Malva Yellows, an Aphid-Transmitted Virus Disease

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Several introduced European species of the genus *Malva* have become naturalized in California and are now common weeds in various parts of the state (7, 8)[4]. In the Salinas Valley, species of this group, known generally as mallow plants, are perhaps among the most prevalent weeds on waysides, along fences, in waste places, and in the fields of many crop plants.

An abnormal yellowing of the foliage of *Malva* weeds, especially cheeseweed (*M. parviflora* L.), has occurred in California for several years. This yellowing was found to result from infection with a virus disease not heretofore described and distinct from others already known to occur in the state. The possibility that this prevalent disease might be of importance in relation to the yellowing complexes of sugar beets and other economic plants led the writers to study it. The results of the investigations on this newly described disease, which has been named "malva yellows," are reported in this paper.

Geographical Distribution

Malva yellows is known to occur in various parts of California. Nothing is known of its occurrence in other parts of the United States, although the host species of *Malva* are widely distributed throughout the country (4).

In areas where malva yellows occurs, the disease may be found on cheeseweed plants at any time of the year. Natural stands of this weed generally show high incidence of the disease, especially large plants growing along fences, roads, or around utility poles and pump houses. It is sometimes difficult to find cheeseweed plants without symptoms even among seedlings of this species that are growing amid crop plants.

Cheeseweed plants are regarded as the most important source of the malva yellows virus. Since this species is also an excellent food and breeding host for the vector, there is always a great chance that the disease might spread from this weed to susceptible crop plants.

Economic Importance

It is obvious that yellows of mallow would be a beneficial disease if its attacks were limited to weeds of this group. The

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[4] Numbers in parentheses refer to literature cited.

disease, however, may affect some ornamental *Malvaceae,* crop plants of Chenopodiaceae, and probably of other families.

In the Salinas Valley, malva yellows has been found to be associated with foliage yellowing in spinach (*Spinacia oleracea* L.) that impairs the value of the crop. However, the economic importance of this disease in relation to the spinach yellows complex has not yet been ascertained.

In a few instances the malva yellows virus was recovered from field-grown sugar-beet (*Beta vulgaris* L.) plants that were showing mild yellowing symptoms. The possibility exists, therefore, that this virus may also cause some yellowing of sugar beet under field conditions.

The malva yellows virus has been recovered from some weeds of the *Cruciferae* family showing yellowing of the lower leaves. Whether the disease may affect crop plants of this family is not yet well established.

Materials and Methods

Except in tests designed to determine the host range, the differential host plant used to study malva yellows was cheeseweed.

In most cases the inoculations were carried out with seedlings that had 2 to 4 foliage leaves. In a general way, younger seedlings were more susceptible to infection than older ones.

Malva yellows virus was obtained from infected cheeseweed or spinach plants collected in the Salinas Valley. Several different collections were made, but little difference was noted among them.

The various species of aphids tested as vectors were reared on appropriate host plants confined in cages within an insectary. Nonviruliferous green peach aphids (*Myzus persicae* (Sulz.)) were reared on radish (*Raphanus sativus* L.).

In routine transmission tests, the insects were permitted to feed on the virus source for 48 hours and then for an equal period on the test plants.

Host Range

Studies on the host range of the malva yellows virus were carried out in two ways: 1) By placing nonviruliferous green peach aphids on various plants collected in areas where the disease was very prevalent among cheeseweed and after 48 hours transferring the insects to healthy cheeseweed seedlings, and 2) by placing at least five seedlings of a number of species in an insectary compartment where a large population of viruliferous green peach aphids was being reared on malva yellows infected cheeseweed. In the latter method, a large number of insects were shaken from the virus source plants onto those to be inoculated and permitted to feed for 48 hours; afterwards, the test plants

Table 1.—List of Species from Which the Malva Yellows Virus Has Been Recovered.

Althaea rosea (L.) Cav. GR, NS[1]	*Malva rotundifolia* L. FR, GR, NS
Amaranthus sp. FR, GR, SQ	*Nicotiana alata* Link & Otto GR, NS
Beta patellaris, Moq. GR, SP	*N. clevelandii* Gray, GR, SP
B. patula Ait. GR, NS	*Petunia hybrida* Vilm. GR, NS
B. vulgaris L. FR, GR, SQ	*Raphanus sativus* L. FR, SQ
Brassica campestris L. FR, SQ	*Rumex crispus* L. FR, GR, NS
B. juncea (L.) Coss. GR, NS	*Senecio vulgaris* L. FR, GR, NS
Capsella bursa-pastoris (L.) Medic. FR, GR, SQ	*Silene gallica* L. GR, NS
Cerastium viscosum L. GR, SQ	*Sonchus oleraceus* L. GR, NS
Erodium cicutarium L'Her. FR, GR, SQ	*Spinacia oleracea* L. FR, GR, SP
Hibiscus esculentus L. GR, NS	*Stellaria media* (L.) Cyr. FR, GR, NS
Lactuca sativa L. FR, SQ	*Tetragonia expansa* Murr. GR, NS
Lamium amplexicaule L. FR, GR, NS	*Urtica urens* L. FR, GR, NS
Lavatera assurgentiflora Kell. GR, SP	*Zinnia elegans* Jacq. GR, NS
Malva parviflora L. FR, GR, SP	

[1] FR—Recovered from field plants; GR—Recovered from greenhouse-inoculated plants; SP—Symptoms present; NS—No symptoms noticed; SQ—Symptoms questionable.

were removed from the insectary, sprayed, and placed in the greenhouse. Since many host plants of the malva yellows virus permit its increase without showing symptoms of infection, recovery was attempted from all inoculated species.

Host plants of the malva yellows virus, based on results from recovery tests from field plants and from greenhouse-inoculated plants, are listed in Table 1. Several species of weeds and economic plants are shown to support increase of the malva yellows virus and some were found to be naturally infected with the virus in the field.

Symptoms

Species that supported increase of the malva yellows virus showed either moderate symptoms, consisting of yellowing of the lower leaves, or were symptomless carriers. The description of the reactions of a selected group of host plants follows:

Cheeseweed

Field-infected plants show strong yellowing in the lower and middle leaves. The yellowing pattern may be sectorial in the early stages of the disease, but in the later stages tends to invade the whole leaf, except for narrow areas along the main veins. Leaves higher on the plant show yellowing symptoms as they become older, but not all such leaves will necessarily develop them. In these leaves, the symptoms generally appear near the margins and progress towards the center of the leaf. Young leaves on the main stem or those on axillary branches do not show yellowing, but may develop such symptoms later. The yellowing symptoms are usually more severe in the early stages of the dis-

ease. A certain degree of recovery is rather common, but a few leaves with symptoms are usually present on affected plants.

The first symptoms of infection in cheeseweed seedlings inoculated in the greenhouse appear 8 to 15 days after inoculation as yellowish areas between the radiating main veins of the leaf. Frequently these yellow areas involve the whole space between two adjoining veins and are sectorial in shape (Figure 1-B). These symptoms possibly represent areas where increase of the virus occurred after its introduction into the plant. These areas increase in size and may occupy the whole leaf, or only part of it. In late stages these and other lower leaves on the plant show a more generalized type of yellowing, only a narrow area along the main veins remaining green. Systemic invasion of the plant seems to be accompanied by a mild veinclearing of the young growth in some instances, but this is not a common feature of the disease. A certain degree of recovery follows the appearance of early symptoms, and infected older leaves may shrivel and die so that the infected plants may appear healthy at times. Recovered plants may remain without symptoms if they are growing slowly or yellowing symptoms may reappear later as the plants age.

So far, cheeseweed has been the best differential host for malva yellows because of the distinctive early symptoms. Although experimental results are not available, observations have indicated that good lighting is favorable for the manifestation of malva yellows symptoms in this species. Another advantage of this species is the fact that it is apparently immune from common field strains of beet yellows, thus providing a good means of differentiating between the two viruses.

Cheeseweed plants growing in the greenhouse frequently show several anomalies that may result in death of the lower leaves. These conditions, whether due to nutritional disturbances, to fumigation, or to other factors, are not yet understood and might be confused with symptoms of malva yellows, thus making the observations on this disease more difficult.

Under certain conditions in the greenhouse, cheeseweed plants that were inoculated with malva yellows developed the symptoms of this disease and in addition leaf malformation and rosette (Figure 2). Similar symptoms were observed on field plants in only two instances, but were rather common in the greenhouse, and seemed to be related to conditions of reduced light during a series of overcast days.

It has not yet been ascertained whether the symptoms of rosette and leaf malformation represent variations in symptoms

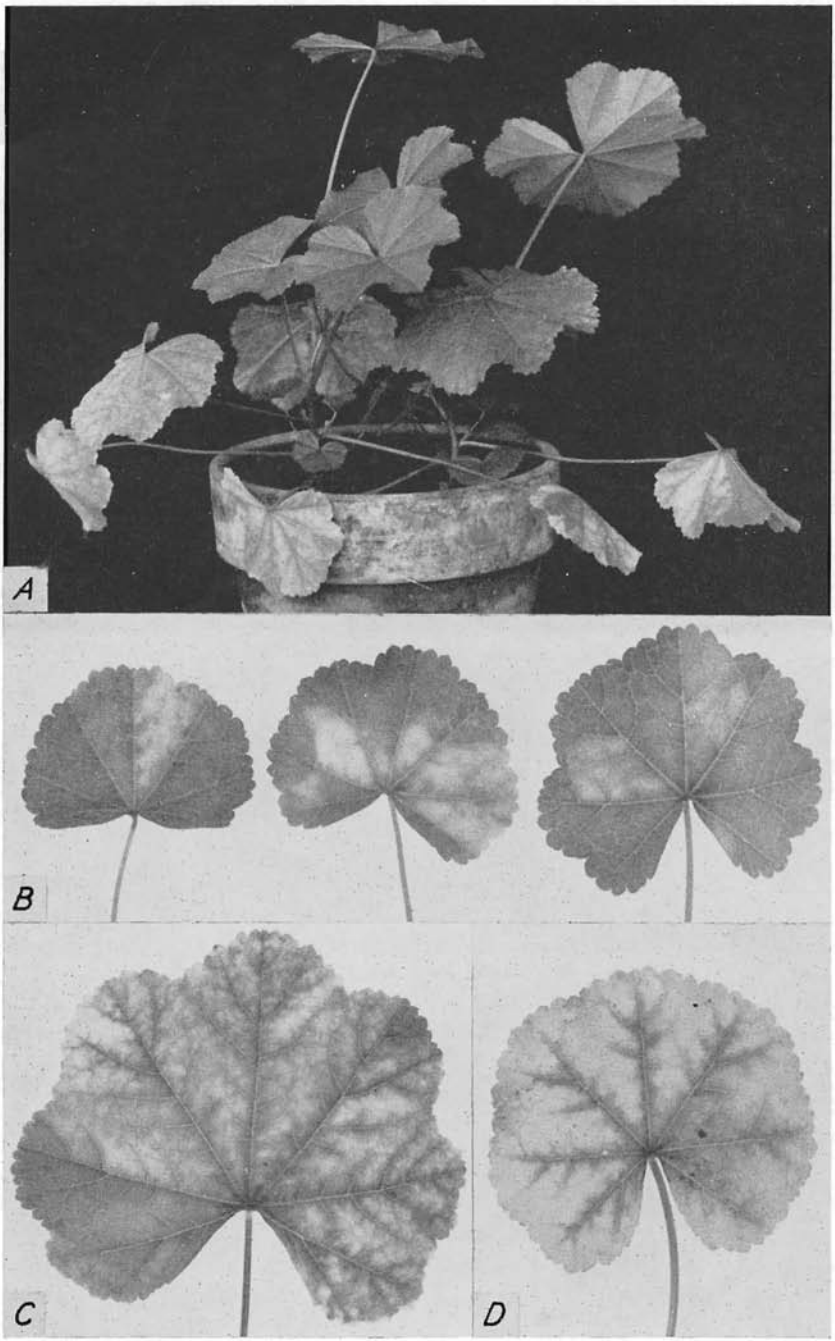

Figure 1.—Symptoms of malva yellows on cheeseweed. A, Greenhouse-infected plants; B, initial leaf symptoms following aphid inoculation; C and D, progressive stages of leaf yellowing.

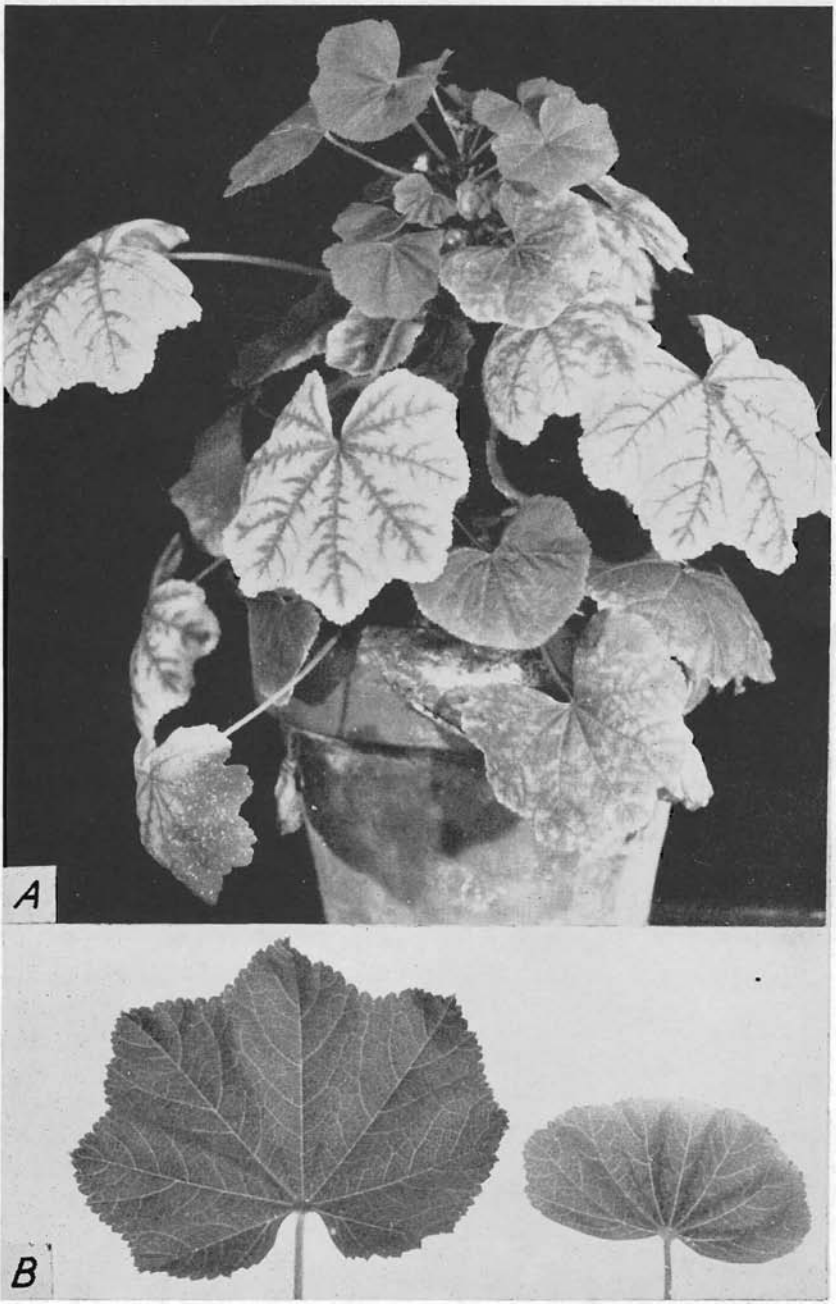

Figure 2.—Symptoms of rosette and malformation accompanying malva yellows. A, Greenhouse-infected cheeseweed plant; B, normal (left) and malformed leaves.

of malva yellows due to environmental conditions, or result from the presence of another virus that causes a disease the symptoms of which are expressed only under such conditions.

Several attempts to obtain the malformation and rosette symptoms in the absence of malva yellows have failed. Neither has it been possible to obtain consistently the manifestation of these symptoms.

Malva rotundifolia

This species can be infected with the malva yellows virus, but does not show symptoms under greenhouse conditions. Nonviruliferous aphids fed on inoculated *Malva rotundifolia* plants produced malva yellows when transferred to cheeseweed seedlings.

Nicotiana clevelandii

Veinclearing or a slight mottling of the young leaves, accompanied by some rugosity was noticed in the infected plants 8 to 12 days after inoculation. As the plants grew older, the lower leaves began to turn yellow and the upper leaves showed interveinal chlorosis. Yellowing of the lower leaves was intensified as the plants aged, and even the upper ones in some instances showed marked symptoms at maturity.

Association of Malva Yellows Virus and Yellowing of Spinach

Spinach plants infected with malva yellows in the greenhouse have not shown definite symptoms. Occasionally, however, the plants have shown mild yellowing of the vein islets in older leaves, mostly near the leaf tips or in the interveinal areas.

Field spinach plants inoculated[5] when approximately half grown showed only mild symptoms about 30 days after inoculation. Symptoms consisted of yellowing of the lower leaves near the tip and around the border (Figure 3). Comparable plants inoculated with strains of the beet yellows virus previously isolated from spinach showed severe yellowing and stunting. Plants inoculated with a mixture of the malva yellows and beet yellows viruses showed symptoms still more severe.

After it was found that spinach plants supported the increase of the malva yellows virus, it became of interest to investigate whether or not it could be associated with the yellowing of spinach foliage present in fall-and winter-planted fields in the Salinas Valley.

Several spinach fields planted in the latter part of 1956 and early in 1957 were surveyed, and a number of plants displaying

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[5] The writers are greatly indebted to F. W. Zink, Associate Specialist in Vegetable Crops, University of California, Davis, California, for preparing the field plots and helping with the inoculations of the spinach plants.

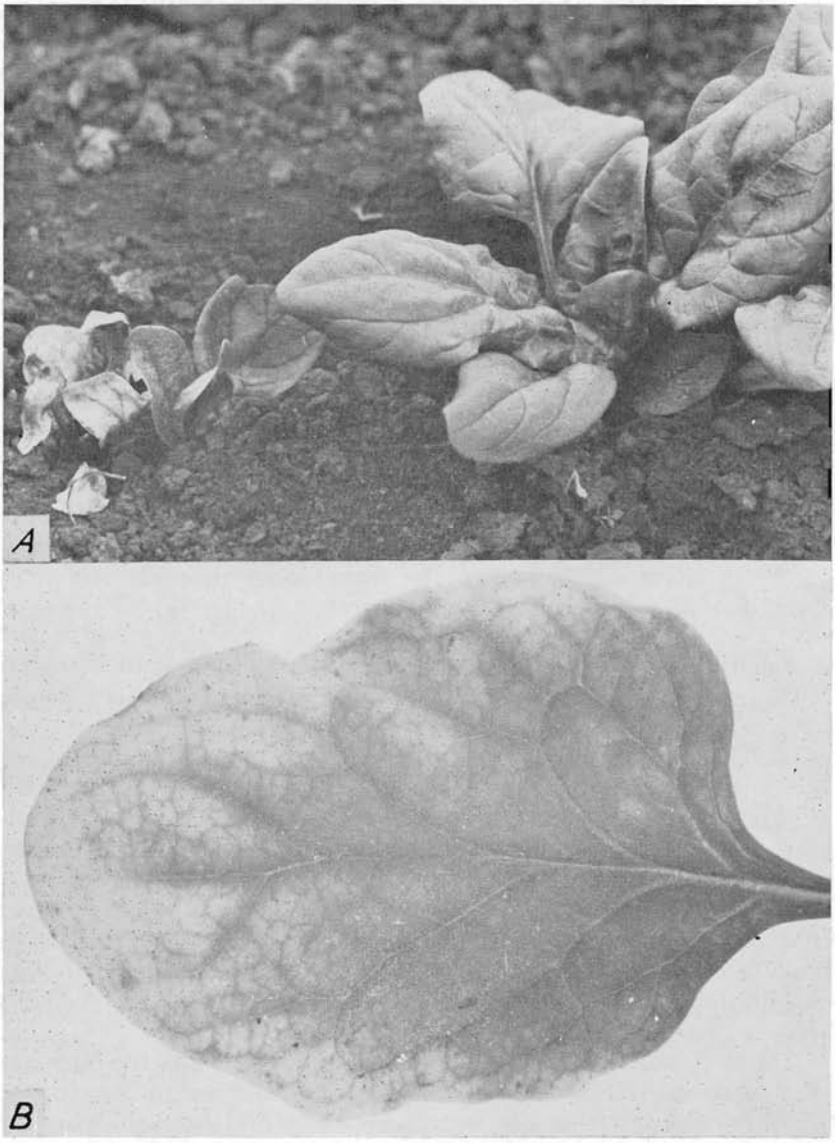

Figure 3.—Symptoms incited by the malva yellows virus on spinach. A, infected and healthy field plant; B, diseased leaf from field inoculated plant.

yellowing were collected at random from each field. The individual plants in each sample were placed with their roots in water and nonviruliferous green peach aphids were fed on them for a period of 48 hours. Afterwards the insects were transferred to healthy cheeseweed and sugar beet seedlings. The results of a number of these recovery tests (Table 2) show that the malva yellows virus was present in the yellow spinach leaves either alone or accompanied by the beet yellows virus in almost all instances. The frequency of plants infected with the two viruses was higher in the samples from the fall crop. Malva yellows virus alone was found much more frequently than beet yellows virus alone.

Table 2.—Results of Virus Recovery Tests from Field Spinach Plants Showing Foliage Yellowing.[1]

Field No.	Number of Plants Tested	Number of Plants from Which the Indicated Viruses were Recovered		
		Malva Yellows Plus Beet Yellows	Malva Yellows Alone	Beet Yellows Alone
1	5	5	0	0
2	10	10	0	0
3	5	3	2	0
4	10	4	5	0
5	15	1	12	0
6	10	5	4	0
7	9	0	7	0
8	5	1	2	2
9	5	5	0	0
10	6	0	5	0

[1] All fields located in Monterey County, California. Fields 1-4, fall-planted; fields 5-10, winter-planted.

Transmission Tests

Seed

No experiment was carried out specifically to determine whether or not malva yellows was transmitted through cheeseweed seeds. However, several thousand seedlings of this species were raised in the greenhouse from seed produced by field plants that were infected by the disease. Careful observation of these seedlings up to the time they were used in tests failed to show any evidence of malva yellows infection.

Tissue Union

A total of 15 cheeseweed seedlings were inoculated by grafting with malva yellows infected scions. Twelve of the seedlings on which the grafts established organic union became infected and showed symptoms of the disease.

Mechanical Transmission

Many attempts were made to transmit malva yellows virus by mechanical inoculation, employing routine methods and some of the usual variations. These included the use of abrasives, extraction of the inoculum in a 0.02 M phosphate buffer solution at pH 7, in 0.02 M sodium sulfite alone or added to the buffer. Inoculum for these tests was obtained mostly from infected cheeseweed or *Nicotiana clevelandii* plants.

A minimum of 5 plants of over 35 species were tested, including those that had been found to be susceptible to malva yellows when inoculated by means of the insect vector. Cheeseweed and *Nicotiana clevelandii* plants were used to a much larger extent than other species in the mechanical transmission tests. Also, in some of the tests with these two species the test plants were conditioned in the dark for 48 hours before inoculation.

None of the plants inoculated mechanically with malva yellows showed symptoms of infection nor was any virus recovered from them.

Insect Vectors

Malva yellows has been found to be an aphid-transmitted virus disease. *Myzus persicae* is a vector of the virus, as well as *M. ornatus* Laing and *Aphis gossypii* Glov. A few attempts to transmit malva yellows virus with other species of aphids were made. The results of the aphid transmission tests except for those of *M. persicae* are presented in Table 3.

Table 3.—Results of Transmission Tests with Malva Yellows Using Plants of *Malva parviflora* and Various Species of Aphids.

Species of Aphid	Number of Plants Infested with the Indicated Aphid Species and Number Becoming Infected	
	Infested	Infected
Aphis fabae Scop.	26	0
A. gossypii Glov.	5	3
A. helianthi Monell	35	0
A. helichrysi Kalt.	30	0
Macrosiphum dirhodum (Walker)	27	0
M. sp. near *erigeronensis* Thos.	16	0
Myzus ornatus Laing	33	21
Rhopalosiphum conii (David.)	13	0
R. fitchii (Sand.)	22	0
Therioaphis maculata (Buckton)	11	0

Virus-Vector-Plant Relationships

Although species of aphids other than the green peach aphid transmitted malva yellows, the virus-vector-plant relationships were studied only with this species.

Number of Insects Per Plant and Percentage of Infection

Viruliferous aphids bred on diseased cheeseweed plants were used in tests designed to determine the efficiency of different numbers of insects per plant in securing infection. The insects were fed on the cheeseweed test plants singly or in groups of 3, 9, and 27 insects per plant. After a 24- or 48-hour feeding period they were killed with a TEPP spray application. The results of these tests are in Table 4. Single insects transmitted malva yellows to more than 50 percent of the plants. Transmission percentages increased with larger numbers of insects.

The number of plants infected from single-insect inoculations (Table 4) is rather low; much better results were obtained in other tests. When the persistence of the virus in the vector was studied with single insects (Table 9), it was found that out of 20 insects bred on diseased plants only 1 failed to infect at least one of the test plants. Also, 24 insects were tested singly in another experiment and they all produced infection in the test plants.

Table 4.—Results of Transmission Tests with Malva Yellows Virus, with Different Numbers of Green Peach Aphids per Plant.

Test No.	Number of Cheeseweed Seedlings Inoculated and Infected, when Colonized with the Indicated Number of Viruliferous Insects Per Plant							
	1		3		9		27	
	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.	Inoc.	Inf.
1	8	3	8	8	4	3	4	4
2	8	6	8	7	4	4	4	4
3	16	6	16	12	8	7	8	8
4	16	11	16	15	8	8	8	8
5	16	10	16	15	8	8	8	8
Percent Transmission	56.3		89.1		93.8		100	

Other tests were carried out to determine the persistence of the virus in the vector as related to the feeding period on the virus source. In these tests insects that were fed on the virus source plant for periods of 6 to 48 hours were considerably less efficient vectors than those bred on diseased plants.

Feeding Time Required by Viruliferous Vectors to Infect Test Plants

These tests were conducted with insects bred on diseased cheeseweed. Groups of 5 insects were placed on each of the test plants and permitted to feed for varying periods. At the end of the feeding period the insects were removed and the plants were sprayed with TEPP. The results of these tests are in Table 5. They show that in most cases the shortest feeding period tried, 1.5 hours, was long enough to secure a fair percentage of transmission.

Table 5.—Feeding Time Required by 5 Viruliferous Green Peach Aphids to Infect Cheeseweed Seedlings with the Malva Yellows Virus.

Test No.	Number of Plants Infected Out of 4 on which the Aphids were Allowed to Feed for the Indicated Period in Hours				
	1.5	3	6	12	24
1	2	..	4	4	4
2	1	.	3	4	4
3	0	0	0	0	2
4	1	2	3	3	3
5	2	3	2	3	3
6	1	2	3	4	4
7	2	1	3	4	4
Percent Transmission	42.9	55.0	64.3	78.6	85.7

Table 6.—Feeding Period on Malva Yellows Virus Sources Required by the Green Peach Aphid to Become Infective.

Test No.	Number of Cheeseweed Seedlings Infected Out of 4 that Were Inoculated with Groups of 5 Insects Fed on the Source of Virus for the Indicated Number of Hours.				
	1.5	3	6	12	24
1	1	..	4	4	4
2	1	.	3	4	3
3	2	1	4	4	4
4	0	0	0	2	2
5	0	1	2	2	4
6	1	0	2	1	4
7	0	0	3	3	2
8	1	3	1	2	2
9	1	0	1	1	1
Percent Transmission	19.4	17.9	55.6	63.8	72.2

Feeding Time on Virus Source Required by the Vector to Become Infective

Radish leaves with green peach aphids from the nonviruliferous stock colonies were placed in a dry container overnight. Next morning the crawling insects were placed on cheeseweed plants infected with malva yellows. After determined feeding intervals, insects were removed from the virus source plants and placed in groups of 5 aphids per plant on cheeseweed seedlings. The insects were permitted to feed 72 hours and were then killed. The results of these tests are in Table 6. The minimum feeding period tried, 1.5 hours, was enough to permit some insects to become viruliferous. As expected, efficiency of the vectors increased with increase in feeding time on the virus source.

The aphids, in the tests described above, were fed on various source plants. When the transmission results were plotted against the virus source plants, it was noticed that the differences between the sources very often outweighed those between feeding periods. From some of the source plants results were very poor, suggesting that virus content in them was extremely low. In a few instances there was difficulty in recovering virus. The same indications were noted in results from single insect inoculations when the vectors were fed on various source plants.

Incubation Period of the Malva Yellows Virus in the Vector

The incubation period of the malva yellows virus in the green peach aphid was studied in a series of tests. Nonviruliferous aphids bred on radish were starved overnight and placed on diseased cheeseweed the next morning. After feeding periods of 3, 6, and 12 hours, groups of 5 insects were removed from the virus source plant and transferred serially to healthy cheeseweed seedlings. The intervals between transfers were such as to permit testing for incubation periods of 6, 12, 24, 48, and 96 hours.

In further tests another technique was utilized in an effort to reduce possible injuries to the insects resulting from the serial transfers and to increase the feeding time on the test plants. The aphids were allowed to feed on the virus source for 3, 6, and 12 hours and were then colonized in groups of 5 per plant on healthy cheeseweed seedlings for the necessary time intervals to permit testing for incubation periods of 6, 12, 24, 48, and 96 hours. The results of these tests are in Table 7.

In other tests, the incubation period of the virus in the vector was studied with single insects. Four feeding periods on the virus source were tried: 6, 12, 24, and 48 hours. Incubation

Table 7.—Results of Tests to Determine the Incubation Period of the Malva Yellows Virus in the Green Peach Aphid.

Test No.	Feeding Period on Virus Source in Hours	Number of Cheeseweed Seedlings Infected Out of 4 Inoculated with 5 Insects in which the Virus had the Following Incubation Periods in Hours				
		6	12	24	48	96
1	3	0	0	0	0	1
	6	..	0	0	1	2
2	3	0	0	0	1	0
	6	..	0	0	2	2
3	6	..	0	1	2	4
	12	..	..	0	2	3
4	3	0	0	0	0	3
	6	..	0	2	1	1
	12	..	..	0	2	2
5	3	0	0	1	1	2
	6	..	0	1	2	2
	12	..	..	0	1	3

Tests 1-3, serial transfers; 4-5, continuous feeding on test plant to reach the time interval under test.

periods up to 144 hours were tested in serial transfers with the single insects. The results of 2 tests are in Table 8.

The data obtained in the tests with single insects or with groups of 5 indicated that the incubation period of the malva yellows virus in the green peach aphid was between 12 and 24 hours.

Table 8.—Results of Tests to Determine the Incubation Period of the Malva Yellows Virus in Individual Green Peach Aphids.

Test No.	Feeding Period on Virus Source in Hours	Number of Cheeseweed Seedlings Infected Out of 8 Inoculated with Single Insects in which the Virus had the Following Incubation Periods in Hours						
		12	24	48	72	96	120	144
1	6	0	1	0	1	2	2	0
	12	..	1	1	3	0	2	1
	24	..	..	1	0	0	1	2
	48	..	..	..	1	2	2	1
2	6	0	0	0	1	0	0	1
	12	..	0	3	4	5	5	5
	24	..	..	2	4	4	3	3
	48	..	..	..	6	5	4	4

Persistence of the Virus in the Vector in Serial Transfers

In a test carried out to study the persistence of the malva yellows virus in the green peach aphid, 20 insects bred on diseased cheeseweed plants were used singly in daily serial transfers on healthy cheeseweed seedlings. Out of 20 insects only 1 failed to transmit the virus. The results obtained with 12 of the aphids that survived longest are in Table 9. The insects were highly efficient in transmitting the virus during the first 10 transfers, but there was a definite loss of transmitting ability in the latest transfers of those insects surviving for 19 days or longer. The longest retention period observed was 18 days.

In another test the persistence of the virus was studied in individual green peach aphids that had fed on the same virus sources for different intervals of time. Four virus sources and 4 feeding periods, 6, 12, 24, and 48 hours, were tried. Two aphids from each time and source plant combination were transferred serially to a new cheeseweed seedling every other day until all aphids died. The results with 32 aphids are in Table 10. The aphids that had fed for only 6 hours on the virus sources gave poor transmission results but transmission was good when the aphids fed for 12 hours or longer, and there was little difference among them. Most of the aphids that acquired the virus remained viruliferous throughout their life. The apparent discrepancy between these results and those obtained on the daily transfers suggested the possibility that virus increase in the plant might occur in 48 hours and that the aphids in this experiment had thus been able to replenish themselves with virus. A series of tests was then carried out to verify this point.

In an early series of tests, healthy cheeseweed seedlings were inoculated with groups of viruliferous green peach aphids. These were removed after 12 or 24 hours and nonviruliferous aphids were then placed on the plants to pick up the virus; the aphids were tested at different intervals after they had started feeding. In one of the tests, the inoculation was carried out with viruliferous winged aphids, nonviruliferous nymphs being placed simultaneously on the plants and tested at intervals. The results of these tests showed that nonviruliferous aphids were able to acquire virus from plants within the 48-hour period after their inoculation, but not in 24 hours. These results could be interpreted as indicating that the virus increased in the plants within the 48-hour period and thus became available to the nonviruliferous insects, or that the amount of virus first introduced by the viruliferous insects had been large enough to permit the nonviruliferous vectors to pick it up.

Table 9.—Cheeseweed Seedlings Infected (+) and Non-infected (—) in Daily Serial Transfers with Single Peach Aphids Reared on a Malva Yellows Source.

Aphid No.	Successive Daily Transfers																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	+	+	+	+	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d
2	+	+	+	+	+	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d
3	—	+	+	+	—	+	+	—	+	+	+	+	—	—	+	—	—	—	—	—	—	—	—	—	—	d	
4	+	+	+	+	—	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	d	
5	+	—	+	+	+	+	—	+	—	+	+	—	+	+	—	+	+	—	—	—	—	—	—	—	d		
6	+	+	+	+	+	—	+	+	+	—	—	—	+	—	—	—	—	+	—	—	—	—	d				
7	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d				
8	+	+	+	+	+	—	—	+	—	+	—	+	—	—	—	—	—	+	—	—	—	—	d				
9	+	+	+	—	+	+	+	+	+	—	+	—	—	—	—	—	d										
10	+	+	+	+	—	+	—	d																			
11	+	+	+	+	+	+	—	d																			
12	+	+	+	+	+	+	d																				

d—Insect died.

Table 10.—Cheeseweed Seedlings Infected (+) and Non-infected (—) in Two-Day Serial Transfers with Single Green Peach Aphids Fed on Malva Yellows Sources for Different Periods of Time.

Feeding Period in Hours	Virus Source	Aphid Number	Successive Serial Transfers
6	A	1	— — + — — — — — — — — — — d
		2	— d
	B	3	— — — — — — — — d
		4	— — — — — — — d
	C	5	+ — — — d
		6	— — — — — — — — — — — — — — — d
	D	7	— — — + d
		8	— — — d
12	A	9	+ + + + + + d
		10	+ + — + + + + — — — — — + — — — — d
	B	11	— + + + + + + + — — — — + — d
		12	— — — d
	C	13	+ — + — + — — — — — — — — — — d
		14	— — — — + d
	D	15	+ + + + + + — + + + + — — + d
		16	— + + + + + + — d
24	A	17	+ — — — — d
		18	— — — — — — — — — — — — — — — d
	B	19	— — — — — — — — — — — — — — d
		20	— — — + — — — — — — — — — — — d
	C	21	+ + + — — — d
		22	+ + + + + + + + + + + — — — — — d
	D	23	— + — + + + — + d
		24	+ + + + + + + + + — + — + — — d
48	A	25	— — — + — — + + + — — — — d
		26	+ + — — — + — — — — — — — — — d
	B	27	+ — — — — + — — — — — — — — — — d
		28	+ + + + + + + + + + + — + — — — — d
	C	29	— + + — + + + + + + + — + — + + d
		30	+ + + + + + + + + + + + + + — — d
	D	31	+ + — + + d
		32	+ + + + + + + + + + + + — — — + d

d—Insect died

In a further series of tests, inoculations were effected with a single viruliferous aphid and recovery of virus was attempted with groups of nonviruliferous insects. Testing of the aphids used for recovery was made separately for those fed on the inoculated leaves and on the upper leaves of the same plants.

Table 11.—Recovery of the Malva Yellows Virus from Inoculated Plants at Short Intervals After Inoculation.

Test No.	Number of Plants Infected Out of 8 that were Inoculated with 20 Green Peach Aphids Per Plant, Fed on Source Plants Previously Inoculated with a Single Viruliferous Aphid the Indicated Number of Days Before					
	2		4		6	
	Aphids Fed on Inocul. Leaves	Aphids Fed on Other Leaves	Aphids Fed on Inocul. Leaves	Aphids Fed on Other Leaves	Aphids Fed on Inocul. Leaves	Aphids Fed on Other Leaves
1	0	0	6	4	8	6
2	0	0	8	8	8	8
3	1	0	7	7	8	7
4	3	0	6	5	8	8

The results of these tests are in Table 11. They show that even when the source plant was inoculated with a single aphid, groups of nonviruliferous vectors could acquire virus from it within 48 hours after inoculation and be able to infect healthy plants on which they were subsequently fed. Only the aphids fed on the inoculated leaves were able to pick up virus within the 48-hour period after inoculation.

Persistence of the Virus in Vectors on an Immune Host Plant

Tests were carried out in which viruliferous insects were transferred to immune tomato (*Lycopersicon esculentum* Mill.) plants and then tested at intervals. Aphids were removed daily from the tomato plants and placed at the rate of 2 insects per plant on 4 cheeseweed seedlings. The results of these tests showed that the virus was retained in the vector up to 7 days, the longest period which the insects survived on tomato. After the tests were terminated, the tomato plants were tested for virus and found to contain none.

Transmission of Malva Yellows and Sugar Beet Yellows Viruses by the Same Insects

The green peach aphid is an efficient vector of the malva yellows virus, a typically persistent virus. The same insect is also an efficient vector of the beet yellows virus, which is considered semi-persistent (11). The possibility that the presence of one of these viruses might interfere with transmission of the other by the same insect seemed worth investigating.

Twenty-four green peach aphids that had been reared on diseased cheeseweed plants were fed for 24 hours on a sugar beet plant infected by beet yellows. After this feeding period the insects were transferred singly to sugar beet seedlings, allowed to feed for 24 hours, and then transferred, also singly, to healthy cheeseweed seedlings.

Out of the 24 insects that were tested, 15 induced beet yellows on the sugar beet seedlings on which they fed; all 24 insects transmitted malva yellows virus to the cheeseweed plants. Transmission of beet yellows virus by individual green peach aphids not carrying malva yellows virus was not attempted in this experiment. It is not possible, therefore, to judge whether transmission of the beet yellows virus was better or worse with insects carrying the malva yellows virus.

Since the beet yellows virus is retained in the vector for only a short period (2, 11, 12, 13), the reversed order of feeding was not attempted.

Name and Description of the Virus

The malva yellows virus appears to be an undescribed virus. Its general characteristics agree with those of the genus *Corium* of the family *Marmoraceae* according to Holmes' system. The following name and description are proposed for the virus.

Corium malvae sp. nov.

Common name: malva yellows virus. An aphid-transmitted, persistent virus commonly found in species of *Malva* and other weeds and in crop plants. *M. parviflora* L. is an indicator plant, showing yellowing of lower and middle leaves. *M. rotundifolia* L. is a symptomless carrier. Other suscepts include *Spinacia oleracea* L., *Beta vulgaris* L., *Nicotiana clevelandii* Gray, *Senecio vulgaris* L., and other plants.

Myzus persicae (Sulz.), *M. ornatus* Laing., and *Aphis gossypii* Glov. are vectors. *M. persicae* is a highly efficient vector, single insects transmitting the virus to more than 50 percent of the plants. The vector may become infective after feeding on virus source for 1.5 hours and may infect healthy plants within an equal period. Incubation period of the virus in the insect is between 12 and 24 hours. Virus is retained by the vector for 18 days in daily serial transfers.

Transmitted by grafting, but not by mechanical inoculation.

Descriptive habitat: widespread on *Malva parviflora* in California.

Discussion

Malva yellows presents many points of similarity with other well known virus diseases of the yellows type, although it can be differentiated from them.

Beet yellows and malva yellows occur in the same areas in California. They are transmitted by the same vector and have some common host plants. Recovery tests have indicated that both viruses can coexist in the field in the same plant, such as in spinach, beet, and chickweed, as well as other weeds and crop plants.

Malva yellows can be distinguished from beet yellows on the basis of being incited by a typically persistent virus that can be retained in the vector for as long as 18 days. Beet yellows virus is semi-persistent (11), being retained in the vector for a much shorter period (2, 11, 12, 13). Also, common field strains of the beet virus cause more severe symptoms on spinach, but do not seem to infect cheeseweed.

Reports from England (14), Ireland (3), and Australia (9) have mentioned mild yellowing diseases of beets somewhat different from regular beet yellows. The viruses responsible for the Irish and Australian diseases failed to show serological relationship with beet yellows. Since species of Malva are common weeds in Europe (4) and Australia (6), it is possible that the malva yellows virus may also occur in these continents, and that its effects on beets and spinach have been confused with those of the beet yellows virus.

Several other yellows diseases have been reported from California. Filaree red leaf (1, 5) is also incited by a typically persistent virus. It differs from malva yellows virus in that it has a restricted host range and is not transmitted by *Myzus persicae*. Sugar beet yellow net (10) is easily differentiated from malva yellows by symptoms on a number of host plants and by being less efficiently transmitted by the green peach aphid.

Climatic and agricultural conditions of the Salinas Valley make this area an excellent breeding ground for aphids and it is consequently an area where aphid-transmitted viruses are of great importance. The writers have observed other types of yellows diseases apparently distinct from those discussed in this paper. These diseases would probably be lumped together with other known diseases for lack of a differential test.

Tests showed that nonviruliferous aphids could acquire the malva yellows virus when feeding on plants that had been inoculated with viruliferous insects only 48 hours earlier. However, within the 48-hour period, only aphids that fed on the same leaf on which the viruliferous aphids had been caged were able to acquire the virus; not those that fed on other leaves of the same plant. Within a 4-day period after inoculation, aphids that fed

on leaves at the growing point were also able to acquire virus. It is not possible, at present, to decide whether these results should be interpreted as evidence that virus increase occurred in the inoculated leaves within 48 hours or whether the nonviruliferous aphids picked up virus introduced by the single viruliferous aphid. Even if virus increase occurred within 48 hours after inoculation, the recovery results seem to indicate that this did not take place in 24 hours, and therefore it can be assumed that in the 24-hour serial transfers the aphids were not able to replenish themselves. The results obtained in the daily transfers are thus considered as indicative that the malva yellows virus can be retained in the vector for 18 days.

Malva yellows is one of the most persistent, aphid-transmitted viruses. Single insects reared on diseased plants are highly efficient in its transmission and practically 100 percent of the aphids are capable of becoming infective. These facts might suggest that *Myzus persicae* supports multiplication of the malva yellows virus, as in the case of some leafhoppers and the viruses they transmit. However, results from single-insect transfers on healthy seedlings have shown that practically all the insects that lived long enough lost their infectivity after a number of transfers. This suggests that there was a depletion of virus in the insect's body when it did not have access to the virus source. It could also be reasoned that virus multiplication could occur only during the early life of the insect and that increase of virus in the vector is followed by a recovery stage in which virus concentration would be reduced or practically disappear. It is felt that the malva yellows virus and its vector provide excellent material to verify some of these assumptions as well as for other studies pertaining to the relationship between the virus and its aphid vector.

Summary

Malva yellows is a newly described virus disease found in California on *Malva parviflora*, spinach, and other plants. Its incidence among susceptible Malva weeds is high at all times of the year. The disease is considered one of the components of the spinach yellowing complex in the Salinas Valley, and is also likely to be a factor in yellowing diseases of other plants.

Symptoms of malva yellows in *Malva parviflora* consist of marked yellowing of the lower and middle leaves. In early stages, yellowing may be limited by the main veins, but late yellowing symptoms affect most of the leaf area, except for narrow bands along the larger veins. Recovery from symptoms is rather common.

Malva yellows is incited by a persistent, aphid-transmitted virus, described under the name *Corium malvae*. *Myzus persicae, M. ornatus,* and *Aphis gossypii* are vectors. The disease was transmitted by grafting but not by mechanical inoculation.

Myzus persicae is an efficient vector, single viruliferous insects transmitting the virus to more than 50 percent of the plants on which they feed and commonly to all of them. The insect may become infective after feeding on the virus source for 1.5 hours; its effectiveness increases with increase in feeding time. Viruliferous green peach aphids can infect healthy cheeseweed in a 1.5-hour feeding period. The incubation period of the virus in the vector is between 12 and 24 hours. Insects bred on diseased plants retained the virus up to 18 days when transferred daily to new plants, but there was a definite loss in efficiency when the insects did not have access to a virus source. Single aphids proved capable of carrying simultaneously the malva yellows and beet yellows viruses.

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