A HIGHLY VIRULENT STRAIN OF CUCUMBER MOSAIC VIRUS OCCURRING IN CUCUMBER IN EASTERN ONTARIO[1]

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Abstract

A strain of cucumber mosaic virus (CMV) was isolated from Cucumis sativus L. at Ottawa, Ontario, that was more virulent than isolates of CMV from Ontario, Maryland, and Wisconsin. The new strain also infected many cucumber varieties than were resistant to CMV 1. Other differences included the length of time the virus remained infective in cucumber and the concentration of the virus in cucumber as shown by assay tests on cowpea.

Introduction

Cucumber mosaic virus (CMV) has for years been one of the most destructive diseases of slicing and pickling cucumbers. Several strains of the virus have been reported by various workers, but the strain most commonly used in breeding for disease resistance has been referred to as cucumber virus 1, and varieties of cucumber described as mosaic resistant have, in most cases, been tested against this strain. Resistance to this strain does not, however, preculde susceptibility to other strains of cucumber mosaic virus. Porter (4) in 1931 described a strain that he referred to as cucumber virus 2 and reported that the cucumber variety Chinese Long, used as a resistant parent in much of the breeding work in North America, was susceptible to it. In 1934 Price (5) described two strains of CMV that produced yellow spots in tobacco leaves; one of the strains was capable of causing systemic infection in cowpea.

During the summer of 1966 fruits of the cucumber variety Armour growing at Ottawa became severely affected by cucumber mosaic. This variety had survived screening for CMV resistance using a strain of the virus obtained from Beltsville, Maryland, in 1965 and one isolated at Ottawa in 1962. subsequently from 1966 to 1970, several reputedly highly tolerant cucumber varieties became severely infected with CMV in the Ottawa area. The work described in this paper was undertaken to determine if a strain of the virus was present in the Ottawa area that was different from the one isolated previously.

Materials and methods

In 1966 isolations were made from wilted 'Marketer' cucumbers (Cucumis sativus L.) growing in the field at Ottawa (isolate O-66) and from severely infected plants in the greenhouse at the Ottawa Research Station (isolate C-66). The infectivity of this isolate was compared with that of the strain of CMV obtained from Beltsville, Md., in 1965 (isolate B-65); an isolation made from cucumbers at Ottawa in 1962 (isolate O-62); and a strain of the virus identified as cucumber virus 1 obtained in 1966 from the University of Wisconsin (isolate W-1). Preliminary infectivity tests failed to show differences between O–66 and C–66 and between B–65 and W-1; therefore isolates O–66, O–62, and W-1 were used in the experimental work reported here.

Inoculum was prepared by grinding systemically infected cucumber leaves and stems in 0.05 M phosphate buffer at pH 7.0 at a rate of 1 gram tissue to 10 ml buffer solution. The inoculum was rubbed lightly into cucumber cotyledons or leaves after dusting them with 600–mesh carborundum powder.

To test for rate of multiplication of the virus within cucumber plants, healthy Marketer plants were inoculated at the second true leaf stage and inoculum was prepared from them 3, 4, 6, 8, 10, 12, 16, 20, and 24 days later. This inoculum was rubbed onto cotyledons of 14–day–old Marketer plants and observations were made 14 days later.

Assay tests were made using the cowpea (Vigna sinensis Savi.) variety Dixielee. Cucumber plants of the variety Marketer were inoculated with each of the virus isolates in the usual manner. At various intervals inoculum was prepared from the infected plants and rubbed onto the leaves of cowpea plants that had been planted at intervals so that each inoculation was made on plants of the same age. Local lesions appeared about 48 hours after inoculation and were counted 4 to 6 days later.

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Results

Each of the three stains 0–66, 0–62, and W-1 caused severe symptoms, including stunting and mottling, on the susceptible Marketer cucumber. There were distinct differences among the isolates in the degree of stunting, the amount of yellowing, and the mosaic pattern on the leaves. 0–66 caused very severe stunting but limited leaf chlorosis whereas 0–62 produced definite yellow blotches on the leaves. W-1 infection resulted in vein clearing and mottling of the type generally described for the disease on cucumbers. All three isolates caused the fruits to become extremely warty accompanied by yellow mottling.

Strain 0–66 produced mild mottling and moderate stunting of plants of the resistant cucumber variety Niagara, but symptoms on the fruits were severe. No symptoms were observed in this variety after inoculation with 0–62 or W-1. The variety Armour became slightly mottled and severely stunted after inoculation with 0–66, whereas W-1 caused mild mottling and 0–62 did not produce symptoms in this variety. Both 0–66 and W-1 caused severe fruit symptoms in Armour. The cucumber varieties Chinese Long and Tokyo Long Green were resistant to all three isolates.

In testing for reaction to infection by the various isolates it was observed that the time interval between inoculation of cucumber and attempts to recover the virus for assay affected the inoculum infectivity. When sap was taken from Marketer plants 16 days after inoculation, 0–66 and W-1 produced severe symptoms in cucumber while only very mild symptoms appeared in those inoculated with 0-62. Plants that had been infected for a shorter period of time were then used for inoculum production, with the result that severe symptoms were produced with 0–66 and 0–62 and mild symptoms with W-1.

Table 1. Time required by three CMV isolates to produce severe symptoms in Marketer cucumber

Days after inoculation	severity ratings* for virus isolates		
	0-66	0-62	W-1
3	+		-
4	+		-
6	+	+	
8	++	+++	+
10	+++	+	++
12	+++	+	++
16	+++	+	+++
20	-	+	+
24		+	

* Severity ratings: - = no infection to +++ = severe symptoms.

Four experiments run over a period of 7 months indicated that isolates 0–66 and W-1 were most infective 12–16 days after inoculation and that 0–62 was most infective 8 days after inoculation (Table 1). There was also some variation among the strains in the time interval during which the plants remained infective.

The differences among the three strains in time required to reach maximum infectivity and in length of the infectivity period were demonstrated in an assay for virus titer using Dixielee cowpea as the local lesion host (Table 2). The concentration of 0-66 in sap from Marketer cucumber was considerably higher than that of the other two strains and

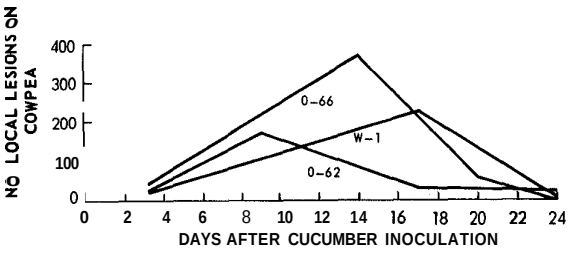

Figure 1. Pattern of infectivity of three isolates of cucumber mosaic virus as determined by assay an 'Dixielee' cowpea.

it remained high over a longer period (Fig. 1). Isolate 0–62 reached maximum titer in 9 days and W-1 in 17 days after inoculation. Inoculum of 0–66 and W-1 prepared 24 days after inoculation did not produce local lesions in cowpea, but inoculum of 0–62 prepared 29 days after inoculation was infective. In four assays conducted over a period of 14 months under slighly different environmental conditions, these differences remained consistent (Table 2).

Discussion

Considerable variation in the incubation time of CMV in cucumber before the appearance of CMV symptoms has been reported. According to Kooistra (2) symptoms of cucumis virus 2 appeared 12–14 days after inoculation. Linnasalmi (3) reported that the mottling symptoms of CMV appeared 10–14 days after inoculation, and symptoms of cucumber green mottle mosaic usually could be observed 3 weeks after inoculation. According to Doolittle (1) symptoms of CMV appeared 6 days after inoculation. Porter (4) found that

Table 2. Recovery of CMV isolates from Marketer cucumber at various times after inoculation as expressed by the number of local lesions produced in Dixielee cowpea

CMV isolate	Days after cucumber inoculation	Average no. of lesions per cowpea leaf				
		Test 1	Test 2	Test 3	Test 4	Averaae
0-62	3	22	12	17	10	15.2
	4	38	52	50	29	42.2
	6	65	96	88	58	64.8
	9	187	150	192	163	173.0
	11	147	121	207	105	145.0
	14	45	67	82	110	76.0
	17		20	17	41	26.0
	20		18	10	26	18.0
	24		22	19	25	22.0
0-66	3	36	20	28	51	33.7
	4	51	73	49	46	53.8
	6	147	122	96	96	115.2
	9	268	148	163	195	193.5
	11	264	282	217	256	254.7
	14	387	415	326	352	370.0
	17		198	302	216	238.7
	20		78	37	51	55.3
	24		2	0	6	2.6
W-1	3	12	19	22	9	15.5
	4	52	23	17	26	29.5
	6	41	27	19	22	27.2
	9	102	111	86	94	98.2
	11	155	129	180	99	140.7
	14	196	217	224	168	201.2
	17		196	271	221	229.3
	20		97	154	137	129.3
	24		3	17	2	7.3

symptoms of CMV1 and CMV2 were produced 4 to 6 days respectively after inoculation.

In the present investigation there was little variation in incubation period within a single strain in spite of the fact that there were variations in environmental conditions during the course of the experiments. There were, however, definite differences in the pattern of infectivity between different strains.

Sill and Walker (6) reported difficulty in producing local lesions in the cowpea variety Black when the inoculum was obtained directly from severely infected cucumber plants, and they found it necessary to use tobacco plants as sources of assay inoculum. Sill and Walker (7) later attributed the lack of production of local lesions in cowpea to the presence of a virus inhibitor in cucumber. In the present study, using the cowpea variety Dixielee, no difficulty was encountered in obtaining local lesions using the sap directly from cucumber plants. The virus titer of sap from plants infected with strain 0-66 was much greater and remained so for a longer time than that of sap from plants infected with either isolate W-1 of cucumber virus 1 or isolate 0-62. It is possible that the inhibitor present in cucumber is less effective against 0-66 than it is against the other strains.

It seems quite evident that 0-66 is a strain of CMV distinct from cucumber virus 1 represented in the experimental work by W-1. The method by which 0-66 became widespread in the Ottawa area is not known. In the field this strain caused a high incidence of rapid

wilting in susceptible varieties such as Marketer, Highmoor, and Straight Eight. The virus isolated from wilted or severely stunted plants in the area from 1966 to 1970 in all cases proved to be of the 0–66 type. Attempts to produce the rapid wilting symptoms in the greenhouse and in growth rooms were unsuccessful so there is no proof that this is a typical symptom of infection with this particular strain. Strain 0–66 failed to cause systemic infection in Chenopodium amaranticolor and did not produce symptoms of any kind in Phaseolus vulgaris. The fact that moderate to severe symptoms appeared on fruits of varieties such as SMR58, Triumph, Saticoy MR, Hiyield MR, Challenger, Gemini, and Spartan Dawn at the Ottawa Research Station is reason for some concern. If these varieties are resistant in other areas in North America, strain 0–66 probably exists only in the Ottawa area; but if this strain is widespread these and many other supposedly resistant varieties will become infected.

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