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RINGSPOT AND ASSOCIATED VIRUS DISEASES OF DAHLIAS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Roland A. Mildner, Jr.

1960

THESIS

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RINGSPOT AND ASSOCIATED VIRUS DISEASES OF DAHLIAS

by

Roland A. Mildner, Jr.

AN ABSTRACT

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1960

Approved by Robert P. Scheffer

ROLAND A. MILDNER, JR.

ABSTRACT

Five virus isolates from commercial dahlias were mechanically transmitted to 16 plant species in 8 families. Isolates 1 and 3 caused ringspots in dahlia seedlings, while the other isolates caused various degrees of mottling and other non-distinctive symptoms. Disease development was rapid when soft, succulent, well-fertilized plants grown at a night temperature of 22° C, reduced light intensity and short day conditions were inoculated. Slow-growing plants were less susceptible. Optimum infectivity was achieved by grinding leaves for inoculum in a 0.05 M phosphate buffer, pH 8.5.

The following physical properties were determined: Isolate 1 - thermal inactivation between 46° and 48° C; dilution end point between 1:100 and 1:150; resistance to ageing in vitro between 6 and 16 hours; resistance to ageing in drying leaves between 96 and 120 hours; withstands rapid desiccation in leaves. Isolate 2 - thermal inactivation between 48° and 50° C; dilution end point between 1:200 and 1:300; resistance to ageing in vitro between 32 and 40 hours; resistance to ageing in drying leaves between 48 and 72 hours; resists rapid desiccation in leaves. Isolate 3 - thermal inactivation 40° to 42° C; dilution end point between 1:10 and 1:20; resistance to ageing in vitro between 8 and 12 hours; resistance to ageing in drying leaves between 48 and 72 hours; inactivated upon rapid desiccation in leaves. Isolate 5 - thermal inactivation 46°

to 48° C; dilution end point 1:200 to 1:250; resistance to ageing *in vitro* between 8 and 12 hours; resistance to ageing in drying leaves between 96 and 120 hours; withstands rapid desiccation in leaves. Isolate 6 - thermal inactivation between 94° and 96° C; dilution end point between 1:1200 and 1:1400; resistance to ageing *in vitro* between 480 and 720 hours; resistance to ageing in drying leaves beyond 720 hours; remains active after rapid desiccation in leaves.

The presence of mixtures of viruses in dahlia source plants was indicated by host range differences before and after single lesion "purifications", which greatly modified the host ranges of isolates 2, 3 and 5. Further evidences of mixtures are the facts that isolates 2 and 5 did not cause ringspots in dahlia seedlings after "purification", although they were originally isolated from plants with such symptoms, and isolate 6 came from petunias inoculated with isolate 5.

Host ranges and physical properties were significantly different from isolate to isolate. Isolates 1 and 5 were similar enough to be tentatively identified as "strains" of the same virus. They have some characteristics in common with tomato spotted wilt virus, but host ranges and properties were somewhat different. Isolate 2, distinctively different from the other isolates, also differed from viruses described in the literature. Isolate 3 also differed greatly from the other isolates and resembled dahlia ringspot virus as described in the literature. Isolate 6 differed

radically from the other isolates and from viruses described by others. Dahlia mosaic, oakleaf and yellow ringspot viruses were not found during the assays, nor was cucumber mosaic virus, which is commonly considered present in the dahlia virus complex.

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INTRODUCTION

Virus diseases are prevalent throughout the world in dahlia stocks, a fact generally recognized by plant pathologists and horticulturists. This is not surprising, since the dahlia has been propagated vegetatively for generations, possibly since its original introduction to Europe from Mexico in 1789. In spite of this, little is known about the diseases or the viruses involved. Possibly the best known is mosaic, which apparently affects only dahlia in nature. Previous investigators have suggested that dahlias carry some wide host range viruses, two of which may cause the common ringspot disease. My work began as an effort to determine whether or not this was true. It soon became apparent that there are viruses closely related to the ringspot viruses, but which do not cause ringspots in the dahlia. Five virus isolates from plants showing typical ringspots were studied carefully to determine their relationships.

REVIEW OF LITERATURE

Several viruses are known to affect dahlias but only dahlia mosaic has received much attention (2). Other viruses found in dahlias are cucumber mosaic virus (3, 11), dahlia ringspot, yellow ringspot, oakleaf (2), tomato spotted wilt virus (4, 5, 10) and potato virus Y (9). Literature concerning viruses that attack dahlias was reviewed by Mildner (8); only ringspot diseases will be reviewed further.

Brierly (2) described virus ringspot symptoms on dahlias as irregular concentric rings and zigzag markings, intricate hieroglyphic patterns and green islands. Much variation was found in width, coloration and area of zones. The virus was transferred by grafting but sap and insect transmission attempts failed. Brierly also described a yellow ringspot which he thought was caused by another virus or by a strain of ringspot virus. Yellow ringspot symptoms are prominent yellow patterns, which contrast with the greenish and yellowish-green patterns of dahlia ringspot.

Tomato spotted wilt virus (TSWV) is often considered to be the most common cause of ringspots in dahlias, especially in Europe. Smith (10) identified TSWV in dahlias and described the symptoms as concentric rings. He transferred the virus from dahlias to Datura stramonium L., Nicotiana tabacum L. and Lycopersicon esculentum Mill., where the usual bronzing TSWV symptoms developed. Holmes (4) isolated TSWV from dahlias and transferred it to

Nicotiana glutinosa L., *Stellaria media* L. and *L. esculentum*. Several varieties of tomato resistant to TSWV were susceptible to the isolate from dahlias. Apparently no attempt was made to reinfect dahlias or other plants. Roland (9) described TSWV symptoms on dahlias as chlorotic concentric rings which may disappear late in the growing season. Holmes (5) apparently succeeded in propagating virus-free plants from plants carrying TSWV by the use of tip cuttings. There is no data to indicate that TSWV differs from Brierly's ringspot virus.

MATERIALS AND METHODS

For most studies plants were grown in 4 inch clay pots containing equal volumes of soil, muck and peat. Plants were fertilized weekly or as required with a commercial fertilizer (Plant Marvel). Night temperatures were kept at 22° to 28° C, depending on the needs of the particular experiment. Light intensity was regulated to a degree by growing plants under a cloth shade during bright summer months and by supplementing shorter days of autumn and winter with incandescent lights. To determine the influence of day length on symptom expression, the light period was reduced to 9 hours during July and August. Plants were covered and uncovered at designated times with an opaque black cloth commonly used for the purpose. Control plants on adjacent benches were not covered. Insect populations were eliminated or kept very low by fumigation with Parathion or by spraying with Malathion at 4 to 6 week intervals.

Dahlias showing ringspots in the field were brought into the greenhouse and grown as sources of viruses for the study. Such material, collected in fields of commercial dahlias in Michigan, served as the original source of all viruses except isolate 1, which was supplied in dahlia leaves by Dr. R. D. Raabe of the University of California. Isolate 2 came from the variety Betty Zane. Isolate 3 came from the variety Sherwood's Peach, and Isolate 5 came from the variety Nicky K. Isolate 4 from the variety

Commodore was lost early in the work and no data are reported. Isolate 6 came from the growing tip of a Petunia hybrida Vilm. plant which developed systemic vein clearing. The petunia plant previously had been inoculated with material from Datura tatula L. carrying Isolate 5. Normally, Isolate 5 causes only local lesions in petunia.

Transfers were made from dahlia hosts to D. tatula, Vigna sinensis (L.) Endl. authority, or Gomphrena globosa L. authority using Yarwood's (12) leaf disk method. The isolates were then "purified" after the method of Jensen (6) by removing a single lesion, adding buffer and carborundum dust and grinding together on a glass slide with a glass spatula. Upon inoculation and subsequent appearance of symptoms, a single lesion was again removed, ground and transferred. This procedure was repeated 4 times, after which all lesions appeared uniform. Isolates thus "purified" were maintained in stock plants and were used in all later experiments. To insure maximum concentration of the viruses, only vigorous young plants showing strong systemic symptoms were used as a source of inoculum.

In all physical property studies only test plants that produced countable lesions (D. tatula or G. globosa)were used. Five plants were inoculated with each sample to compensate for variations in host plant resistance. Leaf sizes were recorded and lesions counted. In most experiments, half leaves were inoculated.

Thermal inactivation tests were conducted by placing 0.5 ml. of sap in 5 x 65 mm. thin wall glass tubes. Tubes containing

the sap sample were attached to a thermometer with a rubber band, positioned so as to place the tube contents adjacent to the thermometer bulb. Thermometer and tube were then placed in a water bath at the desired temperature and 20 seconds was allowed for sap temperature to reach that of the water. After 10 minutes, the tube was plunged in cold water and sap was immediately rubbed on test plants. The equipment used for these studies (Fig. 1) consisted of a 600 ml. beaker heated by a small electric element and stirred with a magnetic stirrer. Temperature was regulated accurately ($\pm$ 0.5° C) and conveniently by the use of a "Powerstat" voltage regulator. Tests with a thermocouple showed that sap in the tube came to the desired temperature within 30 seconds.

RESULTS

Symptoms on dahlia source plants

Isolate 1 was obtained from dahlia leaves with "target" spots of concentric white necrotic rings, about 1 cm. in diameter, around a normal green center. These symptoms apparently are the same as those described by Smith (10) and Brierly (2). Symptoms on dahlia variety Betty Zane, source of isolate 2, were occasional target spots 6-8 mm. diameter, irregular dark green islands and yellow chlorotic spots. Stems and petioles were distorted and some vein banding was evident. On the variety Sherwood's Peach, source of isolate 3 (Fig. 2), symptoms were similar to those from which isolate 1 was obtained. Symptoms on the variety Nicky K, source of isolate 5, were indistinct reddish targets about 4-5 mm. diameter.

The possibility of variations in symptoms with environmental conditions was considered. Virus carrying tubers of 12 different commercial varieties of dahlias were divided, and 1 to 3 plants of each clone were grown at approximately 28°, 22° and 17° C. Eleven weeks after planting most plants were symptomless but a few exhibited various symptoms at all temperatures tested. Eighteen weeks after planting half the plants at all 3 temperatures were symptomless, while symptom expression in the remainder varied and was not correlated with temperature or with variety. Symptom expression is known to vary in the field, but these results suggest that factors other than temperature are involved.

Factors affecting transmission

Sap inoculations from dahlias to dahlias and from dahlias to other plants were unsuccessful but transmission by Yarwood's (12) leaf disk method was effective and reliable. Inoculations to determine host range, physical properties and other tests were made mechanically as follows: infected leaf tissue was ground in a phosphate buffer solution containing a small amount of 400 mesh carborundum. Inoculation was most successful when leaves just short of full expansion were dusted with carborundum and rubbed with a glass spatula or index finger which had previously been dipped in the infectious sap.

D. tatula was the most satisfactory local lesion host for isolates 1, 3, 5 and 6 while Gomphrena globosa L. was most suitable for isolate 2. These plants were used as both donor and acceptor

hosts for all assays. Inoculations were uniformly successful when plants were soft and succulent, well fertilized, grown at a minimum night temperature of 22° C, and under reduced light intensity. Hard, slow-growing plants often escaped infection.

The effect of day length on virus transmission and symptom development was determined. Plants were grown in the greenhouse in July and August under 9 hour days and under normal daylengths before and after inoculation. Isolate 1 caused symptoms 1 to 5 days sooner in short day plants than in long day plants, although final numbers of lesions were about the same. Isolate 2 caused about 300 lesions per leaf under short day conditions and about 100 per leaf with long days, without changing the incubation time. Isolate 5 caused lesions to appear 2 days earlier in short day plants than in long day plants under the conditions used. The short day, isolate 5 combination also caused 2 times more lesions than did the long day, isolate 5 combination.

There were 2 experiments to determine the most favorable buffer concentration for virus transmission. Infected leaves were ground in potassium phosphate buffer at 0.066, 0.050, or 0.040 molar solutions (pH 8.5) and the number of lesions developing were counted. Concentration of buffer had an effect on infectivity of isolates 2 and 5 (Table I), with 0.050 M being optimum for transmission. Data for the other isolates indicated similar trends.

Experiments were conducted to find the best pH level for transmission. Infected leaves were ground in solutions of potassium

TABLE I

EFFECT OF CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER ON DAHLIA VIRUS TRANSMISSION

Molarity	Isolate Number 2	5
0.066	262[a/]	72
0.050	362	161
0.040	254	74

a/ Average number of lesions per ½ leaf obtained from 10 leaves in two separate experiments. D. tatula was used with isolate 2 and G. globosa was the donor and assay plant for isolate 5.

phosphate buffer at pH levels from 5.5 to 9.5. The buffer solutions were adjusted to the lower pH values by adding 0.1 N phosphoric acid and to the higher values by adding 0.1 N KOH. Isolate 1 caused a maximum number of lesions at pH 8.5. Isolate 2 had a maximum effect at pH 6.5 and another high at pH 9.5. Isolate 3 was little affected by pH from 7.5 through 9.5. Isolate 5 may also have a double peak, with maximums at pH 6.5 and 9.5. Complete data are given in Table II. The results show that the isolates are affected differently by pH, which possibly reflects differences in isoelectric points.

When infected leaf tissue was ground with distilled water rather than with buffer, isolates 2 and 6 remained highly infectious, isolate 5 was rarely infectious and isolates 1 and 3 lost infectivity completely. Isolates 2 and 6 were not inactivated by freezing in buffer but isolates 1, 3 and 5 were destroyed by freezing (see later). The distilled water and freezing effects show that isolates 1, 3 and 5 are more labile than are isolates 2 and 6.

Host range

In host range experiments, plants to be tested were usually inoculated in groups of 5. Several groups were then inoculated over a period of several days or weeks, to compensate for environmental influence on transmission. Plants were tested at least 25 times if symptoms did not appear. Isolates made from all plants which developed symptoms were transferred back to the original host where normal symptoms appeared in every trial. Plants without symptoms were not retested, therefore symptomless carriers were not found, except in

TABLE II

EFFECT OF pH ON DAHLIA VIRUS TRANSMISSION

pH[a/]	Isolate Number 1	2	3	5
5.5	4[b/]	164	0	164
6.5	81	171	0	287
7.5	94	54	12	216
8.5	135	72	18	147
9.5	93	121	21	290

a/ Leaves were ground in 0.05 M potassium phosphate buffer solution at the pH values indicated.

b/ Values are average number of lesions per ½ leaf on 10 leaves in 2 separate experiments. G. globosa was the donor and assay plant for isolates 1, 3 and 5. D. tatula was used for isolate 2.

one instance. Control plants of known reactions were used in all host range trials to determine infectivity of the sap preparation. Experiments were discarded when symptoms did not appear on such controls.

Preliminary host range experiments were conducted before the isolates were "purified" by single lesion isolations. These experiments are summarized in Table III. Host ranges of the "crude" isolates were generally wider than were those of the "purified" isolates (Table IV) and symptoms in some hosts differed before and after purification (Fig. 3). Representative differences in host ranges before and after "purification" are shown in Table V.

Marked changes in infectivity occurred when isolates 3 and 5 were "purified". Least change was observed after "purification" of isolate 2, where the host range was decreased by one species and the ability of the virus to infect 3 other species was reduced. Isolate 5 was peculiar in that it gained the ability to cause symptoms in one species, lost the ability to cause symptoms in 2 others, and 3 other species became harder to inoculate. Cucumis sativus L., a host for isolates 2 and 3 but not 5 before "purification", was affected by only isolate 5 after "purification". Phytolacca decandra L. and Sinningia speciosa Benth. & Hook., hosts for all 3 isolates before "purification", were no longer hosts for isolates 3 and 5 after "purification". Such changes in host range indicate that mixtures of viruses were present in the dahlia source plants and that "purification" procedures eliminated some of these. All physical properties were determined on the "purified" isolates (see below).

TABLE III

HOST RANGE OF DAHLIA VIRUSES BEFORE "PURIFICATION" BY SINGLE LESION TRANSFERS

Host Plants	Isolate Number 2	3	5
Calceolaria crenatiflora Cav.	0/4[a]	4/4	0/4
Calendula officinalis L.	25/25	7/7	6/6
Chrysanthemum maximum Ramond	3/6	0/12	0/4
Cucumis sativus L.[b]	3/15	15/48	0/10
Dahlia variabilis Cav.var.Unwin	6/18	0/14	0/19
Datura stramonium L.	21/23	25/27	0/14
Gomphrena globosa L.	32/32	4/6	6/6
Nicotiana glutinosa L.	0/5	0/9	1/9
N. tabacum L.var. Havana 38	0/9	0/11	0/5
N. tabacum L. var. xanthii	0/10	0/10	-
Phytolacca decandra L.	12/13	3/12	2/6
Sinningia speciosa Benth.& Hook.	12/12	18/22	4/9
Stellaria media L.	0/9	0/8	0/6
Verbescina encelioides (Cav.) Benth. & Hook.	4/16	1/5	4/9
Vigna sinensis Savi. var. Black Hog	9/9	5/16	3/7
Vinca rosea L.	0/7	3/6	0/3
Zinnia elegans Jacq.	7/7	5/5	5/5

a/ Number of plants infected/number of plants inoculated. Results are based entirely on visible symptom expression.

b/ Variety names are given in the text.

TABLE IV

HOST RANGE OF DAHLIA VIRUSES AFTER "PURIFICATION" BY SINGLE LESION TRANSFERS

Host Plants	Isolate Number 1	2	3	5	6
Calendula officinalis	3/20[a]	5/15	0/25	5/15	0/25
Capsicum frutescens	13/15	3/36	7/18	18/18	0/25
Chenopodium amaranticolor	0/25	16/16	0/25	0/25	0/25
Cucumis sativus[b]	0/29	0/20	0/25	2/28	25/25
Dahlia variabilis	31/65	18/75	36/125	7/25	10/90
Datura tatula	100/100	4/16	70/100	100/100	100/100
Gomphrena globosa	4/15	100/100	7/15	9/15	15/15
Lycopersicon esculentum	18/30	0/42	12/25	15/20	0/65
Nicotiana glutinosa	8/17	3/26	0/20	5/11	10/10
N. tabacum var. H-38	6/20	9/17	0/25	10/15	15/15
Petunia hybrida	22/25	6/14	2/20	15/15	6/12
Phytolacca decandra	0/26	16/16	0/25	0/25	0/25
Sinningia speciosa	6/17	14/15	0/33	0/25	0/25
Verbescina encelioides	0/25	1/25	0/25	1/25	0/25
Vigna sinensis	8/28	28/29	2/18	7/17	0/20
Zinnia elegans	0/26	2/15	0/25	7/15	5/25

a/ Number of plants infected/number of plants inoculated.

b/ Variety names are given in text.

TABLE V

COMPARATIVE HOST RANGES OF DAHLIA VIRUSES
BEFORE AND AFTER SINGLE LESION "PURIFICATION"

	Isolate Number					
	2		3		5	
Host Plants	before purification	after purification	before purification	after purification	before purification	after purification
Calendula officinalis	25/25[a/]	5/15	7/7	0/25	6/6	5/15
Cucumis sativus[b/]	3/15	0/20	15/48	0/25	0/10	2/28
Phytolacca decandra	12/13	16/16	3/12	0/25	2/6	0/25
Sinningia speciosa	12/12	14/15	18/22	0/33	4/9	0/25
Verbescina encelioides	4/16	1/25	1/5	0/25	4/9	1/25
Zinnia elegans	7/7	2/15	5/5	0/25	5/5	7/15

a/ Number of plants infected/number of plants inoculated.

b/ Variety names are given in text.

In an effort to distinguish between the "purified" isolates and to compare them with other viruses for which host ranges are known, plants of 16 species belonging to 8 families were inoculated. The symptoms of the several isolates on various hosts are given below.

Isolate 1

Amaranthaceae

Gomphrena globosa L. (globe-amaranth) Occasional pale yellow spots and irregular green islands developed. All growth subsequent to infection became distorted.

Compositae

Calendula officinalis L. (calendula)var. Boll Gold Symptoms were yellow spots 1 mm. in diameter. All new growth later became distorted.

Gesneriaceae

Sinningia speciosa Benth. & Hook. (gloxinia) var. unknown. Black necrotic rings 2-3 mm. in diameter and wandering lines were formed. Systemic infection was not apparent.

Leguminosae

Vigna sinensis Savi. (cowpea) var. Black Hog. Symptoms were a few yellow spots 3-4 mm. in diameter on inoculated leaves only. No systemic infection was apparent by visual inspection.

Solanaceae

Datura tatula L. (jimson weed). This plant was a good local lesion host. Many 3-4 mm. diameter necrotic lesions were produced. Later, infection became systemic and caused targets, slight vein clearing and mottle.

Nicotiana glutinosa L. Symptoms were a very few necrotic spots 3-4 mm. in diameter on the inoculated leaves. No systemic reaction was observed.

N. tabacum L var. Havana 38. Symptoms were necrotic spots 3-4 mm. in diameter on the inoculated leaves. No systemic symptoms developed.

Capsicum frutescens L. (tabasco pepper) var. tabasco. Targets 2-3 mm. in diameter developed on the inoculated leaves. Systemic symptoms were vein clearing, epinasty and tip necrosis.

Petunia hybrida Vilm. (petunia) var. Minstrel. A few necrotic black spots, 1-2 mm. in diameter developed on the inoculated leaves. Systemic symptoms were not found.

Lycopersicon esculentum Mill. (tomato) var. Bonny Best. Diseased plants first developed a mottle. Next, there were a few targets and leaf twisting. Later, stems developed necrotic areas and tip necrosis. Plants were stunted (Fig. 4).

Isolate 2

Amaranthaceae

G. globosa (globe-amaranth). Profuse white necrotic local lesions 1-2 mm. in diameter developed on the inoculated leaves. Later, irregular lines and petiole droop developed (Fig. 3).

Chenopodiaceae

Chenopodium amaranticolor Coste and Regn. (pigweed). Symptoms were yellow spots 1 mm. in diameter. Systemic symptoms were not observed.

Compositae

C. officinalis L. (calendula). Rugose new growth and tip necrosis developed. There were no symptoms on inoculated leaves.

Verbescina encelioides Benth. & Hook. (crownbeard). Symptoms were a slight mottle and leaf puckering. There were no symptoms in inoculated leaves.

Zinnia elegans Jacq. (zinnia) var. Brightness. Plants developed small white necrotic spots and irregular light green areas on new growth. No symptoms developed on inoculated leaves.

Gesneriaceae

S. speciosa (gloxinia) var. unknown. Necrotic targets 3-5 mm. in diameter formed. Later, the targets coalesced to cause leaf drop. Still later, tip necrosis occurred.

Leguminosae

V. sinensis var. Black Hog (cowpea). Symptoms were light brown circles 2 mm. in diameter. Later, the circles coalesced, which caused russet leaf and vascular necrosis. Still later, all new growth became badly distorted.

Phytolaccaceae

Phytolacca decandra L. (pokeweed). White rings 1 mm. in diameter developed. No systemic symptoms were observed.

Solanaceae

D. tatula (jimson weed). Systemic mottle and elongated leaves appeared. No symptoms were seen on inoculated leaves.

Nicotiana glutinosa L. A slight mottle appeared on the new growth. No symptoms were found on inoculated leaves.

N. tabacum var. Havana 38. Symptoms consisted of a white dotted line target 2-8 mm. in diameter, puckered leaves and interveinal white necrosis on all new growth. No symptoms were seen on inoculated leaves.

C. frutescens (tabasco pepper). Necrotic brown streaking developed on all new growth. No symptoms were observed on inoculated leaves.

P. hybrida (petunia). Infected plants produced a rugose mottled new growth. Symptoms were not seen on inoculated leaves.

Isolate 3

Amaranthaceae

G. globosa (globe-amaranth). Primary symptoms were reddish spots 2-3 mm. in diameter with a pale yellow halo. Secondary or systemic symptoms were an indistinct mottle and some leaf distortion.

Leguminosae

V. sinensis var. Black Hog (cowpea). Symptoms were a very few targets 2-3 mm. in diameter on the inoculated leaves. No systemic symptoms were seen.

Solanaceae

D. tatula (jimson weed). Many targets 3-4 mm. in diameter were formed. These lesions were useful in infectivity assays. Later, pinpoint necrotic spots or light green spots, rugose leaves, considerable stem twisting, slight stunt and chlorotic interveinal areas developed.

C. frutescens L. (tabasco pepper). Symptoms were white spots 2-3 mm. in diameter which later became targets. Still later, tip necrosis developed.

P. hybrida (petunia). Necrotic spots 3-4 mm. in diameter and a slight systemic mottle developed.

L. esculentum (tomato). Symptoms were a slight mottle. Later, leaves developed necrotic spots and became twisted. The plants were generally stunted and eventually developed necrotic stem areas.

Isolate 5

Amaranthaceae

G. globosa (globe-amaranth). Distorted and necrotic leaf areas developed on inoculated leaves and on new growth.

Compositae

C. officinalis (calendula). Symptoms consisted of white rings 2 mm. in diameter. Later, a systemic mottle developed.

V. enceliodes (crownbeard). Diseased new growth was puckered and deformed. No symptoms developed on inoculated leaves.

Z. elegans (zinnia). Symptoms were a light green mottle. Later, there was leaf distortion, petiole twisting and stem necrosis.

Cucurbitaceae

Cucumis sativus L. (cucumber) var. National Pickling. Symptoms were light green spots 2-3 mm. in diameter with pinpoint necrotic centers on the inoculated leaves only. No systemic symptoms developed.

Leguminosae

V. sinensis (cowpea). Light green spots 1-2 mm. in diameter developed on the inoculated leaves only. No systemic symptoms were observed.

Solanaceae

D. tatula (jimson weed). This plant was a useful local lesion host. Necrotic sunken spots 2-3 mm. in diameter developed on inoculated leaves. Later, small necrotic spots or rings 2-3 mm. diameter, leaf puckering, petiole droop and necrotic flowers developed. Still later, the plants were considerably stunted and usually died.

N. glutinosa. Symptoms were sunken spots 1-2 mm. in diameter on the inoculated leaves only (Fig. 5). No systemic symptoms were found.

N. tabacum var. Havana 38. Symptoms were necrotic spots 2-3 mm. in diameter. Later, mottled new growth developed.

C. frutenscens (tabasco pepper). Plants developed light green targets 2-4 mm. in diameter in inoculated leaves. Later, a slight vein clearing developed.

P. hybrida (petunia). Brown necrotic spots 1-2 mm. in diameter developed on the inoculated leaves.

L. esculentum (tomato). Irregular blotchy areas with necrotic spots, epinasty, vascular necrosis and some wilting were caused. The lower leaves later became chlorotic.

Isolate 6

Amaranthaceae

G. globosa (globe-amaranth). Symptoms were a yellow

mottle and distorted leaves. No symptoms were seen on inoculated leaves.

Compositae

Z. elegans (zinnia). Symptoms were vein clearing and slightly puckered new growth. No symptoms developed on inoculated leaves.

Cucurbitaceae

C. sativus (cucumber). This plant was a symptomless carrier of the virus.

Solanaceae

D. tatula (jimson weed). This was an excellent local lesion host. Yellow spots 1-2 mm. in diameter which later became necrotic were produced in large quantities on the inoculated leaves. The plant was not systemically invaded by the virus.

N. glutinosa. Symptoms were white necrotic lesions 1-2 mm. in diameter on the inoculated leaves. All new growth was symptomless.

N. tabacum var. Havana 38. Symptoms were yellow spots 3-5 mm. in diameter. Later, dark green islands and a systemic mottle of white and yellow areas developed.

P. hybrida (petunia). Vein clearing and slightly puckered new growth developed. No lesions developed on inoculated leaves.

Host ranges are summarized in Table IV.

Symptoms of "purified" isolates on dahlia seedlings

Unwin dahlia seedlings, presumably virus free, were inoculated with the "purified" isolates to determine infectivity and to compare symptoms with the original isolates. When dahlia seedlings were inoculated with isolate 1, indistinct yellow spots 2-3 mm. in diameter developed on the rubbed leaves within 6 to 11 days. Later, some spots became necrotic while others developed concentric target rings. Still later, necrotic stem areas developed, lower leaves died and many plants were lost. Isolate 2 caused yellow or dark green mottle, occasionally followed by necrotic areas. Later, petioles and stems became distorted, leaf tips and margins became necrotic and yellow mottled leaf areas developed. Leaf necrosis followed still later, causing death of all lower leaves. No targets developed. Isolate 3 caused yellow spots 2-3 mm. in diameter 10 to 15 days after inoculation. Later, target rings usually developed around the spots. Still later, hieroglyphics and wandering lines developed around the targets or veins, while other targets developed in the new growth.

Seedlings inoculated with isolate 5 developed white necrotic leaf areas which had a tendency to form wide single rings (not targets). Occasionally, dark green islands were seen. These were followed by large brown necrotic or chlorotic areas. Later, petioles became necrotic, followed by death of the leaves, which remained attached to the plant. Isolate 6 caused non-distinctive symptoms consisting of light green spots 2-3 mm. in diameter on the inoculated leaves followed by an indistinct dark green mottle. These leaves later turned yellow, making the spots very conspicuous. Still later, small

scattered necrotic spots appeared in younger leaves, which then enlarged and coalesced, causing death of the leaves. Leaves remained attached to the plant. After a time, symptoms tended to disappear in all seedlings making detection of infected plants difficult or impossible.

Only isolates 1 and 3 caused target patterns or ringspots similar to those in the source plants. Symptoms caused by the other "purified" isolates differed from those on source plants. Change in symptom pattern after "purification" is considered as further evidence that mixtures of viruses were present in the source plants.

Physical properties

Sap was prepared for thermal inactivation tests in two ways. Isolates 1, 3 and 5 were prepared by grinding 1 gm. of infected leaf tissue with 4 ml. of pH 8.5 or pH 9.5 potassium phosphate buffer solution in a mortar. Isolates 2 and 6 were prepared by freezing tissue and then extracting sap according to the following procedure: Infected leaf tissue was sliced in strips about 5 mm. wide, placed in a rubber stoppered 15 ml. nitrocellulose acetate centrifuge tube and frozen 24 hours. The material was then thawed and the end of the tube was fitted into a hole in a wood block. Sap was then extracted by pressure with a 13 x 200 mm. glass rod fitted with a wooden handle (Fig. 6). Liquid was decanted during the extraction. Liquid thus collected was considered "pure sap" and used without further dilution. Sap was held for 10 minutes at each temperature level used (Table VI). G. globosa was used as

TABLE VI

THERMAL INACTIVATION OF DAHLIA VIRUSES

Isolate Number	Experiment Number 1	2[a]	3	4
1	44/48[b]	44/46	46/48	46/48
2	40/50	48/50	48/50	48/50
3	40/50	40/44	40/42	40/42
5	40/50	40/50	44/46	46/48
6	90/92	90/92	94/96	90/

a/ G. globosa was used as source material and to test infectivity of isolate 2; D. tatula was used with other isolates.

b/ Preparation active after 10 minute exposure to 44° C, but inactive after exposure to 48° C for 10 minutes.

a source of sap and to test infectivity of sap carrying isolate 2, but D. tatula was used with isolates 1, 3, 5 and 6.

Each isolate was used in 4 experiments. For each experiment, 5 plants were inoculated for each temperature level and resulting lesions were counted. Thermal inactivation data (Table VI) indicate that isolate 3 was inactivated at the lowest temperature, between 40° and 42° C for 10 minutes. Isolates 1 and 5 required 46° to 48° C for inactivation. Thermal inactivation rate of isolate 2 was still higher (48° to 50° C), while isolate 6 was most stable to heat, requiring 96° C for inactivation.

Isolates 1, 3 and 5 were prepared for dilution experiments by grinding 1 gm. of infected leaf tissue in 9 ml. of 0.05 M potassium phosphate buffer solution (pH 8.5) in a mortar. The resulting preparation was considered a 1:10 dilution and further dilutions were made with the buffer solution. Isolates 2 and 6 were prepared by the method described for thermal inactivation, using distilled water for further dilutions. In addition, isolate 2 was also diluted with buffer in another series of tests. There were 4 dilution end point experiments for each isolate. In each experiment, 5 plants were inoculated for each dilution. Dilution end points (Table VII) show that isolate 3 was infective at a 1:10 dilution, but not at a 1:20 dilution. Isolate 6 was the most tolerant to dilution, being infective at 1:1200 but not at 1:1400. Other isolates fell between these extremes (Table VII). When water was substituted for buffer, isolate 2 could not be diluted above 1:64 and remain infective. Dilution

TABLE VII

EFFECT OF DILUTION ON INFECTIVITY OF DAHLIA VIRUSES

Isolate Number[a]	Diluent	Experiment Number 1	2	3	4
1	Buffer[b]	100/1000[c]	100/200	100/150	100/150
2	Buffer	100/1000	200/400	200/400	200/300
2	Distilled water	10/100	10/100	60/64	60/64
3	Buffer	10/100	10/50	10/20	10/20
5	Buffer	100/1000	100/200	200/400	200/250
6	Buffer	1000/10000	100/10000	1000/1200	1200/1400

a/ Datura tatula leaves were ground as source material for isolates 1, 3, 5 and 6; D. tatula was used to test infectivity. Gomphrena globosa was similarly used for isolate 2.

b/ The buffer solution was 0.05 M potassium phosphate, pH 8.5

c/ Preparation active when diluted to 1:100, but not active to 1:1000. Values were determined by using 5 plants at each dilution level.

end points of isolates 3 and 6 are sufficiently lower and higher, respectively, to differentiate them from the other isolates. Isolate 2, infectious when diluted with distilled water, can be separated in this manner from isolates 1 and 5, which are not infectious when diluted with water. The results of these experiments further indicate that the isolates are different viruses.

Sap was prepared as described for thermal inactivation experiments in order to determine stability of the virus isolates to ageing in vitro. Sap samples were held in test tubes at 22° C and were removed at intervals and rubbed on test plants. The experiment was repeated 3 times for each isolate, inoculating 5 plants per isolate per time interval in each experiment. Ageing in vitro data (Table VIII) suggest differences among some of the isolates. Ability of isolates 1, 3 and 5 to withstand ageing were the same, being infectious at 6 to 8 hours but not after 12 to 16 hours. Isolate 2 remained infectious longer and was active at 32 hours but not at 40. Isolate 6 remained infectious in crude sap much longer than the other isolates and was still active at 480 hours. Many bacteria were observed in the sap extracts of D. tatula which may have affected the results. Isolate 2, more easily transmitted than isolates 1, 3 and 5, was extracted from G. globosa, making these results not strictly comparable to those on the other isolates. These results, while probably influenced by saprophytic bacteria, original concentration of the various isolates, and other factors, nevertheless do support the idea that isolates 2 and 6 differ from each other and from the other isolates.

TABLE VIII

EFFECT OF AGEING IN SAP ON INFECTIVITY OF DAHLIA VIRUSES

Isolate Number[a]	Experiment Number 1	2	3	4
1	0/8[b]	0/8	8/16	6/8
2	24/48	40/48	32/40	32/40
3	0/12	0/12	8/16	8/12
5	0/24	0/12	8/12	8/12
6	240/	240/	480/720	480/600

a/ D. tatula was used for isolates 1, 3, 5 and 6; G. globosa was used for isolate 2.

b/ Preparation active when aged 0 hours, but not active after 8 hours. Each value was obtained from 5 inoculated plants.

The ability of dry leaves to maintain infectious virus was tested by placing 1 gm. quantities of infected leaf tissue between paper towels on the greenhouse bench, out of direct sunlight. Temperatures ranged from 22° to 32° C during the trials. At appropriate intervals, samples were removed from the towels, ground with 4 ml. of phosphate buffer in a mortar and the resultant "sap" was quickly rubbed on test plants. Source and assay plants were D. tatula for isolates 1, 3, 5 and 6, and G. globosa for isolate 2. Four experiments were conducted for each isolate. In each experiment 5 plants were inoculated for each assay. Results (Table IX) again indicated differences between the isolates. Isolates 2 and 3 were not active at 72 hours, isolate 5 was active at 72 but not at 96 hours, and isolate 1 was infectious at 96 but not at 120 hours. Again, the most stable isolate was 6 which was still infectious at 720 hours. These results clearly separate isolate 6 from the others, but differences between the rest are more subtle. Although differences between the first 5 isolates are small, they remained essentially the same in all 4 experiments which suggests that virus concentrations in the donor plants were uniform from one experiment to another.

When infected leaf tissue was frozen, thawed and assayed for infectivity, only isolates 2 and 6 remained active. Repeated trials produced an occasional infected plant with isolate 5, but isolates 1 and 3 were always inactive after freezing. In a different experiment isolate 2 (the only isolate tested) was still active after being frozen for 14 weeks. Accordingly, freezing infected leaves

provided a useful means of storing isolates 2 and 6 and a further indication that the isolates were not the same virus.

Tests to determine the ability of the isolates to survive quick drying were conducted by placing 5 mm. strips of infected leaf tissue in small beakers. The material was then dried at room temperature and at 0° - 4° C under partial vacuum in desiccators containing calcium chloride. The dry material was pulverized with a glass rod and placed in small vials under the same conditions. Periodically, tissue was removed, ground with phosphate buffer in a mortar and assayed for infectivity on D. tatula or G. globosa. When dried and stored at 0° - 4° C, isolate 1 was found in very low concentration after 30 days. The preparations of isolates 2, 5 and 6 did not seem to be affected by storage for 30 days under the same conditions, while isolate 3 was inactivated by the treatment. Earlier experiments suggested that only isolates 2 and 6 were able to withstand desiccation at room temperature. Isolate 2, desiccated at room temperature, was still active at 16 weeks. Isolate 6, desiccated at room temperature, was still active at 7 weeks. In another test of isolates 2 and 6, the viruses survived freeze drying with no apparent loss of activity. Thus, quick drying further differentiated the isolates and also provided a convenient means of storage.

Cross protection

Cross protection experiments were designed to test the relationship of isolate 6 to the other isolates. Five separate test plants were inoculated with each isolate. Later, leaves showing strong systemic symptoms were inoculated with isolate 6. In every

TABLE IX

EFFECT OF AGEING DRIED HOST LEAVES ON INFECTIVITY OF DAHLIA VIRUSES

Isolate Number[a]	Experiment Number 1	2	3	4
1	96/120[b]	96/120	96/120	96/120
2	48/96	48/72	48/72	48/72
3	48/96	48/72	48/72	48/72
5	72/96	72/96	72/96	96/120
6	240/480	384/	720/	720/

a/ D. tatula was used for isolates 1, 3, 5 and 6; G. globosa was used for isolate 2.

b/ Preparation active at 96 hours, but not after 120. Each value was determined by inoculating 5 plants.

trial, D. tatula and G. globosa infected with isolates 1, 2, 3 and 5 were not protected against later invasion by isolate 6. The usual symptoms of isolate 6 developed on all assay plants, but in a slightly milder form. These results indicate that isolate 6 is not related to the other isolates. The experiment was repeated with the same results.

DISCUSSION

The possible existence of virus mixtures in dahlia plants was considered early in this work. Brierly (2) observed that dahlia ringspot and dahlia mosaic often occurred together. He also described various necrotic and chlorotic spots on dahlia leaves but was unable to transmit the causal agents. Similar observations in the present study indicated the possible presence of virus mixtures in dahlias. Host ranges of the isolates before and after "purification" by single lesion isolates are good evidence that this is true. Reduced host ranges of the "purified" isolates suggest that certain components of mixtures were separated. For example, isolate 3 lost the ability to infect 6 species of 4 families after "purification". Furthermore, isolates 2 and 5 caused mottle rather than ringspot in dahlia plants grown from seed, even though the isolates came from host plants with good target spots. Finally, isolate 6 was obtained from a systemically infected petunia plant inoculated with isolate 5. Isolate 6 was transferred back to dahlia. The only conclusion available to fit these facts is that mixtures of viruses were present in the source plants.

The second important question considered was the systematic relationship of the virus isolates to each other. Differences and similarities soon became apparent. Only isolates 1 and 3 caused ringspot on dahlia seedlings, while the other isolates caused mottle or other non-distinctive symptoms. Many host range differences were found. Of 16 species tested, only 4 plants (dahlia, D. tatula, G. globosa and P. hybrida) were hosts of all the isolates. Phytolacca decandra was a host of isolate 2, but the other isolates did not cause visible symptoms on this plant. Isolate 3 was the only isolate that did not affect N. tabacum var. Havana 38. D. tatula was an excellent local lesion host for isolate 6; other isolates were systemic in this plant. Thus, isolates 2, 3 and 6 can easily be separated from each other and from isolates 2 and 5. Physical property studies add further evidence that isolates 2, 3 and 6 differ from each other and from isolates 1 and 5. Host ranges and symptom expressions were similar between isolates 1 and 5, making them difficult to separate in this way. Although host range differences between isolates 1 and 5 are subtle, other experiments show some differences. When inoculated under short day conditions, isolate 5 produced 2 times more lesions than under long day conditions, while isolate 1 produced the same number of lesions under both conditions. Physical properties also indicate a relationship between isolates 1 and 5, although there were minor differences. The tentative conclusion from this data is that isolates 2, 3 and 6 should be classified as different viruses. Isolates 1 and 5 are tentatively considered as strains of one virus which again differs from the other viruses.

The third important consideration of the work was whether or not the virus isolates are identical with viruses previously described in the literature. It has been suggested that dahlia ringspot virus, tomato spotted wilt virus (TSWV), cucumber mosaic virus (CMV) and perhaps still other viruses may be present in dahlias. Indeed, the concentric rings or "targets" caused in dahlia seedlings by isolates 1 and 3 are essentially the same as those described by Brierly (2), Holmes (4) and Smith (10). Smith (10) identified TSWV in dahlias and by inoculation experiments showed that mottle appeared on new growth, after ringspot symptoms had disappeared.

Although isolates 1, 3 and TSWV cause similar symptoms on dahlias, host ranges (Table X) and some properties differ. In addition to host range differences, isolates 1, 3 and TSWV differ in the symptoms expressed on common hosts. TSWV causes a characteristic leaf bronzing on tomato. This was not observed on tomatoes infected with isolates 1 and 3. N. glutinosa reacts to TSWV by severe systemic invasion which usually kills the plant. Isolate 1 caused only local lesions, while isolate 3 caused no visible effect. Zinnia was not a host of isolates 1 and 3, but is easily infected with TSWV. Petunia is a good host for TSWV, but the virus is seldom systemic; the plant is difficult to inoculate with isolate 3, but systemic invasion was the rule. Thus virus isolates 1, 3 and TSWV have common symptoms on dahlia, but differ in host range. Isolate 1 shares the host range of TSWV more closely than does isolate 3.

Some physical properties of isolates 1, 3 and TSWV are compared in Table XI. Thermal inactivation points and resistance

TABLE X

COMPARATIVE HOST RANGE OF TOMATO SPOTTED WILT VIRUS AND DAHLIA VIRUS ISOLATES 1 AND 3

Host Plants[a/]	Virus 1	Virus 3	TSWV[b/]
Capsicum frutescens	x	x	x
Dahlia variabilis (Unwin hybrid)	x	x	x
Lycopersicon esculentum	x	x	x
Nicotiana glutinosa	x	o	x
Petunia hybrida	x	xx	x
Zinnia elegans	o	o	x

a/ X = easily infected host; xx = difficultly infected host; o = non-host.

b/ Data from Bald and Samuel (1).

TABLE XI

COMPARATIVE PHYSICAL PROPERTIES FOR TOMATO SPOTTED WILT VIRUS (TSWV) AND DAHLIA VIRUS ISOLATES 1 AND 3

Property	Virus 1	Virus 3	TSWV[a/]
Thermal inactivation	46/48[b/]	40/42	42/45
Dilution end point	100/150[c/]	10/20	10,000/100,000
Resistance to ageing *in vitro*	6/16[d/]	8/12	0/5

a/ Data by Bald and Samuel (1).

b/ Thermal inactivation point between 46° and 48° C.

c/ Dilution end point between 1:100 and 1:150.

d/ Resistance to ageing *in vitro* between 6 and 16 hours.

to ageing *in vitro* are similar, but dilution end points vary widely. On the basis of the host range and property differences, isolate 3 probably is not a strain of TSWV, but isolate 1 may be related to TSWV.

Isolate 2 has many hosts in common with TSWV. However, isolate 2 does not affect tomato, goes with difficulty to some species which are excellent hosts for TSWV, and goes easily to species not included in the TSWV host range. Thermal inactivation points for the two viruses are similar, but dilution end point of isolate 2 is much lower and resistance to ageing *in vitro* is greater than that recorded for TSWV. Thus symptomatology, host range and physical properties differentiate isolate 2 from TSWV.

Isolate 5 has many hosts in common with TSWV. However, symptoms differ in some plants such as tomato, in which isolate 5 does not produce the TSWV bronzing symptoms, and *N. glutinosa*, in which isolate 5 produces local lesions. In common with isolate 2, isolate 5 infects species not recorded as hosts of TSWV. Thermal inactivation and dilution end points of TSWV and isolate 5 differ. On the basis of host range and properties, isolate 5 probably is not TSWV.

Repeated attempts to infect tomato with isolate 6 failed. Host ranges and properties of isolate 6 and TSWV differ enough to conclude that isolate 6 is not TSWV.

Isolates 2 and 5 infect some hosts of dahlia mosaic virus (DMV), although the latter has a more restricted host range. There are differences in symptoms, ease of transmission and physical properties. Isolate 6 has only one host plant in common with DMV,

Z. elegans. This isolate also differs from DMV in some physical properties.

Dahlia ringspot (DRV), yellow ringspot and oakleaf viruses have been identified only by symptom expression on dahlias (2). On this basis, isolate 1 or 3 or both could be DRV. Brierly (2) was not able to transmit DRV mechanically, but considering the difficulties involved in transmitting the present isolates from dahlia, this objection does not differentiate them from DRV.

Smith (11) described CMV as causing a mottle of light and dark green patches on dahlia leaves, or light green whorls or ring and line patterns differing from the clear-cut concentric rings of TSWV. Brierly (3) described CMV symptoms as a mild, diffuse mottle. Isolates used in the present study gave similar symptoms, but host ranges and physical properties show that CMV was not included in the isolates.

Other viruses that could be similar to the isolates are Potato Viruses A and Y, Tobacco Streak Virus, and Tomato Ringspot Virus. Host ranges, symptomatology and physical properties also rule out these viruses.

Isolate 6 was found in a petunia plant which developed systemic vein clearing after inoculation with isolate 5 from D. tatula. This indicates that petunia may have acted as a filter, leaving isolate 5 in local lesions and allowing systemic invasion by isolate 6. Possibly D. tatula limits multiplication of isolate 6 but allows isolate 5 to build up. Once free of isolate 5, isolate 6 caused profuse local lesions in D. tatula. There is no direct

evidence for this reasoning, but when first isolated from petunia or dahlia seedlings, isolate 6 caused only a few lesions on D. tatula, indicating a low concentration in the donor. A single subinoculation in D. tatula resulted in many lesions.

SUMMARY

Five virus isolates from dahlias were mechanically transferred to 16 plant species in 8 families. Two of the isolates (1 and 3) caused ringspots on dahlia seedlings but the others caused non-distinctive symptoms. Mixtures of viruses apparently were present in donor plants because there were significant changes in host range before and after single lesion transfer "purification". The isolates were further separated from each other and from known viruses by physical properties. Thermal inactivation points ranged from 40° to 94° C, dilution end points varied from 1:10 to 1:1200, resistance to ageing in vitro ranged from 8 to 480 hours, resistance to ageing in drying leaves ranged from 48 to beyond 720 hours. All isolates resisted desiccation except isolate 3. Host range and physical property experiments led to several conclusions. Isolates 1 and 5 may be related to Tomato Spotted Wilt Virus (TSWV). Isolates 2 and 6 are not readily identified with known viruses. Isolate 3 differs from TSWV and is considered to be dahlia ringspot virus. Other viruses known to affect dahlia were not found.

LITERATURE CITED

1. Bald, J.G. and G. Samuel. 1931. Investigations on spotted wilt of tomatoes II. Austr. Counc. Sci. & Ind. Res. Bull., 54.
2. Brierly, P. 1933. Studies on mosaic and related diseases of dahlia. Contrib. Boyce Thompson Inst. 5: 235-288.
3. Brierly, P. 1951. V lue of index plants for detecting dahlia viruses. Plant Disease Reptr. 35: 405-407.
4. Holmes, F.O. 1948. Resistance to spotted wilt in tomato. Phytopath. 38: 467-473.
5. Holmes, F.O. 1955. Elimination of spotted wilt from dahlias by the propagation of tip cuttings. Phytopath. 45: 224-226.
6. Jensen, J.H. 1933. Isolation of yellow mosaic viruses from plants infected with tobacco mosaic. Phytopath. 23: 964-974.
7. Martin, C. 1952. Insolement d'une Souche de Virus Y de La Pomme de Terre (Marmor upsilon Holmes) à Partir du Dahlia Et de La Tomate. Ann. Inst. Rech. Agron. Paris, Ser. C (Ann. Epiphyt. 3: 394.
8. Mildner, R. A. 1959. Virus Diseases of the Dahlia. Bul. Amer. Dahlia Soc. Ser. XXXII 2: 4-;0, 3: 4-6.
9. Roland, G. 1948. Les virus des taches bronzees de la tomato (Lycopersicum virus 3, Brittlebank) et de la mosaique du concombre (Cucumis virus 1, Doolittle) sur dahlia. Parasitica 4: 98-102.

10. Smith, K. M. 1932. Studies on plant virus diseases. XI. Further experiments with a ringspot virus: Its identification with spotted wilt of the tomato. Ann. Appl. Biol. 19: 305-330.

11. _____. 1945. Dahlias and the problem of virus infection. Gard. Chron. 118: 140-141.

12. Yarwood, C.E. 1953. Quick virus inoculation by rubbing with fresh leaf disks. Plant Disease Reptr. 37: 501-503.

FIGURE 1

EQUIPMENT USED FOR THERMAL INACTIVATION EXPERIMENTS

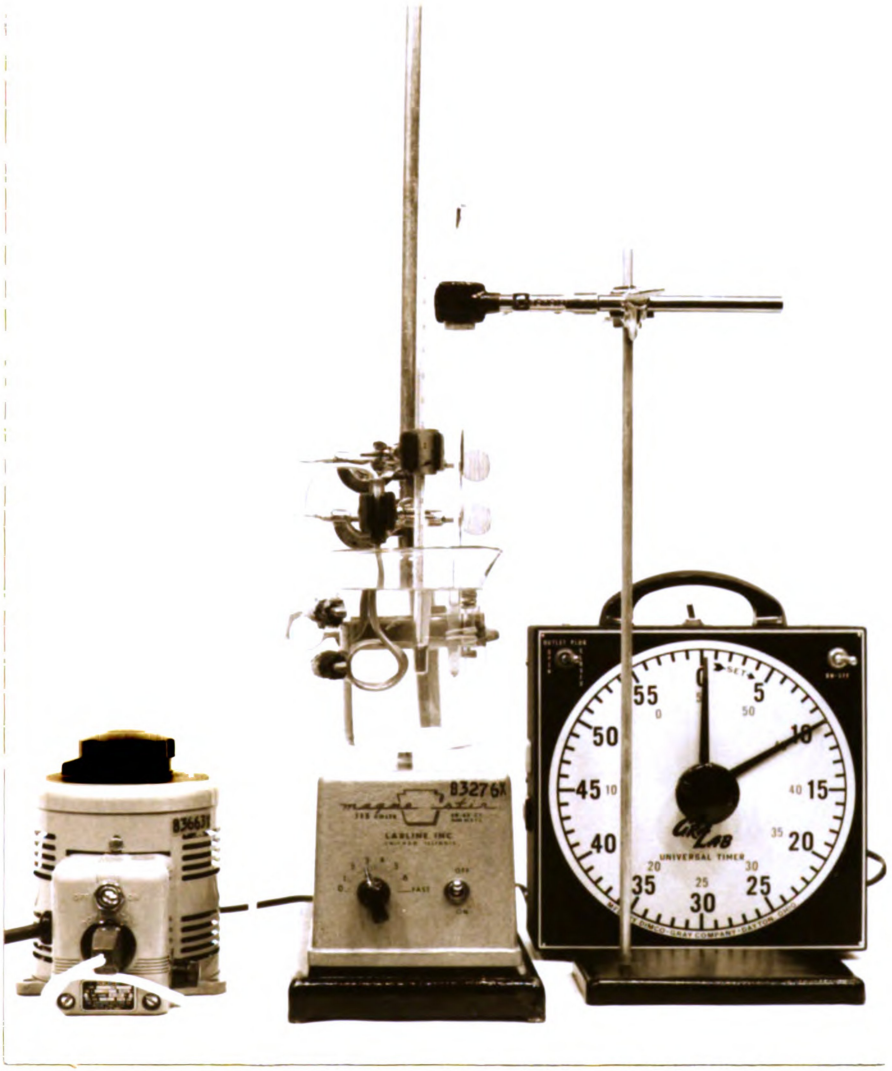
83663
83276
LABLINE INC.
OFF
FAST
UNIVERSAL TIMER

FIGURE 2

VIRUS RINGSPOT SYMPTOMS ON FIELD GROWN
DAHLIA LEAVES WHICH WERE THE SOURCE OF ISOLATE 3

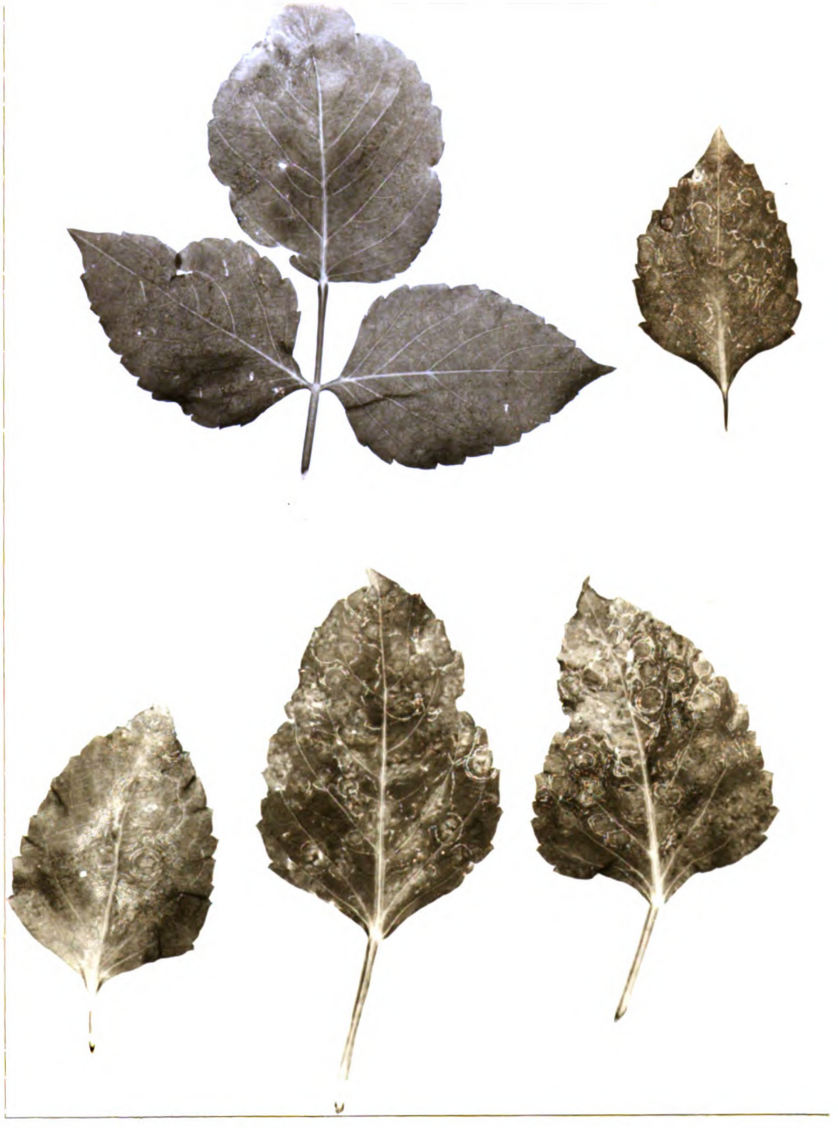

FIGURE 3

VIRUS DISEASE SYMPTOMS ON Gomphrena globosa BEFORE AND AFTER SINGLE LESION TRANSFER "PURIFICATION". A, HEALTHY CONTROL; B, DISEASED PLANT BEFORE "PURIFICATION"; C, HEALTHY CONTROL; D, DISEASED PLANT AFTER "PURIFICATION".

Table 2


A
B


C
D

FIGURE 4

SYMPTOMS PRODUCED BY ISOLATE 1 ON TOMATO.
A, HEALTHY CONTROL; B, DISEASED PLANT.


A
B

FIGURE 5

SYMPTOMS CAUSED BY ISOLATE 5 ON Nicotiana glutinosa.
A, HEALTHY CONTROL; B, DISEASED PLANT.

A

B

FIGURE 6

EQUIPMENT USED FOR EXPRESSING SAP FROM PLANT LEAVES

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