Two Diseases of Dimorphotheca Caused by Lettuce Mosaic Potyvirus and Tomato Spotted Wilt Tospovirus

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In 1997, virus-like symptoms were observed in dimorphotheca plants (*Dimorphotheca sinuata*) at two different locations in Greece. In a greenhouse near Heraklion (Crete), plants showed chlorotic patches mainly in the older leaves, and a mild stunting; near Katerini (Macedonia), garden plants showed chlorotic rings. Sap inoculation of healthy dimorphotheca plants with extracts from diseased plants from the two regions, reproduced each of the two diseases. Examination by electron microscopy (EM) of samples from diseased plants from the first region revealed filamentous particles, whereas samples from the second region showed quasi-spherical particles. The filamentous particles were decorated in EM with antibodies specific to lettuce mosaic potyvirus (LMV). Samples from the second location reacted with antibodies specific to tomato spotted wilt tospovirus (TSWV) in ELISA tests. It is concluded that LMV and TSWV are the causal agents of the disease noticed in Heraklion and in Katerini, respectively.

KEY WORDS: Dimorphotheca; natural infection; lettuce mosaic potyvirus; aphid transmission; tomato spotted wilt tospovirus; plant virus.

INTRODUCTION

Dimorphotheca (*Dimorphotheca* sp. Compositae), also known as 'African Daisy' (*D. sinuata*) or 'Rainy Daisy' (*D. pluvialis*), is widespread in the Mediterranean region. In recent years *D. pluvialis* has been used as a field crop for oil-seed production, particularly in northern Europe (5). In Greece, *D. sinuata* is the most common species and it is used mainly as an ornamental bedding plant. Sowing usually starts in late October and the plants are grown in greenhouses during winter. In winter 1997 in a greenhouse crop in Crete, a severe disease affecting approximately 30% of the cultivated plants was observed. Symptoms consisted of numerous yellow patches appearing mainly on the lower leaves, and yellow rings on the upper and nascent leaves (Fig. 1). Growth of the diseased plants was slightly retarded, but they flowered normally. However, the general appearance of the infected plants made them unsuitable for trade.

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Fig. 1. Dimorphotheca plants from a greenhouse cultivation in Crete, showing symptoms of the disease caused by lettuce mosaic potyvirus.

During the same period, in surveys in northern Greece, several infected plants with symptoms consisting mainly of chloritc rings were noticed in gardens. In this paper we describe our work for the characterization of the causal agents of both diseases, which were identified as lettuce mosaic potyvirus (LMV) and tomato spotted wilt tospovirus (TSWV), respectively.

MATERIALS AND METHODS

Electron microscopy

Leaves from diseased plants were extracted in phosphate buffer (0.07 M, pH 6.5), centrifuged at 8000 x*g* for 2–3 min, and the supernatants examined for virus particles by electron microscopy (EM) (4). For decoration tests, carbon-coated grids were floated on 20 μl drops of LMV antiserum for 1 h at 37°C. Grids were floated overnight at 0–4°C on leaf extracts from diseased plants and then floated on drops of LMV antiserum as described above. Washing with buffer was done between steps. Finally the grids were stained with sodium phosphotungstate 2% (pH 7.0) and examined in an electron microscope.

ELISA

ELISA tests were carried out with the buffer system (2). Polyclonal antibodies raised against the nucleocapsid (N) protein of a Greek isolate of TSWV (GR-34) (13) were used in a double antibody sandwich (DAS) ELISA. An antigen-coated (ACP) ELISA was carried out using mouse monoclonal antibodies specific to recognize members of the genus *Potyvirus* (AGDIA) according to the manufacturer's protocol. The absorbance values at 405 nm were corrected for blank values consisting of only buffer in the sample incubation step. Samples that exhibited values higher than twice the value of the healthy control were considered infected.

Host range studies

Mechanical transmission tests were carried out by grinding leaf material in 0.01 M phosphate buffer, pH 7.2, containing 0.1% sodium sulphite (Na_2SO_3) and inoculating carborundum-dusted cotyledons or leaves of test plants. Inoculated plants were kept for 4 weeks in a glasshouse at 18–24°C and 16 h photoperiod for symptom development.

Aphid transmission

Aphid transmission tests were done using viruliferous wingless adults of *Myzus persicae*. Aphids were starved for 2–4 h before they were given 2–5 min acquisition access on either a dimorphotheca or lettuce leaf with symptoms. Afterwards, they were transferred in groups of five to the cotyledons of 15 test plants (either lettuce or dimorphotheca). Plants were sprayed with an aphicide 24 h later, and transferred into an insect-proof glasshouse for 3–4 weeks to allow symptoms to develop.

RESULTS

Host range and symptoms

Inoculation with sap from plants from the Heraklion region caused numerous chlorotic local lesions in *Chenopodium amaranticolor* and *C. quinoa*, which progressed in necrotic ones, followed by systemic infection causing top distortion and necrosis. Inoculation in *Petunia hybrida* resulted in systemic leaf chlorosis, whereas sap inoculation in *Nicotiana benthamiana* caused a mild mosaic. Symptoms similar to those observed in the field were reproduced in *D. sinuata*, whereas in 13 out of the 15 lettuce varieties tested, the virus caused initially vein clearing followed by a typical mosaic. The infected lettuce varieties were: Parris Island, Atraxion, Bix, Niagara, Saladin, AF2, Gramsi, Attrazione, Verde Degli Ortolani, Vert Maraicher and three locally cultivated (Thessaly) ones of unknown origin. The two remaining varieties tested, namely, Myconos and ex 1801, did not show any signs of disease although they were not tested for latent infection. When the virus was transmitted by *M. persicae* to lettuce variety AF2, it caused local necrotic patches 10 days after inoculation, followed by vein necrosis.

Mechanical inoculation of indicator plants with the virus isolated from Katerini caused symptoms typical for TSWV infection (3). For example, it caused necrotic local lesions on *Petunia hybrida*, *Gomphrena globosa* and *Chenopodium quinoa* 3–4 days after inoculation, chlorotic rings and mosaic on *Nicotiana rustica* and *N. tabacum* cv. Samsun, and top necrosis on *N. benthamiana*.

Aphid Transmission

In two experiments, the virus from the Heraklion region was transmitted from and to dimorphotheca with an efficiency of approximately 60%. In another experiment, with lettuce as a source, the virus was transmitted in five out of ten dimorphotheca, and seven out of ten lettuce test plants.

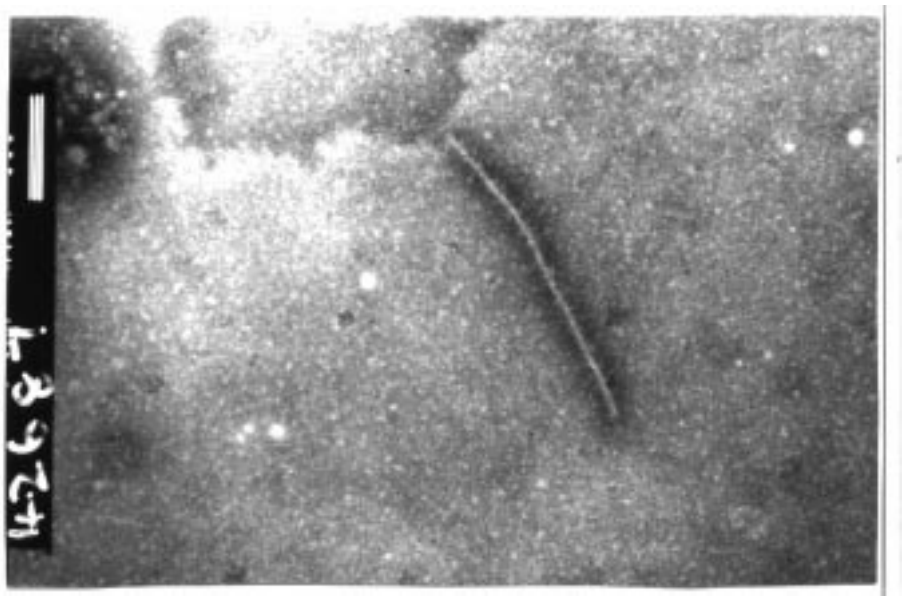

Fig. 2. A potyvirus-like particle decorated with antisera, prepared against lettuce mosaic potyvirus (Bar corresponds to 200 nm).

EM and ELISA

Samples from the Heraklion area revealed potyvirus-like particles 740 nm long and 12 nm wide, which were decorated by LMV antibodies (Fig. 2). In ELISA tests, these samples reacted with the monoclonal antibody specific for the potyvirus genus but none of them reacted with TSWV-specific antisera. The same results were obtained from plants infected by either sap inoculation or aphid transmission. Samples from the Katerini region examined by EM revealed groups of quasi-spherical particles with a diameter of 80–110 nm. Sap from these samples, tested by ELISA, reacted with the antiserum against TSWV N-protein.

DISCUSSION

There have not been many reports on dimorphotheca diseases, probably because this species has little commercial importance. In recent years, however, dimorphotheca has been used as a field crop, for seed oil production, particularly in northern Europe, and knowledge about pathogens infecting it may help to improve its productivity. To date only

five viruses, namely, alfalfa mosaic alfamovirus (AMV), cucumber mosaic cucumovirus (CMV), lettuce mosaic potyvirus (LMV), tomato spotted wilt tospovirus (TSWV) and a carlavirus not yet fully characterized, are known to infect dimorphotheca (7).

The causal agents of the two diseases found in Greece were shown to be LMV and TSWV, using biological and serological tests as well as particle morphology. This study showed that LMV can naturally infect and cause severe disease in dimorphotheca, reducing its commercial value as an ornamental. The way(s) the virus is spread in the greenhouse is not known, but our results show that at least one vector species, the aphid *M. persicae*, is capable of transmitting LMV from either dimorphotheca or lettuce to dimorphotheca plants and from dimorphotheca to lettuce. Vector transmissibility may be more important in open field crops, where the seasonal abundance of aphids is expected to be higher and the number of possible aphid vectors greater.

LMV is seed-transmitted in lettuce and genotype and temperature influence the level of transmission, which ranges from 3% to 10%. The weed *Senecio vulgaris* seems to be of major epidemiological importance (9). Seed transmission of this virus in lettuce is likely to be of major epidemiological importance but there is no information on seed transmission in dimorphotheca.

The strain of LMV present in Crete can also cause severe disease in lettuce. Indeed, 13 out of the 15 lettuce varieties tested were infected by the virus and they were finally killed; the other two varieties showed no symptoms but it is not known whether they were infected or not. The virus has not been found previously on the island of Crete, and it is not known whether it was introduced recently or has been present for a long time in the region. However, a lethal strain of LMV occurs on the mainland (Attica, Macedonia) in considerable frequency, and a semi-lethal LMV isolate was isolated from the ornamental *Tagetes* sp. as well as from the composite *Helminthia echioides* (6) (Katis, unpublished data).

The second virus found to infect dimorphotheca, according to the serological and biological tests, was identified as TSWV, which has spread rapidly in Greece during the last decade. The first report of this virus in Greece was in 1972, in tobacco crops in northern Greece (southeast and central Macedonia) (12). The virus has been spread mainly in tobacco crops for many years by *Thrips tabaci* Lindeman. The invasion of the thrips species *Frankliniella occidentalis* Pergande in 1988, first in Crete (11) and subsequently on the mainland (I. Tsitsipis, D. Lykouressis, personal communication), altered the epidemiology of the virus and resulted in the spread of TSWV among vegetable and ornamental crops. Recent studies (1993–95) of TSWV incidence in several host plants including vegetables and ornamentals, revealed that it is widespread throughout the country (1,8). Thrips isolated from dimorphotheca plants from Katerini were identified as *F. occidentalis.* The isolation of *F. occidentalis* individuals from diseased dimorphotheca plants supports its role in the spread of TSWV in this ornamental species. In the vicinity of dimorphotheca plants found infected by TSWV, many other ornamentals, such as *Dahlia hybrida*, *Chrysanthemum* sp., *Salvia splendens* and *Zinnia elegans* were also infected by TSWV (Chatzivassiliou and Katis, unpublished data). Outbreaks of TSWV in dimorphotheca were also reported in southern France and in northern Italy (10). Because of the losses that TSWV can cause and the difficulties in controlling its vector, the disease should be considered among the most destructive in dimorphotheca production.

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