig	5	25	0	25	0
Celery aphid, *Aphis apii* Theo	8	40	0	35	0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	8	40	0	35	0
Cotton or melon aphid, *Aphis gossypii* Glover	9	45	0	35	0
Erigeron root aphid, *Aphis middletonii* Thos.	5	25	0	25	0
Yellow willow aphid, *Cavariella capreae* (Fab.)	8	40	0	35	0
Lily aphid, *Myzus circumflexus* (Buck.)	8	40	0	35	0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	5	25	0	25	0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	10	50	0	35	0
Total	66	330	0	285	0

TABLE 3

RETENTION OF CELERY-YELLOW-SPOT VIRUS BY LOTS OF 25 NATURALLY INFECTED HONEYSUCKLE APHIDS TRANSFERRED TO SUCCESSIVE HEALTHY CELERY PLANTS DAILY

Number of days after transfer of aphids from infected plants	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
1	25	21	20	17	10	2	20	13	10	7
2	25	9	20	18	10	0	20	13	10	2
3	25	11	20	18	10	1	20	16	10	3
4	25	21	20	18	10	1	20	12	10	8
5	25	20	20	13	10	2	20	10	10	1
6	25	20	20	3	10	0	20	9	10	2
7	..	..	20	0	10	0	20	3	10	0
8	..	..	20	1	10	0	20	2	10	0
9	..	..	..	..	10	0	20	4	10	2
10	..	..	..	..	10	0	20	1	10	3
11	..	..	..	..	..	..	20	1	9	0
12	..	..	..	..	..	..	10	0	7	1
13	..	..	..	..	..	..	3	0	7	0
14	..	..	..	..	..	..	2	0	7*	0

* Continued for 23 days; but no infections resulted after twelfth day.

The results, shown in table 3, indicate that the aphids retain the virus for some time. Apparently, therefore, the mode of transmission differs from that observed for most aphid-transmitted viruses; most aphid vectors fail to transmit after the first day. The honeysuckle aphid retained the virus 5 to 12 days

after being removed from infected poison hemlock. The possibility of aphids' reacquiring virus from plants on which they were allowed to feed for 24 hours was eliminated, since all tests so far conducted with aphids or with mechanical inoculations have failed to recover the virus from infected celery.

DISCUSSION

Judging from the results presented, honeysuckle aphids that have acquired the yellow-spot virus may retain it for 12 days. Soon after being removed from an infected plant, most aphids lose the capacity to produce infection. Several species of them, however, retain viruses for 3 to 29 days. Smith (1929, 1931) and Elze (1927) have shown that the green peach aphid, *Myzus persicae* (Sulzer), the vector of potato-leafroll virus, can feed on immune plants such as cabbage and spinach for 7 to 10 days and then infect healthy potatoes. Bennett (1927) has demonstrated that the raspberry-leaf-curl virus remains active for 2 weeks in the body of the aphid vector, *Aphis rubiphila,* Patch. Osborn (1935, 1938) found that the pea-mosaic virus was retained by the pea aphid, *Macrosiphum onobrachis* (B. d. F.) (*M. pisi* Kalt.), for 29 days and by the potato aphid, *M. solanifolii* (Ashm.) for 21 days. In the experiment of Roland (1939) and Watson (1940) the green peach aphid when transmitting the sugar-beet-yellows virus produced infection on successive healthy plants for a period of 3 days.

Why the aphids were not able to acquire virus from celery plants infected with yellow spot is difficult to explain. A possible reason might be low virus concentration in the celery plant, or plant-tissue relations that make it impossible for aphids to acquire the virus. Hoggan (1929, 1931, 1934) found that aphids did not transmit the tobacco-mosaic virus from one tobacco plant to another, but could acquire it regularly from tomato and transmit it to tobacco. The results obtained with the yellow-spot virus indicate a somewhat similar situation. Recovery of virus from some host plants infected with aster yellows has also proved difficult. Severin and Hassis (1934) were able to infect potato plants with aster-yellows virus by means of the aster leafhopper, *Macrosteles divisus* (Uhl.), but not to recover the virus subsequently from those plants. More recently, however, Severin (1940) reports that aster-yellows virus was recovered from naturally infected potato plants by means of the long-winged aster leafhopper.

SUMMARY

Celery (*Apium graveolens* L. var. *dulce* DC.), poison hemlock (*Conium maculatum* L.), and parsnip (*Pastinaca sativa* L.) were found to be naturally infected with the celery-yellow-spot virus.

The symptoms on celery are irregular areas or spots; or stripes at first pale green, later yellow, and finally white. These spots occur along the veins and scattered over the leaflets.

The virus was recovered from 25 naturally infected poison-hemlock plants, which were symptomless carriers of the disease, and was transmitted by means of the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.), to 126 of 205 celery plants, or 61.5 per cent. Attempts to transmit the virus from infected poison-hemlock plants to healthy plants by mechanical inoculation were unsuccessful.

Attempts to transmit the virus from celery to celery by means of 9 species

of aphids and by mechanical inoculation failed to produce infection in 615 celery plants tested.

Honeysuckle aphids collected on naturally infected poison hemlock transmitted the disease to 37 of 85 celery plants, or 43.5 per cent; and 14 of 54 poison-hemlock plants, or 25.9 per cent.

Experimentally infected poison hemlock proved to be a poor source of virus for aphid-transmission experiments, especially if recovery was attempted after the plant had been diseased for more than a month.

Single specimens of winged honeysuckle aphids proved to be more efficient vectors than mature wingless aphids.

Honeysuckle aphids collected on naturally infected poison-hemlock plants and transferred to successive healthy celery plants daily were able to infect plants for a period of 12 days.

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Plate 1.—*A*, Healthy celery leaflet. *B* to *F*, symptoms of celery yellow spot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *B*, early stage with only a few chlorotic spots between the veins and a few short narrow chlorotic stripes along the veins; *C*, elongated yellow stripes along the veins and chlorotic spotting; *D*, enlarged chlorotic spots, the result of the coalescing of smaller ones; *E*, numerous chlorotic spots with general yellowing of basal portion of leaflets; *F*, coalescing of chlorotic spots to form enlarged yellow areas.

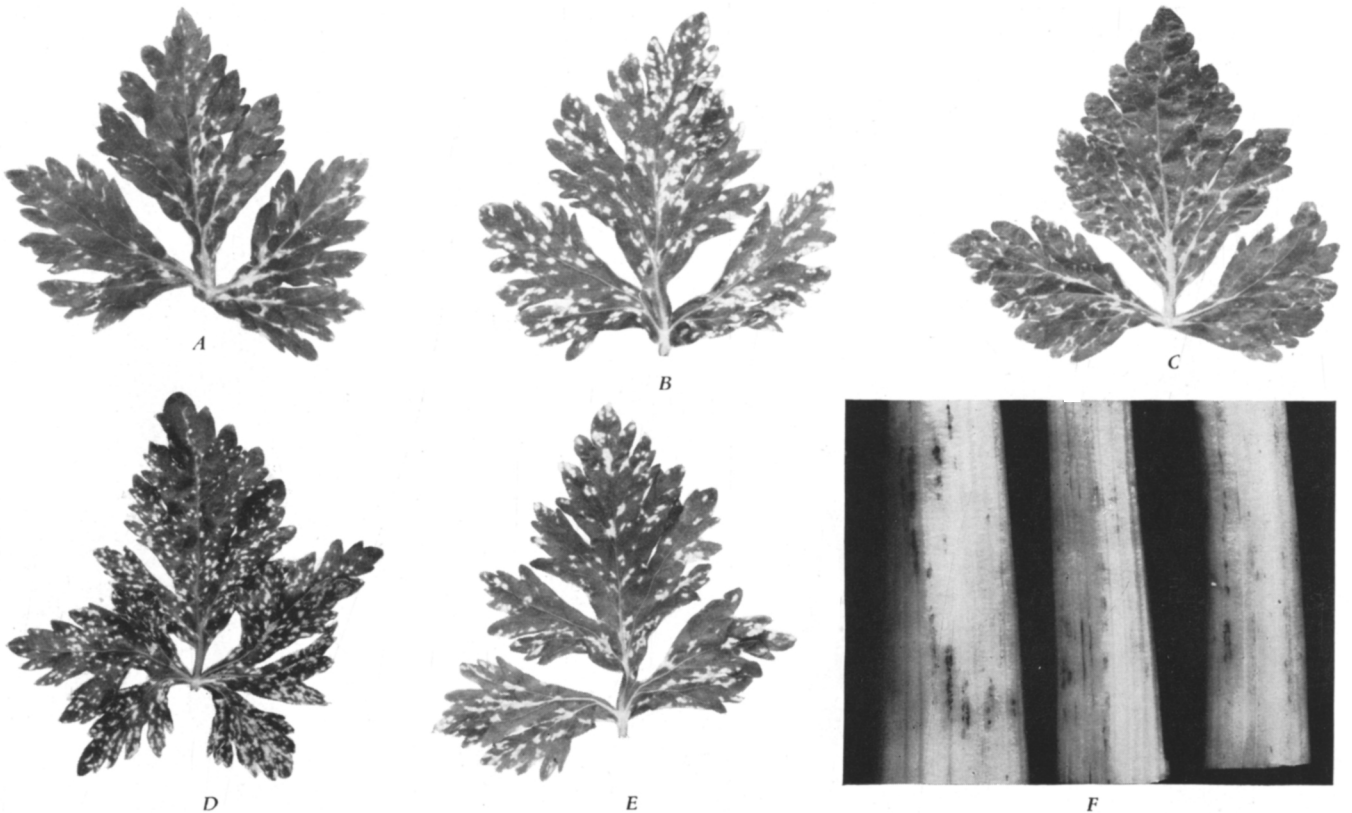

Plate 2.—Symptoms of celery yellow spot on leaflets and petioles of celery taken from naturally infected plants: *A*, yellow spots and stripes along the veins, mostly at basal portion of veinlets where they join main and lateral veins; *B*, irregularly shaped chlorotic spots, many the result of smaller spots coalescing; *C*, small chlorotic spots and stripes along the veins; *D*, numerous small chlorotic spots, some of which are circular; basal portion of leaflet shows enlarged chlorotic spots formed by the coalescing of smaller ones; *E*, chlorotic spotting and elongated stripes along the veins; *F*, epidermis removed from three petioles, showing brown specks along the veins. (Milpitas, November 18, 1934; courtesy M. W. Gardner.)