

SHORT COMMUNICATION
VIRUSES OF CUCURBITS IN PANAMA

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SUMMARY

The main areas of field-grown cucurbit production in Panama were surveyed for the occurrence and distribution of viruses in the growing seasons of 2006 and 2008. Notably, in 2006 only *Melon necrotic spot virus* (MNSV) was detected, while *Papaya ringspot virus* (PRSV) was the most frequent in 2008 in terms of number of fields and samples analysed, followed by *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and MNSV. *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Cucumber green mottle mosaic virus* (CGMMV), *Tomato spotted wilt virus* (TSWV), *Cucumber leaf spot virus* (CLSV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucurbit aphid-borne yellows virus* (CABYV), *Beet pseudo-yellows virus* (BPYV), *Beet yellows virus* (BYV), and *Cucumber vein yellowing virus* (CVYV) were not detected. Single and double (PRSV+WMV; PRSV+ZYMV; ZYMV+MNSV) infections were detected in 60% and 40% of the samples tested, respectively. The occurrence and distribution of each virus varied according to the province and cucurbit species.

Key words: Cucurbitaceae, mixed infection, single infection, virus diseases, survey, diagnosis.

Cultivated cucurbit species [cucumber, *Cucumis sativus*; melon, *Cucumis melo*; squash, *Cucurbita moschata*; watermelon, *Citrullus lanatus*] are important vegetable crops worldwide. Panama is the sixteenth largest producer of melons in the American continent (FAO, 2012). Melon is the most cultivated cucurbit species (3,500 ha; 13,646 t/year) in Panama (FAO, 2012) where virtually the whole production originates from field-grown crops, mainly in the central provinces of the country.

Virus diseases are a worldwide problem for cucurbit production and the cause of severe economic losses. Of the approximately 35 different viruses infecting Cucurbitaceae worldwide (Provvidenti, 1996), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*), *Squash mosaic virus* (SqMV, genus *Comovirus*), *Melon necrotic spot virus* (MNSV, genus *Carmovirus*) and the potyviruses *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) are known to occur in field-grown melons in the Los Santos province (Panama) (García, 1997; Herrera *et al.*, 2006a,b). All these viruses, except for SqMV and MNSV, are transmitted by aphids in a non-persistent manner (Pirone and Harris, 1970). In addition, CMV is transmitted by seeds (Sharma and Chohan, 1974), SqMV by beetles and seeds (Franken *et al.*, 1990) and MNSV by the chytrid fungus *Olpidium bornovanus* (Sahtiyanci) Karling (Campbell *et al.*, 1995) and seeds (Herrera-Vásquez *et al.*, 2009b).

Symptoms caused by MNSV in melons include stem necrosis at the crown level and, less frequently, small necrotic spots that may abscise, leaving holes on the leaves and fruits. Virus-induced wilting and plant death have also been observed (Herrera *et al.*, 2006a). Foliar symptoms caused by the other viruses include mosaic, mottling, chlorotic mottle, vein clearing or vein banding, leaf distortion and malformation, reduction in plant growth and flower abortion. Fruits can present depressed and discoloured areas and abnormal shapes that render them unmarketable. All the aforementioned viruses can cause considerable crop losses when plants are infected during the early growth stages (Nameth *et al.*, 1986).

Despite the importance of field-grown cucurbits in Panama, information on the occurrence of viral diseases is limited to melon in the Los Santos provinces (García, 1997; Herrera *et al.*, 2006a, 2006b). Thus, we surveyed all the other areas of the country where cucurbits are field-grown in order to estimate the occurrence and distribution of 14 important viruses affecting cucurbit crops.

Surveys were conducted during the 2006 and 2008 growing seasons in five provinces of Panama in which melons, squash and watermelons are field-grown (Fig.

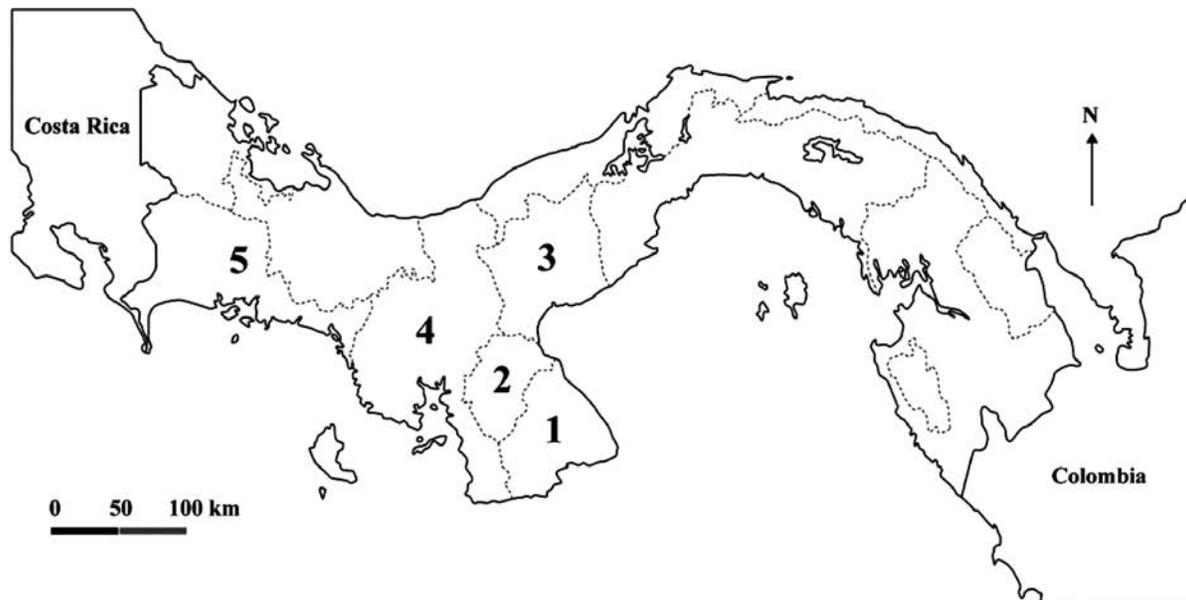


Fig. 1. Los Santos (1), Herrera (2), Coclé (3), Veraguas (4) and Chiriquí (5) provinces surveyed to determine the presence or absence of viruses in cucurbit fields.

1). In each province fields were arbitrarily selected, visited and surveyed prior to harvest, while samples were collected from mature plants. Six melon samples from plants showing the characteristic necrotic symptoms of MNSV infection (Fig. 2a) and 18 with no symptoms, were obtained from 11 fields in 2006, whereas 56 samples of melon, squash and watermelon from plants showing virus like-symptoms (Fig. 2b, c) and 6 with no symptoms, were collected from 26 fields in 2008 (Table 1). The number of samples collected in each field depended on the number of symptomatic plants observed.

Root samples were stored at -20°C , while leaf samples were dried using the silica gel method and kept at room temperature until analysed. Root samples were analysed for MNSV and leaf samples for CMV, SqMV, PRSV, WMV, ZYMV, *Cucumber green mottle mosaic virus* (CGMMV, genus *Tobamovirus*) and *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*) by ELISA using commercial kits by Loewe Biochemica (Germany) for SqMV, CGMMV and TSWV and by Sediag (France) for the rest of the viruses, following manufacturers' instructions. Fresh root or dry leaf samples (0.5 g) were homogenised in a plastic bag containing 10 ml extraction buffer [2% PVP in PBS-Tween, 1:20 (w/v)]. Healthy and virus-infected cucurbit root and leaf samples were included as negative and positive controls, respectively. ELISA reactions were read at 405 nm and a sample was considered positive if the absorbance value was twice higher than the mean of the negative control samples.

Root (for MNSV) and leaf (for other viruses) samples were also analysed by RT-PCR as follows: fresh root or dry leaf samples (0.1 g) were ground in liquid nitrogen,

and total RNA was extracted using the RNAwiz kit (Ambion, UK). Single-step RT-PCR was carried out using SuperScript II RT with the Platinum *Taq* kit (Invitrogen, Spain) and specific primers for MNSV (Herrera *et al.*, 2006a), *Cucumber leaf spot virus* [CLSV, genus *Carmovirus*; Segundo *et al.* (2001)], *Cucurbit aphid-borne yellows virus* [CABYV, genus *Luteovirus* (Juárez *et al.*, 2004)], *Beet pseudo-yellows virus* [BPYV, genus *Crinivirus*; Rubio *et al.* (1999)], *Beet yellows virus* [BYV, genus *Closterovirus*; Kundu and Rysanek (2004)], *Cucurbit yellow stunting disorder virus* [CYSDV, genus *Crinivirus*; Celix *et al.* (1996)] and *Cucumber vein yellowing virus* [CVYV, genus *Ipomovirus*; Cuadrado *et al.* (2001)], while RT-PCR was carried out in a two-step reaction using the Cloned AMV First-Strand Synthesis Kit and Platinum *Taq* DNA Polymerase (Invitrogen, Spain) and universal degenerate primers for potyviruses (PRSV, WMV, ZYMV) (Herrera-Vásquez *et al.*, 2009a), according to the manufacturer's instructions and using the protocol described for each virus by the above mentioned authors.

In 2006, surveys were conducted in 11 commercial melon fields distributed in the Los Santos, Herrera and Coclé provinces (Fig. 1). MNSV, CMV, SqMV, PRSV, WMV, ZYMV, CGMMV, TSWV, CLSV, CABYV, BPYV, BYV, CYSDV and CVYV were looked for in 24 melon samples from these fields. Typical MNSV symptoms were shown by six samples (25% of all melon samples) from Los Santos and Coclé, and the virus presence was confirmed by ELISA and RT-PCR (Table 1). No other symptoms were observed in any of the regions and no additional viruses were detected in any of the melon samples analyzed.

In 2008, surveys were conducted in 26 commercial cucurbit fields in all Panamanian provinces where cucurbits (melon, squash and watermelon) are field-grown. In these areas, MNSV and other virus-like symptoms were observed, and 62 samples from these fields were analysed for the presence of all the above listed viruses. Fifty-six plants (90.3% of all samples collected) from 23 fields (88.5% of all fields surveyed) showed symptoms of virus infection. As shown in Table 1, ZYMV was the prevailing virus (33.3% of melon samples analysed), followed by the PRSV (26.7%), WMV (26.7%) and MNSV (13.3%). PRSV and WMV were distributed in three provinces, while ZYMV and MNSV were only found in Los Santos. PRSV was the most common virus in watermelon (46.2%), followed by WMV (15.4%) and ZYMV (15.4%). PRSV and ZYMV were distributed in four and two provinces, respectively, while WMV was present only in Veraguas. PRSV and ZYMV were found in 33.3% of all squash samples analysed, both being detected only in Los Santos. These samples were ELISA-positive for the specific viruses, and RT-PCR positive for MNSV and/or potyviruses (Table 1).

MNSV was not detected in any of the watermelon and squash samples analysed in our study, and WMV was not found in any squash. CMV, SqMV, CGMMV, TSWV, CLSV, CABYV, BPYV, BYV, CYSDV and CVYV were not detected in any of the cucurbit samples analysed. However, CMV and SqMV have previously been detected in Panamanian melons from Los Santos (García, 1997). A larger number of samples should be tested in a future study for a better assessment of the presence/absence of these viruses in cucurbit species from Panama.

The presence of PRSV, WMV and ZYMV was validated by ELISA, which also allowed the specific identification of each virus. By contrast, the use of RT-PCR with potyvirus degenerate primers, confirmed infection, but was unable to distinguish the individual potyviruses.

Aphis gossypii Glover (Hemiptera:Aphididae) is the most common aphid found in melon crops of Panama (García, 1997). However, the presence of aphids was not noted in any of the provinces and fields surveyed in 2006, but was observed in 2008. This may explain the difference in the presence and incidence of aphid-borne viruses, which were detected in 2008, but not in 2006. The whitefly *Bemisia tabaci* Gennadius (Hemiptera:Aleyrodidae) (primarily biotype B) has also been reported in Panamanian cucurbit crops (Alvarado *et al.*, 2004). However, the presence of whiteflies was not investigated in this study.

Twenty-five species of weeds from 11 families (Amaranthaceae, Asclepiadaceae, Asteraceae, Boraginaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Labiatae, Malvaceae, Papilionaceae, and Solanaceae) growing in melon crops of Los Santos were reported as being

infected by at least one of three potyviruses (PRSV, WMV, ZYMV) (Herrera *et al.*, 2006b). Volunteer cucumber and melon plants and *Momordica charantia* are common in this province. The first couple of hosts had



Fig. 2. A. Symptoms of stem necrosis at the crown level typical of MNSV; B. mottling, blistering and malformation on a leaf infected by PRSV+WMV; C. mottling, blistering and abnormal growth on a melon fruit infect by ZYMV.

Table 1. Occurrence and distribution of viruses (surveys of 2006 and 2008) as determined by ELISA and RT-PCR on cucurbit samples.

Year of survey	Crop	Region	Fields ²	Samples ³	ELISA and RT-PCR detection ¹					% single infections ⁴	% double infections ⁴
					MNSV	PRSV	WMV	ZYMV	Negative		
2006	Melon	Los Santos	2/7	4/16	4 (2)	0	0	0	12	100	0
		Herrera	0/3	0/6	0	0	0	0	6	0	0
		Coclé	1/1	2/2	2 (1)	0	0	0	0	100	0
		Subtotal	3/11	6/24	6 (3)	0	0	0	18	100	0
2008	Melon	Los Santos	4/4	12/12	4 (2)	0	0	10 (4)	2	60	40
		Herrera	2/2	4/4	0	4 (2)	2 (1)	0	0	50	50
		Coclé	0/1	2/4	0	0	2 (1)	0	2	100	0
		Veraguas	1/1	2/2	0	2 (1)	0	0	0	100	0
		Chiriquí	3/3	8/8	0	2 (1)	4 (2)	0	2	100	0
		Subtotal	10/11	28/30	4 (2)	8 (4)	8 (4)	10 (4)	6	75	25
	Watermelon	Los Santos	1/1	2/2	0	0	0	2 (1)	0	100	0
		Herrera	2/2	6/6	0	2 (1)	0	0	4	100	0
		Coclé	2/2	4/4	0	2 (1)	0	0	2	100	0
		Veraguas	4/4	8/8	0	2 (1)	4 (2)	2 (1)	2	66.7	33.3
		Chiriquí	3/3	6/6	0	6 (3)	0	0	0	100	0
		Subtotal	12/12	26/26	0	12 (6)	4 (2)	4 (2)	8	88.9	11.1
	Squash	Los Santos	1/1	2/2	0	2 (1)	0	2 (1)	0	0	100
		Coclé	0/1	0/2	0	0	0	0	2	0	0
		Veraguas	0/1	0/2	0	0	0	0	2	0	0
		Subtotal	1/3	2/6	0	2 (1)	0	2 (1)	4	0	100
	Total			26/37	62/86	10 (5)	22 (11)	12 (6)	16 (7)	36	60

¹Number of plants positive. In parentheses are numbers of fields from which the positive samples were collected. CMV, SqMV, CGMMV, TSWV, CLSV, CABYV, BPYV, BYV, CYSDV and CVYV were not detected in this study. Therefore, these viruses were not included in the Table.

²Fields with symptomatic plants/fields surveyed.

³Symptomatic samples/samples analyzed.

⁴Percentage was calculated over the total number of virus-infected plants.

been found infected by PRSV, WMV and ZYMV – single and mixed infections (Herrera *et al.*, 2006b), both of which were detected also in our study, while *M. charantia* was identified as a reservoir of *Squash vein yellowing virus* (SqVYV, genus *Ipomovirus*) (Adkins *et al.*, 2008), as reported from Florida and Indiana (Adkins *et al.*, 2007; Egel and Adkins, 2007). Infected volunteer cucurbits and weeds constitute year-round viral reservoirs. Their improved management needs to be incorporated into cucurbit management plans.

The presence of CVYV but not of SqVYV was tested for, both of which are transmitted by *B. tabaci* (Adkins *et al.*, 2007; Lecoq *et al.*, 2007). CVYV has been detected in melon plants with mosaic symptoms in France (Lecoq *et al.*, 2007), but not in the New World so far. Since mosaic symptoms are shown by cucurbit crops in Panama, we checked for the presence of this virus, but no CVYV-positive samples were found. Thus, these symptoms could be caused by the mosaic-inducing potyviruses detected in our study (Nameth *et al.*, 1986). SqVYV is present in the US on watermelon and squash displaying a typical vine decline (including vine collapse and wilt and root rot) and vein yellowing, respectively (Adkins *et al.*, 2007; Egel and Adkins, 2007). Since these type of symptoms were not observed in watermelon and squash crops from Panama, the presence of this virus was not looked for. Further investigations are therefore needed to determine the presence or absence of SqVYV in Panama.

The occurrence and distribution of MNSV were reduced in 2008 compared to 2006 so that, in many of the surveyed plots there were no plants with MNSV-like symptoms, in line with the notion that with this virus, whose infections largely depend on environmental conditions, the symptoms observed in the field are not predictable (Herrera-Vásquez *et al.*, 2009b).

Whereas PRSV was the most prevalent and widespread virus, cucurbits were also infected by ZYMV and WMV, which were the second and third most represented, respectively. Previous studies by García (1997) found that PRSV was the most prevalent virus together with CMV, SqMV, WMV and ZYMV, in Panamanian melons from Los Santos. However, our study is the first to report virus occurrence and distribution in all areas in which cucurbits are field-grown in Panama.

PRSV, ZYMV, WMV and MNSV were found in 11 (42.3%), 7 (26.9%), 6 (23.1%) and 2 (7.7%) of the cucurbit fields surveyed in all provinces, respectively (Table 1). Seventeen of 26 fields were infected with at least one of these viruses as determined by ELISA and RT-PCR. Single infections were prevalent in each province and accounted for 75% and 88.9% of virus-infected melon and watermelon plants, respectively. Double infections by ZYMV+PRSV; ZYMV+MNSV; PRSV+WMV were found only in Los Santos, Herrera and Veraguas, accounting for 25, 11.1 and 100% of virus-infected melon,

watermelon, and squash plants, respectively (Table 1). Mixed infections of a potyvirus with a non-potyvirus could have a significant effect on yields since synergistic effects have been reported between these viruses (Poolpol and Inouye, 1986). Mixed infections have also been observed in Spain (Kassen *et al.*, 2007). Twelve samples (four melon and eight watermelon) from symptomatic plants collected in all the five provinces surveyed were ELISA- and RT-PCR-negative for all viruses tested. This may be due to the presence of cucurbit-infecting begomoviruses, that occur in several Central and South American countries (Morales, 2006), or of SqVYV, which has also been recorded in the New World (Adkins *et al.*, 2007; Egel and Adkins, 2007). Both begomoviruses and SqVYV are transmitted by whiteflies (Morales, 2006; Adkins *et al.*, 2007). Testing for begomovirus and for SqVYV should be carried out in the future.

Six plants (two melons from Coclé and four squash from Coclé and Veraguas), in which no virus symptoms were observed, were negative by ELISA and RT-PCR for all the viruses analysed (Table 1).

Surveys of field-grown cucurbits in Panama revealed that MNSV infection is of special concern in Los Santos and Coclé, while infections by mosaic-inducing viruses are significant throughout the country. This information should help establishing a basis for the control of viral epidemics in Panamanian cucurbits.

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