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APHID TRANSMISSION OF MILD MOSAIC VIRUS OF ANNUAL STOCK

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ADDITIONAL VIRUS DISEASES OF SPINACH IN CALIFORNIA

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APHID TRANSMISSION OF MILD MOSAIC VIRUS OF ANNUAL STOCK¹

HENRY H. P. SEVERIN² and C. M. TOMPKINS³

SUMMARY

Five species of aphids, tested in lots of 20, were demonstrated to be vectors of mild-mosaic virus of annual stock; these are:

Bur clover or cowpea aphid, *Aphis medicaginis* Koch

Cabbage aphid, *Brevicoryne brassicae* Linnaeus

Artichoke aphid, *Myzus braggi* (Gillette)

Green peach aphid, *Myzus persicae* (Sulzer)

Turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis)

Of these, only the turnip aphid breeds on annual stock plants under natural conditions. It causes pale-green circular areas around the mouth-part punctures, and, when abundant, dwarfing and yellowing or blanching of the flowers.

The turnip aphid failed to transmit the virus to nine varieties of healthy cauliflower.

Several infections were obtained with single turnip and green peach aphids fasted for 2 hours, fed ½, 1, 2, 5, or 10 minutes on leaves from infected stock plants, and then transferred to healthy plants, 1 aphid per plant. No infections were obtained with several hundred turnip, green peach, and cabbage aphids tested singly without fasting and with longer periods on infected stock plants.

In tests on retention of the virus, turnip aphids, fasted for 30 minutes, then fed singly 5 or 10 minutes on mild-mosaic-infected annual stock and 5 or 10 minutes on 5 or 6 successive healthy stock plants, produced infections only in the first healthy plant.

Lots of 20 turnip aphids had lost their infectivity by the fourth day after transfer from an infected to a previously healthy stock plant. If allowed to remain on the plant from 7 to 13 days, however, the aphids were able to recover the virus from the plant they had inoculated; this was long before symptoms appeared. The incubation period of the disease in the original inoculated plants varied from 16 to 22 days.

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INTRODUCTION

In 1930, commercial growers of annual stock (*Mathiola incana* var. *annua*) observed a serious disease that caused breaking in the color of flower petals. Field infection was severe from 1931 to 1948. The disease destroyed the value of the plants for cut flowers and reduced seed production. The trouble was identified as a virus disease (Tompkins, 1934).⁴ Tompkins (1939) named it mild mosaic of annual stock, and distinguished it from severe mosaic, another virus disease of this host, which causes even more severe breaking and occurs under field conditions in San Pablo, California. He described the symptomatology, transmission, host range, and properties of the two viruses.

Breaking in the color of petals has been reported to result from mechanical inoculation with several crucifer mosaic viruses, in addition to the two stock viruses—namely, turnip mosaic (Tompkins, 1938; Chamberlain, 1939), cabbage black ring (Tompkins, Gardner, and Thomas, 1938), Chinese cabbage mosaic (Tompkins and Thomas, 1938), cabbage mosaic (Larson and Walker, 1938), and horseradish mosaic (Tompkins, 1939). Investigators in various countries have reported breaking in the color of petals or mosaic disease in annual stock, without identifying the causal virus (see Tompkins, 1939).

Annual stock has been demonstrated to be naturally infected with sugar-beet curly top in California (Severin, 1934), and with an unidentified disease resembling California aster yellows; but these do not cause breaking.

In connection with the investigation of other phases of mild and severe mosaics of annual stock by Tompkins (1939), experiments were undertaken in 1934 on various phases of aphid transmission. Tompkins reported early results obtained by the senior author: the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), the cabbage aphid, *Brevicoryne brassicae* Linnaeus, and the green peach aphid, *Myzus persicae* (Sulzer), were demonstrated to be vectors of both viruses. The turnip aphid breeds on stock under natural conditions, the other two do not.

The present paper reports further results of experiments on aphid transmission of mild mosaic of annual stock; phases investigated include transmission of the virus by several aphid species from infected stock plants to healthy stock and cauliflower plants, comparison of transmission of the virus by mechanical inoculation with that by three species of aphids, transmission of the virus by single aphids and during short feeding periods, the retention of the virus, and loss and recovery of infectivity by aphids. Symptoms on foliage and flowers caused by feeding of noninfective aphids were studied and differentiated from those caused by the mild-mosaic virus.

MATERIALS AND METHODS

The virus causing mild mosaic of annual stock was obtained at Montara, San Mateo County. Plants of the Fiery Blood Red variety of annual stock and the February variety of cauliflower grown from seeds were used in all experiments. Methods used in aphid-transmission experiments were similar to those used previously (Severin and Freitag, 1938). The carborundum method (Rawlins and Tompkins, 1936) was used in mechanical inoculations.

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

DISTRIBUTION

Annual stock naturally infected with mild mosaic is generally distributed on seed farms and in home gardens in the coastal districts of California. Stock plants showing breaking in the color of the petals were obtained at Davis and the virus was recovered and transferred by means of the turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis) (plate 1), to healthy stock.

SYMPTOMATOLOGY

Induced by Feeding of Aphids. Noninfective and infective turnip aphids, *Rhopalosiphum pseudobrassicae*, produced pale-green circular areas (plate 2, *B*) around the mouth-part punctures on the leaves. The flowers of stock plants which had large populations of aphids (plate 1) were dwarfed, and frequently the tips of the petals were yellow or white; sometimes most or all of the petals were yellowish green (plate 2, *C*).

Mild Mosaic. Tompkins (1934, 1939) has described the symptoms of mild mosaic on annual stocks. The more important symptoms are briefly reviewed here for comparison with the symptoms induced by the feeding of aphids.

The first symptom of mild mosaic on the younger leaves of annual stock appears 2 to 3 weeks after inoculation, as a clearing of the veins and veinlets (plate 3, *B, C, D*) followed by mottling consisting of pale and dark-green areas (plate 3, *E*). These symptoms rarely occur on naturally infected stock plants. Sometimes the apical leaves are distorted, curled, and puckered. Infected plants are slightly or severely stunted with shortened internodes.

A striking flower symptom is breaking in color of the petals (plate 4, *B, C*). Sometimes the cluster of flowers at the apical end of the stems is apparently normal, while the lower racemes show breaking; usually, however, all racemes show breaking. A reduction of the number and size of the seed pods occurs.

APHID TRANSMISSION OF VIRUS

By Vectors That Do Not Breed on Stock under Natural Conditions. No intensive investigations were undertaken on aphid vectors which do not breed on annual stock plants under natural conditions.

Bur clover or cowpea aphids, *Aphis medicaginis* Koch, collected on lamb's-quarters (white pigweed) (*Chenopodium album*) and on rough pigweed (*Amaranthus retroflexus*) growing among annual stock plants near Montara, were transferred from these weeds to stock infected with mild mosaic. A high mortality of the aphids occurred on annual stock. Three of 6 lots of 20 aphids each, changed from mosaic to healthy annual stock plants, caused infection.

Artichoke aphids, *Myzus braggi* (Gillette), collected on artichokes near El Granada, were transferred to mosaic-infected annual stock, and then to healthy annual stock plants. Three lots of 20 aphids produced 2 infections.

Green peach aphids, *Myzus persicae*, collected on sugar beets, were transferred to naturally infected stock plants and thence to healthy stock and cauliflower plants. The results are given in table 1. The cabbage aphid, *Brevicoryne brassicae*, was also demonstrated to be a vector of this virus.

By a Vector That Breeds on Stock under Natural Conditions. Whenever a large population of the turnip aphid was found on naturally infected

annual stock plants showing breaking in the flowers, lots of 20 aphids were transferred from the mosaic to healthy annual stock plants and to healthy cauliflower seedlings. This aphid transmitted the virus to 60 per cent of the stock plants inoculated, but to none of the cauliflower plants (table 1).

From Experimentally Infected to Healthy Annual Stock Plants and Varieties of Cauliflower. Lots of 20 infective turnip aphids were transferred from

TABLE 1
TRANSMISSION OF MILD-MOSAIC VIRUS FROM NATURALLY INFECTED
TO HEALTHY ANNUAL STOCK BY TWO SPECIES OF APHIDS

Aphid species and district in which infected plants were found	Annual stock		Cauliflower	
	Inoculated	Infected	Inoculated	Infected
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>:				
Alameda County:				
Berkeley.....	10	2	10	0
Berkeley.....	6	5	6	0
San Francisco County:				
San Francisco.....	6	2	6	0
San Mateo County:				
Burlingame.....	12	10	12	0
Montara.....	10	8	10	0
Montara.....	10	8	10	0
Montara.....	10	3	10	0
Yolo County:				
Davis.....	12	6	12	0
Davis.....	6	5	6	0
Total.....	82	49	82	0
Percentage.....	..	60	..	0
Green peach aphid, <i>Myzus persicae</i>:				
Alameda County:				
Berkeley.....	5	4	5	0
Berkeley.....	5	3	5	0
San Francisco County:				
San Francisco.....	5	3	5	0
San Mateo County:				
Burlingame.....	5	2	5	0
Montara.....	5	2	5	0
Montara.....	5	2	5	0
Montara.....	5	2	5	0
Yolo County:				
Davis.....	5	1	5	0
Davis.....	5	1	5	0
Total.....	45	20	45	0
Percentage.....	..	45	..	0

experimentally infected annual stock to healthy annual stock plants and nine varieties of cauliflower. Table 2 shows that 51 per cent of the annual stock plants, but none of the cauliflower plants, were infected. Each cauliflower plant was again inoculated with a lot of 20 infective false cabbage aphids, and again no infections occurred. The fact that no infection of cauliflower was obtained with the mild-mosaic virus of annual stock serves to differentiate this virus from the cauliflower-mosaic virus, a conclusion which Tompkins (1937) reached from his results with mechanical inoculation.

Comparison of Aphid Transmission and Mechanical Inoculation. The transmission of the virus from experimentally infected to healthy stock plants by mechanical inoculation was compared with transmissions by three species of aphids. The virus extract from infected plants upon which the aphids had

TABLE 2
TRANSMISSION OF VIRUS FROM STOCKS EXPERIMENTALLY INFECTED
WITH MILD MOSAIC TO HEALTHY ANNUAL STOCK AND
CAULIFLOWER BY THE TURNIP APHID

Mosaic-infected stock plant no.	Annual stock		Cauliflower		
	Inocu- lated	Infected	Variety	Inocu- lated	Infected
1.	5	3	Danish Perfection.	5	0
2.	5	0	Dryweather Danish Giant.	5	0
3.	5	1	Extra Early Dwarf Erfurt.	5	0
4.	5	2	Hartmans Special.	5	0
5.	5	1	Hartmans Special Medium.	5	0
6.	5	1	Late Pearl.	5	0
7.	5	4	February 759*.	5	0
8.	5	5	Early March 713*.	5	0
9.	5	5	Mission Special 4577*.	5	0
Total.	45	22	45	0
Percentage.	49	0

* Grown from seed from Ferry-Morse Seed Co., San Francisco.

TABLE 3
COMPARISON OF TRANSMISSION OF MILD-MOSAIC VIRUS OF ANNUAL
STOCK BY MECHANICAL INOCULATION AND BY
THREE SPECIES OF APHIDS

Number of plants from which virus was recovered	Mechanical inoculation			Aphid transmission			
	Plants inocu- lated	Plants infected	Per cent infected	Aphid species	Plants inocu- lated	Plants infected	Per cent infected
5	25	20	80	Cabbage aphid, <i>Brevicoryne brassicae</i>	25	1	4
10	50	7	14	Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>	50	22	44
5	25	15	60	Green peach aphid, <i>Myzus persicae</i>	25	13	52

fed was inoculated into healthy plants. The results obtained are given in table 3. The turnip aphid was more efficient in transmitting the virus than was mechanical inoculation, the cabbage and green peach aphids less so.

By Single Aphids. An attempt was made to determine the efficiency of virus transmission by three species of mature aphids fed 1 day on mosaic stock plants, then each fed singly on a healthy stock plant. Equal numbers of winged and wingless aphids were used. Not a single infection was obtained with 300 turnip aphids, 200 cabbage aphids, and 150 green peach aphids.

TABLE 4
SHORT FEEDING TIME ON MILD-MOSAIC STOCK PLANTS
BY SINGLE WINGLESS APHIDS OF TWO SPECIES

Aphid species	Feeding time, minutes									
	0.5		1		2		5		10	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> ..	5	1	5	0	5	0	5	1	5	0
Green peach aphid, <i>Myzus persicae</i>	5	2	5	1	5	1	5	0	5	1

TABLE 5
RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL
STOCKS BY TWO SPECIES OF APHIDS

Aphid species and test no.	Number of aphids on first plant	Number of annual stock					
		Inocu- lated 1st day	Infected 1st day	Inocu- lated 2d day	Infected 2d day	Inocu- lated 3d day	Infected 3d day
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> :							
Test 1.....	5	12	2	12	0	12	0
Test 2.....	10	6	1	6	0	6	0
Test 3.....	20	7	4	7	0	7	0
Test 4.....	20	6	3	6	0	6	0
Test 5.....	20	6	1	6	0	6	0
Test 6.....	20	5	4	5	0	5	0
Test 7.....	20	5	1	5	0	5	0
Test 8.....	20	5	1	5	0	5	0
Test 9.....	20	4	1	4	0	4	0
Total.....	..	56	18	56	0	56	0
Percentage.....	32	..	0	..	0
Green peach aphid, <i>Myzus persicae</i> :							
Test 1.....	20	5	4	5	0	5	0
Test 2.....	20	5	3	5	0	5	0
Test 3.....	20	5	3	5	0	5	0
Test 4.....	20	5	3	5	0	5	0
Test 5.....	20	5	2	5	0	5	0
Test 6.....	20	5	2	5	0	5	0
Test 7.....	20	5	2	5	0	5	0
Test 8.....	20	5	2	5	0	5	0
Test 9.....	20	5	2	5	0	5	0
Test 10.....	20	5	2	5	0	5	0
Test 11.....	20	5	1	5	0	5	0
Test 12.....	20	5	1	5	0	5	0
Test 13.....	20	5	1	5	0	5	0
Test 14.....	20	5	1	5	0	5	0
Total.....	..	70	29	70	0	70	0
Percentage.....	41	..	0	..	0

Because of the failure of the single-aphid tests, further tests of the turnip and green peach aphids were made with a different technique. Noninfective, wingless aphids of these two species were fasted in a phial for 2 hours. Five lots of 5 aphids each were transferred from the phial to a leaf from an infected stock plant and fed $\frac{1}{2}$, 1, 2, 5, or 10 minutes; and then each aphid was transferred to a healthy plant. With the turnip aphid two infections were obtained, at $\frac{1}{2}$ - and 5-minute feeding times, and with the green peach aphid five infections at $\frac{1}{2}$ -, 1-, 2-, and 10-minute feeding times (table 4).

TABLE 6
RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL STOCK BY SINGLE
TURNIP APHIDS, IN SHORT FEEDING PERIODS

Time on diseased annual stock, minutes	Penetration time of stylets in first healthy annual stock, minutes	Feeding time on successive healthy annual stock, minutes*					
		5	10	10	10	10	10
5	7	+	—	—	—	—	—
5	5	+	—	—	—	—	—
10	4	..	+	—	—	—	—
10	3	..	+	—	—	—	—
10	2	..	+	—	—	—	—
Total	4.2	2+	3+	5—	5—	5—	5—

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

Effect on Flowers. Tests were made to determine the effect on the flowers of infecting annual stock plants at various stages of flower-bud development; the turnip aphid was used for transmission. On plants infected when flower buds were large, the apical cluster of flowers sometimes showed no breaking in color of the petals, but the lower flowers developed breaking. Plants infected before flower buds were visible or when buds were small showed breaking in all flowers.

RETENTION OF VIRUS

By Varying Numbers of Aphids. The retention of the virus was determined for the turnip and the green peach aphids. In the preliminary work, lots of 5 and 11 aphids were transferred daily for 20 days to successive healthy plants, and the aphids remained on the last healthy annual stock plant for one week. No infections were obtained after the first day.

In later work (table 5) lots of 20 infective aphids reared on mild-mosaic stock plants were transferred daily for 3 days to successive healthy stock plants. Both species of aphids transmitted the virus from diseased to healthy annual stock during the first day, but none of the lots tested caused infection the second or third days.

By Single Aphids in Short Feeding Times. Noninfective, mature, wingless, turnip aphids were fasted for 30 minutes in a moist chamber, then were transferred singly to annual stock infected with mild mosaic for a feeding time of 5 or 10 minutes, and then to 5 or 6 successive healthy annual stocks for a feeding time of 5 or 10 minutes on each plant. Table 6 shows that 5 aphids tested singly transmitted the virus to only the first annual stock plant. Sixty-six aphids failed to transmit the virus (not included in table 6).

LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHIDS

An experiment was conducted to determine whether the turnip aphid could recover the mild-mosaic virus from annual stock plants before breaking in color of petals appeared. A large population of aphids reared on mild-mosaic-

TABLE 7
LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHID ON
STOCK PLANTS INOCULATED WITH MILD-MOSAIC VIRUS

Original plant number	Results* when a lot of 20 aphids was transferred from the first inoculated plant to a second healthy plant on:											Days to color breaking on petals of original plants
	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
1.....	-	-	-	-	+	-	-	+	-	+	+	37
2.....	-	-	-	-	+	-	-	-	-	+	+	38
3.....	-	-	-	+	-	-	+	+	-	-	+	40
4.....	-	-	-	-	-	-	-	-	+	-	+	37
5.....	-	-	-	-	-	-	+	-	-	+	+	44
6.....	-	-	-	-	+	-	-	-	-	+	+	44
7.....	-	-	-	-	-	+	-	-	-	-	+	44
8.....	-	-	-	-	+	-	+	-	-	-	+	46
9.....	-	-	-	-	-	-	-	+	-	+	-	38
10.....	-	-	-	-	+	-	-	-	-	+	+	40
11.....	-	-	-	-	-	+	-	-	+	+	-	39
12.....	-	-	-	-	+	-	-	-	-	+	-	45
13.....	-	-	-	+	-	-	-	+	+	-	-	34
14.....	-	-	-	-	-	-	+	+	+	-	-	38
15.....	-	-	-	-	-	-	-	-	+	+	-	38
16.....	-	-	-	-	+	-	-	-	-	-	+	39
17.....	-	-	-	-	-	-	-	-	-	+	-	40
18.....	-	-	-	-	-	-	-	-	+	-	+	41
19.....	-	-	-	-	-	-	+	-	+	-	-	46
20.....	-	-	-	-	+	-	+	+	-	-	-	39
21.....	-	-	-	+	-	-	-	+	-	-	-	30
22.....	-	-	-	-	-	+	-	-	-	-	-	42
23.....	-	-	-	-	-	-	-	-	+	-	+	41
24.....	-	-	-	-	+	+	-	-	-	-	+	43
25.....	-	-	-	-	+	+	-	-	-	-	+	44
26.....	-	-	-	-	+	+	-	-	-	-	-	40
27.....	-	-	-	-	-	+	-	-	-	+	+	43
28.....	-	-	-	-	-	+	-	-	-	-	-	44
29.....	-	-	-	-	-	+	+	-	-	-	+	47
30.....	-	-	-	-	-	+	+	-	-	+	+	48
Total +.....	0	0	0	3	11	10	8	7	8	12	17	
Total -.....	30	30	30	27	19	20	22	23	22	18	13	

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

infected stock plants was transferred to 36 large healthy stock plants for 3 days. Daily from the fourth to the fourteenth day, one lot of 20 of these aphids was transferred from each of the plants so inoculated to a healthy stock plant, where it was left for 3 days; then the plant was fumigated to kill the aphids.

As table 7 shows, no infections were obtained with any of the lots transferred from the fourth to the sixth day; the aphids had lost their infectivity after the first transfer. Infections were obtained with some lots transferred

on the seventh and later days, however; so that the aphids must have recovered the virus from the first inoculated plants. Breaking did not appear on these plants until 30 to 48 days after inoculation. Tompkins (1939) reported an incubation period of 16 to 22 days on mechanically inoculated plants. The virus was not recovered from 6 plants (not included in table 7) during the entire period.

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Plate 1. Heavy population of turnip aphids, *Rhopalosiphum pseudobrassicae*, on annual stock. White specks are molted skins. (Davis, California, August 3, 1934.)

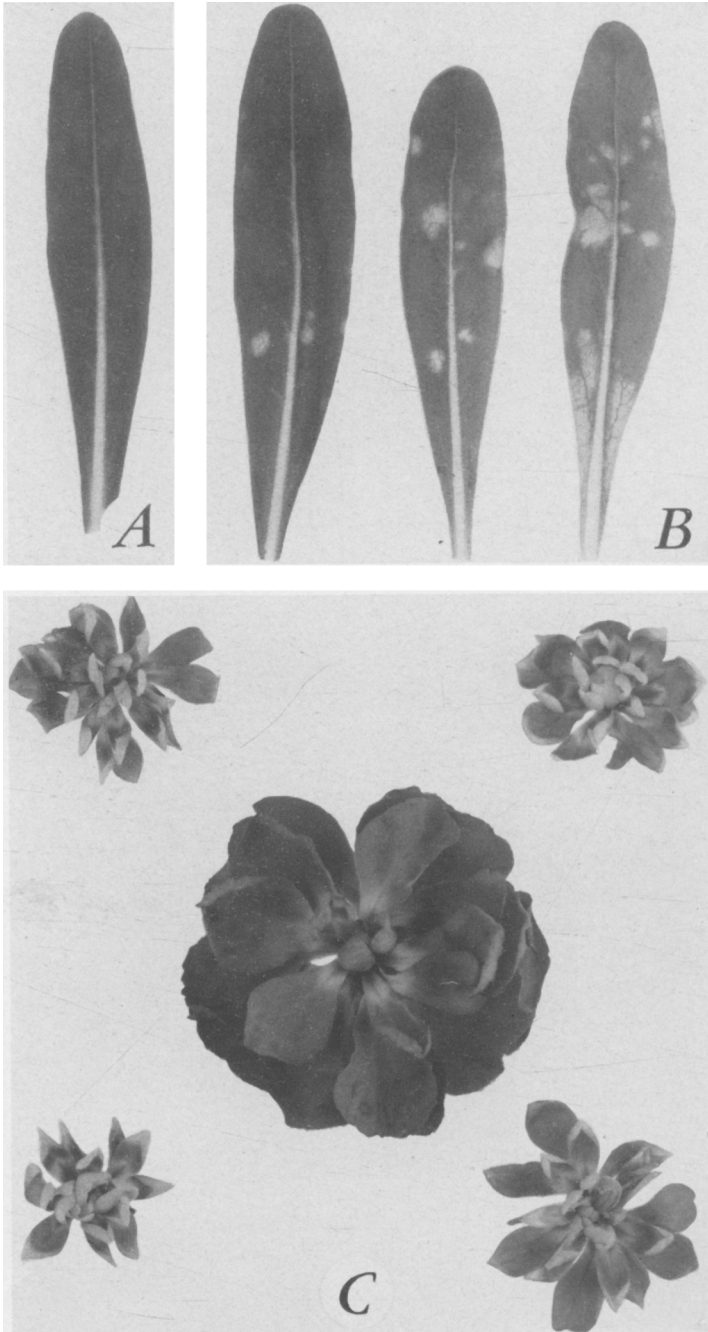


Plate 2. Symptoms produced by feeding of noninfective turnip aphid, *Rhopalosiphum pseudobrassicae*, on annual stock, *Mathiola incana* var. *annua*: *A*, leaf from healthy check or control plant on which no aphids had fed; *B*, pale-green circular areas around mouth-part punctures; *C*, center, normal flower of the Fiery Blood Red variety; grouped around it are four dwarfed flowers with tips of petals yellow or white or with all petals yellowish green.

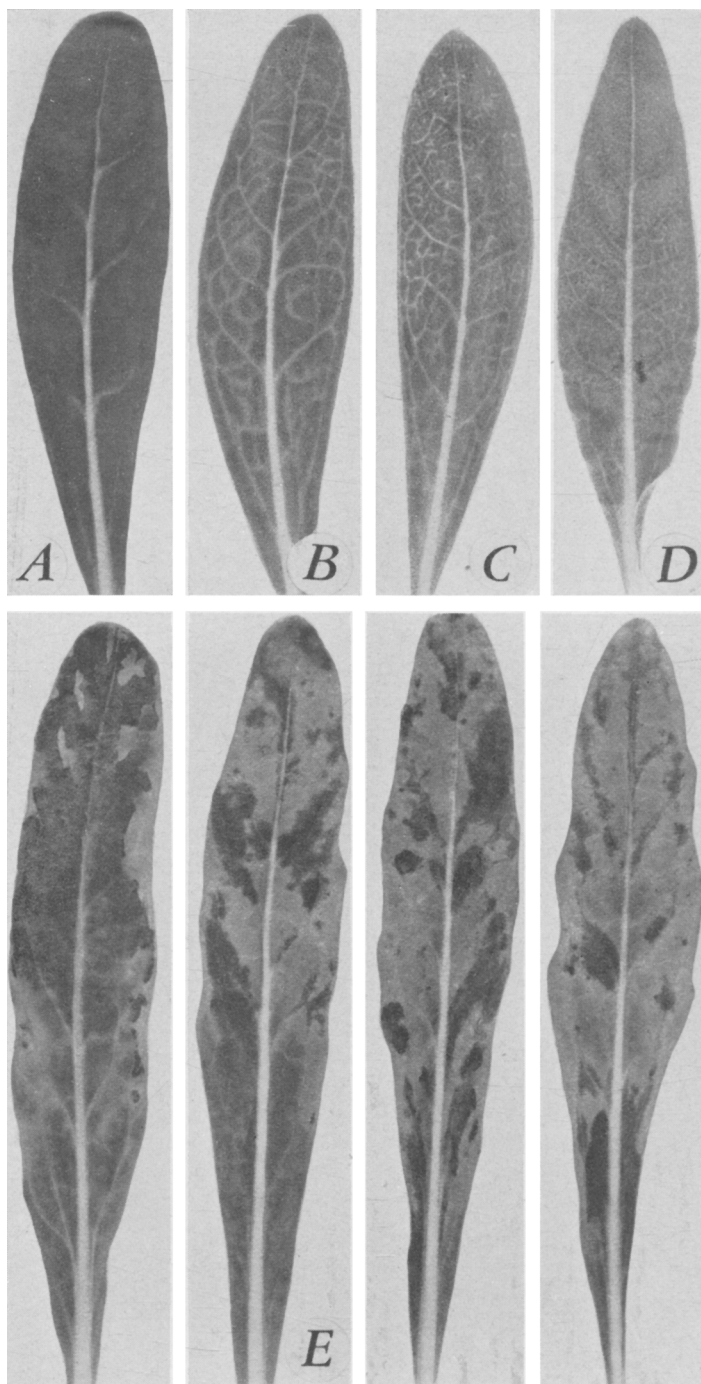


Plate 3. Symptoms produced by mild-mosaic virus on young leaves of annual stock, *Mathiola incana* var. *annua*: *A*, leaf from healthy plant; *B, C, D*, stages in clearing of veins and veinlets; *E*, mottling consisting of irregular-shaped pale and dark-green areas on young leaves (from a plant infected by means of the turnip aphid).

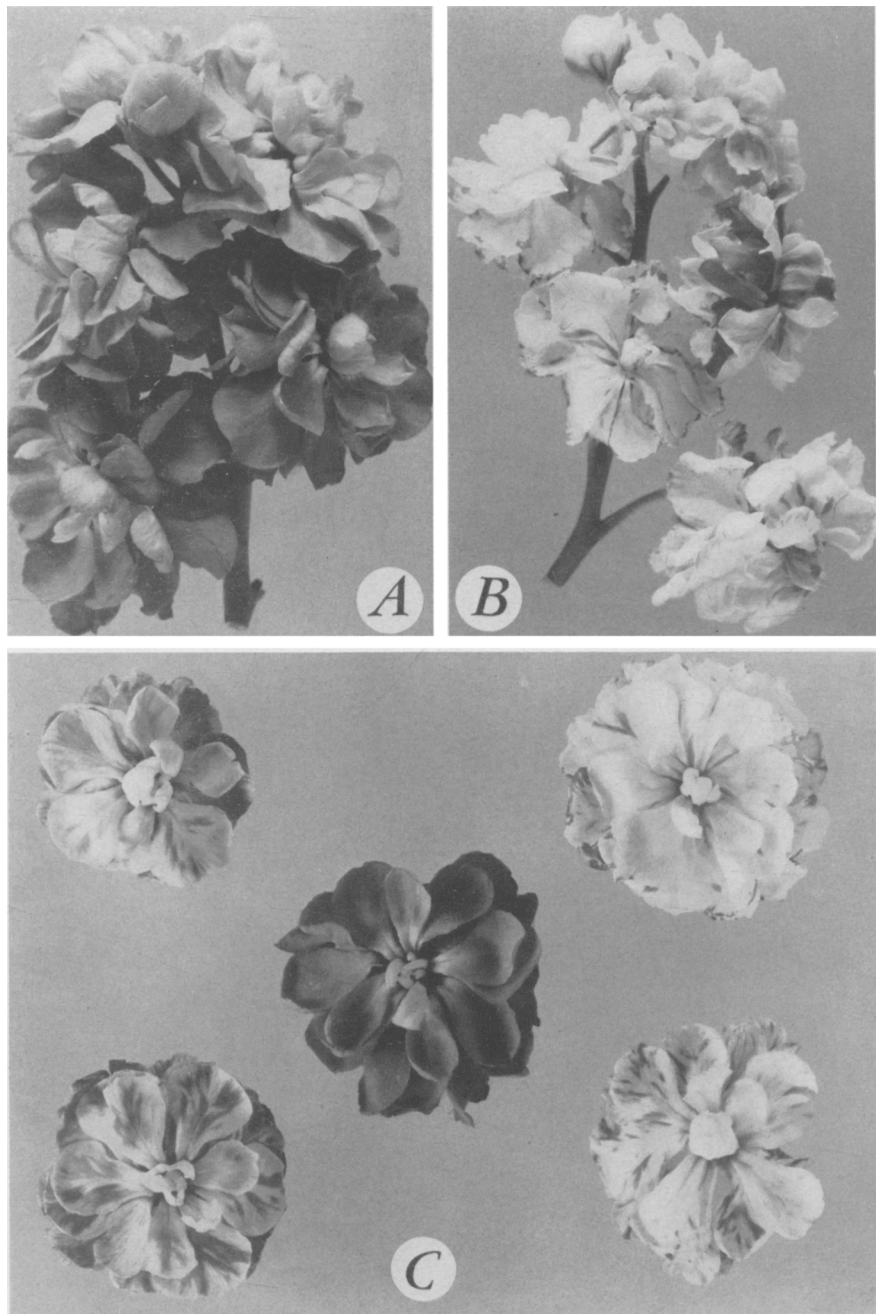


Plate 4. Field symptoms induced by mild-mosaic virus on flowers of naturally infected annual stock, *Mathiola incana* var. *annua*; Fiery Blood Red variety: *A*, apical cluster of flowers from a healthy plant; *B*, apical cluster of flowers showing breaking in color of petals from a naturally infected plant; *C*, center, normal flower; grouped around it are four flowers showing breaking, consisting of white areas and normal color. (Montara, San Mateo County, California, August 20, 1934.)

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