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APHID TRANSMISSION OF SEVERE-MOSAIC VIRUS OF ANNUAL STOCK

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APHID TRANSMISSION OF SEVERE-MOSAIC VIRUS OF ANNUAL STOCK¹

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

INTRODUCTION

IN THE CANYONS of the Montara Mountains and the slopes along the Pacific Ocean, the mild-mosaic virus of annual stock (*Matthiola incana* var. *annua*) induces a conspicuous breaking in the color of the flower petals in all but white and yellow flowering types. The breaking renders these affected varieties unmarketable as cut flowers. A severe mosaic disease of annual stock with a striking color-breaking and conspicuous mottling and malformation of the leaves occurs in home gardens and in a nursery at San Pablo, Contra Costa County.

The turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicæ* (Davis), breeds on annual stock under natural conditions; the cabbage aphid, *Brevicoryne brassicæ* L., and green peach aphid, *Myzus persicæ* (Sulzer), do not.

REVIEW OF LITERATURE

A review of the literature on virus diseases of annual stock has been published by Tompkins (1934, 1939)⁴ and Severin and Tompkins (1948).

A paper by Oliveira and Borges (1944) appeared and was not reviewed in our last contribution. A number of cultivated and wild crucifers, tobacco and *Nicotiana glutinosa* were tested, by juice inoculation, with nine isolates of an annual-stock virus from Lisbon gardens. The symptoms induced by each of the isolates on the differential hosts were mosaic, rugosity, stunting, chlorosis, necrosis, blistering, and streak.

MATERIALS AND METHODS

The virus causing severe mosaic of annual stock was obtained at San Pablo, Contra Costa 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 reported in this paper. All inoculated annual stock plants were held for observation after the flowering stage.

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

The production of colonies of noninfective aphid species has been described in a previous paper (Severin and Tompkins, 1948). The method of transferring aphids has also been published (Severin and Freitag, 1938). The preparation of the virus from crushed, infective aphids was similar to the method described for the beet leafhopper (*Circulifer tenellus* Baker) (Severin and Freitag, 1938). The carborundum method (Rawlins and Tompkins, 1936) was used in mechanical inoculations.

SYMPTOMATOLOGY OF SEVERE MOSAIC

Tompkins (1939) has published a brief description of the foliage and flower symptoms of severe mosaic on annual stock plants. A more detailed description of the successive symptoms follows. The first symptom on the youngest leaves consists of cleared venation (plate 1, *B*) accompanied by a bending downward of the apical end of the stem (plate 1, *A*). Later the veins protrude on the lower surface of the younger leaves (plate 1, *C*). Two or three weeks after inoculation a prominent coarse mottle (plate 2, *B*) appears on the younger leaves, which continues unchanged. The younger leaves are curled, twisted, and puckered (plate 2, *A*) and the intermediate leaves may be deformed (plate 2, *C*). In the advanced stage of the disease the older leaves show a permanent mottling (plate 3, *A*), sometimes with blisterlike elevations (plate 3, *B*). Plants infected shortly after transplanting are sometimes dwarfed.

Breaking in color of the petals consists of a conspicuous, coarse, blotched, normal color within white areas (plate 4, *A*). Examination of individual petals shows irregular white areas scattered in the normal-colored blotches or white streaks encompassing the veins, but often spread out on each side of the veins (plate 4, *B*).

HOST RANGE OF MILD- AND SEVERE-MOSAIC VIRUSES

The host ranges of the mild- and severe-mosaic viruses, as recorded by Tompkins (1939), include 16 species and six species respectively in the family *Cruciferae*; three species and one species respectively in the *Chenopodiaceae*; one species each in the *Resedaceae*; and two species each in the *Solanaceae*.

"Differential hosts serve as one means of differentiating the two mosaic viruses of annual stock. Susceptible to infection with the mild-mosaic virus only are Chinese or leaf mustard, pe-tsai, radish, Chinese radish, Virginia stock, honesty, sweet alyssum, wallflower, *Brassica adpressa*, sowbane or nettle-leaf goosefoot, *Nicotiana glutinosa*, and *N. langsdorffii*. Infection of lambsquarters, spinach, and petunia was obtained only with the severe-mosaic virus." (Tompkins, 1939.)

PROPERTIES OF MILD- AND SEVERE-MOSAIC VIRUS

The properties of the mild- and severe-mosaic viruses of annual stock as determined by Tompkins (1939) are as follows: Mild mosaic, aging *in vitro* five days, severe mosaic seven days at 22° C; thermal inactivation of the two viruses between 58° and 60° C; and dilution one to 4,000 and one to 3,000 with the mild- and severe-mosaic viruses, respectively.

TRANSMISSION OF SEVERE-MOSAIC VIRUS

By Unfasted, Single, Infective Aphids. The efficiency of virus transmission was determined by means of infective, unfasted cabbage and green peach aphids reared on mosaic-infected annual stock plants. Each aphid was fed singly on a healthy annual stock plant. Table 1 shows that single cabbage and turnip aphids transmitted the severe-mosaic virus to 3 and 6 per cent of the plants, respectively, but that none of the plants was infected with single green peach aphids.

Not a single infection was obtained with 200 cabbage aphids, 300 turnip aphids, and 150 green peach aphids tested singly with the mild-mosaic virus of annual stock as reported in a previous paper (Severin and Tompkins, 1948).

TABLE 1
TRANSMISSION OF THE SEVERE-MOSAIC VIRUS OF ANNUAL STOCK BY
SPECIES OF UNFASTED, SINGLE, WINGLESS, INFECTIVE APHIDS
FROM DISEASED TO HEALTHY ANNUAL STOCK PLANTS

Common and scientific name of aphids	Plants inoculated	Plants infected	Per cent infected
Cabbage aphid, <i>Brevicoryne brassicae</i>	100	3	3.0
Turnip aphid, <i>Rhopalosiphum pseudo brassicae</i>	100	6	6.0
Green peach aphid, <i>Myzus persicae</i>	100	0	0.0

By Previously Noninfective, Fasted, Single Aphids in Short Infection-Feeding Periods. Previously noninfective, wingless aphids of three species were starved for two to three hours in a phial, then were fed singly on a mosaic-infected annual stock plant for one-half, one, two, five, or ten minutes, and then transferred to healthy annual stock plants. Table 2 shows that the green peach aphid and the turnip aphid transmitted the severe-mosaic virus to 42 and 12 per cent respectively of the plants inoculated. The cabbage aphid failed to infect any of the healthy plants inoculated after short infective feeding periods.

From Experimentally Infected to Healthy Annual Stock and Cauliflower Plants. Lots of 20 infective aphids of the three species were transferred from experimentally infected annual stock to healthy annual stock and February cauliflower plants. Table 3 shows that 32, 73, and 69 per cent of the healthy annual stock plants were infected by the cabbage, turnip, and green peach aphid, respectively, but that none of the cauliflower plants was infected.

COMPARATIVE EFFICIENCY OF MECHANICAL AND APHID TRANSMISSION OF SEVERE-MOSAIC VIRUS

Transmission of the severe-mosaic virus from experimentally infected to healthy annual stock plants by mechanical inoculation was compared with transmissions by three species of aphids. The virus extract from the infected plants upon which the aphids had fed was mechanically inoculated into healthy plants. In addition, in each test five lots of 20 aphids of each species were transferred from each infected plant to healthy plants. The results are

TABLE 2
TRANSMISSION OF THE SEVERE-MOSAIC VIRUS OF ANNUAL STOCK BY TWO SPECIES OF PREVIOUSLY
NONINFECTIVE, FASTED SINGLE, WINGLESS APHIDS AFTER SHORT INFECTION
FEEDING PERIODS ON VIRUS SOURCE

Aphid species and lot no.	Feeding time, minutes, on diseased leaves										Totals		
	0.5		1		2		5		10				
	Plants inocu- lated	Plants infected	Plants inocu- lated	Plants infected	Plants inocu- lated	Plants infected	Plants inocu- lated	Plants infected	Plants inocu- lated	Plants infected	Plants inocu- lated	Plants infected	Per cent infected
Green peach aphid, <i>Myzus persicae</i> ...	5	4	5	4	5	4	5	2	5	1	25	15	60
Test 1.....	5	4	5	3	5	4	5	2	5	2	25	15	60
Test 2.....	5	4	5	3	5	3	5	0	5	3	25	13	52
Test 3.....	5	2	5	2	5	1	5	2	5	1	25	8	28
Test 4.....	5	1	5	0	5	0	5	0	5	0	25	1	4
Total.....	25	15	25	12	25	12	25	6	25	7	125	52	..
Percentage.....	..	60	..	48	..	36	..	24	..	28	42
Turnip aphid, <i>Rhopalosiphum pseudobrassicæ</i>
Test 1.....	5	0	5	1	5	0	5	1	5	2	25	4	16
Test 2.....	5	0	5	0	5	1	5	1	5	1	25	3	12
Test 3.....	5	0	5	0	5	1	5	1	5	0	25	2	8
Total.....	15	0	15	1	15	2	15	3	15	3	75	9	..
Percentage.....	..	0	..	7	..	13	..	20	..	20	12

TABLE 3

TRANSMISSION OF THE SEVERE-MOSAIC VIRUS FROM EXPERIMENTALLY INFECTED ANNUAL STOCK TO HEALTHY ANNUAL STOCK AND CAULIFLOWER PLANTS BY THREE SPECIES OF APHIDS, 20 IN EACH LOT

Aphid species and lot no.	Mosaic-infected annual stock plant no.	Annual stock		Cauliflower	
		Inoculated	Infected	Inoculated	Infected
Cabbage aphid, <i>Brevicoryne brassicae</i> :					
Lot 1.....	1	5	4	5	0
Lot 2.....	2	5	1	5	0
Lot 3.....	3	5	1	5	0
Lot 4.....	4	5	1	5	0
Lot 5.....	5	5	1	5	0
Total.....	..	25	8	25	0
Percentage.....	32	..	0.0
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> :					
Lot 1.....	1	5	5	5	0
Lot 2.....	2	5	5	5	0
Lot 3.....	3	5	5	5	0
Lot 4.....	4	5	4	5	0
Lot 5.....	5	5	2	5	0
Lot 6.....	6	5	1	5	0
Total.....	..	30	22	30	0
Percentage.....	73	..	0.0
Green peach aphid, <i>Myzus persicae</i> :					
Lot 1.....	1	5	5	5	0
Lot 2.....	2	5	5	5	0
Lot 3.....	3	5	4	5	0
Lot 4.....	4	5	4	5	0
Lot 5.....	5	5	4	5	0
Lot 6.....	6	5	1	5	0
Lot 7.....	7	5	1	5	0
Total.....	..	35	24	35	0
Percentage.....	69	..	0.0

given in table 4. Of the three species of aphids tested, the turnip aphid was the most efficient vector. Mechanical inoculation was more efficient in transmitting the virus: 51.4 per cent of the healthy annual stock plants became infected when mechanically inoculated; 46.7 per cent when the virus was transmitted by aphids.

RETENTION OF SEVERE-MOSAIC VIRUS

By Lots of 20 Aphids in Daily Transfers. The retention of the severe-mosaic virus was determined with three species of aphids reared on mosaic-infected annual stock plants. Lots of 20 aphids of each species were transferred daily for three days to successive healthy plants. As shown in table 5, each of the three species of aphids transmitted the virus from diseased to healthy annual stock plants during the first day only.

By Lots of 20 Aphids with Hourly Transfers. An attempt was made to determine how long the green peach aphid retained the severe-mosaic virus. Each lot of 20 wingless aphids reared on mosaic-infected annual stock plants was transferred hourly to six successive healthy plants. As table 6 shows, one lot of green peach aphids transmitted the virus during the first two hours, eight lots during the first hour only, and one lot during the second hour only.

TABLE 4
COMPARISON OF TRANSMISSION OF THE SEVERE-MOSAIC VIRUS OF
ANNUAL STOCK BY MECHANICAL INOCULATION AND BY
THREE SPECIES OF APHIDS, 20 IN EACH LOT

Number of plants from which virus was recovered	Mechanical inoculation			Aphid transmission			
	Annual stock plants			Common and scientific names of insects	Annual stock plants		
	Inoculated	Infected	Per cent infected		Inoculated	Infected	Per cent infected
7.....	35	28	80.0	Cabbage aphid, <i>Brevicoryne brassicae</i>	35	9	25.7
5.....	25	11	44.4	Green peach aphid, <i>Myzus persicae</i>	25	17	68.0
4.....	45	15	33.3	Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>	45	33	73.3
Total.....	105	54		105	49
Percentage	51.4		46.7

TABLE 5
RETENTION OF THE SEVERE-MOSAIC VIRUS OF ANNUAL STOCK BY
THREE SPECIES OF APHIDS, 20 IN EACH LOT

Common and scientific names of aphids	Aphid lots (20 each) tested			
	Number of lots tested	Number transmitting virus		
		1st day	2d day	3d day
Cabbage aphid, <i>Brevicoryne brassicae</i>	5	4	0	0
Green peach aphid, <i>Myzus persicae</i>	5	5	0	0
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>	5	5	0	0

By Previously Starved Single Aphids in Short Infection-Feeding Periods. Retention of the virus was determined more precisely with two species of aphids. Previously noninfective, wingless aphids were starved for two to three hours in a vial, then were fed singly on a mosaic-infected annual stock plant for intervals of five or ten minutes, and then were transferred at ten-minute intervals to six successive healthy plants. As table 7 shows, the two species of aphids transmitted the severe-mosaic virus during the first ten-minute period only.

Non-persistent Virus. Watson (1939), Watson and Roberts (1940) define non-persistent viruses as those which are easily mechanically transmissible

and whose vectors lose their infectivity within a few hours of removal from the source of infection. The retention time of the virus by aphids is less than the time that the virus is active in extracted sap, *in vitro*. In the Watson and Roberts (1940) interpretation, most emphasis is put upon the effect of starvation on increase of vector efficiency.

TABLE 6
RETENTION OF THE SEVERE-MOSAIC VIRUS OF ANNUAL STOCK BY
TWO SPECIES OF APHIDS TRANSFERRED HOURLY TO SIX
SUCCESSIVE HEALTHY ANNUAL STOCK PLANTS

Aphid species and lot no.	Number of aphids on first plant	Results* on successive plants with hourly transfers						Last infection produced by aphids, hour
		1st	2d	3d	4th	5th	6th	
Green peach aphid, <i>Myzus persicae</i> :								
Lot 1.....	20	+	+	—	—	—	—	2d
Lot 2.....	20	—	+	—	—	—	—	2d
Lot 3.....	20	+	—	—	—	—	—	1st
Lot 4.....	20	+	—	—	—	—	—	1st
Lot 5.....	20	+	—	—	—	—	—	1st
Lot 6.....	20	+	—	—	—	—	—	1st
Lot 7.....	20	+	—	—	—	—	—	1st
Lot 8.....	20	+	—	—	—	—	—	1st
Lot 9.....	20	+	—	—	—	—	—	1st
Lot 10.....	20	+	—	—	—	—	—	1st
..		9+	2+	0+	0+	0+	0+	
..		1—	8—	10—	10—	10—	10—	
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> :								
Lot 1.....	20	+	—	—	—	—	—	1st
Lot 2.....	20	+	—	—	—	—	—	1st
..		2+	0+	0+	0+	0+	0+	
..		0—	2—	2—	2—	2—	2—	

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

LOSS AND RECOVERY OF INFECTIVITY OF TURNIP APHIDS

An attempt was made to determine whether the turnip aphid could recover the severe-mosaic virus from inoculated annual stock plants before the first symptom developed. A large population of aphids reared on infected annual stock plants was transferred in lots of 20 aphids to healthy annual stock plants for four days. Each day, from the fifth to the fourteenth day, one lot of 20 of these aphids was transferred from the plant so inoculated to a healthy annual stock plant.

The results, reported in table 8, show that no infections were obtained with any of the lots transferred from the fifth to the seventh day. The aphids lost infectivity after the first transfer. Infections were obtained with some lots transferred on the eighth, ninth, tenth, twelfth and later days, however; so that the aphids must have recovered the severe-mosaic virus from the first

inoculated plants. The first symptom developed on the original inoculated plants in 13 to 31 days. The results show that the aphids were able to recover the virus from the first original inoculated plants before symptoms developed on them.

TABLE 7
RETENTION OF THE SEVERE-MOSAIC VIRUS OF ANNUAL STOCK BY
SINGLE APHIDS TRANSFERRED AT 10-MINUTE INTERVALS
TO SUCCESSIVE HEALTHY ANNUAL STOCK PLANTS

Aphid species and lot no.	Infective feeding time on diseased annual stock plants, minutes	Feeding time on successive healthy annual stock plants, minutes*					
		10	10	10	10	10	10
Green peach aphid, <i>Myzus persicae</i>							
Lot 1.....	10	+	-	-	-	-	-
Lot 2.....	10	+	-	-	-	-	-
Lot 3.....	10	+	-	-	-	-	-
Total +.....	..	3+	0+	0+	0+	0+	0+
Total -.....	..	0-	3-	3-	3-	3-	3-
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>							
Lot 1.....	5	+	-	-	-	-	-
Lot 2.....	5	+	-	-	-	-	-
Lot 3.....	5	+	-	-	-	-	-
Lot 4.....	10	+	-	-	-	-	-
Total +.....	..	4+	0+	0+	0+	0+	0+
Total -.....	..	0-	4-	4-	4-	4-	4-

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

TABLE 8
LOSS AND RECOVERY OF INFECTIVITY OF TURNIP APHID, *RHOPALOSIPHUM PSEUDOBRASSICAE*, ON ANNUAL STOCK PLANTS
INOCULATED WITH THE SEVERE-MOSAIC VIRUS

Original plant number	Elapsed time in days between inoculation of each plant with infective aphids and transfer, in daily lots of 20, to healthy plants*										Days to the first symptom on original plant
	5	6	7	8	9	10	11	12	13	14	
1.....	-	-	-	+	-	+	+	-	-	+	13
2.....	-	-	-	-	+	-	-	+	-	+	15
3.....	-	-	-	-	-	+	+	-	+	+	25
4.....	-	-	-	-	-	-	-	+	-	+	31

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

MECHANICAL INOCULATION WITH SEVERE-MOSAIC-VIRUS EXTRACT FROM CRUSHED TURNIP APHIDS

An attempt was made to transmit the severe-mosaic virus of annual stock with the extract of crushed, infective turnip aphids by mechanical inoculation of healthy annual stock plants. The virus extract prepared from one gram of infective aphids crushed in 9 cc of sterile distilled water was inoculated in ten healthy annual stock plants. The experiment was repeated five times. A total of 50 plants were inoculated, but no infection occurred.

SUMMARY

Infective, unfasted cabbage aphids (*Brevicoryne brassicae* L.), and the turnip or false cabbage aphid (*Rhopalosiphum pseudobrassicae* Davis), tested singly, transmitted the severe-mosaic virus of annual stock to 3 and 6 per cent of the healthy annual stock plants (*Matthiola incana* var. *annua*) respectively, but in similar tests none of the plants were infected by the green peach aphid (*Myzus persicae* Sulzer).

When the cabbage and turnip aphids were fasted for two to three hours, fed one-half, one, two, five, or ten minutes on the leaves of infected annual stock plants, and then transferred to healthy annual stock plants, the turnip aphid infected 42 per cent and the green peach aphid 12 per cent of the plants inoculated.

The three species of aphids failed to infect cauliflower with the severe stock-mosaic virus.

Mechanical inoculation (51.4 per cent) was more efficient than transmission of the severe-mosaic virus by three species of aphids.

In tests on the retention of the severe-mosaic virus, lots of 20 cabbage, turnip, and green peach aphids transmitted the virus during the first day, but none of the lots tested produced infection during the second and third days. In hourly transfers, one lot of green peach aphids transmitted the virus during the first two hours, eight lots during the first hour only, and one lot during the second hour only. The turnip aphid transmitted the virus during the first hour only. In ten-minute-period transfers, the green peach and turnip aphids transmitted the virus during the first interval only.

The severe-mosaic virus was lost during the fifth day (the first day of transfer) by lots of 20 infective turnip aphids, but was recovered during the eighth to the twelfth and later days, before the earliest symptom developed on inoculated plants, at periods varying from 13 to 31 days. The aphids were able to recover the virus from the first original inoculated plants before symptoms developed on them.

The severe-mosaic virus of annual stock is of the non-persistent type in the aphid vectors.

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PLATES

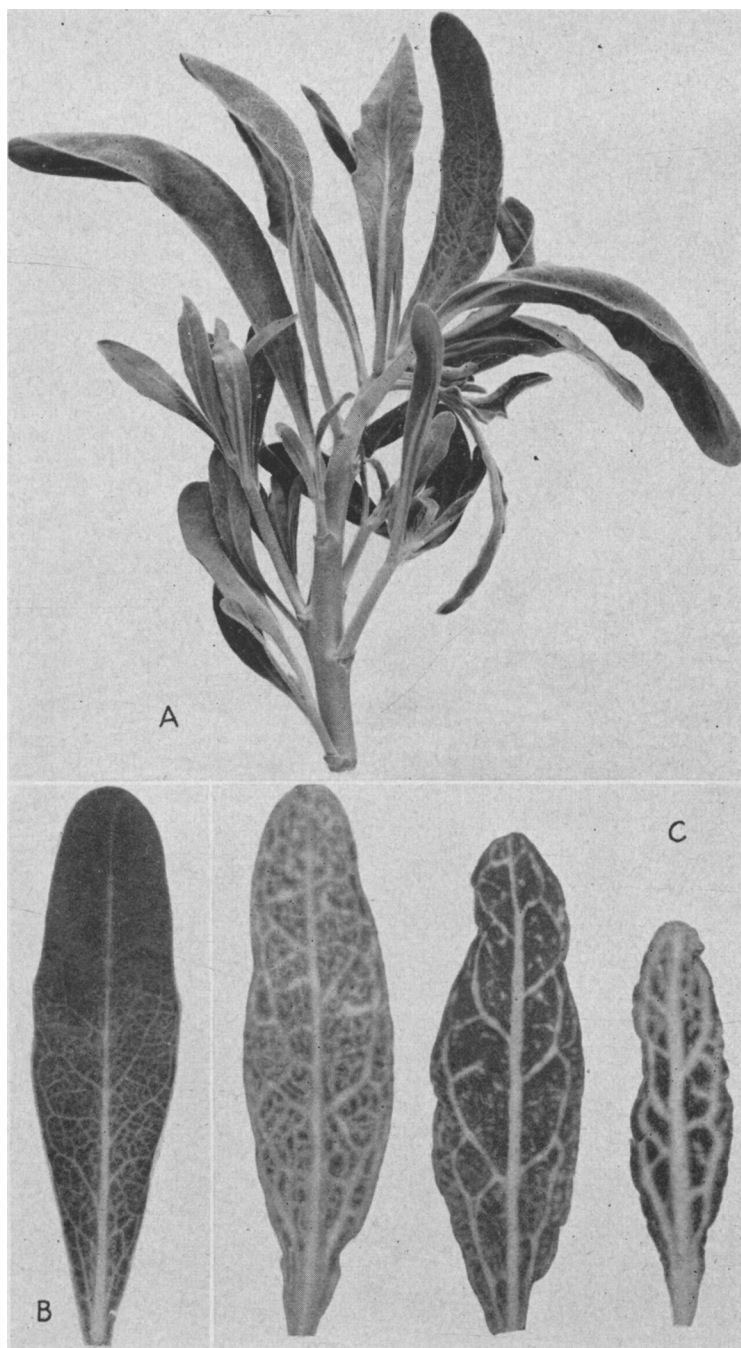


Plate 1. Early symptoms of severe-mosaic virus of annual stock (*Matthiola incana* var. *annua*) on the variety Fiery Blood Red: *A*, downward-bending of apical end of stem; *B*, cleared veinlets on youngest leaf; *C*, lower surface of younger leaves showing protruding veins.

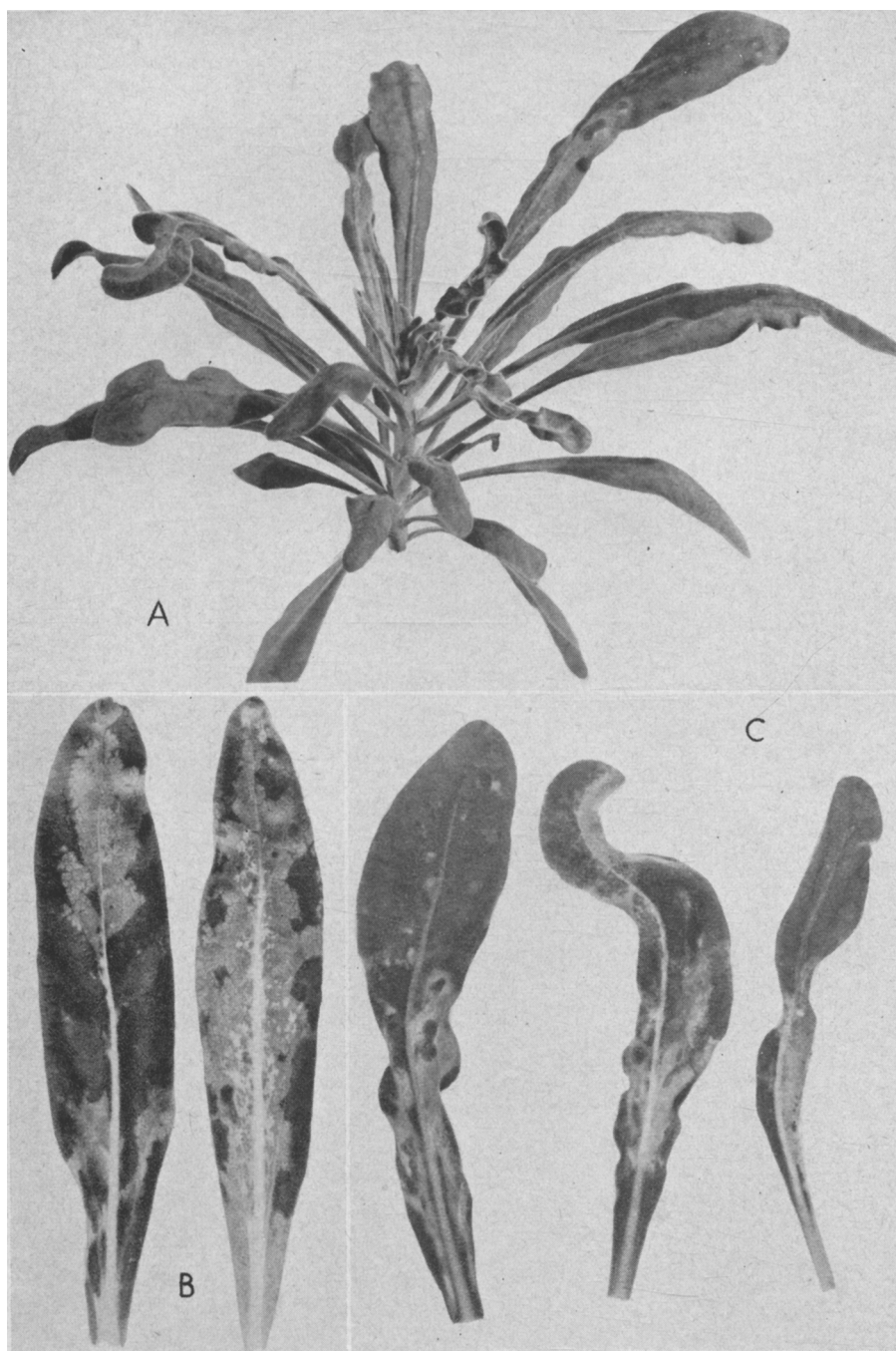


Plate 2. Symptoms induced by severe-mosaic virus of annual stock (*Matthiola incana* var. *annua*) by mechanical inoculation: *A*, infected plant showing deformed intermediate leaves, curled, twisted, and puckered younger leaves, and stunting; *B*, mottling; *C*, deformed leaves.

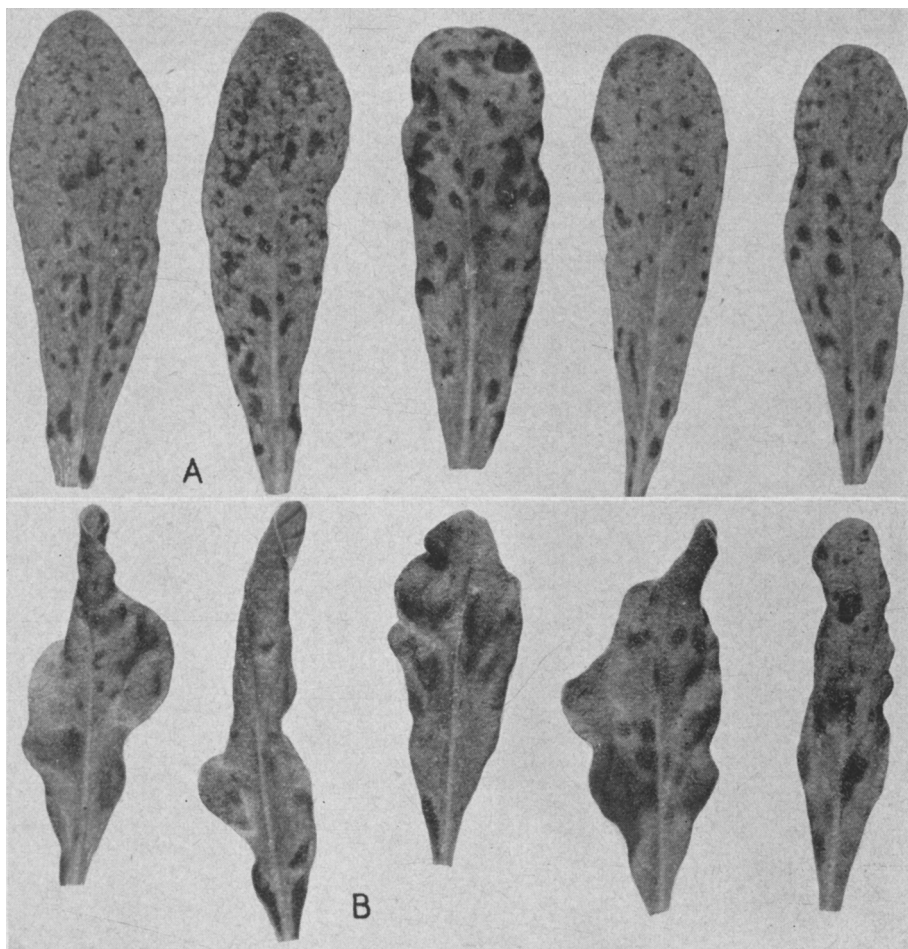


Plate 3. Advanced stage of severe-mosaic virus of annual stock (*Matthiola incana* var. *annua*) on the variety Fiery Blood Red: *A*, leaves showing mottling; *B*, leaves showing blisterlike elevations.

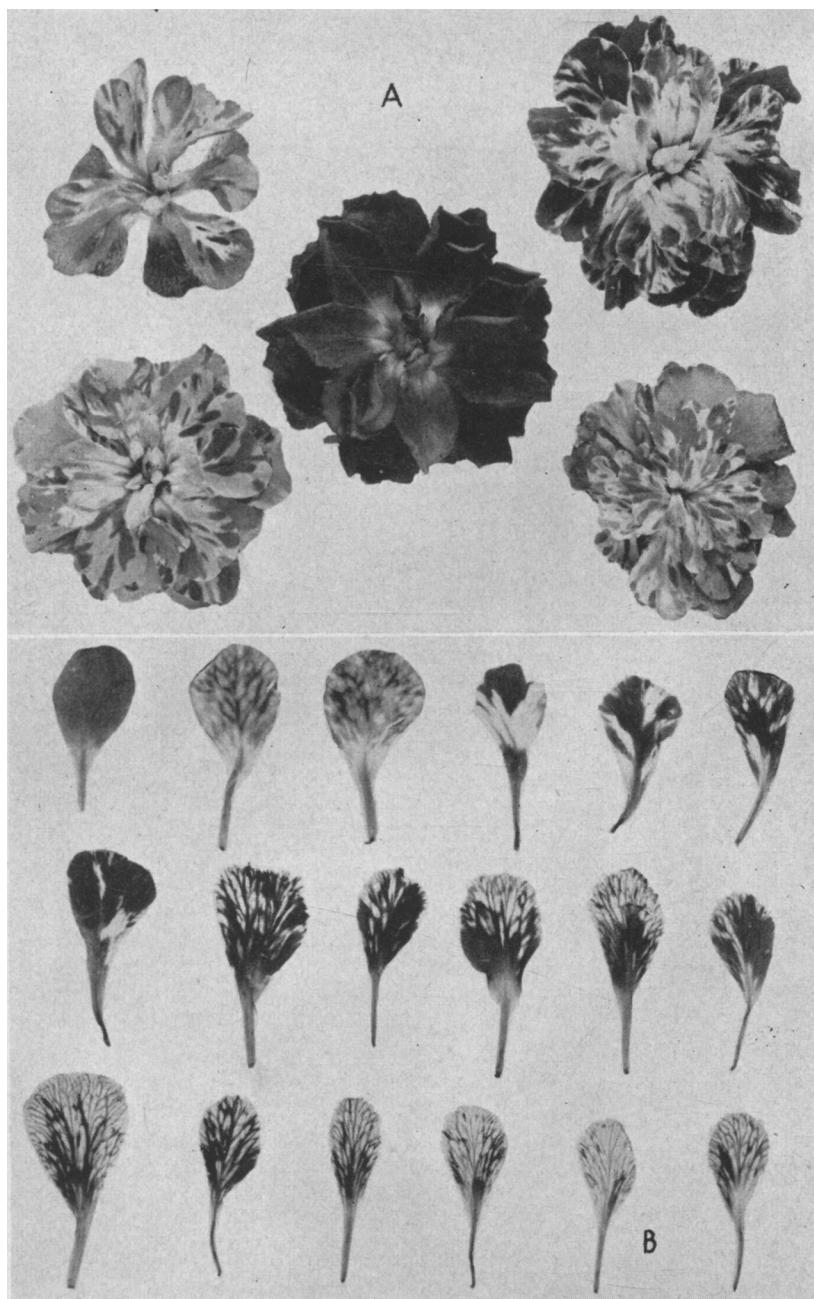


Plate 4. Symptoms induced by the severe-mosaic virus of annual stock (*Matthiola incana* var. *annua*) on flowers of naturally infected annual stock: *A*, normal flower; grouped around it are four flowers showing breaking, consisting of coarse, blotched, normal color with white streaks on petals. *B*, petals showing breaking in color: upper row, irregular white areas scattered in normal color; middle row, white streaks encompassing veins; lower row, white streaks spreading out on each side of veins (San Pablo, Contra Costa County, July 15, 1936).

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