A VIRUS DISEASE OF WATERMELON IN WISCONSIN INCITED BY THE TOBACCO RINGSPOT VIRUS[1]

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INTRODUCTION

In August 1946, marked viruslike symptoms were observed on watermelon (*Citrullus vulgaris* Schrad.) and muskmelon (*Cucumis melo* L.) plants in a field at Merrimac, Wis. Inoculations from infected plants to tobacco (*Nicotiana tabacum* L.), cucumber (*Cucumis sativus L.*), and watermelon demonstrated that the disease was of virus origin. Isolates from watermelon and muskmelon were identical, and one from watermelon was chosen for further study. In 1947 plantings in the same area were surveyed; and again the same virus was recovered from both watermelon and muskmelon, although the incidence was lower than in 1946. In 1948 the disease appeared in much greater proportion than in the two preceding years, affecting about 10 percent of the watermelon plants. Considerable loss resulted. This paper is a description of symptoms of the disease in the field and on certain host plants in the greenhouse, as well as a description and identification of the virus. A preliminary report has already been made (*9*).[3]

MATERIALS AND METHODS

The virus studied was obtained from a single watermelon plant growing at Merrimac, Wis. Stock cultures were kept in tobacco in aphid-proof cages. Inoculations were made by the rubbing method on plants previously sprinkled with carborundum. Greenhouses were fumigated frequently for aphid control. Air temperatures ranged 75° to 80° F. except where otherwise stated. In the host-range study, at least five plants were used in each test inoculation. From each host inoculated, recovery inoculations were made to a suitable test plant to prove the presence or the absence of the virus. Property studies were made according to methods previously detailed (12).

[1] Received for publication January 31, 1949.

[2] Appreciation is expressed to Eugene Herrling for making the photographs used in this manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 658.

EXPERIMENTAL RESULTS

SYMPTOMS AND HOST RANGE

Field Symptoms on Muskmelon and Watermelon

Naturally infected muskmelon plants showed marked stunting and a yellow-green leaf mottle accompanied by leaf malformation. Fruit set and size were greatly reduced. Symptoms were indistinguishable from those caused by viruses of the *Cucumber virus* 1 group, except that after several weeks, symptoms produced by the watermelon virus became considerably masked. Infected watermelon plants were severely stunted and chlorotic, and they produced no marketable fruits. Fruits which set were warty and occasionally exuded small drops of a viscous liquid at necrotic points on the surface. A diagnostic feature of infected vines was the upright position of distal ends of runners compared with the nearly prostrate position of healthy plants. This characteristic was discernible from a considerable distance and provided an easy way of locating infected vines. Leaf symptoms on watermelon consisted of a coarse mottle accompanied by irregular black, necrotic lesions (fig. 1, *A*). Affected leaves, when tattered and necrotic, somewhat resembled symptoms induced by *Colletotrichum lagenarium* (Pass.) E. & H. Internodes were markedly shortened, producing a compact, bunchy growth (fig. 2). Infected leaves and stems were exceedingly brittle. Infected plants showed some recovery toward the end of the season, but symptoms were much more persistent than for muskmelon.

Greenhouse Reaction on Tobacco

Local symptoms on inoculated leaves of Havana tobacco appeared first as faint chlorotic rings and ringspots but later became etched or necrotic, depending upon the temperature. Systemic symptoms appeared as concentric chlorotic rings and ringspots which often were conspicuously yellow and numerous enough to produce a mottled effect. Often a jagged (oak-leaf) pattern was associated with the veins (fig. 3, *B*). Finally, leaves lost the ringspot patterns and developed a conspicuous diffuse yellowing (fig. 3, *A*). Eventually, masking was almost complete, but yellowing was persistent on the lower leaves.

Greenhouse Reaction on Bean and Pea

Every variety of bean tested was found to be susceptible to the virus, but three did not develop pronounced systemic symptoms. The reaction was almost wholly necrotic, occasional varieties showing some mottling. Primary symptoms usually consisted of reddish-brown necrotic flecks, spots, or ringspots with occasional streaking of the veins (fig. 4, *A*). The most characteristic systemic symptom was top necrosis, a necrotic blighting of the growing tip. In practically all varieties in which systemic development occurred, the terminal growing point withered and died within 7 days after inoculation as if killed by steam (fig. 5). Occasionally plants recovered from this top necrosis and from lateral buds produced severely stunted leaves with

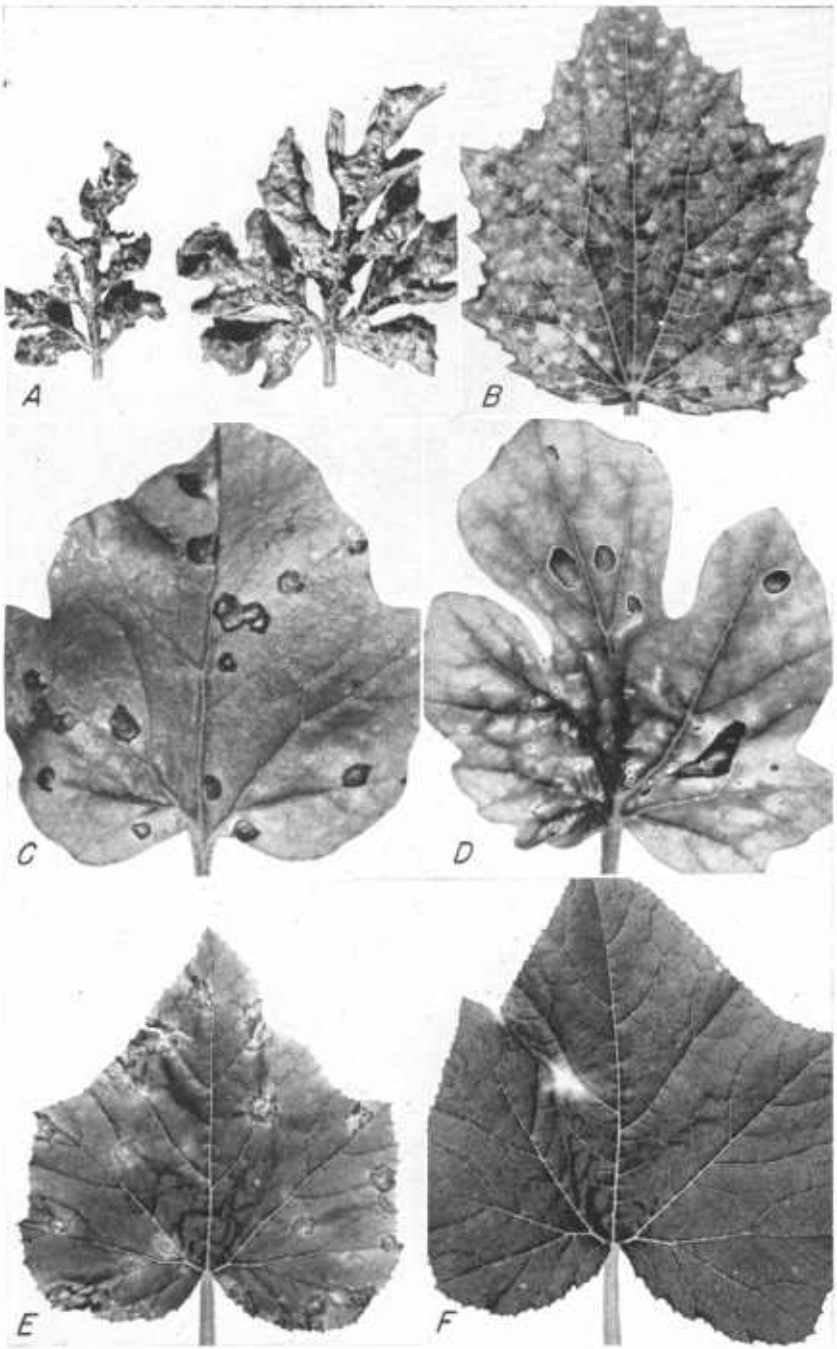

FIGURE 1.—Symptoms produced on various cucurbits by the watermelon virus. *A*, Systemic symptoms of naturally infected watermelon; *B*, systemic symptoms on inoculated cucumber; *C*, *D*, local and systemic symptoms, respectively, on inoculated watermelon; *E*, *F*, local and systemic symptoms, respectively, on inoculated Early Prolific squash.

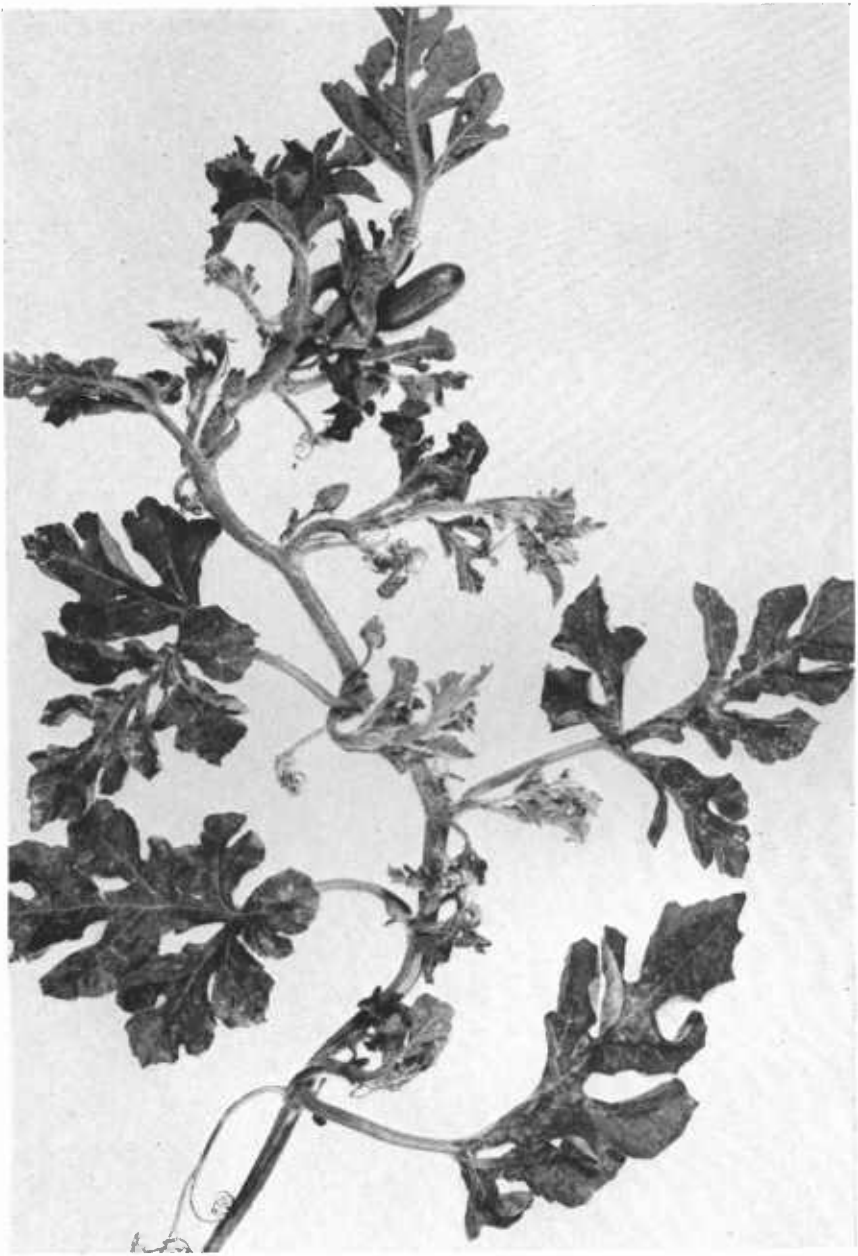

FIGURE 2.—Portion of naturally infected watermelon vine, showing much shortened internodes.

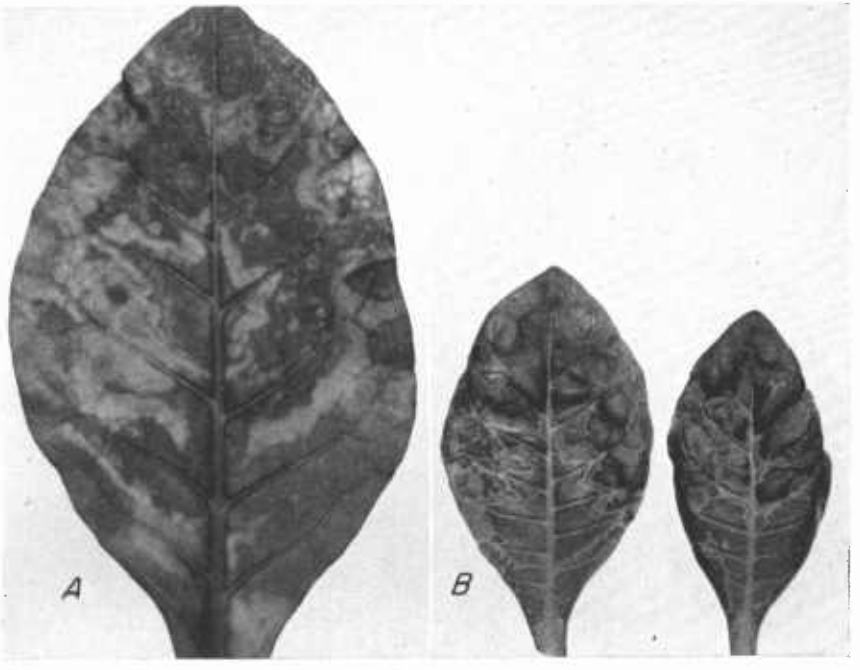

FIGURE 3.—Leaves of Havana tobacco systemically infected with the watermelon virus. *A*, Old leaf showing persistent diffuse yellowing; *B*, young leaves showing ringspotting and oak-leaf pattern.

a coarse mottle (fig. 4, *B*). Other characteristic systemic symptoms were necrotic cankers on main stem, leaf petioles, and necrotic flecking or spotting of leaves (fig. 4, *C*). The following varieties showed systemic development as indicated by tip blight, leaf mottle, and necrosis, and necrotic cankers of petioles and stems: Florida Belle, Stringless Black Valentine, Tenderpod, Tendergreen, Bountiful, Plentiful, Keystonian, Sensation Green Pod, Sensation Refugee 1066, Striped Creaseback, Kentucky Wonder, Blue Lake, Full Measure, Rival, U. I. No. 1, and Pinto. Varieties Michelite, Robust, and Rust Resistant Kentucky Wonder showed no systemic development of tip blight or leaf necrosis but did develop occasional cankers of the main stem above the inoculated leaves. Stringless Green Refugee developed necrotic cankers of petioles and stem and slowly developing necrotic lesions of top leaves, but no tip blight. Rarely did more than one necrotic spot occur on one leaf. All bean varieties were tested at air temperatures of 16°, 20°, and 28° C. Symptom type was not changed by temperature, but incubation periods were shorter and symptoms were more severe at high temperatures.

The pea varieties Alaska, Delwiche Commando, Merit, Pride, and Prince of Wales all showed identical reactions. Inoculated leaves developed irregular necrosis; systemic symptoms were severe stunting, yellow mottling (fig. 4, *D*), and necrotic streaking of the main stem followed by death. In no case did inoculated plants survive infection.

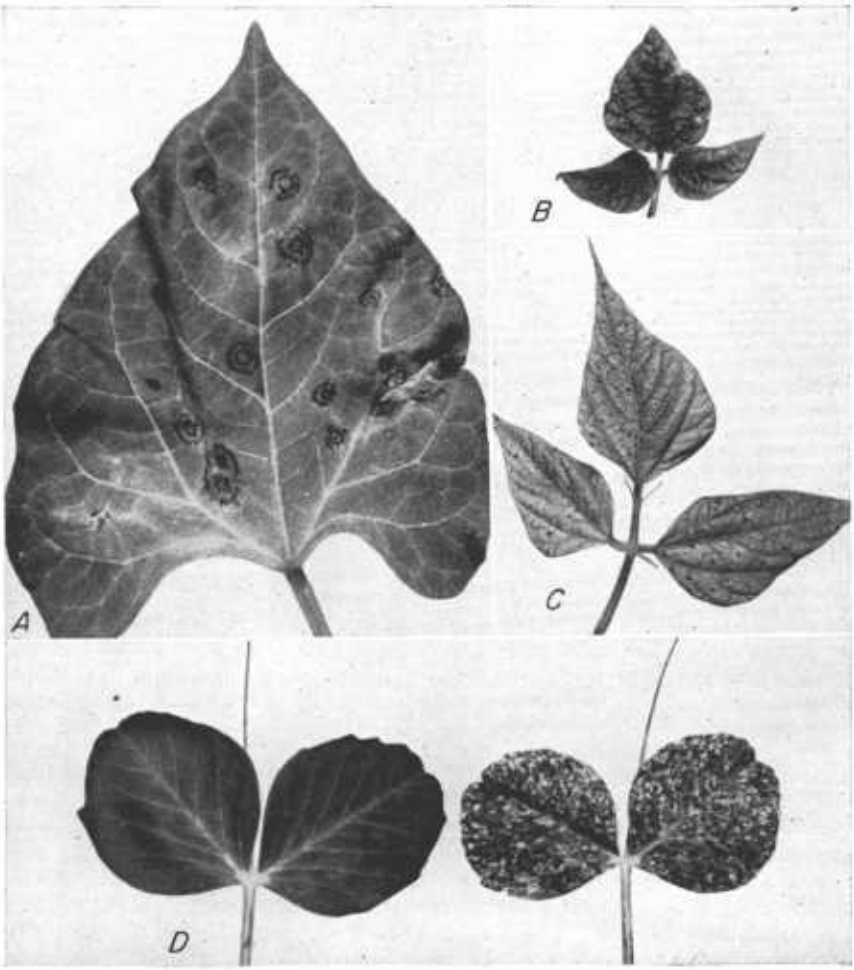

FIGURE 4.—Symptoms induced on bean and pea by the watermelon virus. *A*, local lesions on primary leaf of Pinto bean; *B*, systemic mottle; *C*, systemic necrotic flecking produced on Florida Belle bean; *D*, healthy (left) and systemically infected leaf of Alaska pea.

GREENHOUSE REACTIONS OF OTHER HOSTS TESTED

In table 1 are given the symptoms produced on selected greenhouse plants. In all cases, except where the contrary is indicated, the virus was recovered from each host which produced symptoms. The reactions on cucumber, watermelon, and squash are shown in figure 1.

EFFECT OF TEMPERATURE ON SYMPTOM EXPRESSION

The effect of air temperature on symptom development was studied on tobacco and cucumber. On cucumber, chlorotic rings were pro-

duced on inoculated cotyledons at 28° and 24°, while there was severe bleaching at 20° and 16°. Systemic symptoms developed first at 28° and last at 16°. At all temperatures, conspicuous chlorotic mottling occurred. At the high temperatures there was a marked tendency toward recovery while at low temperatures symptoms were persistent.

On tobacco, local symptoms ranged from chlorotic rings and ringspots at 28° to conspicuous necrotic rings at 16°. Systemic symptoms appeared at 28° and 24° as conspicuous rings and ringspots with considerable stunting and distortion. Chlorotic rings were gradually replaced by a diffuse yellowing which was persistent on old leaves but

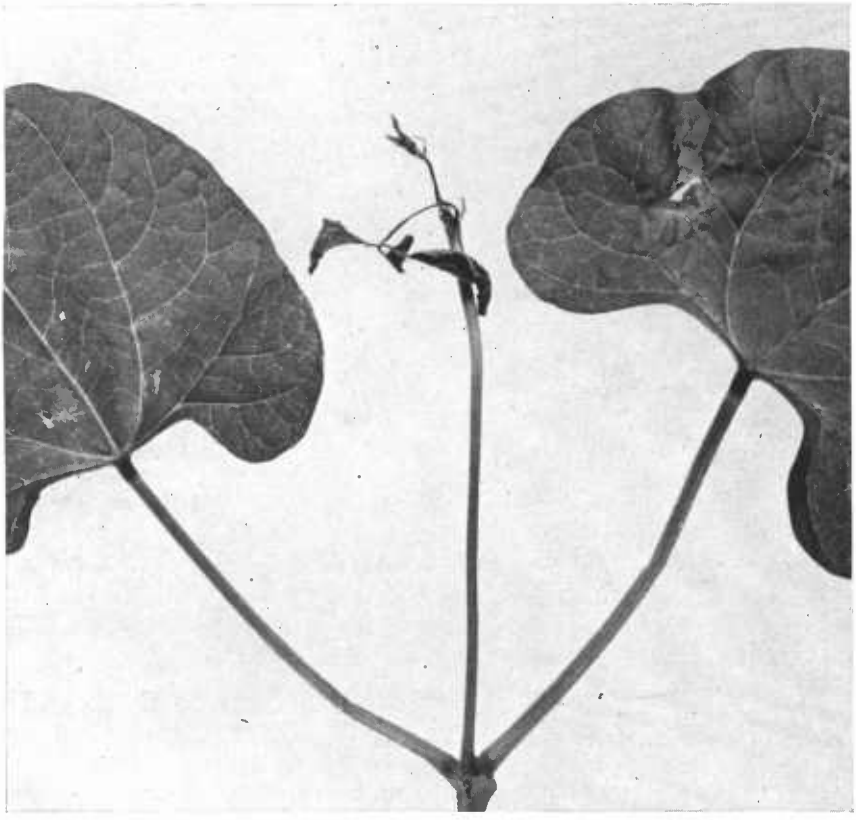

FIGURE 5.—Characteristic top necrosis induced in bean by the watermelon virus.

from which young leaves recovered. At 20° the yellowing was less diffuse and tended to occur as chlorotic spots and blotches. Occasional plants produced necrotic rings. At 16° severe systemic necrosis and very little chlorotic ringing or yellowing occurred. Plants at the low temperatures did not exhibit recovery in the course of the experiment.

TABLE 1.—*Symptoms produced by the watermelon virus on selected greenhouse plants*

Host family and species	Local symptoms	Systemic symptoms
Cucurbitaceae:		
Cucumis sativus L. (cucumber, vars., Maine No. 2, Ohio 31).	Small chlorotic rings followed by general chlorosis of cotyledons; chlorotic lesions on older leaves.	Yellow mottle with distinct chlorotic spots followed by diffuse chlorosis in which lesions lose distinctiveness; severe stunting.
Cucumis melo L. (muskmelon, vars. Golden Delicious, Early Knight).	Chlorotic lesions becoming necrotic and zonate.	Same as above; eventually masked.
Citrullus vulgaris: Schrad. (watermelon, var. Early Kansas).	Irregular to concentrically zonate necrotic lesions.	Severe stunting, distortion of leaves; irregular chlorotic mottle with necrosis.
Cucurbita maxima Duchesne (squash, vars. Early Prolific, Blue Hubbard).	Conspicuous chlorotic lesions followed by extensive necrotic areas.	None for Blue Hubbard and virus not recovered. Occasional single large chlorotic lesions for Early Prolific.
C. pepo L. (pumpkin, vars. Connecticut Field, Sugar).	do	Severe yellow mottle with distortion and stunting. Few plants infected.
Compositae:		
Aster amellus L. (aster)	Necrotic lesions	Yellow-green mottle followed by irregular necrosis.
Calendula officinalis L. (calendula, var. Orange King).	None	None; virus recovered.
Helianthus annuus L. (sunflower, var. Mammoth Russian).	Scattered necrotic lesions	Coarse yellow-green mottle.
Zinnia elegans Jacq. (zinnia, var. Liliput).	Chlorotic lesions	Chlorotic mottle.
Cruciferae:		
Hesperis matronalis L. (dames violet).	None	Slight yellow-green mottle.
Thlaspi arvense L. (pennycress)	do	None; virus recovered.
Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	do	Do.
Brassica napus L. (rape, var. Dwarf Essex).	do	None.
B. oleracea botrytis L. (broccoli, var. Italian Green Sprouting).	do	Do.
B. oleracea gemmifera Zenker (brussels sprouts, var. Long Island Improved).	do	Do.
Leguminosae:		
Pisum sativum L. (pea, vars. Alaska, Pride, Merit, Commando, Prince of Wales).	Irregular necrosis and chlorosis	Severe mottle followed by death of plant.
Melilotus alba Desr. (white sweetclover).	None	None; virus recovered.
M. indica All. (yellow sweetclover).	do	Do.
Phaseolus vulgaris L. (bean. See page 5 for varietal reactions).	Necrotic flecks, streaks or ringspots.	Severe necrosis of growing tip, necrotic flecks and ringspots on leaves and elongate cankers on petioles and stem; severe stunting and mottling on leaves from adventitious buds.
Trifolium pratense L. (red clover).	None	None, virus recovered.
Vigna sinensis Endl. (black cowpea).	Numerous red necrotic lesions	Blotchy mottle with irregular necrosis of stem and petioles; often lethal.
Vicia faba L. (broad bean)	Irregular black necrosis	Black necrosis of leaves and stem lethal.
Solanaceae:		
Capsicum frutescens L. *grissum* Bailey (pepper, var. California Wonder).	None	None.
Lycopersicon esculentum Mill. (tomato, var. Bonny Best).	do	Do.
Nicotiana tabacum L. (tobacco, var. Conn. Havana No. 38).	Scattered etched rings; occasional circular necrotic lesions.	Concentric chlorotic ringing and ringspotting with slight leaf puckering; later replaced by conspicuous diffuse yellowing: in transition from ringing to yellowing marked jagged, lightninglike pattern common; partial masking.
N. glutinosa L.	Very faint chlorotic rings and ringspots.	Faint chlorotic rings and ring spots becoming etchedlike; often no symptoms; complete masking.

TABLE 1.—*Symptoms produced by the watermelon virus on selected greenhouse plants*—Continued

Host family and species	Local symptoms	Systemic symptoms
Solanaceae—Continued		
N. rustica L.	Etchedlike ringing and ringspotting.	Broad, chlorotic rings on young leaves becoming markedly zonate.
Petunia hybrida Vilm. (petunia)	Chlorotic and necrotic lesions	Chlorotic mottle.
Solanum triflorum Nutt.	Scattered necrotic lesions	Chlorotic mottle; stunting and distortion.

PROPERTIES OF THE VIRUS

Maine cucumber plants were used in determining the physical properties. Inoculum in each case was taken from recently infected tobacco plants. The results are given in table 2. The points at which inactivation occurred (dilution, 1 to 100,000; aging in vitro, 5 days at 20° C.; thermal inactivation, 70° for 10 minutes) were similar to those reported for *Cucumber virus* 1 and the tobacco ringspot virus.

TABLE 2.—*Physical properties of the watermelon virus as determined on cucumber*

Type and degree of treatment	Ratio of cucumber plants infected to those inoculated in trial—			
	1	2	3	Total
Dilution:				
0	30/30	20/20	100/100	150/150
1-10	30/30	19/20	100/100	149/150
1-100	29/30	14/20	100/100	143/150
1-1,000	9/30	8/20	9/100	26/150
1-10,000	0/30	2/20	2/100	4/150
1-100,000	0/30	0/20	0/100	0/150
Aging in vitro (hours at 20° C.):				
0	30/30	24/24	50/50	104/104
24	30/30	24/24	50/50	104/104
48	21/30	24/24	43/50	88/104
72	16/30	18/24	31/50	65/104
96	5/30	1/24	0/50	6/104
120	0/30	0/24	0/50	0/104
Thermal inactivation (10 minutes at ° C.):				
Unheated	24/24	24/24	24/24	72/72
45	24/24	24/24	24/24	72/72
50	24/24	24/24	24/24	72/72
55	24/24	24/24	24/24	72/72
60	22/24	24/24	20/24	66/72
65	1/24	7/24	3/24	11/72
70	0/24	0/24	0/24	0/72

Several attempts to transmit the virus from tobacco to cucumber and vice versa by the green peach aphid (*Myzus persicae* L.) were unsuccessful.

IDENTIFICATION OF THE VIRUS

In extensive cross-protection tests, the watermelon virus failed to protect tobacco plants against subsequent infection with *Tobacco virus* 1 and *Cucumber virus* 1, but complete protection was afforded against the tobacco ringspot virus.

The tests of relationship to the tobacco ringspot virus were as follows: Several tobacco plants were inoculated with tobacco ringspot

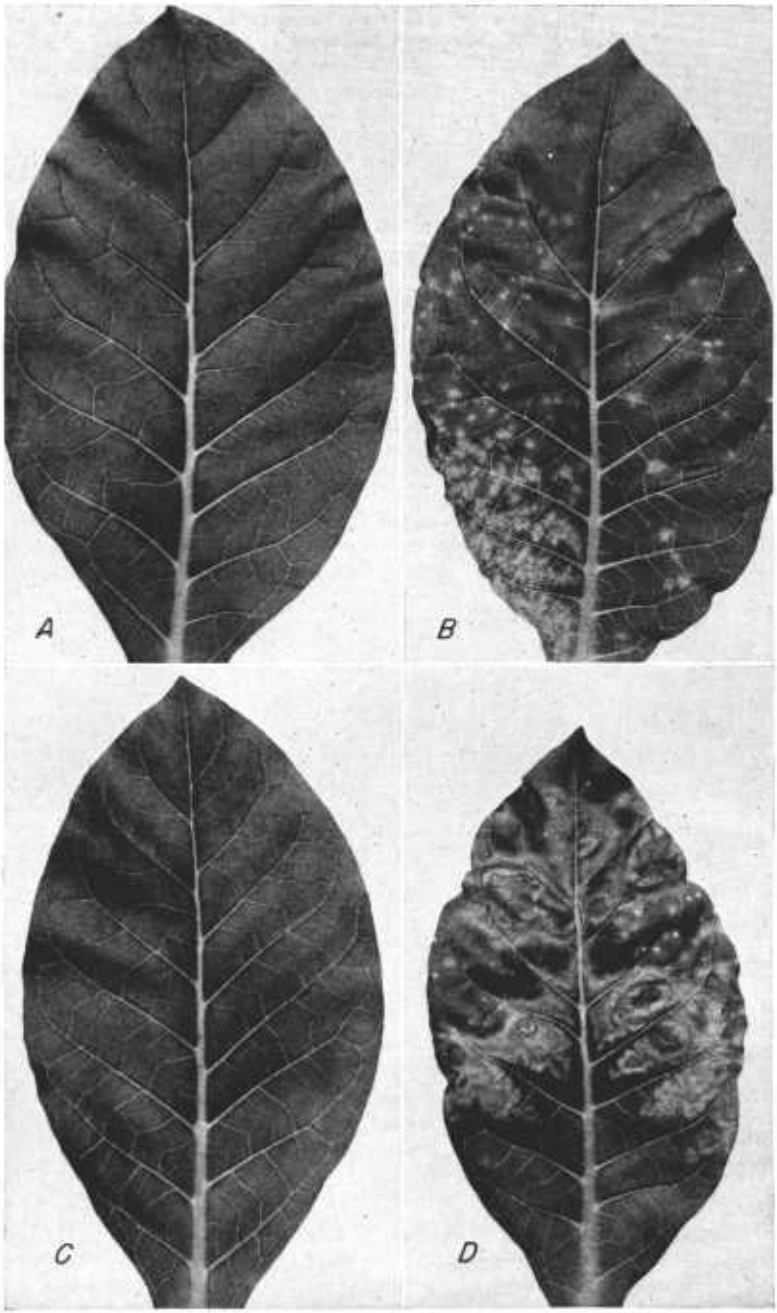

FIGURE 6.—Leaves from plants used in the cross-protection tests with the tobacco ringspot virus. *A*, Inoculated leaf which had recovered from symptoms of tobacco ringspot when reinoculated with the watermelon virus; *B*, leaf of same age which was healthy when inoculated with the watermelon virus; *C*, *D*, leaves from same plants, respectively, but taken above the inoculated leaves shown in *A* and *B*.

virus.[4] After complete recovery occured, the symptomless leaves were reinoculated with the watermelon virus. At the same time healthy plants of the same age were inoculated with the watermelon virus to serve as controls. In all cases (involving six different trials), the watermelon virus produced no symptoms on the ringspot-recovered plants but abundant symptoms on the control plants (fig. 6). This indicated a strain relationship between the watermelon virus and tobacco ringspot virus.

DISCUSSION

Several reports have been made of virus diseases and of viruses affecting watermelon under artificial inoculations. Ainsworth (*1*) reported the infectiousness of *Cucumber viruses* 3 and 4 to watermelon. Porter (*7*) reported that watermelon was highly susceptible to *Cucumber virus* 2. Many strains of *Cucumber virus* 1 produce only local symptoms on watermelon (*3*, *15*), and only rarely have systemic infections of this host been demonstrated. Wellman (*14*) reported systemic infection of watermelon by the celery strain of *Cucumber virus* 1 under greenhouse tests and inferred that mosaic-infected vines observed in nature were of the same cause. Porter and Melhus (*8*) of Iowa, Milbrath (*6*) of California, and Walker (*13*) of Florida reported natural occurrence of watermelon mosaic without identifying the causal virus. The virus producing the watermelon disease described herein was shown to be caused by a yellow strain of the tobacco ringspot virus. It agreed very closely with the latter virus in symptomatology, in host range, properties, and in failure to be transmitted from tobacco to other hosts by the use of the green peach aphid (*Myzus persicae* L.). Furthermore, by host-immunity reactions, its relationship to the tobacco ringspot virus was proved. Wingard (*16*) and others reported the infectiousness of the tobacco ringspot virus to cucurbits in greenhouse tests. Henderson (*4*) reported a natural occurrence of this virus on muskmelon and squash in Virginia, and Valleau (*10*) reported that it caused a mosaic disease of cucumbers in Kentucky.

The regular occurrence of the tobacco ringspot virus in watermelon in Wisconsin is interesting in view of the absence of known insect vectors and its rare occurrence as a disease of proximal tobacco plantings. However, the virus is well-distributed on soybean plantings throughout the North Central States (*2*) and no doubt occurs on many weed hosts as well. Its transmission to watermelon might well be by the same means as its transmission to soybean. Also the possibility of transmission through cucurbit seeds should not be overlooked. Henderson (*5*) and Valleau (*10*) demonstrated that the tobacco ringspot virus is transmitted to a considerable degree in seeds of petunia and tobacco, respectively.

[4] Two different strains of tobacco ringspot were obtained from Dr. R. W. Fulton. One was from infected tobacco in Maryland in 1948. The other came originally from Wisconsin and was shown to immunize against Price's Strain of tobacco ringspot virus.

SUMMARY

A mosaic disease of watermelon and muskmelon in Wisconsin was found to be caused by a yellow strain of the tobacco ringspot virus. The virus was found to be nontransmissible from tobacco to other hosts by the green peach aphid (*Myzus persicae* L.). In physical-property studies, inactivation of the virus occurred at: Dilution, 1 to 100,000; thermal inactivation, 10 minutes at 70° C.; aging in vitro, 5 days at 20°. The watermelon virus effectively protected tobacco plants against subsequent infection with the tobacco ringspot virus. A description of the disease in the field and of symptoms on selected greenhouse hosts is given.

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