

## MANAGEMENT OF VIRUS DISEASES OF SQUASH IN LEBANON

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### SUMMARY

Virus diseases are the major cause of economic losses in commercial cucurbit production in Lebanon. Field tests showed that cross-protection with a mild strain of *Zucchini yellow mosaic virus* (ZYMV-WK) gave significant protection of squash plants against severe mosaic and leaf deformation symptoms induced by viruses and resulted in significant yield increase as compared to the control. The use of grey mulch gave only a low level of protection against such diseases. The highest total yield was obtained with floating row covers that gave good early protection against severe mosaic and leaf deformation symptoms and against the yellowing diseases. Plants showing yellowing symptoms were found to be infected by *Cucurbit aphid-borne yellows virus* (CABYV) or CABYV and *Cucurbit yellow stunting disorder virus* (CYSDV) in mixed infections.

*Key words:* squash, cross-protection, ZYMV, integrated management.

### INTRODUCTION

Cucurbits are among the major vegetables grown in Lebanon. Several virus diseases inducing mosaic symptoms were previously reported including the Potyviruses *Watermelon mosaic virus 2* (WMV-2), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* – watermelon strain (PRSV-W), *Zucchini yellow fleck virus* (ZYFV), and the Cucumovirus *Cucumber mosaic virus* (CMV) (Makkouk and Lesemann, 1980; Lesemann *et al.*, 1983; Katul, 1986). The incidence of leaf mosaic and deformation symptoms on squash and cucumber and the associated yield loss are so high that summer and fall crops have been largely abandoned in the coastal region of Lebanon. More recently severe yellowing symptoms in older leaves of cucumber, melon and

squash were observed in field and greenhouse crops. Viruses causing these yellowing symptoms on cucurbits were identified as the Polerovirus *Cucurbit aphid-borne yellows virus* (CABYV) (Abou-Jawdah *et al.*, 1997) or the Crinivirus, *Cucurbit yellow stunting disorder virus* (CYSDV) (Abou-Jawdah *et al.*, 2000b). These two viruses have been reported in several countries (Lecoq *et al.*, 1992; Wisler *et al.*, 1998).

More than 35 viruses have been isolated from cucurbits (Provvidenti, 1996), presenting complex and dynamically changing problems as described by Nameth *et al.* (1986), and causing important economic losses throughout the world. Several management practices have been reported including the use of different types of plastic mulch (Brown *et al.*, 1993; Summers *et al.*, 1995), floating row covers with fine mesh placed directly over the plants (Perring *et al.*, 1989; Orozco *et al.*, 1994) and/or cross-protection using mild strains of the predominant virus or viruses (Lecoq *et al.*, 1991; Walkey *et al.*, 1992; Perring *et al.*, 1995; Rezende and Pacheco, 1998). However, in cucurbits, the comparative efficacy of cross-protection and other management practices has received limited attention.

This paper presents data on the usefulness of some management practices for the control of virus diseases of squash and reports the existence of mixed infections of CABYV and CYSDV in this crop.

### MATERIALS AND METHODS

**Plant material and mild strain inoculation.** Seeds of squash (*Cucurbita pepo* L.) variety 'California' (Bekaa) were sown in trays and maintained in plastic tunnels protected by insect-proof nets until transplanted. The mild strain ZYMV-WK (Lecoq *et al.*, 1991) was provided by H. Lecoq as lyophilized extracts of infected tissue. In a previous study we observed that fresh inoculum of the mild strain was superior to a lyophilized one (Abou-Jawdah *et al.*, 2000a), and similar results have been reported for PRSV-W inoculum (Rezende and Pacheco, 1997). Therefore, fresh inoculum was used. The lyophilized extract of the mild strain was diluted in

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water and used to mechanically inoculate squash seedlings at the cotyledon stage, maintained in insect-proof cages. Leaves that developed mild mosaic symptoms and tested positive in ELISA (see below) were used for the cross-protection tests. The leaves were ground (1:2 w/v) with 0.03 M Na<sub>2</sub>HPO<sub>4</sub> containing 0.2% sodium-diethyl dithiocarbamate (extraction buffer) using a pre-cooled mortar and pestle. For the cross-protection treatments, seedlings at the cotyledon stage with first leaf just emerged were dusted with carborundum and rub-inoculated with the inoculum.

**Field trial.** A field trial under natural infection conditions was conducted to evaluate the effect of management practices alone or integrated for the control of cucurbit viruses that affect field-grown squash crops. This trial was conducted in Qaa, a major cucurbit production area in the Bekaa plain, during the second cropping season which suffers most from severe virus infections. A randomized complete block design was followed with 5 treatments, four replicates and three rows per replicate. The experimental plot size was 15 x 6.0 m with a space of 2 m between rows, 60 cm between plants within a row and a space of bare soil of 2 m between plots. Each plot contained 75 plants and data were collected from 20 plants in the centre of the plot. The treatments included a control (black plastic mulch), cross-protection with a mild strain of ZYMV, grey plastic mulch (alone or with cross-protection), and floating row covers (FRC) Agryl®. The squash seedlings were transplanted on August 27, 1999, 7 days after inoculation with the mild strain. The FRCs were removed on September 25, 1999. Normal horticultural practices as used by farmers were followed and pesticides were applied only as necessary.

#### Identification of present viruses

**ELISA tests.** On October 23, young leaf samples were collected from plants in all replicates and tested for virus infection by the standard double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA), (Clark and Adams, 1977). IgGs and alkaline phosphatase-conjugated IgGs for five viruses (CMV, CABYV, PRSV-W, WMV-2 and ZYMV) were kindly provided by Dr. H. Lecoq (INRA-France). Leaf samples were extracted (1:10 w/v) in extraction buffer. After addition of the substrate p-nitrophenyl phosphate (Sigma N-2765), the reaction was detected colorimetrically at 405 nm using an ELISA reader (Organon Teknika, Microwell System). Two wells were used per sample. The test was considered positive when the mean absorbance value was over twice that of healthy controls.

**RT-PCR.** Because yellowing symptoms and populations of *Bemisia tabaci* were present, samples from seven plants showing yellowing symptoms were collected and analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) for the presence of CYSDV, using the primers described by Celix *et al.* (1996), as previously described (Abou-Jawdah *et al.*, 2000b).

**Statistical analysis.** Statistical analysis were conducted using MSTAT C (Michigan State University) for all parameters. Data for percent infection were transformed to arcsine of the square root of the proportion ( $\sqrt{x}$ ) before analysis. Mean separation was accomplished using Duncan's Multiple Range Test and all tests of significance were conducted at  $P = 0.05$ .

## RESULTS

**Management of virus diseases.** Symptoms of mosaic and leaf deformation were evident on some plants, three weeks after transplanting (September 9). On October 23, severe yellowing symptoms were observed, differences between treatments were noted and therefore data on the yellowing syndrome were also collected. By the end of the experiment squash plants in the control treatments were severely infected while most cross-protected plants appeared healthy but plants under FRC treatment had the most vigorous growth. The use of FRC delayed the appearance of severe symptoms for over two weeks after removal of the FRC (until 9/10/99). The incidence of severe mosaic and leaf deformation symptoms then increased rapidly and reached 70.0% at the end of the experiment (Table 1). This treatment also gave significant protection against the pathogens inducing the yellowing syndrome (Table 2). Cross-protection with a fresh inoculum of the mild strain of ZYMV gave significant and lasting protection of squash plants, the incidence of severe symptoms in cross-protected plots being significantly reduced as compared to the unprotected control till the end of the growing season (21.3% vs. 93.8%, Table 1).

The grey mulch gave only intermediate protection. On October 2, the incidence of severely infected plants was 35.0% as compared to 48.8% in the control. Combination of grey mulch with cross-protection gave no additive effect.

Harvest extended from October 2 till November 5, 1999. All treatments gave higher yields than the untreated control (Table 1) with the highest yield recorded for the FRC treatment, followed by cross-protection and the grey mulch treatments, 114, 83 and 39% higher than the control, respectively.

**Table 1.** Comparison of management measures for the control of squash viruses. Total yield and percentage of plants showing severe mosaic and leaf deformation virus symptoms at selected dates.

Treatment <sup>y</sup>	% Severely diseased plants <sup>w</sup>						Yield <sup>wx</sup>
	18/9/99	2/10/99	9/10/99	16/10/99	23/10/99	6/11/99	
C	18.8a	48.8a	61.3a <sup>z</sup>	67.5a	73.8a	93.8a	5.2d
MS	2.5b	8.8c	12.5c	16.3c	21.3c	21.3d	9.5b
G	11.3a	35.0b	48.8b	55.0b	60.0b	83.8b	7.2c
FRC	0.0b	0.0d	0.0d	20.0c	61.3b	70.0c	11.2a
MS+G	1.3b	3.8c	8.8c	10.0d	17.5c	20.0d	9.1b

<sup>w</sup> Data shown are means from four replicates.

<sup>x</sup> Total marketable yield (mean number of fruits/plant).

<sup>y</sup> C: control; MS: mild strain inoculated; G: grey mulch; FRC: floating row covers; removed on September 25, 1999.

<sup>z</sup> Means within a column, followed by the same letter(s) are not significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

**Table 2.** Comparison of management measures for the control of squash viruses. Percentage of plants showing severe yellowing symptoms at selected dates.

Treatment <sup>x</sup>	% of plants showing yellowing symptoms	
	23/10/99	6/11/99
C	87.5 a <sup>y</sup>	100.0 a
MS	88.7 a	100.0 a
G	86.2 a	100.0 a
FRC	40.0 b	48.7 b
MS+G	77.5 a	100.0 a

<sup>x</sup> C: control; MS: mild strain inoculated; G: grey mulch; FRC: floating row covers removed on September 25, 1999.

<sup>y</sup> Means within a column, followed by the same letter(s) are not significantly different ( $P < 0.05$ ) according to Duncan's multiple range test.

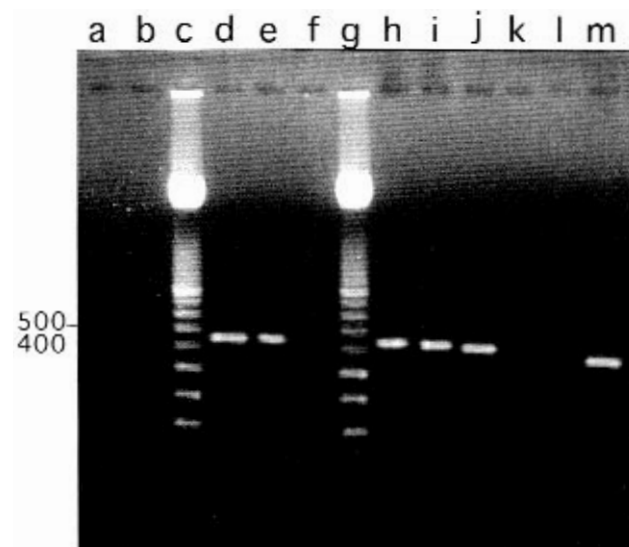
**Identification of present viruses.** ELISAs at the end of the experiment showed a high incidence of ZYMV and CABYV, and a relatively high incidence of CMV, PRSV-W and WMV-2 (Table 3).

Seven samples showing yellowing symptoms were tested by ELISA for the presence of CABYV and by RT-PCR for the presence of CYSDV using CYSDV-specific primers (Celix *et al.*, 1996). ELISA results showed that all seven samples were infected by CABYV. Electrophoretic analysis of the RT-PCR products showed that, in five of the samples tested, the expected 465 bp PCR product was amplified (Fig. 2). Further single strand conformation polymorphism (SS-CP) analysis revealed that Lebanese CYSDV isolates collected from different locations were closely related to the Spanish CYSDV but distantly related to a Saudi CYSDV isolate (data not presented).

**Table 3.** Relative frequencies of squash samples infected by different viruses as determined by ELISA.

no. tested	no. of virus-infected samples				
	CMV*	CABYV	PRSV-W	WMV-2	ZYMV
88	44	59	43	44	76

\* CMV: *Cucumber mosaic virus*; CABYV: *Cucurbit aphid-borne yellows virus*; PRSV: *Papaya ringspot virus*; WMV-2: *Watermelon mosaic virus 2*; ZYMV: *Zucchini yellow mosaic virus*.

**Fig. 1.** Agarose gel electrophoresis of RT-PCR amplified products obtained from total RNA extracts of squash plants showing yellowing symptoms using two CYSDV-specific primers. Lanes a, b and l: healthy squash; lanes d, e, f, h, i, j and k: squash plants with yellowing symptoms; lane m: positive CYSDV control; lanes c and g: molecular marker 100 bp ladder (Promega) – sizes indicated on the left. Only five samples out of seven tested were positive (samples d, e, h, i, j).

## DISCUSSION

Severe virus infections of field-grown squash are a major cause of economic losses for farmers who grow summer and fall squash in Lebanon. The presence of ZYMV, WMV-2 and PRSV-W have been reported (Lesemann *et al.*, 1983). In addition, yellowing symptoms related to CABYV and/or CYSDV infections are present in open fields but are masked by mosaic and leaf deformation symptoms, explaining why these infections passed unnoticed for several years. Only recently attention has been drawn to CABYV and CYSDV (Abou-Jawdah *et al.*, 1997, 2000b).

A recent survey (Abou-Jawdah *et al.*, 2000a) showed that field production of squash in Lebanon is severely affected by ZYMV. There are reports on successful use of cross-protection with a mild strain of ZYMV in France (Lecoq *et al.*, 1991), Taiwan (Wang *et al.*, 1991), the UK (Walkey *et al.*, 1992) and California (Perring *et al.*, 1995). Use of FRC and plastic mulches for preventing or delaying the onset of aphid-borne virus infections is also reported, but comparison of cross-protection with these two treatments and their integration has received little attention.

This study showed that cross-protection with the ZYMV-WK gave significant protection against infection by severe strains under field conditions in Lebanon and resulted in a significantly higher yield of marketable fruits (*ca* 83%). Therefore, as shown in other ecological situations (Lecoq *et al.*, 1991; Wang *et al.*, 1991; Walkey *et al.*, 1992), this mild strain seems to have been effective in reducing disease losses. Lecoq *et al.* (1991) and Walkey *et al.* (1992) reported that a minimum of 10-14 days delay between inoculation with the mild strain and observation of the cross-protection phenomenon are required. In this experiment, the cross-protection treatment gave a significant level of protection even when the cross-protected plants were transplanted only 7 days post-inoculation with the mild strain.

This may be explained by a delay in vector arrival or spread in the field under our experimental conditions during this trial and in previous trials (Abou-Jawdah, *et al.*, 2000a). However, differences may be observed between years and/or between geographical locations. In situations where infective aphids migrating from neighbouring fields may reach a newly planted squash crop earlier, the results may differ and transplanting 7 days post-inoculation may not be effective; a longer period may be required. It was interesting that cross protection with ZYMV-WK gave significant protection even in the presence, towards the end of the growing season, of relatively high incidence of CMV, PRSV-W and WMV (about 50%, Table 3). This indicates that ZYMV is the

major cause of yield loss in squash under the prevailing conditions in Lebanon.

The level of protection obtained with the grey mulch used in this experiment was lower than reported previously for silver mulch (Abou-Jawdah *et al.*, 2000a). Black mulch is commonly used to reduce water use and to control weeds. Therefore, replacement of black mulch by silver mulch may not represent a considerable extra cost to the farmer. But special attention must be paid to the importance of the reflective quality of silver mulch (the grey mulch being less effective).

The FRC treatment gave the most efficient control; plants grown under FRC grew vigorously, and despite the high disease incidence later in the season, gave the highest yield, which did not seem to be severely affected, even though the harvesting period may have been slightly reduced due to late infections. FRC delayed early infection which normally leads to the highest yield reduction. Blua and Perring (1989) reported that infection by ZYMV during the vegetative stage caused 94% reduction in marketable melons while infection after fruit set did not result in significant loss in number or quality of fruit. Furthermore, FRC was the only treatment giving significant protection against the yellowing disease complex shown to be caused by CABYV and/or CYSDV. This may be of great importance for other cucurbit crops in the area, mainly melon and cucumber, because CABYV was reported to cause considerable yield losses (40-50%) in melon and cucumber but not in squash (Lecoq *et al.*, 1992), and CYSDV causes a yield reduction of about 50% in cucumber (Abou-Jawdah *et al.*, 2000b).

In view of the dynamic and complex nature of virus diseases that affect cucurbits in Lebanon, cross protection with ZYMV-WK may be effective for squash, but may not be enough for protection of cucumber and melon due to the yield losses that can be caused by CABYV and/or CYSDV. In these conditions, FRC offers the best alternative but its cost may be prohibitive for some farmers. However, silver mulch and its integration with cross-protection for the control of the cucurbit disease complex may be less expensive and should be considered in further investigations.

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