Neth. J. Pl. Path. 95 (1989) 177-184

Artichoke yellow ringspot nepovirus naturally infecting cucumber in Crete

A.D. AVGELIS[1] and C. VOVLAS[2]

[1] Plant Protection Institute, 71110 Heraklion, Greece
[2] Dipartimento di Patologia Vegetale, Università degli Studi, 70126 Bari, Italy

Accepted 14 April 1989

Abstract

An isometric virus was isolated from cucumber plants growing in a plastic house in Crete and showing stunting and bright yellow mosaic of the leaves. Based on host range, properties in crude sap, behaviour during purification, electron microscopy and serology, the virus was identified as an isolate of artichoke yellow ringspot nepovirus. Ecological data corroborate transmission of the virus via the soil.

Introduction

Cucumber (*Cucumis sativus*) is the main cucurbit species grown in plastic houses on the island of Crete (Greece), where it is the only vegetable crop grown for export. Identification and control of the pathogens causing losses in this crop are therefore of paramount importance.

Six viruses have been reported from cucumber in the plastic houses of Crete, viz. cucumber mosaic cucumovirus (CMV), cucumber leaf spot carmovirus (CLSV) (= cucumber fruit streak virus), zucchini yellow fleck potyvirus (ZYFV), papaya ringspot potyvirus, watermelon strain (PRSV-W), watermelon mosaic 2 potyvirus (WMV2) and cucumber green mottle mosaic tobamovirus (CGMMV) (Avgelis and Vovlas, 1986). Since 1984 virus-like symptoms not resembling those caused by any of these viruses have been observed in a plastic house near Heraklion. Plants were stunted as a consequence of internode shortening and the young leaves showed a bright yellow mosaic while small enations appeared occasionally on the undeside of diseased leaves. Infected plants, distributed patch-wise, creased to grow and were fruitless. The disease appeared during three successive growing seasons (1984 through 1986) at the same sites in the plastic house. The symptoms did not reappear after the soil was treated once with methyl bromide (100 kg ha^{-1}) in July 1987.

Sap from leaves showing symptoms, collected every growing season, inoculated onto indicator plants, produced symptoms distinct from those of known cucumber viruses. Further investigations, to be reported in the present paper, showed the Cretan cucumber virus to be an isolate of artichoke yellow ringspot nepovirus (AYRV) (Rana et al., 1983).

Materials and methods

Host range. The virus was maintained in cucumber cv. Pepinex. Systemically infected

leaves were ground in 0.1 M phosphate buffer pH 7.2 and the expressed sap was used to inoculate celite-dusted leaves of 59 species belonging to 10 families. All tests were carried out in an insect-proof glasshouse at 18-24 °C.

Stability in crude sap. Thermal inactivation, dilution end-point and longevity in vitro were determined according to standard procedures using cucumber and *Chenopodium quinoa* as source of virus and *C. amaranticolor* as assay host.

Aphid-transmission tests. These tests were carried out using non-viruliferous apterous adults of *Myzus persicae* and *Aphis fabae* according to persistent and non-persistent transmission modes. Five aphids were allowed to feed on infected cucumber plants and then transferred to healthy seedlings. Ten test plants per treatment were used for each aphid species.

Soil-transmission tests. Cucumber seeds of cv. Pepinex were sown in a steam-sterilized soil mixture composted of Russian peat, soil and sand (1 : 1 : 1) in 250-ml plastic pots. The following treatments using infectious sap obtained by grinding diseased cucumber leaves in distilled water (1 g tissue/10 ml) were carried out:

a) pots were watered immediately after sowing with infectious sap (20 ml/pot);
b) pots were watered with infectious sap (20 ml/pot) when the seedlings had just emerged;
c) roots of cucumber seedlings at the cotyledonary stage were immersed for 24 h in infectious sap and then planted in the pots.

Control seeds and seedlings were treated with distilled water. The rate of transmission was assessed by visual examination of the seedlings and/or inoculation onto plants of *C. quinoa*.

To determine the presence of the virus in soil leachates from infested soil, 10 cucumber seedlings cv. Pepinex grown in plastic pots were inoculated. Two months later the pots were placed in a glass funnel lined with filter paper and percolated with 300 ml distilled water per pot. The filtrates were centrifuged for 70 min at 105 000 *g*. The resulting pellets were resuspended in 0.5 ml distilled water and inoculated to *C. quinoa* and cucumber plants. Five non-inoculated cucumber plants were used as control.

Purification. Attempts to purify the virus from infected plants of cucumber or of *C. quinoa*, using several of the common extraction and purification procedures, were unsuccessful. Infectivity was already lost after the first low-speed centrifugation because of intensive aggregation of virus particles. The best results were achieved when purification was according to Rana et al. (1980). Infected tissue, mainly from plants of *C. quinoa*, collected before appearance of top necrosis, was homogenised in 0.1 M phosphate buffer pH 7.2 containing 0.5% sodium ascorbate, 4% Triton X-100, 2% sucrose and chloroform (1 ml/g tissue). The homogenate was centrifuged at 8000 *g* for 15 min and the virus was precipitated by adding 10% (w/v) PEG 8000 MW and 1% NaCl and centrifugation at 12 000 *g* for 15 min. The pellets were resuspended in 0.02 M phosphate buffer pH 7.2. The preparations were further purified by two cycles of differential centrifugation (8000 *g* for 20 min and 90 000 *g* for 1 h) in 25% and 12.5% sucrose, respectively. The final pellets containing the virus were resuspended in 0.02 M phosphate buffer pH 7.2 and subjected to a sucrose density-gradient centrifugation (10-40% sucrose)

at 90 000 *g* for 2.5 h. Tubes were scanned using an ISCO 640 density-gradient fractionator. The virus from UV-absorbing zones, dialysed against 0.02 M phosphate buffer pH 7.2, was used for serological tests and for electron microscopy.

Serology. Tests were carried out in double diffusion in agar gels, and virus preparations were treated before gel diffusion tests with 0.3 M ethanolamine pH 10.5 (Shepard and Grogan, 1967) or with 1% SDS in water with the addition of 1% SDS to the agar (Purcifull and Batchelor, 1977). The following antisera to 18 different isometric viruses including nine nepoviruses (*Arabis* mosaic, artichoke Italian latent, artichoke yellow ringspot, chicory yellow mottle, grapevine Bulgarian latent, gravevine fanleaf, grapevine chrome mosaic, raspberry ringspot and strawberry latent ringspot), broad bean wilt comovirus, carnation mottle carmovirus, cucumber mosaic cucumovirus, cucumber leaf spot carmovirus, melon necrotic spot carmovirus, sowbane mosaic sobemovirus, squash mosaic comovirus, tobacco necrosis necrovirus and tomato bushy stunt tombusvirus were placed to react against treated preparations of the Cretan virus. All antisera used were obtained from own stock except for AYRV antiserum kindly supplied by Dr G.L. Rana.

Electron microscopy. Virus particles were observed in crude sap of infected cucumber leaves and in purified preparations stained with 2% neutral potassium phosphotungstate. Decoration tests with AYRV antiserum were carried out as described by Milne and Luisoni (1977).

Results

Hoste range. The virus was transmitted mechanically to 48 plant species belonging to eight different families (Table 1). Symptoms on inoculated plants were similar to those reported for AYRV (Rana et al., 1980) although some remarkable differences were noticed. All tested cvs of *Phaseolus vulgaris* showed reddish local lesions only during winter, while systemic latent infection occurred rarely. *Gomphrena globosa* exhibited local ringspots and systemic mottling, while in *Zinnia elegans* only systemic mosaic appeared. On the other hand, some solanaceous species reacted with ringspots and a line patterns (Fig. 1B), typical of AYRV. Cucumber plants inoculated at the cotyledonary stage exhibited severe stunting and conspicuous enations (Fig. 1A) without the bright yellow mosaic observed in the field.

Stability in sap. In expressed crude sap of both source plant species the virus lost infectivity between 55-60 °C, at a dilution between 10^{-4}-10^{-5} and after 48-50 days at room temperature (18-22 °C).

Transmission tests. All attempts to transmit the virus by aphids failed. Attempts of transmission via roots were also negative as treated cucumber seedlings neither showed symptoms nor could the virus isolated from their roots or leaves. Likewise, infectivity was not detected in soil leachates from pots containing infected cucumber plants.

Purification. The virus has not yet been successfully purified. Yields were extremely low although pellets of the last high-speed centrifugation were highly infectious. After

Table 1. Host range of a cucumber isolate of artichoke yellow ringspot virus.

Hosts	Reactions*	
	local	systemic
Amaranthaceae		
Amaranthus retroflexus	—	— (L)
Celosia cristata	—	Mt-L
Gomphrena globosa	Ch-NRsp	Mt-L
Chenopodiaceae		
Beta vulgaris var. *rapa*	—	— (L)
B. vulgaris	Resp	Resp, D
Chenopodium amaranticolor	Ch	Mt, D, TN
C. quinoa	Ch	Mt, D, TN
Compositae		
Chrysanthemum coronarium	—	L
Cynara scolymus cv. Camus de Bretagne	—	— (L)
cv. Molese	N	— (L)
cv. Cretan local	—	— (L)
Helianthus annuus	—	M-L
Lactuca sativa	—	M-L
Zinnia elegans	—	M-L
Cruciferae		
Brassica oleracea	—	— (L)
B. chinensis	—	— (L)
Raphanus sativus	—	— (L)
Cucurbitaceae		
Benincasa cerifera	—	Mt, D
Bryonia dioica	—	— (L)
Citrullus vulgaris	N	— (L)
Cucumis melo (various cvs)	—	Mt, En (R)
C. metuliferus	N	— (L)
C. sativus (various cvs)	Ch	Mt, St, En, D
Cucurbita ficifolia	Ch-N	— (L)
C. foetidissima	—	— (L)
C. maxima	—	— (L)
C. pepo	—	Mt (R)
Ecballium elaterium	—	— (L)
Lagenaria vulgaris	—	Mt, En, D
Lyffa acutangula	Chsp	Mt (R)
L. cylindrica	Chsp	Mt, En, D
Momordica balsamina	Ch-NPP	Mt, En, D
Sichyos angulatus	—	— (L)
Graminaceae		
Zea mais	—	— (L)

Table 1. (Continued).

Hosts	Reactions*	
	local	systemic
Labiatae		
Ocimum basilicum	—	Mt-L
Leguminosae		
Glycine max cv. Evans	—	— (L)
Phaseolus aureus	—	Nsp (R)
Ph. coccineus	—	— (L)
Ph. vulgaris cv. Pinto	Re	L (R)
cv. La Victoire	Re	L (R)
Pisum sativum	—	Mt, D
Vicia faba cv. major	—	L
cv. minor	Repp	— (L)
Vigna unquiculata	N (R)	— (L)
Malvaceae		
Hibiscus esculentus	—	VC (R)
Malva spp.	—	— (L)
Solanaceae		
Capsicum annuum cv. Lamuyo	—	— (L)
C. chinense cv. Miscucho	—	— (L)
C. frutescens cv. Tabasco	NB	— (L)
Datura metel	NB	Mt (R)
D. stramonium	N	Mt (R)
Lycopersicun esculentum cv. Dombo	—	Mt (R)
Nicotiana benthamiana	—	Mt, LP, N
N. clevelandii	—	Ch-NRsp, LP
N. glutinosa	NB	L
N. megalosiphon	—	Ch-NRsp, LP
N. paniculata	—	ChRsp
N. rustica	—	ChRsp, LP, L
N. tabacum cv. Samsun	Ch-NRsp	ChRsp, LP
cv. White Burley ml	Ch-NRsp	ChRsp, LP
cv. White Burley nl	Ch-NRsp	ChRsp, LP
cv. Xanthi nc	Ch-NRsp, LP	ChRsp, LP, L
Petunia hybrida	—	ChRsp, L
Physalis floridana	NRsp	NRsp
Solanum melongena	—	M-L
S. tuberosum cv. Spunta	Re	— (L)

* Ch = chlorosis, N = necrosis, Rsp = ringspot, Mt = mottle, Re = reddish, sp = spot, D = deformations, TN = top necrosis, M = mosaic, L = latent infection, En = enations, St = stunting, pp = pinpoint, LP = line pattern, (R) = rarely, (L) = no infection as checked by back inoculation to *Chenopodium quinoa*.

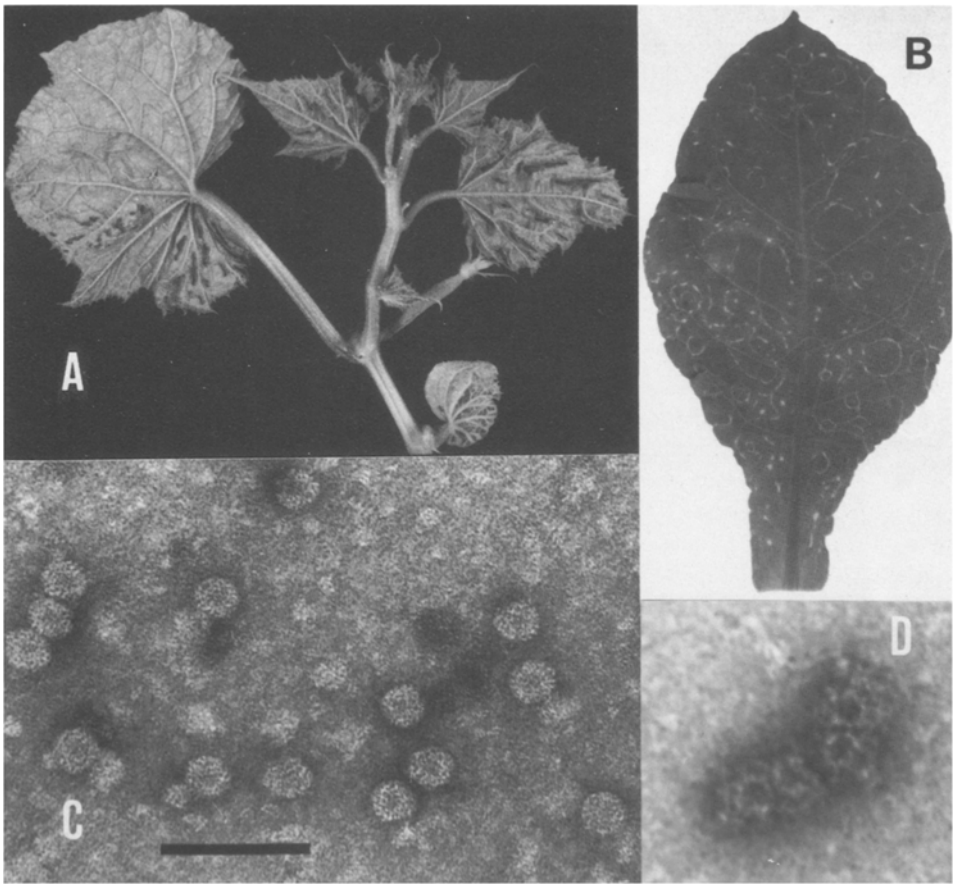

Fig. 1. A) Enations on underside of leaves and stunting of an inoculated cucumber plant. B) Necrotic rings and line patterns in a leaf of *Nicotiana tabacum* cv. Xanthi nc induced by artichoke yellow ringspot virus, cucumber isolate (AYRV-C). C) Electron micrograph of AYRV-C particles, negatively stained with PTA. D) Electron micrograph of particles decorated with antiserum to AYRV. Bar = 100 nm.

sucrose density-gradient centrifugation two prominent peaks were visible in ISCO-sedimentation profiles. The fast sedimenting fraction was more infectious than the slower one and its spectrophotometric curve showed a maximum at 260 nm and a minimum at 242 nm.

Serology. Only purified virus preparations reacted positively in agar double diffusion tests with an antiserum to AYRV (titre 1 : 32).

Electron microscopy. Crude plant sap and purified virus preparations contained isometric particles about 30 nm in diameter (Fig. 1C). They strongly decorated with AYRV antiserum (Fig. 1D).

Discussion

The results obtained, including poor yields at purification, indicate that the virus isolated from cucumber in Crete with bright yellow mosaic and stunting is similar to, if not identical with AYRV. The virus has a wide natural host range (Kyriakopoulou et al., 1985) but was recorded only from North-Eastern Peloponnesus of Greece and from Sicily (Italy) (Rana et al., 1978). Cucumber had already been reported as an artificial host of AYRV having diagnostic value (Rana et al., 1983). The AYRV isolate from Cretan cucumber differs in some host reactions from two other isolates – one from Greek artichoke and another from Sicilian cardoon (*Cynara cardunculus*) – previously studied (Rana et al., 1980).

The occurrence of AYRV in cucumber grown in plastic houses in Crete is not readily explained. Kyriakopoulou et al. (1985) have shown AYRV to be seed- and pollen-borne, but they were unable to find a nematode vector in Greek soils. The results of the transmission tests carried out in this study were also inconclusive. However, the patchy distribution of infected cucumber plants in the plastic house in Crete, the occurrence at the same sites in three consecutive years, its disappearence following methyl-bromide treatment, the inability of cucumber seedlings to acquire the virus from sterilized soil and the absence of virus in soil leachates, throroughly suggest soil transmission and the possible involvement of a biological entity in the natural transmission of AYRV. The absence of infectivity in soil leachates and classification of the virus as a nepovirus (Rana et al., 1983) point to the involvement of a nematode vector.

It seems unlikely that the virus has been introduced into Crete with cucumber seeds, as these are all imported from Central and Northern Europe where AYRV had not yet been reported. AYRV most probably is native to Crete as supported by the recent finding of AYRV-infection in field-grown crops of broad bean (*Vicia faba*) (Katis and Avgelis, unpublished data). Soil- and seed-transmission in a wide range of hosts (Kyriakopoulou et al., 1985) make the virus potentially important.

Acknowledgments

Grateful thanks are due to Dr G.L. Rana and Dr G.P. Martelli (Bari, Italy) for providing antiserum to artichoke yellow ringspot virus and for helpful discussion and revision of the text, respectively.

Samenvatting

Natuurlijke infectie van komkommer met 'artichoke yellow ringspot nepovirus' op Kreta

Uit komkommerplanten in plastic-foliekassen op Kreta werd een bolvormig virus geïsoleerd; de aangetaste komkommerplanten vertoonden dwerggroei en helder geel mozaïek op de bladeren. Gebaseerd op de resultaten verkregen uit onderzoek met het virus naar de waardplantenreeks, de eigenschappen in perssap, zuivering, elektronenmicroscopie en serologie kon het virus worden geïdentificeerd als een strain van het 'artichoke yellow ringspot nepovirus'. Waarnemingen op het gebied van de ecologie wijzen op overdracht van het virus via de grond.

References

Avgelis, A. & Vovlas, C., 1986. Occurrence of cucumber green mottle mosaic virus in the island of Crete (Greece). Phytopathologia Mediterranea 25: 166-168.

Kyriakopoulou, P.E., Rana, G.L. & Roca, F., 1985. Geographic distribution, natural host range, pollen and seed transmissibility of artichoke yellow ringspot virus. Annals Institute Phytopathological Benaki 14: 139-155.

Milne, R.G. & Luisoni, E., 1977. Rapid immune electron microscoy of virus preparations. Methods in Virology 8: 85-101.

Purcifull, D.E. & Batchelor, D.L., 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. University of Florida, Bulletin No 788: 39 pp.

Rana G.L., Rosciglione, B. & Cannizzaro, G., 1978. La maculatura gialla del cardo e del carciofo. Phytopathologia Mediterranea 17: 63-65.

Rana, G.L., Gallitelli, D., Kyriakopoulou, P.E., Russo, M. & Martelli, G.P., 1980. Host range and properties of artichoke yellow ringspot virus. Annals of applied Biology 96: 177-185.

Rana, G.L., Kyriakopoulou, P.E. & Martelli, G.P., 1983. Artichoke yellow ringspot virus. C.M.I./A.A.B. Descriptions of Plant Viruses 271: 4 pp.

Shepard, J.F. & Grogan, R.G., 1967. Serodiagnosis of Western celery mosaic virus by double-diffusion tests in agar. Phytopathology 57: 1136-1137.