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A survey of tobacco viruses in tobacco crops and native flora in Greece

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Abstract

The most important tobacco producing areas in Greece were surveyed for virus presence, from 1997 to 2000. Tobacco seedlings or plants showing virus-like symptoms were randomly collected from seedbeds or fields, respectively, and tested by ELISA, and/or mechanical inoculation onto indicator plants. *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were detected in all sampling areas, with TMV mainly found in oriental varieties. *Tomato spotted wilt virus* (TSWV) consisted a serious endemic virus in Northern Greece (Thrace, Central and Eastern Macedonia), whereas *Alfalfa mosaic virus* (AMV) was mainly found in regions, where alfalfa was cultivated in the vicinity of tobacco crops. *Eggplant mottled dwarf virus* (EMDV) was detected in several areas but always in very low incidence (<0.01%). Surveys were also conducted to assess the potential reservoir hosts of PVY, CMV and AMV among weeds collected from highly infected tobacco fields from 1998 to 2000. Among 3450 samples tested for PVY, plants from 17 species in 10 families were found infected. For CMV, 2891 weed samples were tested and 19 species in 12 families were positive. Assays for AMV infection were made on 961 samples and 12 species in 9 families were identified as hosts of this virus.

Introduction

Tobacco (*Nicotiana tabacum*) is an important field crop in Greece, especially in agricultural areas with low incomes. It is cultivated in approximately 75,000 ha mostly in deficient, mountainous areas. A high proportion (80–90%) of the total tobacco production is exported, thus being one of the major determinants of the Greek gross national product. The main tobacco producing regions are located in Macedonia, Thrace, Thessallia and Aitoloakarnania provinces. In all these areas, a wide range of locally adapted varieties of 'sun cured' (oriental) tobacco can be found mainly in non-irrigated fields, while 'flue cured' and 'light air cured' varieties (Virginia and Burley) are cultivated in irrigated fields (Vasiliadis, 1996).

Tobacco crops are attacked by a wide range of diseases and a great number of viruses can infect them either naturally or experimentally (Shew and Lucas, 1991). In Greece, although a broad range of virus-like symptoms is encountered and serious epidemics are claimed to be caused by viruses, only sporadic information are available on the identity of the causal agents involved (Tsakiridis and Gooding, 1972; Bem, 1987; Katis et al., 1993; Chatzivassiliou et al., 1999; Katis et al., 2000). The thrips-transmitted *Tomato spotted wilt virus* (TSWV, Family: *Bunyaviridae*, Genus: *Tospovirus*), the aphid-transmitted *Potato virus* Y (PVY, Fam-

ily: *Potyviridae*, Genus: *Potyvirus*), *Cucumber mosaic virus* (CMV, Family: *Bromoviridae*, Genus: *Cucumovirus*) and *Alfalfa mosaic virus* (AMV, Family: *Bromoviridae*, Genus: *Alfamovirus*) and the mechanically transmitted *Tobacco mosaic virus* (TMV, Genus *Tobamovirus*) are thought to be the most prevalent ones (Katis et al., 1993). Others such as *Tobacco rattle virus* (TRV, Genus: *Tobravirus*) (Bem, 1987), *Artichoke yellow ringspot virus* (AYRV, Family: *Comoviridae*, Genus: *Nepovirus*) (Kyriakopoulou, 1981), *Eggplant mottled dwarf virus* (EMDV, Family: *Rhabdoviridae*, Genus: *Nucleorhabdovirus*) (Katis et al., 2000) and an unidentified virus belonging in the *Closterovirus* genus (Family: *Closteroviridae*) (Chatzivassiliou et al., 1999) have been reported to occur locally and they seem to be of minor economic importance.

The most destructive viruses for tobacco production in Greece, except TMV (Zaitlin and Israel, 1975) are not seed-borne in tobacco (Francki et al., 1979; Jaspars and Bos, 1980; De Bokx and Huttinga, 1981; Reddy and Wightman, 1988). Several susceptible crops (e.g. cucurbits and solanaceous for CMV, potato, tomato, pepper, eggplant for PVY and alfalfa for AMV) can be implicated in the epidemiology of those viruses, however infected weeds may also play a significant role for their spread to tobacco crops (Duffus, 1971; Zitter, 1977; Thresh, 1981). Surveys have recently identified a large number of alternative TSWV sources among wild flora in Greece (Chatzivassiliou et al., 2001), however similar information for the other viruses is lacking.

Identification of the most prevalent viruses and their alternative sources in a crop allow farm advisors and growers to make better management decisions. In order to achieve this, a survey was conducted to investigate the relative incidence of TSWV, PVY, CMV, AMV and TMV infecting the tobacco crops in Greece. The presence of PVY, CMV and AMV among the wild flora was also investigated, in order to better understand their epidemiology.

Materials and methods

Survey of tobacco viruses

Extensive surveys were performed in tobacco seedbeds and fields and in wild flora in the major tobacco producing areas in Greece (Figure 1). All samples were kept between 0 and 4 °C and immediately tested by ELISA, while stock material was stored at −80 °C for mechanical inoculations. All tobacco samples, originating either from seedbeds or fields, were analyzed for the presence of PVY, CMV, TSWV, and AMV. As TMV infection of the seedlings occurs mainly during transplantation by the virus present in seed coats (Zaitlin and Israel, 1975, only field samples were tested for this virus. Finally, due to its low incidence (Katis et al., 2000), only suspected samples with characteristic symptoms (severe dwarfing, leaf curling and vein clearing) were tested for the presence of EMDV.

More than 9000 samples of the common cultivated varieties were collected from seedbeds during 1998 and 1999, while no seedbed was sampled in 1997. Fifty, and only occasionally fewer, seedlings were randomly collected from each seedbed during April or May. Furthermore, 9556 samples were collected from virus-infected tobacco fields, in samplings performed between July and August, from 1997 to 1999. Before sampling, the percentage of plants showing virus-like symptoms (stunting, leaf distortion, vein clearing or necrosis, mosaic and necrotic patterns) in a random batch of 100 plants was counted in each field. Subsequently, young fully expanded leaves were collected from 10 randomly selected symptomatic plants and tested in order to estimate the relative infection rate of each virus. Fewer plants were sampled in case of low infection rates (<10%).

Finally, samplings of arable weeds and wild plant species were performed during the whole year, in and around tobacco fields, in areas with a known high incidence of the respective virus; for AMV in Larissa and Chalkidiki and for PVY and CMV in Karditsa, Pieria and Pella (Figure 1). Weeds were collected irrespectively of the presence of symptoms, with most of them being symptomless, and the sampling unit consisted mainly the youngest fully expanded leaves of the plants and flowers when present. During 1998, 559 plants were tested for PVY and 961 for AMV infection, while during 1999 and 2000 in total 2891 samples were tested for each of PVY, CMV and AMV.

Virus detection and identification

Samples were analysed in double antibody sandwich enzyme-linked immunosorbent assay (DAS-

Figure 1. Sampling areas of infected tobacco plants in Greece.

ELISA) tests for the presence of TSWV, PVY, CMV and AMV (Clark and Adams, 1977), while bioassays were used for TMV and EMDV detection.

DAS-ELISA tests. They were run using Costar immuno plates (Costar corporation, Cambridge, USA) and polyclonal antibodies from Boeringer Mannheim (Mannheim, Germany) for PVY or non-commercialised antibodies for TSWV (Aristotle University of Thessaloniki, AUTh; Chatzivassiliou et al., 2000a), AMV (AUTh) and CMV (Dr. C. Varveri, Benaki Phytopathological Institute, Athens). Leaves were homogenized to a dilution of 0.1 g ml^{-1} in PBS plus 0.05% Tween 20 for tobacco samples, with the addition of 2% (w/v) polyvinylpyrollidone (MW: 6000) for the weed samples. Due to the low virus incidence in seedbeds, samples were pooled in groups of ten punched together with a leaf puncher of 1 cm in diameter. In order to choose the number of samples grouped, each of five infected field samples, with ELISA readings from 0.5 to 2.0, was analyzed in a group of 5, 10, 20, 30, 40 or 50 plants, together with healthy ones. Those readings did not show any reduction in groups of 10 plants for any of the tested virus (results not shown). When a group of seedbed samples was tested positive, subsequently they were analyzed individually in order to evaluate infection rate. Tobacco field and weed samples were individually processed, but tested in dublicated wells. Absorbance values (A_{405}) were measured, 1 h after substrate addition, using a MPR A4J (Eurogenetics, Tessenderlo, Belgium) plate reader. Samples with readings higher than the average of healthy control readings plus three times their standard deviation were considered to be positive. Two healthy controls were used per plate for tobacco or weeds, respectively; tobacco plants grown in the laboratory, or plants of most of the tested wild species, collected from the farm of the Agricultural Faculty of AUTh, where no infection of the respective virus was found.

Bioassays. Leaf extracts were prepared in a phosphate buffer (pH 7.0) containing either 0.1% sodium sulfite (Na_2SO_3) for tobacco samples or 1% activated charcoal for weed samples. For TMV detection, tobacco extracts were mechanically inoculated onto *N. glutinosa* detached leaves placed on wet filter paper on trays, and covered with wrapping membrane. Field tobacco samples showing typical virus-like symptoms which were found negative in ELISA tests, and samples from previously unknown weed hosts which were tested positive for any of the viruses, were subsequently mechanically inoculated onto *N. tabacum* 'Samsun', *N. glutinosa*, *N. rustica* and *Chenopodium quinoa*. A positive test for TMV was based on the development of typical necrotic local lesions on *N. glutinosa* 2–4 days post inoculation (p.i.) and for EMDV on the development on the *Nicotiana* species tested of chlorotic local lesions 1 week p.i., followed by systemic vein clearing and crinkling 2–3 weeks later. In order to confirm bioassay results for ELISA positive weeds, indicator plants were tested in ELISA tests for the respective virus 3 weeks after inoculation.

Results

Seedbeds surveys

In 1998, all tested viruses were detected in the surveys performed in seedbeds, but in 1999, despite the high number of seedbeds sampled, sporadic low infection rates were recorded (Table 1). PVY and CMV were the most prevalent viruses in the seedbeds; at least one of them was found in 11 of the 12 areas surveyed. The higher virus incidence for oriental varieties was recorded in Drama (13.2%) for PVY and in Rhodopi (6.3%) for CMV, while for Virginia or Burley seedbeds they occurred in Karditsa (55.5%) or Pella (80%), respectively. TSWV infection rates reached 10.7% in Chalkidiki in oriental, but only 2% in Kilkis in Virginia seedbeds. Except those areas, TSWV seedbeds infections were only detected in Kavala and Thessaloniki. In a seedbed in Larissa we detected the higher AMV infection for oriental tobacco (12%), but this rate was only 3% for Virginia, in Kilkis. AMV was also present in seedbeds located in Pieria and Karditsa.

Table 1. Incidence (%) of PVY, CMV, TSWV and AMV in tobacco seedbeds (Burley, oriental, Virginia) sampled in different areas in Greece, during 1998 and 1999

County	1998						1999				
	Variety	No. of samples	PVY	CMV	TSWV	AMV	No. of samples	PVY	CMV	TSWV	AMV
Rhodopi	Oriental	16	6.3	6.3	0	0	20	0	0	0	0
	Virginia	65	0	0	0	0	150	0.7	0	0	0
Xanthi	Oriental	–	–	–	–	–	50	0	0	0	0
Drama	Burley	111	12.6	0	0	0	–	–	–	–	–
	Oriental	121	13.2	0	0	0	2250	0	0	0	0
	Virginia	50	8	0	0	0	–	–	–	–	–
Serres	Oriental	–	–	–	–	–	400	0.8	0	0	0
Kavala	Oriental	–	–	–	–	–	850	0	0	0.2	0
Kilkis	Oriental	50	0	6	0	8	50	0	0	0	0
	Virginia	100	0	5	2	3	800	0	0	0	0
Thessaloniki	Virginia	3	0	0	0	0	–	–	–	–	–
	Oriental	335	0.3	5.4	6.7	0	–	–	–	–	–
Chalkidiki	Oriental	140	0.6	3.6	10.7	0	300	0	0	0	0
Pella	Burley	15	13.2	80	0	0	–	–	–	–	–
	Oriental	141	0	2.1	0	0	150	0	0	0	0
Imathia	Burley	170	1.8	3.6	0	0	–	–	–	–	–
Pieria	Oriental	250	0.4	2.4	0	2	300	0.3	0	0	0
Larissa	Oriental	50	0	2	0	12	150	0	0	0	0
	Virginia	–	–	–	–	–	100	0	0	0	0
Karditsa	Burley	50	0	0	0	0	–	–	–	–	–
	Oriental	52	3.8	0	0	0	750	0.4	0	0	0.3
	Virginia	51	55.5	0	0	0	–	–	–	–	–

Field surveys

The relative frequencies of PVY, CMV, TSWV and AMV sampled during 1997, 1998 and 1999 are shown in Tables 2–4, respectively. For the viruses identified, we considered vein clearing and/or necrosis and top necrosis in oriental and a yellowing with veinal necrosis in the Virginia and Burley plants associated with PVY, while a severe to faint mosaic was associated either with CMV or TMV; CMV infection could also induce the appearance of chlorotic rings and patterns. These symptoms differed considerably from those caused by TSWV; plants were showing from mild mosaic and chlorotic or necrotic ring spots and patterns to severe dwarfing and necrosis of the young plants. Finally, AMV was distinct by causing an apparent yellow mosaic and/or different yellow type of patterns in all varieties. Mixed infections, especially of PVY and CMV were often, resulting in a variation of the symptoms observed.

Virus incidence, as estimated by the number of symptomatic plants present in the field, highly fluctuated depending on the year, tobacco variety and sampling area. Virus identification showed that PVY and CMV were the most commonly found viruses, detected every year in almost all examined varieties and regions, even in 100% of infected plants in some individual fields (results not shown). Those viruses consistently prevailed in Northern and Central Greece. In Pella, their relative incidence among symptomatic plants reached higher rates both in oriental tobacco (81% for PVY and 68% for CMV) in 1997, as well as in Virginia (100% for CMV) and Burley (44% for PVY) in 1999. Close to that area, in Imathia higher infection rates were recorded for CMV in Virginia (100% in 1997) or Burley (74% in 1998) but also in oriental crops in Larissa (49% in 1998) and Karditsa (56% in 1999). Finally in Aitoloakarnania, the higher PVY infection (94%) among oriental tobacco was recorded during the 1999 sampling.

TSWV was mainly found in Northern Greece (Central, East Macedonia and Thrace: Thessaloniki, Kilkis, Drama, Kavala, Xanthi and Rhodopi provinces) where some fields were totally infected. In Thessaloniki we recorded high infection rates

Table 2. Incidence (%) of PVY, CMV, TSWV and AMV in tobacco fields (Burley, oriental, Virginia) sampled in different areas in Greece in 1997

County	Variety	% symptomatic plants	No. of samples	Virus incidence (%)			
				PVY	CMV	TSWV	AMV
Rhodopi	Virginia	80–85	52	15	0	75	0
	Oriental	30–60	66	7	2	86	0
Drama	Oriental	2–30	231	32	25	14	0
Serres	Oriental	2–40	492	14	13	61	0
Kavala	Oriental	5–30	210	61	10	7	0
Thessaloniki	Oriental	80–90	16	0	0	100	0
Pella	Burley	20–80	28	25	61	0	4
	Oriental	2–100	230	81	68	0.4	50
Imathia	Virginia	–[a]	12	58	100	0	0
	Burley	–	32	78	81	0	3
Kozani	Oriental	1–100	231	72	43	0	11
Pieria	Burley	10–70	10	80	70	0	0
	Oriental	2–30	102	45	10	0	0.9
Grevena	Oriental	–	62	39	57	0	5
Larissa	Oriental	1–90	349	13	38	0	5
Trikala	Virginia	50–70	56	40	20	0	0
Karditsa	Virginia	45–60	62	21	61	0	0
	Burley	30–70	101	13	76	0	0
	Oriental	10–65	31	39	65	0	0
Lamia	Virginia	40–70	19	42	32	0	26

[a] Not recorded.

Table 3. Incidence (%) of PVY, CMV, TSWV, TMV and AMV in tobacco fields (Burley, oriental, Virginia) sampled in different areas in Greece in 1998

County	Variety	% symptomatic plants	No. of samples	Virus incidence (%)				
				PVY	CMV	TSWV	TMV	AMV
Rhodopi	Oriental	0.01–34	290	1.0	1.0	82.4	8.6	1.4
Xanthi	Oriental	0.1–65	549	1.8	1.1	79.5	13.5	1.1
Drama	Oriental	0.1–8	70	7.1	1.4	15.7	62.9	0
Serres	Oriental	0.01–66	759	2.2	0.8	76	7.1	0.4
Kavala	Oriental	1–68	486	3.3	4.7	77.6	12.6	1
Kilkis	Oriental	10–40	7	0	14.3	57.1	0	0
	Virginia	1–50	137	2.9	8.8	81	7.3	1.5
Thessaloniki	Oriental	0.01–85	101	2	0	88.1	4	0
	Virginia	0.1–60	166	10.8	2.4	74.1	12.7	1.2
Chalkidiki	Oriental	1–86	154	5.2	0.7	72	5.8	2.6
	Virginia	40	7	0	0	85.6	0	0
Pella	Oriental	0.001–100	399	38.6	21.1	0	7.1	7.8
	Burley	30–80	46	15.2	74	0	0	37
Imathia	Burley	5–75	75	2.7	5.3	0	0	14.7
Kozani	Oriental	10–17	70	31.4	40	0	5.7	8.6
Pieria	Oriental	1–60	242	7.4	14.9	0	57.9	0.8
	Burley	2–80	9	55.5	22.2	0	0	0
Larissa	Oriental	–[a]	51	3.9	49	0	11.8	25.5
Trikala	Oriental	20–100	90	17.8	13.3	0	100	10
	Burley	10	10	0	0	0	100	0
	Virginia	5	2	0	0	0	100	0
Karditsa	Oriental	0.01–100	87	31	13.8	0	21.8	0
	Burley	0.01–10	59	54.2	33.9	0	50.9	0
	Virginia	0.5–2	14	21.4	50	0	57.1	0
Argolida	Oriental	0.001–25	283	11.3	22.6	0	62.5	1.4

[a] Not recorded.

both for oriental (100% and 88.1% in 1997 and 1998, respectively) or Virginia (100% in 1999) crops. High virus incidence for oriental tobacco was also recorded in Kilkis (93.3% in 1999) and for Virginia in Rhodopi (75% in 1997) and Chalkidiki (85.6% in 1998). TSWV was not detected in the surveyed provinces of Western, Central and Southern Greece (Pella, Imathia, Kozani, Pieria, Trikala, Larissa, Karditsa, Aitoloakarnania and Argolida). Despite that, in the summer of 2000, high infection rates of TSWV, ranging from 50% to 80%, were recorded in Western Macedonia (Kastoria and Kozani) causing high crop losses (results not shown).

Among the aphid-borne viruses detected, the most limited spread was recorded for AMV. This virus was found mainly in regions of Central and Northern Greece, in tobacco crops adjacent to alfalfa fields containing a high number of plants showing typical AMV symptoms. Infection of those alfalfa fields was confirmed by testing representative samples by ELISA. In Pella, greatest incidence was recorded for oriental (50% in 1997), Burley (37% in 1998) and Virginia (10% in 1999) crops. Higher rates were also recorded in Larissa (25.5% in 1998) and Karditsa (1.3% in 1999) for oriental and in Lamia (26% in 1997) for Virginia crops. During this survey AMV was not detected in Aitoloakarnania and Argolida provinces.

Field samples collected during 1998 and 1999 were also tested for the presence of TMV. This virus was encountered in all surveyed areas, except Imathia, but predominated in oriental varieties in Argolida, Drama and Pieria, but also in Burley and Virginia in Trikala and Karditsa. All plants, collected in Trikala in 1998 were infected by TMV, irrespectively of the variety. However its incidence was higher in oriental tobacco and most Virginia or Burley fields were symptomless, therefore were not sampled. Top rates in 1999 were found in Argolida (87.8%) for oriental and in Pella (only up to 9.3%) for Burley crops.

Table 4. Incidence (%) of PVY, CMV, TSWV, TMV and AMV in tobacco fields (Burley, oriental, Virginia) sampled in different areas in Greece in 1999

County	Variety	% symptomatic plants	No. of samples	Virus incidence (%)				
				PVY	CMV	TSWV	TMV	AMV
Xanthi	Oriental	3–95	497	5.4	0	64.2	45.1	0
Drama	Oriental	1–22	181	6.6	0.6	21	55.2	0.6
Serres	Oriental	1–80	832	5.6	7.1	48.1	38.9	0.1
Kavala	Oriental	1–82	618	5	1.8	65.4	35.3	0.2
Kilkis	Virginia	40–90	70	5.7	5.7	94.3	5.7	8.6
	Oriental	50–80	30	10	6.7	93.3	0	0
Thessaloniki	Oriental	80–100	52	9.6	5.8	55.8	23.1	0
	Virginia	27	10	0	0	100	0	0
Pella	Virginia	10	10	0	100	0	0	10
	Burley	1–50	86	44	72	17.4	9.3	0
Pieria	Oriental	0.1–4	26	7.7	0	0	26.9	0
Trikala	Oriental	1–2	30	10	33	0	73.3	0
Karditsa	Oriental	2–5	78	77	56	0	16.7	1.3
Aitoloakarnania	Oriental	–[a]	48	94	8.3	0	35.4	0
Argolida	Oriental	0.5–75	433	8.8	8.3	0	87.8	0

[a] Not recorded.

During all sampling years, in numerous field samples showing virus-like symptoms, we could not detect PVY, CMV, AMV, TSWV or TMV. In several of those originated from Kilkis, Drama, Lamia, Karditsa and Rhodopi showing severe stunting, leaf deformation and vein clearing, we detected EMDV. The virus was found during all samplings years in both oriental and aromatic type varieties and cultivars, its incidence was constantly very low (<0.01%), while infected plants were always located in the field edges. In a few tobacco crops in Pieria, plants where found that showed symptoms similar to those caused by TRV, such as stem necrosis and necrotic ring patterns in the leaf lamina, while most plants in Argolida showed severe necrotic spotting and rings symptoms associated with AYRSV. Although from those plants we were able to reproduce the symptoms after mechanical inoculations on *N. tabacum* 'Samsun' plants in the laboratory, we did not detected any of the viruses under study.

Weeds as virus reservoirs

PVY incidence in weeds. Out of the 559 plants sampled during 1998 and tested for PVY, 13 plant species in eight families were found infected (Table 5). In the samplings performed this year, 16 plant species, belonging to 15 families, although sampled, were not found to be infected with PVY (numbers in parenthesis represents total number of plants tested): **Amaranthaceae;** *Amaranthus blitoides* (11), *A. retroflexus* (76); **Apocynaceae:** *Apocynum* sp. (1); **Boraginaceae:** *Heliotropium europaeum* (2); **Chenopodiaceae:** *Chenopodium album* (72); **Compositae:** *Chamomilla suaveolens* (3); **Cruciferae:** *Capsella bursa-pastoris* (4); **Fabaceae:** *Melilotus officinalis* (11); **Gramineae:** *Sorghum halepense* (20); **Labiatae:** *Mentha longifolia* (21); **Onagraceae**: *Epilobium hirsutum* (1); **Plantaginaceae:** *Plantago major* (2); **Polygonaceae:** *Polygonum aviculare* (1); **Rubiaceae:** *Galium aparine* (42); **Solanaceae:** *Datura stramonium* (15) and **Urticaceae**: *Urtica dioica* (1). During the survey performed in 1999 and 2000, PVY was detected by ELISA in 11 species belonging to 7 families (Table 6). In total, during the study among 3450 samples tested for PVY, 17 species in 10 families were identified as hosts of the virus.

CMV incidence in weeds. Weeds were tested for CMV presence only during 1999 and 2000, however results showed that it is the most widespread virus among the native flora. Among 2931 weed samples collected, 19 species from 12 families were positive to CMV in ELISA tests, with most belonging to the Compositae family (Table 6).

Table 5. The number of PVY-infected annual (An), biennial (Bi) and perennial (Pe) weed species sampled in tobacco crops during 1998

Family	Species	Life span	No. of plants infected/tested
Caprifoliaceae	*Sambucus ebulus*[a,b]	Pe	1/17
Compositae	*Cichorium intybus*[a,b]	Pe	7/7
	Cirsium arvense[a]	Pe	2/12
	Conyza canadensis[a,b]	An	6/8
	Lactuca serriola[a,b]	Bi (An)	2/2
	Xanthium spinosum[a]	An	1/1
	Xanthium strumarium[a]	An	5/7
Convolvulaceae	*Convolvulus arvensis*[b]	Pe	3/3
Cyperaceae	*Cyperus rotundus*[a]	Pe	43/47
Dispacaceae	*Scabiosa tennuiis*[a,b]	An (Bi)	10/22
Plantaginaceae	*Plantago lanceolata*	Pe	1/1
Portulacaceae	*Portulaca oleracea*	An	2/22
Solanaceae	*Solanum nigrun*[b]	An	3/44

[a] Possible new hosts.
[b] Infection was confirmed in bioassays.

Table 6. The number of PVY and CMV-infected annual (An), biennial (Bi) and perennial (Pe) weed species sampled in tobacco crops in 1999 and 2000

Family	Species	Life span	No. of tested plants	No. of infected plants	
				PVY	CMV
Amaranthaceae	*Amaranthus blitoides*[b]	An	73	–	1
	Amaranthus retroflexus	An	133	–	1[c]
Boraginaceae	*Heliotropium europaeum*[a,c]	An	40	3[c]	–
Compositae	*Cichorium intybus*[a]	Pe	63	1[c]	4
	Cirsium arvense	Pe	3	–	1
	Chondrila juncea[b]	Pe	13	–	1
	Conyza canadensis[a]	An	118	10[c]	14[c]
	Picris echioides[a]	An	5	2[c]	–
	Senecio vulgaris	An (Bi)	60	–	2[c]
	Sonchus oleraceus	An (Bi)	94	1	5[c]
	Xanthium strumarium[a]	An	65	4	–
Convolvulaceae	*Convolvulus arvensis*	Pe	159	1[c]	1
Cruciferae	*Capsella bursa-pastoris*	Bi	150	–	2[c]
	Raphanus sativus	An	50	–	9[c]
Cyperaceae	*Cyperus rotundus*[a]	Pe	32	3	–
Fabaceae	*Vicia* sp.		37	–	1[c]
Labiatae	*Mentha pulegium*[a,b]	Pe	16	12	14
Malvaceae	*Malva neglecta*[b]	An	57	–	1
Papaveraceae	*Fumaria officinalis*[b]	An	16	–	1
Polygonaceae	*Polygonum persicaria*	Pe	1	–	1
Portulacaceae	*Portulaca oleracea*	An	139	1	9[c]
Rubiaceae	*Galium aparine*	An	30	–	1[c]
Solanaceae	*Solanum nigrum*	An	128	15[c]	7[c]

Possible new hosts [a] PVY or [b] CMV.
[c] Infection was confirmed in bioassays.

AMV incidence in weeds. In 1998, among 961 samples collected from AMV infected tobacco fields and tested for AMV presence, plants from 13 species belonging to 9 families were found infected (Table 7). Thirty four plant species belonging to 20 families although sampled were not infected with

Table 7. The number of AMV-infected annual (An), biennial (Bi) and perennial (Pe) weed species sampled in tobacco crops during 1998

Family	Species	Life span	No. of plants infected/tested
Apiaceae (Umbelliferae)	*Daucus carota*[a]	An (Bi)	51/54
Chenopodiaceae	*Chenopodium album*	An	1/74
Compositae	*Picris echioides*[b]	An	4/16
	Sonchus oleraceus[a]	An (Bi)	2/11
	Xanthium strumarium[a]	An	21/45
Cruciferae	*Cardaria draba*[a]	An	1/17
Cyperaceae	*Cyperus rotundus*[b]	Pe	5/15
Dispacaceae	*Scabiosa tenuiis*[a,b]	An (Bi)	6/80
Plantaginaceae	*Plantago lanceolata*	Pe	1/3
	Plantago major	Pe	1/6
Solanaceae	*Solanum nigrum*[a]	An	1/37
Urticaceae	*Urtica dioica*[a,b]	Pe	2/10

[a] Possible new hosts.
[b] Infection was confirmed in bioassays.

AMV (numbers in parenthesis represents total number of plants tested): **Amaranthaceae**: *Amaranthus albus* (23), *A. retroflexus* (199); **Apiaceae (Umbelliferae):** *Conium maculatum* (1); **Apocynaceae**: *Apocynum* sp. (1); **Aristolochiaceae**: *Aristolochia clematitis* (7); **Boraginaceae**: *Heliotropium europaeum* (4); **Caprifoliaceae**: *Sambucus ebulus* (27); **Compositae**: *Artemisia vulgaris* (2), *Chondrila juncea* (1), *Cichorium intybus* (1), *Cirsium arvense* (4), *Chamomilla suaveolens* (1), *Conyza canadensis* (47), *Lactuca serriola* (29), *Senecio vulgaris* (4); **Convolvulaceae**: *Convolvulus arvensis* (11); **Fabaceae**: *Melilotus officinalis* (11); **Gramineae**: *Cynodon dactylon* (2), *Dactylis glomerata* (1), *Echinochloa crus-galli* (1), *Sorghum halepense* (61); **Hypericaceae**: *Hypericum perforatum* (8); **Labiatae**: *Mentha longifolia* (21); **Malvaceae**: *Abutilon theophrasti* (8), *Malva neglecta* (2); **Onagraceae**: *Epilobium hirsutum* (1); **Polygonaceae**: *Polygonum aviculare* (2), *P. persicaria* (6), *Rumex crispus* (30); **Portulacaceae**: *Portulaca oleracea* (53); **Ranunculaceae**: *Clematis vitalba* (4); **Rubiaceae**: *Galium lucidum* (3); **Scrophulariaceae**: *Verbascum blattaria* (2) and **Solanaceae:** *Datura stramonium* (15).

In the surveys performed during 1999 and 2000 none of PVY, CMV or AMV could be detected by ELISA in plants of 70 species out of 30 families. These species and their families were **Amaranthaceae:** *Amaranthus retroflexus* (28); **Apiaceae:** *Bupleurum* sp. (3), *Conium maculatum* (11), *Pastinaca sativa* (1); **Boraginaceae:** *Anchusa* sp. (3); **Caprifoliaceae:** *Sambucus ebulus* (24); **Caryophyllaceae:** *Cerastium hirsutum* (6), *Stellaria media* (64); **Chenopodiaceae:** *Artiplex prostrata* (14), *Chenopodium album* (122), *Salsora kali* (5); **Compositae:** *Artemisia vulgaris* (46), *Carduus nutans* (1), *Chamomilla recutita* (70), *Crepis setosa* (40), *Crepis* sp. (2), *Lactuca serriola* (40), *Marticaria perforata* (2), *Picris sprengerana* (1); **Cucurbitaceae:** *Ecbalium elaterium* (2); **Cruciferae:** *Cardaria draba* (27), *Raphanus* sp. (34), *Sinapis arvensis* (13), *Sisymbrium irio* (38); **Dispacaceae:** *Scabiosa tenuis* (34); **Euphorbiaceae:** *Euphorbia exigua* (6); *Chrozophora tinctoria* (2); **Fabaceae:** *Vicia* sp. (20), *Trifolium* sp. (22); **Geraniaceae:** *Erodium cicutarium* (5), *Geranium* sp. (5); **Graminae:** *Alopecurus myosuroides* (1), *Cynodon dactylon* (43), *Lolium perenne* (10), *Poa annua* (68), *Phalaris* sp. (5), *Sorgum halepense* (142); **Gentianaceae:** *Centaurium spicatum* (3); **Guttiferae:** *Hypericum perforatum* (2); **Labiatae:** *Ballota nigra* (17), *Lamium amplexicaule* (21), *L. purpureum* (23), *Mentha longifolia* (10), *Stachys* sp. (16); **Malvaceae:** *Abutilon theophrasti* (15); **Onagraceae:** *Epilobium hirsutum* (1), *E. lanceolatum* (2); **Papaveraceae:** *Papaver rhoeas* (66); **Plantaginaceae:** *Plantago lanceolata* (1), *P. major* (16); **Polygonaceae:** *Polygonum* sp. (14), *P. aviculare* (19), *Rumex crispus* (12); **Ranunculaceae:** *Anemone coronaria* (6), *Ficaria anunculoides* (8); **Rosaceae:** *Aremonia agrimonoides* (17), *Potentilla reptans* (4); **Rubiaceae:** *Galium* sp. (11); ***Scrophulariaceae:*** *Kickxia elatine* (10), *Verbascum blattaria* (9), *Veronica hederifolia* (6); *V. officinalis* (11), *V. persica* (3); **Solanaceae:** *Datura stramonium* (42), *Hyoscyamus albus* (8), *Solanum dulcamara* (25); **Urticaceae:** *Urtica dioica* (11); **Verbenaceae:** *Ver-*

bena officinalis (1), *Vitex agnus-castus* (2); **Zygophylaceae:** *Tribulus terrestris* (37).

All plants of the species of *Apocynum* sp., *Crepis setosa*, *Crepis* sp., *Epilobium hirsutum*, *Erigeron* sp., *Malva longifolia*, *Anchusa* sp. and *Verbascum blattaria* gave high OD values for all tested viruses, even when plants of the same species were collected from areas where no any of the viruses under study could be detected. None of those plants showed any virus-symptoms and when representative samples were bioassayed, none of the indicator plants was infected. These plants were, therefore, considered to be healthy and the positive ELISA reactions were explained by a strong non-specific reaction.

Discussion

Surveying the main tobacco producing areas in Greece we have encountered several viruses. Among those, PVY and CMV were present in all tobacco varieties and sampling areas, and therefore can be considered as a prominent viral threat for Greek tobacco industry. Serious problems were encountered mainly in Northern and Central Greece, where crop losses could reach the point of complete crop failure especially because of PVY. Although no strain specific antibodies were used for PVY detection, the symptomatology encountered (leaf yellowing, veinal necrosis) suggests that the necrotic strain (PVY^n) is mainly involved.

Both PVY and CMV, were present in several seedbeds, suggesting that infected seedlings can act as internal virus sources for further spread in the field. As these viruses are not seed transmitted to tobacco (Francki et al., 1979; Jaspars and Bos, 1980; De Bokx and Huttinga, 1981), their incidence highly depends on the presence of alternative virus sources and active vector populations. Large surveys and studies on *Myzus persicae*, an efficient vector of tobacco aphid-transmitted viruses, have shown that anholocyclic genotypes, originating from alternative hosts, prevail in tobacco seedbeds (Margaritopoulos et al., 2002). Furthermore, aphid emergence and population fluctuation, have been associated with the onset and final incidence of CMV and PVY in tobacco crops (Tsitsipis et al., 2001).

Several ornamentals and vegetables, cultivated usually in the vicinity of seedbeds, which are situated in the surroundings of the farmers' house, can contribute to virus epidemiology. Infected potato plants in the vicinity can be significant PVY source (Marte and Bellezza, 1988). In this study we also observed that, in some cases of PVY epidemics, potato volunteer plants were scattered throughout the affected tobacco crop, while alfalfa fields seem to be significant AMV sources for the adjacent tobacco crops. Except cultivated hosts, native flora is widely known as an extensive collection of virus and/or vector sources. Annual species can be primary inoculum during the cropping period, while virus perpetuation can be ensured through seed transmission or on perennial or biannual species (Duffus, 1971; Thresh, 1981). CMV whose epidemiology has been studied in lettuce crops (Rist and Lorbeer, 1991) is a representative virus; it overwinters in several weeds (Quiot et al., 1979) and it is seed transmitted in some of them, such as *Stellaria media* (Tomlinson and Carter, 1970). Interestingly, despite the high incidence of AMV in alfalfa crops (Avgelis and Katis, 1989; this study) and the infection of several weeds adjacent to crops, AMV incidence in tobacco remained low compared to the other aphid transmitted viruses. This confirms that several factors, such as the number of aphid species present and their relative ability to transmit, as well as virus titer in the host plant involved in virus acquisition by its vectors, greatly affect virus spread in the field (Zitter, 1977). Therefore, detailed studies are needed in order to determine the actual role of the alternative reservoirs as virus sources.

Among the species identified as hosts of PVY, CMV and AMV several have not been reported previously. To our knowledge this is the first report of natural infection of *Sambucus ebulus, Cichorium intybus, Conyza canadensis*, *Helliotropium europaeum, Lactuca serriola, Picris echioides* and *Scabiosa tennuiis* by PVY (Schmelzer, 1967; De Bokx and Huttinga, 1981; Katis, 1984; Edwardson and Christie, 1997; Espino de Paz et al., 1997; Škorić et al., 2000; Fletcher, 2001; Kazinczi et al., 2002), of *Fumaria officinalis* by CMV (Douine et al., 1979; Francki et al., 1979; Ford et al., 1988; Rist and Lorbeer, 1989, 1991; Crescenzi et al., 1993; Lavińa et al., 1996; Fletcher, 2001; Rodriguez-Alvarado et al., 2002), and of *Scabiosa tenuiis* and *Urtica dioica* by AMV (Jaspars and Bos, 1980; Fletcher, 2001; Kazinczi et al., 2002). Some others

such as *Cirsium arvense*, *Cyperus rotundus, Mentha pulegium, Xanthium spinosum* and *X. strumarium* for PVY, *Amaranthus blitoides, Chondrila juncea*, *Malva neglecta* and *Mentha pulegium* for CMV, as well as *Cyperus rotundus* and *Picris echioides* for AMV, were found infected for the first time by ELISA, but the virus detected was not readily recoverable in bioassays. Factors such as low virus titre and stability (Rist and Lorbeer, 1989), the presence of inhibitors in sap (Yarwood and Fulton, 1976) or the susceptibility of test plants and their physiological age may have resulted in an unsuccessful inoculation. Although ELISA is undoubtedly practical for large-scale surveys, special attention should be paid on the use of virus-free samples for all species (Rist and Lorbeer, 1989) in order to reduce the effect of non-specific reaction (Chatzivassiliou et al., 2001; this study) and the results must be confirmed by tests of infectivity on indicator plants.

Apart from PVY and CMV, TSWV is also considered an important viral disease in tobacco crops since its first report in Drama in 1972 (Tsakiridis and Gooding, 1972), especially in Central and East Macedonia and Thrace (Chatzivassiliou et al., 2000b). In these areas, the coexistence of TSWV with populations of *Thrips tabaci*, showing high vectoring ability (Chatzivassiliou et al., 2002) has resulted in a significant spread in crops of all tobacco varieties as well as among the native flora (Chatzivassiliou et al., 2001). Under those circumstances, TSWV consists a continuous threat especially during years when environmental conditions favor the increase of *T. tabaci* populations.

Since all cultivated varieties in Greece are susceptible to TSWV (Chatzivassiliou, unpublished results), as expected from the limited available sources of natural resistance (Prins and Goldbach, 1998), the absence of evident spread in West, South and Central Greece, can be firstly attributed to the absence of virus sources and/or its thrips vector. In Pieria, although TSWV was detected in numerous ornamentals and vegetables (Chatzivassiliou et al., 2000a), no infection has been detected in the adjacent tobacco seedbeds. The inability of *Frankliniella occidentalis*, the only vector present in the area, to infest tobacco (Chatzivassiliou, unpublished results) may have prevented TSWV from spreading into tobacco crops. However, tobacco invasion will be inevitable, when *T. tabaci*, will be introduced in the area, as both thrips species share an overlapping host range including TSWV hosts. Afterwards, favorable environmental conditions can lead to a geometrical increase of vector population and result to an extensive virus spread. This can explain the epidemic appearance of TSWV in Western Macedonia during 2000, although its presence remained unnoticed in our surveys.

Among the other viruses commonly encountered in tobacco fields, TMV also occupies an important position. This virus is present throughout the country, but a particularly extensive spread was recorded in oriental tobacco cultivated in Argolida, Trikala and Drama, some of the oldest tobacco producing provinces. Although TMV is known to be transmitted through tobacco seeds (Zaitlin and Israel, 1975) and although certified seeds for all cultivated varieties are available by the National Agricultural Tobacco Research Institute, a large number of farmers continue to use non-certified seed produced locally at least for the oriental varieties. This practice in combination with the mechanical transmission of TMV is of major importance for its spread. In the long run the longstanding presence of TMV in the crop remains (Zaitlin and Israel, 1975) and the short rotation applied (3–4 years) favors an ongoing presence of this virus. The use of certified seed and the cultivation of irrigated fields, where no oriental tobacco has been previously grown, seem to be the main reasons for the lower TMV incidence or the absence of any virus in Virginia or Burley fields in areas where TMV was the main if not the only virus present in oriental tobacco e.g. Trikala.

Another virus identified in this study, that has only recently been reported to infect tobacco (Polverari et al., 1996; Ribeiro et al., 1996), is EMDV. This virus, known also to infect tomato, pepper, eggplant and cucumber in Greece (Kyriakopoulou et al., 1994; Katis et al., 2000), was found in several quite distant tobacco-producing areas. EMDV is generally considered of minor importance, due to a really limited (<0.01%) spread within tobacco fields. However, we cannot speculate its future significance due to a lack of data concerning the association of its only identified vector, the leafhopper *Agallia vorobjevi* (Babaie and Izadpanah, 2003) or other vectors possibly involved with tobacco crops in Greece. Although we encountered symptoms similar to

those caused by TRV in Pieria, where this virus was firstly reported (Bem et al., 1987), and the occurrence of the diseased plants could be associated with the limited spreading ability of TRV's nematode vectors (Robinson and Harrison, 1989), we did not confirm the presence of this virus. Simirarly, the endemic presence of AYRV in artichoke and/or tobacco crops in Argolida (Kyriakopoulou, 1981) and the symptoms encountered in several of the samples from this area tested negative for the viruses under study, suggest that this could be the virus involved. Finally, in some samples displaying virus-like symptoms we could not detect any virus, after mechanical inoculation onto indicator plants suggesting the presence of another agent, virus or phytoplasmas.

Our results on the incidence of viruses in tobacco crops and the associated wild flora, as well as their specific epidemiological properties suggest that the insect transmitted PVY, CMV and TSWV consist the main threat for the Greek tobacco production. Due to the importance of tobacco in the national economy of the country, it is crucial to prevent destructive virus spread and subsequent crop losses. The elimination of weeds that consists the main alternative virus and vectors' reservoirs, is generally suggested as an effective procedure for managing virus epidemics (Duffus, 1971; Thresh, 1981). Even difficult to apply, due to the numerous host species involved, this control measure has been successful in controlling CMV in lettuce and celery crops (Doolittle and Walker, 1926; Wellman, 1937). Alternatively, breeding for resistance still remains the most effective way of combating viral diseases and should be further exploited.

Further studies must be carried out in order to confirm the presence of TRV and AYRV, to look for other viruses present in tobacco crops in Greece and evaluate their impact on tobacco production.

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