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PERENNIAL-DELPHINIUM RINGSPOT

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PERENNIAL-DELPHINIUM RINGSPOT[1]

HENRY H. P. SEVERIN[2] AND R. C. DICKSON[3]

INTRODUCTION

Among the virus diseases encountered in the course of the investigation of celery calico on perennial delphiniums (*5*)[4] was one which caused ringspot of perennial delphinium; this was found on unknown varieties or hybrids at Berkeley, Hillsborough, and Montara, California, the symptoms being identical in the three places. The symptoms of celery calico on delphinium are confined to the basal or intermediate leaves, whereas ringspots occur on all of the leaves.

Work was undertaken on the host range, properties of the virus, and determination of the vectors. The symptoms of the disease were compared with those of ringspot previously reported on delphiniums.

Valleau (*7*) found a virosis of delphinium in Kentucky closely resembling ringspot of tobacco. He later (*8*) reported a delphinium virus causing a "coarse etch" when transferred to tobacco and suggested seed transmission. In another paper (*9*), he described the symptoms in more detail and gave delphinium, tobacco, tomato, and cucumber as host plants of the virus. The symptoms on delphinium consist of chlorotic ring patterns on individual lobes of the affected leaf, sometimes extending into every lobe. He also stated that this disease occurred naturally in tobacco both in Kentucky and in Minnesota and that "this virus corresponds most closely to the typical cucumber viruses."

Johnson (*3*) found patterns on delphinium similar to the large, yellowish, concentric patterns on dark tobacco caused by a tobacco-ringspot virus identical with that described by Fromme, Wingard, and Priode (*2*).

Burnett (*1*) described a virus disease causing dark-brown to black lesions on delphinium leaves. Tobacco plants inoculated from these showed an irregular mottle with ring-and-line patterns. Delphinium plants inoculated from these tobacco plants developed dark-brown ring-and-line patterns during the first season.

MATERIALS AND METHODS

Source of Virus.—The source of ringspot virus used in the work on host range was delphiniums collected at Montara and Berkeley and that of the virus used in the property studies was a single naturally infected

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

delphinium plant obtained in Berkeley. The virus was retained by repeated mechanical inoculation of susceptible host plants.

Virus Extract.—In preparing juice from infected plants, leaves were ground in a sterilized food chopper or in a mortar. The pulp was then placed in two layers of cheesecloth and the juice pressed out by hand.

Mechanical Inoculation.—The carborundum method of mechanical inoculation used was that described by Rawlins and Tompkins (*4*). Shortly after inoculation the carborundum and inoculum were washed from the leaves with water. The virus extract was usually inoculated into lots of 5 healthy plants. Inoculated plants were observed daily for symptoms. If symptoms failed to develop, tobacco plants were held for 21 days, cucumber plants for 30 days, and plants used in the host-range study for 30 to 60 days or longer, before they were discarded.

Recovery of Virus.—Whether or not symptoms appeared, an attempt was made to recover and transfer the virus from each plant or group of 5 plants used in the host-range studies, to lots of 5 healthy Turkish tobacco or White Spine cucumber plants. The inoculum was taken from young leaves and in some cases from the inoculated leaves also.

Noninfective Insects.—Most of the insects tested to determine the vector of the ringspot virus were from virus-free colonies maintained on caged celery plants in the greenhouse, although a few species of insects were collected in the field. Most of the aphids tested for virus transmission were confined in leaf cages clipped to the leaves of diseased plants and after a feeding period of about 18 hours were shaken from the cages and transferred to healthy seedlings by means of a moist camel's-hair brush. Leafhoppers were captured by means of a 10-cc pipette and were fed on diseased and healthy plants either in leaf cages or in large cages enclosing the plant. Mites were transferred from diseased to healthy plants with a moist camel's-hair brush.

HOST RANGE

The natural host range of delphinium ringspot so far determined is limited to perennial delphiniums. The host range of delphinium ringspot as determined by experimental infection by mechanical inoculation includes 11 species, in 8 genera, in 5 families. Some of the infected host plants developed local lesions, in others the infection was systemic, and one symptomless carrier was demonstrated.

RANUNCULACEAE, CROWFOOT FAMILY

Perennial Delphinium.—The symptoms on the younger leaves of naturally infected perennial delphiniums are faint chlorotic rings, frequently irregular in shape, 1 to 5 mm in diameter, enclosing green (plate

1, *A*) or yellow centers with concentric chlorotic and green lines (plate 1, *A*). Small, irregular, chlorotic areas are scattered between the rings (plate 1, *A*). The mature leaves show irregular chlorotic rings frequently 10 mm in diameter enclosing green areas, yellow bands 1 to 2 mm in diameter, and irregular chlorotic areas 1 to 4 mm wide (plate 1, *B*). Numerous small, concentric, chlorotic rings occur near the margin and in the serrations of the mature leaves (plate 1, *B*). The older leaves show large circular or irregular chlorotic areas surrounded by green and yellow lines (plate 1, *C, D*). Numerous faint chlorotic rings enclosing green centers or masses of small, yellow, circular areas cover more than half of the leaf surface (plate 1, *C*). The first symptoms on the inoculated leaves of Blackmore and Langdon delphinium are pale-green areas, which soon develop into zonate, pale-yellow ringspots 3 to 10 mm in diameter (plate 1, *E*). The first symptoms appeared 32 to 42 days after inoculation.

The virus was easily recovered from naturally infected delphiniums and transmitted to other susceptible hosts by mechanical inoculation.

Turban and Persian Buttercups.—Turban and Persian buttercups (*Ranunculus asiaticus*) were symptomless carriers of the delphinium-ringspot virus. Systemic infection occurred and the virus was easily recovered.

CHENOPODIACEAE, GOOSEFOOT OR SALTBUSH FAMILY

Sugar Beet.—Dark-brown necrotic rings, 0.5 to 1.0 mm in diameter, surrounded by semichlorotic areas 2.0 to 3.0 mm in diameter, sometimes encircled by a broken necrotic ring (plate 2, *A*) developed on the inoculated leaves of sugar beet (*Beta vulgaris*) 21 to 35 days after inoculation. Attempts to recover the virus from beets by juice inoculation to Turkish tobacco and White Spine cucumber plants were unsuccessful.

MALVACEAE, MALLOW FAMILY

Acala Cotton.—No symptoms of the disease developed on the inoculated leaves of Acala cotton (*Gossypium hirsutum*), but dark-brown, irregular, necrotic lesions, 5 mm or less in diameter, sometimes with pale centers, appeared on the young leaves 10 to 12 days after inoculation.

The virus was recovered from this host and transmitted to tobacco and cucumber plants.

SOLANACEAE, NIGHTSHADE FAMILY

Tobacco.—The symptoms of delphinium ringspot on Turkish tobacco (*Nicotiana Tabacum*) consist of zonate, necrotic lesions. The first symptoms to appear on the inoculated leaves 3 to 8 days after inoculation are

brown, shining, sunken, necrotic spots, circular or sometimes irregular in shape and 0.3 to 1.0 mm in diameter (plate 3, *A*). The lesions slowly enlarge during the next day or two (plate 3, *B*), and some of them become surrounded by pale-green halos. Narrow, broken, necrotic rings 3 to 4 mm in diameter appear around each spot 6 to 11 days after inoculation. These broken rings become entire within 1 day and form a green ring about 1 mm wide and the included tissue becomes necrotic (plate 3, *B*). The rings enlarge and become necrotic (plate 3, *C*). When the original spots are less than 2 mm apart, the rings coalesce (plate 3, *D*). A few necrotic rings about 3 mm in diameter may appear in previously normal areas of the leaf. The area surrounded by each ring becomes necrotic within 2 days and may enlarge to become slightly irregular in outline and have a diameter of 6 mm.

The recovery of the virus from inoculated Turkish tobacco plants was possible only while the lesions were still developing. Lesions and small rings of tissue surrounding them were cut from Turkish tobacco leaves 5 days after inoculation; the juice was extracted and inoculated into healthy Turkish tobacco. A few infections were obtained in this manner. The lesions were fully developed 10 days after inoculation, and all attempts to recover the virus from them or from other parts of the plant were unsuccessful. Repeated attempts to recover the virus from inoculated Turkish tobacco plants during a period of 5½ months after their inoculation were failures.

It appears that the virus spreads but a short distance from the point of entrance in this plant, and is rendered inactive by the dying and drying of the tissue that it has invaded.

The symptoms of the disease on White Burley tobacco (*Nicotiana Tabacum*) are similar to those described on Turkish tobacco, and the incubation period is the same, but the necrotic rings are somewhat larger. Attempts to recover the virus from this host plant were unsuccessful.

Nicotiana Glutinosa.—In the early stage of the disease, faint, chlorotic rings enclosing green areas (plate 3, *E*) appear on the inoculated leaves of *Nicotiana glutinosa;* later the peripheries of the rings become necrotic (plate 3, *E*). In the late stage of the disease, concentric rings appeared, followed by complete necrosis within the rings (plate 3, *F*). The lesions become dry and brittle and sometimes are broken and fall out. The incubation period of the disease varied from 15 to 18 days. No systemic infection occurred, and attempts to recover the virus were unsuccessful.

Jasmine Tobacco.—Symptoms on the inoculated leaves of jasmine tobacco (*Nicotiana alata* var. *grandiflora*) are similar to those described for Turkish tobacco. In addition, many of the inoculated leaves develop

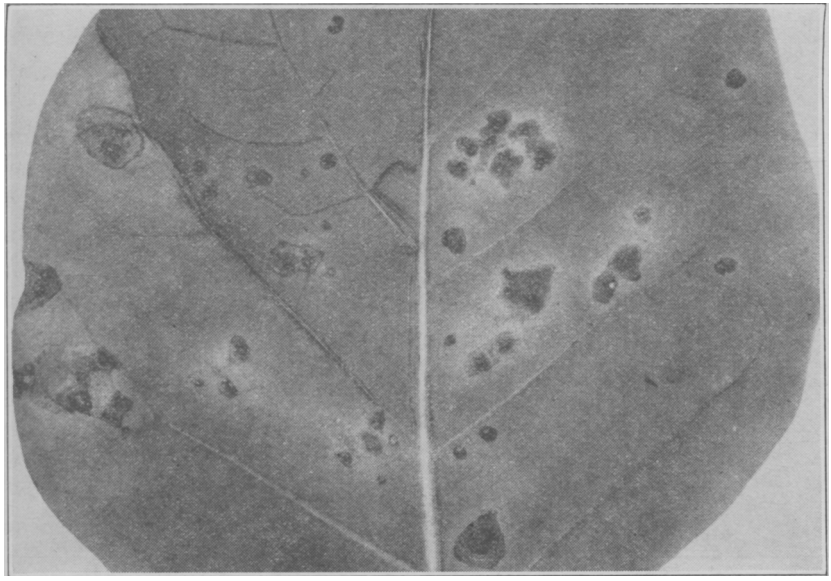

Fig. 1.—*Nicotiana alata* var. *grandiflora:* leaf 40 days after inoculation with delphinium-ringspot virus, showing groups of dark-brown, necrotic lesions and large zonate ringspots.

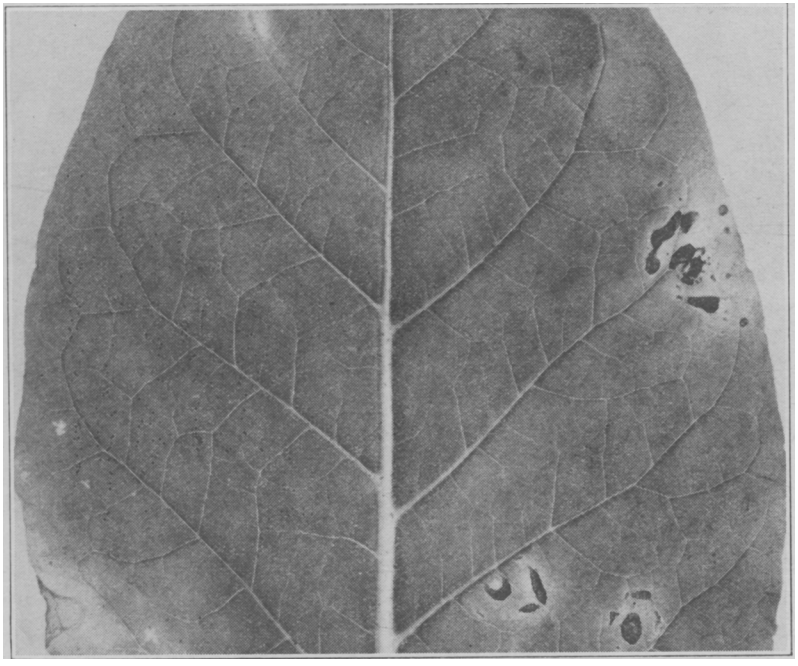

Fig. 2.—*Nicotiana alata* var. *grandiflora:* leaf which developed after inoculation, showing necrotic ringspots surrounded by broken, necrotic rings 40 days after the plant was inoculated with delphinium-ringspot virus.

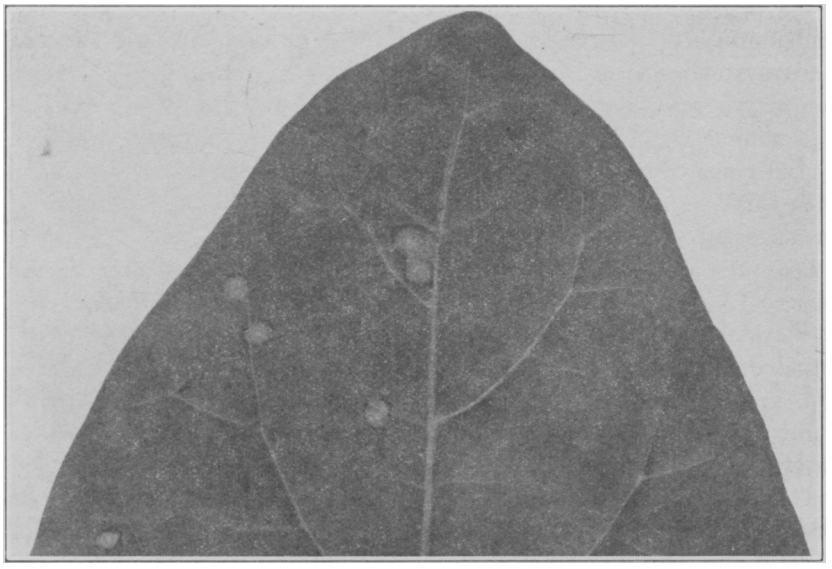

Fig. 3.—Peasant's tobacco (*Nicotiana rustica* var. *humulis*): leaf 9 days after inoculation with delphinium-ringspot virus, showing necrotic rings surrounding necrotic areas.

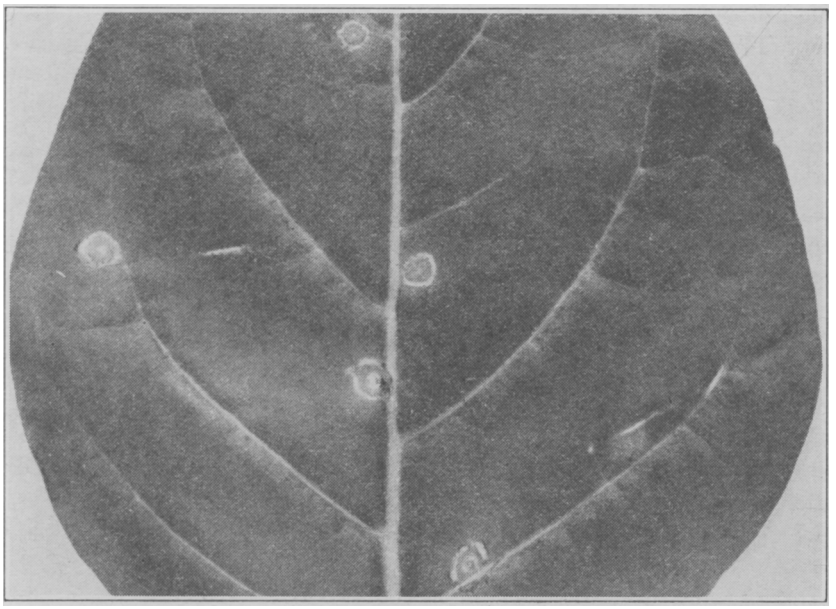

Fig. 4.—Peasant's tobacco (*Nicotiana rustica* var. *humulis*): leaf 11 days after inoculation with delphinium-ringspot virus, showing small target pattern with necrotic center surrounded by alternating green and necrotic rings.

semichlorotic areas surrounding the necrotic lesions. The infection becomes systemic in this host plant, and leaves appearing after inoculation may develop dark-brown, zonate, necrotic lesions (fig. 1) 30 to 60 days after the plant is inoculated. These spots may be surrounded by partial rings of necrotic tissue (fig. 2). Attempts to recover the virus from this host and transmit it to Turkish tobacco and cucumber were unsuccessful.

Peasant's Tobacco.—The early symptoms of the disease on peasant's tobacco (*Nicotiana rustica* var. *humilis*) are similar to those described on Turkish tobacco. Each necrotic lesion or minute ring becomes surrounded by a necrotic ring which also encloses a ring of green tissue (fig. 3). The ringspot may show a small, target pattern with necrotic center surrounded by alternating green and necrotic rings (fig. 4). Later, the entire area within each ring becomes necrotic.

The incubation period of the disease is 5 to 7 days. No recovery was made.

Petunia.—The early symptoms of ringspot on the leaves of Crimson King petunia (*Petunia hybrida*) 5 to 8 days after inoculation are dark-brown, necrotic lesions 1 mm or less in diameter. These enlarge to attain a diameter of 10 mm 2 weeks after inoculation (plate 4, *A, B, C*). Ten to 14 days after inoculation, necrotic lesions (plate 4, *D*), or necrotic spots (plate 4, *E*) or streaks, sometimes along the veins and midrib (plate 4, *F, G*), or rings appear on the young leaves. These symptoms increase in severity so that 30 days after inoculation the leaves are marked by small necrotic lesions or rings (plate 4, *H*), or necrotic concentric rings (plate 4, *I*), or chlorotic rings enclosing brown, dead tissue, or concentric rings (plate 4, *J*), or irregular masses or bands of necrotic tissue (plate 4, *K, L*).

The virus was easily recovered from infected petunia plants and transferred to healthy Turkish tobacco and cucumber plants.

Jimsonweed.—The symptoms on jimsonweed (*Datura Stramonium*) are necrotic, local lesions (plate 2, *B*) similar to those described on Turkish tobacco, but slightly smaller. The development sequence and incubation period are the same. Inoculated leaves which develop many lesions may absciss prematurely. No systemic infection occurs.

CUCURBITACEAE, GOURD FAMILY

Cucumber.—In White Spine cucumber (*Cucumis sativus*), the first symptoms of ringspot which develop on the inoculated cotyledons and true leaves of seedlings, 5 to 10 days after inoculation, are pale-green, circular areas with indistinct margins and each with a white pin-point center, probably marking the point of entrance of the virus. The circu-

TABLE 1

PLANTS UNSUSCEPTIBLE TO DELPHINIUM-RINGSPOT VIRUS

Family and common name	Scientific name	Plants inoculated
		number
Chenopodiaceae, goosefoot or saltbush family:		
Virginia Savoy spinach	*Spinacia oleracea* L.	5
Ranunculaceae, buttercup family:		
Annual larkspur	*Delphinium Ajacis* L.	5
Cardinal larkspur	*Delphinium cardinale* Hook	5
Love-in-a-mist	*Nigella damascena* L.	5
Summer Adonis	*Adonis aestivalis* L.	5
California buttercup	*Ranunculus californicus* Benth.	15
Poppy anemone	*Anemone coronaria* L.	5
Cruciferae, mustard family:		
February cauliflower	*Brassica oleracea* L. var. *botrytis* L.	5
Annual stock	*Mathiola incana* R. Br. var. *annua* Voss	3
Violaceae, violet family:		
King of the Black pansy	*Viola tricolor* L. var. *hortensis* DC.	5
Tropaeolaceae, Tropaeolum family:		
Golden Gleam nasturtium	*Tropaeolum majus* L.	10
Leguminosae, pea family:		
Brabham cowpea	*Vigna sinensis* Endl.	5
Blackeye cowpea	*Vigna sinensis* Endl.	15
Chilean alfalfa	*Medicago sativa* L.	5
Horse bean	*Vicia faba* L.	5
A. & M. Wonder garden pea	*Pisum sativum* L.	5
Lupine	*Lupinus polyphyllus* Lindl.	5
Cucurbitaceae, gourd family:		
Cantaloupe	*Cucumis Melo* L. var. *cantalupensis* Naudin	10
Sugar pumpkin	*Cucurbita Pepo* L.	5
Zucchini squash	*Cucurbita Pepo* L.	10
White Bush Scallop squash	*Cucurbita Pepo* L.	5
Umbelliferae, parsley family:		
Golden Self-blanching celery	*Apium graveolens* L. var. *dulce* DC.	15
Blue laceflower	*Trachymene caerulea* R. Graham	5
Labiatae, mint family:		
Scarlet sage	*Salvia splendens* Ker	3
Solanaceae, nightshade family:		
Seedling potato	*Solanum tuberosum* L.	2
Black Beauty eggplant	*Solanum Melongena* L.	11
Marglobe tomato	*Lycopersicon esculentum* Mill.	58
California Wonder bell pepper	*Capsicum frutescens* L.	5
Scrophulariaceae, figwort family:		
Snapdragon	*Antirrhinum majus* L.	5
Compositae, sunflower family:		
Large Russian sunflower	*Helianthus annuus* L.	5
China aster	*Callistephus chinensis* Nees	7
Romaine lettuce	*Lactuca sativa* L. var. *longifolia* Lam.	8

lar areas become bright yellow in color and vary from 4 to 6 mm in diameter (plate 2, *C*). An indistinct chlorotic area may later extend from each of the circular spots to adjacent parts of the leaf, sometimes followed by necrosis. After the infection becomes systemic, 9 to 18 days after inoculation, numerous chlorotic rings enclosing green centers or

necrotic spots and chlorotic circular areas appear on the leaves (plate 2, *D*).

The interspaces between the veinlets are sometimes filled by small chlorotic areas (plate 2, *E*), and these may coalesce, especially along the margin of the leaf (plate 2, *F*). Frequently the leaves become chlorotic

TABLE 2

THERMAL INACTIVATION OF DELPHINIUM-RINGSPOT VIRUS

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated							
	Unheated control	45° C	50° C	55° C	60° C	65° C	70° C	75° C
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish tobacco:								
1	5	5	5	1	1	0	0	0
2	5	5	3	0	0	0	0	0
From cucumber into Turkish tobacco:								
3	5	5	3	2	0	0	0	0
4	5	5	5	0	0	0	0	0
5	5	3	1	0	0	0	0	0
6	5	5	5	0	0	0	0	0
7	5	5	1	0	0	0	0	0
8	2	2	4	0	0	0	0	0
From cucumber into cucumber:								
6	5	5	4	0	0	0	0	0
7	5	5	4	0	0	0	0	0
8	2	5	4	1	0	0	0	0
Total, all sources	49	50	39	4	1	0	0	0
Percentage	*89.1*	*90.9*	*70.9*	*7.3*	*1.8*	*0.0*	*0.0*	*0.0*

with green vein-banding (plate 2, *F*), or may become almost entirely chlorotic; they are usually recurved. The plants become brittle and stunted and are often killed under greenhouse conditions.

This virus is easily recovered from cucumber plants and transmitted to Turkish tobacco and cucumber plants by juice inoculation.

PLANTS UNSUSCEPTIBLE

No infection was obtained by mechanical inoculation in 28 species of plants representing 26 genera in 12 families as shown in table 1. Attempts were made to recover the virus from each lot of 5 plants.

PROPERTIES OF VIRUS

Thermal Inactivation.—The thermal inactivation of the virus was determined with undiluted, extracted juices from the leaves of a naturally infected delphinium plant and also from the leaves and stems of

TABLE 3

TOLERANCE TO AGING IN VITRO OF DELPHINIUM-RINGSPOT VIRUS AT ROOM TEMPERATURE

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated after various periods of aging															
	Control	4 hours	8 hours	12 hours	18 hours	1 day	1½ days	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish tobacco:																
1	5	5	5	5	2	0	0	0	0	0	0	0	0	0	0	0
From cucumber into Turkish tobacco:																
2	5	5	5	3	4	4	5	5	3	1	0	0	0	0	0	0
3	5	5	5	5	2	4	2	4	2	0	0	0	0	0	0	0
4	5	5	5	5	3	4	4	0	0	0	0	0	0	0	0	0
5	4	3	4	1	1	3	1	0	0	0	0	0	0	0	0	0
6	4	2	4	1	4	3	2	0	0	0	0	0	0	0	0	0
7	5	5	5	5	5	2	0	0	0	0	0	0	0	0	0	0
From cucumber into cucumber:																
2	4	3	5	3	3	3	5	2	3	1	0	0	0	0	0	0
3	4	3	1	2	0	1	3	3	2	0	0	0	0	0	0	0
4	1	4	3	4	4	5	2	0	1	2	0	0	0	0	0	0
Total, all sources	42	40	42	34	28	29	24	14	11	4	0	0	0	0	0	0
Percentage	*84.0*	*80.0*	*84.0*	*68.0*	*56.0*	*58.0*	*48.0*	*28.0*	*22.0*	*8.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

TABLE 4

TOLERANCE TO DILUTION OF DELPHINIUM-RINGSPOT VIRUS

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated, after various dilutions									
	Undiluted control	1:10	1:100	1:500	1:1,000	1:2,000	1:5,000	1:10,000	1:50,000	1:100,000
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish Tobacco:										
1	5	5	5	5	2	0	0	0	0	0
2	5	5	5	3	1	0	0	0	0	0
3	5	5	3	2	0	0	0	0	0	0
4	4	5	3	2	0	0	0	0	0	0
From cucumber into Turkish tobacco:										
5	5	5	3	1	1	0	0	0	0	0
6	5	5	4	1	0	0	0	0	0	0
7	5	5	2	0	0	0	0	0	0	0
8	5	4	2	0	0	0	0	0	0	0
9	5	2	1	0	0	0	0	0	0	0
10	4	5	2	0	0	0	0	0	0	0
From cucumber into cucumber:										
5	5	5	3	0	0	0	0	0	0	0
6	5	5	4	1	0	0	0	0	0	0
10	4	5	0	2	0	0	0	0	0	0
Total	62	61	37	17	4	0	0	0	0	0
Percentage	*95.4*	*93.8*	*56.9*	*26.1*	*6.2*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

experimentally infected cucumber plants. Ten cc of juice from diseased plants was placed in each sterile, thin-walled, test tube by means of a pipette to avoid contamination of the lip of the tube. Each test tube containing the virus extract was immersed in the water bath at the desired temperature for 11 minutes, about 1 minute being required for the heat to penetrate the glass of the test tube. The bath was maintained within 0.5 degree of the desired temperature throughout each test. The water was kept in circulation by an agitator connected to an electric motor. After exposure to the desired temperature, the test tubes were cooled rapidly in running water. After cooling, the juice was poured into a small culture dish and the plants were inoculated without delay. Unheated controls were used in each test. The results obtained are indicated in table 2. The delphinium-ringspot virus was active after heating the expressed juice from delphinium and cucumber plants at 45°, 50°, and 55° C. A single infection was obtained after heating the virus extract from delphinium at 60°. The virus was inactivated by heating to 65°.

Tolerance to Aging in Vitro.—To determine the resistance of the ringspot virus to aging in vitro, test tubes each containing 10 cc of expressed juice from diseased delphinium or cucumber plants were plugged with cotton and kept in the dark at room temperature. Fresh-extract controls were used in each trial. The juice was tested for infectivity by mechanical inoculation after periods of 4, 8, 12, and 18 hours, 1 day, 1½ days, and then daily until 10 days had elapsed. The results are shown in table 3.

It is evident that the virus was active in the cucumber extract in vitro for various periods up to and including 4 days, but was inactivated after 5 days. One virus extraction from delphinium resulted in 2 infections after 18 hours' aging in vitro, but none after 1 day.

Tolerance to Dilution.—The tolerance to dilution of the ringspot virus was determined by diluting the expressed juice from diseased delphinium and cucumber plants with distilled water. The higher dilutions were inoculated first to minimize the danger of accidental infection. Undiluted controls were used in each test. Table 4 indicates the results obtained.

Infections were occasionally obtained at a dilution of 1:1,000 with the extract from delphinium and cucumber plants but not at 1:2,000 or at higher dilutions.

ATTEMPTS TO DETERMINE THE VECTOR

A limited number of insects and mites occur on delphinium under natural conditions in California. The mountain leafhopper (*Thamnotettix montanus* Van D.), the geminate leafhopper (*Thamnotettix gemi-*

TABLE 5

INSECTS AND MITE THAT FAILED TO TRANSMIT RINGSPOT VIRUS

Common and scientific name of insects and mite	Insect or mite			Plants used
	Average number on each plant	Period on diseased plant	Period on healthy plant	
From diseased to healthy delphinium				
	number		*days*	*number*
Aster leafhopper, *Macrosteles divisus* (Uhl.)	15	12 hours	3	10
Geminate leafhopper, *Thamnotettix geminatus* Van D.	24	5 days	24	10
Mountain leafhopper, *Thamnotettix montanus* Van D.	22	6 days	17	10
Celery leaf aphid, *Aphis apigraveolens* Essig	27	18 hours	2	10
Celery aphid, *Aphis apii* Theob.	33	18 hours	2	10
Cotton, or melon, aphid, *Aphis gossypii* Glover	23	18 hours	4	10
Erigeron root aphid, *Aphis middletonii* Thomas	29	18 hours	4	10
Cabbage aphid, *Brevicoryne brassicae* (Linn.)	30	18 hours	3	10
Yellow willow aphid, *Cavariella capreae* (Fabr.)	23	18 hours	2	10
Turnip, or false cabbage, aphid, *Lipaphis pseudobrassicae* (Davis)	29	18 hours	2	10
Onion aphid, *Micromyzus formosanus* Taka	31	18 hours	3	10
Lily aphid, *Myzus circumflexus* Buckton	28	18 hours	6	10
Foxglove aphid, *Myzus convolvuli* (Kalt.)	30	18 hours	8	10
Green peach aphid, *Myzus persicae* (Sulz.)	25	18 hours	7	25
Honeysuckle aphid, *Rhopalosiphum melliferum* (Hottes)	28	18 hours	3	10
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	29	18 hours	2	10
Two-spotted mite, *Tetranychus bimaculatus* Harvey	25	Reared	7	10
From diseased to healthy cucumber plants				
	number	*days*	*days*	*number*
Agallia californica (Baker)	7	2	5	1
Blue-green sharpshooter, *Cicadella circellata* (Baker)	12	2	5	2
Western potato leafhopper, *Empoasca abrupta* De L.	5	2	5	2
Cotton or melon aphid, *Aphis gossypii* Glover	23	2–5	2	20
Erigeron root aphid, *Aphis middletonii* Thomas	20	2	2	10
Cabbage aphid, *Brevicoryne brassicae* (Linn.)	15	4	2	1
Turnip, or false cabbage, aphid, *Lipaphis pseudobrassicae* (Davis)	15	4	2	5
Onion aphid, *Micromyzus formosanus* Taka	8	4	2	2
Lily aphid, *Myzus circumflexus* Buckton	24	2	2	22
Green peach aphid, *Myzus persicae* (Sulz.)	24	2	3	20
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	20	2	2	10
Two-spotted mite, *Tetranychus bimaculatus* Harvey	22	Reared	4	10
From diseased to healthy turban and Persian buttercup plants				
	number		*days*	*number*
Ornate aphid, *Myzus ornatus* Laing	17	Reared	10	15
Cyclamen mite, *Tarsonemus pallidus* Banks	10	Reared	30	10

natus Van D.) and two species of mites—the two-spotted mite (*Tetranychus bimaculatus* Harvey), and the cyclamen mite (*Tarsonemus pallidus* Banks)—were found breeding on delphinium. An occasional green peach aphid (*Myzus persicae* [Sulz.]) and undetermined dead winged aphids were found on this plant under natural conditions. The insects and the mite which failed to transmit the ringspot virus from diseased to healthy delphinium are listed in table 5.

Since the virus is easily transmitted to various host plants by mechanical inoculation, it was assumed that the vector is probably an aphid. Tests were made with colonies of various species of aphids maintained in the greenhouse. Insects which could not be reared on delphinium were fed on diseased plants for periods of from 12 to 18 hours and then were transferred to healthy delphinium seedlings.

Daily observations were made on the mortality of the insects, and after the last insect of a lot was dead (table 5), the seedlings were fumigated. The last living specimen of an average lot of 30 foxglove aphids (*Myzus convolvuli* [Kalt.]) survived on seedling delphinium for 8 days, green peach aphid, (*M. persicae* [Sulz.]) for 7 days, lily aphid (*M. circumflexus* Buckton) for 6 days, and all other species of aphids for 2 to 4 days, as shown in table 5.

No transmission of the virus was obtained with any of the various species of aphids tested.

Unsuccessful attempts were made to transmit the virus by means of leafhoppers, aphids, and mites from White Spine cucumbers infected with the ringspot virus to healthy cucumber plants. A list of insects and mites that failed to transmit the ringspot virus from diseased to healthy cucumber plants is given in table 5.

Transmission of the virus from diseased to healthy turban and Persian buttercups (*Ranunculus asiaticus*) was attempted, with aphids and mites but was unsuccessful. A list of the aphids and mites which failed to transmit the virus is shown in table 5.

DISCUSSION

Valleau (*9*) lists delphinium, Turkish tobacco, tomato, and cucumber as hosts of a virus found in delphinium in Kentucky. Tomato plants could not be infected with the California delphinium-ringspot virus. A total of 58 Marglobe tomato plants were inoculated, but no symptoms developed, and all attempts to recover the virus from these plants were failures.

The symptoms described by Valleau (*9*) on delphinium and Turkish tobacco as produced by the virosis on delphinium in Kentucky are not identical with the symptoms produced on the same host plants by the

ringspot virus in California. It is therefore evident that the two viruses are not identical.

The symptoms of tobacco ringspot on delphinium described by Johnson (*3*) differed from those produced on this host plant by the delphinium-ringspot virus in California. The symptoms of tobacco ringspot on delphinium and cucumber are described in another paper of this series (*6*).

The symptoms described by Burnett (*1*) on delphinium and tobacco also differed from those produced on these host plants by the delphinium-ringspot virus in California.

DESCRIPTION OF DELPHINIUM-RINGSPOT VIRUS

Name: Delphinium ringspot.

Host families: Ranunculaceae, Chenopodiaceae, Malvaceae, Solanaceae, and Cucurbitaceae.

Symptoms of disease: On young leaves of delphinium faint chlorotic rings enclosing green or yellow centers; on mature leaves, irregular chlorotic rings encircling green areas, yellow bands, and irregular chlorotic areas.

Incubation period of disease: 32 to 42 days in the greenhouse.

Property studies: Thermal inactivation 65° C in 10 minutes' exposure, tolerance to dilution 1:1,000, and resistance to aging in vitro 5 days.

Modes of transmission: Mechanical inoculation with expressed juice, in nature vector was not found.

SUMMARY

Perennial or garden delphinium was demonstrated to be naturally infected with an undescribed ringspot virus.

The host range of the ringspot virus as determined by mechanical inoculation includes 11 species of plants in 8 genera belonging to 5 families, as follows:

Ranunculaceae, crowfoot family:
- Blackmore and Langdon perennial delphinium (*Delphinium* sp.), systemic infection, virus recovered.
- Turban and Persian buttercups (*Ranunculus asiaticus*), symptomless carrier, virus recovered.

Chenopodiaceae, goosefoot or saltbush family:
- Sugar beet (*Beta vulgaris*), local infection, virus not recovered.

Malvaceae, mallow family:
- Acala cotton (*Gossypium hirsutum*), systemic infection, virus recovered.

Solanaceae, nightshade family:
- Turkish tobacco (*Nicotiana Tabacum*), local infection, virus recovered only while the lesions were developing.
- White Burley tobacco (*Nicotiana Tabacum*), local infection, virus not recovered.
- *Nicotiana glutinosa*, local infection, virus not recovered.
- *Nicotiana alata* var. *grandiflora*, systemic infection, virus not recovered.
- Peasant's tobacco (*N. rustica* var. *humulis*), local infection, virus not recovered.

Crimson King petunia (*Petunia hybrida*), systemic infection, virus recovered.
Jimsonweed (*Datura Stramonium*), local infection, virus not recovered.
Cucurbitaceae, gourd family:
White Spine cucumber (*Cucumis sativus*), systemic infection, virus recovered.

Twenty-eight species of plants in 26 genera in 12 families were inoculated with the ringspot virus but proved unsusceptible.

The thermal inactivation of the ringspot virus was 65° C in a 10-minute exposure. Inactivation of the virus occurred after the extracted juice from diseased cucumber plants was exposed to the air at room temperature for a period of 5 days. The tolerance to dilution of extracted juice from diseased delphinium and cucumber plants was 1:1,000.

All attempts to find a vector of the ringspot virus were failures.

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PLATES

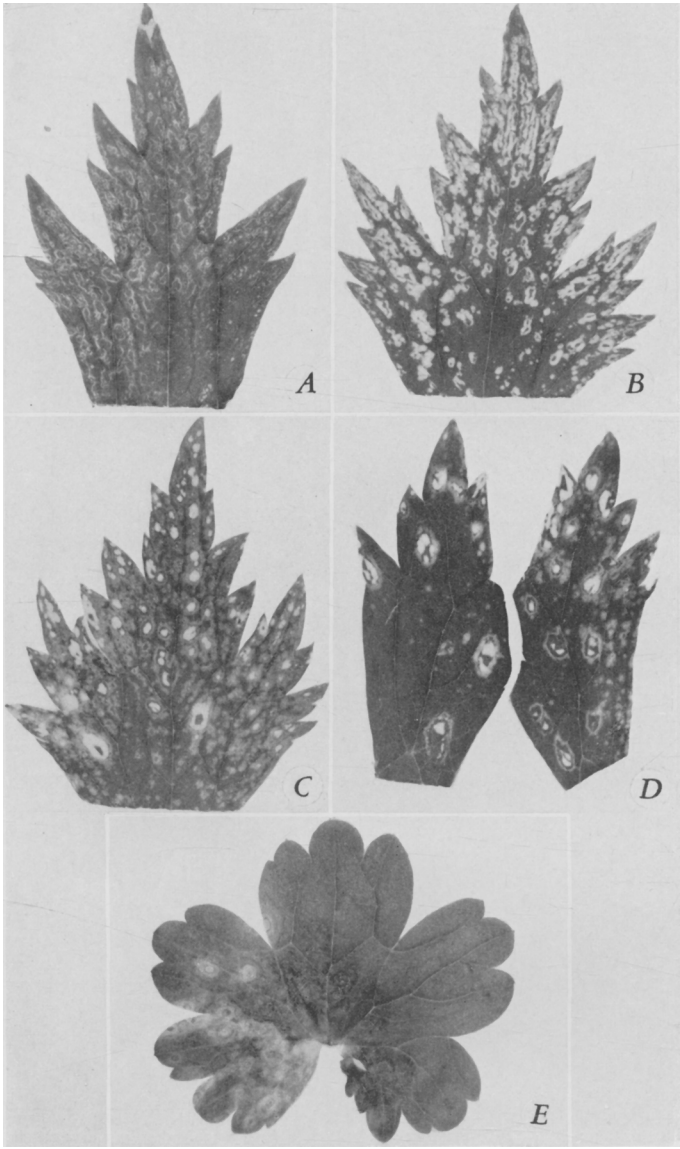

Plate 1.—Perennial delphinium naturally infected with ringspot: *A*, lobe of young leaf showing faint, chlorotic rings enclosing green or yellow centers, and small, irregular, chlorotic areas scattered between the rings; *B*, lobe of mature leaf from the same plant showing irregular, chlorotic rings enclosing green areas, yellow bands, irregular, chlorotic areas, and numerous, small, concentric rings near the margin and in the serrations; *C*, lobe of old leaf from the same plant showing large, circular or irregular, chlorotic areas, each surrounded by a green line, and sometimes with a green center, also masses of small yellow, circular areas, and faint chlorotic rings enclosing green centers; *D*, enlarged lobes of mature leaves from the same plant showing yellow rings enclosing chlorotic or green areas or both; *E*, inoculated leaf from Blackmore and Langdon delphinium showing pale, yellow ringspots with concentric lines.

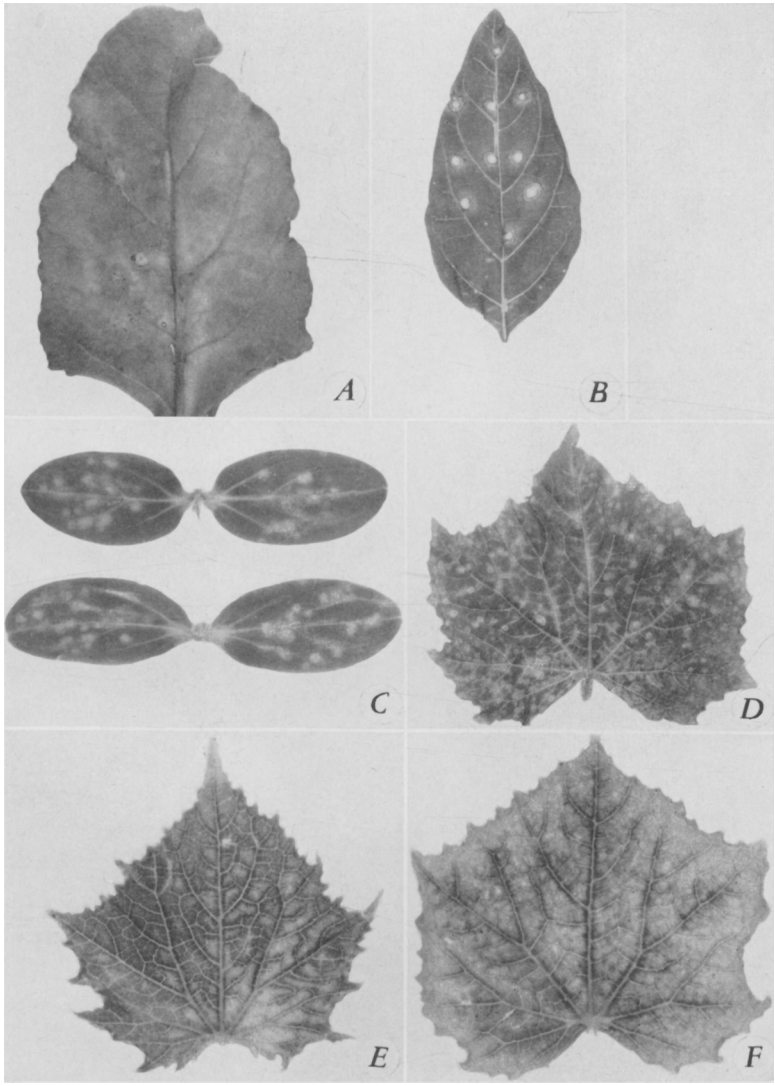

Plate 2.—Symptoms of delphinium ringspot on leaves of various host plants: *A*, leaf of sugar beet (*Beta vulgaris*) showing necrotic rings surrounded by chlorotic areas 24 days after inoculation; *B*, leaf of jimsonweed (*Datura Stramonium*) showing concentric rings 13 days after inoculation; *C*, cotyledons of White Spine cucumber (*Cucumis sativus*) showing circular, yellow areas, each with a white or necrotic pin point in the center, 11 days after inoculation; *D*, leaf of cucumber plant 26 days after inoculation, showing numerous, chlorotic rings enclosing green centers or necrotic spots; *E*, leaf of cucumber plant 23 days after inoculation, showing chlorotic areas in the interspaces of the veinlets; *F*, leaf of cucumber plant showing numerous chlorotic areas which frequently coalesce, and green vein-banding.

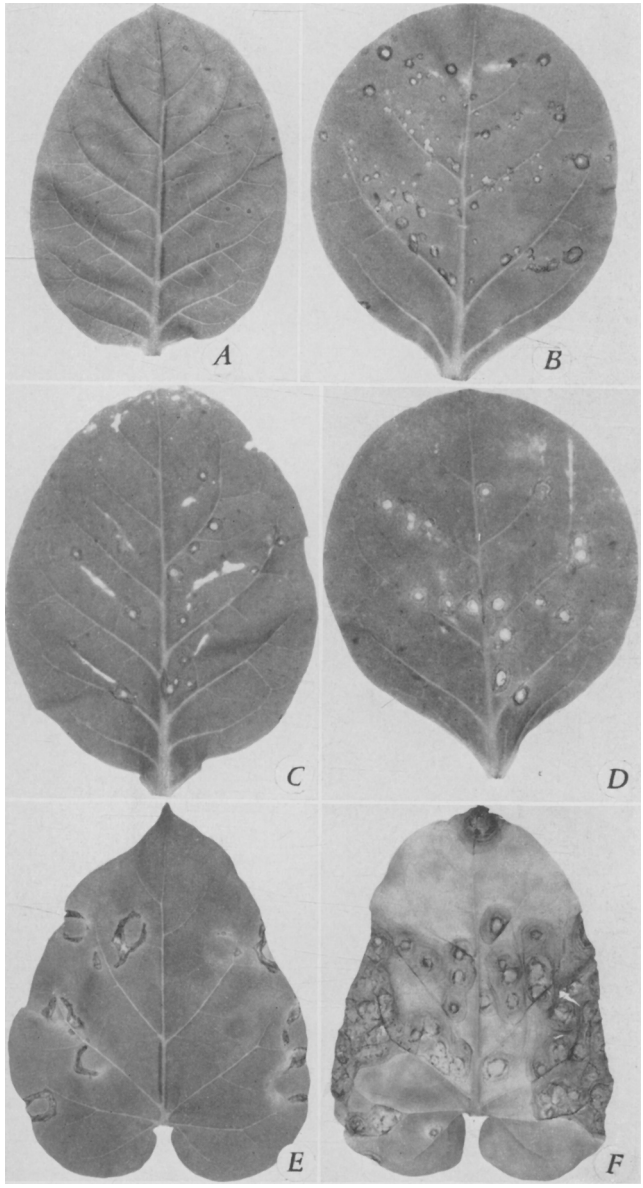

Plate 3.—Symptoms of delphinium ringspot on Turkish tobacco (*Nicotiana Tabacum*) and *N. glutinosa*: *A*, leaf showing small, necrotic lesions 4 days after inoculation; *B*, leaf 6 days after inoculation, showing necrotic areas enlarged and with centers bleached; *C*, leaf 9 days after inoculation, showing necrotic areas surrounded by wider rings—white areas are abrasions caused by inoculation with carborundum; *D*, leaf 10 days after inoculation, showing ringspots, some of which had coalesced; *E*, leaf of *N. glutinosa* showing early stage of ring formation with a faint, wide, chlorotic ring enclosing a green area; later, the periphery of the rings becomes necrotic; *F*, leaf of *N. glutinosa* 4 weeks after inoculation, showing concentric rings and complete necrosis within the rings.

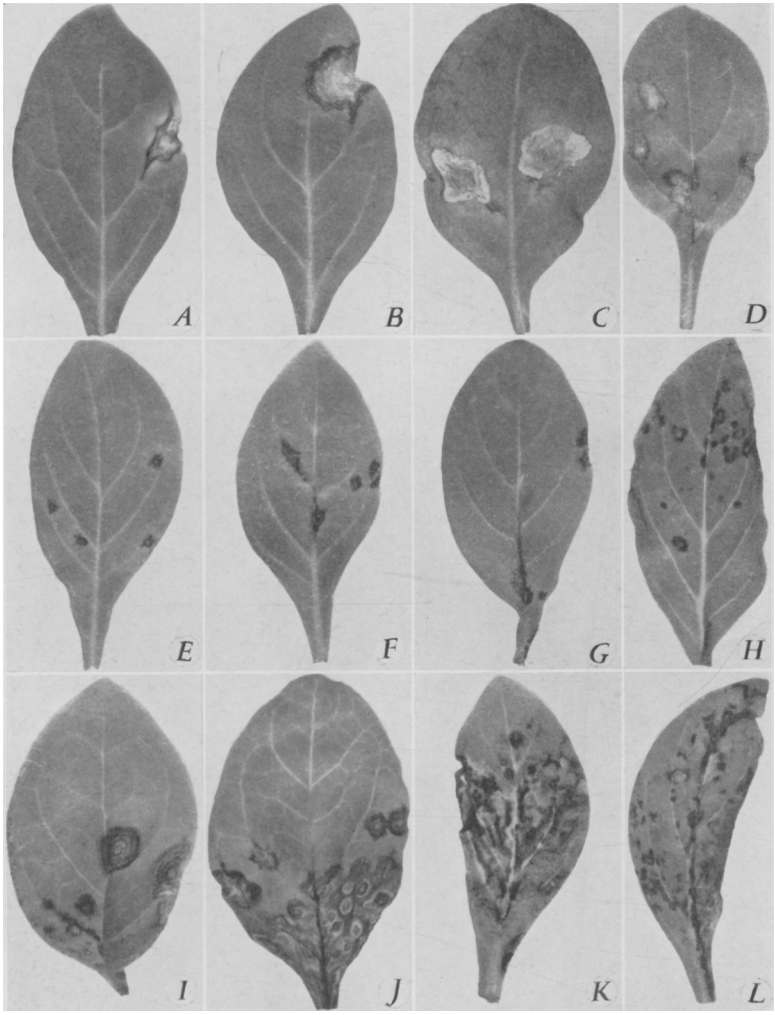

Plate 4.—Crimson King petunia (*Petunia hybrida*) infected with delphinium-ringspot virus: *A*, *B*, *C*, leaves showing large, necrotic lesions 14 days after inoculation; *D*, necrotic lesions on new leaf; *E*, *F*, *G*, necrotic spots or streaks, sometimes along the veins or midrib on young leaves; *H*, small, necrotic rings on leaves which developed 30 days after inoculation; *I*, necrotic, concentric rings; *J*, chlorotic rings enclosing brown, dead tissue; *K*, *L*, irregular masses or bands of necrotic tissue.