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POISON-HEMLOCK-RINGSPOT VIRUS AND ITS TRANSMISSION BY APHIDS TO CELERY[1]

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INTRODUCTION

DURING an attempt to find a weed reservoir of the western celery mosaic, another virus affecting celery was recovered from one of the weeds tested. Poison hemlock, *Conium maculatum L.*, a common umbelliferous weed, was often found naturally infected with a ringspot virus. The symptoms of ringspot on celery have been described briefly in a previous paper (Severin and Freitag, 1938).[4] Celery plants showing symptoms resembling ringspot were collected in celery fields on several occasions, but attempts to recover virus from these apparently naturally infected plants have failed. The ringspot virus occurs commonly on poison hemlock in the Santa Clara, San Benito, Salinas, and Sacramento valleys of California.

Experiments were undertaken during 1936 to determine the symptoms, host range, and insect vectors of the poison-hemlock-ringspot virus. Various phases of its transmission by aphids were studied, including the relative importance of the different species as vectors, transmission during short feeding periods, retention of the virus by aphids, loss and recovery of infectivity by aphids on celery, and ability of aphids to acquire virus from plants after infection. Experiments were conducted on mechanical transmission of the virus.

A number of virus diseases that produced ringspot symptoms have been described on the following host plants:

Tobacco: Fromme, Wingard, and Priode (1927); Henderson and Wingard (1931); J. Johnson (1936); E. M. Johnson (1930); Price (1936); Priode (1928); Valleau (1932); Wingard (1928)

Potato: Burnett and Jones (1931); J. Johnson (1925); J. H. Smith (1928); K. M. Smith (1929, 1931); Valleau and Johnson (1930)

Tomato: Bald and Samuel (1931); Gardner, Tompkins, and Whipple (1935); Samuel, Bald, and Pittman (1930); K. M. Smith (1932)

Delphinium: Burnett (1934); Valleau (1932)

Clover: Henderson (1934); E. M. Johnson (1933)

Rose: Nelson (1930); White (1930)

Sugar beet: Hoggan (1933)

Hyoscyamus niger L.: Hamilton (1932)

Cabbage: Tompkins, Gardner, and Thomas (1938)

Dahlia: Brierley (1933)

Plum and peach: Valleau (1932)

Peony: Whetzel (1915)

Johnson and Valleau (1935) reviewed the literature on virus diseases causing ringspot symptoms, but without mentioning any virus that causes chlorotic or necrotic ring and line patterns on celery.

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

Wingard (1928) could not transmit tobacco-ringspot virus to carrot and parsnip, the only two species of umbelliferous plants tested. However, he experimentally infected a total of 62 species of plants belonging to 38 genera in 17 families with the tobacco-ringspot virus.

Wellman (1934) transmitted tobacco-ringspot virus to celery and described the resultant patterns, consisting of small green and yellow spots, many with a small necrotic area. The plants became yellow; and the leaves were malformed, or "shoestring." When southern-celery-mosaic virus was transmitted to Turkish or Broadleaf tobacco, white spots were produced on the inoculated leaves, and, as the plants became older, chlorotic and necrotic zigzag lines and ring patterns developed.

Gardner, Tompkins, and Whipple (1935) demonstrated that celery is susceptible to tomato-spotted-wilt virus, which produces the ringspot symptoms on various host plants. Severin and Freitag (1938) included a description and figure of celery infected with tomato-spotted-wilt virus, but did not record the development of chlorotic rings.

Tompkins, Gardner, and Thomas (1938) noted in cabbage a virus disease that produces necrotic rings. They were unable to infect celery with the cabbage-black-ring virus.

METHODS AND MATERIALS

The source of the ringspot virus used in the experiments here reported was naturally infected poison hemlock collected at Alvarado, California. The virus was transmitted from these plants to healthy ones by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.) [*R. melliferum* (Hottes)].

The methods used in rearing noninfective aphids and transferring them from one plant to another resemble those reported earlier (Severin and Freitag, 1938).

The mechanical inoculations were performed by dusting leaves with carborundum and then drawing a cotton swab soaked with infective juice over the leaf according to the method of Rawlins and Tompkins (1936).

HOST RANGE

Natural Infection.—Poison hemlock, *Conium maculatum* L., a tall, branching biennial with finely cleft leaflets, is the only plant known to be infected with ringspot in nature. Extremely high populations of the honeysuckle aphid occur on this introduced weed, which is widely distributed throughout California. The umbels or flower clusters are often black with aphids. Honeysuckle aphids, taken from the umbels of naturally infected poison hemlock collected near Alvarado, were used to transmit the virus to healthy celery grown from seed. Lots of 25 to 100 aphids transferred from 15 naturally infected poison-hemlock plants infected 55 of 95 celery plants, or 57.9 per cent.

The virus was recovered from this experimentally infected celery by previously noninfective honeysuckle aphids and transmitted to healthy poison hemlock and celery grown from seed. Lots of 25 aphids were transferred from each of 12 experimentally infected celery plants. They transmitted the virus to 19 of 30 poison-hemlock plants, or 63.3 per cent; and to 29 of 60 celery plants, or 48.3 per cent.

Experimental Infection.—The following economic plants of the family Umbelliferae have been infected by means of the honeysuckle aphid:

Celery, *Apium graveolens* L. var. *dulce* DC.
Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.
Dill, *Anethum graveolens* L.
Salad chervil, *Anthriscus Cerefolium* Hoffm.
Coriander, *Coriandrum sativum* L.
Carrot, *Daucus Carota* L. var. *sativa* DC.
White varieties: Short White, White Belgian, White Mastodon
Yellow variety: Yellow Belgian
Orange varieties: Chantenay, Chantenay Red Cored, Danvers Half Long, Early Scarlet Horn, French Forcing, Imperator, Improved Long Orange, Nantes, and Oxheart
Hollow Crown and Long Smooth parsnip, *Pastinaca sativa* L.
Single, or Plain, parsley, *Petroselinum crispum* Nym. var. *latifolium*
Double Curled, Extra Triple Curled, and Fern Leaf parsley, *Petroselinum crispum* Nym.
Hamburg, or turnip-rooted, parsley, *Petroselinum hortense* Hoffm. var. *radicosum* Bailey

Recovery of Virus.—The virus, recovered from the experimentally infected plants by previously noninfective honeysuckle aphids, was transferred to celery. Attempts were also made to recover it from inoculated plants that failed to develop symptoms.

SYMPTOMS ON VARIOUS HOSTS

Since poison hemlock is naturally infected with this disease and celery is subject to several virus diseases that might be confused with this one, the symptoms on these two plants are described in detail. Those on other host plants are briefly summarized.

Poison Hemlock.—The first symptoms on the intermediate leaves of experimentally infected plants are usually irregular, scattered, small, pale-green areas, which may enlarge. Later some of the pale-green areas become chlorotic (plate 1, *B*) and may develop minute necrotic centers. Many irregular chlorotic areas of various sizes (plate 1, *C*) appear more commonly along the margin or apical region than near the basal portion of the leaves. Numerous chlorotic patterns are observed on the leaves, such as irregular lines or bands (plate 1, *D*) or zigzag lines resembling somewhat the oak-leaf pattern (plate 1, *E*). Often the chlorotic lines enclose green areas (plate 1, *F*), forming ringspots. The line patterns and chlorotic areas may develop simultaneously. The veins of the leaflets usually remain green, often banded with green tissue (plate 1, *C*), although the remaining leaf tissue may be chlorotic or white. The chlorotic areas often become buff-colored, and sometimes a purplish discoloration of the leaves develops. The two symptoms most useful in identifying this ringspot have been the chlorotic areas and the line patterns.

Under natural conditions the infected plants are not stunted, but often show a downward curling of the leaflets along the midrib. They can easily be detected by the mottling of the leaves and by line and ringspot patterns.

Celery.—The symptoms of the disease on celery develop on the older and intermediate leaves, but not on the younger ones. Experimentally infected celery does not appear dwarfed or stunted; it seems to be only mildly affected. The symptoms vary considerably.

Some ringspots are formed by pale-green lines or bands, which later become yellow rings or bands. Imperfect rings in the form of semicircles may occur along the margin of the leaflets (plate 2, *A*); or they may be circular, oval, or irregularly shaped (plate 2, *B*). The ringspots may be few, or they may occur abundantly on all the leaflets (plate 2, *C*).

There are at least four types of ringspot patterns, according to the arrangement of parts. These may be described as follows: yellow ring or band encircling green tissue (plate 2, *A, B*); green ring enclosing a chlorotic center (plate 2, *C*) concentric alternating yellow and green lines surrounding a green area (plate 2, *D;* plate 3, *A*), which is sometimes surrounded by a pale yellow halo (plate 2, *E*).

The chlorotic line patterns also vary. The lines may be broken, composed of dots and dashes, which sometimes surround green tissue (plate 2, *F*); or concentric, broken lines may alternate with parallel green lines of tissue enclosing green areas (plate 3, *A*); or irregular, yellow bands may encircle green areas (plate 3, *B*); or chlorotic tissue may run zigzag, resembling an oak-leaf pattern (plate 3, *C*). Line patterns and ringspots may appear on the same leaflets (plate 2, *D;* plate 3, *A*).

Sometimes on the leaves of celery plants in an advanced stage of the disease, small chlorotic areas are fused, forming large, irregular, yellow areas which surround green spots (plate 3, *D*). Chlorosis may continue (plate 3, *E*), spreading over the leaflets, with the green tissue still remaining (plate 3, *F*). The chlorotic patterns on the outer and intermediate leaves may fade from yellow to white, gradually disappearing as the maturing leaves become entirely chlorotic; and the plant may then show no further symptoms of disease. Previously noninfective aphid vectors, however, readily recover the virus from such symptomless plants and transfer it to healthy ones.

Large Smooth Prague Celeriac.—Numerous large chlorotic rings encircling green areas appear on the outer leaves of infected plants (plate 4, *A*).

Dill.—Although the virus was recovered from a single plant, no ringspots were observed under the binocular microscope on the finely dissected leaves.

Salad Chervil.—The first symptom on the outer leaves was circular or elliptical, chlorotic areas, which coalesced and formed bands. Some plants showed necrotic spots, commonly along the serrated margin of the leaflets, often followed by purpling, browning, and drying of the outer leaves.

Coriander.—Pale-yellow areas, circular or elliptical, developed along the margin of the leaflets, then became deep yellow, and frequently coalesced to form bands. The leaflet tips were often yellow.

Carrots.—On the oldest leaves of carrot varieties examined under the binocular microscope, the symptoms varied somewhat. Most of the varieties showed small, sunken, chlorotic areas. Sometimes a chlorotic ring occurred, with or without a green center surrounded by cleared veinlets; or solid, irregular, yellow areas were present in the depressions.

Hollow Crown Parsnip.—The outer leaves of infected plants showed circular, pale-green areas, usually with no outer chlorotic rings; the rings when present were not sharply defined.

Long Smooth Parsnip.—The oldest leaves of diseased plants developed circular, pale-green areas with no outer chlorotic rings.

Single or Plain Parsley.—The oldest leaves developed a striking pattern—ringspots, broken yellow lines, zigzag lines resembling an oak-leaf pattern, and green or chlorotic veinbanding (plate 4, *D, E*). Each ringspot was composed of an outer chlorotic ring and an inner green ring, enclosing a chlorotic center.

Curled Varieties of Parsley.—Scattered over the oldest leaves, as observed under the binocular microscope, were extremely small chlorotic circular or irregular areas that coalesced, forming irregular blotches.

Hamburg, or Turnip-Rooted, Parsley.—In the early stages of the disease, the outer leaves developed chlorotic rings enclosing green areas (plate 4, *B*); later they showed broken, chlorotic lines composed of dots, dashes, or streaks. Occasionally clusters of dashes or dots surrounded green areas, forming ringspots (plate 4, *C*). Sometimes chlorosis gradually spreads over the leaflets, leaving only the veins banded with green tissue (plate 4, *C*).

TABLE 1

TRANSMISSION OF VIRUS FROM EXPERIMENTALLY INFECTED POISON HEMLOCK TO HEALTHY CELERY BY HONEYSUCKLE APHID AND BY MECHANICAL INOCULATION TO POISON HEMLOCK AND CELERY

Infected poison hemlock no.	Aphid transmission: celery plants infected, of 5 inoculated	Mechanical transmission	
		Poison hemlock infected, of 5 inoculated	Celery plants infected, of 5 inoculated
1	5	0	0
2	5	0	0
3	4	0	0
4	5	0	0
5	5	0	0
6	5	0	0
Totals	29	0	0
Percentage	*96.7*	*0.0*	*0.0*

SYSTEMIC NATURE OF THE VIRUS

Because the symptoms of ringspot on celery appear only on the outer and intermediate leaves, an experiment was undertaken to determine whether the virus was systemic. All leaves showing symptoms were removed from 10 celery plants, and then previously noninfective honeysuckle aphids were fed on these plants for 1 to 7 days. Lots of 25 aphids were then transferred from each infected plant to each of 5 healthy celery plants. The aphids recovered the virus from the symptomless leaves of the 10 plants and transmitted it to 33 of 50 celery plants, a fact indicating that the virus is systemic. After removal of the aphids, 9 of the 10 plants subsequently developed symptoms again on the outer and intermediate leaves.

INOCULATION EXPERIMENTS

Extract from Diseased Poison Hemlock.—An attempt was made to transmit the virus by mechanical inoculation of extracted sap from experimentally

infected poison hemlock to healthy poison hemlock and celery. Transmission by aphids served to prove the presence of the virus. Table 1 gives the results. Mechanical inoculation of 30 poison-hemlock and 30 celery plants with the juice from 6 experimentally infected specimens of poison hemlock had no effect; yet aphids acquired the virus from each one of the 6 sources of inoculum

TABLE 2

TRANSMISSION OF VIRUS FROM EXPERIMENTALLY INFECTED SINGLE, OR PLAIN, PARSLEY TO PARSLEY BY MECHANICAL INOCULATION AND BY THE HONEYSUCKLE APHID TO CELERY PLANTS

Single or plain parsley no.	Mechanical inoculation: parsley plants infected, of 5 inoculated	Aphid transmission: celery plants infected, of 5 inoculated
1	3	3
2	2	3
3	3	2
4	2	1
5	3	2
6	1	1
7	4	0
8	4	0
9	5	0
10	3	0
11	2	0
12	2	0
13	1	0
14	0	4
15	0	4
16	0	4
17	0	2
18	0	4
19	0	3
20	0	4
21	0	2
22	0	2
23	0	1
24	0	1
25	0	3
26	0	1
27	0	1
28	0	1
Total	35	49
Percentage	*25.0*	*35.0*

and transmitted it to 29 of 30 celery plants, or 96.7 per cent. Thus, although the virus from poison hemlock was recovered readily by aphids, it could not then be transmitted to poison hemlock and celery by the carborundum method of mechanical inoculation.

Extract from Diseased Celery.—The ringspot virus could not be transmitted by mechanical inoculation of sap from infected to healthy celery. As tables 3 and 5 show, 500 healthy celery plants were inoculated mechanically with juice from 100 infected plants without the production of a single diseased

plant. Aphid transmission proved that the virus was present in 72 per cent of the celery plants used as a source.

Extract from Diseased Parsley.—Although poison hemlock and celery plants could not be infected with the ringspot-virus extract by mechanical inoculations, nevertheless, the extract from experimentally infected Single, or Plain, parsley inoculated in healthy parsley produced infection in a low percentage of trials. According to table 2, previously noninfective honeysuckle aphids recovered the virus from 21 of 28 infected parsley plants and infected

TABLE 3

TRANSMISSION OF VIRUS BY ELEVEN SPECIES OF APHIDS AND ATTEMPT TO TRANSMIT THE VIRUS FROM EXPERIMENTALLY INFECTED TO HEALTHY CELERY PLANTS BY MECHANICAL INOCULATION

Aphid	Aphid transmission, celery plants			Mechanical inoculation, celery plants		
	Inoculated	Infected	Per cent infected	Inoculated	Infected	Per cent infected
Celery leaf aphid, *Aphis apigraveolens* Essig	25	14	56.0	25	0	0.0
Celery aphid, *Aphis apii* Theo	25	9	36.0	25	0	0.0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	25	6	24.0	25	0	0.0
Cotton or melon aphid, *Aphis gossypii* Glover	25	1	4.0	25	0	0.0
Erigeron root aphid, *Aphis middletonii* Thos.	25	4	16.0	25	0	0.0
Bean or dock aphid, *Aphis rumicis* Linn	25	1	4.0	25	0	0.0
Yellow willow aphid, *Cavariella capreae* (Fab.)	25	7	28.0	25	0	0.0
Lily aphid, *Myzus circumflexus* (Buck.)	25	14	56.0	25	0	0.0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	25	3	12.0	25	0	0.0
Green peach aphid, *Myzus persicae* (Sulzer)	25	5	20.0	25	0	0.0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	25	18	72.0	25	0	0.0
Total	275	82	....	275	0	....

49 of 140 celery plants, or 35 per cent. The virus was recovered from 13 of 28 infected parsley plants and mechanically transmitted by sap inoculation to 35 of 140 parsley plants, or 25 per cent. Virus was successfully recovered by both aphid and mechanical transmission from only 6 of 28 infected parsley plants. Attempts to use parsley as a source of virus and as a test plant to determine the physical properties of the virus were not encouraging; the experiments were discontinued because of erratic results.

APHID TRANSMISSION OF RINGSPOT VIRUS

Vectors Breeding on Celery.—According to previous observations (Severin and Freitag, 1938) at least 11 species of aphids breed on celery under natural conditions in California, and all these are vectors of the western-celery-mosaic virus. The 11 species (listed in table 3) were tested for transmission of ringspot virus by transferring previously noninfective aphids of each species to 5 infected celery plants. The aphids fed on this material for about 7 days before being transferred to 5 healthy celery plants in lots of 25 per plant. Table 3 presents the results obtained with aphid transmission and also with attempted mechanical transmission.

All 11 aphid species proved capable of transmitting the virus from celery

to celery, but their efficiency varied greatly (table 3). The honeysuckle aphid was found to be the most efficient vector. The melon or cotton aphid, *Aphis gossypii* Glover, and the bean or dock aphid, *Aphis rumicis* Linn., were inefficient, transferring the virus to only 1 plant of 25, or 4 per cent.

As table 3 indicates, 275 celery plants were mechanically inoculated with sap from infected plants used as a virus source in the aphid experiments; but, again, none of them developed symptoms.

Single Aphids as Vectors.—Single specimens of winged and mature wingless aphids of 2 species that breed naturally on poison hemlock, the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.), and the rusty-banded aphid, *Aphis ferruginea-striata* Essig, were tested for their ability to transmit the virus. The winged honeysuckle aphids infected 28 out of 100 celery plants; the mature wingless, only 16 out of 100. With the rusty-banded aphids, the situation was reversed: the winged specimens infected only 2 out of 100 celery plants; the mature wingless, 19 out of 100.

Vectors That Do Not Breed on Poison Hemlock and Celery.—An attempt was made to transmit the ringspot virus by means of 5 species of aphids that do not breed on poison hemlock and celery plants under natural conditions in California. To celery plants infected with ringspot virus, the black peach aphid, *Aphis persicae-niger* Smith, was transferred on peach leaves; the mealy plum aphid, *Hyalopterus arundinis* (Fab.), on prune; and the black cherry aphid, *Myzus cerasi* (Fab.), on cherry. As the leaves wilted and became dried, the aphids moved from them to the foliage of infected celery. Next day the aphids on the infected plants were transferred to 5 healthy celery plants in lots of 25 per plant. Previously noninfective specimens of the cabbage aphids, *Brevicoryne brassicae* (Linn.), and the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), reared in the greenhouse, were also used in attempts at transmission. The 5 species tested survived on celery for only a few days, and 4 of the 5 failed to transmit the virus. The false cabbage or turnip aphid infected only 1 of 25 plants inoculated. The 4 aphid species that failed as vectors might possibly produce infections if additional tests were undertaken.

Transmission by Honeysuckle Aphids during Short Feeding Periods.—Efforts to transmit the ringspot virus by feeding each of 112 single honeysuckle aphids 5 minutes on diseased and then 5 minutes on healthy celery were all failures.

Since single honeysuckle aphids did not transmit the virus during 5-minute feeding periods on each diseased and healthy plant, it was decided to test lots of 25 feeding for short periods. The noninfective honeysuckle aphids were fasted for a half hour. Lots of 25 were then fed for 5, 10, and 15 minutes on the infected celery and immediately transferred to healthy plants, where they were fed for the same intervals. At each of the three short feeding periods, 100 lots of 25 aphids were tested. These lots infected only a low percentage of the celery—2 of the 100 celery plants during the 5-minute, 3 of the 100 plants during the 10-minute, and 2 of the 100 plants during the 15-minute feeding periods.

The small number of transmissions obtained by feeding lots of 25 honeysuckle aphids on the diseased celery for short periods made it desirable to

lengthen the period to 1 hour; the aphids were then transferred hourly to 9 successive healthy plants. As table 4 shows, 10 of 12 lots transmitted the virus during the first hour. Two lots failed to infect the first healthy plant; one of these lots infected the second plant, and the other the third. Three lots infected two successive plants on which they fed during the first and second hours. Two lots transmitted the virus once, then failed to transmit it to several successive healthy plants, but finally produced infection during the eighth hour.

Judging from the results, the honeysuckle aphid can infect healthy plants immediately after feeding for short periods on diseased ones. There is no

TABLE 4

TRANSMISSION OF VIRUS BY TWENTY-FIVE PREVIOUSLY NONINFECTIVE HONEYSUCKLE APHIDS FED ONE HOUR ON INFECTED CELERY AND THEN TRANSFERRED HOURLY TO NINE SUCCESSIVE HEALTHY CELERY PLANTS

Lot no.	Infections produced*									Number of celery plants infected
	2d hour	3d hour	4th hour	5th hour	6th hour	7th hour	8th hour	9th hour	10th hour	
1	+	−	−	−	−	−	−	−	−	1
2	+	−	−	−	−	−	−	−	−	1
3	+	+	−	−	−	−	−	−	−	2
4	+	−	−	−	−	−	−	−	−	1
5	+	+	−	−	−	−	−	−	−	2
6	+	−	−	−	−	−	+	−	−	2
7	+	−	−	−	−	−	−	−	−	1
8	+	−	−	−	−	−	−	−	−	1
9	+	+	−	−	−	−	−	−	−	2
10	+	−	−	−	−	−	−	−	−	1
11	−	+	−	−	−	−	+	−	−	2
12	−	−	+	−	−	−	−	−	−	1
Total +	10	4	1	0	0	0	2	0	0	....
Percentage	*83.3*	*33.3*	*8.3*	*0.0*	*0.0*	*0.0*	*16.7*	*0.0*	*0.0*	....

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

indication that a certain length of time must elapse between the acquiring of the virus and the infection of a plant. In the last experiment, the honeysuckle aphid usually infected only one or both of the first two healthy plants on which it fed, then lost the capacity to produce infection; it never retained the virus for more than 8 hours. If the tests had been more extensive and had continued somewhat longer, there might have been infections after 8 hours.

Retention of Virus by Aphids.—Two experiments were conducted to determine how long the honeysuckle aphid retained the ringspot virus. The first experiment tested 9 species of aphids that breed on celery under natural conditions. The species tested are listed in table 5. In each test 25 infective aphids reared on diseased celery in the greenhouse were transferred daily to 3 successive healthy celery plants. The aphids were confined on the third celery plant for a week, at the end of which they were killed by fumigation with Nico-fume tobacco paper. With each species, 25 tests were made.

As table 5 shows, the aphids generally lost the infective power during the first 24 hours of the experiment. Of the 225 celery plants on which they fed the

TABLE 5

RETENTION OF VIRUS BY NINE SPECIES OF APHIDS TRANSFERRED DAILY TO THREE SUCCESSIVE HEALTHY CELERY PLANTS, AND ATTEMPT TO RECOVER VIRUS BY MECHANICAL INOCULATION FROM INFECTED CELERY ON WHICH APHIDS WERE REARED

Aphid	Aphid transmission									Mechanical inoculation		
	1st day			2d day			3d to 9th day					
	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
Celery leaf aphid, *Aphis apigraveolens* Essig	25	15	60.0	25	0	0.0	25	0	0.0	25	0	0.0
Celery aphid, *Aphis apii* Theo	25	19	76.0	25	4	16.0	25	0	0.0	25	0	0.0
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	25	12	48.0	25	1	4.0	25	0	0.0	25	0	0.0
Erigeron root aphid, *Aphis middletonii* Thomas	25	18	72.0	25	1	4.0	25	0	0.0	25	0	0.0
Yellow willow aphid, *Cavariella capreae* (Fab.)	25	1	4.0	25	0	0.0	25	0	0.0	25	0	0.0
Lily aphid, *Myzus circumflexus* (Buck.)	25	18	72.0	25	1	4.0	25	0	0.0	25	0	0.0
Foxglove aphid, *Myzus convolvuli* (Kalt.)	25	14	56.0	25	0	0.0	25	0	0.0	25	0	0.0
Honeysuckle aphid, *Rhopalosiphum conii* (Dvd.)	25	17	68.0	25	1	4.0	25	0	0.0	25	0	0.0
Cotton or melon aphid, *Aphis gossypii* Glover	25	1	4.0	25	0	0.0	25	0	0.0	25	0	0.0
Total	225	115	....	225	8	....	225	0	....	225	0	....

first day, 115, or 51.1 per cent, became infected. The aphids succeeded in infecting only 8, or 3.5 per cent, of the 225 plants the second day; and none of the 9 species infected any celery the following week. The celery aphid was the most efficient vector, whereas the cotton or melon aphid and the yellow willow aphid were the least efficient, infecting only 1 plant out of 25 tested the first day.

According to table 5, no infection resulted when juice from the infected celery used as a source of virus for the aphids was mechanically inoculated

TABLE 6

RETENTION OF VIRUS BY LOTS OF TWENTY-FIVE INFECTIVE HONEYSUCKLE APHIDS REARED ON DISEASED CELERY AND TRANSFERRED HOURLY TO TEN SUCCESSIVE HEALTHY CELERY PLANTS

Lot no.	Infections produced*										Number of plants infected
	1st hour	2d hour	3d hour	4th hour	5th hour	6th hour	7th hour	8th hour	9th hour	10th hour	
1	+	−	+	+	−	−	−	−	−	−	3
2	+	+	+	+	+	−	−	−	−	−	5
3	+	−	+	−	−	−	−	−	−	−	2
4	+	−	−	−	−	−	−	−	−	−	1
5	+	+	+	−	−	−	−	−	−	−	3
6	−	−	+	+	+	−	−	−	−	−	3
7	−	+	+	−	−	−	−	−	−	−	2
8	+	+	−	−	+	−	−	−	−	−	3
9	−	+	+	+	−	−	−	−	−	−	3
10	+	+	+	−	−	−	−	−	−	−	3
11	+	−	−	−	−	−	−	+	−	−	2
12	+	+	−	+	+	−	−	−	−	−	4
13	+	+	−	−	−	−	−	−	−	−	2
14	+	−	+	−	−	−	−	−	−	−	2
15	+	+	−	−	−	−	−	−	−	−	2
16	−	+	−	−	−	−	−	−	−	−	1
17	+	−	−	−	−	−	−	−	−	−	1
18	−	+	−	−	−	−	−	−	−	−	1
Total +	13	11	9	5	4	0	0	1	0	0	..
Percentage	*72.2*	*61.1*	*50.0*	*27.8*	*22.2*	*0.0*	*0.0*	*5.5*	*0.0*	*0.0*	..

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

into 225 healthy celery plants. The failure of mechanical inoculation has already been discussed under "Inoculation Experiments" (p. 394).

In the second experiment 18 lots of 25 honeysuckle aphids reared on infected celery were transferred hourly to 10 successive healthy plants. As table 6 shows, the aphids infected a higher percentage of celery the first hour than during any later period. The results demonstrate a gradual loss of the virus by the aphids: one lot in this experiment infected a celery plant the eighth hour; none infected any during the ninth and tenth hours.

Loss and Recovery of Infectivity by Honeysuckle Aphids on Inoculated Celery.—An experiment was conducted to determine whether the honeysuckle aphid could recover the ringspot virus from celery before symptoms developed. Five celery plants were inoculated by lots of 100 aphids reared on in-

fected celery. Single aphids were transferred daily for 21 days from 1 inoculated plant to each of 10 healthy ones, and lots of 25 aphids from each of the remaining 4 inoculated plants to each of 4 healthy ones. This experiment was repeated three times, with the results indicated in table 7. The same aphids that were used to infect the plant were used to recover the virus. Previous experiments (p. 399) indicated that the aphids failed to retain the virus for more than two days and, therefore, that any infectivity demonstrated after the second day would be evidence of recovery of virus from the plant rather than retention by the aphids.

TABLE 7

LOSS AND RECOVERY OF INFECTIVITY BY HONEYSUCKLE APHIDS ON CELERY INOCULATED WITH RINGSPOT VIRUS

Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated			Lots of 25 aphids: plants infected of 4 inoculated		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
2	0	0	0	0	0	0
3	1	1	0	2	1	0
4	1	0	0	2	0	1
5	0	1	1	3	1	2
6	4	2	3	4	2	3
7	4	4	0	4	3	3
8	2	2	0	4	2	2
9	1	1	0	4	1	4
10	3	1	1	4	3	3
11	4	2	3	3	3	3
12	3	1	2	3	2	2
13	6	5	2	4	3	4
14	1	4	5	4	3	4
15	3	5	2	4	4	3
16	5	3	2	3	4	4
17	4	4	5	4	4	4
18	2	5	5	4	3	3
19	4	3	2	3	2	2
20	3	5	3	3	2	3
21	3	5	5	3	3	4
Total	54	54	41	65	46	54
Percentage	*27.0*	*27.0*	*20.5*	*81.3*	*57.5*	*67.5*

As table 7 shows, single aphids and lots of 25 recovered the virus from the original inoculated celery the third day and transferred it to healthy celery. They acquired the virus more readily the sixth and seventh day after inoculation. The symptoms in the original inoculated plants required 21 days to develop. In other experiments the incubation period of the disease varied from 7 to 33 days, with an average of 16.4 days. The results show that the aphids acquired the virus from inoculated plants before symptoms appeared.

Availability of the Virus to Honeysuckle Aphids after Infection.—Experiments were conducted to test whether the virus is more available to the aphids in recently infected celery, or in plants that had been infected for a long time. The methods followed resembled those described under the preceding subheading. Tests were made with single aphids and with lots of 25, and for the

TABLE 8

Ability of Single and Lots of Twenty-five Honeysuckle Aphids to Acquire and Transmit Virus from Celery Plants at Intervals Following Infection

Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated	Number of days after plants were inoculated	Single aphids: plants infected of 10 inoculated	Lots of 25 aphids: plants infected of 4 inoculated
2	0	0	61	0	2	121	1	2	181	0	3
3	0	0	62	0	1	122	4	4	182	1	3
4	0	1	63	3	4	123	3	3	183	1	2
5	1	2	64	0	1	124	6	3	184	0	3
6	3	3	65	1	1	125	0	2	185	2	3
7	0	3	66	1	4	126	2	4	186	0	3
8	0	2	67	1	2	127	0	4	187	0	1
9	0	4	68	1	3	128	0	4	188	5	2
10	1	3	69	4	2	129	1	4	189	1	3
11	3	3	70	0	2	130	2	3	190	3	3
12	2	2	71	1	4	131	1	3	191	1	2*
13	2	4	72	1	3	132	0	4	192	0	0*
14	5	4	73	1	3	133	2	3	193	2	2*
15	2	3	74	3	1	134	3	3	194	0	1*
16	2	4	75	3	3	135	3	0	195	3	1*
17	5	4	76	1	1	136	2	4	196	5	2*
18	5	3	77	2	3	137	3	3	197	2	2*
19	2	2	78	3	3	138	3	2	198	1	2*
20	3	3	79	4	3	139	2	4	199	2	0*
21	5	4	80	0	0	140	2	4	200	1	2*
Total	41	54	Total	30	46	Total	40	63	Total	30	40
Percentage	*20.5*	*67.5*	*Percentage*	*15.0*	*57.5*	*Percentage*	*20.0*	*78.8*	*Percentage*	*15.0*	*66.7*

* After the 190th day, only 2 plants were inoculated each day, so that the total number of plants inoculated for the period from 181 days to 200 days was 60 instead of 80 as in other periods.

following four periods after infection: 2 to 21 days, 61 to 80 days, 121 to 140 days, and 181 to 200 days.

Judging from the percentage of transmission shown in table 8 the virus was equally available during the four intervals following infection. Single aphids recovered it regularly during each of the four periods and infected 15.0 to 20.5 per cent of the celery. Lots of 25 infected 57.5 to 78.7 per cent of the celery during the four periods without showing significant differences in ability to acquire the virus.

The aphids while feeding and multiplying on the infected celery under test would repeatedly reinfect the plants. Since, however, the virus is not known to undergo any changes or multiplication within the body of the insect, this fact should not affect the results. The aphids would merely return virus to the leaf from which they had obtained it. To prevent reinfection, the periods during which they were allowed to feed on infected celery would have to be very short; but the low percentage of transmissions obtained during short feeding periods makes such experiments impractical.

SUMMARY

Poison hemlock, *Conium maculatum* L., is the only plant demonstrated to be naturally infected with ringspot.

The host range of poison-hemlock-ringspot virus is limited to the family Umbelliferae. The following plants have been experimentally infected by means of the honeysuckle aphid; celery, *Apium graveolens* L. var. *dulce* DC.; Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC.; dill, *Anethum graveolens* L.; salad chervil, *Anthricus Cerefolium* Hoffm.; coriander, *Coriandrum sativum* L.; varieties of carrots, *Daucus Carota* L. var. *sativa* DC.; Hollow Crown and Long Smooth parsnip, *Pastinaca sativa* L.; and horticultural and botanical varieties of parsley, *Petroselinum crispum* Nym. and *P. crispum* Nym. var. *latifolium*.

Symptoms of the disease consisted mainly of chlorotic line and ring patterns.

The ringspot virus was demonstrated to be systemic in the celery plant. The virus was mechanically transmitted from parsley to parsley in 14.1 per cent of the tests, but could not be thus transmitted from poison hemlock and celery.

Eleven species of aphids that breed on celery under natural conditions were demonstrated to be vectors of ringspot virus.

Four of the 5 aphid species tested that do not breed on celery or poison hemlock failed to transmit ringspot virus.

The honeysuckle aphid transmitted the virus in a low percentage of trials during short feeding periods of 5, 10, and 15 minutes on each diseased and healthy plant.

Previously noninfective honeysuckle aphids, fed for 1 hour on a diseased celery plant and then fed hourly on 9 successive healthy celery plants, usually infected the first healthy plant on which they fed, but only a low percentage of the later plants. Two plants were infected during the eighth hour, but none during the ninth and tenth hours.

Experiments conducted to determine how long the aphids retained the virus demonstrated that 4 species lost it during the first 24 hours on healthy plants, whereas 5 species infected a low percentage of plants during the second 24-

hour period, but none infected any after 48 hours. Infective honeysuckle aphids reared on diseased celery and then transferred hourly to 10 successive healthy celery plants infected the highest percentage of healthy plants during the first hour and gradually lost the infective power. All aphids failed to transmit the virus during the ninth and tenth hours.

The same honeysuckle aphids that had infected celery were able to recover virus from plants 3 days after inoculation. Since the minimum incubation period of the disease in the plant was 7 days, this experiment demonstrated that the aphids could acquire the virus before symptoms developed.

Experiments were conducted to determine the ability of aphids to acquire ringspot virus from celery during four 20-day periods after infection. The aphids acquired the virus as readily during the fourth period, 181 to 200 days after infection, as during the first 20 days.

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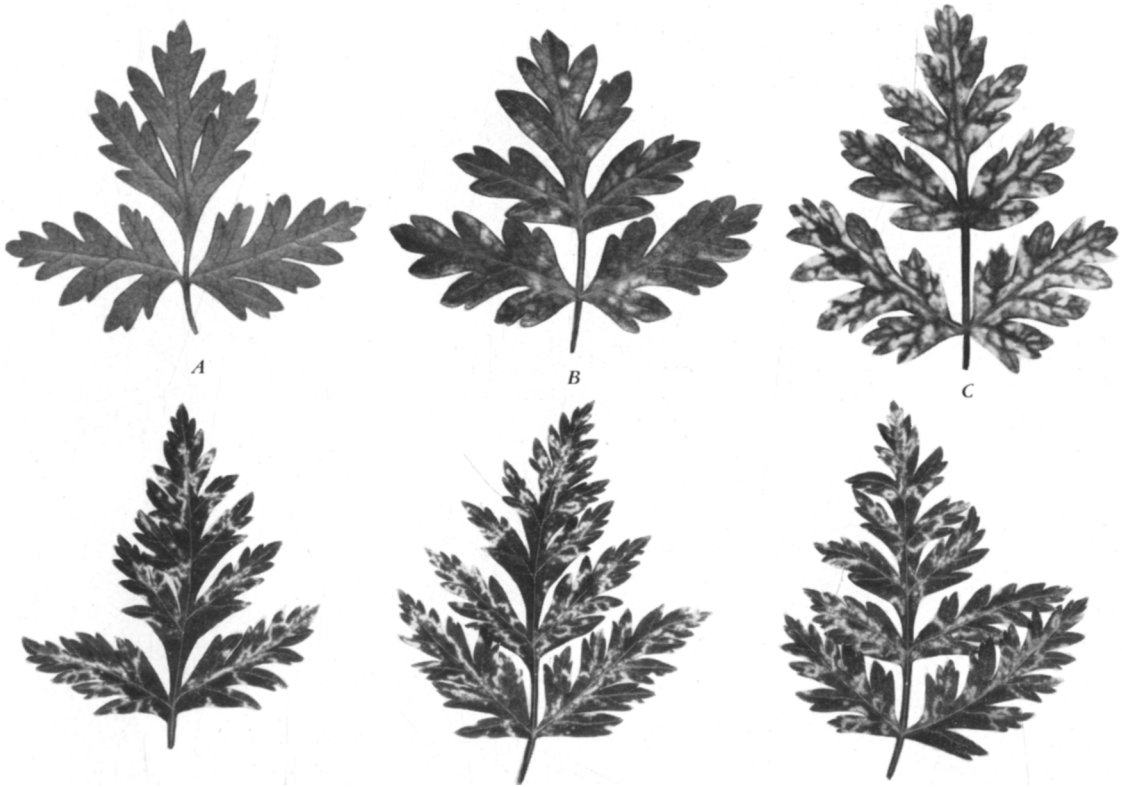

Plate. 1—Poison hemlock, *Conium maculatum* L., ringspot: *A,* leaflets from healthy control plant grown from seeds; B, chlorotic areas scattered irregularly on the leaflets; *C,* leaflets showing irregular chlorotic areas of various sizes, and green veinbanding; *D,* leaflets showing irregular line patterns, *E,* zigzag lines on some of the leaflets, resembling oak-leaf pattern; *F,* leaflets showing ringspots, each composed of a chlorotic ring encircling a green area.

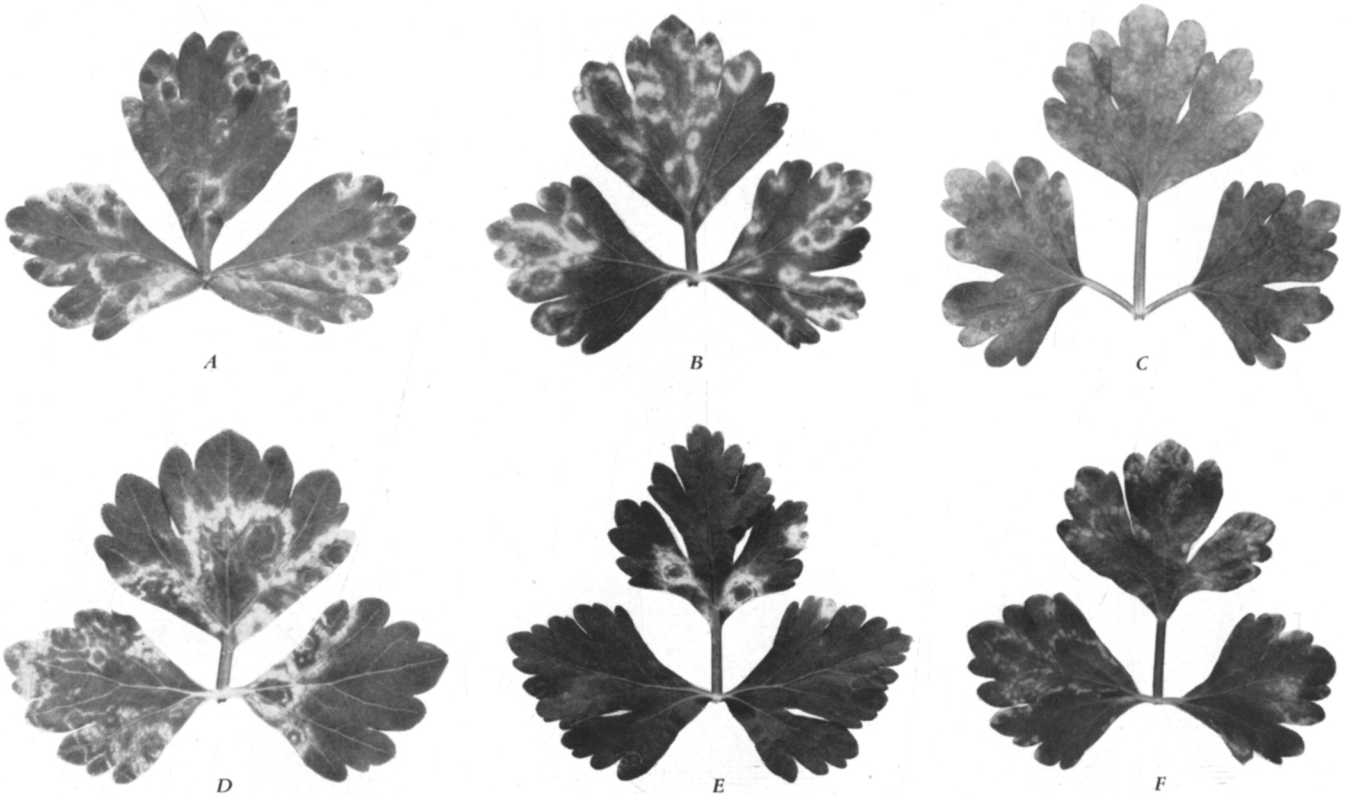

Plate 2.—Symptoms of poison-hemlock ringspot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *A*, chlorotic rings encircling green areas and imperfect rings in the form of semicircles along the margin. *B*, Various-shaped ringspots such as circular, oval, or irregular. *C*, Numerous ringspots with green rings enclosing yellow centers. *D*, Several types of ringspots on the leaflets—on the apical leaf, concentric alternating yellow and green lines surrounding green areas; on the leaflet at left, line pattern and ringspot with chlorotic ring encircling green tissue; on the leaflet at right, outer chlorotic ring, inner green ring with a chlorotic center on the basal margin of the leaflet, ringspot with green area embedded in chlorotic tissue near the attachment of the leaflet, and two adjacent ringspots with green rings enclosing chlorotic centers. *E*, Concentric alternating yellow and green lines surrounding a green area within a pale yellow halo. *F*, Broken-line pattern composed of dots and dashes, sometimes enclosing large areas of green tissue.

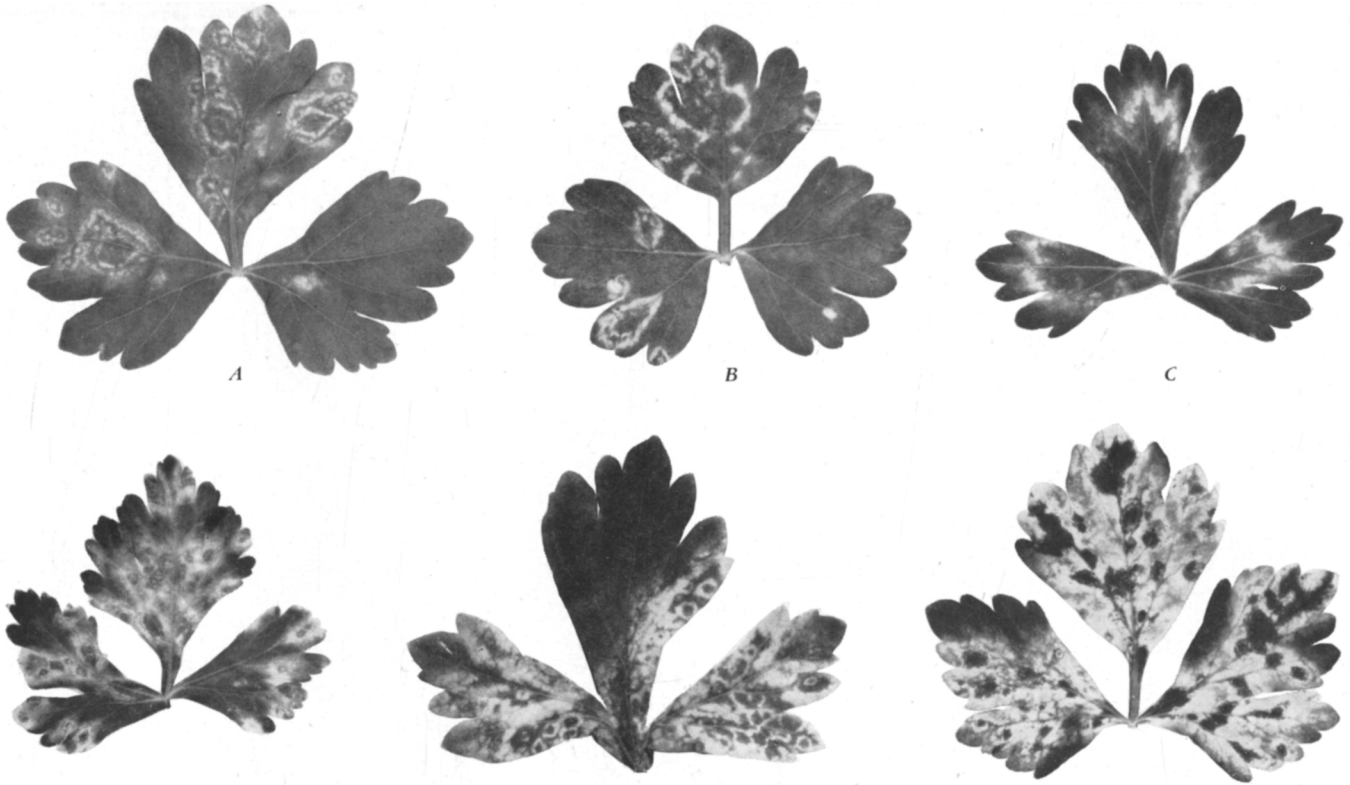

Plate 3.—Symptoms of poison-hemlock ringspot on leaflets of celery plants experimentally infected by the honeysuckle aphid, *Rhopalosiphum conii* (Dvd.): *A*, concentric broken lines alternating yellow with green lines, surrounding large green areas and ringspots with outer chlorotic ring and inner green ring with chlorotic center; *B*, irregular yellow bands enclosing green tissue and chlorotic spots; *C*, zigzag chlorotic tissue resembling oak-leaf pattern; *D*, advanced stage of disease, showing fusion of small chlorotic areas into enlarged irregular yellow tissue in which green spots are embedded; *E*, *F*, advanced stages of disease, showing chlorosis spreading over the leaflets, with green areas remaining.

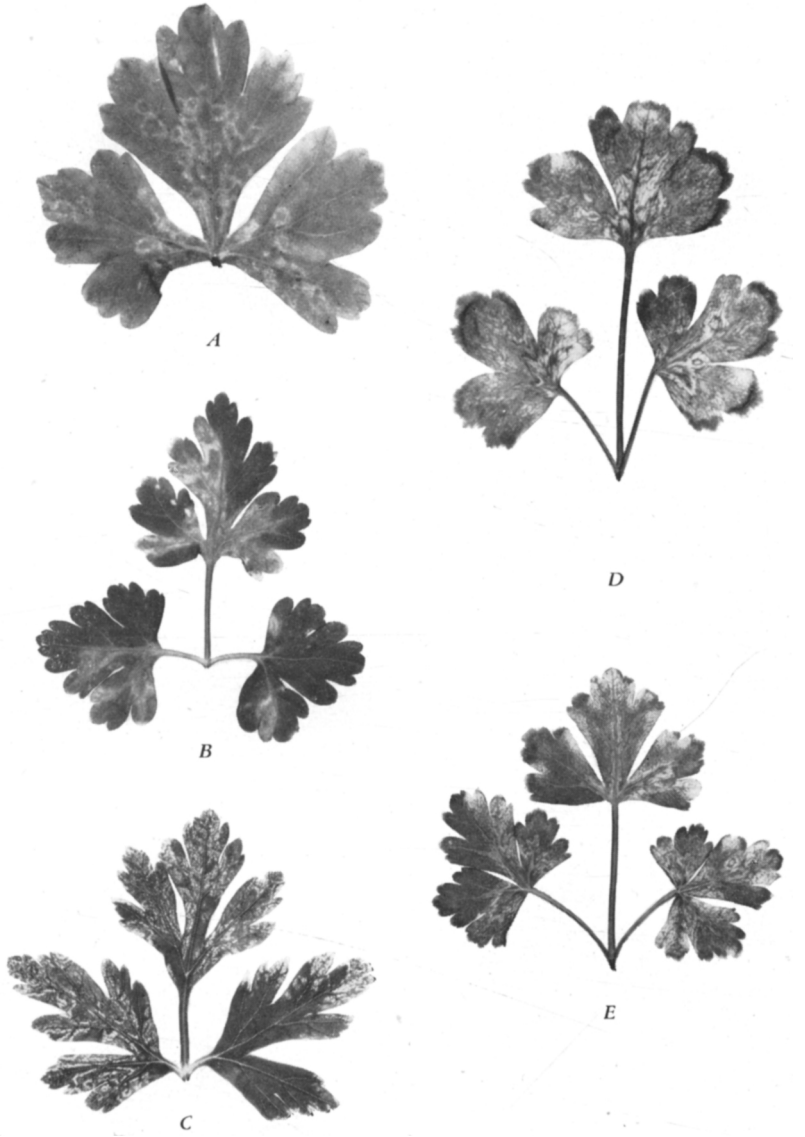

Plate 4.—Symptoms of poison-hemlock ringspot: *A*, leaflets from Large Smooth Prague celeriac, *Apium graveolens* L. var. *rapaceum* DC., showing numerous large chlorotic rings encircling green areas; *B*, early symptoms on leaflets from Hamburg or turnip-rooted parsley, *Petroselinum hortense* Hoffm. var. *radicosum* Bailey, showing chlorotic areas; *C*, later symptoms, showing a few ringspots composed of clusters of chlorotic dots or dashes enclosing green centers and numerous interveinal chlorotic dots, dashes, and streaks often arranged to form broken lines, also green veinbanding; *D*, leaflets from Single or Plain parsley, *Petroselinum crispum* Nym. var. *latifolium*, showing ringspots, each with an outer chlorotic ring, an inner green ring enclosing a chlorotic center, and also chlorotic areas; *E*, leaflets showing a single ringspot, also broken yellow lines, zigzag lines resembling an oak-leaf pattern on the leaflet at the left, and green or chlorotic veinbanding.