Whiteflies Other Than *Bemisia tabaci* as Vectors of Plant Viruses

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Abstract

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Whiteflies other than *B. tabaci* were first recognized as virus vectors in the Sixties and include until to date the species *Trialeurodes vaporariorum*, *T. abutilonea* and possibly, *Parabemisia myricae*. The viruses transmitted, whose type-member may be considered beet pseudo-yellows virus, have been reported from the USA, Europe, Japan and Australia but not so far from Africa, South America or continental Asia. They mostly infect plants in the families Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae and Solanacease, causing symptoms such as chlorotic spots, yellowing or chlorosis, and thickening, brittleness and downward curling of leaves. The closterolike virus particles, 900-1000 nm long and 12 nm wide, with an ssRNA genome, have not been fully characterized at the molecular level but studies on some of the viruses are in progress. Experimentally, transmission has been obtained by grafting but not by sap or aphid inoculation, by contact or through seed. The whitefly vectors can transmit with acquisition and inoculation feedings of at least 5 min but the transmission rate increases as both feeding periods are increased. There is no clear evidence for the presence of a latent period, although infectivity can be retained for up to 6 days. Some epidemiological aspects of these viruses are discussed with particular emphasis on those that have been detected in Italy.

Key words: Insect transmission, geminiviruses, closteroviruses.

Introduction

The whiteflies (Homoptera: Aleyrodidae) are small piercing and sucking insects that feed on a wide range of wild and cultivated plants through their stylets. Eggs are laid on the leaf undersurface and there are four nymphal instars which remain firmly attached to the host plant, except for a short period during the first instar. The adults are winged and fly actively from plant to plant. Both nymphs and adults penetrate plant tissues intracellularly with their stylets, and feed in the phloem. Of the about 1,200 species described in the Family *Aleyrodidae*, only a few are considered agricultural pests. They cause damage either directly, by feeding punctures and by extraction of phloem nutrients, or by acting as vectors of pathogenic viruses (3, 18).

Bemisia tabaci has long been the only whitefly known as vector of diseases prevalent in the tropics and are characterized by symptoms such as vein yellowing or mosaic, dwarfing, and curling and distortion of the leaves. Such diseases have been shown to be caused by geminiviruses (8). More recently, *B. tabaci* has also been found to transmit filamentous viruses which induce yellowing type of symptoms in plants. Many such viruses have been identified as closteroviruses on the basis of particle morphology and cythopathological characteristics (2). Two other whitefly species, *Trialeurodes abutiloneus* (= *T. abutilonea*) (10), and *T. vaporariorum* (4) are

capable of transmitting viruses of this type and another species, *Parabemisia myricae*, has been shown very recently to transmit a new virus of citrus (13).

A few other species of *Bemisia*, two of *Aleurotrachelus*, and one of *Trialeurodes* were reported by Heinze (9) as plant virus vectors. All these, however, are either synonyms of the above whitefly species or have not been confirmed as vectors (18). In conclusion, the whitefly species other than *B. tabaci* presently known as virus vectors, and discussed in this paper, are *T. vaporariorum*, *T. abutilioneus* and *P.myricae*.

Viruses Transmitted by *T. vaporariorum*

1. Beet pseudo-yellows virus (BPYV).

This virus was first found in California in beet (*Beta vulgaris*) plants showing yellowing, thickening and brittleness of leaves (4), and then also reported from France, The Netherlands, Australia, Tasmania, and Italy (6, 16, 19, 21). In Europe, glasshouse lettuce was the crop affected most severely, reacting with yellowing and thickening of the basal leaves, and failure of heading. Before BPYV had been identified as its causal agent, the disease was described as lettuce yellowing ('Jaunisse de la laitue', 'Giallume della lattuga') that should not be confused with lettuce infectious yellows, a disease induced by another filamentous virus (LIYV) transmitted by B. tabaci in California (7). As BPYV is the first discovered and best characterized whitefly-borne closterovirus (1, 15), it can be considered the prototype of this group.

BPYV has filamentous particles approximately 12 nm wide and 1,500-1,800 nm long. It was purified from infected Nicotiana clevelandii by clarification with chloroform, precipitation by polyethylene glicol (PEG, MW 6,000), low- and high-speed centrifugation and cesium sulfate density gradient centrifugatin. The purified virus was used to prepare a polyclonal antiserum with a homologous titer of 1/32 in microprecipitin tests (15). Recently, cDNA clones to dsRNA purified from BPYV-infected cucumbers were produced but appeared insufficiently sensitive to detect infection-specific RNA in dot and northern blots of crude nucleic acid extracts. However, a knowledge of the sequence of such clones has been used to synthesize oligonucleotides which have been used for PCR amplification of specific sequences from both purified dsRNA and infected plants, acting as sensitive diagnostic probes (1).

BPYV infects a wide range of plants in the families Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Geraniaceae, Linaceae, Malvaceae, Portulacaceae, Ranunculaceae, Solanaceae, Umbelliferae, and Urticaceae (5). The best indicator plants are *Capsella bursa-pastoris*, lettuce, *Chenopodium capitatum*, *Nicotiana glutinosa* and muskmelon which show, in general, symptoms of stunting, interveinal yellowing, and/or chlorotic spotting. The virus could be transmitted experimentally by using single *T. vaporariorum* as vectors but increasing the number of infective insects per plant improved the rate of trasmission considerably. BPYV can be both acquired from infected plants and trasmitted to test plants by its vector with feeding periods of 1 h or longer. The presence of a latent period in the vector is uncertain, and virus-exposed insects can retain infectivity for up to 7 days after acquisition.

In Italy, BPYV was first found in Campania, near Naples, in chichory, endive and lettuce (19), then appeared in Liguria (Italian Riviera) causing serious epiphytotics in autumn-sown glasshouse crops of lettuce from 1982 to 1985. In this area, the virus was found to infect both lettuce and some wild species (*Capsella bursa-pastoris*, *Sonchus* spp.) in the open, and then to spread rapidly in protected crops that were massively invaded by whiteflies as the outside temperature started to fall (M. Conti, unpublished). BPYV is presently endemic in Liguria but epiphytotics in glasshouse lettuce have no longer been observed since 1986.

2. Viruses of cucurbits.

With the only exception of BPYV, all the other filamentous viruses trasmitted by *T. vaporariorum* were isolated from cucurbits. They are: cucumber yellows (23), muskmelon yellows (17); cucumber infectious chlorosis (11), melon yellows (12, 20), and cucumber chlorotic spot (22).

2a. Cucumber yellows virus (CYV). This virus was isolated in Japan from either cucumbers (*Cucumis sativus*) or melons (*C. melo*) with symptoms of severe chlorosis and yellowing of the leaves (23). CYV has a narrow host-range, and is not mechanically transmissible. The virus particles, 1,000 nm long and 12 nm wide,were detected in sieve tubes and companion cells, and occasionally in the xylem. They were regularly seen in the cell cytoplasm, and rarely in the nuclei. The cytopathological alterations caused by CYV in its hosts include phloem necrosis, accumulation of starch grains in the chloroplasts of mesophyll cell, and characteristic inclusions consisting of membranous bodies containing virus particles.

2b. Muskmelon yellows virus (MMYV). The symptoms caused by this virus in muskmelon were pinpoint chlorotic spots on the young leaves, that also showed greasy flecks on their lower surface, and interveinal

yellowing of the old leaves. The disease has been noted in Provence, southeastern France, since 1980 (17). The virus has labile filamentous particles about 1,000 nm long, which occur in the phloem parenchyma and companion cells. Aggregates of virus particles, and vesicles containing both fibrous material and electron-dense granular structures, closely resembling viroplasms, were present in the infected cells. Phloem necrosis was also frequent. MMYV could also be transmitted by *T. vaporariorum* to cucumbers which reacted with leaf yellowing and stunting. The virus was experimentally transmissible by grafting but not by sap or aphid inoculation. Using the vector whitefly, it was shown that MMYV is transmitted most efficiently when the acquisition feeding period is more than 16 h, the inoculation feeding period is 36-48 h, and 20-40 insects are used to inoculate each test plant (17).

2c. Cucumber infectious chlorosis virus (CICV). This name was given to the agent of a serious disease of glasshouse cucumbers first noted in Bulgaria in 1981 (11). The symptoms consisted of mild chlorotic or dark green spots on young leaves, and slight curling of old leaves. The cucumber cultivar Sandra appeared to be particularly sensitive to infection, with losses of up to 50% in 1982. CICV could be transmitted to lettuce test plants by single *T. vaporariorum* whiteflies, and the minimum efficient periods of feeding for both virus acquisition and inoculation could be as short as 5 min. Serial transfer experiments showed that the vector can retain infectivity up to the 6th day after the acquisition. CICV was also transmitted experimentally to lettuce and *N. glutinosa* which both reacted with chlorotic spots and interveinal chlorosis (11). No information is available on virus morphology or the cytopathology of infected host cells.

2d. Melon yellows virus (MYV). MYV has been reported to occur in melon crops under plastic tunnels in Spain since 1982 (20). The typical symptoms caused were very small, yellow spots on the old leaves, and later on the young leaves; diffuse yellowing of the interveinal leaf tissue due to progressive increase in size of the yellow spots, which finally coalesce; persistence of the normal green colour along the leaf veins. The disease was transmitted experimentally by both grafting and *T. vaporariorum* but not by sap inoculation or through melon seed. The host-range, determined by inoculating at least ten seedlings of each test species with fifty virus-exposed whiteflies, includes the following species: *Beta vulgaris*, *Capsella bursa-pastoris*, endive, cucumber, muskmelon, *Cucurbita maxima*, *C. moschata*, *Sonchus oleraceus*, *Taraxacum officinale*, and possibly *Phaseolus vulgaris* (12, 20). The particles of MYV are flexuous filaments with clear helical structure, 12 nm wide and most frequently 900-950 nm long. Among different procedures tested to purify the virus, the most successful included: extraction in liquid nitrogen with 0.1 M Tris-HCl buffer pH 7.8 containing 1 mM cysteine, clarification by stirring with 2% activated charcoal, precipitation by PEG (MW 6,000), and low and high speed centrifugation. Virus suspensions obtained in this way were used to prepare a polyclonal antiserum with a homologous titer of 1/64, that was suitable for detection of MYV by ELISA (12).

2e. Cucumber chlorotic spot virus (CCSV). This was isolated from cucumbers grown in France and showing chlorotic spots and diffuse leaf chlorosis or interveinal yellowing. The genome organization of this closterovirus was studied in the Netherlands by nucleotide sequencing (22). CCSV was partially purified, and a cDNA library constructed and screened with probes prepared from purified dsRNA. A set of overlapping clones covering over 16 kb of viral RNA (corresponding to at least 95% of the genome) was obtained. Search for similar known sequences revealed close relationships to beet yellows closterovirus (BYV). The genome organization of CCSV, however, was found to be clearly different from that of any previously analized plant virus (22).

Viruses transmitted by *T. abutiloneus*

1. Sweet potato yellow dwarf virus (SYDV).

The name " yellow dwarf "was given to a disease of sweet potato, component of the "Feathery mottle virus complex" reported from the USA since 1945 (10). The other two components of the complex, namely the "internal cork" and "leafspot" of sweet potato, were both transmitted by aphids. Hildebrand (10) observed that *T. abutiloneus*, the whitefly vector of SYDV, was abundant on the Indian Mallow weed, *Abutilon theophrasti*. Severe epiphytotics of SYDV in sweet potato occurred when hot, dry weather in summer allowed the whiteflies to increase so much that they defoliated the weed host, and then migrated to the sweet potato crops. No information is available on the morphological or biological characteristics of SYDV.

2. Abutilon yellows virus (AYV).

This virus was found in California, in **Abutilon** sp. plants affected by severe leaf chlorosis. It has filamentous, clostero-like particles resembling those of BPYV, CYV and similar viruses, and is trasmitted by *T. abutiloneus* in the semipersistent manner but not by sap inoculation (7). The other properties of AYV have so far not been determined.

3. Diodia vein chlorosis virus (DVCV).

This virus was isolated in south eastern USA from the Virginia buttonweed, *Diodia virginiana*, with symptoms of vein clearing and chlorosis. The virus has filamentous, flexuous particles 12 nm wide, and causes in its host cells the formation of fibril-containing vesicles deriving from the vacuolar membrane. In addition, greatly proliferated tubular membranes, and unique, double membrane-bound bodies were observed in the cytoplasm and within the vacuoles of the infected cells, respectively. DVCV was trasmitted both by *T. abutiloneus* and by grafting but not by sap inoculation. Double-stranded RNA isolated from virus infected Diodia and subjected to polyacrylamide gel electrophoresis migrated as three bands with estimated Mr of 4.6, 4.3 and 1.9 x 10^6, similar in size and number to those of closteroviruses (14).

The virus is probably the same as that found in *D. maritima* in California and provisionally named Diodea (= *Diodia*) yellow vein virus (7).

Viruses transmitted by *Parabemisia myricae*

P. myricae has been identified as a virus vector only recently, during investigations on citrus viruses in Turkey (13). The virus was found to infects grapefruit, lemon, mandarin and sweet orange, this latter species being less severely affected than the others. The symptoms, consisting of warping, pocketing and flecking of the young leaves,resemble those induced by citrus variegation virus (CVV), although they can be distinguished because the new citrus virus also causes reduction of the leaf size on all varieties, and severe leaf drop on ' Eureka' lemon.

The new virus seems presently to cause the most serious disease of citrus trees in the eastern mediterranean region of Turkey. Its presence, however, still seems to be restricted to two citrus-growing areas, near Icel and Adana respectively. The very high incidence of the disease in the former area suggests that the virus might have originally appeared there, then spread to other areas over long distances.

Discussion and Conclusions

With the exception of the above-mentioned new virus of citrus, which seem to be unique regarding both its vector and host plant species, all the other viruses transmitted by whiteflies other than *B. tabaci* (i. e. *T. vaporariorum* and *T. abutiloneus*) cause the same type of symptoms. These consist typically of yellow spots on the young leaves, interveinal yellowing or chlorosis, and mild downward curling of the mature leaves. In some cases, leaf-vein yellowing (DVCV) and phloem necrosis (CYV, MMYV) may also occur.

The particles of the viruses associated with the different diseases have been detected only in some cases. In all instances, they were flexuous filaments, 12 nm wide and about 1,000 nm long, with the only exception of BPYV which seems to have particles of 1,500-1,800 nm. Electron microscopic observations have revealed that, in infected plants, the virus particles occur only in the phloem sieve tubes and companion cells, and occasionally in the xylem. The cytopathological alterations observed include: cytoplasmic particle aggregates, particle-containing vesicles which apparently derive from vacuolar membranes, and viroplasm-like structures.

On the basis of particle morphology and cytopathological characteristics, such whitefly-borne viruses have been assigned to the closterovirus group (2).

This assignement has recently been confirmed by two findings: first, that close similarities exist between the genomes of CCSV and BYV, the type member of the closterovirus group (22); second, that the three bands obtained by polyacrylamide gel electrophoresis of dsRNA from DVCV-infected plants were very similar to those typical of closteroviruses (14).

It is not known whether the various diseases trasmitted by *T. vaporariorum* or *T. abutiloneus* are caused by different viruses or not. Serological relationships between the viruses that have been isolated and partially characterized have yet not been found. This is a consequence of the fact that few antisera are at the moment available (12, 15); probably because the whitefly-borne closteroviruses occur in relatively low concentration in plants, are restricted to the phloem, and possess labile particles that are difficult to purify.

Whiteflies are very difficult insects to control as virus vectors because they multiply rapidly and fly actively to plants, and their immature forms are quite resistant to insecticides. The following cultural practices, however, can be adopted to reduce virus incidence in crops: promptly destroy the old crops to prevent infected whiteflies from dispersing; avoid planting in proximity of

infected crops which may act as virus reservoirs; use perforated polyethylene covers or polypropylene sheets (AGRYL P17; Sodoca, France) to protect the nurseries from incoming insects; mulch the crops with sawdust, straw or yellow polyethylene sheets, that attract the whiteflies and kill them by heat. These and other control practices, including insecticide sprays, roguing or treating with herbicides before planting have recently been reviewed (8).

الملخص

كونتي، موريزيو. 1994.أنواع الذباب الأبيض غير *Bemisia tabaci* كناقل للفيروسات النباتية. مجلة وقاية النبات العربية: 12 (2): 121-126

أمكن في الستينات تحديد أنواع من الذباب الأبيض غير *Bemisia tabaci* تسهم بدور الناقل لعدد من الفيروسات وتشمل *Trialeurodes vaporariorum*، *T. abutilonea* وربما *Parabemisia myricae*. أما الفيروسات التي تنتقل بواسطة هذه الأنواع من الذباب الأبيض، والتي يمثلها فيروس الإصفرار الكاذب للشوندر، فقد سجلت في الولايات المتحدة الأمريكية- أوربا، اليابان، وأستراليا الاّ أنها لم توجد حتى الآن في افريقيا، أمريكا الجنوبية أو آسيا. وتصيب هذه الفيروسات نباتات تتبع الفصائل الرمرامية، المركبة، الصليبية، القرعية والباذنجانية مسببة أعراضاً مثل البقع الصفراء، الإصفرار الكامل، تثخن الأوراق والتفاف الأوراق الى الأسفل. ان الجزيئات الفيروسية التي تصاحب هذه الأمراض، والتي تشابه فيروسات الكلوستيرو، طولها 900-1000 نانومتراً (ن م) وعرضها 12ن م، وتحتوي على حمض نووي RNA وحيد السلسلة، ولم يتم دراستها على المستوى الجزيئي بعد، الا أن هناك تقدم في هذا الاتجاه. ولقد تم نقل هذه الفيروسات تجريبياً بواسطة التطعيم الا أنها لم تنقل بواسطة الإلقاح الميكانيكي، أو بحشرات المنّ أو بواسطة البذور. وتستطيع حشرات الذباب الأبيض نقل هذه الفيروسات بعد تغذي الحشرة، لمدة خمسة دقائق، لاكتساب الفيروس أو إلقاحه، وتزداد كفاءة الانتقال باطالة هذه الفترة . ولايوجد دليل واضح لوجود فترة حضانة داخل الحشرة، بالرغم من أنها تحتفظ بإمكانية النقل لستة أيام بعد اكتساب الفيروس. وسيتم مناقشة بعض النواحي الوبائية لهذه الأمراض مع تركيز خاص على الفيروسات التي تم كشفها في إيطاليا.

كلمات مفتاحية: النقل بالحشرات، الفيروسات التوأمية، الفيروسات الخيطية.

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