Neth. J. Pl. Path. 92 (1986) 147-152

Viruses of tomato in plastic houses in Crete

A.D. AVGELIS

Plant Protection Institute, 71110 Heraklion, Crete, Greece

Accepted 1 April 1986

Abstract

Tomatoes grown in plastic houses in Crete have been inspected since 1980 for virus diseases. Plants with virus-like symptoms were checked by sap inoculation to test plants and the isolated viruses were identified by host reaction and serology. The most common viruses were, in order of frequency, tomato mosaic virus (ToMV), potato virus X, tomato bushy stunt virus (TBSV), potato virus Y and cucumber mosaic virus. The large use of ToMV-resistant cultivars reduces gradually the importance of ToMV while TBSV tends to become a serious problem of tomato in Crete.

Additional keywords: cucumber mosaic virus, potato virus X, potato virus Y, tomato bushy stunt virus, tomato mosaic virus.

Introduction

Tomatoes grown in plastic houses are an important crop in Crete. At present the acreage of the crop is about 1650. In the past, 'Earlipak' was the prevalent cultivar, but at the beginning of the 80s new cultivars, mostly with Tm genes, began to be introduced. They are planted in early autumn as a main crop, or in February as a second one; both are harvested up to the middle of June. Production is directed towards the local markets although results of attempts to export tomatoes to Europe have been encouraging. Virus diseases affecting tomato in Crete have not been intensively surveyed in the past and current literature is very limited. Tobacco mosaic virus (TMV) and 'double streak' (TMV+potato virus X) have been reported to affect tomato in plastic houses (Kyriakopoulou and Bem, 1977). Recently, tomato mosaic virus (ToMV) and tomato bushy stunt virus (TBSV) were reported to infect ToMV-resistant cultivars (Avgelis, 1981a, 1983). However, relatively little is known about the importance and distribution of these viruses on the island. In order to establish the geographical distribution of the known virus diseases, to identify new strains and/or new viruses and to determine the behaviour of ToMV-resistant cultivars, surveys were undertaken in the main tomato-growing areas of the island during the years 1980 to 1984. This paper summarizes the results.

Materials and methods

Survey procedures. Six main tomato-producing areas were surveyed: the Messara Valley, Timpaki, Paleochora and Ierapetra areas of the southern coast, the Platanos area of the western coast and the northern area near Rethimno (Fig. 1). Samples were

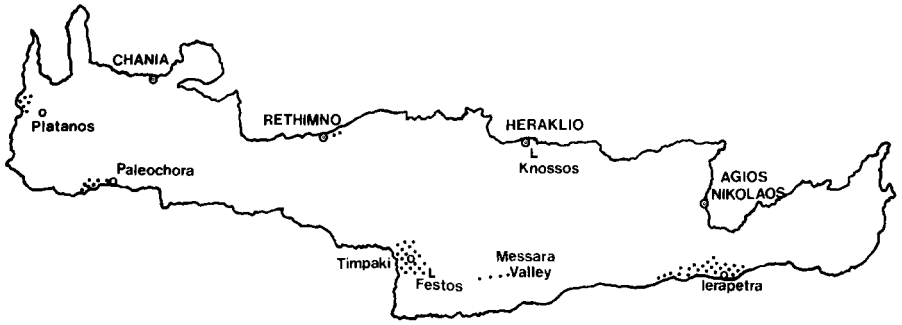

Fig. 1. Surveyed tomato-growing areas in the island of Crete (.= 10 ha of plastic houses).

collected from tomato plants suspected of being infected with a virus and were taken to the Plant Protection Institute (Heraklion) for identification of the causal agent. Until their check they were preserved at −20 °C. Specimens submitted to the Institute for diagnosis from tomato growers were also included.

Virus identification. Over 600 samples, consisting of leaves and fruits, were checked routinely for the presence of virus, using indicator plants and serology. Tissue to be tested was ground in 0.1 M phosphate buffer (pH 7.2) and the extract was rubbed onto celite-dusted leaves of test plants including: *Gomphrena globosa, Chenopodium quinoa, Cucurbita pepo, Capsicum annuum* 'Cleopatra', *Datura stramonium, Lycopersicon esculentum* 'Dombo', 'Dombito' and 'Carmelo', *Nicotiana benthamiana, N. glutinosa* and *N. rustica.* Additional hosts were used to characterize ToMV and potato virus Y (PVY) isolates including isogenic lines of tomato 'Craigela' +/+, Tm-1/Tm-1, Tm-2/Tm-2, Tm-2^2/Tm-2^2, pepper cultivars resistant to PVY and *Physalis floridana,* respectively. Mechanical inoculation tests were carried out in a glasshouse with a temperature fluctuating from 18 to 26 °C. Serological tests in agar or agar-SDS plates using crude extracts of infected tomato 'Dombo' were routinely carried out for ToMV and TBSV and occasionally for potato virus X (PVX), PVY and cucumber mosaic virus (CMV). Antisera to ToMV and TBSV have been previously prepared (Avgelis, 1981a, 1983) and antisera to PVX, PVY and CMV were provided by Dipartimento di Patologia Vegetale, Bari, Italy.

Evaluation of new cultivars. The suitability of three new cultivars recently introduced in Crete was estimated by direct observations in the plastic houses of growers. Inspections concerned a few holdings in each surveyed area. In each plastic house some rows were selected at random. Plants with virus symptoms were counted and samples were collected for virus identification. Moreover, newly released and commercial cultivars, resistant to ToMV were tested for susceptibility to ToMV and TBSV. Five plants of each cultivar were inoculated at the five-leaf stage with sap from TBSV-infected plants of *N. benthamiana* and were then transplanted in a plastic house. As regards tests for ToMV strain 1, four plants of each cultivar were transplanted in a plastic house, in which in the middle of each row a ToMV strain 1-infected plant of cv Earlipak was placed. Plants were grown as usual in local commercial production. If no evident symptoms appeared till fruit bearing, the presence of ToMV and TBSV was tested by inoculations on *N. glutinosa* and *C. pepo,* respectively.

Results

Throughout the island, the acreage of 'Earlipak', without resistance factors, drastically decreased so that during the 1984-85 growing season it represented about 10% of tomatoes grown in plastic houses. As its replacement three cultivars viz. Dombo C_2 VF_2, Dombito TmC_2 F_2 and Carmelo TmVFN, seem to be predominant at present in Crete. It is worthwhile to note that the latter two cultivars are heterozygous regarding resistance to ToMV (Dombito: Tm-2^2 Tm-1/++ and Carmelo: Tm-2^2/+). Plants with virus symptoms appeared to be mainly concentrated in plastic houses with a history of virus and on 'Earlipak' and 'Dombo'.

The predominant virus infecting tomato in plastic houses was ToMV (in about 77% of the samples). In most holdings the disease spread to all plants in the middle of the growing season. Mosaic mottling or leaf deformation was the main symptom and frequently internal necrosis in fruits developed when the days were short and the temperature was low. On the ToMV-resistant cultivar Carmelo, necrosis of fruits and leaves due to ToMV infection was often noticed (Fig. 2A). These serious damages occurred more frequently when high temperatures prevailed and susceptible tomatoes infected by ToMV were grown at a short distance.

PVX was the second widespread virus which in combination with ToMV caused severe losses mainly on 'Earlipak' (16% of the total number of samples). Double streak symptoms were also noticed on 'Dombo'. Single infection of PVX was rarely detected on ToMV-resistant cultivars which exhibited only moderate mosaic symptoms.

About 6% of the samples contained TBSV which in tomatoes causes chlorotic rings

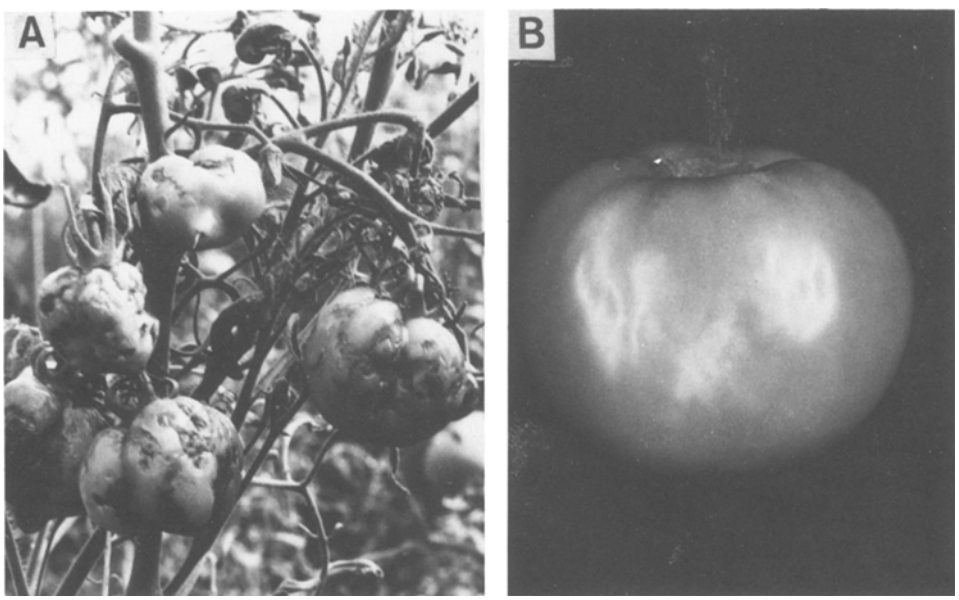

Fig. 2. A) Tomato plant of a resistant cultivar (Tm-2^2/+) with necrosis following natural infection by ToMV. B) Chlorotic rings on tomato fruit induced by TBSV.

on the fruits (Fig. 2B), while the entire plant stays nearly normal. The virus seemed to be confined to the Paleochora and Rethimno areas, infecting 'Carmelo' and 'Dombito'.

PVY and CMV were detected in 0.6 and 0.2% of the samples, respectively and appeared to be of minor importance in tomatoes grown in plastic houses.

The host plant reactions to the viruses detected during this work are shown in Table 1. More than one isolate of each virus was studied but as most of them gave similar reactions, the results are combined. Two strains of ToMV, 0 and 1, could be distinguished by reactions on tomato 'Craigella'. Strain 1 was easily identified because it infected 'Craigella' Tm-1/Tm-1 (Pelham, 1972; Hollings and Huttinga, 1976). It appeared to be the most widely spread (86% of the samples infected by ToMV) and was detected in all 'Carmelo' samples. Isolates of PVY did not infect peppers resistant to pathotypes 0 and 1, and *Physalis floridana* exhibited systemic necrosis. These responses agreed well with Y^0 group reactions (De Bokx and Huttinga, 1981). Isolates of TBSV could not be distinguished by host range symptoms and the serological tests were not sufficiently extensive to identify strains (Martelli et al., 1971). CMV isolates proved to be very similar to a strain previously described from Crete and adapted to Solanaceae (Avgelis, 1981b). Serological tests successfully provided a quick means of identifying virus mixtures and supported data obtained in the host reaction studies.

Cultivars Earlipak and Dombo mostly appeared to be very sensitive to ToMV and considerable losses were suffered in all surveyed areas. The resistant cultivar Carmelo heterozygous for the $Tm\text{-}2^2$ gene showed a considerable incidence of systemic necrosis

Table 1. Response of test plants to mechanical inoculation with isolates of tomato mosaic virus (ToMV) strain 0 and 1, potato virus X (PVX), tomato bushy stunt virus (TBSV), potato virus Y (PVY) and cucumber mosaic virus (CMV), isolated from protected tomato.

Test plant	ToMV-0	ToMV-1	PVX	TBSV	PVY	CMV
Gomphrena globosa	LL SN	LL SN	LL	LL SN	–	LL
Chenopodium quinoa	LL	LL	LL	LL	–	LL
Cucurbita pepo	–	–	–	LL	–	SM
Capsicum annuum 'Cleopatra'	LL	LL	LL	LL SM	SM	SM,LN
Datura stramonium	LL	LL	SM	LL SM	–	SM
Lycopersicon esculentum 'Dombo'	SM	SM	SM	LL SM	SM	SM,LN
Lycopersicon esculentum 'Dombito'	LL	LL	SM	LL SM	SM	SM,LN
Lycopersicon esculentum 'Carmelo'	LL	LL SN	SM	LL SM	SM	SM,LN
Nicotiana benthamiana	LL	LL	SM	LL SM	SVC	SM
N. glutinosa	LL	LL	SM	LL	SVC	SM,LN
N. rustica	LL	LL	SM	LL	SVC	LL SM,LN
Lycopersicon esculentum						
'Craigella' +/+	SM	SM			nt	
'Craigella' Tm-1/Tm-1	–	SM			nt	
'Craigella' Tm-2/Tm-2	LL	LL			nt	
'Craigella' $Tm\text{-}2^2/Tm\text{-}2^2$	LL	LL			nt	
Capsicum annuum						
'No 720 PVY(0)R'	nt	nt			–	
'No 210 PVY(0-1)R'	nt	nt			–	
Physalis floridana	nt	nt			LL SN,D	

LL = local lesions, SN = systemic necrosis, SM = systemic mosaic, SVC = systemic vein clearing, LN = leaf narrowing, D = leaf distortion, – = no symptoms, nt = not tested.

due to strain 1 of ToMV, mainly when in the holding 'Earlipak' was also grown. Later on growers quickly abandoned the system of growing 'Carmelo' together with 'Earlipak' and therefore serious losses were less frequently observed in the last two years. On the other hand the resistant cultivar Dombito heterozygous for the Tm-2^2 Tm−1 genes appears immune until now.

No differences were noticed between the susceptibility of the cultivars Carmelo and Dombito to TBSV in the inspected holdings.

Tests in plastic house with 16 cultivars, distinguished for their adaptation to local conditions (Petrakis, 1981) proved that TBSV could infect all of them and that ToMV strain 1 did not overcome the resistance of cultivars heterozygous for the genotype combination Tm-2^2 Tm-1 or homozygous for the Tm-2^2 gene.

Discussion

The use of a selected host range combined with serological tests deservingly provided a means for identifying a number of viruses and determining their distribution in the main tomato-growing areas of Crete. ToMV seems to be the predominant virus in tomatoes grown in plastic houses although its incidence decreases gradually as ToMV-susceptible cultivars are replaced by resistant ones. However, resistance due to the Tm-2^2 gene frequently was overcome in heterozygous cultivars such as Carmelo. This might be due to: 1. the high temperature prevailing for a long time in plastic houses and 2. the fact that they were grown next to susceptible cultivars as Earlipak or Dombo (Laterrot, 1973; Pilowsky, 1981; Avgelis, 1981a). On the contrary, on heterozygous cultivars as Dombito possessing both Tm-2^2 and Tm-1 genes symptoms were not noticed until now by infection due to ToMV, although strain 1 is able to produce necrosis on Tm-2^2 Tm-1/++ genotype at high temperatures (Pelham, 1972; Laterrot, 1973). Thus cultivars possessing more than one resistant gene seem to be better protected against ToMV in the local conditions. It may be worth pointing out that Greek customers prefer large tomatoes. So growers have to choose from susceptible and few resistant cultivars. Due to the widespread occurrence of ToMV in Crete and the failure of cross-protection in local conditions (Avgelis, 1985), the use of resistant cultivars seems to be the only possibility. However large fruit tomato cultivars, ToMV resistant, are usually heterozygous for the Tm-2^2 gene. A moderate change in the preference of customers towards smaller tomato fruits will allow the growers to select from numerous cultivars possessing the Tm-2^2 Tm1/++ genotype. In addition Tm-2^2/Tm-2^2 cultivars were proved to be immune to ToMV infection but they produced rather small fruits. At present TMV has not been isolated in tomatoes although it occurs in local peppers (A.D. Avgelis, unpublished data). The other viruses most frequently found were PVX and TBSV. As regards PVX, which in mixed infection with ToMV caused severe losses on susceptible cultivars, it can be stated that it does not seem to be a serious problem of newly grown tomato cultivars. On the contrary, TBSV, although found to infect tomato in two areas only, must be regarded as an important pathogen because of its epidemiological characteristics (soil-borne, seed-borne, 'alimentary resistant') (Martelli and Quacquarelli, 1982; Tomlinson et al., 1982) and may represent a serious threat to the local tomato industry. Moreover, TBSV was recently isolated from eggplants grown in a few plastic houses in the Ierapetra area (Koenig and Avgelis, 1983). For aphid-transmitted viruses such as PVY and CMV the

results suggest that in protected tomato they are of very little economic importance in Crete.

Samenvatting

Virussen van tomaten geteeld in plasticfolie-kassen op Kreta

Van 1980-1984 werden op Kreta de in plasticfolie-kassen geteelde tomaten geïnventariseerd op de aanwezigheid van virussen. Verdachte planten werden door sapinoculatie op toetsplanten onderzocht. De daarbij geïsoleerde virussen werden daarna via de symptomen op de waardplanten en serologisch geïdentificeerd. In volgorde van belangrijkheid werden de volgende virussen het meest aangetroffen: tomatemozaïekvirus, aardappelvirus X, tomatedwerggroeivirus, aardappel Y virus en komkommermozaïekvirus. De laatste jaren is door de toenemende teelt van tomaterassen met resistentie tegen tomatemozaïekvirus het belang van dit virus sterk verminderd, terwijl het tomatedwerggroeivirus een steeds ernstiger probleem lijkt te worden.

References

Avgelis, A.D., 1981a. ToMV-necrosis of resistant tomato cultivar (Tm-2^2/+) under plastics. Pan. Congr. Geot. Res., Halkidiki, Greece, 121 (Abstr.).

Avgelis, A.D., 1981b. Virus diseases of vegetable crops in Crete. 3: A yellow mosaic of eggplant. Agric. Res. 5: 183-190.

Avgelis, A.D., 1983. Tomato bushy stunt virus on tomato crops in Crete. 1st Hellenic Congr. Pl. Dis., Athens, Greece. 24 (Abstr.).

Avgelis, A.D., 1985. Effectiveness of cross-protection against tomato mosaic virus in tomatoes grown under plastic houses. Agric. Res. (in press).

De Bokx, J.A. & Huttinga, H., 1981. Potato virus Y. CMI/AAB Descr. Pl. Viruses 242: 6 pp.

Hollings, M. & Huttinga, H., 1976. Tomato mosaic virus. CMI/AAB Descr. Pl. Viruses 156: 6 pp.

Koenig, R. & Avgelis, A., 1983. Identification of a virus similar to the BS3 strain of tomato bushy stunt virus in eggplant. Phytopath. Z. 106: 349-353.

Kyriakopoulou, P.E. & Bem, F.R., 1977. Some virus and virus-like diseases of cultivated plants noticed in Greece during the years 1971 and 1972. Proc. 1st Greek Agric. Res. Symp. B-II: 409-419.

Laterrot, H., 1973. Résistance de la tomate au virus de la mosaïque du tabac. Difficultés rencontrées pour la selection des variétés résistantes. Annls Amél. Pl. 23: 287-313.

Martelli, G.P., Quacquarelli, A. & Russo, M., 1971. Tomato bushy stunt virus. CMI/AAB Descr. Pl. Viruses 69: 4 pp.

Martelli, G.P. & Quacquarelli, A., 1982. The present status of tomato and pepper viruses. Acta Hortic. 127: 39-64.

Pelham, J., 1972. Strain genotype interaction of tobacco mosaic virus in tomato. Ann. appl. Biol. 71: 219-228.

Petrakis, M., 1981. Improved tomato cultivars. Cyclostyled paper. Inst. Veget. crops Flor., Heraklion, Greece 4 pp. (in Greek).

Pilowsky, M., 1981. Factors affecting the incidence of systemic necrosis in F_1 hybrid tomato plant resistant to tobacco mosaic virus. Pl. Dis. 65: 684-686.

Tomlinson, J.A., Faithfull, E., Flewett, T.H. & Beards, G., 1982. Isolation of infective tomato bushy stunt virus after passage through the human alimentary tract. Nature 300: 637-638.