Artichoke Yellow Ringspot Virus Infecting Vetch (*Vicia sativa*) in Greece

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A seed-transmitted virus was isolated from vetch seedlings showing severe stunting, reduced lamina and leaf mottling. Identification was based on host range, seed transmission, coat protein and nucleic acid analysis, electron microscopy and serology. This is the first record of vetch as a natural host of artichoke yellow ringspot virus (AYRSV).
KEY WORDS: Artichoke yellow ringspot nepovirus; vetch; seed transmission.

During a survey of seedborne viruses infecting local cultivars of fodder legumes, a seed-transmitted virus was isolated from a few vetch (*Vicia sativa*) seedlings. The virus was studied extensively and shown to be an isolate of artichoke yellow ringspot virus (AYRSV) (7), referred to here as AYRSV-V.

AYRSV-V was maintained in cucumber and *Chenopodium amaranticolor* plants. The experimental host range included 49 species in eight plant families, mainly Fabaceae and Solanaceae. Many hosts developed symptoms similar to those reported previously for AYRSV, for example, production of enations in cucumber, mottling and top necrosis in *Chenopodium quinoa,* and systemic ringspots and line patterns in leaves of *Nicotiana* species (1,2,6).

Myzus persicae and *Aphis fabae* failed to transmit the virus in a non-persistent or persistent manner (10 aphids/plant, 5 plants/replication, three replications).

Seedlings grown from seed collected from naturally infected vetch and from experimentally inoculated indicator host species were observed for symptoms and tested individually by inoculations to *C. amaranticolor.* The virus was detected in three out of 23 seedlings resulting from seeds of a naturally infected vetch plant and showing stunting and leaf mottling. It was also seed-transmitted in *C. amaranticolor* (89%), *C. quinoa* (4.2%), *Nicotiana benthamiana* (1.6%), *N. megalosiphon* (3.1%), *N. rustica* (5.5–11%) and *Trigonella foenum-graecum* (26.4%). In Fabaceae, seed transmission tests were limited by the severe symptoms in inoculated plants that remarkably reduced seed production. AYRSV-V showed no reduction in percent transmission through seeds of *C. amaranticolor* after storage for 2 years at room temperature. Treatments to eradicate virus from *C. amaranticolor* seeds (100 seeds/treatment × 2 replications) using 10% Na_3PO_4 for 10 min, at 60°C for 2 days and at 70°C for 3 days, were unsuccessful.

Virus particles were purified from systemically infected leaves of *C. amaranticolor* according to the method of Steere (9). When partially purified virus preparations were centrifuged through sucrose density gradients, they sedimented as three components. Such virus preparations had absorption spectra typical of nucleoproteins, with a maximum at A_{260nm} and a minimum at A_{240nm}; without correction for light scattering, the A_{260}/A_{280} absorption ratio varied from 1.71 to 1.90.

Received June 7, 2001; received in final form Oct. 1, 2001; http://www.phytoparasitica.org posting Jan. 13, 2002.
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The molecular weight of the virus coat protein was determined by electrophoresis of samples from purified virus particle preparations in SDS-polyacrylamide gels (4). Virus protein preparations contained a major polypeptide with an estimated molecular weight of 54,000.

Nucleic acid was extracted from purified virions by the SDS-phenol method (10) and electrophoresed in 1.2% agarose gel. Viral nucleic acid, assumed to be ssRNA, occurred as two species with molecular weights of 2.54×10^6 (RNA-1) and 1.8×10^6 (RNA-2).

Sap from diseased plants or purified virus preparations was stained with 2% sodium phosphotungstate, pH 6.0, and examined in an electron microscope. Isometric particles ~28 nm in diameter were noticed. In immune electron microscopy, done according to Roberts (8) by using AYRSV antiserum, many more particles were seen on grids coated with AYRSV antiserum than on grids without the antiserum.

In tests for serological relationships, the plate-trapped antigen procedure of ELISA was used (5). Antisera to the following nepoviruses held at the Scottish Crop Research Institute were used: Arabis mosaic, artichoke Italian latent, artichoke yellow ringspot, cherry leaf roll, chicory yellow mottle, crimson clover latent, grapevine Bulgarian latent, grapevine chrome mosaic, grapevine fanleaf, olive latent, raspberry ringspot, satsuma dwarf, strawberry latent ringspot, tomato black ring and tomato ringspot. AYRSV-V reacted clearly with antiserum of AYRSV (6), but failed to react specifically with any of the 14 other antisera to nepoviruses tested.

In conclusion, AYRSV was found to be the virus infecting seedlings of vetch in Greece. AYRSV-V was found to have some minor host range and/or symptom differences compared with isolates from artichoke (6), cucumber (2) and broad bean (1). As regards determinations on coat protein and nucleic acid, small differences occurred between AYRSV-V and artichoke isolate (6,7). However, the poor quality of available antisera (reaction with healthy sap even after cross absorption) did not allow us to conclude whether the vetch isolate was different from other described isolates. It should be noted that in all AYRSV isolates reported, comparison studies were unsuccessful as the tendency of virus particles to aggregate during purification could not be overcome.

AYRSV was shown to have a wide natural and experimental host range and to be seed-borne in four natural hosts and in 14 test plant species (3). In our investigations, AYRSV-V was also found to have a wide experimental host range and to be seed-transmitted in vetch and in six test plant species. Among them, *N. benthamiana*, *N. megalosiphon* and *T. foenum-graecum* are reported here for the first time. These properties seem to play a major role in survival and perpetuation of AYRSV. The virus persists in seeds of *C. amaranticolor* for at least 2 years without a decline in the level of seed transmission. Attempts to eradicate the virus using thermotherapy and chemical treatments were unsuccessful, suggesting that the seed embryo is infected, as has been reported previously (1). If so, the virus may persist in seeds as long as they remain viable.

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