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TITLE

CHARACTERIZATION AND CONTROL OF CUCURBIT VIRUSES

SUBMITTED BY
Inst. for Plant Protection, The Volcani Center, ARO, Israel
Dept. of Plant Protection, Autonomous University
San Cristobal, Dominican Republic

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Principal Investigators

Dr. Benjamin Raccah
Dept. of Virology
The Volcani Center
P.O.Box 6, Bet-Dagan 50-250
Israel.

Prof. Lucas Grullon Medina
Dept. of Plant Protection
Autonomous University
Apto. 24, San Cristobal
Dominican Republic

Cooperating Investigators:

ISRAEL

Dr. Ruth Marcus, Dr. Yechezkel Antignus, and Dr. S. Cohen.

DOMINICAN REPUBLIC

Ing. Agr. Faustino Diaz, Ing. Agr. Vinicio Escarran, Ing. Agr. Aridia Figueroa, and Ing. Agr. M.Sc.

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CHARACTERIZATION AND CONTROL OF CUCURBIT VIRUSES

INTRODUCTION

The first period of this research was devoted in both countries to hiring personnel, collection of virus isolates and establishment of the research system both in laboratory and in the field.
The strategy of the study was to collect virus isolates in nature and in laboratory and to compare their characteristics as for host and serological reactions well as for their transmissibility by aphids. In the meantime, purification procedures were adapted for cucurbit viruses ands sera were prepared against one of the common wild isolates of the virus. A system for recognition of the isolates was developed which used biological and serological characteristics.
Control studies were initiated by comparing a number of pyrethroids which are are available and test their effect on the take off of alates from cucurbit treated leaves.

RESULTS AND DISCUSSION

1. Collection of cucurbit potyviruses:

Transmissible Cucurbit potyviruses were collected in the Gilgal Research Station in Jordan Valley (JV-strain). Non transmissible isolates were obtained by repeated mechanical transmission in the laboratory. The virus collected showed symptoms which were typical to what was described in literature as the zucchini yellow mosaic virus (ZYMV) (Table 4).

2. Host range and transmissibility of cucurbit potyviruses

Transmissibility by aphid vectors was used for the segregation of cucurbit potyviruses. C-13 differ from other cucurbit potyviruses while MV, MC and JV in the ability to inoculate peas. C-13 and MV are non aphid transmissible while both MC and JV are highly transmissible by aphids (Table 3 and 4).

3. Purification of the zucchini yellow mosaic virus:

The virus was propagated in zucchini plants for 4 weeks. The virus was purified according to the method of Moghal and Francki (1976). However, it was found polyethylene glycol (PEG) tended to increase the accumulation of slime-like pellet when the virus was purified from zucchini. Leaves were harvested ca. 4 weeks after being mechanically inoculated. They were homogenized in borate buffer containing thioglycolic acid, chloroform and CCl_4. After the separation of the crude leaf material and organic solvent layer, the aqueous fraction was further clarified by a second low speed centrifugation followed by a high speed one on a cushion of 20% sucrose. The pellet was resuspended overnight by gentle stirring in the cold, and layered on a 10-40% sucrose gradient the next day. The virus band was concentrated and tested for purity both spectrophotometry and by the electron microscope.

4. Preparation of polyclonal antiserum and serological tests against the Israeli ZYMV

Antisera against a the JV strain were prepared as described elsewhere (Purcifull and Batchelor, 1977).

The serological reaction of several cucurbit potyviruses collected

in Israel to the antiserum against JV was compared by enzyme linked immunosorbent assay (ELISA) and by serological specific electron microscopy (SSEM). Two non transmissible isolates and one transmissible isolated reacted positively with the anti JV serum, while the MC isolate reacted only with the antiserum prepared against watermelon mosaic 2 (WMV2) kindly provided by Dr. Dan Purcifull, from the University of Florida (Table 1 and 2).

The antiserum obtained in Israel, will serve in the second half of 1989, for detection of cucurbit potyviruses in the Dominican Republic.

5. Laboratory studies on the effect of mineral oils and pyrethroids on the transmission of ZYMV

A series of experiments were conducted to determine the effect of pyrethroids on aphid behavior. The leaves to be tested were formerly dipped in water, or in an emulsion of Telstar or Maverick (at a concentration of 0.15%) in water. the leaves were allowed to dry for about two hours before being tested. Groups of 1-12 alates of *A. gossypii* or *Myzus persicae* were placed on the leaves and the take-off was monitored for a period of 10-20 minutes. In other cases, the treated plants were maintained for up to 7 days after application and the take off was monitored then. Four pyrethroids commonly used in Israel were tested for their effect on desertion of the treated leaves by alates (Table 5). It is apparent that all four pyrethroids inflicted an effect on the aphids. Maverick was selected for further studies for being of little effect on bees. Hives are intentionally placed in cucurbit plots to improve fertilization. Maverick was compared with Telstar as for the effect of desertion of alates at various times after application to the cucumber leaves (Tables 5 and 7). It is apparent

that even after 5 and 7 days, the pyrethroids are still active on the plates. The effect on the departure from treated leaves on the day of application is presented in Fig. 1.

Although ZYMV is a typical non persistent virus which can be inoculated within seconds, the likelihood of infection, increases with time during the first minutes. The fact that an effect was noticed immediately after the first minutes, explain the difference between the efficiency of pyrethroids and other insecticides for prevention of virus transmission (Pirone et al. 1988).

Control experiments in laboratory and in the field will be implemented in coming spring season in the Dominican republic on the basis of the findings in Israel.

FUTURE PLANS

In the second half of the first year we intend to expand research in the following directions:

1. Develop cDNA probes for distinctions among cucurbit potyviruses which cannot be distinguished by serology.
2. A field experiment in Zafaria is now set to record the control of virus spread using oils and pyrethroids.
3. The same plot will also be sued to study the pattern of spread and the role of aphid species trapped in the plot on the incidence of infection.
4. Sera produced in Israel against cucurbit potyviruses will be sent to Prof. L. Medina to carry out serological tests there. A visit of a Dominican Republican researcher will be coordinated.

References

1. Pirone, T.P., Raccah, B. and Madden, L.V. (1988) Suppression of aphid colonization by insecticides: Effect on the incidence of potyviruses in tobacco. Phytopathology 78: 350-353.

2. Purcifull, D.E. and Batchelor, D.L. (1977) Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions, Bull. No. 788. Agric. Res. Sta. IFA, Univ. of Florida, Gainsville, Florida.

Table 1. Absorbance (a405) of the ELISA reaction of JV antiserum against four cucurbit potyviruses

IgG (ug/ml)	Conjugate Dilution	Virus Isolate			
		MC	MV	13	JV
1	1:500	0.0176	0.9458	1.4362	1.4307
1	1:1000	0	0.4712	0.7792	0.7782
2	1:1000	0.0247	0.9217	1.35	1.4742

The results represent means of 8 replicates for each treatment.

Table 2. Mean number of virus particles per electron microscope field as an indication of the trapping capacity of JV-AS for four cucurbit potyviruses using.

Virus isolate	Mean Number + SD	
MC	0.75	1.69
MV	58.95	9.32
ZY	40.6	6.70
13	92.6	10.78

The means represent fields (4 fields in each of 5 grids). A dilution of 1:2000 of the JV-AS in 0.1M phosphate buffer pH 7, was used for coating the grid, prior to trapping the virus particles. Antigen was used at 1g/1ml in 0.1M phosphate buffer pH 7

Table 3. Transmission of potyvirus isolates in Israel by *A. gossypii* and *M. persicae*.

Aphid species	No. of tests	Virus Isolates C-13	JV	MV	MC
Aphis gossypii	8	0/60	34/55	0/60	23/68
Myzus persicae	8	0/65	17/66	0/65	42/62

Three aphids were confined on each test plant

Table 4. Host reaction, transmissibility and serological reaction of four cucurbit potyviruses from Israel.

Isolate	Reaction on peas	transmissibility by aphids	Serological reaction to ZYMV-AS	to WMV2-AS
13	-	-	+	-
MV	+	-	+	-
MC	+	+	-	+
JV	+	+	+	-

Table 5. The number of *A. gossypii* alates taking off from pyrethroid treated cucumber leaves.

Time (min.)	Control	Cymbush	Smash	Telstar	Maverick
1	0	0	0	0	0
3	0	9	5	9	0
6	0	37	54	31	4
9	0	61	76	64	17
12	2	84	87	78	35
15	4	89	90	89	56
18	6	94	92	92	76
21	6	98	94	93	84
Number of alates	112	98	97	96	96
Percent take-off	5.3	100.0	96.9	96.9	87.5

Table 6. The number of *A. gossypii* alates taking off from Maverick treated cucumber leaves 0, 3, and 5 days after application.

Time (min.)	Days after application 0	3	5
1	0	0	0
6	4	5	8
12	35	31	23
15	56	48	42
18	76	68	59
Number of alates	96	97	97

Table 7. The number of *A. gossypii* alates taking off from Telstar treated cucumber leaves 0, 3, 5 and 7 days after application.

Time (min.)	Days after application 0	3	5	7
1	0	0	0	0
6	31	4	3	15
12	78	46	33	40
18	92	62	46	62
Number of alates	96	77	58	67

Percent of A. gossypii alates taking-off from treated leaves

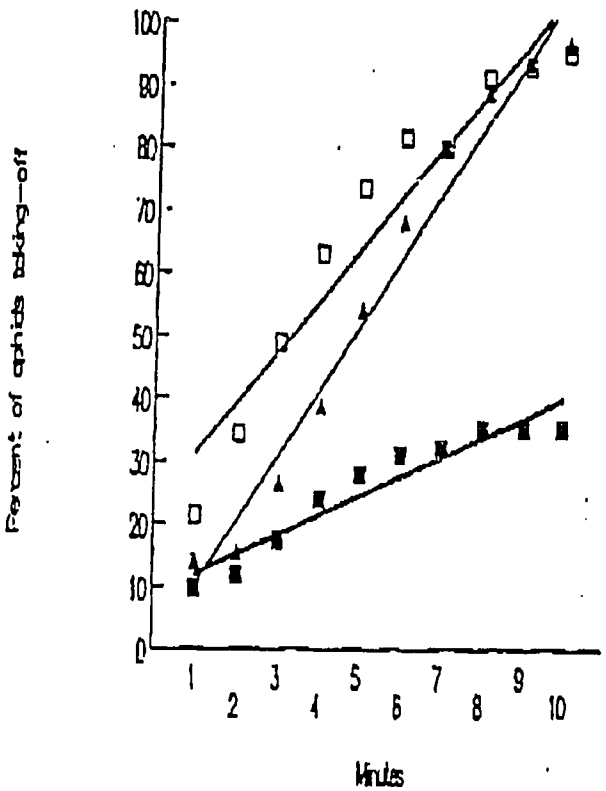

Fig. 1

Figure 1.

Percent of *Aphis gossypii* alates taking off from treated leaves. The triangles represent Maverick at 0.15% in water and the empty rectangles represent 0.15% Telstar. The dark rectangles represent aphids exposed to leaves treated with water alone.