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REVIEW

Zucchini yellow mosaic virus

C. DESBIEZ *and* H. LECOQ
INRA, Station de Pathologie Végétale, 84143 Montfavet Cedex, France

Zucchini yellow mosaic potyvirus (ZYMV), first isolated in Italy in 1973, described in 1981, and then identified in all continents within a decade, is one of the most economically important viruses of cucurbit crops. It is efficiently aphid-transmitted in a nonpersistent manner and it is also seed-borne in zucchini squash, which could have contributed to its rapid spread worldwide. Biological variability has been observed among ZYMV isolates, concerning host range, symptomatology and aphid transmissibility. More recent studies also revealed a serological and molecular variability. The survival of ZYMV in areas where cucurbits are not grown throughout the year remains to be elucidated, because very few natural over-wintering hosts have been identified so far. Partial control of ZYMV can be achieved by limiting transmission of the virus to the crops by aphids, using adapted cultural practices. Cross-protection with a mild strain has been shown to be effective against most ZYMV isolates. Resistance genes found in cucurbit germplasms are currently being introduced into cultivars with good agronomical characteristics. Pathogen-derived resistance strategies using the expression of ZYMV genes in transgenic plants have also been developed and appear promising. Nevertheless, the high biological variability of ZYMV justifies a careful evaluation of the deployment of genetic control strategies in order to increase their durability.

INTRODUCTION

More than 20 viruses have been described as infecting cucurbit crops in the major growing areas. Among them, cucumber mosaic cucumovirus (CMV), watermelon mosaic potyvirus 2 (WMV2), papaya ringspot potyvirus type W (PRSV-W, formerly WMV1), squash mosaic comovirus (SqMV) and melon necrotic spot carmovirus (MNSV) are the most prevalent and have been identified for decades (Lovisolo, 1980). More recently, 'new' virus diseases were reported to cause severe epidemics in cucurbit crops in different parts of the world. Such is the case for lettuce infectious yellows closterovirus (LIYV) in California (Duffus & Flock, 1982), cucurbit aphid borne yellows luteovirus (CABYV) (Lecoq *et al.*, 1992), zucchini yellow fleck potyvirus (ZYFV) (Vovlas *et al.*, 1981; Gilbert-Albertini & Lecoq, 1993) and zucchini yellow mosaic potyvirus (ZYMV).

ZYMV is probably one of the best examples of an 'emerging' plant virus in the recent literature. First described in Europe in 1981, it was associated with severe symptoms on squash and melon and with very destructive epidemics in Italy and France. Within 5 years, the virus was reported worldwide in the most important cucurbit growing areas, including several islands. The way ZYMV was disseminated within such a short period of time remains a very intriguing epidemiological question to be elucidated. This review presents the recent data acquired on ZYMV and describes the diverse approaches explored presently to control the virus.

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DISCOVERY

In 1973, a severe viral disease was observed in zucchini plants in Northern Italy (Lisa *et al.*, 1981). The symptoms were different from those caused by the known cucurbit-infecting viruses CMV, WMV2 and PRSV-W. Infected plants exhibited severe stunting and yellowing symptoms, with leaf and fruit deformations. Lisa *et al*. (1981) identified the causal agent as a new potyvirus that they named zucchini yellow mosaic virus (ZYMV). In 1979, many muskmelon crops in France were devastated by an apparently new virus disease. Plants exhibited yellowing, leaf deformation and stunting, with a diversity of symptoms on the fruits (mottle and hardening of the flesh, cracks on the fruits). These symptoms were shown to be caused by a potyvirus tentatively named muskmelon yellow stunt virus (MYSV) (Lecoq *et al.*, 1981). MYSV was soon shown to be identical to ZYMV, and the name

Table 1 World distribution of ZYMV, and host and year of first description

Country	First description	Reference
Europe		
Bulgaria	1994	Dikova B (1994)
Czechoslovakia	Squash	Chod *et al.* (1991)
England	Zucchini 1987	Walkey (1992)
France	Muskmelon 1979	Lecoq *et al.* (1981)
Germany	1983	Lesemann *et al.* (1983)
Greece	Squash 1989	Kyriakopoulou & Varveri (1991)
Italy	Zucchini 1973	Lisa *et al.* (1981)
Jersey	Zucchini 1983	Wright *et al.* (1984)
Netherlands	Cucumber, zucchini 1983	Schrijnwerkers *et al.* (1991)
Portugal	Zucchini	de Sequeira O (1997), personal communication
Spain	Zucchini 1982	Lecoq & Pitrat (1983)
Africa		
Algeria	Muskmelon 1989	Belkhala & Lecoq (1990)
Egypt	1983	Provvidenti *et al.* (1984b)
Madagascar	Squash 1990	F. Gilbert-Albertini & H. Lecoq (1990), unpublished data
Mauritius		Bos and Dossa *in* Lisa & Lecoq (1984)
Mayotte	Squash 1992	H. Lecoq & B. Reynaud (1992), unpublished data
Morocco		Hafidi & Lockart i*n* Lecoq & Lisa (1983)
Nigeria	*Cucumeropsis edulis* 1978	Igwegbe (1983)
Reunion	*Momordica charantia* 1984	H. Lecoq & M.J. Michel (1984), unpublished data
Sudan	Several cucurbits 1992	Lecoq *et al.* (1994)
Swaziland	Scallop squash, zucchini 1994	H. Lecoq & C. Desbiez (1994), unpublished data
Tunisia		Cherif & Ezzaier (1987)
Asia and Middle East		
China	1986	Zheng & Dong (1989)
Japan	Pumpkin	Ohtsu *et al.* (1985)
Malaysia	Pumpkin 1984	Fujisawa *et al.* (1986)
Nepal		Dahal (1992)
Pakistan	Squash 1991	S. Khalid & H. Lecoq (1992), unpublished data
Singapore	Cucumber 1989	Wong & Lee (1992)
Taiwan	Cucumber 1982	Hseu *et al.* (1985)
Turkey	Squash 1983	Davis & Yilmaz (1984)
Iran	Squash, muskmelon 1988	Ghorbani (1988)
Israel	Cucumber 1982	Antignus *et al.* (1989)
Jordan	Melon 1987	Al-Musa *et al.* (1989b)
Lebanon	Cucumber 1979	Lesemann *et al.* (1983)
Saudi Arabia		Abdulsalam *et al.* (1988)
Syria		Katul & Makkouk (1987)
Yemen	Vegetable marrow 1986	Alhubaishi *et al.* (1987)

ZYMV was retained (Lecoq *et al.*, 1983). Within a few years (1981–85) ZYMV was identified, using serological techniques, in many countries in the world, always associated with severe symptoms and important yield reduction.

GENERAL CHARACTERISTICS

ZYMV is a member of the potyvirus genus (Hollings & Brunt, 1981; Murphy *et al.*, 1995). The flexuous filamentous particles, 750 nm long (Lisa *et al.*, 1981), consist of a single-stranded RNA about 9600 nucleotides long (Balint *et al.*, 1990) with a 5′ viral protein genome linked (VPg) and 3′ poly(A) tail encapsidated in a 36 kDa coat protein. The RNA is translated as a single polyprotein cleaved by three viral proteases (for a review on potyvirus molecular biology see Riechmann *et al.*, 1992; Shukla *et al.*, 1994). Cylindrical inclusions (pinwheels) induced by ZYMV in infected plants are generally of type 1

Table 1 continued

Country	First description	Reference
Oceania		
Australia	Pumpkin, zucchini 1981	Greber *et al.* (1987)
Guam	Watermelon	Yudin *et al.* (1990)
Hawaii	Zucchini 1988	Ullman *et al.* (1991)
New Caledonia	Zucchini 1994	H. Lecoq & D. Bordat (1994), unpublished data
New Zealand	Squash 1996	Fletcher (1996)
America		
USA		
Florida	Squash 1981	Purcifull *et al.* (1984)
Connecticut	Yellow squash 1982	Provvidenti *et al.* (1984)
New York	Cucumber 1983	Provvidenti *et al.* (1984)
California	Squash 1983	Provvidenti *et al.* (1984)
Oregon	Squash 1984	Nameth *et al.* (1985)
South Carolina	Yellow squash 1981	Sammons *et al.* (1989)
New Jersey	Squash 1985	Davis & Mizuki (1987)
Washington	Squash 1986	Crosslin *et al.* (1988)
Louisiana	Several cucurbits 1988–89	Fernandes *et al.* (1991)
Arkansas	Zucchini 1981	Wickizer *et al.* (1985)
Canada	Cucumber 1989	Stobbs & Van Schagen (1990)
Mexico	1984	Nameth *et al.* (1985)
Martinique	Several cucurbits 1992	Lecoq *et al.* (1994)
Dominican Republic	Squash 1989	H. Lecoq & H. Lot (1989), unpublished data
Guadeloupe	Several cucurbits 1994	H. Lecoq, C. Wipf-Scheibel and C. Desbiez (1994), unpublished data
Venezuela		Hernandez *et al.* (1989)
Costa Rica	Melon	Rivera *et al.* (1993)
Brazil	Watermelon 1991	Vega *et al.* (1992)
Honduras	Melon 1993	H. Lecoq (1993), unpublished data
Puerto Rico	Squash 1996	L. Wessel Beaver & H. Lecoq (1996), unpublished data

according to the classification of Edwardson & Christie (1978) (Lecoq *et al.*, 1981; Lisa & Lecoq, 1984). These cytoplasmic inclusions appear as fibrillar masses using the orange-green stain for light microscopic detection of viral inclusions (Christie & Edwardson, 1986).

PURIFICATION

Most protocols used for ZYMV purification derive from the one of Lisa *et al.* (1981). The virus is extracted from leaves of zucchini squash (Lisa *et al.*, 1981), muskmelon (Lecoq & Pitrat, 1985) or pumpkin (Wong *et al.*, 1994) plantlets 2–4 weeks after inoculation. After homogenization in phosphate buffer and low speed centrifugation, the virus is sedimented by high speed centrifugation, and further purified by sucrose density gradient (Lisa *et al.*, 1981), or caesium sulphate gradient (Lecoq & Pitrat, 1985). Virus concentration is estimated spectrophotometrically by using an approximate extinction coefficient $E_{260\,nm} = 2.5$. The purification yields usually range from 10 to 200 mg of virus per kilogram of fresh infected leaves, depending on virus strain and purification method. (Lisa *et al.*, 1981; Lecoq & Pitrat, 1985; Huang *et al.*, 1989).

$A_{260/280}$ and A_{max}/A_{min} were estimated to be 1.13 and 1.07, respectively (Lisa *et al.*, 1981).

GEOGRAPHICAL DISTRIBUTION

ZYMV is present worldwide in almost all countries where cucurbits are grown, under temperate, subtropical and tropical conditions. It has been detected in cucurbit fields or greenhouses in several countries of Europe and Asia, Africa and the Middle East, North and South America, and Oceania (Table 1). The virus is very damaging in highly mechanized production areas as well as in more traditional agroecosystems.

Table 2 Experimental host range of zucchini yellow mosaic virus outside the Cucurbitaceae

Family, species	Infection	
	Local	Systemic
Aizoaceae		
Tetragonia expansa [a]	L/lat.	–/lat.
Amaranthaceae		
Gomphrena globosa [b]	L	–/+
Chenopodiaceae		
Chenopodium amaranticolour[b]	L	–
C. quinoa [b]	L	–/+
Spinacia oleracea[a]	lat.	–
Compositae		
Senecio vulgaris [b]	L	–
Labiatae		
Lamium amplexicaule [b]	lat.	lat.
Leguminosae		
Phaseolus vulgaris [b]	L/–	–
Pisum sativum [c]	lat.	–
Trigonella foenum–graecum [a]	lat.	+/lat.
Ranunculaceae		
Ranunculus sardous [b]	lat.	lat.
Scrophulariaceae		
Torenia fournieri [b]	+	+
Solanaceae		
Nicotiana clevelandii [a]	lat.	–
N. benthamiana [c]	lat.	lat./–
Umbelliferae		
Ammi majus [a]	lat.	lat.

L, chlorotic or necrotic local lesions; +, virus multiplication with symptoms; lat, latent infection (virus multiplication without symptoms); –, no symptoms, no virus detected.
[a]from Lisa *et al.* (1981).
[b]from Lecoq *et al.* (1981).
[c]from Provvidenti *et al.* (1984).

EXPERIMENTAL HOST RANGE

The experimental host range of ZYMV includes members of 11 families of dicotyledons (Table 2), although natural infection has been reported mostly in the Cucurbitaceae. More than 20 members of the Cucurbitaceae were found to be susceptible to the virus, including the main cultivated species *Cucumis melo*, *C. sativus*, *Cucurbita pepo*, *C. moschata*, *Citrullus lanatus* (Lisa *et al.*, 1981; Lecoq *et al.*, 1981). Experimental hosts outside the Cucurbitaceae usually present local lesions or latent infections. *Chenopodium amaranticolour* and *C. quinoa* are useful local lesion assay hosts. *Sesamum indicum* (sesame) presents severe mosaic and deformation symptoms when mechanically inoculated with ZYMV (Mahgoub *et al.*, 1997).

The infection of some experimental hosts (*Phaseolus vulgaris*, *Nicotiana benthamiana*) is strain-specific, as detailed in the section 'Biological variability'.

FIELD SYMPTOMATOLOGY AND ECONOMIC INCIDENCE

Symptoms of ZYMV on cultivated crops are often very severe and induce significant yield reduction. In addition, fruits produced on infected plants exhibit severe deformations and colour alterations, which render them unmarketable. A diversity of symptoms are observed on susceptible hosts, according to the species or the cultivar.

In zucchini squash (*Cucurbita pepo*) (Fig. 1A), leaves develop a yellow mosaic and become severely blistered and laciniated. Fruits are distorted with prominent lumps (Lisa & Lecoq, 1984), and in yellow fruit cultivars, fruits may stay green with glossy yellow knobs (Provvidenti *et al.*, 1984a).

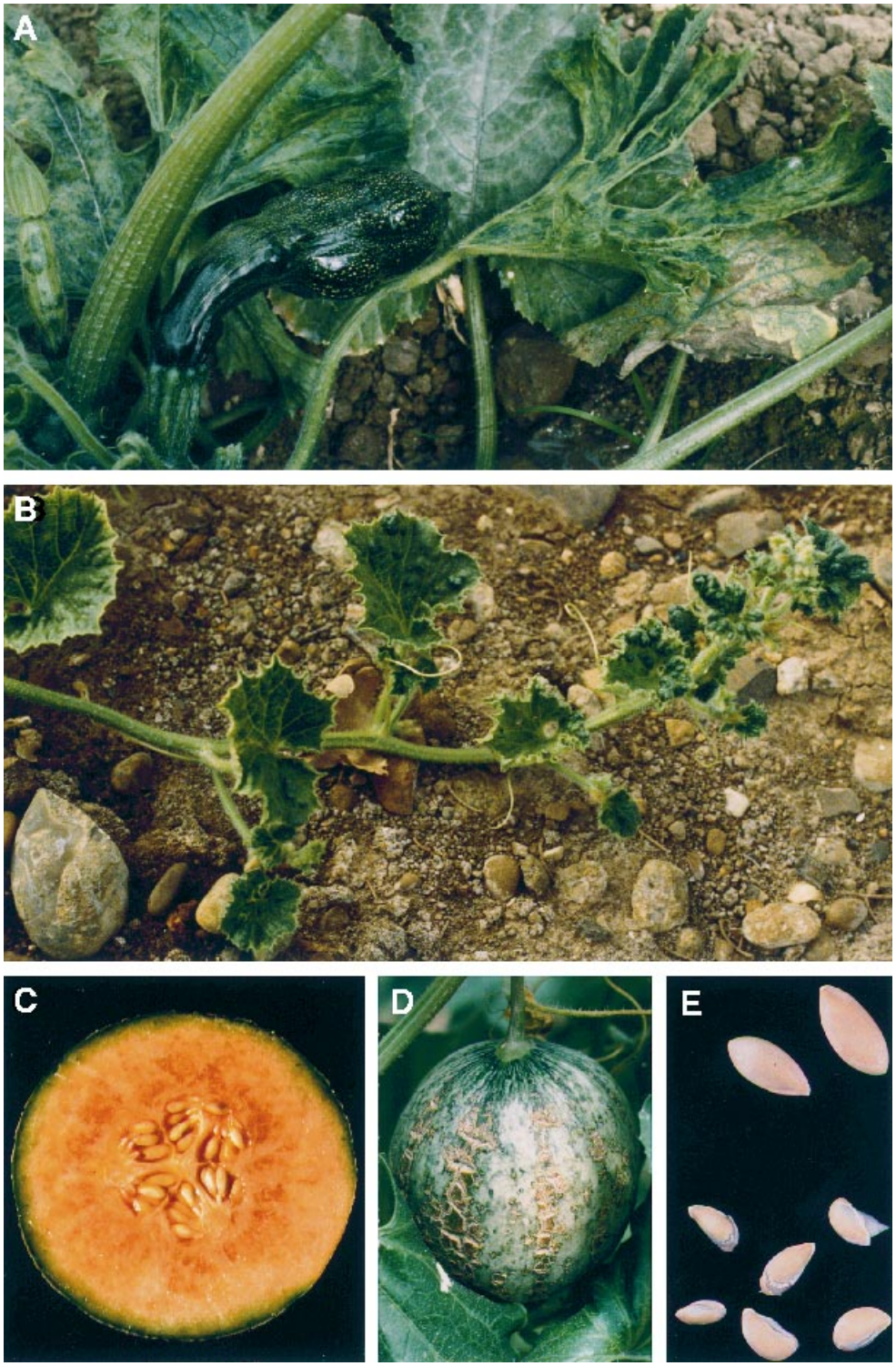
In other squash types (*C. pepo*, *C. moschata*, *C. maxima*) symptoms may vary from mottle to severe mosaic with occasional recovery. Fruits may also be severely distorted.

In melon (*Cucumis melo*) early symptoms on leaves are vein clearing and yellow mosaic. Leaves are subsequently reduced in size, deformed, often with serrated edges and dark green blisters or enations, contrasting with the yellow or light green colour of the rest of the lamina (Fig. 1B). Branches develop short internodes and usually exhibit an erect habit. Discolourations and raised patches are observed on fruits, occasionally associated with internal marbling and hardening of the flesh (Fig. 1C) or superficial cracks with corky edges (Fig. 1D) (Lecoq *et al.*, 1981; Nameth *et al.*, 1985). Seeds are deformed and have low germination rates (Fig. 1E). Some ZYMV isolates induce in melon cultivars possessing the *Fn* gene a sudden wilting followed by a general necrosis of the plant (Lecoq & Pitrat, 1984).

In cucumber (*Cucumis sativus*) severe mosaic and deformations are observed on leaves and on fruits.

In watermelon (*Citrullus lanatus*) mottle, mosaic and leaf filiformism are commonly observed. Fruits may present irregular colouration and slight to severe deformations.

Fig. 1 Symptoms induced by ZYMV in cucurbits (A) leaf and fruit deformation in zucchini squash. (B) to (E) symptoms in muskmelon: (B) leaf deformation and discolouration, shortening of the internodes; (C) internal marbling and hardening of the flesh; (D) external cracking of the fruit; (E) seed deformation (top: seeds produced by a healthy plant).


A
B
C
D
E

Cucurbit crops infected at an early stage are severely affected by ZYMV. Blua & Perring (1989) showed that early ZYMV infection can cause as much as 94% reduction of marketable cantaloupe, but that the effect of ZYMV on melon yield is low if the epidemics start after production of the first fruits. A similar effect was observed in zucchini squash mechanically inoculated with a severe strain at different times after the seedling stage; the earlier the inoculation the lower the total number of fruit per plant (Walkey *et al.*, 1992). Quantitative losses ranged from 64 to 85% in greenhouse-grown cucumbers inoculated with ZYMV, and 95% of the infected fruits were unmarketable (Al-Shahwan *et al.*, 1995).

Environmental conditions might also influence symptom expression. Symptoms produced by 25 ZYMV isolates in zucchini squashes grown in growth chambers at different temperatures were compared. At 15–25°C plants developed mottle or mosaic with slight deformations, while at 25–35°C symptoms were very severe, with extreme laciniation and shoe stringing (H. A. Mahgoub & H. Lecoq, 1995, unpublished data). The effect of temperature on laciniation symptom intensity might be responsible for the frequent occurrence, in naturally infected zucchini squash, of groups of leaves with very severe symptoms alternating on the same plant with groups of leaves with milder symptoms.

Symptoms can be more severe when ZYMV is present in mixed infections with another virus, particularly CMV (Lecoq *et al.*, 1981). In this case, more severe symptoms are correlated with increased CMV concentration, but reduced ZYMV level in infected plants (Poolpol & Inouye, 1986). Mixed infections with CMV were also associated in Japan with a lethal wilt of cucumber plants grafted onto squash rootstock (Iwasaki & Inaba, 1988), while such synergism was not observed in nongrafted cucumbers. Plants with a mixed infection of ZYMV and CABYV also developed symptoms more severely than plants infected by only one of these viruses. CABYV concentrations were significantly increased (2–20 times) while those of ZYMV were unchanged (Bourdin & Lecoq, 1994).

TRANSMISSION AND SPREAD

Aphid transmission

Like other potyviruses, ZYMV is efficiently transmitted by aphids in a nonpersistent manner (Lisa *et al.*, 1981). Transmission by one *Myzus persicae* was estimated to 30% (Lisa *et al.*, 1981). *M. persicae* and *Aphis gossypii* transmit the virus at a frequency of 70–90% with 3 viruliferous aphids per plant (Lecoq *et al.*, 1981). *Macrosiphum euphorbiae* (Lisa & Lecoq, 1984) and *Aphis citricola* (Purcifull *et al.*, 1984) are also vectors of ZYMV.

Adlerz (1987) found that alate *Aphis middletonii*, *A. citricola*, *M. persicae*, *Lipaphis erysimi*, *Aphis craccivora* and *Acyrthosiphon pisum*, trapped alive in Florida, transmitted ZYMV to *C. pepo* with a mean efficiency of 28.4%. In a similar study conducted in California, *M. persicae* and *A. gossypii* were found to transmit ZYMV with 41% and 35% efficiencies, respectively, while *Acyrthosiphon kondoi*, *A. pisum*, *Aphis spiraecola* and *L. erysimi* transmitted ZYMV with less than 10% frequency. Field-collected alate aphids transmitted the virus more efficiently than the laboratory-derived alates (Castle *et al.*, 1992).

Blua & Perring (1992) observed a modification of the colonization and feeding behaviour of *A. gossypii* on ZYMV-infected zucchini plants: the longevity and fecundity of aphids were higher, and more alate aphids were produced in the early stage of infection. In late infections, the yellow colour of infected plants is more attractive for aphids, but their feeding behaviour is modified: more probing events and fewer phloem contacts are observed than on healthy plants, and aphids stay for a shorter time on the plants. All these characteristics might indirectly favour the spread of ZYMV.

Two viral proteins are required for aphid transmission of potyviruses: the coat protein (CP) and a nonstructural protein, the helper component (HC) (Pirone, 1991).

In vitro transmission experiments using purified ZYMV, PRSV and WMV2 virions and heterologous HCs revealed some degree of specificity in the virus–HC interaction although in all cases some transmission occurred (Lecoq & Pitrat, 1985). Strains of ZYMV deficient for aphid transmission either in their CP or HC can be aphid-transmitted when present in mixed infection with another potyvirus that provides the functional complementary protein. *In vivo* 'heteroassistance' was observed in the case of mixed infections with WMV2 (Lecoq *et al.*, 1991a). Aphid transmission of ZYMV-NAT, a CP-deficient aphid nontransmissible strain, has also been described in presence of PRSV. In this case, heterologous encapsidation of the ZYMV RNA by PRSV CP was responsible for the aphid transmission of ZYMV (Bourdin & Lecoq, 1991). 'Heteroencapsidation' also occurred when ZYMV-NAT infected transgenic *N. benthamiana* plants expressing the CP of an aphid

transmissible strain of plum pox virus (PPV) (Lecoq *et al.*, 1993).

Ecology and dissemination of the virus

The presence of ZYMV worldwide raises the question of its means of dissemination and conservation when susceptible cultivated cucurbit crops are not grown.

Very few potential reservoirs of the virus have been identified so far, although some weeds (*Ranunculus sardous*, *Lamium amplexicaule*) or crops (*Sesamum indicum*) were reported to be sytemically infected in experimental conditions (Lecoq *et al.*, 1981; Mahgoub *et al.*, 1997, in press). ZYMV was even found to be seed-transmitted from mechanically inoculated *Ranunculus sardous* (Al-Musa, 1989a). ZYMV was isolated from the wild perennial cucurbit *Melothria pendula* in Florida (Adlerz *et al.*, 1983). Some other wild cucurbit species were also reported to be infected by ZYMV in the USA (Perring *et al.*, 1992) or Sudan (Maghoub *et al.*, 1997, in press). In Jordan, *Moluccella laevis* was described as a natural reservoir of ZYMV (Al-Musa, 1989a). No natural reservoirs of ZYMV have been found so far in temperate regions, despite extensive searching (Lecoq, 1990; H. Lecoq, 1996, unpublished data). The extension of the period of cucurbit cultivation in the Mediterranean basin, with the development of plastic tunnels or glasshouses, might play an important role for overwintering of ZYMV. Indeed, with these conditions early plantings may grow alongside late infected crops. In the desert valleys of California, sources of ZYMV were clearly identified to be old cucurbit crops or volunteer plants surviving in residential areas (Perring *et al.*, 1992).

Once ZYMV is introduced into a cucurbit planting, its spread to the rest of the field is generally very rapid. This can occur concomittantly with the spread of other aphid borne viruses. A recent study showed that non colonizer aphids (such as *A. craccivora*) had both a higher transmission efficiency and propensity to disseminate ZYMV than *A. gossypii*, which settles on cucurbits (Yuan & Ullman, 1996). This corroborates observations made in California, where intense ZYMV spread was associated with heavy aphid colonization of noncucurbit crops growing nearby (Perring *et al.*, 1992).

Although potyviruses are aphid-transmitted in a nonpersistent manner, Fereres *et al*. (1992) observed a ZYMV transmission rate of 1%, 30 h after acquisition by *M. persicae*, and 10–20 h after acquisition by *A. gossypii*. This could contribute to the long-distance spread of ZYMV by aphids carried by the wind, as described for maize dwarf mosaic potyvirus (MDMV) in the USA (Zeyen *et al.*, 1987).

Another factor that might contribute to the rapid dissemination of ZYMV is seed transmission.

Table 3 Seed transmission of ZYMV

Host	Number of seeds	Transmission (%)	Reference
Cucurbita pepo	1400	0.00	Nameth *et al.* (1985)
	1298	18.95[a]	Davis & Mizuki (1986)
	1000	0.00	Greber *et al.* (1987)
	100	1.00	Greber *et al.* (1988)
	6800	0.00	Gleason & Provvidenti (1990)
	4196	0.05	Schrijnwerkers *et al.* (1991)
	10 888	0.00	Robinson *et al.* (1993)
	127	0.00	Wong *et al.* (1994)
	7892	0.00	H. Lecoq (1997), unpublished data
Cucurbita maxima	1000	0.00	Greber *et al.* (1987)
	506	0.00	Robinson *et al.* (1993)
Cucurbita moschata	423	0.00	Robinson *et al.* (1993)
Cucumis melo	1000	0.00	Lecoq *et al.* (1981)
	2700	0.00	H. Lecoq & C. Desbiez (1997), unpublished data
	434	0.00	Provvidenti & Robinson (1987)
	200	0.00	Greber *et al.* (1988)
Cucumis sativus	11 475	0.00	Robinson *et al.* (1993)

[a]Transmission detected serologically but without typical symptoms on seedlings.

Several experiments were conducted in different laboratories with conflicting results (Table 3). Schrijnwerkers *et al*. (1991) showed that ZYMV was seed-transmissible in *C. pepo*, although at a very low rate (0.047%). ZYMV seems to be present externally on the squash seeds (Schrijnwerkers *et al.*, 1991), so seedling infection might occur when the seeds germinate. ZYMV-infected plants usually produce very few viable seeds, but even a small number of virus-transmitting seeds could provide a primary inoculum sufficient to initiate devastating epidemics. No seed transmission has been reported so far in *C. melo* or *C. sativus*.

BIOLOGICAL VARIABILITY (STRAIN)

Symptomatology

Since its first descriptions, ZYMV appeared to present important biological variability: field isolates from the South-west of France induced milder symptoms than isolates from the south-east of France, and were different from the type strain from Italy (Lisa & Lecoq, 1984). A similar variability was reported among isolates from different parts of the USA (Provvidenti *et al.*, 1984a). Some isolates induce symptoms strongly resembling those of PRSV-W or WMV2, preventing a reliable field diagnosis based on symptomatology.

In 1986, a mild isolate was recovered from a mechanically inoculated melon plant presenting an axillary branch with attenuated symptoms. (Lecoq *et al.*, 1991a; Lecoq & Purcifull, 1992). Viral multiplication of this weak strain, named ZYMV-WK, estimated by ELISA tests, is equivalent to that of severe strains. ZYMV-WK is used for cross-protection at an economical scale (Lecoq *et al.*, 1991a; Wang *et al.*, 1991; Walkey *et al.*, 1992).

Some strains also differed in their ability to induce rapid and lethal wilting on muskmelon cv. 'Doublon' possessing the *Fn* gene (Lecoq *et al.*, 1981; Lecoq & Pitrat, 1984). Two pathotypes, F (wilting) and NF (nonwilting), were defined according to the reaction of 'Doublon'. This reaction was observed with many other cultivars, because the *Fn* gene is frequent in germplasm collections (Pitrat *et al.*, 1996). The ratio between F and NF pathotypes is similar in groups of ZYMV isolates originating from temperate as well as subtropical or tropical regions (Lecoq & Purcifull, 1992; Desbiez *et al.*, 1996).

Host range

ZYMV strains present some variability in their experimental host range. Some strains can infect systemically cultivars of *Pisum sativum* (Lesemann *et al.*, 1983; Antignus *et al.*, 1989) without any visible symptoms. *Phaseolus vulgaris* cv. 'Pinto' is systemically infected by a Lebanon strain of ZYMV (Lesemann *et al.*, 1983), but not by other strains from France and the USA, whose infection is limited to the inoculated leaves, with or without induction of local lesions (Lecoq *et al.*, 1981; Provvidenti *et al.*, 1984a). ZYMV strains induce latent infection of *Nicotiana benthamiana* either systemic or limited to the inoculated organs (Lesemann *et al.*, 1983; Wang *et al.*, 1992). Recently, an isolate inducing severe mosaic and leaf deformation was observed on greenhouse-grown, mechanically inoculated *N. benthamiana* (H. A. Mahgoub & H. Lecoq, 1995, unpublished data).

In addition, some variability has been observed in the interactions with some resistant cucurbit lines. Three pathotypes can be defined regarding the ability of the strains to infect the muskmelon PI414723 possessing the *Zym* resistance gene. Strains from pathotype 0 induce no systemic infection and no symptoms, or only local lesions, on inoculated cotyledons; pathotype 1 strains induce chlorotic or necrotic lesions on systemically infected leaves, while pathotype 2 strains cause severe systemic symptoms of mosaic, stunting and leaf deformations (Lecoq & Pitrat, 1984). Provvidenti (1991) also described resistance in *Citrullus lanatus* to ZYMV that was specific to a Florida strain of the virus.

Aphid transmission

Isolates of ZYMV differing in aphid transmissibility have been described (Antignus *et al.*, 1989; Lecoq *et al.*, 1991a). Loss of aphid transmissibility can result from a deficiency of the CP (Antignus *et al.*, 1989; Lee *et al.*, 1993) or from the lack of biologically active HC (Lecoq *et al.*, 1991a; Granier *et al.*, 1993).

Sequence comparisons between the coat proteins of aphid-transmissible and aphid nontransmissible potyvirus strains suggested that an amino-acid triplet Asp-Ala-Gly (DAG) at the N-terminal part of the coat protein is required for aphid transmissibility (Harrison & Robinson, 1988). Atreya *et al*. (1990) showed that an A to T mutation in the DAG triplet could abolish aphid transmission of tobacco vein mottling potyvirus (TVMV). Two natural aphid nontransmissible ZYMV strains, with a DAG to DTG mutation, were described (Gal-On *et al.*, 1990; Lee *et al.*, 1993). Synthesis of an

infectious complementary DNA (cDNA) of the ZYMV genome (Gal-On *et al.*, 1991) allowed a more accurate study, at the molecular level, of the role of this sequence. Gal-On *et al*. (1992) showed that a mutation from T to A in the DTG triplet could restore aphid transmissibility of the virus.

Strains of ZYMV were described that were either poorly or non-aphid transmissible but could be complemented for their transmission by extracts of plants infected with aphid-transmissible strains containing active helper component (Lecoq *et al.*, 1991a). These strains, named PAT (poorly aphid transmissible), are deficient in their helper component activity. Granier *et al*. (1993) compared the helper component sequences of two PAT strains to that of the highly aphid transmissible (HAT) strains from which they derived. They observed in one case a K to E mutation in the N-terminal part of the HC similar to that observed in the PVC aphid nontransmissible variant of potato virus Y (PVY) (Thornbury *et al.*, 1990). The same mutation was also found in a helper-deficient strain of ZYMV from Connecticut (Grumet *et al.*, 1992). For another ZYMV isolate, two mutations were found between the HAT and PAT strains, one of them occurring in a conserved cluster of amino-acids Pro-Thr-Lys (PTK) (Granier *et al.*, 1993). Huet *et al*. (1994) modified the PTK of ZYMV to PAK in an infectious cDNA clone and observed a total loss of HC activity in aphid transmission. An amino-acid exchange (R to I) in another conserved box (the FRNK box) resulted in more than 50% reduction in aphid transmission, but did not completely abolish transmission (Huet *et al.*, 1994).

METHODS FOR IDENTIFICATION AND DETECTION

Assay on indicator hosts

ZYMV in single infection can be easily distinguished from other common cucurbit-infecting viruses using differential diagnostic species (Lisa & Lecoq, 1984). However, unequivocal identification of the virus in field samples is difficult, because of the frequent mixed infections with other viruses, which can mask or render difficult the interpretation of differential host reactions.

Chenopodium amaranticolour is a useful local lesion assay host for ZYMV. It can be used for single local lesion transfers, but because of inhibitors, back inoculation to cucurbits is erratic. *Chenopodium quinoa* might be a useful intermediate host. Zucchini squash or melon seedlings are very convenient systemic assay hosts.

Serological techniques

Polyclonal antisera raised against the virions of an Italian ZYMV isolate (Lisa *et al.*, 1981) and a French ZYMV isolate (Lecoq *et al.*, 1981) were obtained, with titres up to 1:4096 in the slide precipitin test (Lecoq *et al.*, 1983). Detection of ZYMV was also possible using Ouchterlouny gel double-diffusion tests in a medium containing 0.8% agar, 1% sodium azide and 0.5% sodium dodecyl sulphate (SDS-ID) as described by Purcifull & Batchelor (1977). This method contributed to the rapid and practical detection of ZYMV in several countries (Lecoq *et al.*, 1983; Greber *et al.*, 1987). SDS-ID is also useful to detect other mosaic-inducing viruses in cucurbits (Purcifull *et al.*, 1988) and is therefore very convenient for establishing diagnosis in a limited number of samples. However, the relatively large amount of antiserum required for each test makes it inappropriate for large-scale detection.

A current widely used technique for large-scale detection of ZYMV is enzyme-linked immunosorbent assay (ELISA) (Clark & Adams, 1977). Double antibody sandwich (DAS)-ELISA is the most commonly used variant of this method, because of its specificity and reproductibility. The cross reactions often observed with the nonprecoated indirect ELISA can result in misdiagnosis of ZYMV, for instance with the serologically related WMV2 (Somowiyarjo *et al.*, 1988), but this method can be improved by the use of cross-absorbed antisera (Sasaya & Yamamoto, 1995) or monoclonal antibodies (Somowiyarjo *et al.*, 1988). Menassa *et al*. (1986) described the detection of ZYMV in intact leaf disks by direct or indirect ELISA tests; attempts to detect ZYMV in viruliferous aphids were not satisfactory. Dietzgen & Herrington (1991) used a semiquantitative biotin-streptavidin ELISA, with a sensitivity increased 4–8 times compared to DAS-ELISA.

As an alternative to ELISA tests, serological assays using nitrocellulose membranes were used. Direct ‘tissue printing’ of whole infected leaves on the membrane can provide information relating to viral concentration and can be used to some extent to map virus distribution on the leaf surface (Polston *et al.*, 1991). The specificity of the reactivity with differential monoclonal antibodies, for leaf surfaces or petiole sections, was the same in ‘tissue printing’ as in ELISA tests and could be an interesting alternative for serotyping isolates (C. Desbiez, 1994, unpublished data). Crude extract preparations of leaf samples ground in usual ELISA buffers could also be tested on nitrocellulose

membranes ('dot blots'), using cross-absorbed polyclonal antibodies (Somowiyarjo *et al.*, 1989) or a highly specific monoclonal antibody (Somowiyarjo *et al.*, 1988). Somowiyarjo *et al.* (1987) used latex flocculation (LF) and protein A-coated latex-linked antisera (PALLAS) tests to detect ZYMV in pumpkin extracts, the latter method being more efficient. A two-step method employing immunofilter paper assay was also used for diagnosis of multiple virus infections (Multi-RIPA), and was applied successfully to ZYMV (Tsuda *et al.*, 1993).

Serological variability of ZYMV

Some serological variability was observed by ID-SDS tests among ZYMV isolates. Using an antiserum raised against ZYMV-E9 from France, the precipitation line formed by ZYMV-E9 formed a definite spur with the precipitin bands produced by isolates from Reunion island (H. Lecoq & D. E. Purcifull, 1986, unpublished data). Similarly, differences were observed with an isolate from Taiwan (Huang *et al.*, 1989).

Monoclonal antibodies (MAbs) raised against the coat protein of strains from Japan (isolate 169) (Somowiyarjo *et al.*, 1988), Florida (ZYMV-FL) (Wisler *et al.*, 1989; Wisler, 1992), France (ZYMV-E9) (Desbiez *et al.*, 1996), Israel (NAT) and Reunion island (R5 A) (C. Desbiez & H. Lecoq, 1995, unpublished data) were produced. According to the results obtained in triple antibody sandwich (TAS) ELISA tests with the MAbs raised against the France, Israel and Reunion strains, a collection of ZYMV isolates from different geographical regions could be classified in 15 serotypes (Table 4). Strains from Reunion island showed an important serological variability, in agreement with their sequence divergence from the type strains (Baker *et al.*, 1992). Interestingly, these isolates were more closely related to isolates from other islands of the Indian Ocean (Madagascar, Mauritius, Mayotte) than to isolates of other origins. Serotype I was the most frequently observed (Table 4). It is the serotype of the type strain from Italy and was found in Europe, the Middle East, Australia, the USA and Africa. Serological variability using this set of monoclonal antibodies was observed at different geographical levels: field, region, country. No correlation has been found so far between serological variability and biological properties, such as host range and aphid transmission (Desbiez *et al.*, 1996; C. Desbiez, C. Wipf-Scheibel & H. Lecoq, 1997, unpublished data).

Serological relationships with other viruses

Using antisera against virions and SDS-immunodiffusion, no serological cross-reaction was observed between ZYMV and PRSV, WMV-Morocco, bean yellow mosaic (BYMV), ZYFV, clover yellow vein (ClYVV), lettuce mosaic (LMV), and wisteria vein mosaic (WVMV) potyviruses (Lisa *et al.*, 1981; Lisa & Lecoq, 1984) but some cross-reactions were consistently detected between ZYMV and WMV2 antisera (Lecoq *et al.*, 1981; Lisa *et al.*, 1981; Davis, 1986; Greber *et al.*, 1987; Somowiyarjo *et al.*, 1989). This cross-reactivity depends on the antiserum used for the tests. Antisera produced from early bleedings are usually more specific than those from late bleedings (Lecoq *et al.*, 1981; Shukla *et al.*, 1992).

Polyclonal antibodies raised against nonstructural proteins (P1, cytoplasmic inclusions) of ZYMV often cross-reacted with other potyviruses (Suzuki *et al.*, 1990; Wisler *et al.*, 1995). Western blot analysis revealed that ZYMV-CI antiserum cross-reacted with WMV2-CI more than with PRSV-CI (Suzuki *et al.*, 1990), in correlation with results obtained for CP antisera. PRSV-CI and WMV2-CI antisera also reacted with ZYMV CI in western blot (Suzuki *et al.*, 1990). Antisera to tobacco etch virus (TEV) nuclear inclusions NIa and NIb and to PVY and TVMV helper component cross-reacted in immunoprecipitation tests with *in vitro* translation products of ZYMV (Hiebert *et al.*, 1984; in Purcifull & Hiebert, 1992). A polyclonal antiserum and a monoclonal antibody to PRSV-W amorphous inclusion protein (AI) could also detect ZYMV HC in ELISA tests using plant extracts or purified protein (Baker, 1989; in Purcifull & Hiebert, 1992).

Electron microscopy

The 750 nm long, flexuous ZYMV particles present at high concentration in plant tissues are usually easily observed in crude plant extracts using the leaf dip assay (Brandes, 1957). ZYMV also induces the presence of tubular scroll-like cytoplasmic cylindrical inclusions (CIs) of type 1 according to the classification of Edwardson & Christie (1978) (Edwardson, 1992), but some isolates were found to induce CIs of types 3 and 4 (pinwheels, scrolls, bundles and laminated aggregates) (Petersen *et al.*, 1991). Unambiguous identification of ZYMV particles in infected leaves can be achieved by immunosorbent electron microscopy (ISEM): virus particles are first trapped on a grid activated by antisera (Derrick, 1973) and subsequently decorated specifically by a homologous antiserum. Virus

Table 4 Serological variability of 735 ZYMV isolates (including 480 from France), revealed by a set of monoclonal antibodies

	Antibodies															
Serotype	CH10	CE11	BC2	AF4	BG1	DD3	ED3	AB6	CC11	AE11	DD2	DE6	CG4	CC1	Frequency	Strains
I	++[a]	++	++	++	++	++	++	++	++	++	++	++	+	−	81.5%	France, UK, Italy, Greece, Spain, Pakistan, Sudan, Algeria, Syria, Israel, Australia, the USA
II	++	++	++	++	++	++	+	++	++	++	++	−	−	−	10.8%	Martinique, Sudan, France, Spain, Nepal, Australia, Italy
III	++	++	++	++	++	++	++	++	+	−	++	−	−	−	0.4%	Martinique
IV	++	++	++	++	++	++	++	++	−	−	++	−	−	−	0.1%	Martinique
V	++	++	++	++	++	++	−	+	++	++	−	−	−	−	0.1%	Martinique
VI	++	−	+	++	++	++	−	++	+	−	++	++	+	−	0.4%	Guadeloupe
VII	++	++	+	++	++	++	+	−	+	−	++	++	+	−	0.3%	Guadeloupe
VIII	++	++	+	++	+	+	+	++	−	−	++	++	+	−	1.6%	Dominican Republic
IX	++	++	++	++	++	++	++	++	−	−	++	++	+	−	0.1%	Germany
X	++	++	++	++	++	++	++	++	++	++	−	++	+	−	0.5%	Turkey, Sudan
XI	++	++	++	+	++	++	−	−	−	++	−	++	−	−	0.5%	China
XII	++	++	++	++	++	++	++	−	−	−	−	−	++	++	1.0%	Réunion, Madagascar
XIII	++	++	++	++	++	++	++	−	−	−	−	−	−	++	2.0%	Réunion
XIV	++	−	+	++	++	++	−	−	−	−	−	−	++	++	0.1%	Mauritius
XV	++	++	++	++	−	−	++	−	−	−	−	−	−	−	0.5%	Mayotte

[a]Absorbance values at 405 nm (A). + +, A > 0.5; +, 0.1 < A < 0.5; −, A < 0.1 (considered as the background level).

particles appear coated by a 'halo' of antibody molecules (Milne & Luisoni, 1977). ISEM procedures provide a high sensitivity and allow detection of viruses present in mixed infections, even at very low concentrations, as well as the establishment of serological relationships between strains or viruses (Lesemann *et al.*, 1983; Wong *et al.*, 1994).

Molecular techniques

Polymerase chain reaction (PCR) has been developed as an efficient diagnostic tool. In the case of ZYMV, reverse-transcription (RT)-PCR was used successfully to amplify viral fragments of the 3' terminal part of the genome, from extracted total plant RNA (Thomson *et al.*, 1995). The amplified fragment is then directly available for further molecular analysis. Immunocapture of the virus from crude plant extracts followed by RT-PCR avoids the time-consuming step of RNA extraction. Immunocapture (IC)-PCR followed by restriction fragment length polymorphism (RFLP) analysis was used to differentiate serologically indistinguishable isolates of ZYMV (Barbara *et al.*, 1995).

A dot-blot hybridization system using digoxigenin-labelled probes was also used successfully for detection of ZYMV; extraction of the viral nucleic acid was required for effective virus detection (Harper & Creamer, 1995).

MOLECULAR DATA

The genome of ZYMV has been totally sequenced for strains from California (Balint *et al.*, 1990), and Reunion (Baker *et al.*, 1992). Sequence data for the 3′ terminal part of the genome, including the coat protein coding region, have been obtained for strains from Connecticut (Grumet & Fang, 1990), Florida (Quemada *et al.*, 1990), Israel (Gal-On *et al.*, 1990), and Singapore (Lee *et al.*, 1993). The sequence of the N-terminal part of the coat protein coding region was established for three strains from Australia (Thomson *et al.*, 1995) and 15 strains from Martinique (Desbiez *et al.*, 1996). Sequences of the HC gene are also available for the Israel strain, and for French strains (Granier *et al.*, 1993, Huet *et al.*, 1994). Wisler *et al.* (1995) sequenced the P1 coding region of three Florida isolates. Amino acid sequence identity between the CP of distinct ZYMV strains is over 90%, as reported by Shukla *et al.* (1994) for strains of potyviruses (Table 5). In contrast, Singapore and Reunion strains are more divergent from the other strains, particularly in the N-terminal part of the coat protein coding region, one of the most variable parts of potyvirus genome (Shukla *et al.*, 1988). Sequence analysis of the 3′ extremities of the genome of ZYMV strains suggested that recombination events between strains might have occurred, although not enough data are available to confirm this (Revers *et al.*, 1996).

Classification of potyviruses based on the coat protein gene sequence indicated that ZYMV is a distinct potyvirus, related to WMV2, peanut stripe virus (PStV) and passionfruit woodiness virus (PWV) (Ward *et al.*, 1992).

A full-length cDNA of the NAT isolate of ZYMV was obtained by Gal-On *et al.* (1992). It was introduced into a construct allowing direct inoculation of plants with the cDNA, without a transcription step, using a particle gun (Gal-On *et al.*, 1995). Shooting the plants with a particle gun improved significantly the cDNA inoculation procedure (Gal-On *et al.*, 1995).

CONTROL

During the last two decades many efforts have been made to reduce the incidence of ZYMV in cucurbit crops. Among the different control measures some are non specific to ZYMV, and will prevent the

Table 5 Amino acid sequence identity (%) of coat proteins of ZYMV strains

	California	Connecticut	Israel	Florida	Reunion	Singapore
California	–	97.6	95.1	84.9	54.2	58.5
Connecticut	99.6	–	92.7	80.5	48.8	56.1
Israel	99.3	98.9	–	78.0	48.8	58.5
Florida	96.8	96.4	96.1	–	41.5	46.3
Reunion	91.0	90.7	90.7	89.6	–	65.9
Singapore	92.8	92.5	92.8	90.7	93.9	–

Figures above the diagonal: N–terminal part of the protein (41 amino acids). Figures below the diagonal: total of the coat protein (279 amino acids).

dissemination of other aphid borne viruses as well, while others will be effective only against ZYMV. However, the control of ZYMV should be integrated within a more general framework to control aphid borne viruses in cucurbits.

Control of virus spread

ZYMV is very efficiently transmitted by aphids and some control methods are intended to limit the contact of viruliferous aphids with the crops.

Weeding to remove virus or aphid sources near planting has been shown to delay slightly the spread of CMV in melons (Lecoq & Pitrat, 1983). This would probably have little direct effect on ZYMV infection, because, in contrast to CMV, reservoirs of ZYMV are very rare (if any) around cultivated fields. It might, however, decrease vector populations in the vicinity of the crop. Avoiding overlapping crops in the same area, particularly by removing old infected crops before planting any new ones in the vicinity, might reduce the sources for early contamination of the young crops.

Insecticide applications generally reduce significantly the aphid population colonizing a crop, but they were not effective in reducing ZYMV spread within a crop. This is probably because insecticides do not kill viruliferous alate aphids quickly enough to prevent virus transmission, and because the most efficient ZYMV vectors are noncolonizers (Webb & Linda, 1993; Perring & Farrar, 1993).

Mineral oil sprays (Makkouk & Menassa, 1985; Webb & Linda, 1993), in association with pyrethroids (Raccah, 1985), might provide temporary protection under certain ecological conditions, but applications must be repeated frequently with adapted machinery to be effective. Mineral oil seems to interfere with retention of potyvirus particles on the aphid stylet, thus limiting aphid acquisition and transmission of the viruses (Wang & Pirone, 1996). Perring & Farrar (1993) showed that pyrethroid treatment did not lower the rate of ZYMV infection of field-grown cantaloupes, but had a significant positive impact on plant growth and yield.

Plastic mulches were shown to be efficient as aphid repellents (Giunchedi *et al*., 1991; Lecoq, 1992a, 1992b; Brown *et al.*, 1993), and to delay virus spread. However, they have two major drawbacks; their efficiency is progressively decreased when plant growth covers their surfaces and they generally need to be removed and disposed of in a landfill after use. Sprayable silver film mulches proved to be as efficient in delaying the onset of ZYMV in zucchini squash, and, being watersoluble and biodegradable, they might be incorporated into the soil at the end of the season (Summers *et al.*, 1995).

Covers of different types (unwoven, perforated plastic. . .) were also efficient in preventing ZYMV transmission, but they must be removed at the flowering stage to allow insect pollination (Lecoq, 1992a; Reyd *et al.*, 1993; Tomassoli *et al.*, 1993).

All these methods are more efficient when used in association, but seldom give complete control at an economical cost.

Cross-protection

An alternative method for ZYMV control until agronomically acceptable resistant cultivars are available is the use of the mild ZYMV-WK strain (Lecoq *et al.*, 1991b) for cross-protection against severe challenging strains. This protection method has been used successfully under greenhouse and field conditions in south-eastern France (Lecoq *et al.*, 1991b), and Taiwan (Wang *et al.*, 1991). Increase in marketable production of cross-protected plants was up to 14.7 times that of unprotected zucchini squash plants under natural infection conditions in France (Lecoq *et al.*, 1991b), whilst in mechanically inoculated fields under high inoculum pressure, yield increase for cross-protected plants vs. unprotected was 1256% (Wang *et al.*, 1991). Cross-protection using the ZYMV-WK strain was also applied successfully in Hawaii (Cho *et al.*, 1992), the United Kingdom (Walkey *et al.*, 1992), Turkey (Yilmaz *et al.*, 1994), Israel (Singer *et al.*, 1994), California (Perring *et al.*, 1995), and Italy (V. Lisa, 1995, personal communication). Artificial aphid inoculations of the severe challenging strain at different times after inoculation with the mild strain showed that about 14 days of incubation were required to provide protection against subsequent infection with a severe strain (Walkey *et al.*, 1992). Cross-protection was not efficient against strains from Reunion or Mauritius in greenhouse tests, in relation to the very great molecular divergence of these strains (H. Lecoq, 1993, unpublished data).

Resistance by conventional methods

The most convenient way to control viral diseases is the use of resistant cultivars when they are available. The importance of the economic losses associated with ZYMV infection and the difficulty to limit the efficient dissemination of the virus make the search for resistance genes a priority in breeding programs for cucurbits. Lecoq *et al*. (1979)

described resistance to virus transmission by *A. gossypii* in melon PI161375. This resistance, governed by a single dominant gene (Pitrat & Lecoq, 1981) is efficient against ZYMV, but is specific to *A. gossypii* (Risser *et al.*, 1981). In the field, this resistance was not efficient to protect plants against ZYMV, probably because the virus was being spread by noncolonizing aphid species (H. Lecoq, 1995, unpublished data).

Resistance genes against ZYMV have been described for most cultivated cucurbit species (Table 6, modified from Provvidenti & Hampton, 1992). These resistances are usually found in wild accessions, and breeding programs are necessary to introduce them into agronomically acceptable cultivars. Most of these resistances are governed by single genes, and viral variability might result in some of them being rapidly 'overcome'. This is the case for melon, where some ZYMV isolates from the field or obtained in the laboratory after successive inoculations on resistant plants totally overcome the *Zym* resistance gene of muskmelon PI414723. However, most field isolates are either controlled by the *Zym* gene (pathotype 0) or induce systemic chloronecrotic lesions (pathotype 1) (Lecoq & Pitrat, 1984). A resistance to ZYMV in watermelon was also found to be strain-specific (Provvidenti, 1991).

Resistance genes were found in germplasms of different geographical origin, mainly in the supposed areas of diversification of cucurbits: Asia for the genus *Cucumis*, America, Africa and Europe for *Cucurbita*, Africa for *Citrullus*. Genes for resistance to ZYMV are often described in accessions exhibiting multiple resistance to other viruses. The cucumber accession TMG was resistant to ZYMV, WMV2, PRSV-W and ZYFV (Provvidenti, 1987; Gilbert-Albertini *et al.*, 1995). In *C. moschata* 'Menina', the *Zym* resistance gene was found to be identical or closely linked to the gene for resistance to WMV2 (Gilbert-Albertini *et al.*, 1993). Melon accession PI414723 is also resistant to PRSV and CABYV (Dogimont *et al.*, 1996). These characteristics are of interest for selection of multiresistant commercial cultivars. Seed companies are introducing some of these resistances into commercial cultivars, but it will take several years before they are available to farmers, in all of the cultivated cucurbit species.

Pathogen-derived resistance

During the last 10 years, the concept of pathogen-derived resistance has attracted much attention. It depends upon the expression of viral genes in transgenic plants in order to obtain resistance against the homologous virus. Namba *et al.* (1992) expressed the coat protein of ZYMV in *Nicotiana benthamiana* plants (not a natural host of ZYMV) and observed a range of protection levels against seven different potyviruses (WMV2, BYMV, pea mosaic virus (PeaMV), pepper mottle virus (PeMV), PVY, ClYVV, and (TEV) dependent of the virus and the inoculum concentration. A symptom delay of 1 to more than 16 days was observed. Symptoms were usually less severe in transgenic than in control plants. Fang & Grumet (1993) introduced several constructs derived from the ZYMV coat protein gene into muskmelon and tobacco plants: the full-length coat protein gene, a conserved 'core' portion of the gene, and an antisense version. Transgenic melon plants expressing the full-length coat protein were highly resistant to ZYMV infection. Transgenic plants expressing only the core part of the coat protein showed a limited protection against ZYMV. The antisense construct allowed variable levels of protection, correlated with transcript level. The

Table 6 Sources of resistance to ZYMV in cucurbits

Species	Resistance gene(s)	Geographical origin	Reference
Citrullus lanatus		Africa (Zimbabwe)	Boyhan *et al.* (1992)
C. lanatus[a]	*zym*	Africa (Nigeria)	Provvidenti (1991)
C. colocynthis		Africa (Nigeria)	Provvidenti (1986)
Cucumis melo[a]	*Zym*	Asia (India)	Pitrat & Lecoq (1984)
Cucumis sativus	*zym*	Asia (Taiwan)	Provvidenti (1987)
Cucurbita moschata	*Zym*	Europe (Portugal)	Paris *et al.* (1988)
C. moschata	1 dominant	Africa (Nigeria)	Munger & Provvidenti (1987)
C. equadorensis	*Zym*	America (Ecuador)	Robinson *et al.* (1988)
Lagenaria siceraria		Asia (India)	Provvidenti *et al.* (1984a)

[a]Strain-specific resistance.

different constructs also allowed limited protection against two heterologous viruses, TEV and PVY, in tobacco plants.

More recently, transgenic squash hybrids containing combinations of the ZYMV, WMV2 and CMV coat protein coding regions were obtained by Asgrow Seed Co., Kalamazoo, Michigan, USA, as well as cantaloupe containing the coat protein coding regions of the three viruses (Clough & Hamm, 1995). The plants were tested in the greenhouse and field for resistance against the Florida strain of ZYMV (Quemada *et al.*, 1990), used for obtaining the transgenic plants, and against the homologous strains of WMV2 and CMV. Transgenic lines containing single CP constructs showed no or only partial resistance, whereas ZW-20 plants expressing CP constructs of ZYMV and WMV2 had a high level of resistance to both viruses (Fuchs & Gonsalves, 1995; Tricoli *et al.*, 1995). All plants were sensitive to the unrelated PRSV. It is of interest that ZYMV-resistant squashes were the first such pathogen-derived resistant plants to be deregulated in the USA, and will probably be the first commercialised ones. This can be related to the important losses caused worldwide by this virus, and the limited efficiency of most control strategies.

CONCLUSION

ZYMV, first observed in Italy in 1973, was detected worldwide within the last 20 years. The reasons for the sudden appearence of the virus are still largely unknown, as is the case for most plant or animal 'emerging' viruses. The availability of sera since 1981 has made possible the rapid identification of the virus concomitantly in several countries. This revealed that ZYMV spread rapidly in the decade from 1980 to 1990, but this could be the result of either an epidemic of a 'new' virus, or an outburst of an existing virus present in localized areas where it remained undetected. ZYMV was once thought to be a 'new' virus originating from mutations or recombinations of other potyviruses (WMV2, PRSV…) but this could not be confirmed when genome sequences were made available. On the other hand, ZYMV could have been an endemic virus in geographically limited areas for a long time. For instance, in 1955 Tarr observed symptoms on cucurbits grown in Sudan very similar to those caused by ZYMV. However, in Europe, such symptoms were not described, and it seems unlikely that a severe disease like that caused by ZYMV could have gone unobserved for many years.

The rapid spread of ZYMV could result from changes in transmission. However, the hypothesis of changes in vector transmissibility can probably be ruled out, because many aphid species can transmit ZYMV, as is the case for many other potyviruses. Seed transmission might have contributed to ZYMV spread, although transmission rates observed so far are very low and inconsistent. Finally, evolution of cultural practices applied to cultivated cucurbits in the last 30 years might also have favoured the survival of the virus under winter conditions, and its subsequent increased occurrence in the field during the growing season.

ZYMV is now present worldwide, and is responsible for dramatic losses in cucurbit crops. Control strategies have been developed, and resistant plants (obtained by conventional breeding programs, or pathogen-derived strategies) should be available within a few years for all of the cultivated cucurbits. However, the important potential of variability of the virus reveals that some resistances might be rapidly overcome by ZYMV isolates, and that control programs will have to integrate several strategies in order to remain effective.

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