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Transmission of the agent causing a melon yellowing disease by the greenhouse whitefly Trialeurodes vaporariorum in southeast Spain

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Abstract

The agent causing a yellowing disease of melon (*Cucumis melo*), which results in severe losses in crops under plastic on the coastal plains of southeast Spain, was shown to be transmitted in a semipersistent manner by the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood). The agent was transmitted by grafting, but not by mechanical inoculation or through seeds. The agent was acquired in the minimum period tested (2 h) and could infect plants in an infection feeding interval of 6 h.

Capsella bursa-pastoris, *Cucumis melo*, *C. sativus*, *Cucurbita moschata*, *Cichorium endivia*, *Lactuca sativa* and *Taraxacum officinale* were found susceptible.

Results suggest that the yellowing disease affecting melon crops in the southeast of Spain is due to a pathogen similar to beet pseudo yellows virus, but this has to be confirmed by serology.

Additional keywords: *Cucumis melo*, beet pseudo yellows virus.

Introduction

Since 1982, crops of melon (*C. melo*) grown under plastic in Spain, have been seriously damaged due to the increasing incidence of an unidentified yellowing disease.

The symptoms characteristically begin with the appearance of very small, yellow spots on the old leaves, but soon the younger leaves are involved. The chlorotic spots increase in size and fuse together until the whole leaf surface, except the veins, is yellow. The veins maintain their colour until an advanced state of the disease. The onset of the disease can also be signalled by a yellow colouring of the bases of the leaf petioles (Cuartero et al., 1985).

The symptomology and the close correlation between the incidence of the yellowing disease of melon and the presence of the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) (Esteva et al., 1987) suggested that it may be similar to beet pseudo yellows virus as described by Duffus (1965).

The present work is part of a larger study aimed at developing genotypes to be used to introduce resistance to the causal agent of the melon yellowing disease in the several varieties grown commercially in this area. It was designed to evaluate four possible ways of transmitting the disease, namely; vector transmission, using *T. vaporariorum*; mechanical inoculation; seed transmission, and graft transmission.

Material and methods

Wooden boxes with glass fronts and backs, covered with muslin were used to breed colonies of virus-free *T. vaporariorum*. The parent whiteflies of the virus-free colonies were collected from tomato (*Lycopersicon esculentum*) plants in the field and placed on virus-free melon plants.

All the melon plants used in the experiments were grown in special containers designed to keep them free of whiteflies at all times.

Vector transmission using T. vaporariorum. Nonviruliferous whiteflies were placed for 48 h on melon plants showing clear symptoms of the yellowing disease. Then, they were transferred in groups of 40 to 16 healthy melon seedlings at the 1 to 2 true leaf stage. The infection feeding-period was 72 h, after which the whiteflies were destroyed and the plants were transferred to a whitefly-free greenhouse to await the development of symptoms.

Similar experiments were carried out with two other plant species as indicator hosts, *Taraxacum officinale* and *Capsella bursa-pastoris*, to observe the symptoms of the disease in these species and to evaluate the possibility of employing them as indicators of infection in future studies.

As a control, virus-free *T. vaporariorum* were placed on healthy melon plants for 48 h and then transferred in groups of 40 to healthy melon, *Taraxacum* and *Capsella* seedlings for a period of 72 h.

Other controls were healthy plants in a whitefly-free greenhouse.

Twenty days after inoculation the leaves on which the whiteflies had fed were removed to avoid the development of whitefly nymphs.

The experiments were carried out at temperatures which ranged from 25 °C (maximum) to 11 °C (minimum) with a humidity of C. 70%.

Mechanical inoculation. A tissue extract was prepared from melon plants showing clear symptoms of the yellowing disease by cold maceration in 0.1 M, pH 7.0 phosphate buffer and in 0.08 M disodium phosphate (pH 7.2) with 0.05 M sodium bisulfite and 0.02 M sodium diethyldithiocarbamate. Two 10-plant groups of healthy melon plants were prepared. One group was inoculated on the cotyledon leaves, the other group was inoculated on the first true leaf.

The control were two blocks of 5 seedlings each inoculated with buffer solution only. The plants of one block were inoculated on the cotyledon leaves, and those of the other, on the first true leaf.

Transmission by seeds. Two groups, one with twenty healthy melon plants, and the other with twenty melon plants previously subjected to controlled infection and showing clear symptoms of yellowing disease, were cultivated in separate greenhouses provided with whitefly-proof screens. In each greenhouse, the twenty plants were self-fertilized and later observations were made of 100 seedlings from each block cultivated in whitefly-free greenhouses.

Pathogen-vector relationships. several parameters were studied at temperatures ranging from 15 °C to 24 °C and C. 70% relative humidity.

– *Relation of insect numbers and pathogen transmission.* After a 48-h acquisition feeding on whitefly-inoculated melon plants showing yellowing, whiteflies were transferred singly and in groups of 5, 10, 30 and 40 to healthy melon seedlings for a 72-h infection feeding.
– *Acquisition feeding period.* Groups of 40 whiteflies were allowed acquisition feedings on the inoculum sources for periods ranging from 2 to 54 h, and later transferred to healthy melon seedlings for a 72-h infection feeding interval.
– *Infection feeding period.* Groups of 40 whiteflies were allowed acquisition feeding intervals on the inoculum source for 48 h and then, transferred to healthy melon seedlings for infection feeding periods ranging from 6 to 72 h.
– *Persistence.* Whiteflies were reared on whitefly infected melon plants with symptoms, and then transferred daily in groups of 40 insects to new healthy melon seedlings.

Host range. The host range was determined by inoculating at least ten seedlings of each species with 50 whiteflies fed on infected melon for 48 h. Plants showing indication of infection were used as an inoculum source for a further inoculation of that species and to indicator melon plants.

Results

Whitefly transmission. Melon plants inoculated with whiteflies fed on the inoculum source began to show clear yellowing 20 days after inoculation. After 45 days, 23 of the inoculated plants showed symptoms. Yellowing was not observed in melon plants inoculated with whiteflies fed on healthy plants or in noninoculated controls (Table 1). These results indicated the *T. vaporariorum* is an effective vector of the causal agent of the yellowing disease in melon crops.

Table 1. Plants showing symptoms after inoculation with 40 *T. vaporariorum* fed on source melon plants and placed on *C. melo* (CM), *T. officinale* (TO), and *C. bursa-pastoris* (CB).

Days after inoculation	Source[1]								
	muskmelon with yellowing disease			healthy muskmelon			control, no exposure to whiteflies		
	CM	TO	CB	CM	TO	CB	CM	TO	CB
20	1/16[2]	0/5	2/5	0/16	0/5	0/5	0/10	0/2	0/5
25	2/16	0/5	3/5	0/16	0/5	0/5	0/10	0/2	0/5
30	2/16	0/5	5/5	0/16	0/5	0/5	0/10	0/2	0/5
35	3/16	1/5	5/5	0/16	0/5	0/5	0/10	0/2	0/5
40	10/16	3/5	5/5	0/16	0/5	0/5	0/10	0/2	0/5
45	13/16	3/5	5/5	0/16	0/5	0/5	0/10	0/2	0/5

[1] Whiteflies fed on the infected melon source for 48 h and then transferred to test plants for 72 h.
[2] Numerator: number of plants with symptoms; denominator: number of plants inoculated.

T. officinale was a host for the causal agent. The symptomology displayed by this species appears to be identical to that shown when it is infected by BPYV (Duffus, 1965). *C. bursa-pastoris* shows a progressive yellowing of the oldest leaves which curl in a characteristic way and turn brittle. As the disease progresses, the younger leaves show the same symptoms. The symptoms appear to be identical to those induced by BPYV on this host (Duffus, 1965).

Mechanical inoculation. Neither the inoculated melon plants nor the controls showed disease symptoms; so it can be concluded that under the conditions of these experiments the disease is not transmitted mechanically.

Transmission by seeds. No symptoms of the yellowing disease were observed in the 200 plants kept in whitefly-free greenhouses after two and a half months following seeding. It is concluded that this specific disease is not transmitted by seeds.

Graft transmission. Sixteen of 20 grafts of healthy and yellows infected tissue were successful and 37% of the initially healthy plants became infected.

Table 2. Relation of whitefly numbers and causal agent transmission.[1]

Number	Test number		
	1	2	3
1	2[2]	1	2
5	3	3	6
10	4	4	6
20	8	6	8
30	10	8	9
40	10	7	10

[1] Nonviruliferous whiteflies were given 48 h acquisition feeding on melon plants infected with the melon yellowing agent and then transferred singly or in groups to healthy melon seedlings for a 48 h inoculation feeding.
[2] Number of plants infected out of 10 plants inoculated.

Table 3. Results of tests to determine the time required for nonviruliferous whiteflies to become infective with the melon yellowing agent.

Test number	Melon seedlings infected out of 10 inoculated with groups of 40 insects fed on the virus source for the indicated acquisition period in h				
	2	6	18	48	54
1	1	3	9	9	9
2	2	1	5	9	8
3	0	4	6	10	10

Table 4. Results of tests to determine the feeding time required by 40 viruliferous whiteflies to infect melon seedlings with the melon yellowing agent.

Test number	Plants infected out of 10 on which the whiteflies were allowed to feed for the indicated period in h				
	6	12	24	48	72
1	3	8	8	8	10
2	5	5	8	7	9
3	1	2	8	7	9

Pathogen-vector relationships

– *Relation of insect numbers and causal agent transmission.* Single greenhouse whiteflies are capable of transmitting the agent. The efficiency of transmission increased when larger numbers of insects were used, and maximum transmission occurred when groups of at least 30 whiteflies were used (Table 2).

– *Acquisition feeding period.* Two hours feeding on the inoculum source were enough for the vector to acquire the causal agent. Transmission efficiency increase with an increase in feeding time on the source (Table 3).

– *Infection feeding period.* Six hours feeding on the healthy plants were sufficient for the vector to transmit the causal agent. Transmission efficiency increased as longer infection feeding periods were used (Table 4).

– *Persistence.* Our results showed that *T. vaporariorum* loses its transmitting ability in 6 days or less (Table 5).

Host range. In addition to *Cucumis melo*, *Taraxacum officinale*, and *Capsella bursa- pastoris*, *Cucumis sativus*, *Cucurbita moschata*, *Cichorium endivia*, *Lactuca sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Lycopersicon esculentum* and *Tropaeolum majus* were tested as experimental hosts.

The results showed that *C. melo*, *T. officinale*, *C. bursa-pastoris*, *C. sativus*, *C. moschata*, *C. endivia* and *L. sativa* are susceptible. Infected plants exhibited interveinal yellowing symptoms. *Phaseolus vulgaris* plants showed slightly chlorotic areas; such symptoms were not reproduced on the same species in recovery attempts and no yellowing symptoms appeared on melon plants. Some plants of *T. majus* showed yellow spotting on the leaves and yellowing progressed, but these symptoms could not be reproduced on the same species and no yellowing symptoms appeared on melon plants. Plants of *P. sativum* and *L. esculentum* did not show any indication of infection.

Discussion

Whitefly transmitted yellowing viruses of cucurbits have been reported from: the USA, BYPV by Duffus (1965), and lettuce infectious yellows virus (LIYV) by Duffus et al. (1986); Japan, cucumber yellows virus (CYV) by Yamashita et al. (1979); France, BPYV by Lot et al. (1980, 1983), and muskmelon yellows virus (MYV) by Lot et al. (1983); the Netherlands, BPYV by Van Dorst et al. (1980); Bulgaria, BPYV by Hristova

Table 5. Melon seedlings infected (+) and noninfected (−) in daily serial transfers using groups of 40 greenhouse whiteflies reared on a melon yellowing source plant.

Test number	Whitefly colony number	Successive daily transfers						
		1	2	3	4	5	6	7
1	1	+	+	+	+	−	−	−
	2	+	−	−	−	−	−	−
	3	−	−	−	−	−	−	−
	4	+	−	−	−	−	−	−
	5	+	−	−	−	−	−	−
	6	+	−	+	+	−	−	−
	7	+	+	+	+	−	−	−
	8	+	+	+	+	−	−	−
	9	+	+	+	+	−	−	−
	10	+	−	+	−	−	−	−
2	1	+	+	+	−	+	−	−
	2	+	+	+	−	−	−	−
	3	−	−	−	−	−	−	−
	4	−	−	−	−	−	−	−
	5	−	+	+	+	+	−	−
	6	+	−	−	−	−	−	−
	7	+	+	−	−	−	−	−
	8	−	+	+	+	+	+	−
	9	+	+	+	+	−	−	−
	10	+	+	−	−	−	−	−
3	1	−	−	+	−	−	−	−
	2	+	+	−	+	−	−	−
	3	−	+	−	+	+	−	+
	4	+	−	+	−	−	+	−
	5	+	+	+	+	−	−	−
	6	−	−	+	−	−	−	−
	7	−	+	−	−	−	−	−
	8	+	+	+	−	−	−	−
	9	+	+	+	−	−	−	−
	10	−	+	−	−	−	−	−

and Natskova (1986); and the United Arab Emirates, an unidentified *Bemisia* transmitted virus by Hassan and Duffus (1991).

Two of the viruses, LIYV from the USA and the causal agent of the yellowing and stunting disorder (YSD) from UAE are *Bemisia tabacci* (Gennadius) transmitted. Both have flexuous rod shaped particles and are serologically distinct from each other and from BPYV.

Three of the viruses (BPYV, CYV and MYV) are transmitted by *T. vaporariorum*. Recent evidence indicated that the insect transmission and host range characteristics of CYV have been shown to be identical to BPYV (Zenbayashi et al., 1988). So this

virus, reported from Japan, should probably be considered BPYV. MYV was reported as thought to be restricted to the *Cucurbitaceae* but the authors indicated that improvement of transmission techniques could perhaps modify the results (Lot et al., 1983). Thus, the causal agent of the melon yellowing disease in Spain seems to be very similar to BPYV based on the *Trialeurodes* vector, insect relationships and host reactions on melon, cucumber, lettuce, *Taraxacum* and *Capsella*.

Although this yellowing disease is able to affect several species in Spain, losses are very important on melon crops growing in greenhouses in some areas where the largest whitefly populations have been found. Nurseries should be kept free of whiteflies and seedlings should be protected after transplanting by insect-proof covers until they reach enough size and vigor to avoid a severe infection. Overlapping crops of melon, cucumber, or flower crops susceptible to the agent should be avoided.

Breeding for resistance is undoubtedly the best control measure for the yellowing diseases of melon, but there are no reports of yellowing resistance in *C. melo*. In searching for resistance sources, 70 different accessions of this species as well as other wild species of *Cucumis* have been tested using whitefly transmission. Two species have been selected thus far and crosses have been done to introduce this resistance into *C. melo* (Soria et al., 1990).

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