Transmission of Pelargonium Flower Break Virus (PFBV) in Irrigation Systems and by Thrips

G. KRCZAL, Landesanstalt für Pflanzenbau und Pflanzenschutz, Mainz, Germany; J. ALBOUY, I. DAMY, C. KUSIAK, and J. M. DEOGRATIAS, Station de Pathologie Végétale; J. P. MOREAU, Station de Zoologie, INRA (Institut Nationale de la Recherche Agronomique) Versailles, France; and B. BERKELMANN and W. WOHANKA, Forschungsanstalt Geisenheim, Germany

ABSTRACT

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Pelargonium flower break virus (PFBV) has become an important disease in glasshouses in Western Europe. In studies of the spread of PFBV via recirculating nutrient solutions, PFBV was found in nutrient solutions used with PFBV-infected *Pelargonium* plants 2 wk after starting the culture. In previously uninfected plants, PFBV was detected after 6 wk in culture. By 14 wk, 100% of the plants were infected. When the nutrient solution was decontaminated by means of slow sand filtration, infection of the plants was delayed by 6 wk, and the final percentage of infected plants was reduced to about one third. PFBV was also transmitted by thrips (*Frankliniella occidentalis*), and 30% of plants became infected when thrips were caged with PFBV-infected and healthy *Pelargonium* plants. When pollen from PFBV-infected plants was dusted onto the leaves of plants that were then caged with about 15 *F. occidentalis* per plant, about 40% of the *Pelargonium* plants became infected. No infection occurred with thrips or pollen alone. Pollination and inoculation by rubbing with pollen from PFBV-infected *Pelargonium* plants also resulted in virus-infected plants.

Pelargonium flower break virus (PFBV) was first described by Stone and Hollings (22). PFBV is now the most common viral pathogen in *Pelargonium* species and has reached high levels of incidence during the last few years. Little is known about the spread of the virus other than that PFBV is transmitted vegetatively and by infected cutting knives (15). These methods of transmission are inefficient (Albouy and Krczal, *unpublished*), so other means may have contributed to the recent increase in PFBV infections. The use of hydroponic systems with recirculating nutrient solutions may facilitate virus transmission. Tomato mosaic virus (9,14,17), cucumber green mosaic virus, tobacco necrosis virus, and lettuce big vein virus (14) are known to spread through circulating nutrient solutions. Carnation mottle virus, a member of the Carmovirus group like PFBV, was found to occur in rivers and lakes (11); therefore, we suspected that PFBV might be transmitted in irrigation systems.

Thrips-facilitated transmission of Ilarviruses has previously been reported (4–8, 21). Virus adhering to the outer surface of the pollen is transmitted by thrips (3,10). Since the western flower thrips *Frankliniella occidentalis* (Pergande) (23) was introduced to European greenhouses at the same time as PFBV infections increased, the possibility of PFBV transmission by thrips was investigated.

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This work was done to determine if PFBV could spread in cultivation systems with recirculated nutrient solution and if PFBV associated with virus-infected pollen could be transmitted from *Pelargonium* to *Pelargonium* by *F. occidentalis*.

MATERIALS AND METHODS

PFBV infection in recirculating nutrient solutions. Cultivation channels (Batu Rinnen, length 250 cm, width 15 cm) covered with polyethylene-foil were placed on four separate tables, and the solution was pumped from three separate nutrient supply units to drip-irrigate the plants. Excess nutrient solution was returned to the containers via slightly sloped table tops (Fig. 1). In one experimental unit, the nutrient solution was treated by slow sand filtration (25). All plants were raised in rock wool cubes (10 × 10 × 10 cm) and 24 plants, six of each cultivar, were placed on each table. The cultivars were Tango (*P.* × *zonale*), Schöne Helena (*P.* × *zonale*), Rio (*P.* × *zonale*), and Belladonna (*P.* × *peltate*). There was no root or leaf contact between plants during the experiment.

The nutrient solution consisted of 0.4–0.85% ammonium nitrate and a fertilizer mixture (Flory 9 Basisdünger). The pH was kept between 6 and 7 by different forms of nitrification. When pH was above 7, nitrification was done with ammonium sulfate; when pH was below 6, nitrification was done with calcium nitrate.

Infected plants were obtained by grafting 12-wk-old *Pelargonium* plants with scions from PFBV-infected *Pelargonium* shoots. Infection was verified 3 mo later by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) according to Clark and Adams (2) with PFBV-specific polyclonal antibodies (Loewe Biochemica, Otterfing, Germany). Cuttings were taken from the infected plants, rooted, and placed on the first table.

Beginning 2 wk after starting the culture, 200-ml samples of nutrient solution were taken from each container (i.e., control unit and units with and without filter) at weekly intervals. The samples were centrifuged at 100,000 *g* for 3 hr, and the resulting pellet was resuspended

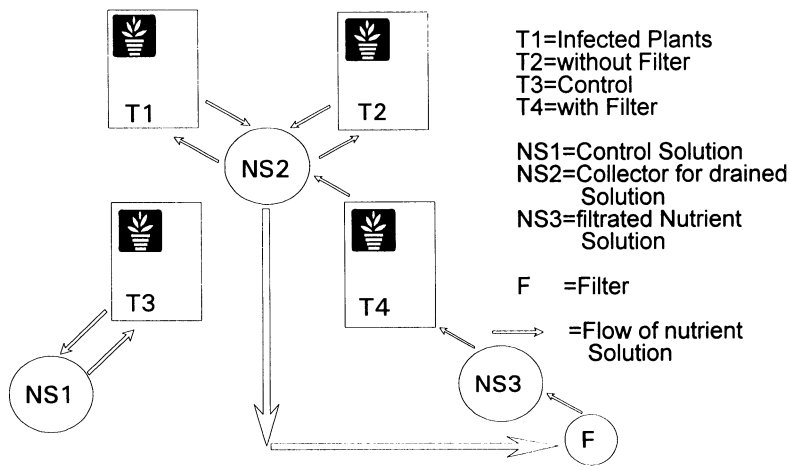

Fig. 1. Experimental setup for testing the spread of Pelargonium flower break virus (PFBV) in recirculating nutrient solutions.

in 1 ml of 0.1 M phosphate-buffered saline-Tween (0.05% Tween, 2% polyvinylpyrrolidone, 0.2% bovine serum albumin) and tested for virus by DAS-ELISA. Samples for DAS-ELISA were leaf and flower tissue collected from each plant every 2 wk on and after the 5th wk of the culture. The plant material was ground in the buffer used to resuspend the pellet after centrifugation and tested by DAS-ELISA. Root material was also tested by DAS-ELISA at the end of the experiment. Infectivity was tested by adding Carborundum as an abrasive to samples of the nutrient solution, which was then rubbed on leaf surfaces of *Chenopodium quinoa* L.

Two consecutive experiments were conducted. During the first experiment that began in summer (August), virus testing was performed once; the nutrient solution was tested in the 2nd wk and plant material in the 12th wk. During the second experiment, which began in winter (December), plant material was tested six times at intervals of 2 wk, beginning in the 5th wk. The nutrient solution was examined in the second and every subsequent week until the end of the experiment.

Virus was purified from PFBV-infected *C. quinoa* plants by saccharose gradient centrifugation (10–40%, 100,000 *g*, 3 hr). The virus zone was identified by light scattering and removed with a syringe and needle. The extinction coefficient of 1 mg/ml of virus was found to be 4.5 at 260 nm in accordance with Bouwen and Maat (1). This value was used to estimate virus concentration of the nutrient solution.

Transmission by pollen and thrips. To determine if pollen was contaminated with virus, pollen from PFBV-infected *Pelargonium* plants was washed five times with 0.1 M phosphate buffer (0.05% Tween, 2% polyvinylpyrrolidone) (1:200, w/v) after which no more virus could be detected in the washing solution by ELISA. Pollen was then crushed in the same buffer and the homogenate was tested by ELISA. Sampling of pollen from PFBV-infected *Pelargonium* plants was also done by gently brushing opened flowers with ripened anthers over leaves of test plants (*Pelargonium*) which were then mechanically inoculated with or without abrasive and without any buffer.

Pollination of healthy *Pelargonium* plants was done with pollen from PFBV-infected *Pelargonium* plants by confining the pollen or a mixture of pollen and an abrasive with the aide of a brush to the fully opened stigma of the flowers of test plants.

For transmission experiments, *F. occidentalis* were collected from healthy or PFBV-infected *Pelargonium* plants by brushing them onto a sheet of white paper. Thrips were also reared on French beans (*Phaseolus vulgaris* L.) and *Scaevola plumieri* L. at 25 C and 80% RH.

To test for PFBV transmission, 15 nonviruliferous *F. occidentalis* were confined to the inflorescence of a PFBV-infected *Pelargonium*. The assembly was completed by caging the plant together with five healthy *Pelargonium* plants. Controls consisted of a combination of healthy plants and thrips.

In addition, groups of 10 to 15 adult thrips were deposited onto pollen-dusted or nondusted young individual healthy *Pelargonium* test plants in cylindrical plastic cages that were pushed into the soil. Controls consisted of untreated *Pelargonium* plants, plants caged with virus-contaminated pollen only, and plants caged with thrips only.

In the last experiment, adult and larval *F. occidentalis* were left for an acquisition access feeding period on PFBV-infected *Pelargonium* plants for several days and then transferred to plastic cages containing healthy test plants. Symptoms on all test plants were observed for 12 wk, and the plants were tested for PFBV in duplicate by DAS-ELISA and dot blot ELISA according to Kusiak et al (12), where the antigen is applied to a nitrocellulose membrane with subsequent serological detection with PFBV-specific antibodies.

RESULTS

Spread and infectivity of PFBV in recirculating nutrient solutions. In both experiments, when virus particles were detected in the nutrient solutions by DAS-ELISA, a positive result was obtained in infectivity tests.

In both experiments, infective virus particles could be detected in the nutrient solutions after 2 wk. The virus titer in nutrient solution not passed through sand increased until the 4th wk after starting the culture. Virus titer of the filtered solution increased steadily until the end of the experiment, but it never reached the concentration of the unfiltered solution (Fig. 2).

In the second experiment, in plants grown in the unfiltered nutrient solution, the first PFBV-infected *Pelargonium* plant was detected in the 7th wk, and the number of infected plants increased between the 11th and 13th wk. At the end of the experiment at 15 wk, all plants cultured in the unfiltered nutrient solution were positive for PFBV. In the sand-

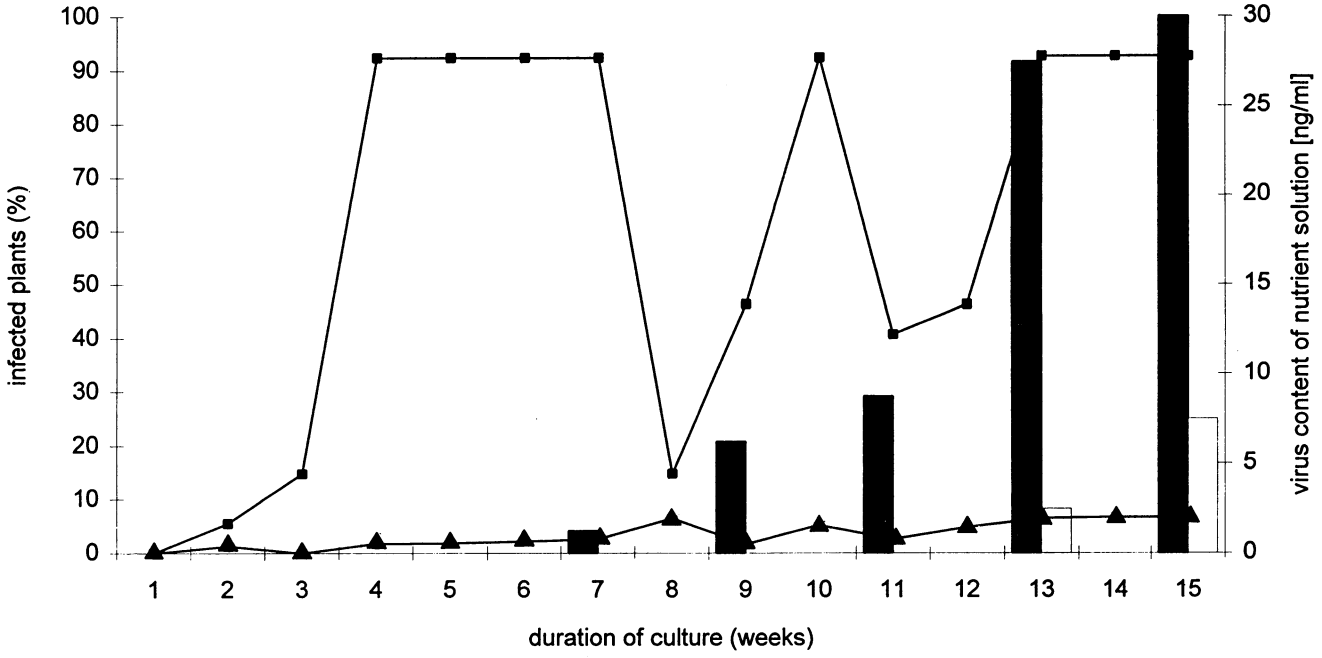

Fig. 2. Spread of Pelargonium flower break virus (PFBV) in *Pelargonium* cultures with recirculating nutrient solutions. Virus content of the nutrient solution, with (▲) and without (■) treatment of by slow sand filtration, and percentage of infected plants (bars) are shown.

filtered nutrient solution, infected plants were first detected in the 13th wk, and only 25% of the *Pelargonium* plants became PFBV-infected (Fig. 2).

In the first experiment (not shown) the percentage of infected plants detected in the 12th wk was comparable to that detected in the second experiment at about the same time. In the first experiment, 58.3% of the *Pelargonium* plants grown in the nutrient solution not sand-filtered were positive for PFBV after 12 wk. In the second experiment, 29.2 and 91,6% of the plants were positive for PFBV in the 11th the 13th week, respectively.

The percentage of infected plants irrigated with water treated by slow sand filtration was 37.5% at the end of the first experiment, compared with 25% in the second experiment.

No infection was detected in control plants in either of the experiments.

The virus was found to be unevenly distributed in the plant. When the leaf, flower, or root from PFBV-infected *Pelargonium* plants was tested, sometimes only one of the tissues was positive for PFBV. It is therefore recommended that mixed samples from different tissues of the same plant should be tested. A similar uneven distribution of a virus in *Pelargonium* plants was previously reported for *Pelargonium* line pattern virus (1).

Pollen transmission. Viral antigen could be detected by DAS-ELISA in the washing solution of intact pollen from PFBV-infected *Pelargonium* plants. Virus was not detected in pollen by DAS-ELISA after the pollen was washed five times (Fig. 3). When pollen was crushed after the last washing step, no increase in the optical density in the ELISA test could be observed.

Virions were easily removed from intact pollen by one washing. When test plants were mechanically inoculated with the addition of an abrasive, 100% of the plants were ELISA-positive after 6 wk. When pollen alone was brushed onto the leaves, no transmission occurred.

Of the *Pelargonium* plants pollinated with a mixture of infectious pollen and abrasive, 71.4% became infected in 3 to 6 wk compared with 14.3% pollinated without abrasive.

Transmission of PFBV by *F. occidentalis*. No test plants became infected when groups of *F. occidentalis* that had been fed on PFBV-infected *Pelargonium* were transferred to *Pelargonium* test plants.

When a PFBV-infected *Pelargonium* was caged together with healthy test plants and *F. occidentalis*, 12 of 40 test plants became infected in 4 to 6 wk. When both pollen and 10 to 15 thrips were placed on each *Pelargonium* plant, five of 12 test plants became infected. Omitting thrips, using thrips that were not loaded with virus-infected pollen, or using pollen in the absence of thrips did not result in any PFBV infections.

DISCUSSION

This is the first report of transmission of PFBV by recirculating nutrient solution and *F. occidentalis*. Although infectious virus particles could be detected in the nutrient solution 2 wk after the start of the culture, systemic infections in the tops of plants were detected after 7 wk. Root infections may have occurred earlier, but roots were only tested at the end of the experiment. The delay of about 5 wk between the first record of virus particles in the nutrient solution and systemically infected *Pelargonium* plants may represent the incubation period of PFBV in the plants. That both systemic top and root infection were similarly detected after 15 wk of culture indicates that virus readily moved from root to top, as do other viruses transmitted in this way (14). This may be due to the high concentration of virions in the nutrient solution. There are indications from other work (16) that high inoculum levels in soil result in a high percentage of systemic infections.

These results show that plants cultivated in systems with circulating nutrient solution are prone to infection when the virus becomes resident in the system and viruses are able to infect plants through the roots, without any vector.

To avoid virus spread in systems with recirculating nutrient solution, plants should be grown under hygienic conditions. Nutrient solutions should be treated to eliminate pathogens, and only healthy mother plants should be used for propagation as described below. Slow sand filtration recently applied to glasshouse cultivation systems proved to clean recirculated nutrient solutions reliably from fungal and bacterial pathogens (18). Our investigations demonstrated, however, that infection by PFBV was only reduced and delayed by 6 wk. Ultrafiltration and heat treatment at more than 90 C were effective in eliminating viruses from drain water (19). Both methods are expensive and only profitable when the cultivation area covers more than 1 ha (25).

Pollen from PFBV-infected *Pelargonium* plants was an effective source of inoculum, and *Pelargonium* plants could be infected by mechanical inoculation with pollen carrying the virus. PFBV seems to adhere to the outer surface of *Pelargonium* pollen.

Both thrips fed on PFBV-infected plants but free of pollen and pollen alone brushed on plants failed to cause PFBV infections; thus, both are required for PFBV infection. The pollen is scattered by the thrips, and infection may occur by virus entering leaf cells damaged by thrips feeding. Pollen transport by thrips may be limited because of their small size and tendency to preen before flying away. This effect is reflected in the lower transmission rates in thrips transmission experiments (30–40%) compared with the infection rates achieved by mechanical inoculation with PFBV-infected pollen. Systemic infection resulting from pollination with infected pollen occurred only to a small extent compared with pollination in the presence of an abrasive. It is not known if pollination with pollen derived from PFBV-infected *Pelargonium* plants results in PFBV-infected seeds, as may occur in Prunus necrotic ringspot virus (PNRV)-infected cherry trees (20). If it did happen, it would be of of minor importance in *Pelargonium* plants, which are propagated vegetatively.

The results of PFBV transmission by pollen and thrips indicate that transmission is mainly mechanical. This is supported by the fact that rub-inoculation of *Pelargonium* plants with PFBV-infected pollen requires the addition of an abrasive and that thrips do not become viruliferous just by feeding on the cell content of PFBV-infected *Pelargonium* plants. Also, infection caused by pollination with pollen-carrying virus particles occurs more often when an abrasive is added prior to

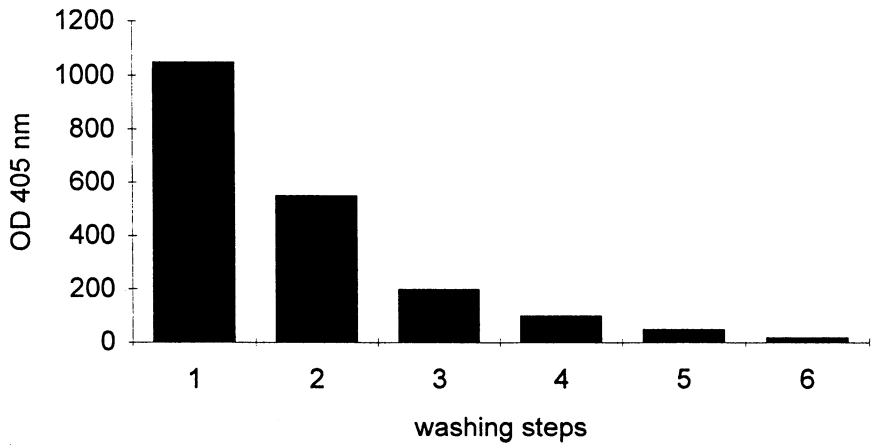

Fig. 3. Absorbance values obtained in enzyme-linked immunosorbent assays with subsequent washes of intact pollen (1–5) and ground pollen (6) from Pelargonium flower break virus (PFBV)-infected *Pelargonium* plants.

pollination.

This study indicates that the epidemic spread of PFBV seems to be related to the introduction of *F. occidentalis* to greenhouse cultures in western Europe. Tomato spotted wilt virus also became a glasshouse pest after the appearance of *F. occidentalis* in these regions (13,24). Thus, thrips management is important for virus control in glasshouses. Changes in cultivation systems and the introduction of *F. occidentalis* to glasshouses in western Europe resulted in an increase of PFBV infections in *Pelargonium* cultures. Means of dealing with the effects of these changes on management of virus diseases in glasshouses need to be devised.

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LITERATURE CITED

1. Bouwen, I., and Maat, D. Z. 1992. *Pelargonium* flower-break and *Pelargonium* line pattern viruses in the Netherlands. Neth. J. Plant Pathol. 98:141-153.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Cole, A., Mink, G. I., and Regev, S. 1982. Location of Prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathology 72:1542-1545.
4. Costa, A. S., and da Costa Lima Neto, V. 1976. Transmissao do virus da necrose branca do fumo por *Frankliniella* sp. Congr. Soc. Bras. Fitopathol. no.9
5. Greber, R. S., Klose, M. J., Milne, J. R., and Teakle, D. S. 1991. Transmission of Prunus necrotic ringspot virus using plum pollen and thrips. Ann. Appl. Biol. 118:589-593.
6. Greber, R. S., Klose, M. J., Teakle, D. S., and Milne, J. R. 1991. High incidence of tobacco streak virus in tobacco and its transmission by *Microcephalothrips abdominalis* and pollen from *Ageratum houstonianum*. Plant Dis. 75:450-452.
7. Greber, R. S., Teakle, D. S., and Mink, G. I. 1992. Thrips-faciliated transmission of prune dwarf and Prunus necrotic ringspot viruses from cherry pollen to cucumber. Plant Dis. 76:1039-1041.
8. Kaiser, W. J., Wyatt, S. D., and Pesho, G. R. 1982. Natural hosts and vectors of tobacco streak virus in eastern Washington. Phytopathology 72:1508-1512.
9. Kegler, H., Griesbach, E., and Skadow, K. 1982. Ausbreitung von Pathogenen beim Tomatenanbau im NFT-Verfahren. Arch. Gartenbau 7:325-337.
10. Kelly, R. D., and Cameron, H. R. 1986. Location of prune dwarf and Prunus necrotic ringspot viruses associated with sweet cherry pollen and seed. Phytopathology 76:317-322.
11. Koenig, R., and Lesemann, D.-E. 1985. Plant viruses in German rivers and lakes. Phytopathol. Z. 112:105-116.
12. Kusiak, C., Louanchi, M., Wang, W. C., and Albouy J. 1992. Evaluation of rapid ELISA procedures for detecting viruses in propagation stock of *Pelargonium, Dahlia* and orchids. Symp. Virus Diseases Ornamental Plants, Prague. Acta Hortic. (In press).
13. Marchoux, G. 1990. La transmission de virus par *Frankliniella occidentalis* et autres thrips. Phytoma 422:40-45.
14. Paludan, N. 1985. Spread of viruses by recirculated nutrient solutions in soilless cultures. Tidsskr. Planteavl 89:467-474.
15. Paludan, N., and Begtrup, J. 1987. *Pelargonium* flower-break virus: Infection trials, symptomatology and diagnosis. Tidsskr. Planteavl 91:183-193
16. Pares, R. D., and Gunn, L. V. 1989. The role of non-vectored soil transmission as a primary source of infection by pepper mild mottle and cucumber mosaic viruses in glasshouse-grown *Capsicum* in Australia. J. Phytopathol. 126:353-360.
17. Pares, R. D., Gunn, L. V., and Cresswell, G. C. 1992. Tomato mosaic virus infection in a recirculating nutrient solution. J. Phytopathol. 135:192-198.
18. Renker, G. 1993. Die Wirkung der Langsamfiltration gegen verschiedene phytopathogene Erreger. Diplomarbeit Fachhochschule Geisenheim/Wiesbaden, Fachbereich Gartenbau. 58 pp.
19. Runia, W. Th. 1988. Elimination of plant pathogens in drainwater from soilless cultures. Proc. Int. Congr. Soilless Cult. 7:429-443.
20. Schimanski, H.-H. 1988. Samenübertragbare Viren bei generativ vermehrten Veredlungsunterlagen für Steinobst und die Erzeugung virusfreien Saatguts. Nachrichtenbl. Pflanzenschutz. DDR 8:153-155.
21. Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by *Thrips tabaci*: A new method of plant virus transmission. Plant Pathol. 36:377-380.
22. Stone, O. M., and Hollings, M. 1973. Some properties of *Pelargonium* flower-break virus. Ann. Appl. Biol. 75:15-23.
23. zur Strassen, R. 1986. *Frankliniella occidentalis* (Pergrande 1985) ein nordamerikanischer Fransenflügler (*Tysanoptera*) als neuer Bewohner europäischer Gewächs-häuser. Nachrichtenbl. Deut. Pflanzenschutzdienstes. Braunschweig 38:86-88.
24. Vaira, A. M., Lisa, V., and Luisoni, E. 1992. Diffusione di une ceppi di tomato spotted wilt virus in Liguria. Inf. Fitopatol. 2:59-63.
25. Wohanka, W. 1991. Wasserentkeimung bei der Hydrokultur von Zierpflanzen. Gartenbau 38:43-46.